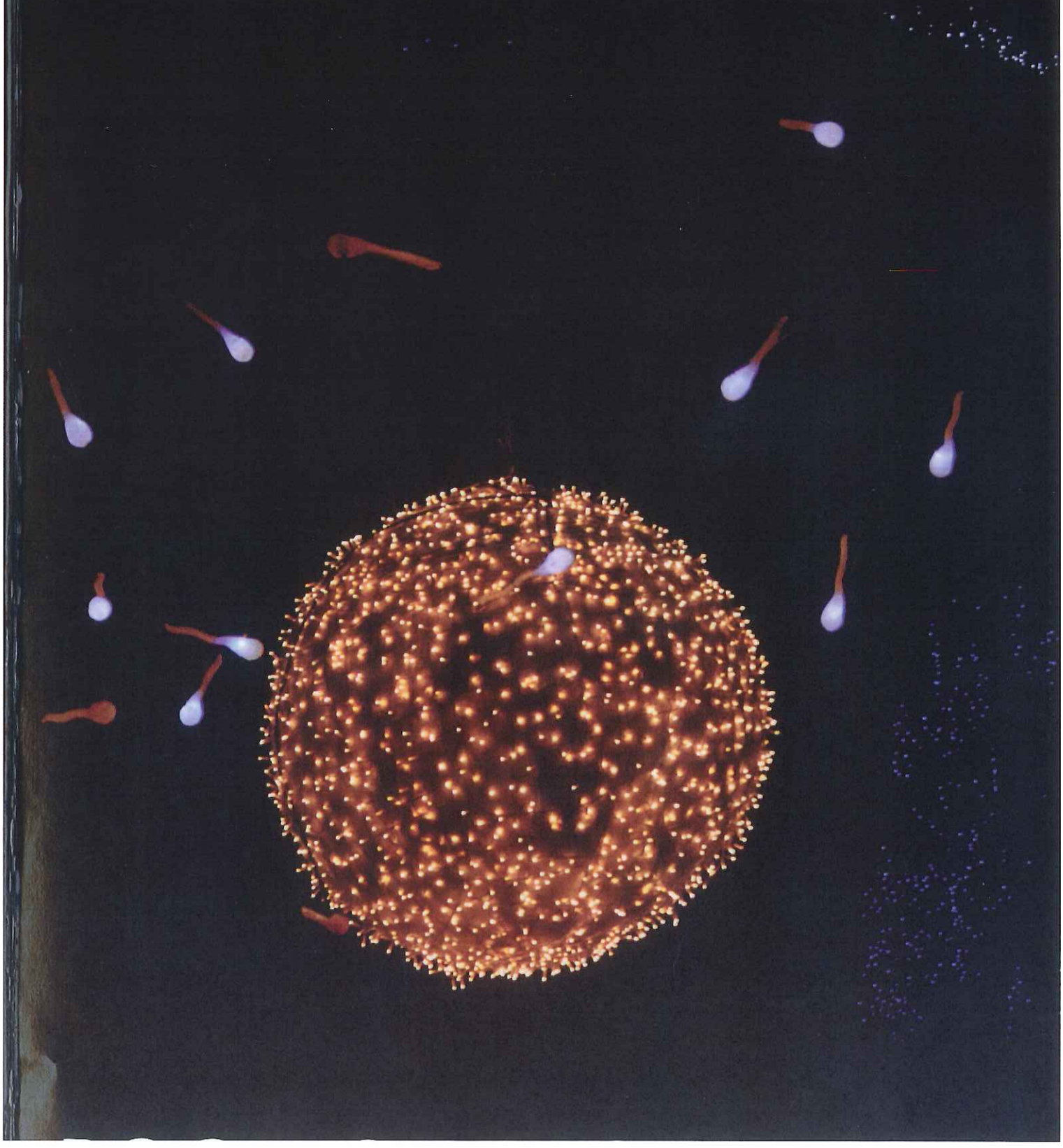
FEPS 2009

12 - 15 November, Ljubljana, Slovenia



FEPS 2009

The physiology meeting organized by *The Slovenian Physiological Society, The Austrian Physiological Society, and The Federation of European Physiological Societies.*

BOOK OF ABSTRACTS

12 - 15 November 2009, Ljubljana, Slovenia

Editors:
Marko Kreft
Nina Vardjan
Robert Zorec

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Stritarjeva ulica (Stritar street) in Ljubljana adorned with the winter street lighting project The Path of Life as a Microcosm, conceived by the artist Zmago Modic.
Photo: Marko Kreft

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CONTENTS

SUPPORTED BY:

- The Slovenian Physiological Society
- The Austrian Physiological Society
- FEPS - Federation of European Physiological Societies
- Faculty of Medicine, University of Ljubljana
- University Medical Centre Ljubljana
- LN-MCP, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana
- Cell Engineering Laboratory, Celica Biomedical Center, Ljubljana
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SCOPE OF THE CONFERENCE

The meeting will cover a broad spectrum of topics in basic and applied physiology. The main goal is to bring together key scientists with various backgrounds and expertises in order to discuss the main developments, achievments and perspectives in physiology. Young researchers are especially welcome.

The meeting will be comprised of symposia, which will typically include four invited lectures. All physiological societies composing FEPS were invited to formulate the scientific programme by submitting symposia proposals. Symposia topics were selected from submitted proposals.

Programme and Organizing Committee

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INVITED LECTURERS

(ALPHABETICALLY)

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Manchester, UK

Laura Ballerini
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Daniele Bano
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Wouter van Marken Lichtenbelt
Maastricht, The Netherlands

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Dortmund, Germany

Juergen Winkler
Erlangen, Germany

Ming Zhang
Chicago, USA

Hans H. Zingg
Montreal,Canada

Robert Zorec
Ljubljana, Slovenia

Antonio Zorzano
Barcelona, Spain

EYPS Invited Speakers:

Gertrudis Perea
Madrid, Spain

Iman S. Gurung
Cambridge, UK

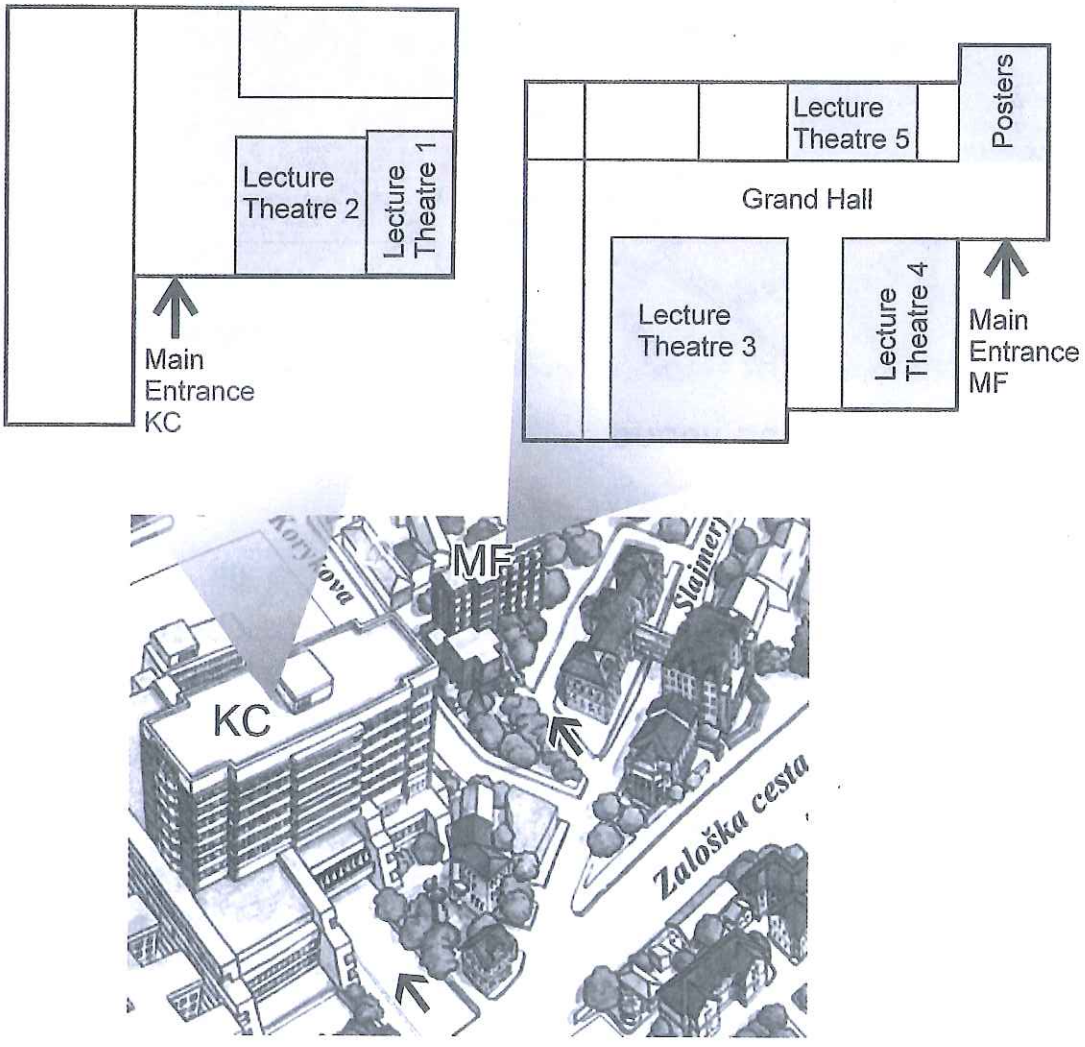
Meeting Venue

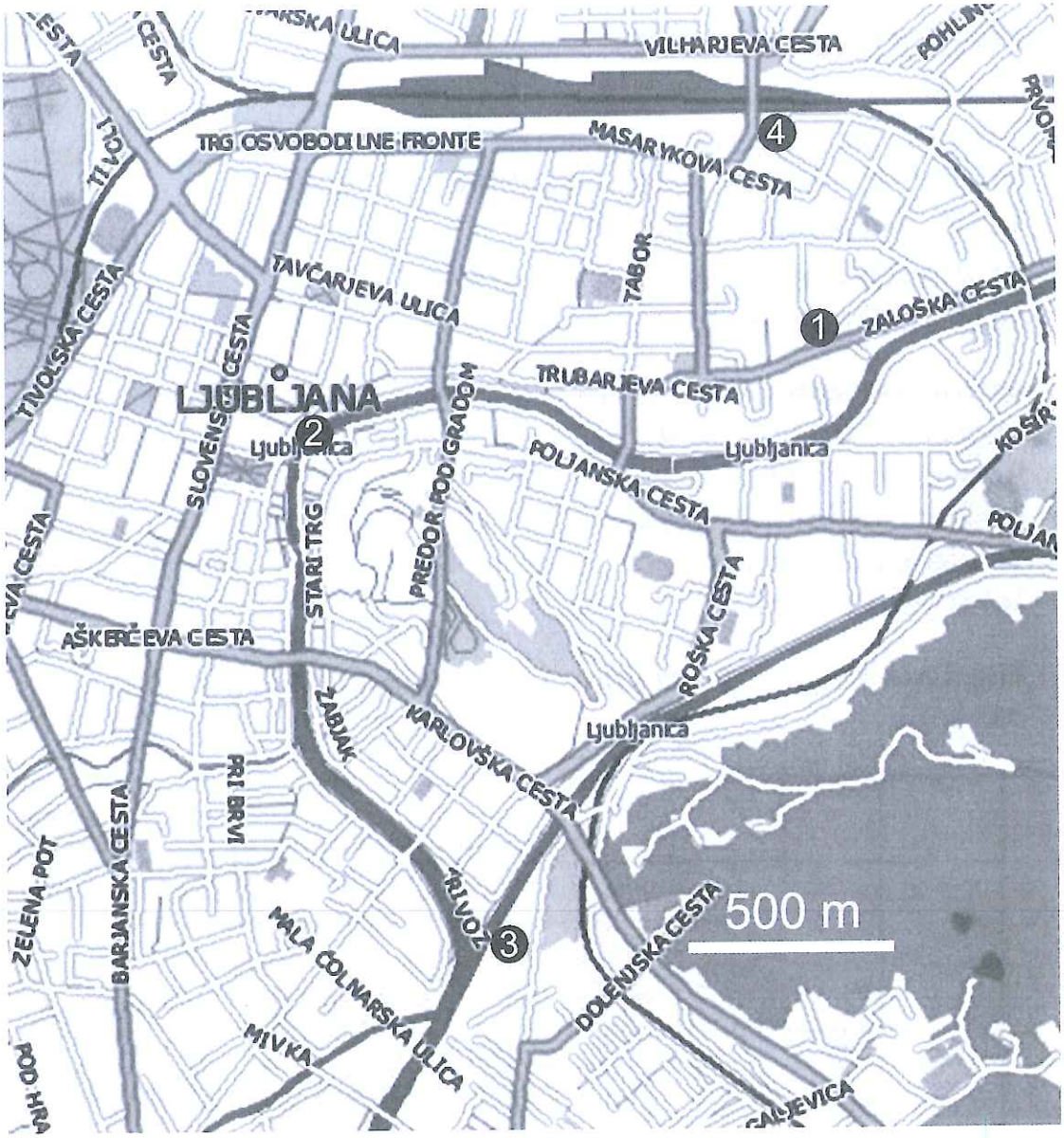
The Meeting will be held at:
Faculty of Medicine, Korytkova 2, and
University Medical Centre Ljubljana, Zaloška 2,
SI-1000 Ljubljana, Slovenia

At the University Medical Centre (KC):
Lecture Theatres 1, 2

At the Faculty of Medicine (MF):
Lecture Theatres 3, 4, 5
Registration Desk
Poster Communications
Trade Exhibition
Internet Lounge

The two buildings are connected by an underground passage.





1 – The meeting venue

2 – The Three Bridges (City centre)

3 – The Special Event will be held on 14th Nov. 09 at 20:00 in Ljubljana Music and Ballet School, Ižanska cesta 12. Walking tour to the special event will be organized from The Three Bridges (location 2 on the map). Departure at 19:30.

4 – The Young Physiologisits' Party will be held on 13th Nov. 09 at 20:30 in Orto Bar, Grablovičeva Ulica 1.

Programme Overview

Thursday, 12. November 2009:

Grand Hall MF	Lecture Theatre 1	Lecture Theatre 2	Lecture Theatre 3	Lecture Theatre 4	Lecture Theatre 5
	10:00-11:20 EYPS				
	11:20-11:40 Break				
	11:40-12:55 EYPS				
	12:55-13:55 Lunch				
	13:55-15:10 EYPS	14:00-15:15 Educational Workshop			
	15:10-15:30 Break	15:15-15:45 Break			
	15:30-16:45 EYPS	15:45-16:45 Educational Workshop			
			17:00-17:30 Opening Ceremony		
			17:45-19:00 Keynote Lecture		
19:00- Welcome and get-together					

FRIDAY, 13. NOVEMBER 2009:

GRAND HALL MF	LECTURE THEATRE 1	LECTURE THEATRE 2	LECTURE THEATRE 3	LECTURE THEATRE 4	LECTURE THEATRE 5
	09:00-11:00 SESSION IIA	09:00-11:00 SESSION IIB	09:00-11:00 SESSION IIC	09:00-11:00 SESSION IID	09:00-11:00 SESSION IIE
11:00-11:30 BREAK					
		11:30-13:00 SESSION IIIA	11:30-13:00 SESSION IIIB	11:30-13:00 SESSION IIIC	11:30-13:00 SESSION IIID
13:00-14:00 LUNCH					
14:00-15:30 POSTER SESSION IV & EXHIBITION					
			15:30-16:30 KEYNOTE LECTURE		
16:30-17:00 BREAK					
	17:00-19:00 SESSION VA	17:00-19:00 SESSION VB	17:00-19:00 SESSION VC	17:00-19:00 SESSION VD	17:00-19:00 SESSION VE
20:00- DEPARTURE TO YOUNG PHYS. PARTY OR WINE TASTING					

20:30 - YOUNG PHYSIOLOGISTS' PARTY: ORTO BAR, GRABLOVIČEVA ULICA 1.

SATURDAY, 14. NOVEMBER 2009:

GRAND HALL MF	LECTURE THEATRE 1	LECTURE THEATRE 2	LECTURE THEATRE 3	LECTURE THEATRE 4	LECTURE THEATRE 5
	09:00-11:00 SESSION VIA	09:00-11:00 SESSION VIB	09:00-11:00 SESSION VIC	09:00-11:00 SESSION VID	09:00-11:00 SESSION VIE
11:00-11:30 BREAK					
			11:30-13:00 SESSION VIIB	11:30-13:00 SESSION VIIC	11:30-13:00 SESSION VIID
13:00-14:00 LUNCH					
14:00-15:30 POSTER SESSION VIII & EXHIBITION					13:30-15:30 FEPS COUNCIL MEETING
			15:30-16:30 KEYNOTE LECTURE		
16:30-17:00 BREAK					
	17:00-19:00 SESSION IXA	17:00-19:00 SESSION IXB	17:00-19:00 SESSION IXC	17:00-20:00 SESSION IXD	17:00-19:00 SESSION IXE

20:00 THE SPECIAL EVENT: LJUBLJANA MUSIC AND BALLET SCHOOL, IŽANSKA CESTA 12.

SUNDAY, 15. NOVEMBER 2009:

GRAND HALL MF	LECTURE THEATRE 1	LECTURE THEATRE 2	LECTURE THEATRE 3	LECTURE THEATRE 4	LECTURE THEATRE 5
			09:00-10:30 SESSION XB	09:00-10:30 SESSION XC	09:00-10:30 SESSION XD
10:30-11:00 BREAK					
	11:00-13:00 SESSION XIA	11:00-13:00 SESSION XIB	11:00-13:00 SESSION XIC	11:00-13:00 SESSION XID	
13:00-14:00 LUNCH					
14:00-15:30 POSTER SESSION XII & EXHIBITION					
			15:30-16:00 FEPS BEST POSTER AND BEST LECTURE AWARD		

SATURDAY, 14. NOVEMBER 2009:

GRAND HALL MF	LECTURE THEATRE 1	LECTURE THEATRE 2	LECTURE THEATRE 3	LECTURE THEATRE 4	LECTURE THEATRE 5
	09:00-11:00 SESSION VIA	09:00-11:00 SESSION VIB	09:00-11:00 SESSION VIC	09:00-11:00 SESSION VID	09:00-11:00 SESSION VIE
11:00-11:30 BREAK					
			11:30-13:00 SESSION VIIB	11:30-13:00 SESSION VIIC	11:30-13:00 SESSION VIID
13:00-14:00 LUNCH					
14:00-15:30 POSTER SESSION VIII & EXHIBITION					13:30-15:30 FEPS COUNCIL MEETING
			15:30-16:30 KEYNOTE LECTURE		
16:30-17:00 BREAK					
	17:00-19:00 SESSION IXA	17:00-19:00 SESSION IXB	17:00-19:00 SESSION IXC	17:00-20:00 SESSION IXD	17:00-19:00 SESSION IXE

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GRAND HALL MF	LECTURE THEATRE 1	LECTURE THEATRE 2	LECTURE THEATRE 3	LECTURE THEATRE 4	LECTURE THEATRE 5
			09:00-10:30 SESSION XB	09:00-10:30 SESSION XC	09:00-10:30 SESSION XD
10:30-11:00 BREAK					
	11:00-13:00 SESSION XIA	11:00-13:00 SESSION XIB	11:00-13:00 SESSION XIC	11:00-13:00 SESSION XID	
13:00-14:00 LUNCH					
14:00-15:30 POSTER SESSION XII & EXHIBITION					
			15:30-16:00 FEPS BEST POSTER AND BEST LECTURE AWARD		

MEETING PROGRAMME

THURSDAY, 12. NOVEMBER 2009:

10:00-16:45 **Session I - EYPS**

LECTURE THEATRE 1

European Young Physiologist Symposium

Chairs: *Jernej Jorgačevski, Elisa Luin*

Key note EYPS: Astrocytes potentiate hippocampal synaptic transmission

Gertrudis Perea

Feasibility of non-quantal release of acetylcholine from the postganglionic parasympathetic nerves in the right atrium of rat

Denis V. Abramochkin

Role of potassium currents in the repolarization of canine ventricular myocardium under action potential clamp conditions

Balazs Horvath

TMEM16B is a novel Ca^{2+}-activated Cl^- channel candidate in vertebrate olfactory transduction

Michele Dibattista

Break 11:20-11:40

Chairs: *Michele Dibattista, Giada Cellot*

The significance of lipid raft/caveolae microdomains in several aspects of the NK1-R biology

Alenka Hrovat

Actin-dependent volume recovery of embryonic cell subjected to hypoosmotic shock

Maria Pogorelova

The role of reactive oxygen species on proliferation and Ca^{2+} signalling of skeletal muscle cells during *in vitro* ageing

Elisa Luin

Embryonic organotypic cultures: An *in vitro* model for excitotoxicity-due spinal cord injury

Graciela L. Mazzone

Nicotine enhances sub threshold resonance behavior of oriens-lacunosum moleculare interneurons in the mouse hippocampus

Marilena Griguoli

Lunch 12:55-13:55

Chairs: *Gertrudis Perea, Denis V. Abramochkin*

Key note EYPS: Role of substrates, metabolites and transcriptional regulation of metabolism in cardiac arrhythmia

Iman S. Gurung

Differential molecular consequences of excitotoxicity and metabolic dysfunction after in vitro experimental spinal cord injury

Anujaianthi Kuzhandaivel

Effect of free oxygen radicals on excitability of rat hypoglossal motoneurons *in vitro*

Francesca Nani

Early in postnatal development, tonic activation of kainate receptors by ambient glutamate reduces GABA release from mossy fiber terminals

Maddalena Delma Caiati

Break 15:10-15:30

Chairs: *Iman S. Gurung, Balazs Horvath*

The effect of gender and hand skin perfusion on tissue insulation during cold exposure

Boris RM Kingma

Loss of LTD at MF-CA3 synapses overexpressing GLT-1

Victoria Safiulina

Neuronal functional differentiation in mammalian postnatal stem/precursor cells

Alberto Montalbano

Carbon nanotubes direct interactions with neuronal membranes ignite post spike excitability

Giada Cellot

Gephyrin regulates both GABAergic and glutamatergic synaptic transmission in cultured hippocampal neurons

Rocco Pizzarelli

14:00-16:45 LECTURE THEATRE 2

Educational Workshop (organised by FEPS):

Reappraisal of basic sciences in the construction of medical curricula and how to evaluate study progress by portfolio

Chairs/Organizers:

Liisa Peltonen, Helsinki, Finland
Luc Snoeckx, Maastricht, The Netherlands.

What science should be included within integrated curricula?

Karen Mattick, Exeter, UK

Teaching in physiology and pathophysiology in the medical curriculum

Brigitte Velkeniers, Brussels, Belgium

Break 15:15-15:45

Portfolio-based assessment of medical and generic competencies in the physician – clinical investigator master program at the Maastricht University

Sylvia Heeneman, Maastricht, The Netherlands

Perspectives on test-enhanced learning and basic science

Charlotte Ringsted, Copenhagen, Denmark

17:00-17:30 LECTURE THEATRE 3

FEPS 2009 Opening Ceremony

17:45-19:00 LECTURE THEATRE 3

FEPS Keynote Lecture

Measuring and manipulating second messenger levels in cellular organelles of living cells

***Tullio Pozzan**, Padova, Italy*

19:00-

Welcome and get-together party

FRIDAY, 13. NOVEMBER 2009:

09:00-11:00 **Session IIa - Symposium**

LECTURE THEATRE 1

Astrocyte dynamics in health and disease

Chair/Organizer:

Milos Pekny, Gothenburg, Sweden

Glial fibrillary acidic protein and vimentin are negative regulators of the neurogenic niche

Milos Pekny

Reactive astrogliosis and post neurotrauma recovery: Human neural stem cell-reactivated proprioneuronal circuitry in GFAP$^{-/-}$Vimentin$^{-/-}$ mice following spinal cord injury

Yang (Ted) D. Teng

Astrocyte subtypes and GFAP isoforms in the developing and adult brain

Elly M. Hol

Properties of regulated exocytosis and vesicle trafficking in astrocytes

Marko Kreft

09:00-11:00 **Session IIb - Symposium**

LECTURE THEATRE 2

Assessment and consequences of asynchronous activation of the ventricles

Chair/Organizer:

Frits W. Prinzen, Maastricht, The Netherlands

The quest for ventricular pacing sites that preserve cardiac contractility and efficiency

Frits W. Prinzen

Contraction patterns in dyssynchronous hearts

Borut Kirn

Pacing-induced protection in the embryonic chick heart model

Eric Raddatz

Role of ventricular activation for arrhythmogenesis in the CAVB dog model

Thom R.G. Stams

09:00-11:00

LECTURE THEATRE 3

Session IIc - Symposium

The physiology of endocrine pancreas

Chair/Organizer:

Marjan Rupnik, Maribor, Slovenia

The physiology of rodent beta-cells in pancreas slices

Marjan Rupnik

Ion channels and the regulation of insulin secretion: of mice and men

Patrik Rorsman

Protein serotonylation modulates insulin secretion

Diego J. Walther

Cell swelling-mediated stimulus-secretion-coupling and actions of insulin in clonal insulinoma cells

Martin Jakab

09:00-11:00

LECTURE THEATRE 4

Session IId - Symposium

Serpins: A family of proteins regulating a variety of physiological processes

Chair/Organizer:

Margarethe Geiger, Vienna, Austria

Structure and function of serpins in blood coagulation system

Wei Li

Protein C inhibitor: A serpin with multiple functions

Margarethe Geiger

Serpin in cancer: Maspin as a paradigm

Ming Zhang

09:00-11:00 **Session IIe – Symposium**

LECTURE THEATRE 5

Molecular Physiology of Hearing

Chair/Organizer:

Tina Pangršič, Göttingen, Germany

Ca^{2+} signals regulating hair cell transmitter release

Tobias Moser

Pathology of cochlear ion transport: Insights from deaf mice and humans

Thomas J. Jentsch

Roles for prestin in amplification and frequency tuning in the cochlea

Ian Russell

Molecular perturbation of a large CNS auditory synapse yields new insights into transmitter release and active zone function

Ralf Schneggenburger

11:30-13:00 **Session IIIa - Educational Workshop**

LECTURE THEATRE 2

Computer models in physiology

Chair:

Luc Snoeckx, Maastricht, The Netherlands

An interactive model for oxygen transport and acid base physiology in human body

Vito Starc

High altitude adaptation in the respiratory control system. A mathematical model simulation

Vito Starc

11:30-13:00 **Session IIIb - Oral Presentations**

LECTURE THEATRE 3

Exercise physiology

Chair:

Jørgen Jensen, Oslo, Norway

Changes in the venous blood oxygen stores at the onset of exercise

Maria Pia Francescato

Effects of grape seed extract on oxidative damage and antioxidant defense induced by acute and chronic exercise in rats

Hakkı Gökbel

The effect of interval training combined with external pressure on thighs on maximal and submaximal performance

Michail E. Keramidas

The levels of leptin, ghrelin and resistin after chronic exercises in rats: The effect of caffeic acid phenethyl ester (CAPE) on these parameters

H. Serdar Gergerlioğlu

11:30-13:00

LECTURE THEATRE 4

Session IIIc - Oral Presentations

Diabetes, obesity, pancreas-physiology

Chair:

Jan Kopecky, Prague, Czech Republic

The role of endocrine tissue network topology in beta-cell bursting oscillations patterns

Dean Korošak

Cardiac ryanodine receptors: A novel therapeutic target in diabetic cardiomyopathy

Belma Turan

The contribution of Stim1 and Orai1 to Ca^{2+} homeostasis and insulin secretion in INS1-R9 β-cells

Ismene Fertschai

Effects of grape seed extract supplementation on plasma oxidative stress and antioxidant defense markers in diabetic rats

Nilsel Okudan

Possible involvement of leptin-AMPK axes in induction of muscle nonshivering thermogenesis by high-fat diet in mice: Association with obesity-resistance

Jan Kopecky

Body composition and body fat percentage of students at the University of Latvia in 2007-2009

Liga Ozolina-Moll

11:30-13:00

LECTURE THEATRE 5

Session IIId - Oral Presentations

Respiratory and cardiovascular physiology

Chair:

Ger J. van der Vusse, Maastricht, The Netherlands

The impact of family history for vascular pathology on the autonomic cardiovascular function in young normotensive individuals

Nina Belova

Lung mechanics measurements by the end-inflation occlusion method in mice

Alessandro Rubini

Hemifusion states of variable duration precede exocytotic fusion pores in alveolar type II cells

Pika Miklavc

Postnatal changes in the cardiomyocyte- contractility and calcium transients

Sarah Martin

14:00-15:30

POSTER AREA MF

Session IV Poster Session and Exhibition

15:30-16:30

LECTURE THEATRE 3

Keynote Lecture

The Glial Regulation of Sleep and Memory

***Philip Haydon**, Boston, USA*

Break 16:30-17:00

17:00-19:00

LECTURE THEATRE 1

Session Va - Symposium

Glia in neurodegenerative processes

Chair/Organizer:

José Julio Rodríguez Arellano, Manchester, UK

Astroglial atrophy and hypertrophy in the hippocampus and entorhinal cortex during the progression of Alzheimer's disease: A combined process

José Julio Rodríguez Arellano

Diffusion parameters of the extracellular space in health and disease

Eva Sykova

Glutamate and ATP excitotoxicity in white matter damage

Carlos Matute

Microglial responses to acute spinal cord injury in vivo

Frank Kirchhoff

17:00-19:00

LECTURE THEATRE 2

Session Vb - Symposium

Calcium signals in cell death and disease

Chairs/Organizers:

Alexei Verkhratsky, Manchester, UK

Ole H. Petersen, Liverpool, UK

Physiology and pathophysiology of calcium signalling in pancreatic acinar cells

Ole H. Petersen

Alteration of nucleocytoplasmic transport occurs during calcium-mediated cell death

Daniele Bano

Aberrant calcium signalling in amyotrophic lateral sclerosis

Julian Grosskreutz

Dynamic calcium stores, calcium signalling and neurodegeneration

Alexei Verkhratsky

17:00-19:00

LECTURE THEATRE 3

Session Vc - Symposium

Regulation of synaptic transmission

Chair/Organizer: *Stephen Smith, Portland, USA*

Regulation of nerve terminal function by the extracellular Calcium-sensing receptor

Stephen M. Smith

Trafficking and function of v-SNARE protein VAMP4 at central synapses

Jesica Raingo

Quantal size and multivesicular exocytosis at the hair-cell synapse: Paired recordings with membrane capacitance measurements

Henrique von Gersdorff

Regulation of synaptic transmission by the ubiquitin-proteasome system

Felix E. Schweizer

17:00-19:00

LECTURE THEATRE 4

Session Vd - Symposium

Histamine and CNS

Chair/Organizer: *Mojca Kržan, Ljubljana, Slovenia*

Is brain histamine involved in the response to antidepressant drugs?

Patrizio Blandina

The role of neuronal histamine and H3 receptors in alcohol-related behaviour

Pertti Panula

Interactions between histaminergic and cannabinoid systems; a potential therapeutic target for cognitive and feeding behaviour impairments?

Maria Beatrice Passani

Histamine as a regulator of astrocyte function

Metoda Lipnik-Štangelj

Astrocytes as a site for histamine inactivation

Mojca Kržan

17:00-19:00 **Session Ve - Symposium**

LECTURE THEATRE 5

Obesity and cardiac cellular physiology

Chair/Organizer:

M.-Saadeh Suleiman, Bristol, UK

What has MR told us about the heart in obesity and diabetes?

Kieran Clarke

Mice fed high-fat diet display altered metabolic characteristics of their hearts and myocytes and increased vulnerability to cardiac insult

Anabelle Chase

Differential analysis of the cardiac proteome of rats artificially selected for either low or high aerobic capacity

Jatin Burniston

High-Fat diet, coronary disease and myocardial protection

M.-Saadeh Suleiman

20:30- **Young Physiologists' Party**

Optional Wine Tasting 20.00

SATURDAY, 14. NOVEMBER 2009:

09:00-11:00

LECTURE THEATRE 1

Session VIa - Symposium

AMP-activated protein kinase: Regulation of energy supply at cellular and whole body level

Chair/Organizer:

A. Mark Evans, Edinburgh, UK

AMP-activated protein kinase and hypoxia-response coupling in the carotid body and pulmonary artery

A. Mark Evans

Role of hypothalamic fatty acids, AMPK and ghrelin in the regulation of food intake

Miguel López

5 AMP-activated protein kinase (AMPK): A role in female reproduction?

Joëlle Dupont

AMP-activated protein kinase serves as a universal regulator of autophagy

Maria Høyer-Hansen

09:00-11:00

LECTURE THEATRE 2

Session VIb - Symposium

Ion transporters in cell migration and apoptosis

Chair/Organizer:

Markus Ritter, Salzburg, Austria

The Na^+/H^+ exchanger NHE1 generates a pericellular pH-nanoenvironment required for cell migration

Christian Stock

Ion channels keep mobile cell on the go

Albrecht Schwab

The functional role of the non-gastric H^+/K^+-ATPase ATP12A (ATP1AL1) as anti-apoptotic ion transporter

Markus Ritter

Interaction of Bax with a mitochondrial potassium channel is crucial for its action in apoptosis

Ildikò Szabò

09:00-11:00

LECTURE THEATRE 3

Session VIc - Symposium

The physiology of neural stem cells in the healthy and diseased brain

Chair/Organizer:

Ludwig Aigner, Salzburg, Austria

Stem cells and neurogenesis in the adult brain

Ludwig Aigner

The physiology of neural stem cells in the healthy and diseased brain

Jürgen Winkler

The rise of cancer stem cells in high-grade gliomas – a matter of a pathological stem cell niche?

Peter Hau

Glutamatergic excitation of mature and newly generated young hippocampal granule cells

Josef Bischofberger

09:00-11:00

LECTURE THEATRE 4

Session VId - Symposium

Physiology of deconditioning
Supported by European Space Agency (ESA)

Chair/Organizer:

esa

Igor Mekjavic, Ljubljana, Slovenia

Effects of prolonged bed rest on mechanical properties of peripheral blood vessels

Ola Eiken

Title to be announced

Per Tesch

Determinants of bone loss during immobilization

Jörn Rittweger

Physiology of deconditioning – nutritional aspects

Ian A. Macdonald

09:00-11:00 **Session VIe - Symposium**

LECTURE THEATRE 5

Exocytosis and fusion pore physiology

Chair/Organizer:

François Darchen, Paris, France

What drives fusion pore enlargement?

François Darchen

Different roles of the SNARE-complex in neuronal exocytosis: From vesicle docking to the fusion pore

Jakob B. Sørensen

Ca dependence of exo and endocytotic coupling at a glutamatergic synapse

Takeshi Sakaba

Fusion pore regulation of peptidergic vesicles

Robert Zorec / Nina Vardjan

11:30-13:00 **Session VIIb – Discussion forum**

LECTURE THEATRE 3

Organized by Zeiss: Discussion forum on high resolution microscopy

Practical superresolution microscopy: Theory and applications of PAL-M and SR-SIM

Annette Bergter

11:30-13:00 **Session VIIc - Oral Presentations**

LECTURE THEATRE 4

Endocrinology

Chair:

Marjan Rupnik, Maribor, Slovenia

Expression of 11β-HSD1 and H6PDH during ontogeny

Lenka Rehakova

The adaptive response of leydig cells to immobilization stress: Stimulation of PKA and StAR protein

Tatjana S. Kostic

Intracellular chloride ions modulate secretory activity of mouse chromaffin cells

Jurij Dolenšek

GPCR-mediated signaling-induced paracrine transactivation of CB1 receptor, an interaction between the effects of calcium-mobilizing hormones and cannabinoid system

László Hunyady

Lysophospholipids modulate voltage-gated calcium channel currents in pituitary cells; effects of lipid-stress

Itzhak Nussinovitch

Behavioural effects of growth hormone replacement therapy in aged Wistar rats

Rubén V. Rial

11:30-13:00

LECTURE THEATRE 5

Session VIId - Oral Presentations

Neurophysiology and sensory system

Chair/Organizer: *Tina Pangršič, Goettingen, Germany*

Habitual characteristics of auditory Go-P3 and NoGo-P3 responses are different

Rezan Hatungil

Arrestin in *Drosophila* phototransduction

Gregor Belušič

Dose dependent nicotine effects on brain excitability in immature rats

Dana Maresova

Otoferlin, a synaptotagmin-like calcium sensor?

Ellen Reisinger

Function of prestin as a bicarbonate-chloride antiporter

Pavel Mistrík

14:00-15:30

POSTER AREA MF

Session VIII

Poster Session and Exhibition

15:30-16:30

LECTURE THEATRE 3

Keynote Lecture:

Structure-function relationship of the endocrine pancreas at a glance

***Per-Olof Berggren**, Stockholm, Sweden*

17:00-19:00 **Session IXa - Symposium**

LECTURE THEATRE 1

Human cutaneous microcirculation in health and disease

Chair/Organizer:

Helena Lenasi, Ljubljana, Slovenia

Skin perfusion and human heat exchange: How, why and use

Wouter van Marken Lichtenbelt

Local regulation of human cutaneous microcirculation: Impact of endothelium-dependent vasodilatation

Helena Lenasi

Skin vasomotion investigation in different human pathological conditions

Marco Rossi

Skin microcirculation in the upper and lower extremities of diabetic patients with and without large vessel disease

Vilma Urbančič-Rovan

17:00-19:00 **Session IXb - Symposium**

LECTURE THEATRE 2

Rhythmic oscillations of spinal networks in health and disease models

Chair/Organizer:

Andrea Nistri, Trieste, Italy

Pathophysiology of experimental spinal injury *in vitro*

Andrea Nistri

Cellular and network mechanisms of rhythm generation in spinal cord circuits

Jürg Streit

Temporal evolution of spontaneous Ca^{2+} signals generated by ventral neurons is a marker of spinal cord maturation in vitro

Laura Ballerini

Exploring the early damage to the locomotor circuits with an in vitro model of acute Spinal Cord Injury

Giuliano Taccola

17:00-19:00

LECTURE THEATRE 3

Session IXc - Symposium

Vasopressin and Oxytocin receptors: Looking for new tools, pharmacology, physiology and therapeutic agents.

Chair/Organizer:

Govindan Dayanithi, Prague, Czech Republic

Vasopressin receptor-mediated calcium signals and peptide release in the supraoptic nucleus neurons: Contradictions and compromises

Govindan Dayanithi

Functional consequences of vasopressin and corticoliberin receptors co-expression in native and heterologous models

Gilles Guillon

Oxytocin receptor coupling to different G-proteins: Role in receptor trafficking

Bice Chini

Novel oxytocin receptor-linked signaling networks

Hans H. Zingg

17:00-20:00

LECTURE THEATRE 4

Session IXd - Symposium

Muscle and fat: Molecular mechanisms of signaling and crosstalk

COST BM0602 WG3-meeting

Chair/Organizers:

Jørgen Jensen, Oslo, Norway

Marko Kreft, Ljubljana, Slovenia

Molecular mechanisms of the fat and muscle crosstalk

Juergen Eckel

Bridging mitochondrial dynamics and metabolism in muscle

Antonio Zorzano

PIKfyve regulates glucose uptake in skeletal muscles

Jørgen Jensen

Reduced AMP-activated protein kinase activity in mouse skeletal muscle does not exacerbate the development of insulin resistance with obesity

Sebastian Beck Jørgensen

Redox regulation of glucose metabolism

Tarja Kokkola

Zooming out on AMPK substrates: A phosphoproteomic approach

Didier Vertommen

17:00-19:00 **Session IXe - Symposium**

LECTURE THEATRE 5

Ca^{2+}, a miraculous messenger - an update

Chair/Organizer:

Wolfgang Graier, Graz, Austria

Can we reveal an agonist-induced Ca^{2+} entry hidden by the ubiquitous store-operated Ca^{2+} entry in endothelial cells?

Maud Frieden

Endoplasmic reticulum-mitochondria crosstalk and cellular stress signaling

Gyorgy Szabadkai

Mitochondrial Ca^{2+} uptake is differentially determined by the Ca^{2+} source and the expression-level of the novel uncoupling proteins UCP2 and UCP3

Roland Malli

Spatial Ca^{2+} Signaling in Cardiac Myocytes

Jens Kockskämper

20:00- **Special Event**

SUNDAY, 15. NOVEMBER 2009:

09:00-10:30 LECTURE THEATRE 3

Session Xb - Oral Presentations

Gravitational physiology

Chair/Organizer: *Nandu Goswami, Graz, Austria*

Patterns of cardiovascular control during repeated tests of orthostatic loading

Jerry Joseph Batzel

Multiparametric structural analysis of retinal vessels induced by pathological states: Possible space flight applications?

Laszlo Simon

Continuous epinephrine infusion modulates passive head-up tilt induced cardiovascular responses

Andreas Roessler

Predicting pulsatile variations in finger arterial pressure using a novel cardiovascular system model

Aurelio de los Reyes V

Cardio-postural interactions: Wavelet analysis of gastrocnemius electromyographic activity and blood pressure variation with respect to postural sway during quiet stance

Andrew P. Blaber

Effects of mental challenge applied before passive head up tilt on orthostatic neurohormonal responses

Nandu Goswami

09:00-10:30 LECTURE THEATRE 4

Session Xc - Oral Presentations

Tissue/cell damage and apoptosis

Chair/Organizer: *Markus Ritter, Salzburg, Austria*

The lysosomal pathway of apoptosis: A complex biological network

Veronika Stoka

The non-gastric H^+/K^+-ATPase ATP12A exerts an anti-apoptotic effect on butyrate-treated myelomonocytic HL60 cells

Sabine Schmidt

The possible effects of topically administired carvacrol on physiological mechanisms in wound healing

M. Yalçın Günal

Apoptosis without karyorrhexis in microglia

Barbara Klein

Role of cell swelling-induced peptide secretion in ischemia-reperfusion injury and preconditioning

Vladimír Štrbák

09:00-10:30

LECTURE THEATRE 5

Session Xd - Oral Presentations

Neurophysiology and sensory system

Chair/Organizer: *Marjan Rupnik, Maribor, Slovenia*

Nicotine protects against kainic acid induced hippocampal damage

Vladimir Riljak

Distinguishing between the sub- and superthreshold regime of neuronal firing

Aleš Škorjanc

Maturation and functional plasticity of cellular respiratory apparatus in flies' eyes

Gregor Zupančič

Improving effects of chronic melatonin treatment on 5-HT neurotransmission and spatial memory task in old rats

Susana Esteban

11:00-13:00

LECTURE THEATRE 1

Session XIa - Symposium

Gravitational Physiology

Chairs/Organizers: *Helmut Hinghofer-Szalkay, Graz, Austria & Nandu Goswami, Graz, Austria*

Gravity, the hydrostatic indifference concept, and the heart

Helmut Hinghofer-Szalkay

Cardiovascular responses to the upright posture
Neil H. Secher

Cardiovascular control, exercise and gravity
Dag Linnarsson

Neuroendocrine responses to orthostasis combined with other stress stimuli
Daniela Jezova

11:00-13:00 LECTURE THEATRE 2

Session XIb - Symposium

Osmoregulation, osmosensing and mechanotransduction

Chair/Organizer:
Markus Ritter, Salzburg, Austria

Ion and water chanels involved in cell volume control: Regulation and physiological roles
Else Kay Hoffmann

The hypertonicity-induced cation channel (HICC) in human hepatocytes: Role in proliferation vs. apoptosis and molecular characterization
Frank Wehner

Osmosignaling in the liver
Dieter Häussinger

Kinetics of force-induced cell reorganization depends on microtubules and actin
Ralf Kemkemer

11:00-13:00 LECTURE THEATRE 3

Session XIc - Symposium

Physiology and pathophysiology of purinergic signalling

Chair/Organizer:
Elsa Fabbretti, Nova Gorica, Slovenia

Overview of the pathophysiology of purinergic signalling
Geoffrey Burnstock

ATP-mediated signalling in trigeminal neurons in a migraine animal model
Elsa Fabbretti

Nucleotide receptors at the neuro-vascular interface

Stefan Boehm

Purinergic signaling in the pulmonary neuroepithelial body microenvironment unraveled by live cell imaging

Dirk Adriaensen

Extracellular ATP and P2 receptors in neurodegenerative diseases: P2X7 is an obligate component of microglia response to amyloid beta

Francesco Di Virgilio

11:00-13:00

LECTURE THEATRE 4

Session XId - Symposium

Pain: Role of glutamate and GABA metabotropic receptors

Chairs/Organizers: *Marcella MOTTA, Milano, Italy &*

Valerio MAGNAGHI, Milano, Italy

Transcriptional regulation of type-2 metabotropic glutamate receptors: A potential strategy for chronic pain treatment

Ferdinando Nicoletti

GABAb impairment in the dorsal horn of neuropathic rats: Possible roles for associated proteins

Marc Landry

Metabotropic GABA-B receptor-mediated effects in nociception

Valerio Magnaghi

Development of potential antihyperalgesic drugs targeting group III mGluRs

Cyril Goudet

14:00-15:30

POSTER AREA MF

Session XII

Poster Session and Exhibition

15:30-16:00

LECTURE THEATRE 3

FEPS Best Poster and Best Lecture Award

FEPS 2009

The physiology meeting organized by *The Slovenian Physiological Society, The Austrian Physiological Society, and The Federation of European Physiological Societies.*

BOOK OF ABSTRACTS

12 - 15 November 2009, Ljubljana, Slovenia

THURSDAY, 12. NOVEMBER 2009:

SESSION I - EYPS

ASTROCYTES POTENTIATE HIPPOCAMPAL SYNAPTIC TRANSMISSION

Perea G, Araque A

Instituto Cajal. CSIC. Madrid, Spain. gperea@cajal.csic.es

Accumulating evidence indicates the existence of bidirectional communication between astrocytes and neurons. However, the effects of astrocytes on action potential-evoked synaptic transmission at single synapse level are largely unknown. We investigated the neuromodulatory role of astrocytes on synaptic physiology at single hippocampal synapses. Using electrophysiological and Ca^{2+} imaging techniques on rat hippocampal slices, we performed paired recordings from CA1 pyramidal neurons and single astrocytes. Astrocytes were loaded with the Ca^{2+} indicator Fluo 4 (50 μM) and the Ca^{2+}-cage NP-EGTA (5mM) and were selectively stimulated by UV-flash photolysis (2Hz, 5s). Single synapses of Schaffer collaterals were stimulated at 0.5 Hz.
We found that:
The selective elevation of Ca^{2+} in single astrocytes transiently increased the synaptic efficacy due to the potentiation of the probability of transmitter release, without affecting the amplitude of synaptic currents.
This form of short-term plasticity was due to SNARE protein- and Ca^{2+}-dependent release of glutamate from astrocytes, that activates presynaptic type I metabotropic glutamate receptors (mGluRs).
The concurrent activity of astrocyte Ca^{2+} elevation and postsynaptic neuron caused the persistent potentiation of synaptic transmission. Therefore, the temporal coincidence of neuronal and astrocytic signals induced the long-term potentiation (LTP) of hippocampal synaptic transmission.
LTP was independent of NMDA receptor activation and postsynaptic intracellular Ca^{2+}. However, LTP was prevented by blockage of presynaptic type I of mGluRs and synthesis of nitric oxide.
We conclude that astrocytes potentiate synaptic transmission playing an active role in the transfer and storage of synaptic information by the nervous system.

Supported by: MICINN *(BFU2007-64764) and European Union (Health-F2-2007-202167).*

FEASIBILITY OF NON-QUANTAL RELEASE OF ACETYLCHOLINE FROM THE POSTGANGLIONIC PARASYMPATHETIC NERVES IN THE RIGHT ATRIUM OF RAT

Denis V. Abramochkin[1,3], Leniz F. Nurullin[2], Eugen E. Nikolsky[2], Leonid V. Rosenshtraukh[3]

[1]*Moscow State University, Moscow, Russia*
[2]*Kazan Institute of Biochemistry and Biophysics, Kazan, Russia*
[3]*Institute of Experimental Cardiology, Moscow, Russia*

Although parasympathetic regulation is extremely important for normal functioning of the mammalian heart, little is known about its secretion from the parasympathetic nerve terminals in the myocardium. It is accepted that in the neuromuscular junction acetylcholine (ACh) may be released from the nerve terminal by quantal or non-quantal type of secretion. The non-quantal release in the neuromuscular junction may be detected due to the slight depolarization of the muscle fiber during inhibition of acetylcholinesterase (AChE). Nothing is known about the existence of non-quantal release in the heart.

We have used the conventional microelectrode technique to register changes of action potential (AP) configuration in the isolated preparation of rat right atrium during the application of AChE inhibitors. AChE inhibitors armin (10^{-7}-10^{-5}M) and neostigmine (10^{-7}-$5\cdot10^{-6}$M) provoked typical cholinergic effects: reduction of AP duration and prolongation of the cycle length. These effects were abolished by atropine, therefore they are mediated by ACh, accumulated in the myocardium during AChE inhibition. These results beg the question of what is the mechanism of AChs release in isolated atrium preparation, where the impulse activity of vagus is absent. Three ways of mediator secretion are known at present: evoked quantal release associated with excitation of neuron, spontaneous quantal release and finally, non-quantal release.

Putative block of postganglionic neurons impulse activity by tetrodotoxin ($2\cdot10^{-7}$M) and hexomethonium (10^{-5}M) as well as the block of all forms of quantal release with botulinic toxin type A (50 U/ml) didn't alter effects of armin. Experiments with lipophilic fluorescent dye FM1-43 confirmed the presence of endocytosis in cholinergic fibers, crucial for the effective block of exocytosis by botulinic toxin. Therefore, accumulation of ACh doesn't depend on evoked or spontaneous quantal release. Selective inhibitor of choline uptake system hemicholinium III (10^{-5}M), which blocks non-quantal release in the neuromuscular junction, suppressed all effects of AChE inhibitors. Therefore, accumulation of ACh in the myocardium during inhibition of AChE is likely to be caused by the non-quantal release of ACh from the cholinergic terminals.

Thus, non-quantal release of ACh, shown earlier in the neuromuscular junction, is present in the cholinergic postganglionic fibers of the rat heart in addition to quantal release.

ROLE OF POTASSIUM CURRENTS IN THE REPOLARIZATION OF CANINE VENTRICULAR MYOCARDIUM UNDER ACTION POTENTIAL CLAMP CONDITIONS

Balazs Horvath, Tamas Banyasz, Janos Magyar, Norbert Szentandrassy, Gabor Harmati, Peter P. Nanasi

Department of Physiology, University of Debrecen, Medical and Health Science Centre Debrecen, Hungary

Aim: The aim of the present study was to give a parametric description of the most important potassium currents flowing during canine ventricular action potentials.
Methods: Our experiments were carried out on enzymatically isolated canine cardiomyocytes. Inward rectifier potassium current (I_{K1}), rapid delayed rectifier potassium current (I_{Kr}), and transient outward current (I_{to}) were measured under action potential clamp conditions using $BaCl_2$, E-4031, and 4-aminopyridine, respectively.
Results: The maximum amplitude of I_{to} was 3.0±0.23 pA/pF and its integral was 29.7±2.5 fC/pF. The current peaked 4.4±0.7 ms after the action potential upstroke and rapidly decayed to zero with a time constant of 7.4±0.6 ms. I_{Kr} gradually increased during the plateau, peaked 7 ms before the time of maximum rate of repolarization (V^{-}_{max}) at −54.2±1.7 mV, had peak amplitude of 0.62±0.08 pA/pF, and integral of 57.6±6.7 fC/pF. I_{K1} began to rise from -22.4±0.8 mV, peaked 1 ms after the time of V^{-}_{max} at −58.3±0.6 mV, had peak amplitude of 1.8±0.1 pA/pF, and integral of 61.6±6.2 fC/pF. Good correlation was observed between peak I_{K1} and V^{-}_{max} (r=0.93) but none between I_{Kr} and V^{-}_{max}. Neither I_{K1} nor I_{Kr} was frequency-dependent between 0.2 and 1.66 Hz. Congruently, I_{Kr} failed to accumulate in canine myocytes at fast stimulating rates.
Conclusion: Terminal repolarization is dominated by I_{K1}, but action potential duration is influenced by several ion currents simultaneously. As Ito was not active during the plateau, and neither I_{K1} nor I_{Kr} was frequency-dependent, other currents must play important role in the frequency dependence of action potential duration.

TMEM16B IS A NOVEL Ca^{2+}-ACTIVATED Cl^- CHANNEL CANDIDATE IN VERTEBRATE OLFACTORY TRANSDUCTION

Simone Pifferi, Michele Dibattista, Anna Menini

International School for Advanced Studies, Scuola Internazionale Superiore di Studi Avanzati, SISSA, and Italian Institute of Technology, SISSA Unit, Trieste, Italy

The olfactory system detects a large variety of volatile molecules. Odorant molecules enter into the nasal cavity and bind to odorant receptors in the cilia of olfactory sensory neurons, activating a transduction cascade that involves the opening of cyclic nucleotide-gated channels and the entry of Ca^{2+} in the cilia. Ca^{2+} activates Cl^- channels that, given the unusually elevated intracellular Cl^- concentration, produce an efflux of Cl^- ions and amplify the depolarization. Ca^{2+}-activated Cl^- channels contribute up to 90% of the total current but their molecular identity is still unknown. Some members of the protein family named transmembrane 16 (TMEM16; also known as anoctamin) have been recently proposed to function as Ca^{2+}-activated Cl^- channels. At least one member of this family, TMEM16B, is present in the mouse olfactory ciliary proteome (Mayer et al. Proteomics, 2009; Stephan et al. PNAS, 2009). We investigated the functional properties of the mouse TMEM16B (mTMEM16B) after expression in human embryonic kidney (HEK) 293T cells. Currents were measured with the patch-clamp technique both in the whole-cell configuration and in inside-out excised patches.
In whole-cell, a current induced by mTMEM16B was activated by intracellular Ca^{2+} diffusing from the patch pipette, released from intracellular stores through activation of a G-protein coupled receptor, or photoreleased from caged Ca^{2+} inside the cell. In inside-out excised patches, bath application of different Ca^{2+} concentrations rapidly activated a current, indicating that mTMEM16B is directly gated by Ca^{2+}. The Ca^{2+}-induced current was anion selective, displayed a Ca^{2+}-dependent rectification, and was blocked by the chloride channel blocker niflumic acid. In inside-out patches, the Ca^{2+} concentration for half-maximal current activation decreased from 4.9 μM at -50 mV to 3.3 μM at +50 mV, while the Hill coefficient was >2. Currents showed a time-dependent decrease (inactivation) at -50 mV in the presence of a constant high Ca^{2+} concentration and, moreover, an irreversible rundown, not observed in whole-cell recordings, indicating that some unknown modulator was lost upon patch excision. We compared the electrophysiological properties of native Ca^{2+}-activated Cl^- currents in mouse olfactory sensory neurons with those of mTMEM16B-induced currents in HEK cells. We found that dose-response relations for Ca^{2+} activation, estimated single channel conductance, rectification properties, Ca^{2+}-dependent inactivation and irreversible rundown are remarkably similar indicating that, at present, mTMEM16B is the best candidate for being the main molecular component of the native Ca^{2+}-activated Cl^- channel in the cilia of vertebrate olfactory sensory neurons.

THE SIGNIFICANCE OF LIPID RAFT/CAVEOLAE MICRODOMAINS IN SEVERAL ASPECTS OF THE NK1-R BIOLOGY

Alenka Hrovat[1], Robert Frangez[1], Azra Pogacnik[1], Marjeta Sentjurc[2], Anders Heding[3], Milka Vrecl[1]

[1]*Veterinary Faculty, Ljubljana, Slovenia*
[2]*Institute for biophysics, Institute Jozef Stefan, Slovenia*
[3]*7TM Pharma, Hørsholm, Denmark*

The ability of seven transmembrane receptors (7TM receptors) to interact with a diverse set of protein partners in a rapid and kinetically favorable way could result from an effective compartmentalization of the receptor/protein components in the plasma membrane microdomains. One such microdomain may be lipid rafts and their subdomain caveolae that were recently proposed to have an important role in efficient signaling and receptor-protein partner interactions. We therefore examined the impact of lipid raft/caveolae microdomains on receptor micro-localization within the plasma membrane, the receptor-protein partner interactions and receptor signaling properties. For the purpose of the study neurokinin type 1 receptor (NK1-R) was stably expressed in chinese hamster ovary (CHO) cell lines, CHO K_1 and mutant CHO 215 that have a defect in the synthesis of cholesterol and consequently the formation of lipid rafts in those cells is inhibited. We employed electron paramagnetic resonance (EPR) with spin labeling to study the domain structure of the plasma membrane and receptor microlocalization. A fitting procedure called GHOST was utilized that provides a means for quantitative characterization of complex bio-membrane systems. We showed that NK1-R was distributed in a well-ordered domain of the CHO-K_1 representing lipid raft/caveolae microdomains, whereas in CHO-215 cells lacking lipid rafts/caveolae the NK1-R plasma-membrane distribution was impaired. We then assessed receptor signaling properties and employed bioluminescence resonance energy transfer ($BRET^2$) to obtain data of relevance for receptor/beta-arrestins interactions. Our results showed that receptor compartmentalization within the plasma membrane caveolae/lipid rafts microdomains influence the efficacy of receptor signaling as well as agonist-induced receptor/beta-arrestin 2 interactions resulting in a decreased desensitization. In this aspect, our findings could provide a new framework to study the functional importance of caveolae/lipid rafts in the context of 7TM receptors and their interacting protein partners.

ACTIN-DEPENDENT VOLUME RECOVERY OF EMBRYONIC CELL SUBJECTED TO HYPOOSMOTIC SHOCK

Maria Pogorelova[1,2], Alexander Pogorelov[2], Vladimir Golichenkov[1]

[1] Biological Faculty, Moscow State University, Leninskie gory 1/12, Moscow, 119991, Russian Federation
[2] Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Moscow province, 142290, Russian Federation

The early embryo membrane is highly permeable for water. It makes the cell very sensitive to osmotic shock. The cell volume alteration plays an important role in regulation of key cellular functions, including metabolism, protein synthesis, gene expression, proliferation and cell death. A fundamental property of animal cells is the ability to regulate their own volume. Even under hypotonic stress the cells can readjust their volume after transient osmotic swelling by a mechanism known as regulatory volume decrease (RVD). The nature of the regulatory mechanisms governing the volume changes in embryonic cell during RVD is not clear.

Osmotic adaptation in a blastomere of two-cell mouse embryo has been studied employing the direct measurement of cell volume with laser scanning microscopy followed by three-dimensional reconstruction. The hypotonicity was created by replacing 140mM NaCl in Dulbecco`s solution with 70mM NaCl. The keeping of the intact volume of the embryo compartments was based on freeze-drying technique. A Z-stack of optical slices was obtained in a confocal microscope (Zeiss, Germany). 3-DR was performed in the 3ds max medium.

Our data indicate that a long-term hypoosmotic shock results in the embryo volume recovery. Exposure of embryonic cells to the hypotonic K^+-free medium does not influence on the kinetics of cell osmotic behavior. Blastomere's volume recovery is abolished after incubation in hypotonic Dulbecco`s with Cytochalasin B.

Na^+ /K^+ -ATPase inhibition does not influence on RVD of the embryonic cells, but volume recovery specific for hypotonic shock is blocked by the alteration of F-actin organization.

THE ROLE OF REACTIVE OXYGEN SPECIES ON PROLIFERATION AND Ca^{2+} SIGNALLING OF SKELETAL MUSCLE CELLS DURING *IN VITRO* AGEING

Elisa Luin[1], Paola Lorenzon[1], Rashid Giniatullin[2], Marina Sciancalepore[1]

[1]*Dept. of Life Sciences, University of Trieste, Italy*
[2]*Dept. Neurobiology, A. I. Virtanen Institute, Kuopio, Finland*

Reactive oxygen species (ROS) are involved in control of many principle cellular processes, such as cell growth, metabolism, differentiation and apoptosis. In skeletal muscle, damaging action of ROS could be one reason for age-dependent sarcopenia. On the other hand, ROS could play a physiological role as second messengers modulating proliferative process. Molecular mechanisms underlying physiological or pathological action of ROS in skeletal muscle are still unknown. Since cultured muscle cells represent a convenient model to explore molecular mechanism of ROS signalling, the main aim of the current study was to investigate the action of ROS on the proliferative potential of myoblasts and their ability to differentiate into myotubes.

To this end, murine satellite cells obtained from young animals were cultured and aged *in vitro*. The proliferative capacity and the efficiency of differentiation of young and old cells were evaluated after the treatment with the diffusible and cell permeable ROS H_2O_2. A single 30 min long episode of oxidative stress induced by 3 μM H_2O_2 stimulated proliferation of young cells. Interestingly, the same concentration of ROS decreased the proliferation of old myoblasts. The higher (100 μM) concentration of H_2O_2 did not change the proliferative ability of young cells but decreased the proliferation of the old ones. Both concentrations of H_2O_2 did not affect significantly the differentiation of myoblasts into myotubes.

Experiments performed using patch-clamp technique in current clamp mode indicated that H_2O_2 increased spontaneous electrical activity of myotubes and facilitated anode break excitation.

Using Ca^{2+} imaging experiments with Fura-2AM we found that 5 min application of 100 μM H_2O_2 induced fast Ca^{2+} transients in a fraction of young myoblasts and elicited slow long-lasting signals in old myoblasts. Only old but not young myotubes responded to H_2O_2. Our data indicate differential effects of H_2O_2 on young versus old muscle cells, supporting the contribution of ROS signalling in skeletal muscle ageing. Long lasting Ca^{2+} signals could potentially underlie the damaging action of ROS in old cells.

EMBRYONIC ORGANOTYPIC CULTURES: AN *IN VITRO* MODEL FOR EXCITOTOXICITY-DUE SPINAL CORD INJURY

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Embryonic organotypic cultures represent a useful model to investigate neuronal maturation and physiology of the spinal cord. In fact, organotypic slices maintain the basic cytoarchitecture of the spinal cord, including the dorsal-ventral orientation of the spinal segments, and the fundamental properties of network dynamics. Furthermore, they allow long term studies of their developmental processes and their ability to adjust to changes in the local environment (Sibilla and Ballerini, Progress in Neurobiology 2009, in press). Thus, these cultures may offer the opportunity to investigate certain mechanisms underlying the response of spinal neurons to injury in terms of alterations in molecular, cellular and circuit characteristics following experimental conditions that mimic a spinal cord lesion.

The main purpose of the present study was to evaluate the consequences of the glutamate analogue kainate on survival of embryonic organotypic spinal cord cultures and the potential use of drugs to limit any toxic effects. Indeed, it is widely believed that hyperactivation of glutamate receptors during the early phase of spinal cord injury represents a major contributor to subsequent damage even if the precise mechanisms of this effect remain poorly understood.

Previous studies by our group, using the *in vitro* spinal cord of the neonatal rat, show that kainate (1 mM) applied for 1 h largely destroyed neurons around the central and ventral gray matter with only modest white matter damage (Taccola et al., Neuroscience 2008, 155:538-555). Nevertheless, the *in vitro* spinal cord preparation is unsuitable for investigating long term consequences of the initial excitotoxicity, a phenomenon which is better explored with organotypic cultures.

Embryonic (E13) rat organotypic spinal slices at 22 days *in vitro* (DIV) were sensitive to kainate treatment (1 h) that significantly increased the percentage of pyknotic nuclei (observed with DAPI nuclear staining). Moreover, the damage appeared to be an ongoing process because 4 and 24 h later (despite extensive washout of kainate), histological examination demonstrated further intensification of cell loss. To characterize the topographical distribution of pyknotic nuclei, slices were divided into three regions, namely dorsal, central, and ventral. The largest number of pyknotic nuclei was predominantly found in dorsal areas. Immunohistochemistry with the neuron specific marker NeuN confirmed strong disappearance of neurons. These findings suggest that kainate-mediated injury to organotypic cultures is a reliable model to study molecular and cellular process regarding spinal injury and may be represent an advantageous test system for exploring neuroprotective drugs.

NICOTINE ENHANCES SUB THRESHOLD RESONANCE BEHAVIOR OF ORIENS-LACUNOSUM MOLECULARE INTERNEURONS IN THE MOUSE HIPPOCAMPUS

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A large variety of distinct GABAergic interneurons are present in the hippocampus. By releasing GABA into principal cells and interneurons, they exert a powerful control on neuronal excitability and are responsible for network oscillations crucial for information processing. Among these, the oriens-lacunosum moleculare (O-LM) interneurons have been intensively studied. These cells exhibit a prominent sag in electrotonic potentials induced by hyperpolarizing current pulses of increasing intensity, mediated by the time-dependent inwardly rectifying cationic current I_h.

Here, whole cell patch clamp recordings in current and voltage clamp mode were used to study whether nicotine, the active component of tobacco, is able to modulate I_h and the oscillatory activity of O-LM interneurons in the CA1 region of the hippocampus. To target these neurons we used hippocampal slices from transgenic mice expressing EGFP in a subset of somatostatin containing interneurons. In a first set of experiments, the sub threshold resonance behavior of these cells was characterized by measuring voltage responses to sinusoidal currents of constant amplitude and linearly increasing frequency (ZAP stimulus), before and during bath application of a low concentration (1 μM) of nicotine, close to that present in the smoker's blood immediately after smoking a cigarette. In the presence of nicotine, at -90 mV (but not at -70 mV), a frequency-selective enhancement of the voltage response in the range of 2-3 Hz was found. This was associated with a mean impedance increase of 439 % (439 ± 143 %; P=0.02; n=6). This effect was mimicked by ZD7288 (100 μM), indicating that it was due to a reduction in amplitude of I_h. Nicotine applied to neurons voltage clamped at -50 mV, strongly reduced I_h in a voltage-dependent way (nicotine-induced conductance change, measured at the end of the voltage step, was 89 $\pm$ 10 % at -70 mV and 49 $\pm$ 13 % at -90 mV; n=7; P=0.01 at -90 mV). Dose-response experiments revealed an IC_{50} of 60 nM. Bath application of DHβE (50 μM) or mecamyllamine (100 μM) failed to prevent nicotine effects on I_h suggesting that the action of nicotine was independent on nAChR activation. The blocking effect of nicotine on I_h was mimicked by the nicotine analogue epibatidine (300 nM) but not by acetylcholine (100 μM).

In conclusions, it is likely that, in O-LM interneurons, nicotine reduces I_h current by directly interfering with the HCN channel pore. This may influence the hippocampal oscillatory activity and rhythmogenesis in smokers.

ROLE OF SUBSTRATES, METABOLITES AND TRANSCRIPTIONAL REGULATION OF METABOLISM IN CARDIAC ARRHYTHMIA

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Human heart is physiologically and metabolically one of the most active organs. It consumes 5 kg ATP per day, of which fatty acids provides two third and glucose and lactate provide one third. Thus, metabolic substrates and metabolites, by participating in bioenergetic pathways, are considered to play important role in cardiac electrophysiology. In many physiological investigations metabolites are often considered as passive bystanders whose sole purpose is generating ATP. Increasing evidence, however, suggests that alteration of metabolic substrates or accumulation of metabolites in heart, particularly during metabolic disorders such as insulin resistance, obesity and diabetes, could be independent risk factors for electrophysiological conditions such as atrial fibrillation and ventricular tachycardia. This is also supported by recent findings that the mutation in metabolic gene causes Brugada syndrome which was previously thought to be exclusively ion channel related disorder. Thus, studying cardiac electrophysiology in context of metabolic alteration could provide important clues to understanding mechanisms of arrhythmia. Here, by combining single cell physiological studies (patch-clamp and Ca^{2+} imaging) and *in vivo* and *ex vivo* monitoring of cardiac rhythm, we provide evidence to suggest that altering metabolic substrates (glucose, lactic acid and pyruvate) could have important physiological consequences, particularly in a system with impaired electrophysiological conditions. Furthermore, some intracellular metabolites such as NAD^+/NADH, ATP/ADP and lipid intermediates were found to exert unexpected electrophysiological effects in heart. Finally, our experimental evidence derived from multidisciplinary approaches of gene expression, metabolomic and lipidomic in parallel with electrophysiological studies show that genetic ablation of transcriptional regulator of metabolic gene causes cardiac arrhythmia. Our studies provide new insights into the mechanism of cardiac arrhythmia that could explain increase cardiac mortality in metabolic diseases such as diabetes, insulin resistance and obesity.

DIFFERENTIAL MOLECULAR CONSEQUENCES OF EXCITOTOXICITY AND METABOLIC DYSFUNCTION AFTER IN VITRO EXPERIMENTAL SPINAL CORD INJURY

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Damage to the spinal cord, whether caused by injury or disease, cannot currently be repaired. The molecular mechanisms underlying the early pathophysiological stages of spinal cord injury remain largely unknown. After the initial insult, the tissue damage considerably spreads (secondary damage) because of further destruction of neuronal and glial cells caused by metabolic dysfunction (including ischemia), excitotoxicity, reactive oxygen radicals and neuroinflammatory reactions. We wished to explore the temporal evolution of such processes using, as a model, the thoracic-lumbar spinal cord of the neonatal rat maintained in vitro for up to 24 h. We have recently observed that excitotoxicity and severe metabolic perturbation differentially contribute to the dysfunction of locomotor networks, spinal reflexes and intrinsic network rhythmicity (Taccola et al., Neuroscience 2008, 155:538-555). Kainate evoked excitotoxicity suppresses fictive locomotion irreversibly, while a pathological medium (containing free radicals and hypoxic/aglycemic conditions) slows down fictive locomotion. Histological analysis shows significant cellular damage around the central canal after kainate treatment, while the pathological treatment induces preferential damage to white matter.

To clarify the molecular mechanisms underlying these distinct types of acute spinal cord dysfunction, we analyzed mRNA and protein content of the neonatal rat spinal cord at various times after applying either kainate or pathological medium (1 h application). Gene expression levels of different cell type markers (GFAP for astroglia, Mac 1 for microglia), the neuronal injury marker ATF-3, and various genes involved in neuroinflammation (interleukin 1b, IL-1b; serpine-1), and cell proliferation (egr-1) were studied using Real-Time PCR or large-scale Superarrays. Our data indicate early (4 h) upregulation of ATF-3, egr-1, IL-1b and serpine-1 expression after kainate treatment. Conversely, following pathological medium, there was significant downregulation of GFAP and MAC1 expression starting already at 4 h after treatment. Western blots and immunohistochemistry (anti-NeuN, anti-GFAP and anti-SMI-32) strongly suggested large neuronal damage by kainate treatment, whereas predominant glial damage by the pathological medium.

Our results validate distinct molecular consequences of excitotoxicity and metabolic dysfunction after experimental spinal cord injury, indicating different cellular sensitivity to acute damage. Combination of different strategies might therefore be necessary to treat the various components contributing to early spinal cord damage.

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EFFECT OF FREE OXYGEN RADICALS ON EXCITABILITY OF RAT HYPOGLOSSAL MOTONEURONS *IN VITRO*

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive loss of spinal cord and brainstem motoneurons. Although the pathogenesis is incompletely understood, oxidative stress and excitotoxicity are suggested to play a pivotal role. In 25 % of cases, ALS has a predominantly bulbar onset primarily affecting the nucleus hypoglossus, responsible for tongue muscle innervation. Since it is unclear how hypoglossal motoneurons (HMs) respond to oxidative stress in functional terms, the present study sought to investigate it by using, as a model, a slice preparation of the neonatal rat brainstem.

HMs were recorded with whole-cell patch clamping before and during application of H_2O_2 as a donor of free oxygen radicals and also examined (with confocal microscopy) for their oxidative stress condition and damage.

In voltage clamp configuration (holding potential = -70 mV), bath application of 1 mM H_2O_2 (30 min) produced a slowly developing inward current in addition to a significant rise in motoneuron input resistance and large depression of all synaptic events. The effects elicited by H_2O_2 were insensitive to blockers of excitatory or inhibitory synaptic transmission. Miniature synaptic events frequency did not change, while the voltage-activated persistent Ca^{2+} current (I_{CaP}) evoked by depolarizing ramps was reduced. Under current clamp, H_2O_2 induced a slow depolarization accompanied by increased spike threshold and firing frequency in response to current injection from similar baseline membrane potential. These effects were also resistant to blockers of fast synaptic transmission and were poorly reversible on washout. Oxidative stress condition induced by H_2O_2 application was assessed on the basis of the rhodamine 123 marker. Propidium iodide and hoechst 33342 stainings were instead used as indices of injured and viable cells, respectively.

These data suggest that even a short application of H_2O_2 to HMs could evoke a persistent electrophysiological deficit associated with a demonstrable oxidative stress, without altering short-term cell survival. Oxidative stress paradoxically made motoneurons more isolated from their network because of impaired synaptic transmission, yet more intrinsically excitable. One can surmise that this sequence of events might be occurring at an early stage of ALS and lead to severe cell dysfunction.

EARLY IN POSTNATAL DEVELOPMENT, TONIC ACTIVATION OF KAINATE RECEPTORS BY AMBIENT GLUTAMATE REDUCES GABA RELEASE FROM MOSSY FIBER TERMINALS

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Mossy fibers (MF), the axons of granule cells in the hippocampus dentate gyrus, which in adulthood are glutamatergic, in the immediate postnatal period release GABA (Safiulina et al., 2006). Here we show that presynaptic kainate receptors (KARs) localized on MF terminals exert a powerful inhibition on GABA release. GABA-A mediated postsynaptic currents (GPSCs) evoked by minimal stimulation of granule cells in the dentate gyrus were recorded from CA3 pyramidal cells using the patch clamp technique (whole cell in voltage clamp configuration). Bath application of AMPA receptor antagonist 4-(8-Methyl-9H-1,3-dioxolo[4,5-h][2,3]benzodiazepin-5-yl)-benzenamine hydrochloride (GYKI, 30 μM) did not modify the amplitude or the kinetics of GPSCs. Addition of the GluK1 KAR antagonist, (*S*)-1-(2-Amino-2-carboxylethyl)-3-(2-carboxylbenzyl)pyrimidine-2,4-dione (UBP 302, 10 μM) to GYKI, significantly increased the amplitude of GPSCs from 48.2 ± 8.2 pA to 83.7 ± 14.8 pA (n = 19; $P<0.001$). In contrast, the GluK1 KAR agonist (*RS*)-2-Amino-3-(3-hydroxy-5-*tert*-butylioxazol-4-yl)propanoic acid (ATPA, 1 μM), significantly reduced GPSC amplitude from 54.3 pA ± 9.3 pA to 21.8 ± 3.7 pA (n = 11; $P<0.01$), indicating that GluK1 KAR are involved in modulation of GABA release. These receptors were activated by glutamate present on the extracellular space as demonstrated by the observation that the enzymatic glutamate scavenger system (glutamic-pyruvic transminase [GPT] + pyruvate) was able to mimic the effect of UBP 302. Pertussis toxin, which inhibits G proteins, fully prevented the potentiating action of UBP 302, suggesting that GluK1 KAR has a metabotropic function. This effect was on presynaptically localized KARs because using GDPβs into the patch pipette failed to block the effects of UBP 302. Furthermore, both ATPA and UBP 302 decreased and increased the probability of antidromic firing in granule cells (recorded in current clamp conditions), respectively.

These data strongly indicate that presynaptic GluK1 KARs, activated by ambient glutamate play a crucial role in regulating GABA release from MF terminals, an effect that involves a metabotropic type of KA receptor.

Safiulina VF, Fattorini G, Conti F & Cherubini E (2006) GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. J Neurosci. 26:597–608.

THE EFFECT OF GENDER AND HAND SKIN PERFUSION ON TISSUE INSULATION DURING COLD EXPOSURE

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Background: To maintain a stable core temperature the human body balances heat production and heat loss. Heat from the core to the skin is primarily transported through conduction and blood flow. Fat has low heat conductive properties; therefore humans with a high fat precentage show high tissue insulation. The magnitude of skin blood flow greatly influences heat loss to the environment. Especially glabrous skin, rich in arteriovenous anastomoses, is capable of facilitating high and low perfusion rates close to the skin surface. Hence, these areas are well adapted for regulating heat loss. This study for the first time explored the relation between gender and perfusion of glabrous skin of the hand to tissue insulation during cold exposure. Methods: Measurements of energy expenditure (indirect calorimetry), skin temperature (wireless thermistors), core temperature (telemetric pill), perfusion (laser Doppler flowmetry), fat percentage (DXA-scan) and surface area (DXA-scan) were performed on 16 young adults (18-28 yrs; 8M 8F). All females were measured during the pre-follicular phase of their menstrual period. Tissue insulation was calculated according to deGroot and Kenney $I=Area*(T_{core}-T_{skin})/(M_{net}+S)$ (°Cm^2/W), where M_{net} is metabolic rate corrected for respiratory heat loss and S is heat storage. During a 15 minute baseline period skin temperature was clamped at 33.5°C by a water perfused suit; next subjects were mildly cooled for 15 minutes by progressively lowering water temperature in the suit. Results: Females had a significantly larger fat percentage than males ($p<0.001$). At baseline tissue insulation between both genders differs significantly (males: 0.08±0.01 vs. females: 0.11±0.01 ($p<0.05$)). Tissue insulation did not correlate significantly with fat percentage. After 15 minutes of cooling no significant difference in tissue insulation between genders is observed anymore. No significant gender difference in vasoconstriction is observed. Log linear regression between tissue insulation and perfusion at glabrous skin of the hand shows highly significant relations for both genders (males: $r^2=0.89$, $p<0.001$; females: $r^2=0.87$, $p<0.001$). Conclusion: The results indicate that during baseline female tissue insulation is significantly higher than in men, which is not significantly correlated to body fat percentage. During whole body cooling the gender effect in tissue insulation disappears. Regression analysis indicates that variation in tissue insulation can mainly be explained by vasoconstriction at glabrous skin of the hand.

LOSS OF LTD AT MF-CA3 SYNAPSES OVEREXPRESSING GLT-1

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Glutamate transporters are responsible for clearing synaptically released glutamate from extracellular space. By this action, they maintain low levels of ambient glutamate, thus preventing excitotoxic damage, and contribute to shape synaptic currents. We show that up-regulation of the glutamate transporter GLT-1 by ceftriaxone severely impaired mGluR-dependent long-term depression (LTD), induced at rat mossy fibers-CA3 synapses by repetitive stimulation of dentate gyrus granule cells. This effect involved GLT-1, since LTD was rescued by the selective GLT-1 antagonist dihydrokainate (DHK). DHK *per se* produced a modest but consistent decrease in fEPSP amplitude that rapidly regained control levels after washing out the drug. Moreover, the degree of fEPSP inhibition induced by the low affinity glutamate receptor antagonist γ-DGG was similar during basal synaptic transmission but not during LTD, indicating that, in ceftriaxone-treated animals, LTD induction did not alter synaptic glutamate transient concentration. Postembedding immunogold studies in rats showed an increased density of gold particles coding for GLT-1a in astrocytic processes and in mossy fiber terminals; in the latter, gold particles were located near and within the active zones. In both CEF-treated KO mice and KO mice the density of gold particles in MF terminals was comparable to background levels. The enhanced expression of GLT-1 at release sites may prevent activation of presynaptic mGluRs and LTD induction. Similarly, in ceftriaxone treated animals, the magnitude of LTP induced at MF-CA3 synapses (but not at Schaffer collateral-CA1 synapses) by high frequency stimulation of granule cells in the dentate gyrus was significantly reduced. These results reveal a novel mechanism by which GLT-1 regulates synaptic plasticity in the hippocampus.

NEURONAL FUNCTIONAL DIFFERENTIATION IN MAMMALIAN POSTNATAL STEM/PRECURSOR CELLS

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Neural stem cells have been found in neurogenic brain regions like the hippocampus, subventricular zone (SVZ), olfactory bulb and in some non-neurogenic regions, *i.e.* spinal cord. Novel stem cell niches, hosting stem/progenitor cells with neural differentiation potential have also been identified in rat leptomeninges. Immunocytochemical data have revealed nestin-positive cells that did not follow the astrocyte (glial fibrillary acidic proteins, GFAP-), or vascular (integral membrane chondroitin sulphate proteoglycan, NG2-) profiles. Such cells are present from embryonic stages up to adulthood. Tissue extracts with nestin-positive cells can be cultured and expanded *in vitro*, both as neurospheres and cell populations with stem cell features. Neurospheres are similar to the SVZ-derived neurospheres in terms of multipotency and gene expression. Cultured cells also have the capacity to form synaptic connections. Preliminary electrophysiological profiles of expanded cells were examined at 15 days *in vitro*. Whole-cell patch-clamp recordings, revealed mostly incomplete functional development, with an early neuron-like appearance. Small rudimentary action potentials were generated by the majority of cells in response to incremental injected depolarizing current steps, starting from a holding potential of -60 mV. Fast overshooting action potentials with a mature configuration characterize mature neurons. Current-voltage relationships revealed various electrophysiological properties, but mainly outward membrane rectification.
Based on the presence or absence of rectification when depolarizing step currents were applied, two basic cell types were observed: the majority of cells were classified as Type I, primarily associated with outward rectification; Type II cells had linear responses to step currents. MAP2-positive cells expressed GluR2 subunits of the ionotropic AMPA-glutamate receptor as well as the glutamate decarboxylase (GAD67), marker of GABAergic neurons. Exogenously-applied glutamate or GABA at 100 μM elicited postsynaptic responses. These data suggest that rat leptomeningeal stem cells can differentiate into fully functional neurons.

CARBON NANOTUBES DIRECT INTERACTIONS WITH NEURONAL MEMBRANES IGNITE POST SPIKE EXCITABILITY

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Nanotechnology enters into the realm of basic biological units by its ability to functional integrate with bio-systems. In recent years we reached an increased interest and improved understanding of such interactions with biological systems at a sub-cellular level. This latter feature can be understood and engineered with a high degree of specificity. One of the more attractive materials employed to develop nano-bio hybrid systems is represented by carbon nanotubes (CNT). CNT, due to their unique range of thermal, electronic and structural properties, have been rapidly developing as a technology platform for biological and medical applications, including those designed to develop novel neuro-implantable devices. CNT have been applied with the aim of probing or augmenting cell behaviour, of tracking subcellular components, or addressing the growth and organization of neural networks. Recently, we reported, for the first time, that the growth of cultured neuronal circuits on a conductive CNT meshwork was always accompanied by a significant enhancement in the efficacy of neural signal transmission. Is this network enhancement ultimately linked to the nanoscale physical interactions between CNT and neurons? Here we show, by patch clamping single neurons and by electron microscopy analysis, that CNT-substrate direct interactions with neuronal membranes affect single cell activity potentiating calcium electro-genesis due to action potential (AP) back propagation and measured via the presence of a somatic depolarization in response to repetitive APs. Our results highlight a potentially hitherto unrecognized CNT-mediated mechanism that exploits the targeting of neuronal integrative properties.

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GEPHYRIN REGULATES BOTH GABAERGIC AND GLUTAMATERGIC SYNAPTIC TRANSMISSION IN CULTURED HIPPOCAMPAL NEURONS

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Gephyrin is a scaffold protein that ensures the correct localization and clustering of glycine and GABA-A receptors (GABA-ARs) at postsynaptic sites. In this study, gephyrin-specific single chain antibody fragments (scFv) containing nuclear localization signals were used to perturb endogenous gephyrin clustering in rat hippocampal neurons in culture (Zacchi et al., J. Mol. Neurosci. 34**:** 141-148, 2008). Immunocytochemical experiments on scFv-transfected cells revealed a significant reduction in the density of gephyrin clusters, as compared to EGFP transfected controls (9.7 ± 1.5 in scFv *vs* 15.9 ± 1.4 in EGFP clusters/100μm^2; $p<0.01$; n=22). The density of γ2-containing GABA-AR clusters co-localizing with the vesicular GABA transporter VGAT (expressed as percentage of the total γ2 immunoreactivity) was also reduced (4.8 ± 1.7 % in scFv *vs* 17.0 ± 2.8 % in EGFP; $p<0.01$; n=11). Electrophysiological experiments demonstrated that these effects were associated with a reduction in frequency (0.18 ± 0.03 Hz in scFv *vs* 0.28 ± 0.04 Hz in EGFP; $p<0.05$; n=23) and amplitude (29.8 ± 1.7 pA in scFv *vs* 42.6 ± 3.0 pA in EGFP; $p<0.01$; n=23) of spontaneous GABA-A-mediated miniature inhibitory postsynaptic currents. In addition, hampering gephyrin function with scFv produced a significant decrease in the frequency of AMPA-mediated glutamatergic events (0.26 ± 0.06 Hz in scFv *vs* 1.2 ± 0.34 Hz in EGFP; $p<0.01$; n=14), without any modification in their amplitude (32.8 ± 4.5 pA in scFv *vs* 34.3 ± 4.6 pA in EGFP; $p>0.05$; n=14). Immunocytochemical analysis of vesicular glutamate transporter VGLUT resulted in a two-fold reduction in immunoreactive puncta in scFv as compared to controls (n=5) indicating a loss of glutamatergic innervation. This may be due to a homeostatic compensatory mechanism and may involve specialized cell adhesion molecules such as neuroligins and neurexins which, bridging the cleft, provide a direct link between the pre and the postsynaptic site of the synapse. Preliminary experiments on scFv-transfected neurons using antibodies against Neuroligin-2 which is predominantly localized at GABAergic synapses revealed a three-fold decrease in Neuroligin-2 immunoreactivity as compared to controls (n=4). It is likely that gephyrin interacts with neuroligin-neurexin complexes to regulate both GABAergic and glutamatergic transmission in the hippocampus. This would ensure the appropriate inhibitory-excitatory balance critical for the correct functioning of neuronal networks.

THURSDAY, 12. NOVEMBER 2009:

EDUCATIONAL WORKSHOP

WHAT SCIENCE SHOULD BE INCLUDED WITHIN INTEGRATED CURRICULA?

Karen Mattick

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Integrated medical programmes are coming under increasing scrutiny to ensure that students are learning science in the context of medicine and in a way that prepares them for their future career as doctors. This scrutiny has led to questions and concerns about 1) the ways that learning outcomes for the sciences underpinning medical practice are determined and 2) the ways that science is taught to medical students.

This talk will give an overview of how decisions are made about what science should be included within integrated curricula, with an emphasis on medical curricula. It will draw on the published literature relating to science within medical curricula, and particularly on a systematic review we have performed recently (Bull and Mattick 2009 "What biomedical science is included in undergraduate medical courses and how is this decided?"). The literature will be supplemented with the findings of our recent empirical research in this area, including a Higher Education Academy-funded study called "Is Anatomy Different?" (de Bere and Mattick) and a National Teaching Fellowship-funded study that takes an innovative and challenging approach to identifying learning outcomes for the sciences underpinning medical practice (Bull and Mattick).

At the end of the talk, I will offer some views on the potential role of portfolio or video taping in the teaching, learning and assessment of the sciences within integrated medical curricula.

TEACHING IN PHYSIOLOGY AND PATHOPHYSIOLOGY IN THE MEDICAL CURRICULUM

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Teaching in physiology and physiopathology requires transferring insights and concepts in integrated systems. This will form the necessary platform for a similar approach to the patient and his state of health or disease. It will itself depend on a bird's eye view on cellular and molecular biologic processes and their sound understanding. Medical education benefits from a global approach of normal and abnormal processes at the cellular, organ and body level.

In the medical curriculum at the Brussels Free University-VUB, the courses of physiology and physiopathology are conceived as individual entities that are not subdivided in independent organ-based modules. Cutting physiology into separate courses on the different organs rapidly leads to a "reductionism" view of physiology, and subsequently of medicine.

Lectures in an auditorium are considered as a crucial instrument in this form of teaching as long as it stimulates interactions with the students. It is therefore important to limit the number of students per lecture session. It is the task, and challenge, of the lecturer to clarify and illustrate the basic mechanisms and insights with relevant examples from the life and medical sciences. This will not only force the teacher to seek the most attractive and convincing tools and illustrations but will also stimulate students into further reading and thinking. Professionalism of the teachers will be the key in this mode of education.

In order to involve learners in their own learning needs, the development of a portfolio may promote intrinsic motivation to deepen the knowledge and to stimulate appropriate scientific "curiosity".

The teacher can propose the framework of a topic, followed by the context application to stimulate clinical understanding. Also, case studies can be used to gain understanding of the physiopathological processes involved.

The portfolio is a tool to actively involve students and learners, with regular reciprocal evaluations.

Finally, the learner should be able to explore experiential phenomena in their "real "life settings: theory building of concepts in physiology does not reduce the wide range of interindividual variability. On the contrary it allows articulating these concepts with the influence of a wide range of internal and external factors influencing health and disease.

In order to achieve these goals, schools of medicine will have to ensure the scientific and medical significance of their biomedical departments.

PORTFOLIO-BASED ASSESSMENT OF MEDICAL AND GENERIC COMPETENCIES IN THE PHYSICIAN – CLINICAL INVESTIGATOR MASTER PROGRAM AT THE MAASTRICHT UNIVERSITY

Sylvia Heeneman

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Various disciplines in university education have used portfolios as a learning and assessment tool for competence-based education. In medical education, portfolios can be used to accommodate effective learning of medical knowledge and skills, as well as generic competencies, such as effective communication, organization, team-work and professionalism. At the Maastricht University, we have implemented a assessment system, partly based on a portfolio, for medical and generic competencies in a 4-year physician-clinical investigator master program (MD-Msc). In this program, the medical degree is combined with a clinical investigator degree, focusing on translational medicine to bridge the gap between research and care delivery by translating questions from patient care into research, and applying research results to care. In the assessment program, the portfolio is used to integrate a wide range of module assessments, progress tests, medical skills assessment and professional behaviour to assess development and progress in 4 competency domains (medical experts, scientist, health care worker and person). Students reflect regularly on strengths and weaknesses in each of the competency domains and formulate learning objectives (formative portfolios) which are discussed with a personal counselor (mentor). Throughout the program, 4 summative portfolios are assembled, which are assessed by an independent assessment committee. A drawback of our current approach is that competency-assessment is still mixed, thus regular assessments (exams) are combined with portfolio-assessment, which still skews student priority towards the regular exams. Currently, we are in the process of re-evaluating the assessment program and examining the possibility of a program-wide assessment program that is based on the portfolio alone.

PERSPECTIVES ON TEST-ENHANCED LEARNING AND BASIC SCIENCE

Charlotte Ringsted

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Learning can be defined as a relatively permanent change in a person's capability to perform a task, that being a cognitive task such as clinical reasoning or a skill such as physical examination or resuscitation. Learning amounts to encoding information in long-term memory and being able to appropriately retrieve and apply the information stored. Traditionally, learning is usually tested immediately after a study period or a course. However, permanent and sustainable learning is best demonstrated by a person's performance on retention and transfer tests, i.e. testing some time after the teaching or training, and testing application on dissimilar, but related tasks or problems. It is well-know that retention and transfer of basic science is a problem and a variety of instructional strategies such as integrated curricula and problem-based learning aims at ameliorating this problem. Yet, until now curriculum level comparisons show no dramatic advantages of these strategies and there is a concern that basic science learning will be too shallow.

In this presentation I will give some perspectives of the importance of basic science knowledge to clinical practice with examples from Anaesthesiology. Secondly I'll describe how testing can be used to enhance learning both as an intrinsic effect stimulating information retrieval and as an extrinsic driving force to ensure learning in basic science.

THURSDAY, 12. NOVEMBER 2009:

FEPS KEYNOTE LECTURE

MEASURING AND MANIPULATING SECOND MESSENGER LEVELS IN CELLULAR ORGANELLES OF LIVING CELLS

Tullio Pozzan

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Cell-to-cell communication evolved as a fundamental process to coordinate the physiological behavior in multicellular organisms. How cell behavior is regulated by the diverse signalling pathways, what mechanisms the cells use to distinguish the input from different receptors activating the same signals, are key questions in modern biomedical research that need to be addressed for a better understanding of physiology and physiopathology. To gain insights into the complexity of the spatio-temporal patterns of the signals that govern cell communication, we need tools that permit the visualization and quantitative measurement of such signals by fluorescent microscopic imaging in living cells. The spatial complexity of the cell and the spatiotemporal heterogeneity of signals require not only the quantitative determination of such signals in the cytoplasm of living cells, but also within subcellular compartments and organelles. Here I will discuss the approaches that have been developed in my laboratory to study second messenger heterogeneity, in particular Ca^{2+} and cyclic nucleotides, and the novel information obtained by the use of this methodologies in a few examples relevant to human pathology. Finally, I will concentrate on the mechanisms regulating second messenger homeostasis in mitochondria, an organelle that in the last few years has been at the centre of biomedical research for its participation not only in cell energy production, but also for its role as master regulator of programmed cell death.

FRIDAY, 13. NOVEMBER 2009:

SESSION II

GLIAL FIBRILLARY ACIDIC PROTEIN AND VIMENTIN ARE NEGATIVE REGULATORS OF THE NEUROGENIC NICHE

Maryam Faiz[1], Åsa Widestrand[1], Ulrika Wilhelmsson[1], Daniel Andersson[1], Yolanda de Pablo[1], Sofia Linde[1], Marika Hietamäki[3], Peter L. P. Smith[1], Anders Ståhlberg[1], Marcela Pekna[2], Cecilia Sahlgren[3], Milos Pekny[1]

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Adult neurogenesis is restricted to two specific neurogenic niches: the subgranular zone (SGZ) of the hippocampus and the subventricular zone of the lateral ventricles. The cellular players within these niches are important for the regulation of the neural stem/progenitor cell development and coordination of cell genesis. Increasing evidence suggests an important role for astrocytes in the neurogenic niche. Previously we showed that ablating the intermediate filament proteins glial fibrillary acidic protein (GFAP) and vimentin facilitates regeneration and increases plasticity after injury and neural grafting. Here, we investigated the role of GFAP and vimentin in the regulation of endogenous neurogenesis. Ablation of GFAP and vimentin reduced Notch signaling from astrocytes and increased the number of newly born neurons. These findings correlated with enhanced learning and memory and greater plasticity in the adult hippocampus in response to injury and running. We conclude that intermediate filament proteins in astrocytes are important for astrocyte-mediated control of neurogenesis and that this control is at least partially mediated by Notch signaling. Thus, changing the intrinsic properties of astrocytes by modulating intermediate filament proteins affects the efficiency and plasticity of the neurogenic niche.

REACTIVE ASTROGLIOSIS AND POST NEUROTRAUMA RECOVERY: HUMAN NEURAL STEM CELL-REACTIVATED PROPRIONEURONAL CIRCUITRY IN GFAP$^{-/-}$VIMENTIN$^{-/-}$ MICE FOLLOWING SPINAL CORD INJURY

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Clinical therapy for traumatic spinal cord injury (SCI) still remains elusive. Though new opportunities to treat brain and spinal cord trauma have been provided by marked advance made in the field of stem cell research, the progress is hindered by the complex post lesion pathology which diminishes effective donor engraftment. Using the *GFAP$^{-/-}$ Vimentin$^{-/-}$* (GV) mouse model we previously demonstrated that integration of human neural stem cells (hNSCs) seeded in poly-lactic-*co*-glycolic scaffolds was improved in the GV spinal cord manifesting mitigated reactive gliosis following SCI. SCI GV mice receiving transplantation of hNSCs consequently showed significantly augmented functional recovery. These findings are consistent with our earlier reports that scaffolded hNSC engraftment improves locomotion in rats. Based on recent work of others and our own, indicating that plasticity of spinal intersegmental networks can trigger pivotal functional changes following SCI in rodents, we have now tested our hypothesis that post-SCI gliosis may hinder reorganization of the propriospinal neural circuits that mediate locomotor improvement after SCI by examining the impacts of double or single (d/s) knock-out of astrocytic GFAP and Vimentin on the therapeutic effects of hNSCs implant after penetrating lesion to the thoracic spinal cord. Specifically, we performed selective transection of the corticospinal tract at T2 and excitotoxic chemical lesion of propriospinal neurons at T7 in d/s KO and wildtype mice receiving scaffolded hNSC treatment. Our data indicate that recovery of locomotion in the treated GV mice largely results from reactivation of the propriospinal neuronal participation in locomotor pattern generation, which is markedly enhanced by genetic ablation of GFAP and vimentin plus the engraftment of hNSCs. The results suggest that reactive gliosis negatively affects neuroplasticity following neurotrauma, and that attenuation of reactive gliosis enhances NSC-based neuroanatomic and functional repair of traumatically injured central nervous system.
Supported by grants from NIH R21NS053935, VA Biomedical and Laboratory R&D merit grant, Massachusetts SCI Cure merit grant, the Swedish Research Council (11548), and ALF Göteborg and Hjarnfonden.

ASTROCYTE SUBTYPES AND GFAP ISOFORMS IN THE DEVELOPING AND ADULT BRAIN

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The intermediate filament (IF) network is a key component of the cell's cytoskeleton that warrants cell integrity and resilience. Recently, important novel IF network functions have emerged: it transduces biomechanical and molecular signals and it controls many associated proteins. Astrocytes express the IF protein Glial Fibrillary Acidic protein (GFAP). Over the last years, we identified novel GFAP splice isoforms, which are expressed in different subtypes of astrocytes in the developing and the adult human brain [1, 2]. GFAPδ is specifically expressed in radial glial cells and in subventricular zone (SVZ) neural progenitors in the developing human cortex and in SVZ astrocytes in the adult human brain. The out-of-frame splice variants that can be detected by the GFAP+1 antibody are mainly expressed in a small number of astrocytes containing very long processes. These specific isoforms are not expressed during brain development or in brains of young healthy adults. Interestingly, we have observed that the expression of GFAP+1 correlates positively with the number of amyloid plaques in brains of Alzheimer patients. The exact function of the specialized IF network in the neurogenic SVZ astrocytes and the astrocytes near amyloid plaques is still elusive. The research in my group is currently aimed at understanding the functional changes in these cells induced by the specific composition of the IF-network. We have set up astrocyte and neurosphere cultures isolated from post-mortem human brains to enable to study the different astrocyte subtypes and the function of the different GFAP isoforms. We ultimately aim to develop novel therapeutic approaches by targeting reactive astrocytes and the astrocytic neural stem cells.

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[2] Roelofs, R.F., Fischer, D.F., Houtman, S.H., Sluijs, J.A., van Haren, W., van Leeuwen, F.W. and Hol, E.M. *Glia* 52 (2005) 289-300.

PROPERTIES OF REGULATED EXOCYTOSIS AND VESICLE TRAFFICKING IN ASTROCYTES

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The important process of communication between astrocytes and neurons is exocytotic release of gliotransmitters (such as glutamate, neuroactive peptides and ATP) from membrane-bound vesicles into the extracellular space. To explore whether stimuli that increase $[Ca^{2+}]_i$ trigger vesicular ATP release from astrocytes, we used HEK-293T cells transfected with P_2X_3 receptor, used as sniffers for ATP release from astrocytes. Glutamate stimulation of astrocytes was followed by an increase in the incidence of small transient inward currents in sniffers, reminiscent of postsynaptic quantal events observed at synapses. Their incidence was highly dependent on extracellular Ca^{2+}. This indicates that glutamate-stimulated ATP release from astrocytes was most likely exocytotic. Prior fusing with the plasma membrane in the process of exocytosis, membrane-bound vesicles are transported through the cytoplasm. Their trafficking and consequently release of their content may be changed in altered physiological conditions, therefore affecting the physiological status of neurons. We studied the prefusion mobility of fluorescently labeled peptidergic ANP vesicles (atrial natriuretic peptide; ANP) in the cytoplasm of single rat and mouse astrocytes in culture. We found out that delivery of vesicles to the plasma membrane for exocytosis involves an interaction with the cytoskeleton, in particular microtubules and actin filaments, which is similar to neurons and excitable secretory cells. Some of the membrane-bound vesicles are retrieved from the plasma membrane to be recycled back into the cytosol. Postfusion transport of glutamatergic and peptidergic vesicles was studied by labeling vesicles with antibodies against specific membrane or luminal vesicle proteins. Trafficking velocity of prefusion and postfusion (recycling) vesicles differ in velocity for one order of magnitude and the traffic of diverse vesicles appears to be differently regulated by calcium ions.

THE QUEST FOR VENTRICULAR PACING SITES THAT PRESERVE CARDIAC CONTRACTILITY AND EFFICIENCY

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Introduction: Conventional right ventricular (RV) apex pacing is associated with asynchronous activation, reduced left ventricular (LV) pump function and increased risk for development of heart failure. We investigated whether pacing at alternative sites can maintain a better cardiac pump function as well as mechano-energetic efficiency of the heart.
Methods: After AV-nodal ablation, mongrel dogs were randomized to receive 16 weeks of VDD pacing at the RV apex (RVa, n = 9), RV septum (RVs, n=7), LV apex (LVa, n = 7), or LV septum (LVs, n = 8). LVs paced animal received a modification of the Medtronic 3830 pacing lead, with extended helix) which was introduced transvenously and, after positioning against the RV septum using a pre-shaped guiding catheter, driven into the interventricular septum by rotation of the screw-in lead until the LV endocardium ; a trans-ventricular septal approach). During atrial pacing (AP) with normal ventricular conduction and after 1-3 hours and 16 weeks of ventricular pacing we measured contractility (dP/dtmax, normalized to instantaneous pressure), relaxation (time constant of LV pressure fall) and stroke work (SW) using conductance catheters. At these times also myocardial oxygen consumption (MVO2) was measured. At 16 weeks, MRI tagging was performed to measure mechanical dyssynchrony and discoordination from myocardial strains.
Results: While acute and chronic RVa and RVs apex pacing significantly reduced contractility and relaxation, LVs and LVa pacing maintained these parameters near AP levels. RVa and RVs pacing also reduced mechanical efficiency (SW/ MVO2) and increased mechanical dyssynchrony and discoordination., whereas LVs and LVa pacing maintained the normal efficiency and coordination.
Conclusions: Chronic LVa and LVs pacing maintain LV contractility and mechano-energetic efficiency near normal levels, and at a higher level than RVa and RVs pacing. Therefore, using a good site of pacing the heart can pump better at a higher efficiency.

CONTRACTION PATTERNS IN DYSSYNCHRONOUS HEARTS

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In dyssynchronous hearts contraction patterns (CP) are heterogeneous due to non-simultaneous activation of the cardiac muscle. Any stress generated in one part of the wall reflects in all other parts of the wall therefore, the earlier activated region causes stretch in later activated regions. The CP are measured non-invasively with MRI-tagging or Ultrasound speckle tracking. Most informative are mid-wall circumferential strains, which are determined for given location in the LV wall.

CP are studied to obtain maps of the LV activation time and parameters of global ventricular function. The maps and the parameters are used to improve selection of patients for cardiac resynchronisation therapy (CRT). Maps are obtained from the prominent events in the CP like the onset of shortening time, peak shortening time and magnitude of shortening.
The parameters of global ventricular function consider interplay of CP in different regions to evaluate the coherence and the reserve capacity of the LV contraction. One of them is internal stretch fraction (ISF), which is defined as the amount of stretch relative to the amount of shortening during ventricular ejection. ISF is better predictor of reverse remodeling after CRT than differences in time to onset and time to peak shortening.

An alternative to mapping prominent events in CP is decomposition of patterns with respect to activation time. For this purpose, a lumped parameter model (CircAdapt) is used to simulate CP in dyssynchronous hearts. The activation time map is determined with reverse modelling by using measured CP.
In the cardiac muscle, on top of altered activation times also the contractility may differ in regions. This adds a new dimension in the complexity of CP and imposes challenges in mapping both simultaneously.

PACING-INDUCED PROTECTION IN THE EMBRYONIC CHICK HEART MODEL

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Introduction.
We have previously shown in the embryonic chick heart model that chronic ventricular pacing leads to structural, hemodynamic, and metabolic remodeling. We also aimed at determining to what extent the L-type Ca^{2+} channel, the sarcolemmal ($sarcK_{ATP}$), and the mitochondrial KATP ($mitoK_{ATP}$) channels are involved in the pacing-induced protection against anoxia-reoxygenation.
Methods and results.
Hearts of 4-day-old chick embryos were paced in ovo during 12-48 hours using asynchronous intermittent ventricular stimulation at 110% of the intrinsic rate. Sham operated and paced hearts were then submitted in vitro to anoxia (30 minutes) and reoxygenation (60 minutes). ECG disturbances and alterations of atrial and ventricular electromechanical delay (EMD) reflecting excitation-contraction (E-C) coupling were systematically investigated. Baseline functional parameters were stable during at least 2 hours. Anoxia induced tachycardia, followed by bradycardia, atrial ectopy, first-, second-, and third-degree atrio-ventricular blocks and, finally, transient electromechanical arrest. Reoxygenation triggered also Wenckebach phenomenon and ventricular escape beats. At the onset of reoxygenation QT, PR, and ventricular EMD increased by 68%, 70%, and 250%, respectively, whereas atrial EMD was not altered. These hearts were exposed to L-type Ca^{2+} channel agonist Bay-K-8644 (BAY-K) or blocker verapamil, nonselective K_{ATP} channel antagonist glibenclamide (GLIB), $mitoK_{ATP}$ channel agonist diazoxide (DIAZO), or antagonist 5-hydroxydecanoate. Under normoxia, heart rate, QT duration, conduction, EMD, and ventricular shortening were similar in sham and paced hearts. During reoxygenation, arrhythmias ceased earlier and ventricular EMD recovered faster in paced hearts than in sham hearts. In sham hearts, BAY-K (but not verapamil), DIAZO (but not 5-hydroxydecanoate) or GLIB accelerated recovery of ventricular EMD, reproducing the pacing-induced protection. In contrast, none of these agents further ameliorated recovery of the paced hearts.
Conclusion. The protective effect of chronic asynchronous pacing at near physiological rate on ventricular E-C coupling appears to be associated with subtle activation of L-type Ca^{2+} channel, inhibition of $sarcK_{ATP}$ channel, and/or opening of $mitoK_{ATP}$ channel.

ROLE OF VENTRICULAR ACTIVATION FOR ARRHYTHMOGENESIS IN THE CAVB DOG MODEL

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For years, the anesthetized dog with chronic, complete AV block (CAVB) is used to screen (non-)cardiovascular drugs for proarrhythmic properties. This model is very sensitive and specific for drug-induced Torsade de Pointes: proarrhythmic drugs show TdP incidences upwards to about 80%, whereas not all QT-prolonging drugs will initiate TdP. Creation of AV block will lead abruptly to a) bradycardia (decrease in heart rate from 110 towards 50 bpm), b) AV-dyssynchrony and c) altered (variable) ventricular activation. In most animals, the idioventricular rhythm (IVR) will originate from the left ventricular (LV) low septum. In time, ventricular remodeling will occur that can be seen as a) electrical (prolongation of repolarization duration and increase of beat-to-beat variability of repolarization duration), b) contractile (doubling of the peak rate of LV pressure rise (LV dP/dtmax) to preserve cardiac output) and c) structural (biventricular hypertrophy) remodeling.

To control ventricular remodeling and arrhythmogenesis, we have set out a number of experiments to investigate the relevance of ventricular activation. In the first group (9 dogs), a pacemaker lead was implanted in the high septal region (4 weeks CAVB) and dofetilide was used as the arrhythmogenic challenge. It was shown that, compared to normal CAVB dogs with IVR, maintenance of normal activation with high septal pacing (HSP) at lowest captured ventricular rate decreased baseline electrical remodeling (LV MAPD increase: 14% in HSP vs. 39% in IVR dogs) and TdP-susceptibility with about 50%: TdP incidence was lowered from 7/9 (78%) towards 4/9 animals (44%). In a second group (n=8), right ventricular apex (RVA) pacing at lowest captured ventricular rate was maintained for 3 weeks. Electrical remodeling was attenuated (LV MAPD increase in baseline: 5%), whereas the incidence of dofetilide-induced TdP was 5/8 animals (63%). After dofetilide, the decreased repolarization reserve was unmasked.

This set of experiments showed that physiologic activation (HSP) diminished TdP-susceptibility. Altered ventricular activation results in more proarrhythmia after dofetilide, whereas the origin of ventricular activation is less important.

THE PHYSIOLOGY OF RODENT BETA-CELLS IN PANCREAS SLICES

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Beta-cells are excitable cells that in pancreatic islets form complex syncytia. Sufficient cell-to-cell electrical coupling seems to ensure coordinated depolarization pattern and insulin release that can be further modulated by rich innervation. The complex structure and coordinated action develop after birth during fast proliferation of the endocrine tissue. These emergent properties can be lost due to various reasons later in life and can lead to glucose intolerance and diabetes mellitus. Pancreas slice is a novel method of choice to study the physiology of beta-cells still embedded in their normal cellulo-social context.

We present major advantages, list drawbacks and provide an overview on recent advances in our understanding of the physiology of beta-cells using the pancreas slice approach, with an emphasis on the Ca^{2+} sensitivity of the exocytotic activity. We are addressing the role of protein phosphorylation in modulation of the sensitivity of the secretory machinery to Ca^{2+}.

ION CHANNELS AND THE REGULATION OF INSULIN SECRETION: OF MICE AND MEN

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The β-cells of the pancreatic islets secrete insulin. Insulin secretion is regulated by changes in β-cell membrane potential that culminate in the opening or closure of voltage-gated calcium channels. Work on rodent β-cells have resulted in a fairly complete picture of the cellular control of insulin secretion. A major discovery was the discovery of the ATP-regulated potassium channels (K_{ATP}-channels) that constitute the resting membrane conductance of the β-cells. These channels close in response to glucose stimulation and this results in membrane depolarization. The K_{ATP}-channels are the target of hypoglycaemic sulphonylureas and they stimulate insulin secretion by direct interaction with the channel protein. Glucose- or tolbutamide-induced inhibition of the K_{ATP}-channels (via acceleration of glucose metabolism and increased ATP production) leads to the initiation of action potential firing in the beta-cell. During these action potentials, voltage-dependent Ca^{2+}-channels activate leading to an increased intracellular calcium concentration that triggers the fusion of insulin-containing secretory vesicles.

The ion channels involved in beta-cell action potential firing have been characterized in some detail. Thus, in mouse β-cells the upstroke of the action potential depends principally on activation of L-type Ca^{2+}-channels of the α1C subclass. In addition, R-type and P/Q-type Ca^{2+}-channels mediate some limited calcium influx. Action potential repolarization is mediated by activation of voltage-dependent delayed rectifying K^+-channels (Kv2.1).

The regulation of insulin secretion from human β-cells has widely been assumed to be very similar (if not identical) to that of rodent cells. Increased availability of human islet cells (as a by-product of islet transplantation programmes) has challenged this view. Like their rodent counterparts, human β-cells are equipped with K_{ATP}-channels and their closure triggers electrical activity. However, the complement of the voltage-gated Ca^{2+}-channels involved in action potential firing exhibits marked differences. Thus, action potential firing in human β-cell involves both voltage-gated Na^+-channels and low-threshold T-type Ca^{2+}-channels; two types of ion channels not playing any role at all in mouse β-cells. The L-type Ca^{2+}-channels that are essential for insulin secretion in rodent β-cells are not all involved in insulin secretion in human beta-cells but are rather required for action potential firing. Insulin secretion instead depends on Ca^{2+}-influx through P/Q-type Ca^{2+}-channels. Finally, action potential repolarization in human beta-cells results from opening of large-conductance Ca^{2+}-activated K^+-channels (channels that rarely activate in mouse beta-cells) whereas delayed rectifying Kv2.1/2.2 channels play a relative minor role. These differences between human and mouse β-cells are summarized in Table 1.

It is unlikely that the differences are limited to those already described and confined to ion channels. Our work so far has demonstrated the potential pitfalls of extrapolating rodent data to the situation in man. More work is required to understand the molecular and cellular causes of *human* diabetes (rodent diabetes is a very rare clinical condition!). Detailed knowledge about the genes expressed in human beta-cells is also essential for the interpretation of the genetic data that are now emerging. Clearly, understanding the

functional consequences of a given gene polymorphism linked to diabetes depends critically on whether the gene is expressed in the beta-cell or not.

Table 1

Channel	**EFFECTS OF PHARMACOLOGICAL INHIBITION ON INSULIN SECRETION**	
	Mouse islets	**Human islets**
K_{ATP}	⇧	⇧
L-type Ca^{2+}-channels	⇩ (by inhibiting exocytosis; α_{1C})	⇩ by inhibiting action potential firing; α_{1D})
R-type Ca^{2+}-channels	⇩	Not expressed
P/Q-type Ca^{2+}-channels	≈ (current small)	⇩ (by inhibiting exocytosis)
Voltage-gated Na^{+}-channel	≈ (channels not active)	⇩
Large conductance Ca^{2+}-activated (BK) K^{+}-channel	≈ (channels not active)	⇧
Kv2.1/Kv2.2 delayed rectifying K^{+}-channel	⇧	≈
	⇧=stimulation, ⇩=decrease, ≈ no effect	

PROTEIN SEROTONYLATION MODULATES INSULIN SECRETION

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Diabetes mellitus is one of the most important metabolic disorders and is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both. However, many aspects of insulin secretion remain elusive for the complex machinery involved, in particular the role of serotonin (5-HT) in endocrine pancreatic β-cells. It is known for more than three decades that 5-HT co-localizes with insulin in the secretory granules of these cells and it is co-released when pancreatic islets are stimulated with glucose. Nevertheless, the function of 5-HT in this context was not identified so far. 5-HT is a neurotransmitter of major importance as well as a peripheral hormone that regulates essential mood-related, metabolic and cardiovascular processes. The serotonergic system is unique amongst monoaminergic (neuro-)hormonal systems for its biosynthesis *via* two distinct rate-limiting tryptophan hydroxylases in the brain (TPH2) and peripheral tissues (TPH1).
The generation of *Tph1*-/- mice has allowed us to identify a previously unknown receptor-independent signalling mechanism of 5-HT in thrombocytes, which we have termed "serotonylation". This transglutaminase-dependent mechanism constitutively activates small GTPases by covalent binding of 5-HT to a glutamine residue of their catalytic centre, thereby triggering the exocytosis of α-granules from thrombocytes. Mice lacking peripheral 5-HT biosynthesis have a wide spectrum of physiological phenotypes, such as impaired primary haemostasis and liver regeneration, and diabetes. However, reduced 5-HT levels also exert protective effects within the cardiovascular system, e. g. in primary pulmonary hypertension.
In the presentation it will be shown that serotonylation of small GTPases of the Rab family modulates insulin secretion from pancreatic β-cells. *Tph1*-/- mice show altered insulin secretion, resulting in glucose intolerance as well as mild fasting hyperglycaemia. Together with its early onset and the inheritable pattern, this phenotype can be classified as maturity-onset diabetes of the young. Thus, monoaminylation (serotonylation) of small GTPases seems to be a crucial mechanism in the fine tuning of insulin secretion. This is the third microserotonergic system that has so far been described to depend on this posttranslational protein modification, adding strong evidence to our view, that monoaminylation plays a decisive modulatory role in signalling within a broad variety of tissues under physiological conditions.

CELL SWELLING-MEDIATED STIMULUS-SECRETION-COUPLING AND ACTIONS OF INSULIN IN CLONAL INSULINOMA CELLS

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Stimulus-secretion-coupling (SSC) in beta cells involves glucose uptake and metabolism, closure of ATP-sensitive K^+ (K_{ATP}; KIR 6.2/SUR1) channels, membrane depolarization and stimulation of insulin exocytosis by Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Ca_v). In various secretory cells cytoplasmic swelling is a potent stimulus for hormone secretion. In native and clonal beta cells glucose uptake induces cell swelling and activation of swelling-dependent Cl^- currents (termed ICl_{swell}, $ICl_{glucose}$ or ICl_{islet}). In clonal INS-1E cells this process is paralleled by cytosol to membrane translocation of ICln, a protein which is crucially involved in generating ICl_{swell}. ICl_{swell} activation contributes to membrane depolarization and insulin release and therefore provides a synergistic, under certain circumstances independent, alternative pathway of SSC. Since ICl_{swell} activation requires intracellular ATP, this current seems to be of critical importance for insulin secretion under conditions where the classical SSC pathway is silenced. The phytostilbene resveratrol reversibly inhibits Ca_v and ICl_{swell} and counteracts secretagogue- and hypotonicity-induced depolarization, and electrical activity. Short-term (1 hour) as well as prolonged (24-48 hours) exposure to resveratrol significantly inhibits glucose-induced and basal insulin release and exerts an antiproliferative/proapoptotic effect as evidenced by cell cycle arrest, apoptotic volume decrease, breakdown of phosphatidylserine asymmetry and caspase activation. Likewise, reduction of the medium glucose concentration results in growth arrest. These events are accompanied by decreased phosphorylation of Akt (PKB), a downstream target in insulin receptor signaling which is crucial for beta cell survival. The inhibition of Akt phosphorylation is significantly attenuated by addition of insulin to the culture medium, thus unraveling the significance of auto/paracrine insulin signaling in the regulation and maintenance of beta cell mass.

STRUCTURE AND FUNCTION OF SERPINS IN BLOOD COAGULATION SYSTEM

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Serpins are a family of serine protease inhibitors that share a unique three dimensional fold containing 3 beta sheets, 8-9 helices and a flexible reactive centre loop (RCL) which acts as the bait for the protease. The inhibition mechanism of serpins involves the initial recognition of the protease followed by the protease cleaving the RCL, concomitant with the conformational change in serpin to form the covalently-linked final serpin-protease complex, where the protease is destroyed due to active site deformation. The native serpin is metastable and contains a 5-stranded beta sheet A, and upon cleavage, RCL inserts into the beta sheet A, resulting in a much more stable 6-stranded form. Work in our lab focus on the serpins in the blood coagulation system, namely antithrombin, heparin cofactor II and protein C inhibitor. Over the past few years, we have solved a number of crystal structures of native serpins and serpin-protease complexes which provide us the molecular insight into how these serpins are activated and how specificity is achieved. In blood, serpins are generally circulating in a state with low activity, with high activity conferred by binding to cofactors at the appropriate time and place. The inhibition specificity can be regulated by interactions in the RCL or exosite through either allostery or bridging.

PROTEIN C INHIBITOR: A SERPIN WITH MULTIPLE FUNCTIONS

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The human serpin (*ser*ine *p*rotease *in*hibitor) protein C inhibitor (PCI) is a secreted glycoprotein with broad protease reactivity and wide tissue distribution. Target proteases of PCI include proteases of the blood coagulation and fibrinolytic systems (e.g. thrombin, activated protein C, factor Xa, urokinase), tissue kallikreins, and the sperm protease acrosin. PCI is present in blood, urine, and most other body fluids. The highest PCI concentrations are found in seminal plasma. These data together with our finding that male homozygous PCI-deficient (knockout) mice are infertile (Uhrin et al. 2000) due to abnormal spermatogenesis, suggest that PCI is important for the regulation of male reproduction. However, studies in mice are limited by the fact that in adult mice PCI is exclusively expressed in the reproductive tract. In the human system PCI may therefore have additional, different functions.

PCI is a heparin-binding serpin, and in *vitro* glycosaminoglycans modulate not only its activity but also its target enzyme specificity. We have recently shown that PCI also binds to negatively charged/oxidized phospholipids. Phospholipid binding involves the heparin-binding site (H-helix) of PCI, and PCI-binding phospholipids influence PCI activity in a way similar to heparin (Malleier et al. 2007). Furthermore, PCI can be internalized by cells via a non-conventional mechanism involving the phospholipid phosphatidylethanolamine (Baumgärtner et al. 2007). Internalized PCI can translocate to the nucleus, and this nuclear targeting requires basic amino acids of the H-helix. By using yeast-2-hybrid screening we have identified JFC-1 (synaptotagmin-like protein 1, Slp1) as an intracellular partner of PCI. JFC1 is a phospholipid-binding protein and an effector molecule of the small GTPase Rab27a, which is involved in vesicular transport processes in the cell. Additional intracellular interaction partners of PCI are CSN5 and CSN6, both components of the Cop9 signalosome, which colocalize with PCI in the nucleus of lymphocytes.

In order to determine the biological role of PCI we therefore have to consider its interaction not only with glycosaminoglycans, but also with phospholipids, which may act as locally inducible regulators of PCI activity and specificity e.g. at sites of inflammation and/or apoptosis. Additionally phospholipids may regulate the inhibition of transmembrane serine proteases by PCI, and may also present a link to so far unrecognized intracellular actions of PCI.

SERPIN IN CANCER: MASPIN AS A PARADIGM

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Maspin is a unique serpin with diverse biological functions. Initially identified from human normal mammary epithelial cells, maspin expression is either reduced or completely silenced in breast cancers. Numerous studies have implicated maspin function in cancer progression and angiogenesis. Maspin has also been targeted for breast cancer gene therapy. Recently, transgenic and gene knockout mouse models have been used by our laboratory to identify the biological functions of maspin *in vivo*. In this report, I will summarize the multiple functions of maspin in tumor progression and mouse development. Maspin knockout mice have been generated to address its function. Beginning with embryo development, maspin is required for the appropriate cell-matrix interactions to occur so that a healthy and viable embryo develops. In adult mouse, maspin is present in the epithelial cells of most tissues. Our laboratory has begun to unveil the role of maspin in the development of mammary gland and several other organs. These data demonstrate that maspin not only plays a role in tumor progression and metastasis but also is a key regulatory molecule for normal mammary gland and embryonic development.

Ca^{2+} SIGNALS REGULATING HAIR CELL TRANSMITTER RELEASE

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The hair cell ribbon synapse is specialized for temporally precise and long-lasting synaptic transmission. I will present current insights into the Ca^{2+} signals regulating exocytosis at this synapse. Presynaptic Ca^{2+} influx occurs at ~ 400 nm sized clusters of ~80 Cav1.3 channels, whose trafficking and function depend on Cavbeta2. Both, Cavalpha1.3 and Cavbeta2 are essential for hearing. The Cav1.3 channels of hair cells show little inactivation due to efficient antagonism of calmodulin-mediated CDI by Ca^{2+} binding proteins. Lack of the cytosolic Ca^{2+} binding proteins parvalbumin-alpha, calbindin and calretinin speeds up CDI and augments sustained exocytosis while leaving fast exocytosis unchanged. Synaptic Ca^{2+} influx causes the build-up of Ca^{2+} domains within few milliseconds that reach low micromolar average concentrations and submicrometer size. The voltage-dependence and amplitude of these Ca^{2+} microdomains display remarkable heterogeneity even within individual IHCs. Analysis of the apparent Ca^{2+} dependence of exocytosis during Ca^{2+} influx indicates that Ca^{2+} nanodomains around one or few channels - that sum-up to the observable microdomains - are the relevant signal triggering exocytosis of synaptic vesicles. This Ca^{2+} influx-secretion coupling supports coding of sounds with high temporal precision already at weak sound intensities.

PATHOLOGY OF COCHLEAR ION TRANSPORT: INSIGHTS FROM DEAF MICE AND HUMANS

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Ion homeostasis is essential for the hearing process. Depolarizing K influx into sensory hair cells through apical mechanosensitive channels requires a high K concentration (150 mM) in the scala media. It is generated by the epithelium of the stria vascularis which needs basolateral Cl channels for the recycling of Cl taken up by NaK2Cl cotransport. These channels are ClC-K/barttin heteromers. Mutations in barttin lead to Bartter syndrome IV, which combines severe renal salt loss with congenital deafness. We have generated a mouse model in which barttin is selectively inactivated in the inner ear, avoiding early lethality due to salt and fluid loss. These mice display congenital deafness. Endocochlear K^+ concentration was normal, but endocochlear potential was reduced. This sufficed to suppress otoacoustic emissions from outer hair cells. Outer hair cells also degenerated progressively. K leaves outer hair cells (OHCs) through basal KCNQ4 K channels. We have discivered that KCNQ4 mutations lead to slowly progressive hearing loss in humans and have generated appropriate mouse models. After exiting OHCs, K must be removed by supporting Deiter's cells which express the K-Cl cotransporters KCC3 and KCC4. Knock-out of either cotransporter leads to deafness in mice, with KCC3 disruption resulting in a slowly progressing hearing loss that is associated with hair cell degeneration. In addition, KCC4 KO leads to renal tubular acidosis, while the mice lacking KCC3 display severe neurodegeneration similar to patients with Anderman syndrome who also lack KCC3.

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ROLES FOR PRESTIN IN AMPLIFICATION AND FREQUENCY TUNING IN THE COCHLEA

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The term "Cochlear Amplifier" was coined by Hallowell Davies for "an active process" which "somehow provides additional energy that enhances the vibration of a narrow segment of the basilar membrane near the apical foot of the familiar, travelling wave envelope". Currently there are two putative candidates for the cochlear amplifier. These are voltage-dependent, somatic, motility mediated by the unique outer hair cell motor protein prestin and calcium-mediated, hair-bundle, motility, which is a ubiquitous feature of hair cells. We have attempted to distinguish between these possibilities by measuring basilar membrane displacements from *Tecta* mice in response to acoustic and electrical stimulation. At low stimulus levels basilar membrane displacements at and around the peak of the travelling wave are dominated by electromechanical amplification from the outer hair cells. In response to acoustic stimulation this amplification is mediated through sensory transduction caused by displacements of the outer hair cell hair-bundles through their interaction with the tectorial membrane, to which they are attached. Electrical stimulation of the cochlea bypasses sensory transduction and drives directly both outer hair cell somatic and hair-bundle motility. We measured basilar membrane displacements in response to acoustic and electrical stimulation in wild type $Tecta^{+/+}$ mice, which have a tectorial membrane that will permit electrically elicited hair-bundle movements to interact with it. This opportunity is precluded to the hair bundles of $Tecta^{\Delta ENT/\Delta ENT}$ mice where the residual tectorial membrane is detached from the organ of Corti. Basilar membrane frequency tuning measured from $Tecta^{+/+}$ mice in response to acoustic stimulation and from $Tecta^{+/+}$ and $Tecta^{\Delta ENT/\Delta ENT}$ mice in response to electrical stimulation are very similar at threshold when OHC activity dominates the electromechanical properties of the cochlea. At low stimulus levels, electrically elicited hair-bundle motility cannot amplify basilar membrane responses of $Tecta^{\Delta ENT/\Delta ENT}$ mice since OHC sensory bundles cannot react against the tectorial membrane, a prerequisite for exerting forces on the basilar membrane. Thus, amplification and compression of basilar membrane responses of $Tecta^{\Delta ENT/\Delta ENT}$ mice in response to near-threshold electrical stimulation (similar to near-threshold acoustically and electrically elicited responses from $Tecta^{+/+}$ mice) cannot be due to hair-bundle motility.

If time permits, evidence will be presented for a role for prestin in the frequency tuning of cochlear mechanical responses and their transmission to neural excitation.

MOLECULAR PERTURBATION OF A LARGE CNS AUDITORY SYNAPSE YIELDS NEW INSIGHTS INTO TRANSMITTER RELEASE AND ACTIVE ZONE FUNCTION

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Transmitter release from active zones of presynaptic nerve terminals is the most important means of fast information transfer between neurons in the brain. In recent years, significant progress in our understanding of the molecular mechanisms of presynaptic active zone function has been achieved by creating and analyzing k.o. mouse models deficient for various presynaptic proteins. However, important aspects of the coupling between Ca^{2+} channels and readily-releasable vesicles, as well as of the Ca^{2+} triggering function in transmitter release have remained obscure, maybe because of the inaccessibility of many small synapses to direct presynaptic electrophysiology. The calyx of Held is a large synapse in the auditory brainstem of mammals at which precise control of the nerve terminal $[Ca^{2+}]_i$ concentration can be achieved (Schneggenburger & Neher 2005, *Curr. Op. Neurobiol.*). However, molecular perturbation of this large brainstem synapse has been difficult, since many k.o. mouse models of presynaptic proteins are perinatally lethal, and since rescue of presynaptic function at the calyx of Held in a k.o. mouse model has not yet been obtained. Here, in a first study on the role of Synaptotagmin in Ca^{2+}-triggered vesicle fusion, we have established a functional rescue of Ca^{2+} triggered release at the calyx of Held in Synaptotagmin-2 k.o. mice, by using adenovirus-mediated overexpression of Syt-2. This has allowed us to perform a structure-function analysis of Syt-2 function under direct control of presynaptic $[Ca^{2+}]_i$. In a second study, we have established a tissue-specific k.o. of all known RIM 1/2 isoforms (Rab3a-interacting molecule) at the calyx of Held, using recently generated floxed mouse lines for RIM1 (Kaeser et al. 2008, *J. Neuroscience*) and RIM2, crossed with an auditory-brainstem specific Cre mouse line. This approach avoids perinatal lethality, and promises to yield new insights into the roles of RIM's in docking and priming of readily-releasable vesicles, and in targeting Ca^{2+} channels to the active zone.

FRIDAY, 13. NOVEMBER 2009:

SESSION III

AN INTERACTIVE MODEL FOR OXYGEN TRANSPORT AND ACID BASE PHYSIOLOGY IN HUMAN BODY

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Our interactive computer model for the oxygen transport uses demographic physiologic data combined with individual clinical and laboratory data to simulate various physiological and pathophysiological states. Besides for teaching purposes it can be also used for integration of multiple clinical and laboratory measurements. Thus, the model enables to to predict the arterial oxygen saturation and blood CO_2 levels by interactively changing different parameters that are accessible from clinical and laboratory measurements or alternatively replaced by the corresponding demographic data. In the model it is possible to vary the gas composition and the barometric pressure, alveolar ventilation, diffusion capacity of the lungs, blood hemoglobin concentration, plasma protein concentration (both affect pH), blood perfusion of different tissues (so far we use 6 different tissues), specific tissue metabolism and the tissue capillary density. It is possible to change the acid-base status by adding bicarbonate and nonvolatile acids, and thus to change base excess. The model is based on known kinetic principles to describe transport of blood gases in human body from the outer atmosphere to different tissues. It is composed of four compartments for transport of blood gases (O_2 and CO_2): airways, alveolo - capillary interface, the blood and the blood vessels, and the Krogh cylinder for blood gas exchange in tissues, including chemical regulation of breathing. It is a steady state model, and does not provide transients. The components of the model were validated using published data from different experiments, such as those of Siggaard Anderson for the acid-base status of the blood.

HIGH ALTITUDE ADAPTATION IN THE RESPIRATORY CONTROL SYSTEM. A MATHEMATICAL MODEL SIMULATION

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Key words: hypoxia, chemoreflex control of breathing, acid-base balance, strong ion difference, 2,3 DPG.

Introduction: Our interactive personal computer based mathematical model for the oxygen transport uses clinical and laboratory data for the purpose of simulation of physiological and pathophysiological states, and is based on the known kinetic principles to describe transport of blood gases in human body from the outer atmosphere to different tissues[1]. Recently, the model was upgraded to include the influence of acid-base balance in the chemoreflex control of breathing[2], as well as the influence of hypoxia on the peripheral blood flow[3]. With such model we simulated adaptation of breathing to the prolonged hypoxia to find which of the known compensatory mechanism is most effective.

Material and methods: The model is composed of four compartments for transport of blood gases (O2 and CO2): airways, alveolo - capillary interface, the blood and the blood vessels, and the Krogh cylinder for blood gas exchange in tissues. With the model, it is possible to change interactively in real-time different parameters that are accessible from clinical and laboratory measurements to monitor the arterial oxygen saturation and blood CO_2 levels. Thus, it is possible to vary the gas composition and the barometric pressure, alveolar ventilation, diffusion capacity of the lungs, blood hemoglobin concentration, plasma protein concentration (both affect pH), blood perfusion of different tissues (so far we use 6 different tissues), specific tissue metabolism and the tissue capillary density. In addition, it is possible to vary strong ion difference (SID) in the blood plasma and cerebrospinal liquor, or to change the concentration of 2,3DPG in the erythrocytes.

Results: The most effective adaptation measure to reduce effects of hypoxia were found to be changes of brain SID and acidification of blood due to bicarbonate excretion by kidneys, whereas substantial changes in 2,3 DPG in the blood inexpectedly reduced the efficincy oxygen transport to tissues, particularly after acid-base adaptation has already reached

Conclusions: The model enables understanding of integrative function of respiratory adaptation and suggests direction of the experimental studies connected with hypoxia.

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CHANGES IN THE VENOUS BLOOD OXYGEN STORES AT THE ONSET OF EXERCISE

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At the start of exercise oxygen uptake (VO_2) lags behind the energy requirement giving rise to an oxygen deficit. This is a measure of the amount of energy which has to be covered by other sources, mainly: 1) utilization of O_2 already stored in the body and 2) phosphocreatine (PCr) breakdown.
The present work was carried out to quantify the changes in the body oxygen stores from transient or steady state measurements.
Alveolar VO_2, cardiac output (Q; obtained from the signals of a photoplethysmographic system), and PCr signal, by means of ^{31}P-MR spectroscopy, were measured non-invasively on 8 healthy volunteers (1.78±0.06 m; 70.1±9.7 kg) at rest and during plantar flexion exercise (8.6±2.4 W). A non-linear iterative mono-exponential fitting procedure was applied to determine the time constants of the O_2 uptake (τ_{VO2}) and the PCr splitting (τ_{PCr}) kinetics, respectively. Changes in the venous blood O_2 stores were calculated: 1) as the product of the alveolar steady state VO_2 above resting and the difference between the time constants τ_{VO2} and τ_{PCr} ($\Delta O_2^s\big|_\tau$) and 2) as the product of the estimated venous blood volume and the difference in the arterio-to-mixed-venous blood oxygen content between rest and steady state exercise ($\Delta O_2^s\big|_Q$). τ_{PCr} (25.0±4.9 s) was significantly shorter than τ_{VO2} (40.3±6.3 s; paired t-test, $p<0.001$, $n=8$). The $\Delta O_2^s\big|_Q$ (35.8±14.7 mL) were not statistically different from $\Delta O_2^s\big|_\tau$ (35.3±19.5 mL; paired t-test, p=n.s., $n=8$) and were linearly related to each other ($R^2=0.595$; $p<0.05$; $n=8$). The Bland–Altman plot between $\Delta O_2^s\big|_Q$ and $\Delta O_2^s\big|_\tau$ shows: 1) no trend in the data as the mean venous blood oxygen store increases, 2) a 95% confidence interval from –25.3 to +24.3 mL, and 3) a bias of –0.57 mL.
Present results support the view that difference in the arterio-to-mixed-venous blood oxygen content between rest and steady state provide a good estimate of the changes in the venous blood O_2 stores at exercise onset, thus showing that it is possible to obtain information on variables characterizing the metabolic transient from data obtained at steady state, which can be considered the "memory" of the events having occurred at exercise onset.

EFFECTS OF GRAPE SEED EXTRACT ON OXIDATIVE DAMAGE AND ANTIOXIDANT DEFENSE INDUCED BY ACUTE AND CHRONIC EXERCISE IN RATS

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The aim of the present study was to investigate the effects of grape seed extract (GSE) on oxidative stress and antioxidant defense markers in acutely and chronically exercised rats.

The study protocol was approved by Ethics Committee of Experimental Medicine Research and Application Center, Selçuk University. Sixty-four adult male Sprague-Dawley rats, weighing 200-300 g at the beginning of the experiment were used. Rats were housed cages with 12/12 h light/dark cycle at 21±2 ^{o}C and 50 % humidity. Rats were assigned randomly to six groups: Control (C), Control Chronic Exercise (CCE), Control Acute Exercise (CAE), GSE-supplemented Control (GC), GSE-supplemented Chronic Exercise (GCE) and GSE-supplemented Acute Exercise (GAE). Chronic exercise consisted of treadmill running at 25 m.min^{-1}, 45 min.day^{-1}, 5 days per week for 6 weeks. Rats in the acute exercise groups were run on the treadmill at 30 m.min^{-1} until exhaustion. Rats in the GSE supplemented groups received GSE (100 mg.kg^{-1}.day^{-1}) in drinking water for 6 weeks. Rats were sacrificed by cardiac puncture immediately after the exercise in acute exercise groups and 24 h after the last exercise in chronic exercise groups. Control rats were sacrificed under similar conditions. Plasma were separated from the blood for the analysis of malondialdehyde (MDA), xanthine oxidase (XO), adenosine deaminase (ADA), nitric oxide (NO), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Plasma MDA levels were higher in acute exercise groups and lower in chronic exercise groups compared to the controls. MDA levels were lower in GSE-supplemented groups compared to their controls. XO and ADA activities were higher in CAE group compared to the other groups. Although NO level increased with chronic exercise and with GSE supplementation, it was not different in acute exercise groups compared to the controls. SOD and GPx activities were lower in acute exercise group and higher in chronic exercise group compared to their controls. Antioxidant enzyme activities were higher in GSE-supplemented groups compared to the controls.

We concluded that oxidative stress increases with acute exercise and decreases with chronic exercise and antioxidant enzyme activities decrease with acute exercise and increase with chronic exercise. Oral grape seed extract supplementation prevents exercise-induced oxidative stress by preventing lipid peroxidation and increasing the antioxidant enzyme activities in rats.

THE EFFECT OF INTERVAL TRAINING COMBINED WITH EXTERNAL PRESSURE ON THIGHS ON MAXIMAL AND SUBMAXIMAL PERFORMANCE

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It is well known that a moderate reduction of muscle blood flow (low body positive pressure of 50 mmHg) during endurance continuous exercise training enhances the local aerobic muscle adaptations. The present study investigates the effect of interval training combined with thigh cuffs pressure of +90 mmHg on maximal and submaximal performance. Twenty untrained individuals (age = 23.0 ± 4.2 yrs; stature = 168.3 ± 9.6 cm; body mass = 64.2 ± 15.0 kg) were assigned either to control (CON) or to experimental (CUFF) training group. All participants carried out an incremental exercise test to exhaustion (VO_{2max}), a 6-min constant test at 80% of VO_{2max} (Sub_{80}) and a constant power test (TF_{150}) pre and post training. Furthermore, they obtained an incremental exercise test with cuffs inflated to external pressure of +90 mmHg (VO_{2max}Press) pre-training in order to be determined the training intensity. Both groups performed interval training on cycle-ergometers 3 $d \cdot wk^{-1}$ for 6 wks. The CON group trained on cycle ergometer without cuffs, whereas the CUFF group trained with cuffs on thighs pressurized to +90 mmHg (cuffs depressurized during active recovery) at the same relative intensity. In particular, each training session consisted of 2-min work bout at 90% of VO_{2max} or VO_{2max}Press: 2-min active recovery bout at 40% of VO_{2max}. Despite the unchanged VO_{2max}, both groups increased significant PPO (CON: Pre = 207.2 ± 60.6 Watts, Post = 237.7 ± 77.2 Watts; CUFF: Pre = 182.3 ± 40.8 Watts, Post = 227.0 ± 37.7 Watts; $P \leq 0.05$) that was accompanied by higher deoxygenation (ΔStO_2) (CON: Pre = -15.3 ± 1.3%, Post = -29.1 ± 1.3%; CUFF: Pre = -21.6 ± 1.5%, Post = -42.4 ± 1.6%; $P \leq 0.05$) measured with near infrared spectroscopy (NIRS); the deoxygenation was more pronounced on CUFF group ($P \leq 0.05$) at the same relative PPO. Moreover, both groups reduced VO_2 ($P \leq 0.05$) during Sub_{80} without concomitant changes in ΔStO_2. Also, CON and CUFF group improved TF_{150} by ~40% and ~32%, respectively; but there were no differences between training groups. It seems that 6-wks interval training combined with thigh cuffs pressure of +90 mmHg on exercised legs at the same relative intensity does not provide any additive effect on maximal and submaximal performance. However, despite the lower absolute training intensity of CUFF group, the enhanced PPO that was accompanied by higher ΔStO_2 may reveal improvement of peripheral aerobic factors transferring and consuming O_2.

THE LEVELS OF LEPTIN, GHRELIN AND RESISTIN AFTER CHRONIC EXERCISES IN RATS: THE EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE) ON THESE PARAMETERS

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Objective: We aimed to investigate the effects of and caffeic acid phenethyl ester (CAPE) on adipokine (leptin, ghrelin, resistin) levels and lipid parameters in rats after chronic exercise.
Methods: This study was performed on 60 Sprague-Dawley male rats at 6 weeks. Groups were formed as follows: Group 1(sedantary+saline), group 2 (exercise+ saline), group 3 (sedantary+ ethanol), group 4 (exercise+ ethanol), group 5 (sedantary+ CAPE), group 6 (exercise+ CAPE). Leptin, ghrelin, and resistin levels were determined with ELISA technique and lipid concentrations (TG, TK, HDL-K) were measured with colorimetric methods in serum samples obtained from rats.
Results: Significantly decreased ghrelin levels in CAPE groups ($p<0.05$, $p<0.01$) were observed. Triglyceride levels were found to be significantly increased in sedantary+CAPE groups.
Conclusion: According to our findings we concluded two suggestions a) exercise alone can't be sufficient on weight loss. b) exercise is not the only factor which influence the studied parameters (leptin, ghrelin, resistin, TG, TK, HDL-K ve LDL-K). As a result, it can be suggested that exercise application with dietary restriction might more efficient in weight control.

THE ROLE OF ENDOCRINE TISSUE NETWORK TOPOLOGY IN BETA-CELL BURSTING OSCILLATIONS PATTERNS

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Often a specialized physiological task, like control of nutrient storage, is entrusted to a limited number of cells in our body. When this cell task force fails we can face a serious health problem. An appropriate example is insulin-secreting tissue organized into groups or islets of beta-cells scattered within pancreas. Electrical activity in beta-cells exhibits a characteristic bursting pattern, which consists of slow oscillations in membrane potential between a depolarized plateau, on which calcium action potentials are superimposed, and a hyperpolarized electrically silent interval. The gap junctional coupling between pancreatic beta-cells is critical for synchronous glucose-dependent insuling secretion and bursting electrical activity. While the heterogeneity of beta-cells was shown to be important in modelling of electrical response of coupled beta-cell clusters most models consider only nearest neighbors coupling between the cells in specified geometries thus excluding any paracrine effects.

Here we take a different route and propose to introduce coupling between beta-cells based on the spatially embedded complex network cytoarchitecture model of an intact living islet. We used a fitness network model approach to construct complex networks connecting the beta-cells based on their electrophysiological state representing the ability to communicate with other beta-cells in the islet. The physiology of beta-cells was studied in cytoarchitectural intact islets using pancreas slice method. Under fixed electrophysiological conditions bursting oscillations of the membrane potential of beta-cells were computed in islets modeled as regular lattices with nearest neighbor interactions and as networks with complex topologies. We show that long range communication between beta-cells emerging from the scale-free topology of the islet cytoarchitecture can have important consequences for the beta-cells bursting patterns.

CARDIAC RYANODINE RECEPTORS: A NOVEL THERAPEUTIC TARGET IN DIABETIC CARDIOMYOPATHY

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Abnormal intracellular Ca^{2+} ($[Ca^{2+}]_i$) handling by the sarcoplasmic reticulum (SR) is a critical factor in the development of heart failure (HF). Excitation-contraction coupling is predominantly controlled by Ca^{2+} release from the SR via the ryanodine receptor (RyR2). Release of Ca^{2+} via RyR2 is induced by Ca^{2+} influx through L-type Ca^{2+} channels and is modulated by multiple factors, including phosphorylation of RyR2 by protein kinase A, calmodulin kinase II and FKBP12.6. Hyperphosphorylation of RyR2 induces Ca^{2+} leak during diastole, which can cause fatal arrhythmias and lead to HF. This makes RyR2 an important therapeutic target.

We studied and confirmed the defective $[Ca^{2+}]_i$ signaling with both lower amplitude and slower kinetics of Ca^{2+} transients as well as the decreased SR Ca^{2+} load in diabetic rats. Furthermore we clearly established that these defects could be attributed to anomalous RyR2 behavior, as revealed by the spatio-temporal properties of Ca^{2+} sparks that especially exhibited slower kinetics. The reduced amount of RyR2 and FKBP12.6 levels and the PKA-dependent phosphorylation of RyR2 could be responsible for most of these observations. A lower SR Ca^{2+} load reinforced these defects in Ca^{2+} release channels.

We demonstrated that a beta-adrenergic receptor blocker, timolol treated diabetic rats exerted a protective action against anomalous Ca^{2+} homeostasis that was attributable to reduced RyR2 phosphorylation level, and recovery of protein levels of both RyR2 and FKBP12.6. Similar recoveries are also obtained by treating the diabetic rats with another beta-adrenergic receptor blocker, propranolol. We also showed that treatment of diabetic rats with these two beta-blockers restored the altered kinetic parameters of Ca^{2+} transients, depressed Ca^{2+} loading of SR and basal $[Ca^{2+}]_i$. It is concluded that a detailed understanding of the basic structure and function of RyR2 will provide us their involvement in heart diseases, and the development of drugs to prevent RyR2 malfunction.

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THE CONTRIBUTION OF STIM1 AND ORAI1 TO Ca^{2+} HOMEOSTASIS AND INSULIN SECRETION IN INS1-R9 β-CELLS

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Ca^{2+}-homeostasis plays a critical role for insulin-secretion in pancreatic β-cells. There are two main sources of Ca^{2+}, the extracellular space and intracellular stores. In pancreatic β-cells the L-type voltage gated Ca^{2+}-channel is the main influx pathway upon stimulation by glucose and the main mechanism for insulin secretion. Ca^{2+}-signaling is further modified by Ca^{2+} release from internal stores and the accompanied Ca^{2+} entry via store-operated Ca^{2+}-channels (SOCs). In the last few years it was shown in a number of different cell types that the stromal interacting molecule 1 (Stim1) and the Orai proteins represent the key-proteins responsible for SOCE. It was already demonstrated that in mouse MIN6 cells (1) Stim1 is expressed and translocated from the ER to the peri-PM space upon ER depletion. The aim of this study was to investigate the role of Stim1 and Orai1 for basal Ca^{2+}-homeostasis and SOCE in rat pancreatic β-cells and to elucidate their possible contribution to insulin secretion. The expression of Stim1 and Orai1 mRNA in the rat β-cell line INS1-R9 was positively tested with RT-PCR. Subsequently, changes in the Ca^{2+} homeostasis under resting conditions and upon cell stimulation in β-cells, that transiently overexpressed Stim1 and Orai1, were assessed. In Stim1 and Orai1 overexpressing cells, an increase in resting cytosolic Ca^{2+} and an enhanced Ca^{2+}-entry upon store-depletion with different agonists (carbachol, caffeine, BHQ) was found, while Ca^{2+}-influx via L-type voltage gated Ca^{2+}-channels upon depolarization was unmodified. Down-regulation of Stim1 supports the data mentioned above. The increase of basal cytosolic Ca^{2+} was not accompanied by a significantly increased depletion of the ER in Ca^{2+} containing medium. Furthermore we have indications that knockdown of Stim1 reduces the ER leak, which is known to be strong in β-cells. These data point to a Stim1/Orai1-dependent basal Ca^{2+} cycling in β-cells that may contribute not only to Ca^{2+} homeostasis but also for insulin secretion.

(1) Tamarina N.A. et al.; Cell Calcium, 44, 2008.

EFFECTS OF GRAPE SEED EXTRACT SUPPLEMENTATION ON PLASMA OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE MARKERS IN DIABETIC RATS

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The aim of the study was to investigate the effects of grape seed extract (GSE) supplementation on oxidative stress and antioxidant defense markers in diabetic rats.
The study protocol was approved by Ethics Committee of Experimental Medicine Research and Application Center, Selçuk University. Thirty-seven adult male Sprague-Dawley rats weighing 200-300 g were used. Rats were housed cages with 12/12 h light/dark cycle at 21±2 ^{o}C and 50% humidity. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 50 mg.kg^{-1} body weight). On the 7th day after administration of STZ, diabetes was confirmed by measuring blood glucose levels using a glucometer. Rats with blood glucose level 300 mg.dl^{-1} or higher considered to be diabetic. Four groups were formed: Control (C, n=8), GSE supplemented control (GC, n=10), diabetic (D, n=6) and GSE supplemented diabetic (GD, n=7). Beginning on the 7th day after STZ injection the rats in GC and GD groups were administered GSE (100 mg.kg.day^{-1}) in drinking water for 6 weeks. At the end of the 6th week, rats were sacrificed by cardiac puncture. Plasma separated from the blood were stored at -80 ºC until the time of analysis of malondialdehyde (MDA), xanthine oxidase (XO), adenosine deaminase (ADA), nitric oxide (NO), superoxide dismutase (SOD) and glutathione peroxidase (GPx).
GSE supplementation did not affect blood glucose levels in diabetic rats. Plasma MDA levels increased in diabetic rats and GSE supplementation ameliorated this elevation. XO and ADA activities were higher in diabetic rats compared to other groups. Plasma NO levels decreased in diabetic rats and GSE supplementation normalized that decrease. Antioxidant enzyme activities (SOD and GPx) decreased in diabetic rats compared to the controls. GSE supplementation increased antioxidant enzyme activities both diabetic and healthy rats.
In conclusion, 6 weeks of oral grape seed extract supplementation prevents oxidative stress and improves antioxidant status in diabetic rats.

POSSIBLE INVOLVEMENT OF LEPTIN-AMPK AXES IN INDUCTION OF MUSCLE NONSHIVERING THERMOGENESIS BY HIGH-FAT DIET IN MICE: ASSOCIATION WITH OBESITY-RESISTANCE

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Obesogenic effect of high-fat (HF) diet can be counterbalanced by stimulation of energy expenditure and lipid oxidation in response to the meal. Aim of this study was to reveal whether muscle nonshivering thermogenesis could be stimulated by HF diet. The other aim was to support the hypothesis of leptin and AMP-activated protein kinase (AMPK) involvement in this mechanism. Male mice of obesity-resistant A/J and obesity-prone B/6J strains, born and maintained at 30 °C, were used. At 4 weeks of age, mice were randomly weaned onto a low-fat (LF) or HF diet. At the age of 6 weeks, indirect calorimetry and cold tolerance test were performed and tissues for *in-vitro* experiments, biochemical and gene expression analysis were collected. The A/J LF mice were cold-sensitive, while the A/J HF, B/6J LF and B/6J HF mice were cold-tolerant. Cold-sensitivity of the A/J LF mice was associated with low energy expenditure, which was normalized by HF diet. Only in A/J mice, oxygen consumption, total content and phosphorylation of AMPK, and AICAR-stimulated palmitate oxidation in soleus muscle was increased by the HF diet in parallel with significantly increased leptinemia. Gene expression data in soleus muscle of the A/J HF mice indicate a shift from carbohydrate to lipid oxidation. Our results suggest a role of muscle nonshivering thermogenesis and lipid oxidation in the obesity-resistant phenotype of A/J mice and indicate that HF diet could induce thermogenesis in oxidative muscle, possibly by the leptin-AMPK axis.

BODY COMPOSITION AND BODY FAT PERCENTAGE OF STUDENTS AT THE UNIVERSITY OF LATVIA IN 2007-2009

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BACKGROUND: A positive correlation is well established between obesity and predisposition of health risk to develop metabolic syndrome, hypertension, insulin resistance and other functional disorders.
OBJECTIVE. To characterize the body composition, body fat percentage (%BF) and somatotype of the students of University of Latvia and document the percentage of overweigh, obese, and underweight students.
METHODS: To estimate the body composition, %BF and somatotype, we examined 489 students (ages 18 –25) of University of Latvia: 146 men and 343 women. Underweight, overweight and obesity was assessed by anthropometric and weight measurements, body mass index (BMI), skin folds measurement, and bioelectrical impedance analysis. Analyses were performed separately in men and women adjusted for age. Results were assessed according to the criteria of World Health Organization and American Council of Exercise.
RESULTS and DISCUSSION: The results show that by using different methods to establish the underweight, overweight and obesity, the results differ. BMI characterizing underweight (under 18,5) was obtained in 1.3% men and 7.5% women. BMI characterizing overweight and obesity (over 25) was obtained in 13.74% men and 12.7% women.
Though individual values of BF% determined by skin folds measurement and bioelectrical impedance analysis methods differ, our results show a correlation between body fat percentage determined by these both methods ($r=0.79$ for men, $r=0.68$ for women, $p<0.001$). Such difference in results using several measurement methods is acceptable. And each method's results are comparable using each method's evaluation scale.
The BF% criteria evaluated 31.8% of men and 32.3% of women as being slightly to acutely underweight. The BF% criteria evaluated 17.0% of men and 22.3% of women to have a %BF that indicates overweight. The BF% criteria evaluated 5.6% of men and 1.8% of women respectively as obese.
The leading somatotype estimated by anthropometric method for men as well for women is *ectomorphic-mesomorph*.
CONCLUSION: More than half of those tested have BF% evaluations outside of normal body fat level. Such results suggest either an increased health risk to more than half of those tested or it is necessary to specify the individual somatotypic feature in the evaluation of increased health risk factors. We suggest that a potential health risk group include only persons with an extreme increased or decreased BF% which characterizes obesity or malnutrition (approximately 5% of those studied). To specify the amount of health risk additional studies are required.

THE IMPACT OF FAMILY HISTORY FOR VASCULAR PATHOLOGY ON THE AUTONOMIC CARDIOVASCULAR FUNCTION IN YOUNG NORMOTENSIVE INDIVIDUALS

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Coronary and cerebral vascular pathology results from the interaction of many hereditary and environmental factors acting at cellular, organ and system level. Our aim was to evaluate the autonomic cardiovascular function in healthy offspring of individuals with vascular pathology related to essential hypertension, metabolic disorders or to its outcome (myocardial infarction, cerebral stroke).
The assessment of the autonomic cardiovascular function was based on the measurement of heart rate variability (HRV), blood pressure variability (BPV) and baroreflex sensitivity (BRS). In addition pulse wave velocity (PWV) was measured as a marker of arterial rigidity. Two cardiovascular function tests were applied: orthostatic test (OT) as a sympathetic challenge and Valsalva maneuver for BRS evaluation. Continuous recording of electrocardiogram, noninvasive blood pressure (Finapress, Ohmeda 1300) and photoplethysmogram was performed using Biopac MP100 digitizer. Data were analyzed using Acknowledge and MIS2000 software.
The studied individuals were divided into 2 groups: with family history for vascular pathology (N+) and their age matched controls (C).
HRV parameters in N+ demonstrated a predominance of the sympathetic influences on cardiac function, e.g. HRV index of autonomic balance was markedly higher in N+ as compared to C both at rest (2.7 vs. 0.8) and during OT (7.5 vs. 2.4). BPV was greater in N+ (17 vs. 9 $mmHg^2$ in C at rest; 63 vs. 41 $mmHg^2$ during OT). Accordingly, BRS was lower in N+ (3.8 ms/mmHg) as compared to C (12.7 ms/mmHg) at rest and 2 vs. 8.6 ms/mmHg after OT performance. Modified abPWV was higher in N+ individuals as compared to controls – 4.2 vs. 3.3 m/s.
Our data revealed the existence of discreet alterations of the autonomic cardiovascular function in young healthy individuals with family history for vascular pathology. Those alterations were manifested by slight changes in HRV, BPV, BRS and PWV, which were typical for the predominance of the sympathetic efferent influences on the cardiovascular effectors. Hence, these noninvasive tests are suitable for screening individuals at risk and for primary prevention of cardiovascular pathology.

Keywords: Heart rate variability; Baroreflex sensitivity; Blood pressure variability; Pulse wave velocity; Cardiovascular autonomic function.

This study was supported by Grant N Bg-SK 201 of the Bulgarian Fund for Scientific Research.

LUNG MECHANICS MEASUREMENTS BY THE END-INFLATION OCCLUSION METHOD IN MICE

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11 female mice (mean weight 30.8±0.6 g) were anesthetized (chloralose 50 mg/100 g i.p.), paralyzed (cis-atracurium 0.5 mg/100 g i.p.) and trachetomized. A cannula was inserted into the second tracheal ring, and positive pressure ventilation (tidal volume 0.4 ml, breathing frequency 120/min) was started. The thorax was open, and the lungs exposed to athmospheric pressure. The tracheal cannula was connected to a constant flow pump set to deliver an inflation volume of 0.4 ml at a constant flow rate of 1 ml/sec. The time for the rise and the fall of flow was about 30 msec. Lateral tracheal pressure was monitored and recorded. The pressure tracings allowed the measurements of lung mechanics according to the end-inflation occlusion method. The mean value of static lung compliance (Cst,l) resulted 0.043±0.002 ml/cmH_2O and of total lung resistance (Rmax,l) 1.59±0.06 cmH_2O/ml sec^{-1}. The latter includes the newtonian lung resistance due to the movement of airflow and lung's tissue (Rmin,l = 0.38±0.03 cmH_2O/ml sec^{-1}), and the pressure dissipation due to stress-relaxation and time constants inhomogeneity within the lung (Rvisc,l = 1.21±0.05 cmH_2O/ml sec^{-1}). Equipment resistance (Req) was separately measured and subtracted from results, which hence represent intrinsic values. Confirming what observed in rats and humans, the component of visco-elastic pressure dissipation in mice lung is higher than the newtonian component. Mice lung mechanics measurements by the end-inflation occlusion method are scanty in the literature. Present results are generally comprised in the range of previously reported values for normal mice. Thus, present data validate our measurement technique. We conclude that the end-inflation occlusion method is suitable for mechanics measurements in the small mice lungs.

HEMIFUSION STATES OF VARIABLE DURATION PRECEDE EXOCYTOTIC FUSION PORES IN ALVEOLAR TYPE II CELLS

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Hemifusion, the merger between two opposing membrane leaflets, is considered a key step for fusion pore formation. However, the model of fusion through hemifusion has not yet been directly demonstrated for exocytosis in living cells. Using high resolution darkfield and fluorescence microscopy in alveolar tape II pneumocytes containing large (> 1 µm) secretory vesicles (lamellar bodies), we show that lamellar body fusion events with the plasma membrane were accompanied by a mostly bi-phasic *s*cattered (darkfield) *l*ight *i*ntensity *d*ecrease (SLID) originating from the border of the fusing lamellar body. Correlation of SLID with the diffusional behaviour of various fluorescence markers for either content or membrane mixing revealed that the onset of the fast, second, phase of SLID corresponded to the formation of a fusion pore, followed by lamellar body swelling. In contrast, the slow, first, phase of SLID preceded pore formation but could still be accompanied by diffusion of farnesylated DsRed, a marker for the inner plasma membrane leaflet, or nile red. We conclude that the first phase of SLID reflects the hemifusion state of exocytotic lamellar body fusion, while the second phase reflects fusion pore opening, fluid entry and lamellar body swelling. These data indicate that exocytotic fusion proceeds through hemifusion intermediates. Furthermore, hemifusion may last up to 8 s. SLID is a highly sensitive and non-invasive approach by which hemifusion can be detected independently of membrane markers, thereby providing a new tool to investigate the regulation of hemifusion in living cells.

POSTNATAL CHANGES IN THE CARDIOMYOCYTE-CONTRACTILITY AND CALCIUM TRANSIENTS

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The myocardium undergoes many functional and structural changes during postnatal development. These changes allow organization of proteins and organelles to adapt to conditions that are best for the efficient function of the growing heart. The age-related functional changes of the cardiac pump are known. However, nothing is known about contractile characteristics of single myocytes and whether such characteristics are also associated with changes in Ca^{2+} mobilization. Measurement of contractility and Ca^{2+} mobilisation can therefore provide a useful insight into the cell function and Ca^{2+} cycling.

Contractile changes were studied in freshly isolated cardiomyocytes from 14, 21, 28 day old and adult male rats. The cardiomyocytes were perfused with HEPES buffer (2ml/min) in a chamber and field stimulated at 34°C. An edge detecting device was used to measure changes in myocyte length upon contraction. Isolated cardiomyocytes were also loaded with the Ca^{2+} fluorescent dye Fura-2 to measure Ca^{2+} transients (excitation at 340 and 370nm and emission at 520nm).

Cardiomyocyte size varied significantly between all age groups in length, width and surface area.

Contractility results showed a significant difference between all age groups, with the maximal percentage cell shortening seen in adult, minimal at 14 days. This agrees with changes in intact heart.

Stimulation-induced Ca^{2+} transients were measured in myocytes of all age groups. The amplitude of Ca^{2+} transients were greatest in the adult myocytes, which decrease with decreasing age. The results also showed that the time to peak for Ca^{2+} transients decreases with age. The diastolic and systolic Ca^{2+} concentrations of the cardiomyocytes also showed significant differences with $[Ca^{2+}]$ increasing with age.

These results indicate that in older myocytes Ca^{2+} mobilization is more efficient than in younger cells. It is suggested that the role of L-type Ca^{2+} channels in excitation-contraction coupling becomes more important with age. The opposite is supposed to occur for Na-Ca exchanger. Therefore entry on the exchanger will likely be slower than when the channels open in adult. Indeed, the sarcoplasmic reticulum of the immature rat myocytes, although present and functional is not fully developed. In fact is has been shown that ryanodine receptor inhibitors have very little effect upon neonatal cell contraction. Additionally it has been suggested that the Ca^{2+} sensitivity of cardiac myofilaments increases over development reaching adult levels at approximately 15 days of age. This work shows that there are significant transitional changes in myocyte contractility and Ca^{2+} transients occurring between 2-3 weeks of age.

FRIDAY, 13. NOVEMBER 2009:

KEYNOTE LECTURE FRIDAY

THE GLIAL REGULATION OF SLEEP AND MEMORY

Philip Haydon

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Astrocytes modulate neuronal activity by releasing chemical transmitters via a process termed gliotransmission. The role of this process in the control of behavior is unknown. Since one outcome of SNARE-dependent gliotransmission is the regulation of extracellular adenosine and because adenosine promotes sleep, we genetically inhibited the release of gliotransmitters and asked if astrocytes play an unsuspected role in sleep regulation. Inhibiting gliotransmission attenuated the accumulation of sleep pressure, assessed by measuring the slow wave activity of the EEG during NREM sleep, and prevented cognitive deficits associated with sleep loss. Since the sleep-suppressing effects of the A1 receptor antagonist CPT were prevented following inhibition of gliotransmission and because intracerebroventricular delivery of CPT to wild-type mice mimicked the transgenic phenotype, we conclude that astrocytes modulate the accumulation of sleep pressure and its cognitive consequences through a pathway involving A1 receptors.

FRIDAY, 13. NOVEMBER 2009:

SESSION V

ASTROGLIAL ATROPHY AND HYPERTROPHY IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX DURING THE PROGRESSION OF ALZHEIMER'S DISEASE: A COMBINED PROSESS

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Astrocytes, the most numerous cells of the brain, are essential for neuronal activity and are also the fulcrum of the brain diseases such as neurodegenerative processes like Alzheimer's disease (AD). Extensive literature points towards a hypertrophic reaction of astrocytes during ageing, neurodegenerative processes and AD. Here, we have studied the astrocytic changes and modifications that appear throughout the progression of AD within the hippocampus. For this we have used an immunohistochemical approach that has allowed us not only to determine the complexity (surface/volume ratio) of the glial cytoskeleton but also their relationship and/or association with one of the major pathological hallmarks of AD: the neuritic plaques. We studied this in a recently developed triple transgenic mouse model of AD (3xTg-AD) that mimics the temporal evolution and progression of AD.

Surprisingly and apposed to what has been described till now we found that from early ages (3 months of age; 1.31%), it starts to appear an astrocytic atrophy (reduction of complexity, volume and surface) which becomes markedly significant at 12 and 18 months of age (5.04% and 8.33% reduction respectively) in the dentate gyrus of the hippocampus when we compared 3xTg-AD to non-Tg animals. We observed similar atrophic reaction within the CA1 hippocampal sub-field but later at 18 months of age but accounting for a 3.56% decrease. This atrophy also appears in the entorhinal cortex but with earlier incidence, being significant from 1 month of age.

However, we found that whilst this is a generalised process, astrocytes surrounding plaques show a cytoskeleton hypertrophy as revealed by an increase of complexity (7.76%), volume (98.19%) and surface (118.69%) of GFAP labelling. Despite these changes in cytoskeleton organisation, the numerical density of astrocytes in both areas remain constant.

Therefore, our results suggest that there are differential effects of AD on astrocytic subpopulations depending on their association with amyloid plaques which will account for the progressive glial and neuronal alterations in synaptic connectivity and neurotransmitters imbalance that appear in AD and that are responsible for the observed mnesic and cognitive impairments.

DIFFUSION PARAMETERS OF THE EXTRACELLULAR SPACE IN HEALTH AND DISEASE

Eva Sykova

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Although synaptic transmission is an important means of communication between neurons themselves, neurons and glia also communicate by extrasynaptic "volume" transmission, which is mediated by diffusion in the extracellular space (ECS). The ECS of the central nervous system (CNS) is the microenvironment of neurons and glial cells. The composition and size of the ECS changes dynamically during neuronal activity as well as during pathological states. Following their release, a number of neuroactive substances, including ions, mediators, metabolites and neurotransmitters, diffuse via the ECS to targets distant from their release sites. Glial cells affect the composition and volume of the ECS and therefore also extracellular diffusion, particularly during development, aging and pathological states such as ischemia, injury, X-irradiation, gliosis, Alzheimer´s disease, demyelination diseases, epilepsy and tumors. A decrease in ECS volume and an increase in diffusion barriers were found during ageing in the rat and mouse cortex, corpus callosum and hippocampus. These changes were accompanied by a learning deficit, astrogliosis, the rearrangement of astrocytic processes and a loss of extracellular matrix (ECM). An increase in diffusion barriers, manifested also as a decrease in the apparent diffusion coefficient (ADC), due to astrogliosis as well as due to an increase in chondroitin sulphate proteoglycans was also found after cortical injury and in grafts of embryonic tissue. Measurements in mice deficient for the ECM glycoprotein Tenascin-R revealed not only an increase in ADCs, but also a smaller ECS volume fraction, while APP23 mice with excessive amyloid plaque deposition had a larger ECS volume fraction. Similarly in tumors where we found a large increase in ESC volume, ADCs are decreased due to the large deposition of ECM. The ECM, besides its apparent importance in tissue anisotropy, is therefore important for maintaining a relatively large ECS volume (Sykova and Nicholson, Physiol. Rev., 2008).

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GLUTAMATE AND ATP EXCITOTOXICITY IN WHITE MATTER DAMAGE

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Glutamate is the principal excitatory neurotransmitter in the central nervous system (CNS), but it is also a potent neurotoxin that can kill nerve cells. Glutamate damages oligodendrocytes, like neurons, by excitotoxicity which is caused by sustained activation of AMPA, kainate and NMDA receptors. Glutamate excitotoxicity depends entirely on calcium overload of the cytoplasm and can be initiated by disruption of glutamate homeostasis. Thus, inhibition of glutamate uptake in isolated oligodendrocytes *in vitro* and in the optic nerve *in vivo*, is sufficient to trigger cell death, which is prevented by glutamate receptor antagonists. In turn, activated, but not resting microglia, can compromise glutamate homeostasis and induce oligodendrocyte excitotoxicity, which is attenuated by AMPA/kainate antagonists or by the blockade of the system x_c^- antiporter present in microglia. On the other hand, non-lethal, brief activation of glutamate receptors in oligodendrocytes rapidly sensitizes these cells to complement attack. Intriguingly, these effects are exclusively mediated by kainate receptors which induce calcium overload of the cytosol and the generation of reactive oxygen species.

In addition, ATP signaling can trigger oligodendrocyte excitotoxicity via activation of calcium-permeable P2X7 purinergic receptors expressed by these cells. Sustained activation of P2X7 receptors *in vivo* causes lesions that are reminiscent of the major features of MSplaques, i.e., demyelination, oligodendrocyte death, and axonal damage. In addition, treatment with P2X7 antagonists of chronic experimental autoimmune encephalomyelitis (EAE), a model of MS, reduces demyelination and ameliorates the associated neurological symptoms. Together, these results indicate that ATP can kill oligodendrocytes via P2X7 activation and that this cell death process contributes to EAE. Importantly, P2X7 expression is elevated in normal-appearing axon tracts in MS patients, suggesting that signaling through this receptor in oligodendrocytes may be enhanced in this disease. Thus, P2X7 receptor antagonists may be beneficial for the treatment of MS.

In conjunction, these observations reveal novel mechanisms by which altered glutamate and ATP homeostasis can trigger oligodendrocyte death. This knowledge may generate new therapeutic avenues to protect white matter from acute and chronic damage.

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MICROGLIAL RESPONSES TO ACUTE SPINAL CORD INJURY *IN VIVO*

Frank Kirchhoff

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To understand the pathomechanisms of spinal cord injuries will be a prerequisite to develop efficient therapies. By investigating acute lesions of spinal cord white matter in anesthetized mice with fluorescently labeled microglia and axons using *in vivo* two-photon laser-scanning microscopy (2P-LSM), we identified the messenger nitric oxide (NO) as a modulator of injury-activated microglia.
Local tissue damages evoked by high-power laser pulses provoked an immediate attraction of microglial processes. Spinal superfusion with NO synthase and guanylate cyclase inhibitors blocked these extensions. Furthermore, local injection of the NO-donor SPNO or the NO-dependent second messenger cGMP induced efficient migration of microglial cells towards the injection site.
High tissue levels of NO, achieved by uniform superfusion with SPNO and mimicking extended tissue damage, resulted in a fast conversion of the microglial shape from ramified to ameboid indicating cellular activation. When the spinal white matter was preconditioned by increased, ambient ATP (known as microglial chemoattractant) levels, the attraction of microglial processes to local NO release was augmented, while it was abolished at low levels of tissue ATP. Since both signaling molecules, NO and ATP, mediate acute microglial reactions, coordinated pharmacological targeting of NO and purinergic pathways will be an effective mean to influence the innate immune response after spinal cord injury.

PHYSIOLOGY AND PATHOPHYSIOLOGY OF CALCIUM SIGNALLING IN PANCREATIC ACINAR CELLS

Petersen OH

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The pancreatic acinar cell continues to be a very useful object for Ca^{2+} signalling studies due to its highly polarized structure and the relative ease of isolation. High-resolution confocal and two-photon studies of the dynamics of [Ca^{2+}] in the cytosol, all major organelles and the immediate extracellular environment of these cells have provided a detailed understanding of physiological (Petersen & Tepikin *Annu Rev Physiol* 70, 273-299, 2008) and pathological (Petersen et al. *Cell Calcium* 45, 634-642, 2009) Ca^{2+} signalling processes. The most important information will be reviewed together with very recent data about the role of IP3 receptors in pathophysiology (Gerasimenko et al. *PNAS* 106, 10758-10763, 2009) and the roles of STIM1 and Orai1 in regulating Ca^{2+} entry (Lur et al. *Curr Biol* 2009 in press).

ALTERATION OF NUCLEOCYTOPLASMIC TRANSPORT OCCURS DURING CALCIUM-MEDIATED CELL DEATH

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Selective loss of nuclear permeability has been associated with virus-mediated and caspase-dependent cell death. In apoptosis, processing of several components of the envelope is downstream of caspase activation and thereby downstream of the mitochondrial signals for caspase activation. Disassembly of the nuclear envelope, intranuclear proteins and chromosomal DNA are likely to play an important role in promoting disposal of the nucleus and its content. Probably because nuclear permeabilization is thought to be a late event in death programmes, only a handful of studies have examined functional and structural alterations of Nuclear Pore Complex (NPC) during neuronal injury and death. We study the redistribution of proteins across the nuclear envelope during excitotoxicity in rodent neurons and in *C.elegans deg-3* mutant. Through a combination of fluorescent confocal imaging and biochemical studies, we have ordered the events that promote alterations in nucleocytoplasmic transport and loss of nucleoporins. We unveil a new mechanism that shows changes in NPC function and we propose that the signalling between the nucleus and the rest of the cell may have a relevant function in the early phases as well as in the execution of caspase-independent cell death.

ABERRANT CALCIUM SIGNALLING IN AMYOTROPHIC LATERAL SCLEROSIS

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Calcium dependent processes have long been implicated in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS), a progressive neurodegenerative disease which leads to death on average after 3-4 years due to painless generalized weakness without sensory loss eventually causing respiratory failure. In histopathology, upper and lower motoneurons die with signs of mitochondrial calcium overload, and fragmentation of the Golgi apparatus and the endoplasmic reticulum (ER) while cytosceletal proteins and misfolded proteins aggregate into intracytoplasmic inclusion bodies. Surrounded by widespread astrogliosis subcortical, intracortical, transcallosal and corticospinal tracts connecting the motor cortex degenerate. Spinal motoneurons retract from the neuromuscular junction and undergo axonal swelling before disintegration. ALS muscle fibres paradoxically seem to increase non-mechanical energy consumption, and liver mitochondria display disruption similar to neuromuscular endplates suggesting a systemic disease process which mainly targets selectively vulnerable motoneurons. In laser-captured spinal human post mortem motoneurons posttranslational editing of the GluR2 subunit of the AMPA receptor was impaired, supporting the notion of chronic calcium dependent AMPA receptor mediated excitotoxicity as one of the cell death mechanisms. The disease spares oculomotor nuclei and Onuf's nucleus which both express high levels of calcium buffering protein in contrast to spinal motoneurons sensitive to the disease process. More recently, intracellular calcium deregulation as key pathway in motoneuron degeneration is being recognized with increased understanding of ER – mitochondria calcium shuttling. 5-10% of ALS patients have a positive family history of mostly autosomal dominant heredity with SOD1 mutations and TDP43 mutations present in a large portion of familial ALS. Mutant SOD1 seems to be displaced into mitochondria causing reduced calcium uptake capacity which in motoneurons reduces the main calcium buffering mechanism. Other effects of mutant SOD1 have been observed on the ER where protein processing seems to become more error prone and chronic calcium depletion of the ER may lead to exhaustion of the protective unfolded protein response (UPR). From these findings the hypothesis of a toxic shift of calcium from the ER to mitochondria within the ER-mitochondria calcium cycle (ERMCC) has been formed which may represent a key signalling pathway in the low calcium buffered motoneurons vulnerable to the ALS pathway. ERMCC modulators may therefore play an important role in treating this devastating disease, and possibly other neurodegenerative diseases like Parkinson's disease and Alzheimer's disease.

DYNAMIC CALCIUM STORES, CALCIUM SIGNALLING AND NEURODEGENERATION

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Imbalances in Ca^{2+} are the most common examples of death routines using physiological signalling systems. Deregulation of Ca^{2+} signalling is involved in many types of cell death: it triggers excitotoxicity in neurones and muscle cells, it initiates apoptosis in many excitable and non-excitable tissues, and it acts as activator and executor of necrotic cell death. All these death subroutines use existing molecular systems, which are responsible for physiological Ca^{2+} signalling. As tissue plasticity and remodelling became a fundamental step in evolution of complex organisms, biochemical programmes involving complex cascades leading to cell disassembly have also developed. Intracellular calcium stores, the endoplasmic reticulum (ER) and mitochondria represent multifunctional organelles, intimately involved in various aspects of cellular activity. The disruption of Ca^{2+} homeostasis in both the ER and mitochondria can be involved in neurodegenerative disorders such as diabetic peripheral neuropathies and Alzheimer disease.

REGULATION OF NERVE TERMINAL FUNCTION BY THE EXTRACELLULAR CALCIUM-SENSING RECEPTOR

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The increase in synaptic terminal [Ca^{2+}] that triggers exocytosis is coupled to a decrease in extracellular [Ca^{2+}] ([Ca^{2+}]$_o$). While a fall in cleft [Ca^{2+}] should reduce subsequent synaptic transmission, sustained fast transmission is a common feature in the cerebral cortex. This led us to hypothesize that at excitatory synapses, a fall in cleft [Ca^{2+}] may provide a retrograde compensatory signal to the nerve terminal. Using a modified patch clamp technique we previously demonstrated that falls in [Ca^{2+}]$_o$ activate, a novel, non-selective cation channel (NSCC) at neocortical nerve terminals. Using a combination of pharmacological probes and mutant mice we recently identified the receptor in the Ca sensor-NSCC signaling pathway as the calcium-sensing receptor (CaSR), a G-protein coupled receptor that has been localized to nerve terminals. We tested if this receptor could compensate for falls in external [Ca^{2+}]. Using paired recordings from neocortical neurons we showed that activation of CaSR inhibited evoked excitatory transmission and that the probability of release was increased in neurons from reduced function CaSR mutants.

Spontaneous release is also dependent on [Ca^{2+}]$_o$ but the mechanisms regulating spontaneous vesicle fusion remain unclear. Furthermore, these events and evoked release may arise from distinct vesicle pools. Using patch-clamp methods we have investigated possible mechanisms for the modulation of spontaneous glutamate release by [Ca^{2+}]$_o$ from cultured neocortical neurons. We hypothesized that Ca^{2+} entry via voltage-activated Ca^{2+} channels triggered spontaneous release. However blockade of voltage-gated calcium channels had no effect on the enhancement of miniature excitatory postsynaptic current (mEPSC) frequency by elevation of [Ca^{2+}]$_o$. We next tested if Ca entry via the sodium/calcium exchanger or the plasma membrane calcium ATPase mediated the effect of [Ca^{2+}]$_o$. Inhibition of the sodium/calcium exchanger or the plasma membrane calcium ATPase enhanced the facilitation of mEPSC frequency by [Ca^{2+}]$_o$ indicating the involvement of an alternative signaling pathway. We tested if CaSR could mediate this response and found that CaSR agonists increased mEPSC frequency, and that neurons from mice with mutant CaSR displayed altered mEPSC frequency dependence on [Ca^{2+}]$_o$. We propose that CaSR links calcium in the synaptic cleft to spontaneous glutamate release. Together these data indicate that CaSR regulates evoked and spontaneous release of glutamate at neocortical nerve terminals.

TRAFFICKING AND FUNCTION OF V-SNARE PROTEIN VAMP4 AT CENTRAL SYNAPSES

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SNARE proteins are required for membrane fusion in various intracellular compartments of all eukaryotic cells. At synapses v-SNARE Synaptobrevin-2/VAMP-2 forms tight fusogenic assembly termed core complex with t-SNAREs Syntaxin 1 and SNAP-25. The function of each of the three SNAREs is essential for synaptic vesicle exocytosis and normal synaptic transmission. Another v-SNARE protein, VAMP4 is also present on synaptic vesicles and can form core complex with Syntaxin 1 and SNAP-25 in vitro, however synaptic function of VAMP4 is not known. This presentation will focus on our recent work on the trafficking and putative role of VAMP-4 in central synapses. We found that VAMP-4 is widely expressed in neurons and internalized at the synapses upon stimulation. Also, despite VAMP-4 can form SNARE- complexes *in vitro*, it failed to rescue synaptic vesicle release in VAMP-2 knock-out neurons. In order to understand the fundamental differences between VAMP-2 and VAMP-4 we manipulated their structure and monitored their trafficking and ability to support neurotransmitter release. To date, our results suggest a role for VAMP-4 in regulation of endocytosis rather than exocytosis providing an example of functional selectivity among vesicular SNARE proteins.

QUANTAL SIZE AND MULTIVESICULAR EXOCYTOSIS AT THE HAIR-CELL SYNAPSE: PAIRED RECORDINGS WITH MEMBRANE CAPACITANCE MEASUREMENTS

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The excitatory postsynaptic currents (EPSCs) recorded from eighth-nerve afferent terminals include many large signals thought to represent the simultaneous release of several vesicles. We performed paired whole-cell recordings of the hair cell and its afferent fiber in the auditory amphibian papilla of the adult bullfrog. The amplitudes of spontaneous EPSCs had a broad, Gaussian distribution with a mean of about -130 pA. The largest signals, with amplitudes exceeding -200 pA, displayed kinetics similar to those only a quarter as large, suggesting that the former stem from the highly synchronized release of multiple vesicles. Holding the hair cells at -80 mV eliminated the large spontaneous EPSCs, leaving a narrow amplitude distribution with a mean of -55 pA; the inclusion of 0.4 mM Cd^{2+} in the bath solution to block Ca^{2+} channels had a similar effect. During voltage-evoked exocytosis, we compared the synaptic charge transfer into an afferent terminal with the simultaneous increase in membrane capacitance of a hair cell. The resultant regression line has a linear slope that can used to calculate a single-vesicle capacitance of 50 aF, a value in close agreement with the electron-microscopic estimate of 45 aF. These results strongly support the hypothesis that the large spontaneous EPSCs are multiquantal and due to the synchronous release of several vesicles from a single synaptic ribbon active zone. We propose that these large events originate via a compound or coordinated mechanism of multivesicular release. Their physiological significance will be discussed.

REGULATION OF SYNAPTIC TRANSMISSION BY THE UBIQUITIN-PROTEASOME SYSTEM

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Synaptic transmission, the linchpin in neuronal circuits, is exquisitely controlled by protein synthesis and possibly by protein degradation via the ubiquitin proteasome system (UPS). In addition to altering protein life-time, ubiquitination can also alter protein function. Recent data suggests a role for the UPS in regulation of presynaptic function, but it remains unclear whether ubiquitination at the synapse serves mainly as degradative signal. We used two distinct classes of UPS blockers to determine the effect of UPS inhibition on synaptic function in cultured hippocampal neurons. We find that proteasome inhibitors as well as E1 ubiquitin-activating enzyme inhibitors trigger, within minutes, a several-fold increase in the frequency of excitatory and inhibitory miniature postsynaptic currents. Taken together with the finding that protein synthesis inhibitors do not block the UPS inhibition-mediated increase in mini frequency, these data suggest that ubiquitination at the presynaptic terminal not only regulates the half-life, but also the functional state of proteins. We conclude that, beyond the classic degradatory pathway, the UPS serves as an important and powerful regulator of synaptic activity in the presynaptic terminal by modulating protein function.

IS BRAIN HISTAMINE INVOLVED IN THE RESPONSE TO ANTIDEPRESSANT DRUGS?

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SSRIs are mainstays in treatment of depression, and their highly specific actions enhancing 5-HT neurotransmission appears to explain their safety profile. Despite their claimed selectivity, however, SSRIs interact, either directly or indirectly, with various neurotransmitter systems. The tuberomammillary nucleus (TMN) is the only source of histamine (HA) fibers, and receives serotonergic inputs from the raphé. To learn whether SSRIs affect the activity of HA neurons, citalopram was administered to rats and HA release was determined by microdialysis. SD male rats (250 g) were implanted with one probe in the TMN, and one in either the nucleus basalis magnocellularis or nucleus accumbens. HA output from the two probes, perfused with Ringer at 2-µl/min, was measured in 15-min samples by HPLC-fluorometric detection. Rats were conscious and moved freely in the cage. Spontaneous HA release from all regions was stable, ranging 0.05-0.08 pmol/15min (N=17). Intra-TMN administration of citalopram (1-10 µM) for 60 min increased significantly HA release up to about 100% of basal value from all areas ($P<0.05$, ANOVA/Fisher's test). Pretreatment with methysergide (10 µM), a 5-HT2 receptor antagonist, completely abolished the effect of 10 µM citalopram. These results suggest that citalopram activates HA neurons by increasing the extracellular levels of endogenous 5-HT, and provide evidence of functional connections between HA and 5-HT neuronal systems. Accordingly, earlier studies reported that local perfusion with 5-HT increased HA release from the anterior hypothalamus in anesthetized rats [1], and that 5-HT depolarized HA neurons [2]. Some effects elicited by SSRI may rest on the interaction of 5-HT with the histaminergic system. Mice unable to synthesize histamine due to targeted disruption of the histidine decarboxylase (HDC) gene were used to critically test histamine role in mediating acute behavioural changes elicited by citalopram or reboxetine. To this end, we used the tail suspension test in WT and HDC-KO mice. Both citalopram (SSRI) and reboxetine (NSRI) reduced immobility in WT mice. Reboxetine was effective at reducing immobility also in HDC-KO mice, which, surprisingly, failed to respond to the behavioural effects of citalopram. These data show that histamine plays an important role in mediating acute behavioural and neurochemical actions of citalopram , the most selective SSRI.

[1] Laitinen et al., Eur J Pharmacol 1995; 285:159-164.
[2] Erikson et al., Neuropharmacology 2001; 40:345-351.

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THE ROLE OF NEURONAL HISTAMINE AND H3 RECEPTORS IN ALCOHOL-RELATED BEHAVIOUR

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The histaminergic system in the mammalian brain consists of neurons in the caudal hypothalamic tuberomamillary nucleus and their projections to essentially all parts of the brain. However, the density of histaminergic fibers varies in brain regions. Histamine is involved e.g. in regulation of sleep and wakefulness and hypothalamic endocrine control. Many brain structures related to addictive behavior receive moderate or high density of histaminergic fibers. The role of histamine and its receptors in addiction-related behaviors has not been studied extensively. Studies from our laboratory using ethanol-preferring rats suggest a role for neuronal histamine and its H3 receptor in ethanol-related behaviors. Histamine content is very high in alcohol-preferring rats in contrast to normal or alcohol non-preferring rats. Behavioral studies showed that the acute ethanol intake of alcohol-preferring rats was bidirectionally regulated in a concentration-dependent manner on H3 receptor ligands. Male histidine decarboxylase knockout (HDC KO) mice were then used to study the role of histamine in alcohol-induced stimulation of locomotor activity, impairment of motor coordination and conditioned place preference. HDC KO mice showed a weaker stimulatory response to acute ethanol than wild type (WT) mice. The HDC KO mice showed stronger ethanol-induced place preference than WT mice. Ethanol induced stimulation of locomotor activity in normal mice with a peak effect seen at 5 min post administration. H3R antagonist ciproxifan inhibited this alcohol-evoked stimulation. In conditioned place preference paradigm ciproxifan alone did not result in the development of place preference, but it potentiated the alcohol reward. These data support a role for brain histamine in the mechanisms of alcohol effects. Histaminergic neurotransmission seems to be necessary for the stimulatory effect of alcohol to occur, whereas lack of histamine leads to changes that enhance the conditioned reward by ethanol. Our findings on both rats and mice also suggest a role for histamine H3 receptor in modulation of the ethanol stimulation and reward.

INTERACTIONS BETWEEN HISTAMINERGIC AND CANNABINOID SYSTEMS; A POTENTIAL THERAPEUTIC TARGET FOR COGNITIVE AND FEEDING BEHAVIOUR IMPAIRMENTS?

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Functional studies have recently demonstrated that activation of the central histaminergic system alters CNS functions in both behavioral and homeostatic contexts, which include sleep and wakefulness, learning and memory, anxiety, locomotion, feeding and drinking, and neuroendocrine regulation. These actions are achieved through interactions with other neurotransmitter systems, and the interplay between histaminergic neurons and other neurotransmitter systems are becoming clear. Endocannabinoids and histaminergic neurons exert complex actions on neurotransmitter networks involved in cognitive processes, locomotion, appetite and, interestingly, they command several, similar behavioural states. Until recently, though, no information was available on the possible interactions between these two systems. Recently we demonstrated that agonists of the cannabinoid receptor (CB)1 augment histamine release in rat brain regions of freely moving animals involved in the acquisition and consolidation of memories and in the control of locomotion. In these areas, augmented histamine release improves rats performance in cognitive tests whereas cannabinoids have deleterious effects on cognitive processes. These results are apparently counterintuitive, because augmented histamine release is also an indicator of stress and it is conceivable that protracted occupancy of CB1 receptors, as produced by administering cannabinoid agonists, disrupts the spatiotemporal specificity of histamine release in different brain regions, contributing to maladaptive behavioral responses. The hyperhistaminergic state produced by cannabinoids, though, may have relevance in controlling food related behavior. Brain histamine is involved in feeding physiology by integrating inputs from neurotransmitters and hormones that drive or inhibit feeding. Endogenous cannabinoids regulate satiety and body weight and recent evidence indicates that histamine drives the appetitive rather than the consummatory phase of feeding. In this regard, preliminary data in our laboratory indicate that oleylethanolamide, a peripherally generated endocannabinoid that signals satiety, decreases histamine release from the hypothalamus. These results are better reconciled with the hypothesis of a histaminergic system engaged in the preparatory phases of food condumption.

Hence, understanding in what circumstances endocannabinoids are released and activate histaminergic cells warrants further investigations and may provide interesting hints to develop new therapeutic strategies in the treatment of food intake disorders.

HISTAMINE AS A REGULATOR OF ASTROCYTE FUNCTION

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Astrocytes are the most numerous cell types within the central nervous system and perform a variety of tasks, from axon guidance and synaptic support, to the control of the blood brain barrier and blood flow. They contribute in maintaining homeostasis at the synapse, regulating neuronal signalling, protecting neurons from oxidative damage, and determining the fate of endogenous neural precursors. Astrocytes produce trophic factors, and eliminate neurotoxins and thus fulfil important protective and reparative functions. In addition, astrocytes regulate the recruitment and activity of infiltrating haematogenous cells through their expression of cytokines, proteases, protease inhibitors, adhesion molecules, and extracellular matrix components.

The regulation of astrocyte function is complex. Histamine importantly contributes in this process, acting on histamine H_1- and H_2-receptors, expressed on the astrocyte surface. Activation of histamine H_1-receptors leads to depolarisation and degradation of membrane inositol-lipids and production of inositol-3-phosphate and diacylglycerol and activation of protein-kinase C. Activation of histamine H_2-receptors succeed to hyperpolarisation, activation of adenilat-cyclase and production of cAMP. It has been found that histamine stimulates glycogen breakdown in astrocytes by activation of both H_1- and H_2-receptors. Thus, the histamine-induced glycogen breakdown may involve increases in cAMP formation, and in intracellular Ca^{2+} levels, this latter resulting mainly from H_1-mediated extracellular Ca^{2+} uptake.

Histamine enhances astrocytes proliferation and differentiation via activation of protein-kinase C, and changes of cytoskeleton and morphology of astrocytes. Via activation of histamine H_1-receptors, histamine stimulates production of neurotrophic factors like nerve growth factor. Histamine also importantly influences the immune response of astrocytes. It modulates the production of certain cytokines and interacts with them in their actions.

ASTROCYTES AS A SITE FOR HISTAMINE INACTIVATION

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Synthesis of histamine in histaminergic neurons is catalyzed by histidine decarboxylase. Later on, synthesised histamine is taken up into synaptic vesicles by the vesicular monoamine transporter 2 and released into the synaptic cleft upon depolarization stimuli. The released neurotransmitter histamine is metabolised by the enzyme histamine-N-methyltransferase (HNMT) producing N^{τ}-methylhistamine. In order to be enzymatically degraded or recycled, histamine must be transported either into the presynaptic neuron or into surrounding glial cells. Unlike with other neurotransmitters, the mechanisms by which histamine content is regulated within the brain remain unresolved.

Histamine is a double protonated molecule with corresponding pKa values of 5,8 and 9,4 Therefore, at physiological pH histamine exists as an equilibrium mixture of tautomeric cations, monocation making 96% and dication only 3% and the rest is nonprotonated histamine. As a protonated molecule histamine most probably use a carrier protein in order to cross the cell membrane. Moreover, the protonation states of histamine most probably change during the transport process because of different dielectric properties at different sites of the transport protein. As a preliminary step to molecular simulation of the transport process we ab initio calculated histamine pKa values in conjunction with a microscopic salvation model. We found that histamine can be transported into cultured astrocytes by two processes, active transport which is Na^+- but not Cl^--dependent and bidirectional with Michaelis constant (K_m) of 3.5 ± 0.8 μM and a maximal rate (V_{max}) of 7.9 ± 0.3 pmol/mg protein/min. Aside from this cultured glial cells take up histamine also by electrodiffusion, which is not neither temperature nor Na^+-dependent and ouabain-sensitive. This step, which shows some characteristics of $uptake_2$, might occur by the aid of one of organic cation transporters or plasma membrane monoamine transporters. Taken up histamine can be metabolized in astrocytes due to expression of HNMT within.

Taken together astrocytes can represent the major inactivation site for histamine, but some facts remain to be unresolved – like the existence of specific histamine transporter and the possible release of histamine and/or its metabolites from astrocytes.

WHAT HAS MR TOLD US ABOUT THE HEART IN OBESITY AND DIABETES?

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Obesity and diabetes are major and growing public health issues that affect all Western countries. Cardiac high-energy phosphate metabolism, measured non-invasively as the phosphocreatine (PCr)/ATP ratio using ^{31}Phosphorus magnetic resonance spectroscopy, is abnormal in patients with obesity and/or diabetes. However, the cellular mechanism causing low cardiac PCr/ATP is unclear and whether low energetics are related to contractile dysfunction is unknown. Plasma norepinephrine concentrations are increased in both conditions. Activation of the sympathetic nervous system causes adipose tissue lipolysis, which elevates circulating free fatty acid concentrations. High circulating free fatty acid concentrations are associated with a shift in substrate preference towards free fatty acid oxidation, with greater myocardial oxygen consumption, in the obese or diabetic heart. We have studied the link between abnormal circulating free fatty acid concentrations and low cardiac PCr/ATP ratios in obese and diabetic patients and found that they were correlated. These results suggest that the increased circulating free fatty acid concentrations lead to increased myocardial free fatty acid uptake, which uncouples oxidative phosphorylation in mitochondria and thereby impairs energy metabolism in heart. Thus, changes in substrate availability may lead to a myopathy in patients who are obese or have diabetes. A therapy that reduces circulating free fatty acid concentrations or inhibits mitochondrial free fatty acid uptake and/or oxidation may enhance high-energy phosphate metabolism and thereby prevent cardiac contractile dysfunction.

MICE FED HIGH-FAT DIET DISPLAY ALTERED METABOLIC CHARACTERISTICS OF THEIR HEARTS AND MYOCYTES AND INCREASED VULNERABILITY TO CARDIAC INSULT

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We have recently shown that feeding apolipoprotein E knockout ($apoE^{-/-}$) mice high-fat diet accelerates progression of coronary disease and induces changes to cardiac metabolism and function. These changes could be due to coronary disease and/or direct effect of high-fat feeding. This work aims to investigate whether high-fat diet induces cardiac changes in wild type mice, independent of coronary disease. Male C57/129 wild-type mice were weaned onto a normal rodent diet, and then at approximately 8 weeks old, animals were either switched onto high fat, Western-type diet (21% fat; 0.15% cholesterol) or were maintained on normal rodent diet. Animals were fed their respective diets for 6 months. Histological examination of hearts confirmed absence of coronary disease in both groups. Isolated hearts from both groups were used to measure cardiac metabolites and function. Isolated myocytes were used to measure mitochondrial flux (NAD^+/NADH), contractile function and calcium transients. Mice fed high-fat diet had significantly increased levels of cardiac lactate (from 52 ± 4 to 76 ± 6 nmol/mg protein) and decreased glycogen content (from 0.09 ± 0.005 to 0.06 ± 0.008 mg/g wet weight) but had similar levels of energy rich phosphates. Evidence of metabolic stress in high-fat fed hearts was confirmed in isolated perfused myocytes which showed increased NAD^+/NADH ratio (0.34 ± 0.02 vs. 0.29 ± 0.01). These metabolic differences did not alter functional characteristics of isolated perfused myocytes and hearts. However the rate of rise and decline of calcium transients were slower in high-fat fed myocytes. Intact perfused hearts from high-fat fed animals were significantly more vulnerable to reperfusion injury than those from animals fed normal diet. In conclusion, high-fat diet has profound metabolic effects on the myocardium that is independent of coronary disease. These changes appear to alter vulnerability to reperfusion injury.

DIFFERENTIAL ANALYSIS OF THE CARDIAC PROTEOME OF RATS ARTIFICIALLY SELECTED FOR EITHER LOW OR HIGH AEROBIC CAPACITY

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Artificial selection at the extremes of a trait concentrates contrasting allelic variation from one generation to the next and can produce informative genetic models. In particular, selection on running capacity has created rat phenotypes of intrinsically low capacity runners (LCR) and high capacity runners (HCR). Previous studies demonstrate that HCR exhibit enhanced cardiac and skeletal muscle function and are relatively protected from disease, whereas, LCR are dyslipidaemic, insulin resistant, hypertensive and relatively susceptible to cardiac ischaemia.

Molecular differences that regress with the divergence in running capacity may be mechanistically responsible for the differences in correlated traits and disease risk. We performed proteomic analysis on the hearts of HCR and LCR rats (n = 6, in each group) from generation 23. Homogenates of left ventricle were labelled with cyanine minimal fluorescence dyes Cy2, Cy3 or Cy5. HCR and LCR samples and a pooled internal standard were combined equivalently and resolved using large-format 2-dimensional electrophoresis (i.e. Difference in-gel electrophoresis; DIGE). Gel spots were robotically excised from preparative gels and proteins identified using database searches of their tryptic peptide mass fingerprint and fragment ion spectra collected by matrix-assisted laser desorption ionisation tandem time of flight (MALDI-TOF/TOF) mass spectrometry.

Consistent with earlier reports using this model, the intrinsic running capacity of HCR was 4-fold greater than LCR. DIGE resolved 957 gel spots and protein expression profiling detected 68 statistically significant ($P<0.05$; false discovery rate <10 %, calculated using q-values) differences between HCR and LCR hearts. Proteins were unambiguously identified in 369 gel spots, including 54 of the spots differentially expressed between HCR and LCR. The proteomic analysis identified robust differences in the expression of proteins involved in mitochondrial metabolism and oxidative stress response. This agrees closely with previous microarray data, but the majority of gene products were resolved as multiple isoelectric species. Thus, some of the differences in spot expression represent changes in post-translational modification not evident in microarray investigations. The 54 gel spots differentially expressed between HCR and LCR represent 37 gene products, 20 of which are associated with metabolism. In particular, there was robust modulation of isoelectric species of each enzyme of the beta-oxidation pathway.

Our data suggest that artificial selection on low running capacity altered the metabolic profile of the heart, diminishing its utilisation of fatty acids. The fact that some enzymes of beta-oxidation were modified at the post-translational level suggests novel mechanisms may exist that regulate mitochondrial fatty acid metabolism.

HIGH-FAT DIET, CORONARY DISEASE AND MYOCARDIAL PROTECTION

M-S Suleiman

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Coronary artery disease with resultant myocardial ischaemia is responsible for major clinical complications including acute myocardial infarction, cardiac arrhythmias, and can lead to sudden cardiac death. The burden of ischaemic heart disease on the health service is on the increase as a result of an ageing population and an increase in survival rate after cardiovascular events (e.g. myocardial infarction). A number of clinical interventions (e.g. thrombolysis, percutaneous coronary angioplasty and coronary bypass surgery) are used to reperfuse the acutely or chronically ischemic myocardium. Reperfusion of the ischaemically diseased heart can induce myocardial injury which can be further compounded when coronary revascularization involves cardio-pulmonary bypass surgery and cardioplegic ischaemic arrest. Therefore myocardial protection strategies are continuously being designed, tested but with relatively low success rate. It has been suggested that the "use of animal models that do not adequately approximate the clinical setting" is one major barrier to translation into clinical therapy.

We have recently demonstrated that our validated apolipoprotein E knockout ($apoE^{-/-}$) mouse model of atherosclerosis and plaque rupture is also a relevant model of coronary artery disease and sudden death. Hearts of $apoE^{-/-}$ mice fed high-fat diet for 24 weeks are ischaemic, show extensive coronary disease and infarcts in complete contrast to their littermates, $apoE^{-/-}$ mice fed normal diet. In some animals the lesions caused significant occlusion of the lumen of the vessel and there was evidence of myocardial infarction primarily at the apex of the heart. In contrast, none of the sections from hearts of $apoE^{-/-}$ mice fed normal diet for the same duration showed any sign of occlusive coronary artery disease or myocardial infarction. $ApoE^{-/-}$ diseased hearts had increased pro-survival signalling (a higher ratio of phospho-Akt/total Akt) and were more resistant to cardiac insults than those from normal $apoE^{-/-}$ mice. Differences cannot be due simply to high cholesterol as both control $apoE^{-/-}$ and diseased $apoE^{-/-}$ mice are grossly hypercholesterolaemic. The possibility that the increased resistance of diseased $apoE^{-/-}$ hearts to ischaemia and reperfusion could be simply due to high-fat diet feeding has been excluded using wild-type mice. Our results show the opposite; hearts from wild-type mice fed a high-fat diet had increased vulnerability to ischaemia and reperfusion injury.

Progression of coronary disease in $apoE^{-/-}$ mice or high-fat feeding of wild type mice induce significant changes in myocardial calcium cycling which may explain some of the differences observed and can be used in the design of suitable myocardial protection strategies.

SATURDAY, 14. NOVEMBER 2009:

SESSION VI

AMP-ACTIVATED PROTEIN KINASE AND HYPOXIA-RESPONSE COUPLING IN THE CAROTID BODY AND PULMONARY ARTERY

A. Mark Evans

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Vital homeostatic mechanisms monitor O_2 supply and adjust respiratory and circulatory function to meet demand. The pulmonary arteries and carotid bodies are key systems in this respect. Hypoxic pulmonary vasoconstriction aids ventilation-perfusion matching in the lung by diverting blood flow from areas with an O_2 deficit to those rich in O_2, while a fall in arterial pO_2 increases sensory afferent discharge from the carotid body to elicit corrective changes in breathing patterns. We discuss here the new concept that hypoxia, by inhibiting oxidative phosphorylation, activates AMP-activated protein kinase (AMPK) leading to consequent phosphorylation of target proteins, such as ion channels, that initiate pulmonary artery constriction and carotid body activation. Consistent with this view, AMPK knockout mice fail to adjust their ventilation rate in response to hypoxia. Thus, AMPK may be sufficient and necessary for hypoxia-response coupling and may regulate O_2 and thereby energy (ATP) supply at the whole body as well as the cellular level.

ROLE OF HYPOTHALAMIC FATTY ACIDS, AMPK AND GHRELIN IN THE REGULATION OF FOOD INTAKE

Miguel López

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The "classical" hypothalamic neuropeptide view of feeding regulation has been extensively reviewed and revised during the last few years. Accumulating evidence indicate that pharmacological and genetic modulation of lipogenesis *de novo* in the hypothalamus, through selective pharmacologic and genetic manipulation of acetyl-CoA carboxylase (ACC), AMP-activated protein kinase (AMPK), carnitine palmitoyltransferase-1 (CPT1), fatty acid synthase (FAS) and malonyl-CoA decarboxylase (MCD) enzymes, has a severe impact on food intake and body weight homeostasis. Furthermore, since these manipulations alter the hypothalamic pool of lipids, such as malonyl-CoA and/or long chain fatty acids-CoA (LCFAs-CoA), the concept of lipids as signals of nutrient abundance able to modulate feeding in the hypothalamus has recently re-emerged.

Despite these pharmacological and genetic data, confirmation that it is a physiologically relevant regulatory system of feeding is still incomplete. Our current investigations have revealed that hypothalamic FAS, AMPK and CPT-1 expression and activities are modulated by peripheral signals regulating feeding, such as ghrelin, specifically in the ventromedial nucleus of the hypothalamus (VMH). Our data also identify the fatty acid biosynthetic pathway in the VMH as a potentially important physiological mediator of feeding behaviour of relevance for the understanding and treatment of obesity.

5 AMP-ACTIVATED PROTEIN KINASE (AMPK): A ROLE IN FEMALE REPRODUCTION?

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5 AMP-activated protein kinase (AMPK) is a serine/threonine kinase that acts as a fuel gauge in regulating energy metabolism by switching on catabolic pathways and switching off anabolic pathways. A role for AMPK in reproductive functions has recently emerged. We have showed that AMPK is expressed in granulosa cells and cumulus-oocyte complexes (COCs) in the ovary of several species (rat, cow and women). In vitro, pharmacological activation of AMPK by AICAR or metformin, two known activators of AMPK, reduced progesterone and/or œstradiol secretion in granulosa cells of these species. These effects are mediated through a regulation of the steroidogenic enzyme 3 beta-HSD (3 beta-hydroxysteroid dehydrogenase) expression and the Mitogen-activated protein kinase ERK1/2 signaling pathway. Moreover, AMPK might directly regulate oocyte maturation in different species. These studies suggest a role for AMPK in female fertility through a direct action at the ovary level. However, AMPK is also present in the nervous system central and particularly in the hypothalamus. Thus, we have recently investigated the role of this kinase in the central regulation of female reproduction. We have first determined the effect of one intracerebroventricular (i.c.v) injection of AICAR on the oestrous cyclicity in rat. Injection of AICAR i.c.v specifically increased hypothalamic AMPK activation and reduced the interval between two oestrous stages in vivo. We have also examined the effects of metformin and AICAR on GnRH secretion in immortalized GnRH neurones (GT1-7 cells). We have demonstrated that treatment with either metformin or AICAR significantly inhibits GnRH release in the presence and absence of GnRH in GT1-7 cells. Furthermore, these effects were abolished by the specific AMPK inhibitor compound C, suggesting that AMPK is involved in metformin- and AICAR-induced inhibition of GnRH release. Taken together, our findings suggest a role for AMPK in the ovarian and central regulation of female reproduction.

AMP-ACTIVATED PROTEIN KINASE SERVES AS A UNIVERSAL REGULATOR OF AUTOPHAGY

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Autophagy is a catabolic pathway whereby the cells self-digest proteins and intracellular organelles. It is an evolutionarily conserved, genetically controlled process that starts with a membrane nucleation in the cytoplasm. The membrane elongates around its cargo until the edges fuse forming an autophagosome. The autophagosome fuses with a lysosome allowing the lysosomal hydrolases to degrade the content to building blocks that can be re-used for de novo synthesis of macromolecules or ATP generation. Thus, autophagy serves as a temporary survival mechanism during periods of starvation both in a single-cell and in a whole organism. In addition to starvation numerous stresses, such as anti-cancer treatment, hypoxia, growth factor withdrawal, bacterial infection activates autophagy above the low basal level. In unstressed cells autophagy is kept down by mTORC1 which regulates cell growth and protein synthesis in response to nutrient and growth factor availability. In starved cells LKB1 activates AMPK that inhibits mTORC1 via a pathway involving TSC1/2 and its substrate Rheb. We recently showed that AMPK inhibits mTORC1 and induces autophagy also in non-starved cells. Various calcium mobilizing agents and cytokines activated AMPK via activation of CaMKK-beta or TAK-1, respectively, resulting in inhibition of mTORC1 even in nutrient rich conditions. Thus, we suggest that AMPK is a more general regulator of autophagy than previously expected.

THE Na^+/H^+ EXCHANGER NHE1 GENERATES A PERICELLULAR pH-NANOENVIRONMENT REQUIRED FOR CELL MIGRATION

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High rates of metastasis correlate with a poor prognosis of cancer. Two major steps of the metastatic cascade are cell migration/invasion into and degradation/reorganization of the extracellular matrix. Hypoxia, the predominating situation in solid tumors, forces the tumor cells to perform anaerobic metabolism. The tumor cells cope with the resulting acid load by extruding the excess protons via the Na^+/H^+ exchanger NHE1. A characteristic of numerous tumor cells is the overexpression of NHE1 and/or its upregulation.

In various migrating cells such as fibroblasts, epithelial and tumor cells, NHE1 accumulates at the cell front where its activity is needed for (i) cell adhesion, (ii) directed locomotion and (iii) extracellular matrix digestion. Thus, inhibition of NHE1 reduces the invasiveness of murine melanoma cells in rat liver parenchyma by about 50%.

Human melanoma cells establish an NHE1-dependent pH-nanoenvironment at their surface with a higher proton concentration at the cell front. This pH gradient at the cell surface is stabilized by the glycocalyx and is mirrored by a reciprocal, also NHE1-generated pH gradient in the cytosol. Destroying the cell surface pH-gradient by partial removal of the glycocalyx impairs cell migration substantially, however, can be compensated for by stimulating NHE1 activity. Vice versa, knocking down β1 integrins that mediate cell adhesion and migration in a pH-dependent manner makes the cell surface pH-gradient functionally inefficient.

NHE1 activity creates a pericellular, glycocalyx-stabilized pH-nanoenvironment that promotes both the digestion of the extracellular matrix and the establishment of focal adhesion contacts at the cell front including their release at the cell rear. At the same time, the intracellular pH gradient with more alkaline pH at the leading edge controls cytokeletal remodeling and contractility. In summary, NHE1 regulates cell migration extracellularly by modulating cell-surface interactions and by remodeling the extracellular matrix, and intracellularly by fine-tuning the migratory machinery. These findings point to the therapeutic potential of NHE1 inhibition in the prevention of metastasis.

ION CHANNELS KEEP MOBILE CELL ON THE GO

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Cell migration is a process that plays an important role throughout the entire lifespan. It starts early on during embryogenesis and contributes to shaping our body. Migrating cells are involved in maintaining the integrity of our body for instance by defending it against invading pathogens. On the other side, migration of tumor cells may have lethal consequences when tumors invade surrounding tissue and spread metastatically. Research of the past decade provided a strong body of evidence that ion channels are an integral part of the cellular migration machinery that not only complement and regulate other components of the cellular motor, but that they are also part of the cellular compass during directed migration in a chemotactic gradient. Thus, ion and water flow is required for optimal migration, and the inhibition or genetic ablation of channels leads to a marked impairment of (directed) migration. These observations also imply that ion channels have to be targeted properly to the cell membrane in order to fulfil their physiological functions in cell migration. We will present work from our group on TRPC1 and $K_{Ca}3.1$ channels showing their role in directional migration and their endocytic recycling during migration, respectively.

THE FUNCTIONAL ROLE OF THE NON-GASTRIC H^+/K^+-ATPASE ATP12A (ATP1AL1) AS ANTI-APOPTOTIC ION TRANSPORTER

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The non-gastric H^+/K^+-ATPase ATP12A (ATP1AL1) is expressed in a broad variety of tissues, but the knowledge about its function is sparse. We found by RT-PCR and/or western blotting, measurements of intracellular pH, electron microprobe analysis, cell volume (CV) measurements and flow cytometry that ATP12A is expressed in human myelomonocytic HL60 cells, human polymorphonuclesr leucocytes (PMN), rat insulinoma Ins-1E cells and human pancreatic islets, as well as in normal and cancerous human prostate tissue. Activation of PMN by the chemotactic factor fMLP leads to cell swelling by activation of the Na^+/H^+-exchanger NHE1 and the H^+/K^+-ATPase and inhibition of either ion-transporter inhibits cell migration. Treatment of HL60 cells with low (1mM) concentrations of butyrate leads to differentiation towards the monocytic lineage whereas higher (5-10mM) concentrations induce apoptosis as assessed by flow cytometric determination of CD86 expression, CV, cell granularity, caspase activity, phosphatidyserine exposure on the outer plasma membrane leaflet, cell cycle analysis and cell proliferation. Similar, Ins-1Ecells undergo apoptosis upon treatment with the polyphenol resveratrol or by glucose starvation. Ins-1E cells and the fraction of HL60 cells bearing the common leucocyte antigen CD45 (CD45+) exhibit apparent apoptotic volume decrease (AVD). Untreated CD45+ cells have a greater volume than CD45- cells which also lack AVD. Transcriptional up-regulation of ATP12A is evident during both butyrate-induced differentiation and apoptosis in HL60 cells and during resveratrol-induced apoptosis in Ins-1E cells. Inhibition of the H^+/K^+-ATPase by the K^+-competitive antagonist SCH28080 (10-100μM) leads per se to induction of apoptosis in differentiated HL60 cells and untreated Ins-1E cells and accelerates the time course of apoptosis in these cells if induced by high concentrations of butyrate or resveratrol, respectively. This is accompanied by acceleration and/or aggravation of AVD. Taken together, these results demonstrate that ATP12A is functionally active in PMN, differentiated HL60 cells and Ins-1E cells and that it plays a role during apoptosis at least in part by counteracting AVD. In line with this is the observation, that ATP12A gene expression is stage dependently up- or down regulated in cancerous tissue from human prostate cancer.

INTERACTION OF BAX WITH A MITOCHONDRIAL POTASSIUM CHANNEL IS CRUCIAL FOR ITS ACTION IN APOPTOSIS

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The voltage-gated potassium channel Kv1.3, the major plasma membrane channel in lymphocytes (Chandy KG et al, 2004 *Trends Pharm. Sci.,* 25, 280), has been located also to the inner mitochondrial membrane in these cells (Szabò et al, 2005, *J.Biol. Chem.*, 280, 12790). Mouse and human cells genetically deficient for Kv1.3 or transfected with siRNA suppressing Kv1.3-expression resisted apoptosis induced by several stimuli, while retransfection of Kv1.3 restored death. Pro-apoptotic Bax directly interacted with and functionally inhibited mitochondrial Kv1.3. Incubation of Kv1.3-positive isolated mitochondria with recombinant Bax or channel inhibiting toxins triggered hyperpolarization, formation of reactive oxygen species, release of cytochrome c and depolarization. In Kv1.3-deficient mitochondria these changes did not occur upon incubation with Bax and the toxins. Mutation of Bax at K128, which corresponds to a conserved lysine in Kv1.3-inhibiting toxins, abrogated its effects on Kv1.3 and mitochondria. Likewise, a single point mutation turned Bcl-xL pro-apoptoptic. To test the function of the mutant Bax *in vivo*, $Bax^{-/-}$ $Bak^{-/-}$ mouse embryonic fibroblasts (DKO MEFs) were transfected with either wild type Bax or Bax(K128E). Staurosporine-induced apoptosis was defective in Bax(K128E)-transfected cells, indicating that Bax mediates apoptosis in lymphocytes at least in part via interaction with mitochondrial Kv1.3.

STEM CELLS AND NEUROGENESIS IN THE ADULT BRAIN

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The last two decades of neuroscience were revolutionized by the fact that stem and progenitor cells exist in the adult brain throughout lifetime of mammals including humans. At least within the two classical neurogenic regions, the dentate gyrus in the hippocampus and the subventricular zone / olfactory bulb system, the stem and progenitor cells generate new neurons spontaneously and thus provide a continuous source for cellular homeostasis. In addition, it becomes more and more evident that progenitors outside of the typical neurogenic regions contribute to the generation of new cells in the brain. Hippocampal neurogenesis is tightly linked to memory function, the functional role of subventricular zone neurogenesis is less clear. The fact that neurogenesis can be modulated by molecular and cellular means, makes it an attractive target for neuro-regenerative approaches. This presentation will review the current knowledge on neural stem cells of the adult brain. It will touch basic science and clinical aspects of adult neurogenesis.

THE PHYSIOLOGY OF NEURAL STEM CELLS IN THE HEALTHY AND DISEASED BRAIN

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Neurogenesis, the generation of new neurons, persists in the adult mammalian brain in the subventricular zone (SVZ) / olfactory bulb system and in the hippocampus. Importantly, there is evidence that neurogenesis also takes place in humans. Newborn cells arising in the SVZ normally migrate along the rostral migratory stream to the olfactory bulb where they differentiate into GABAergic and dopaminergic neurons. Neurogenesis involves three crucial steps. Asymmetric cell division of a stem cell, resulting in one daughter stem cell and one cell which can develop into a neuron. The second step entails the migration of the newborn cell to its final and appropriate destination in the brain. The third and final step involves maturation of the cells into a neuron that forms both efferent and afferent connections within the brain. Importantly, aggregation disorders such as synucleinopathies may interfere with these different steps. It is important to note that cognitive dysfunction, depression and olfactory deficits are important pre-motor signs in Parkinson disease (PD), the most prevalent human synucelinopathy, and may be linked to an impaired hippocampal and olfactory bulb neurogenesis. Interestingly, a decreased proliferation of neural precursor cells was described in the SVZ and the hippocampus of PD patients as well as in PD animal models and specifically in mouse models of synucleinopathies. Using different transgenic synuclein models (different promoter such as PDGF and Thy1 as well as tetracycline dependent expression of synuclein) we have precisely characterized at which stage and how synuclein interferes the generation of adult newly generated cells. This knowledge may help us (1) to better understand the impact of protein aggregation in regions of cellular plasticity and (2) to develop novel strategies to maintain the capacity of the adult brain to generate new neurons.

THE RISE OF CANCER STEM CELLS IN HIGH-GRADE GLIOMAS – A MATTER OF A PATHOLOGICAL STEM CELL NICHE?

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Recently, as in many solid tumors, cancer stem cells (CSC) or tumor progenitor cells have been defined as the probable source of human high-grade gliomas, including the most malignant type, glioblastoma. CSCs can be isolated from most of the primary glioblastomas using adequate culture conditions and show features of true stem cells like infinite self-renewal, clonal properties and differentiation in several lines of differentiated cells. There is some evidence that CSCs develop from neural stem cells (NSC), though definite proof for this assumption is lacking at this point. However, there is much evidence that CSCs can only develop in a proper microenvironment, the tumor niche. This tumor niche shares many properties with a similar niche, the stem cell niche, where NSCs are situated. Any kind of a physiological or pathological niche consists of several cellular components (neurons, astroglia, oligodendrocytes, endothelial cells, microglia, immune cells and several progenitor cells) that secrete soluble factors of different origin, and the extracellular matrix. In addition, the regional metabolism plays an important role that has been underestimated until recently. The so called Warburg effect describes a phenomenon of aerobic glycolysis found in many solid tumors, including glioblastomas, which has not been completely understood to date, but which might explain the transition from a normal NSC niche to a pathological CSC niche. It seems plausible that CSCs develop in such a niche and re-generate the tumor bulk that mainly consists of differentiated tumor cells. This would further explain the failure of many of the classical therapies that mostly target differentiated tumor cells. The talk will summarize the available evidence for the outlined hypothesis and provide strategies for the future investigation of the phenomenon.

GLUTAMATERGIC EXCITATION OF MATURE AND NEWLY GENERATED YOUNG HIPPOCAMPAL GRANULE CELLS

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In the dentate gyrus of the mammalian hippocampus, new neurons are generated throughout life (Gage 2000, Science, 287:1433-38). The newly generated young neurons were suggested to express functional glutamate receptors from very early stages on already during the first few days after cell division. Furthermore, the activation NMDA (N-methyl-D-aspartate) - receptors was reported to modulate neuronal differentiation and survival. However, not much is known about the functional properties of AMPA and NMDA receptors in the newly generated young neurons.
Therefore, we analysed the functional properties of AMPA and NMDA receptors in nucleated membrane patches excised from mature and newly generated young granule cells in acute hippocampal brain slices of juvenile rats. Young and mature granule cells were identified according to their electrical input resistance, which was shown to be > 1 GOhm and < 400 MOhm for young and mature cells, respectively (Schmidt-Hieber et al. 2004, Stocca et al. 2008). Brief pulses of glutamate (5-100 ms) were applied with the fast application technique using a piezo-driven application device. All neurons tested showed AMPA- and NMDA-receptor mediated currents identified by the sensitivity to CNQX and D-AP5, respectively. However, the peak amplitude of AMPA-R mediated currents was substantially different with an average amplitude of ~500 pA and ~2000 pA in young and mature cells, respectively. The difference in NMDA-R mediated current density was smaller, resulting in a decrease of the NMDA to AMPA ratio with development. Fiber tract stimulation of the medial perforant path in the presence of gabazine could evoke EPSPs and APs in most young GCs tested. In conclusion, the expression density of glutamate receptors is low in young neurons and strongly increases during the development of newly generated granule cells. However, functional glutamatergic synapses can effectively discharge newly generated granule cells already very early in development.

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EFFECTS OF PROLONGED BED REST ON MECHANICAL PROPERTIES OF PERIPHERAL BLOOD VESSELS

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Prolonged periods of recumbancy, as well as long space missions, induce orthostatic intolerance. Deconditioning of several biloogical functions may contribute to such orthostatic intolerance. The present studies concerns deconditioning of the pressure resistance of peripheral blood following prolonged bedrest (BR). In the upright body position longitudinally oriented blood vessels are aligned with the gravitational force field, resulting in substantial intravascular pressure gradients along the vessels. The question was whether blood vessels of the lower body need the regular increments of local intravascular pressures, associated with habitually assuming erect posture, to maintain their pressure resistance. Several series of experiments were conducted in healthy test subjects to investigate the *in vivo* pressure distension in arteries, arterioles and veins in the arm or the leg. Each subject was positioned either seated or supine in a hyperbaric chamber with either one arm or a lower leg protruding through a hole in the chamber door. Increased pressure in the vessels of the arm/leg was accomplished by increasing chamber pressure. Vessel diameter and flow were measured using Doppler ultrasonography. Measurements were conducted before and after 5 weeks of sustained horizontal BR. Before BR, distensibility was considerably larger in the arteries, arterioles and veins of the arm than in the corresponding vessels of the leg. BR increased vascular distensibility, especially in the arteries and arterioles of the leg, an effect that could be counteracted by intermittently (3 x 40 min/week) increasing local transmural pressure by exposing a lower leg to subatmospheric pressure of 90 mmHg. It thus appears the pressure resistance of arteries, arterioles and veins adapts to meet the demands imposed by the local pressure acting on the vessel wall. The mechanisms governing such local adaptation of vascular wall stiffness are largely unknown. In separate experiments we have found that intravascular pressure provocations induce local release of the vasoconstrictor endothelin-1 (ET-1). After BR, pressure-induced local release of ET-1 was more pronounced in the lower leg that had intermittently been exposed to transmural pressure increments than in the control leg. Thus, decreased local production/release of vasoconstrictors, including ET-1, may be one mechanism underlying the BR-induced increase in leg vessel distensibility.

DETERMINANTS OF BONE LOSS DURING IMMOBILIZATION

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Bone losses occur in clinical cases of immobilization, such as stroke and spinal cord injury. In those cases, the rate of bone loss is initially high and levels off after 2-5 years. Experimental bed rest, which is often used as a model of deconditioning effects, is also associated with bone losses from the legs, but not from the arms. Similar effects can be elicited by unilateral lower limb suspension, which disfavours the hypothesis that increased venous pressure can prevent immobilization-induced bone loss. Adequate resistive exercise, conversely, has the potential to act as a countermeasure against bone losses. Data suggest that resistive exercise may be more effective when combined with whole body vibration.

There seems to be a wide variation in the time course of bone losses from immobilized limbs, but probably not in the amount of bone losses. The question arises as to what determines this biological variation. Recent data show that bone structure can partly explain different rates of bone loss. Moreover, there is a possibility that the kidney's ability of calcium excretion is a limiting factor and might hamper immobilization-induced bone losses.

PHYSIOLOGY OF DECONDITIONING – NUTRITIONAL ASPECTS

Ian A Macdonald

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A variety of clinical and environmental conditions can reduce the use of skeletal muscle and give rise to a state of deconditioning. The most common circumstances in which this happens are illness or surgical operations which lead to the individual being confined to bed for prolonged periods of time, or after fractures of the spine/lower limb where whole-body or limb immobilisation is necessary to promote healing. A less common example of this deconditioning is the microgravity associated with spaceflight, where a variety of different strategies to prevent loss of muscle mass and function (countermeasures) have been developed.

Deconditioning of skeletal muscle is accompanied by a reduction in physical activity energy expenditure. When this occurs during recovery from illness/surgery, or after a fracture, then there is often no alteration in appetite such that positive energy balance can occur. This would lead to an increase in body fat content such that body weight may not decrease even though muscle mass is reduced. The consequence of this is a need to not only improve muscle mass and function after recovery from the illness/fracture, but also to decrease body fat content.

The situation in spaceflight appears to be somewhat different, with a reduction in appetite and loss of weight being reported to accompany the decrease in muscle mass. There has been substantial interest in the possibility that nutritional supplements may be an effective countermeasure to prevent the loss of muscle mass and function during microgravity. However, in a recent study in women using prolonged head down tilt (bed-rest) as a model of microgravity, protein supplementation did not prevent the loss of muscle mass during the bed-rest period. By contrast, a different study of prolonged bed-rest in men which assessed the combined effects of resistance training and essential amino acid supplementation showed a beneficial effect on muscle mass and strength when the amino acids were consumed immediately before the exercise bouts.

Less is known about the influence of diet composition or nutritional supplements on muscle mass and function seen with illness, surgery or fracture, or during recovery from the deconditioning which occurs in these situations. Recent work has indicated that older individuals may be more dependent of the quantity and timing of protein intake for optimal improvement in muscle function during training, but little has been done in the more clinical situations.

WHAT DRIVES FUSION PORE ENLARGEMENT?

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Exocytotic events monitored in adrenal chromaffin cells by means of carbon fiber amperometry can be categorized in two groups according to the rate and extent of fusion pore expansion. The probability of fast fusion increases with intracellular calcium levels. It is also dependent on a short intermembrane distance, mediated by tight zippering of the SNARE complex. We also found that the GTPase cdc42 is needed for rapid expansion of the pore. The components of the fusion machinery are thus not the only determinants of fusion pore dilation.

DIFFERENT ROLES OF THE SNARE-COMPLEX IN NEURONAL EXOCYTOSIS: FROM VESICLE DOCKING TO THE FUSION PORE

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The neuronal SNARE complex is at the heart of neuronal exocytosis. It is an extended coiled coil consisting of four alpha-helical SNARE-motifs, provided by syntaxin-1, synaptobrevin-2 and SNAP-25. The formation energy of the complex is assumed to overcome the energy barrier for fusion, but it has been unclear during which phases leading up to neuronal exocytosis the SNARE-complex forms. Here, I present recent data on the role of the SNARE-complex in vesicle docking, spontaneous release and fusion pore formation. Experiments in chromaffin cells show that two SNARE-complex members, syntaxin-1 and SNAP-25, but not synaptobrevin-2, are involved in docking vesicles to the plasma membrane. The vesicular counterpart is synaptotagmin-1, better known as the calcium censor for exocytosis, which binds to an 1:1 SNAP25:syntaxin acceptor complex on the plasma membrane. This docking reaction does not require Munc18-1, but Munc18-1 plays an indirect role, by stabilizing acceptor complexes and stimulate SNARE-complex assembly. Next, the N-terminal end of the synaptobrevin-2 SNARE-motif joins the complex, driving vesicle priming, the reaction that makes the vesicle releasable. Fusion triggering is executed by calcium binding to synaptotagmin, which causes the C-terminal end of synaptobrevin-2 to 'zipper up' to the remaining complex. This part of the reaction leads to rapid fusion, and to fusion pore formation. Thus, we find that C- but not N-terminal mutations in synaptobrevin-1 change fusion pore duration. The last piece of evidence concerns the origin of spontaneous release events in neurons, which have been attributed to another fusion machinery, or to separate vesicle pools. Recent data obtained in autaptic neurons indicate that spontaneous release events can be inhibited or stimulated by C- and N-terminal mutations in the SNARE-complex, respectively. This indicates that spontaneous release events are driven by the same fundamental machinery as evoked release. The SNARE-complex helps shape the energy landscape for fusion so as to favour evoked release. In sum, our data allow the synthesis of a model for vesicle fusion from the initial docking of vesicles to the plasma membrane until the formation of a fusion pore.

Ca^{2+} DEPENDENCE OF EXO AND ENDOCYTOTIC COUPLING AT A GLUTAMATERGIC SYNAPSE

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The triggering of endocytosis by exocytosis and its regulation by intracellular calcium concentration ($[Ca^{2+}]_i$) are well-established phenomena. Here we show that the mechanism linking these two processes is dependent on microdomain-$[Ca^{2+}]_i$ similar to that which triggers exocytosis. We further show that compensatory endocytosis is eliminated by blocking proteins that mediate endocytosis (dynamin and AP2) as well as by disrupting exocytotic protein synaptobrevin/VAMP. Furthermore, these manipulations all have a limited, retrograde action on exocytosis, delaying recruitment of release-ready vesicles and enhancing short-term depression. The latter effect sets in so rapidly, that it cannot be explained by the non-availability of recycled vesicles. Rather, we postulate that perturbation of a step linking exocytosis and endocytosis temporarily prevents new vesicles from docking at specialized sites for exocytosis.

FUSION PORE REGULATION OF PEPTIDERGIC VESICLES

Nina Vardjan[1,2], Jernej Jorgačevski[2,1], Matjaž Stenovec[1,2] , Marko Kreft[2,1], Robert Zorec[2,1]

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Neurotransmitter and hormone release requires the fusion of secretory vesicles with the plasma membrane of neurons and neuroendocrine cells (*i.e.* exocytosis). Exocytosis begins with the formation of a fusion pore, an aqueous channel between the vesicle and the plasma membrane through which cargo molecules diffuse out of the vesicle lumen to the cell exterior. After the fusion pore formation the pore either closes (transient fusion, "kiss-and-run" exocytosis), it fluctuates between an open and a closed state (fusion pore flickering, the pulsing fusion pore), or it fully opens leading to the merger of the vesicle with the plasma membrane (full fusion exocytosis).
Spontaneous hormone discharge from a single lactotroph vesicle of the anterior pituitary cell is 10-20 times slower than stimulated discharge because of the kinetic constraints of fusion pore flickering. To see whether the slow release at rest reflects also a relatively narrow fusion pore we analyzed the permeation of FM 4-64 dye (molecular diameter = 1 nm) and HEPES (molecular diameter = 0.5 nm) molecules through fusion pores in lactotroph vesicles expressing a pH-dependent fluorescent fusion marker synaptopHluorin. Confocal imaging showed that in 50% of the spontaneous exocytotic events fusion pore openings were associated with a change in synaptopHluorin fluorescence, indicating the efflux of protons, but the pore was impermeable to FM 4-64 and HEPES. These findings, confirmed with capacitance measurements, indicate a fusion pore diameter <0.5 nm, smaller than the neuropeptides stored in these vesicles (molecular diameter = 5.2 nm). In high potassium-stimulated cells, >70% of exocytotic events exhibited a larger pore permeable to FM 4-64 (>1 nm). Capacitance measurements showed that the majority of exocytotic events in spontaneous and stimulated conditions were transient. However, stimulation increased the frequency of transient events and their fusion pore dwell-time, but decreased the fraction of events with lowest measurable fusion pore.
Thus in lactotrophs transient mode of exocytosis is the dominant mode of peptidergic hormone release. Under stimulation, a pre-formed fusion pore may retain the transient nature, but with a prolonged dwell-time, increased frequency of re-openings and an increased fusion pore diameter. All of these changes facilitate the vesicle cargo release.

SATURDAY, 14. NOVEMBER 2009:

SESSION VII

PRACTICAL SUPERRESOLUTION MICROSCOPY: THEORY AND APPLICATIONS OF PAL-M AND SR-SIM

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Superresolution microscopy, i.e. light microscopy beyond the traditional diffraction limit, is rapidly changing its face. From avant-garde method development research, it is currently being transformed into a tool for biomedical research by pioneer users and engineers.
The talk will give an introduction to the superresolution technologies that Carl Zeiss MicroImaging focuses on: Photoactivated Localization Microscopy (PAL-M) and Super Resolution Structured Illumination Microscopy (SR-SIM).
PAL-M is a single molecule imaging method based on the strategy to photoactivate (or photoswitch) only few fluorophores at a single timepoint. A mathematical process afterwards localizes precisely their position by a Gaussian fit. The effective lateral resolution down to 20nm for cells and tissues opens up completely new dimensions of quantitative analysis of complex biological specimens.
In SR-SIM the sample is illuminated with precisely modulated excitation light. This known line pattern is superimposed to the sample´s structure and creates due to interference a Moiré pattern. From this interference pattern in the raw data an algorithm computes the superresolution data that reaches up to twice the resolution in all three dimensions compared to confocal microscopes. SR-SIM is a flexible technique that can be applied on all fluorophores on fixed as well as living samples.
With the data dimensions that these novel imaging methods open up, they also bring up new questions about optimal fluorescence probes, sample preparation methods and data presentation. In this regard, each superresolution technology presents its unique advantages and challenges. Exemplary use cases and successful experiment design will be adressed in a second part of the talk.

EXPRESSION OF 11B-HSD1 AND H6PDH DURING ONTOGENY

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Glucocorticoids are known to modulate immunological processes by influence of development and effector functions of immune system. The ability of the cells to respond to glucocorticoid hormones depends not only on their plasma concentration, the responsiveness of the target cells but also on the expression of their receptors and local metabolism of glucocorticoids that is predominated by 11β-hydroxysteroid dehydrogenases type 1 and 2 (11β-HSD1, 11β-HSD2). 11β-HSD2 operates strictly as an oxidase that converts biologically active glucocorticoids (cortisol, corticosterone) to their inactive 11-keto forms (cortisone, 11-dehydrocorticosterone) whereas 11β-HSD1 reduces predominantly the 11-keto forms to active glucocorticoids. It is suggested that NADPH, which is used by 11β-HSD1 to function as a reductase, is generated by hexose-6-phosphate dehydrogenase (H6PDH) in the lumen of endoplasmic reticulum. Because evidence suggests that glucocorticoids play essential roles during development and differentiation of some kinds of immune cells, the aim of our study was to determine whether the developmental changes of immune organs are accompanied with changes of the local metabolism of glucocorticoids. We studied, therefore, the expression of 11β-HSD1 and H6PDH in immune organs like thymus, spleen, and lymph nodes and in liver of Wistar male rats during suckling, weaning, prepubertal and adult period of life. The abundance of mRNAs was measured by qRT-PCR using TaqMan probes. PCR reaction was performed as a duplex measurement of the gene of interest and the housekeeping gene (GAPDH). Splenic and hepatic 11β-HSD1 mRNA levels were increasing significantly during suckling and weaning period and the highest values reached in adulthood. In contrast, 11β-HSD1 transcript in thymus and lymphatic nodes decreased after weaning. The abundance of H6PDH mRNA increased in spleen and liver of sucklings and weanlings, was not changed in thymus and decreased in lymphatic nodes. In adulthood, the levels of H6PDH mRNA were higher than in early postnatal life only in spleen, and lower in liver and lymphatic nodes. These results show that the developmental patterns of both enzymes are different in the investigated organs and that the capacity of the local metabolism of glucocorticoids might be on different levels during ontogeny.

The project was supported by Czech Science Foundation GACR 305/07/0328.

THE ADAPTIVE RESPONSE OF LEYDIG CELLS TO IMMOBILIZATION STRESS: STIMULATION OF PKA AND StAR PROTEIN

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The ability of immobilization stress (IMO) to decrease Leydig cell steroidogenesis and serum androgens level has been previously observed, but the possible mechanism(s) involved in the adaptation to prolonged or repeated stress have not been identified. In this study we investigate weather the Leydig cells obtained from adult rats subjected to acute (15 min, 30 min or 2h) or repeated (2 or 10 days, 2 hours daily) IMO causes mechanism(s) as adaptive response to stress-impaired steroidogenesis. Results showed that basal and hCG-stimulated cAMP production of Leydig cells isolated from animals exposed to both acute and repeated IMO was significantly reduced. Despite the reduced cAMP production, immunoblot analysis revealed increased immunoreactivity for both protein kinase A (PKA) and steroidogenic acute regulatory (StAR) protein in Leydig cells obtained from animals repeatedly exposed to IMO. Also, the PKA-dependent phosphorylation and production of mature StAR protein was evident during exposure of animals to repeated IMO treatment. The physiological significance of the presented results was suggested by the recovery of serum androgen level, also affected by 2 h or 2x2 h IMO, but which starts to recover after 10x2 h IMO, and significantly increased *in vitro* androgen and progesterone production in presence of cholesterol (the steroid substrate transported into mitochondria by StAR). Aditionaly, increase of stress-reduced androgen production after repeated IMO is not related with steroid gene expression, since real time RQ-PCR analysis reviled that both acute and repeated IMO decreased P450scc, 3betaHSD and P450c17 gene expression. In contrast, the level of StAR transcript was progressively increased in all IMO groups. Thus, PKA-mediated phosphorylation of StAR protein is an important cascade in the adaptive response of Leydig cells to repeated immobilization stress.

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INTRACELLULAR CHLORIDE IONS MODULATE SECRETORY ACTIVITY OF MOUSE CHROMAFFIN CELLS

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We investigated modulatory role of cytoplasmic chloride ion concentration ($[Cl^-]_i$) on secretory activity in mouse adrenal slices. Slow photo-release of caged Ca^{2+} (NP-EGTA) was used to activate Ca^{2+} -dependent exocytosis, measured as membrane capacitance changes (C_m) using whole-cell patch-clamp technique. Slow, ramp-like changes in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$, Fura-6F photometry) enabled high temporal resolution and more precise determination of Ca^{2+} dependency of the elicited exocytotic bursts. Intracellular clamp to high $[Cl^-]_i$ (155 mM) resulted in a significant change in the amplitude as well as maximal rate of C_m change, however Ca^{2+}-sensitivity of the exocytotic process has not been affected. At 155 mM $[Cl^-]_i$, the amplitude of the secretory bursts were approximately two-times larger compared to low $[Cl^-]_i$ (14 mM). Similarly, the maximal rate of C_m change during bursts was significantly faster at high $[Cl^-]_i$ (400 ± 44 fF/s) compared to the low $[Cl^-]_i$ (233 ± 38 fF/s). The sensitivity of the exocytotic machinery did not depend on $[Cl^-]_i$ as Ca^{2+} concentration required for the half maximal exocytotic activity was comparable (EC_{50} = 0.4 μM). Taken together, the influence of $[Cl^-]_i$ on secretory amplitude and rate but not on its sensitivity for Ca^{2+} corroborates the modulatory role of chloride ions in the promotion of vesicular maturation.

GPCR-MEDIATED SIGNALING-INDUCED PARACRINE TRANSACTIVATION OF CB1 RECEPTOR, AN INTERACTION BETWEEN THE EFFECTS OF CALCIUM-MOBILIZING HORMONES AND CANNABINOID SYSTEM

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CB_1 cannabinoid receptor (CB_1R) belongfs to the G-protein-coupled receptor (GPCR) superfamily and couples to $G_{i/o}$ heterotrimeric proteins. We have shown previously that CB_1R can be transactivated by stimulation of the calcium-mobilizing GPCR, AT_1 angiotensin receptor (AT_1R) coexpressed in the same cells. Since these two receptors can be expressed in adjacent cells, we asked if paracrine transactivation of CB_1R is possible. In Chinese hamster ovary (CHO) cells CB_1R activity can be monitored by bioluminescence resonance energy transfer (BRET) between G protein subunits (α-subunit tagged with renilla luciferase and ß-subunit with yellow fluorescent protein, YFP). Activation of CB_1 receptors leads to decrease of BRET signal, whereas treatment with inverse agonist causes an elevation. AT1R stimulation with angiotensin II (Ang II) caused CB_1R activation when the two receptors were expressed in separate set of CHO cells and mixed prior to measurement, indicating endocannabinoid release. Paracrine transactivation also occurred when M_1, M_3, M_5 muscarinic-, B_2 bradykinin-, α_1-adrenergic- or V_1 vasopressin receptors were expressed and stimulated. As activated GPCRs bind ß-arrestins, their activity can be also detected by translocation of ß-arrestins to the membrane detected by confocal microscopy. ß-arrestin2-GFP translocated to cell membrane after stimulation of CB_1Rs with agonist. ß-arrestin binding was increased, when DRY motif in second intracellular loop was mutated to AAY in the receptor. Cells transfected with CB_1R(DRY/AAY)-RFP and ß-arrestin-GFP were mixed with cells expressing AT_1R-YFP. When cells were stimulated with Ang II, ß-arrestin-GFP translocated to the membrane in CB_1R(DRY/AAY)-RFP expressing cells.

In order to investigate the link between GPCR-induced effects and cannabinoid system also in the vasculature, rat skeletal muscle arterioles (SMA) were isolated, pressurized and subjected to microangiometry. In SMA calcium-mobilizing hormones Ang II and noradrenaline (NE) induced vasoconstriction by ca. 30 % and 40 % for 10^{-8} M Ang II and 10^{-6} M NE, respectively. CB_1 receptor inhibition with a neutral antagonist significantly augmented Ang II-induced vasoconstriction (by ~60 % at 10^{-8} M) and noradrenaline (NE)-induced vasoconstriction as well (by ~80 % at 10^{-6} M); and also completely inhibited the CB_1-agonist WIN55-induced vasodilation (from 15±2 %).

These results show a link between calcium-mobilizing GPCR-induced signaling and the cannabinoid system. We found, that stimulation of AT_1Rs or other calcium mobilizing receptors leads to endocannabinoid release and paracrine transactivation of CB_1Rs in CHO cells. In skeletal muscle arterioles CB_1Rs are involved in the adjustment of Ang II- and NE-induced vascular tone.

LYSOPHOSPHOLIPIDS MODULATE VOLTAGE-GATED CALCIUM CHANNEL CURRENTS IN PITUITARY CELLS; EFFECTS OF LIPID-STRESS

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Voltage-gated calcium channels (VGCC) are osmosensitive. To test the hypothesis that this property of VGCCs stems from their susceptibility to alterations in the mechanical properties of the bilayer, we use native VGCCs in pituitary cells and reversibly perturb the bilayer with lipids that alter bilayer stress, i.e. cone-shaped lysophospholipids (LPLs). LPLs of different head group size and charge were used: lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), lysophosphatidylserine (LPS) and lysophosphatidylethanolamine (LPE). Phosphatidylcholine (PC) and LPC (C6:0) were used as controls. We show that partition of both LPC and LPI into the membrane of pituitary cells suppressed L-type calcium channel currents (I_L). This suppression of I_L was slow in onset, reversible upon washout with BSA and associated with a depolarizing shift in activation (~ 8mV). In contrast to these effects of LPC and LPI on I_L, LPS, LPE, PC and LPC (C6:0) exerted minimal or insignificant effects. This difference may be attributed to the prominent conical shape of LPC and LPI compared to the shapes of LPS and LPE (which have smaller headgroups), and to PC (which is cylindrical). The similar effects of LPC and LPI on I_L, despite differences in the structure and charge of their headgroups, suggest a common lipid stress mechanism in their action. It is plausible that after slow incorporation of these cone-shaped lipids into the membrane of pituitary cells, bilayer mechanics and consequently lipid-protein interactions are different, in a way that suppresses calcium channel voltage sensor motion and thus positively shifts voltage dependence of activation.

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BEHAVIOURAL EFFECTS OF GROWTH HORMONE REPLACEMENT THERAPY IN AGED WISTAR RATS

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Growth Hormone (GH) plays an important role in young animals promoting growth and development but its secretion decreases in adulthood, a fact which has been considered to be in part responsible of the degenerative changes observed in aged individuals. Accordingly, the GH replacement therapy has successfully used in rats to revert many biochemical, histological and anatomical traits associated to old age, improving skin structure, vascular endothelia, immune system and causing a powerful neuronal rescue after hipoxy-ischemic lesions in the hippocampus of rats older than 20 months. However, the functional consequences of the GH replacement remain largely unexplored.

The present reports aims at recording the effect of acute and chronic GH administration (2 mg/k b.w/24 h) in the learning and motor capacity of old (24 months) and young (12 weeks) Wistar rats. Young and old saline controls were also studied. All animals were tested for place learning in a 8 arms radial maze and for motor coordination in a rota-rod. Perfomance was tested just after the first GH injection, to record acute effects of GH and after four weeks of GH administration to record the chronic effects.

Both acute and chronic injections of GH caused significant effects in young and old animals, although the maximal effects were observed after chronic GH administration in old animals which reached performance levels similar to those of young injected with saline. The results confirm the usefulness of GH replacement to recover the age associated behavioural deficits observed in cognitive and motor capabilities.

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HABITUAL CHARACTERISTICS OF AUDITORY GO-P3 AND NOGO-P3 RESPONSES ARE DIFFERENT

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The event-related potentials (ERPs) are voltage changes recorded from the human scalp that is time-locked to a sensory, motor, or cognitive process. Go-NoGo task has been widely used to evaluate response activation and inhibition in normal subjects as well as patients with neuro-psychiatric disorders. NoGo-P3 was thought to be related to response inhibition associated with frontal cortex activity. Accordingly, the NoGo-P3 was found maximal at the fronto-central sites, whereas Go-P3 was found maximal at the centro-parietal sites. The aim of the present study was to investigate habituation of event related potential responses to auditory Go and NoGo stimuli. Thirty-eight healthy male volunteers (ages between 18 and 23 years) participated in the study. ERPs were recorded with 30 electrode sites (international 10/20 system) using an auditory Go-NoGo paradigm. Go tones (1000 Hz) and NoGo tones (2000 Hz) with 50% probabilities were binaurally presented by headphones at 70 dB SPL. The tones were presented in a random series with interstimulus intervals (ISI) of 2 s. The low frequency tones were the target (Go stimuli), and subjects were required to make a button press response to each target with the right index finger. Obtained EEG data from the responses to auditory stimuli in the first and the last half of the task were averaged separately. The amplitudes and latencies of the ERP responses to Go and NoGo stimuli were measured for each task period. The differences between two periods were analyzed by repeated measures analyses of variance (ANOVA). The statistical analyses of the present study show that the NoGo-P3 potential amplitudes were significantly lower in the last half of the task compared to the first half of the task at all leads ($p<0.01$) while Go-P3 potential amplitudes were not significantly different between the two periods ($p>0.05$). Also, interaction of the two periods of the task and antero-posterior distribution of the NoGo-P3 potential amplitudes was significant: decrease of the amplitude of NoGo-P3 potential at the fronto-central areas was bigger than the parietal area at the last half of the task compared to the first half of the task ($p<0.05$). There were no significant differences in latency and amplitude values of N1, P2 and N2 potentials response to auditory Go and NoGo stimuli between the first and the last half of the task ($p>0.005$). Our results indicate that the NoGo-P3 potential was habituated whereas the Go-P3 potential did not undergo any habitual changes.

ARRESTIN IN *DROSOPHILA* PHOTOTRANSDUCTION

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The phototransduction process of animal photoreceptors starts with the absorption of light by visual pigment, rhodopsin, resulting in the transformation of the rhodopsin into an active form, metarhodopsin. After several biochemical steps, this results in a quantum bump, a transient change in the membrane voltage. The life time of the metarhodopsin state, which triggers the phototransduction chain, determines the temporal resolution of the visual process. Metarhodopsin life time depends on the binding of arrestin molecules, which inactivate (arrest) the active metarhodopsin state. The arrestin concentration and its binding constant thus are crucial factors determining the light sensitivity, frequency response as well as the speed of light-adaptation of the photoreceptors.

We have studied the wavelength, intensity and arrestin dependence of inactivation of photoreceptors of white-eyed wild-type *Drosophila* and the hypomorphic arrestin mutant ($w^{-};arr2^{3}$) by simultaneously measuring visual pigment conversions, via metarhodopsin fluorescence, and the elicited electrophysiological responses, via the electroretinogram (ERG). We have implemented the measured data in a kinetic model of the rhodopsin-arrestin cycle, allowing us to estimate the active metarhodopsin as a function of light intensity. Arrestin reduction in the mutant increased the light sensitivity by a factor of 3.5. We present a steady-state stochastic model that quantitatively explains the different dependencies on light intensity of the prolonged depolarizing afterpotentials (PDA) in the wild type and the hypomorphic mutant. We discuss the feasibility of different experimental methods for the estimation of the PDA and arrestin content.

DOSE DEPENDENT NICOTINE EFFECTS ON BRAIN EXCITABILITY IN IMMATURE RATS

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The aim of our study was to test the hypothesis, that nicotine treatment (in dose of 1 mg/kg or 0.75 mg/kg respectively) can influence brain susceptibility to seizures in young immature rats. Experiments were performed on freely moving 12- and 25-day-old male Wistar rats. 15 minutes after nicotine i.p. administration, cortical afterdischarges (ADs) were elicited by six-time repeated stimulation of the right sensorimotor cortex. The duration of evoked ADs and the shape of evoked graphoelements were monitored. Administration of nicotine (both doses) resulted in significantly longer ADs duration in 12-day-old rats after the first stimulations compared to control group. There was a significant shortening after the 3rd stimulation (higher nicotine dose), after the 4th stimulation (lower nicotine dose), after the 5th stimulation (both doses) and after the 6th stimulation (lower dose of nicotine). Analysis of ADs duration in 25-day-old animals revealed the prolongation of ADs duration after the 2nd stimulation (lower dose) and after the 3rd stimulation (higher dose of nicotine). The results revealed that nicotine modulates brain excitability in age and dose dependent manner.

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OTOFERLIN, A SYNAPTOTAGMIN-LIKE CALCIUM SENSOR?

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Otoferlin, a multi C_2 domain protein shown to be essential for a late step in exocytosis of the auditory hair cells, is currently discussed to replace synaptotagmin (syt) at this excitatory synapse. This hypothesis is based on (i) otoferlin having 6 or 7 C_2 domains, of which 3 are predicted to bind Ca^{2+}, (ii) the absence of syt 1, 2 and 3 at the first auditory synapse (Safieddine and Wenthold, 1999), (iii) the interaction of otoferlin with syntaxin 1 and SNAP-25 in immunoprecipitation assays and (iv) the absence of fast vesicle release in $Otof^{-/-}$ hair cells (Roux et al, 2006).
In this study, we transduced auditory inner hair cells of $Otof^{-/-}$ mice with syt 1 and tested exocytosis by patch-clamp capacitance measurements, but could not restore Ca^{2+}-triggered exocytosis. Next, we transfected the developing otocysts of $Otof^{-/-}$ embryos at E12 with syt 1 and measured hearing by auditory brainstem response in 3 week old animals. In comparison to the untransfected ear, no difference in hearing could be detected. Further, we analyzed exocytosis in autaptic cultures of syt1-deficient hippocampal neurons, but found no effect when overexpressing otoferlin.
Together, this study suggests that the mechanism of otoferlin function is different from syt action.

FUNCTION OF PRESTIN AS A BICARBONATE-CHLORIDE ANTIPORTER

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The lateral membrane of mammalian cochlear outer hair cells contains a membrane protein, prestin, which can act as a fast actuator driving electromotility and ultimately cochlear amplification. On the basis of its amino-acid sequence, prestin (SLC26A5) belongs to the solute carrier 26 family of transporters which exchange halides for SO_4^{2-} or HCO_3^-. The original electrophysiological analysis of mammalian prestin (Oliver et al. 2001) suggested that such transport functions are minimal; more recent experiments with radioactively labeled substrates also failed to identify a significant HCO_3^- transport (Bai et al. 2009). We have employed sensitive intracellular pH fluorescence probes as an alternative approach to assess the possibility that prestin transports HCO_3^-. A DNA coding sequence of super-ecliptic pHluorin, a pH sensitive variant of GFP, was attached to the C-terminus of prestin and the resulting DNA construct overexpressed in the CHO cell line. As a control for endogenous transport, pHluorin was targeted to the membrane intracellularly using a myristilation-targeting peptide. The experimental data indicate that in the presence of extracellular HCO_3^- the intracellular pH recovers from the CO_2-induced acidification 4 times faster in cells transfected with prestin. This acceleration requires low (4 mM) extracellular Cl^- consistent with prestin transporting HCO_3^- intracellularly in exchange for Cl^-. The process was significantly reduced by extracellular application of 10 mM salicylate, confirming the prestin specificity. As a pHluorin-independent assay, recovery (i.e. HCO_3^- loading) was also only found in those cells expressing prestin using BCECF as a cytoplasmic pH probe. Preliminary quantitative modelling of this system produce pH time courses that mirror the experimental data under reasonable assumptions about the appropriate rate constants. These data therefore suggest that prestin can act as a weak HCO_3^-/ Cl^- antiporter although the effects are anticipated to be greater in OHCs than in expression systems due the higher prestin copy number.

SATURDAY, 14. NOVEMBER 2009:

KEYNOTE LECTURE SATURDAY

STRUCTURE-FUNCTION RELATIONSHIP OF THE ENDOCRINE PANCREAS AT A GLANCE

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Blood glucose homeostasis is controlled by a well-concerted secretion and action of pancreatic hormones from the various cell types in the endocrine pancreas, the islets of Langerhans. The secretion of insulin, glucagon and somatostatin from the pancreatic β-cells, α-cells and δ-cells, respectively, is regulated very precisely by nutrients as well as by autocrine, endocrine, paracrine and nervous signals. The islet of Langerhans has an important role in integrating these various signals. If hormone release is disturbed diabetes develops. The molecular mechanisms involved in the regulation of insulin secretion from the β-cell are relatively well understood and involve an exocytotic apparatus that in principle resembles the well studied presynaptic machinery mediating exocytosis from synaptic vesicles in neurons. Much less is known about the detailed mechanisms regulating exocytosis of glucagon from the α-cell and somatostatin from the δ-cell. Whereas there is a wealth of information about the physiology of rodent islets *in vitro*, the biology of the human islet both *in vitro* and *in vivo* remains poorly understood. Our studies have suggested that the human pancreatic islet has a unique structure-function relationship. I will discuss a systems biology approach where signal-transduction can be investigated in innervated and vascularized human pancreatic islets *in vivo* at single cell resolution. This will allow us to define eventually the molecular machinery involved in regulating hormonal output from the human endocrine pancreas and thereby maintenance of adequate glucose homeostasis.

SATURDAY, 14. NOVEMBER 2009:

SESSION IX

SKIN PERFUSION AND HUMAN HEAT EXCHANGE: HOW, WHY AND USE

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Cold is a vasoconstrictor, while heat is a vasodilator. In general, these statements hold for skin perfusion. However, the variation in circumstances, between skin sites and between individuals is large. Moreover, the regulation of blood perfusion is complex, making understanding the relation between heat exchange and skin perfusion an appealing task. Approximately 90 % of our heat production is lost via the skin, the body heat mainly transported to the skin by the blood. Therefore, it is remarkable that relatively little attention is given to skin blood flow and its regulation in human physiological metabolic studies and in physiological models of thermoregulation.
Metabolic studies in general measure energy expenditure by means of indirect calorimetry. In a small subset of studies skin and core temperatures are being measured simultaneously, but skin blood flow measurements of different skin types are rarely taken into account.
This overview deals with those studies that combine heat production and heat loss with special attention to individual variation. Studies range from those using direct and indirect calorimetry to those measuring local skin perfusion. The different techniques will be discussed briefly.
Special attention will be given to whole body and local cooling in young compared to elderly and between different skin sites (glabrous versus nonglabrous skin). During cold exposure, a decrease in skin perfusion is the first line of defense in human thermoregulation. Control of skin perfusion is via both central and local mechanisms. Generally both mechanisms act simultaneously. With increase in age there is an attenuation of the cutaneous vasoconstrictor (VC) response to whole body cooling, predisposing the older population to risk of hypothermia. Innervation and topology of cutaneous microcirculation is not homogenous. For instance, glabrous skin (palms, soles, lips) is relatively well adapted for regulating heat loss. Moreover, the hands and the face are the areas of the skin that are actually exposed to the environment, making them the primary heat exchangers of the human body. Therefore, studies will be presented on local and reflex mediated mechanisms of vasoconstriction in whole body and local cooling at glabrous and non-glabrous skin sites in male young adults and elderly.
Finally, we describe a human thermophysiology model in which the regulation of blood perfusion will be incorporated.

LOCAL REGULATION OF HUMAN CUTANEOUS MICROCIRCULATION: IMPACT OF ENDOTHELIUM-DEPENDENT VASODILATATION

Helena Lenasi

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In recent years, the cutaneous microcirculation has gained increasing interest as it represents an easily accessible and potentially representative vascular bed to examine the mechanisms of microcirculatory function and dysfunction. Apart from maintaining nutrition to the skin tissues, it is decisively engaged in thermoregulation. Although the quantitative measurement of skin blood flow (SkBF) remains a problem, rough estimates of SkBF range between 200 to 500 ml/l at rest and may attain up to 7-8 l/min during maximal vasodilatation. It is obvious that the regulation of SkBF is a complicated phenomenon; SkBF is regulated by a complex interplay of centrally mediated neural mechanisms as well as by local humoral and metabolic factors.

The endothelium plays a crucial role by releasing a number of vasodilators and vasoconstrictors in response to different physical (shear stress, pulsatility) and pharmacological stimuli. The most investigated vasodilators are nitric oxide, prostaglandins and endothelium-derived hyperpolarizing factor. In spite of several investigations, the exact interplay of these mechanisms is still not well defined, specially not in human skin microcirculation. The phenomenon is more complicated by the fact that the contribution of each of these mediators to the endothelium-dependent vasodilatation varies depending on the measuring site (glabrous vs. nonglabrous area) as well as on the agonist used to stimulate the endothelium. Elucidating the role of endothelial vasodilators in human skin microcirculation is important also from the clinical point of view as early detectable endothelial dysfunction might precede clinical manifestation of the disease states. On the other hand, it has been shown that endurance training leads to an enhancement of endothelium-dependent vasodilatation in human microcirculation.

Using laser Doppler flowmetry, cutaneous microvascular responses to physiological and pharmacological stimuli are currently being investigated as indices of vascular function. The stimuli mostly used include postocclusive reactive hyperemia, whole body heating and/or cooling, local heating/cooling, and the application of specific pharmacologic agents by iontophoresis, intradermal microdialysis or microinjection. The unpleasant limitation of studying human skin microcirculation is the demand of noninvasiveness. The overview will present some new insights in the physiology and pharmacology of skin microcirculation with emphasis on endothelial function as well as potentially beneficial adaptations of skin micorcirculation to exercise.

SKIN VASOMOTION INVESTIGATION IN DIFFERENT HUMAN PATHOLOGICAL CONDITIONS

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Skin vasomotion is the rhythmic variation of skin microvessel diameter responsible for skin microcirculatory blood flow oscillation, the so called blood flowmotion. Experimental and clinical findings suggest that vasomotion depends on different mechanisms, such as the endothelial activity, the spontaneous myogenic activity of the microvascular wall and the local sympathetic activity. Since some skin LDF oscillatory components reflect skin vasomotion, we can indirectly investigate this microvascular behavior in humans by means of the spectral analysis of skin laser Doppler flowmetry (LDF) tracing. In particular, it has been demonstrated that LDF oscillations in the frequency range of 0.01-0.02 Hz, 0.02-0.06 Hz and 0.006-0.2 Hz are respectively related to the endothelial-dependent, the sympathetic-dependent and the myogenic-dependent vasomotion. The spectral amplitude of each of these LDF oscillations has been suggested to reflect the efficiency of the corresponding vasomotion mechanism. Several studies have been performed using this method in patients with different pathological conditions, such as peripheral obstructive arterial disease, arterial hypertension, chronic renal failure, hypercholesterolemia, diabetes or systemic sclerosis. When analyzed with tests such as skin post-ischemic hyperaemia or acetylcholine iontophoresis, these pathological conditions resulted to be characterized by a perturbed skin vasomotion. The results of these studies will be presented and their usefulness in understanding the pathophysiology of the investigated diseases, will be discussed.

SKIN MICROCIRCULATION IN THE UPPER AND LOWER EXTREMITIES OF DIABETIC PATIENTS WITH AND WITHOUT LARGE VESSEL DISEASE

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Background and aims: Diabetic foot problems are among the most serious consequences of diabetes mellitus. Lower limb amputations in diabetic patients represent almost 50% of all non-traumatic lower extremity amputations. Upper extremities are affected much less frequently than the lower. While the role of peripheral obliterative macroangiopathy in the pathogenesis of diabetic gangrene is well established, the role of microangiopathy is less equivocal. The rhythmic variations of skin microvascular blood flow reflect the influence of heartbeat, respiration, intrinsic myogenic activity, neurogenic factors and endothelial activity. The aim of our study was to test the hypothesis that basal skin blood flow (BSBF) and its dynamic components differ 1) among diabetic patients and non-diabetic control subjects without large vessel disease and with it, and 2) among the upper and lower extremities.
Patients and methods: 69 diabetic patients (D) and 78 control subjects (C) entered the study. Peripheral arterial obliterative disease (PAOD) was defined according to ankle/brachial index (ABI). All subjects were classified into three groups: 1 (PAOD +): ABI< 0.9 (21 D, 16 C); 2 (PAOD -): ABI 0.91 – 1.3 (43 D, 36 C); and 3 (mediocalcinosis): ABI > 1.3 (5 D, 26 C). BSBF at 4 recording sites with predominantly nutritive capillary circulation (right and left caput ulnae, right and left medial malleolus) was measured by laser Doppler flowmetry (LDF). The oscillatory components of the LDF signal were analyzed by wavelet transform.
Results: In absolute terms, mean flow at all recording sites was highest in group 2, but the differences among the groups were not statistically significant. The values in the upper extremities were significantly higher than in the lower, except for groups D3 and C1. In addition, significant difference was found between the two arms in D2 and between the two legs in C1 (Table 1). In D, the spectral component of microvascular flow associated with endothelial activity was in significant positive correlation with systolic pressures on brachial and dorsal pedal artery (p=0.001 and 0.010, respectively).
Conclusion: Our results indicate that mean BSBF and its oscillatory components do not change with diabetic PAOD; however there is a strong correlation between systolic pressure and the oscillatory components of BSBF related to endothelial activity manifested in the frequency interval 0.0095 – 0.02 Hz. The differences between the upper and lower extremities provide a possible explanation for the higher prevalence of ulceration and gangrene on the lower extremities in comparison to the upper.

Table 1: Mean flow at 4 recording sites in diabetic patients and control subjects.

	N	F1 R arm	F1/F2	F2 R leg	F1/F3	F3 L arm	F3/F4	F4 L leg	F2/F4
D	69	36.33 ± 29.93	0.000	18.03 ± 11.13	ns	31.89 ± 25.75	0.000	16.59 ± 11.93	ns
1	21	31.13 ± 18.00	0.001	17.66 ± 8.86	ns	34.45 ± 29.85	0.011	15.00 ± 8.04	ns
2	43	40.1 ± 34.99	0.000	18.43 ± 12.42	0.027	31.1 ± 24.02	0.000	17.57 ± 13,63	ns
3	5	25.64 ± 17.50	ns	16.11 ± 9.17	ns	27.92 ± 26.38	ns	14.82 ± 10.78	ns
C	78	45.4 ± 34.95	0.000	23.6 ± 19.47	ns	48.62 ± 38.52	0.000	24.77 ± 17.15	ns
1	16	37.34 ± 25.68	ns	17.6 ± 10.63	ns	37.94 ± 23.01	ns	25.53 ± 19.43	0.004
2	36	47.64 ± 37.50	0.000	23.97 ± 19.81	ns	53.3 ± 46.03	0.000	24.11 ± 16.41	ns
3	26	47.27 ± 36.68	0.001	26.76 ± 22.73	ns	48.7 ± 34.38	0.001	25.20 ± 17.37	ns

PATHOPHYSIOLOGY OF EXPERIMENTAL SPINAL INJURY *IN VITRO*

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Spinal cord injuries are a major source of persistent disability with limited chance of full recovery. It would, therefore, be important to understand the initial mechanisms underlying spinal cord injury to protect patients from damage intensification. In fact, acute spinal cord injury evolves rapidly to produce secondary damage even to initially spared areas with consequential loss of locomotion. Regardless of their aetiology (trauma, vascular or cancer origin, etc), spinal lesions are thought to include the combined effects of excitotoxicity and stroke-like metabolic perturbations. To clarify the relative contribution by excitotoxicity and toxic metabolites to dysfunction of locomotor networks, we used, as a model, the in vitro thoraco-lumbar spinal cord of the neonatal rat treated (1 h) with either kainate (a powerful glutamate receptor agonist) or a pathological medium (containing free radicals and hypoxic/aglycemic conditions), or their combination. After washout, electrophysiological responses were monitored for 24 h and cell damage analyzed histologically. While kainate suppressed fictive locomotion irreversibly, intrinsic network bursting induced by synaptic inhibition block persisted. This result was associated with significant neuronal loss around the central canal and the ventral grey matter. Comparatively less damage was found in the white matter. Combining kainate with the pathological medium evoked extensive, irreversible damage to the spinal cord and no electrophysiological response. The pathological medium alone slowed down fictive locomotion and intrinsic bursting: these oscillatory patterns, however, remained throughout without regaining their control properties. This phenomenon was associated with depression of synaptic transmission and preferential damage to glial cells, while neurons were comparatively spared. Our model suggests distinct roles of excitotoxicity and metabolic dysfunction in the damage of locomotor networks, indicating that different strategies might be necessary to treat the early pathological processes involved in the acute spinal cord lesion.

CELLULAR AND NETWORK MECHANISMS OF RHYTHM GENERATION IN SPINAL CORD CIRCUITS

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The generation of rhythmic electrical activity is a prominent feature of spinal cord circuits that is used for locomotion and also for circuit refinement during development. The mechanisms involved in rhythm generation in spinal cord networks are not fully understood. It is for example not known whether spinal cord rhythms are driven by pacemaker neurons and if yes, which neurons are involved in this function. We studied the mechanisms involved in rhythm generation in slice cultures from fetal rats that were grown on multielectrode arrays (MEAs). We combined multisite extracellular recordings from the MEA electrodes with intracellular patch clamp recordings from single neurons. We found that spatially restricted oscillations of activity appeared in most of the cultures spontaneously. Such activity was based on intrinsic activity in a percentage of the neurons that could activate the spinal networks through recurrent excitation. The local oscillator networks critically involved NMDA, AMPA and GABA / glycine receptors at subsequent phases of the oscillation cycle. Intrinsic spiking in individual neurons (in the absence of functional synaptic coupling) was based on persistent sodium currents. Intrinsic firing as well as persistent sodium currents were increased by 5-HT through 5-HT2 receptors. Comparing neuronal activity to muscle activity in co-cultures of spinal cord slices with muscle fibers we found that a percentage of the intrinsically spiking neurons were motoneurons. These motoneurons were electrically coupled among each other and they could drive the spinal networks through cholinergic recurrent excitation. These findings open the possibility that during development rhythmic activity in motoneurons is not only involved in circuit refinement downstream at the neuromuscular endplates but also upstream at the level of spinal cord circuits.

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TEMPORAL EVOLUTION OF SPONTANEOUS Ca^{2+} SIGNALS GENERATED BY VENTRAL NEURONS IS A MARKER OF SPINAL CORD MATURATION IN VITRO

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Developing spinal networks undergo dramatic structural and/or functional rearrangements to achieve the maturation of motor control. Intracellular calcium changes are crucial signals in spinal network development, since transient elevations of intracellular Ca^{2+} might detail the emergence of specific phenotypes or guide the formation of cellular connectivity. Embryonic spinal neurons maintained in organotypic slice culture are known to mimic certain maturation-dependent signaling changes. Organoptypic spinal slices recapitulate during in vitro growth many molecular and physiological events of spinal networks formation in vivo, therefore they are ideally suited to investigate the dynamic of intracellular calcium signaling in clusters of neurons during their maturation. With such a model we investigated, in embryonic mouse spinal segments, the age-dependent spatio-temporal control of intracellular Ca^{2+} signaling generated by neuronal populations in ventral circuits and its relation with electrical activity. We used Ca^{2+} imaging to monitor areas located within the ventral spinal horn at 1 and 2 weeks of in vitro growth. Primitive patterns of spontaneous neuronal Ca^{2+} transients (detected at 1 week) were typically synchronous. Remarkably, such transients originated from widespread propagating waves that became organized into large scale rhythmic bursts. These activities were associated with the generation of synaptically-mediated inward currents under whole cell patch clamp. Such patterns disappeared during longer culture of spinal segments: at 2 weeks in culture, only a subset of ventral neurons displayed spontaneous, asynchronous and repetitive Ca^{2+} oscillations dissociated from background synaptic activity. We observed that the emergence of oscillations was a restricted phenomenon arising together with the transformation of ventral network electrophysiological bursting into asynchronous synaptic discharges. This change was accompanied by the appearance of discrete calbindin immunoreactivity against an unchanged background of calretinin positive cells. It is attractive to assume that periodic oscillations of Ca^{2+} confer a summative ability to these cells to shape the plasticity of local circuits through different changes (phasic or tonic) in intracellular Ca^{2+}.

EXPLORING THE EARLY DAMAGE TO THE LOCOMOTOR CIRCUITS WITH AN IN VITRO MODEL OF ACUTE SPINAL CORD INJURY

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In the vast majority of injuries to the spinal cord, the neuronal circuits responsible for locomotion, located in the lumbar region, are not directly impaired by the primary damage although they may result more or less completely disconnected from the supraspinal centers due to the severance of the fibers passing across the lesion site.
In the present study, we have investigated how the lumbar locomotor networks are affected by a focal lesion involving only few spinal segments above.
For this purpose, we applied a toxic solution (recently developed in our laboratory) that mimics the principal factors responsible for the secondary injury and that we named pathological medium plus kainate (indicated as PM + KA).
We have reported that PM + KA, if applied to the whole spinal cord, is able to reproduce the histological and functional outcomes of a spinal cord lesion. The functional consequences are monitored for up to 24 hours by recording from ventral roots the reflex responses as well as the presence of locomotor-like oscillatory cycles. At the end of the experiments, samples were fixed for histological analysis.
Preliminary experiments confirm that the experimental procedures needed for limiting the lesion did not damage spinal cord activity.
In the current experiments, while PM + KA (applied focally) acted on only few segments located in the thoracic region, spinal neurons in remote segments showed simultaneous depolarization that eventually faded away to pre treatment baseline.
The efficacy of synaptic transmission was recorded above, within and below the lesion site, while the activity of locomotor circuits in the lumbar portion was measured by eliciting a fictive locomotion rhythm with neurochemicals or trains of repetitive stimuli to dorsal root afferents.
A limited number of surviving axons crossing the injured portions could still functionally connect segments above and below the lesion with appropriate electrical stimuli.
After the lesioning procedures, the operativity of lumbar networks, despite the minimal cell damage to the areas not directly exposed to the toxic, was impaired: chemically and electrically induced fictive locomotion was differentially affected by the lesion.
In fact, while the alternated oscillations elicited by neurochemicals, after a transient early suppression, could reappear following extensive wash out, the dorsal root elicited ones were completely and irreversibly lost.
The amplitude of the cumulative depolarization evoked by trains of repetitive stimuli was comparable for both post-lesional and control preparations, but responses summed up at a significantly lower speed, that was unable to trigger alternating oscillations.
The present study casts light on the very first events that alter the locomotor circuits following an experimental spinal cord injury.
The present data might have relevant implications for neurorehabilitation targeted to exploit the residual automatic capacity of the lumbar networks to gain functional benefits for spinal cord injured persons.

VASOPRESSIN RECEPTOR-MEDIATED CALCIUM SIGNALS AND PEPTIDE RELEASE IN THE SUPRAOPTIC NUCLEUS NEURONS: CONTRADICTIONS AND COMPROMISES

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Vasopressin (AVP) and oxytocin (OT) magnocellular neurons of the hypothalamic supraoptic nuclei autocontrol their electrical activity through the somatodendritic release of their respective peptides. These neurons belong to specific networks, respond to distinct physiological stimuli, display highly characteristic electrical activities and influence the release of AVP and OT at axon terminals in the neurohypophysis as well. The membrane mechanisms supporting AVP and OT action on the electrical activity of SON neurons involve specific autoreceptors and induce an increase in $[Ca^{2+}]_i$ in these cells. AVP involves the phospholipase C and adenylate cyclase intracellular pathways that are activated, in the periphery, by V1 (V1a and V1b)- and V2-type vasopressin receptors, respectively. In AVP neurons, $[Ca^{2+}]_i$ measurements in response to V1a and V2 agonists and antagonists revealed the functional expression of these two types of receptors, but V2-type mRNA is not detectable within AVP cells. While investigating the intracellular messengers, using various second messenger pathway activators and inhibitors, involved in the $[Ca^{2+}]_i$ response to V1a and V2 agonists, $[Ca^{2+}]_i$ measurements and AVP release experiments revealed the activation in hypothalamic supraoptic neurons of V1a receptors involved in both the phospholipase C and the adenylate cyclase pathways. Similarly, both signal transduction pathways are involved following V2 receptor activation, suggesting the functional expression of these two types of receptors in AVP neurons. Together, the physiological responses and pharmacological profiles of AVP receptors in the SON neurons are atypical and do not fit with what has been described in the peripheral system. Therefore, there is a need to further characterize the AVP receptors using newly developed tools to understand the physiology of AVP receptors in the CNS neurons.

FUNCTIONAL CONSEQUENCES OF VASOPRESSIN AND CORTICOLIBERIN RECEPTORS CO-EXPRESSION IN NATIVE AND HETEROLOGOUS MODELS

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In mammals, Vasopressin (VP) and corticoliberin (CRF) co-regulate ACTH and insulin release by acting in synergism at the pituitary and pancreas levels respectively. Using a new selective fluorescent V1b analogue and a selective anti-CRHR1 antibody, we demonstrate that such synergism implies V1b and CRHR1 receptors co-localization. Yet, the molecular mechanisms involved remain partially unknown.

We first extended the VP and CRF synergism to the bovine adrenal chromaffin gland. We showed that VP and CRF both stimulate catecholamines secretion and also act in synergism. Such potentiation may be related to modifications of second messenger cascade. Thus, we observed a clear potentiation of VP-stimulated Inositol Phosphates (IPs) accumulation by CRF and a weak but significant effect of VP on CRF-stimulated cAMP production.

To go further in these mechanisms, we transfected HEK293 cells with functional tagged V1b and CRHR1 receptors. We first demonstrated by BRET and co-immunoprecipitation experiments that these 2 receptors heterodimerized in living cells. Then, we explored the potential modifications of V1b and CRHR1 receptors pharmacology upon receptor co-transfection. We found that CRF might alter VP binding at high doses. More interestingly, we showed that VP potentiated the CRF-stimulated cAMP accumulation. This effect was dose- dependent, receptor-mediated and partially due to PKC activation. Yet, 25% of this potentiation effect was insensitive to a full PLC antagonist suggesting another mechanism of synergy. CRF may also significantly potentiate VP-stimulated IPs accumulation.

In conclusion, we show that VP/CRF potentiation seems to be a general phenomenon in native tissues. We also bring evidence that, beside second messengers crosstalk, heterodimerization of V1b/CRHR1 receptors may also be involved in such a phenomenom.

OXYTOCIN RECEPTOR COUPLING TO DIFFERENT G-PROTEINS: ROLE IN RECEPTOR TRAFFICKING

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As in the case of most G-protein coupled receptors (GPCRs), agonist stimulation of human oxytocin receptors (OTRs) leads to desensitization and internalization. We examined OTR internalization in HEK293T cells where, upon agonist activation, the receptors were almost completely sequestered inside intracellular compartments. Binding and fluorescence assays showed that almost 85% of the internalized receptors returned to the cell surface after four hours, by which time cell responsiveness to the agonist was completely restored. Finally, investigations of receptor recycling pathways showed that OTRs were located in vesicles containing the Rab5 and Rab4 small GTPases (markers of the "short cycle"), whereas there was no co-localization with Rab11 (a marker of the "long cycle") or Rab7 (a marker of vesicles directed to endosomal/lysosomal compartments). Taken together, these data indicate that OTRs are capable of very efficient and complete resensitization due to receptor recycling via the "short cycle".

The oxytocin receptor (OTR) is a promiscuous G-protein coupled receptor that couples to both Gαq and Gαi and whose stimulation leads to the activation of different intracellular signaling pathways. OT –derived peptides that activate selectively either the Gαi or Gαq pathways were characterized in our laboratory (Reversi et al 2005 J Biol Chem). In order to assay the capability of these analogs to promote receptor internalization we developed two fluorescent agonists: dLVT-Alexa568 and atosiban-Alexa568). After stimulation of the cells with dLVT-Alexa568, that promotes both OTR/Gαq and OTR/Gαi coupling, we observed internalization of both the receptor and the fluorescent peptide. The two molecules were initially colocalized in vesicles, but this colocalization was lost after two hours when the receptor and the ligand were found in different vesicles, suggesting that they followed different trafficking pathways. On the contrary, atosiban-Alexa568, a selective OTR/Gαi, did not lead to any change in receptor localization at the plasma membrane even after one hour of stimulation. Since OTRs are expressed in the CNS in neuronal and glial cells, we finally used a neuronal cell line (the mouse neuroblastoma Neuro2A cells) transiently tranfected with human OTR to further investigate OTR trafficking. Both the OTR-EGPF and OTR tagged at its N-terminus with an HA epitope were localized at the plasma membrane as expected, but, again, receptor internalization was observed only after stimulation with dLVT-Alexa568. All together, these data suggest that Gαq activation plays an important role in OTR internalization.

NOVEL OXYTOCIN RECEPTOR-LINKED SIGNALING NETWORKS.

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The oxytocin receptor (OTR) is a Gq-coupled GPCR that fulfills central and peripheral functions, including myometrial contractions at labor. The great diversity of the expression sites and proposed functions of the OTR is paralleled by a diversity of its signaling pathways, many of which have still remained unexplored.
By means of a phosphoproteomics approach, we have detected that OTR stimulation induces activation of eukaryotic translation factor eEF2, a key regulator of protein synthesis, thus defining a novel mechanism by which OT assumes a trophic function. We found that this effect is not mediated by any of the known pathways known to induce eEF2 dephosphorylation (mTOR, ERK1/2 or p38) but by protein kinase C.
Using phosphoERK antibodies, we discovered that OTR stimulation induced not only ERK1/2 activation but also phosphorylation of "big MAP kinase1" or ERK5. ERK5 is part of a distinct MAPK cascade, promotes expression of the myosin light chain gene and plays an obligatory role in muscle cell development. ERK5 knock down by specific siRNAs led to a significant reduction in oxytocin (OT)-induced COX-2 expression, prostaglandin F2alpha secretion and myometrial contraction, indicating that ERK5 is an essential mediator of OT-mediated prostaglandin secretion and contractions. Preliminary evidence indicates that ERK5 activation involves both G protein-dependent and –independent pathways.
The OTR interacts also with other GPCRs co-expressed in myometrial cells. Heterodimerization with the β2 adrenergic receptor (β2AR) was supported by BRET experiments. We also obtained evidence that this interaction has functional consequences: β2AR antagonists are able to modulate allosterically OTR signalling and OTR trafficking and, vice versa, an OT antagonist can modulate β2AR signalling.

MOLECULAR MECHANISMS OF THE FAT AND MUSCLE CROSSTALK

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Chemerin is a newly described adipokine that affects adipogenesis and glucose homeostasis in adipocytes and increases with BMI in humans. This study aimed at investigating the regulation of chemerin release and its effects on glucose metabolism in skeletal muscle cells.
Human skeletal muscle cells were treated with chemerin to study its effects on insulin signalling, glucose uptake and activation of stress kinases. The release of chemerin was analyzed in human adipocytes and adipose tissue explants from lean and obese patients.
Chemerin induces insulin resistance in human skeletal muscle cells at the level of Akt and GSK3 phosphorylation and glucose uptake. Furthermore, chemerin activates p38 MAPK, NF-κB and ERK1/2. Inhibition of ERK partially prevents chemerin-induced insulin resistance pointing to participation of this pathway in chemerin action. Human adipocytes express chemerin and CMKLR1 differentiation-dependently. Fully differentiated fat cells secrete chemerin (15 ng/ml from 10^6 cells). This process is slightly but significantly increased by TNFα and markedly inhibited by over 80 % by PPARγ activation. Adipose tissue explants from obese patients are characterized by significantly higher chemerin secretion as compared to lean controls (21 ng and 8 ng from 10^7 cells, respectively). Chemerin release is correlated with BMI, waist-hip-ratio and adipocyte volume. Furthermore, higher chemerin release is associated with insulin resistance at the level of lipogenesis and insulin-induced antilipolysis in adipocytes.
Adipocyte-derived secretion of chemerin may be involved in the negative crosstalk between adipose tissue and skeletal muscle contributing to the negative relationship between obesity and insulin sensitivity. A novel role might be assigned to chemerin in glucose and lipid metabolism in both adipose tissue and skeletal muscle.

BRIDGING MITOCHONDRIAL DYNAMICS AND METABOLISM IN MUSCLE

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Eukaryotic cells exhibit the operation of mitochondrial fusion and fission processes, which are catalyzed by distinct protein machineries. Mitochondrial fusion requires the activity of mitofusin-1 (Mfn1), mitofusin-2 (Mfn2) and OPA1, and mitochondrial fission depends on Drp1, and Fis1. Some of these proteins are mutated in certain human diseases, and it is likely that other proteins may participate in those processes.
The mitochondrial fusion protein Mfn2 has been reported to show additional cellular functions. More specifically, changes in Mfn2 in nonmuscle and muscle cells modulate the capacity of cells to oxidize substrates (glucose, pyruvate, fatty acids), and in parallel modulate the expression of subunits of the OXPHOS system.
Recent evidence indicated that proteins participating in mitochondrial fusion or fission also participate in metabolism. The mitochondrial fusion protein mitofusin 2 (Mfn2) stimulates respiration, substrate oxidation and OXPHOS subunits expression in cultured cells. In this regard, skeletal muscle of obese subjects and of type 2 diabetic patients shows reduced Mfn2 expression. Therefore, alterations in the activity of proteins involved in mitochondrial dynamics, and particularly Mfn2, may participate in the reduced mitochondrial function present in skeletal muscle in obesity and type 2 diabetes. Mfn2 expression in skeletal muscle is subject to regulation and conditions characterized by reduced mitochondrial activity, such as obesity or type 2 diabetes, are associated with repressed *Mfn2*. In contrast, cold-exposure, treatment with β_3-adrenergic agonists or exercise induce the expression of this gene in muscle. ERRα transcription factor is a key regulator of Mfn2 transcription and recruits nuclear co-activators PGC-1β and PGC-1α. These two nuclear co-activators are potent positive regulators of Mfn2 expression in muscle cells, and ablation of PGC-1β causes Mfn2 down-regulation in skeletal muscle and in heart. We propose that PGC-1β is a regulator of normal expression of Mfn2 in muscle, whereas PGC-1α participates in the stimulation of Mfn2 expression under a variety of conditions characterized by enhanced energy expenditure.

PIKfyve REGULATES GLUCOSE UPTAKE IN SKELETAL MUSCLES

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It is widely assumed that PI 3-kinase is not involved in contraction-stimulated glucose uptake but plays a key role in insulin-stimulated glucose transport. However, it has been difficult to develop specific evidence to document that class 1A PI 3-kinase mediate insulin-stimulated glucose uptake in skeletal muscles. PI 3-kinases are a family of 8 enzymes that are capable of phosphorylating the D-3 position of the inositol head group of phosphoinositides. Class 1A PI 3-kinase produces PtdIns(3,4,5)P3, which is believed to be the lipid signaling molecule recruiting PKB to the membrane. Global gene knockouts of the two major isoforms of PI 3-kinase (p110α and p110β) are lethal; knockin that creates a kinase dead p110α are also embryonic lethal. Data on insulin action has only been obtained from studies of heterozygous mice; these mice are insulin resistant and do have defects in insulin-induced activation of Akt/PKB but the direct effect on insulin-induced glucose transport in muscle and fat has not been reported. Indeed, overexpression of p110α or p110β induces glucose transport and GLUT-4 translocation, but overexpression of PI 3-kinases does not prove a particular PI 3-kinase isoform is involved. In particular forced overexpression of p110 causes not only large increases in PtdIns(3,4,5)P3 but also in the other D-3 inositides, so it is possible that the effects seen are due to the increase in PtdIns(3)P, PtdIns(3,4)P2 and PtdIns(3,5)P2. We have used isoform specific PI 3-kinase inhibitors against p110α, p110β, p110γ and p110δ to study their role in regulation of insulin-stimulated glucose uptake and PKB phosphorylation in skeletal muscles. Wortmannin and LY294002 completely blocked insulin-stimulated glucose uptake and PKB phosphorylation. However, the isoform specific PI 3-kinase inhibitors were unable to reduce glucose uptake significantly despite that PKB phosphorylation was reduced by more than 50 %. Instead, inhibition of PIKfyve, which phosphorylates the D-5 position of the inositol ring, blocked insulin-stimulated glucose uptake. Our data do not support that class 1A PI 3-kinase mediate insulin-stimulated glucose uptake in skeletal muscles, but suggest that PIKfyve is an important mediator of insulin-stimulated glucose uptake in skeletal muscles.

REDUCED AMP-ACTIVATED PROTEIN KINASE ACTIVITY IN MOUSE SKELETAL MUSCLE DOES NOT EXACERBATE THE DEVELOPMENT OF INSULIN RESISTANCE WITH OBESITY

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HYPOTHESIS: Obesity-related insulin resistance is associated with accumulation of bioactive lipids in skeletal muscle. The AMP-activated protein kinase (AMPK) regulates lipid oxidation in muscle by inhibiting acetyl-CoA carboxylase-2 (ACC2) and increasing mitochondrial biogenesis. We investigated whether reduced levels of muscle AMPK promote lipid accumulation and insulin resistance during high-fat feeding. METHODS: Male C57/BL6 wild-type mice and transgenic littermates overexpressing an alpha2AMPK kinase-dead (KD) in muscle were fed control or high-fat diet. Whole-body glucose homeostasis was assessed by glucose and insulin tolerance tests, and by measuring fasting and fed serum insulin and glucose. Insulin action in muscle was determined by measuring 2-deoxy-[(3)H]glucose uptake and Akt phosphorylation in incubated soleus and extensor digitorum longus muscles. Muscle triacylglycerol, diacylglycerol and ceramide content was measured by thin-layer chromatography. Mitochondrial proteins were measured by immunoblotting. RESULTS: KD mice had reduced skeletal muscle alpha2AMPK activity (50% in gastrocnemius and >80% in soleus and extensor digitorum longus) and ACC2 Ser228 phosphorylation (90% in gastrocnemius). High-fat feeding increased body mass and adiposity, and impaired insulin and glucose tolerance; however, there were no differences between wild-type and KD littermates. High-fat feeding impaired insulin-stimulated muscle glucose uptake and Akt-phosphorylation, while increasing muscle triacylglycerol, diacylglycerol ($p = 0.07$) and ceramide, but these effects were not exacerbated in KD mice. In response to high-fat feeding, mitochondrial proteins were increased to similar levels in wild-type and KD muscles. INTERPRETATION: Obesity-induced lipid accumulation and insulin resistance were not exacerbated in AMPK KD mice, suggesting that reduced levels of muscle alpha2AMPK do not promote insulin resistance in the early phase of obesity-related diabetes.

REDOX REGULATION OF GLUCOSE METABOLISM

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Impaired insulin-mediated glucose uptake in skeletal muscle is believed to be the primary defect leading to the development of type 2 diabetes. Our research aims at finding means to improve peripheral glucose metabolism by manipulating thioredoxin and thioredoxin-interacting protein (TXNip).

Thioredoxin system is composed of the small oxidoreductase thioredoxin, thioredoxin reductase and NAPDH. This system is essential for life in mammals, as it regulates cellular redox status and maintains proteins in their reduced state. TXNip is a pro-apoptotic protein that binds to thioredoxin with high affinity, inhibiting its activity. TXNip-deficiency gives protection against diabetes by preventing beta-cell death and enhancing glucose uptake in fat and skeletal muscle cells, whereas TXNip overexpression has an inhibitory effect on glucose uptake. Blood glucose level and insulin have opposite roles in regulating the TXNip protein in the skeletal muscle. High glucose increases the expression of TXNip, which further increases the glucose level in blood by inhibiting glucose uptake. In contrast, insulin reduces TXNip expression. TXNip expression is found to be consistently higher in the muscles of patients with type 2 diabetes or prediabetic condition, characterized by insulin resistance and elevated blood glucose level.

We have developed rat L6 skeletal muscle cell lines where thioredoxin or TXNip have been stably overexpressed / silenced. According to our recent findings, overexpression of thioredoxin downregulates endogenous TXNip in L6 myoblasts, and we seek to elucidate the mechanisms by which thioredoxin is regulating TXNip expression. We are also characterizing how changes in thioredoxin / TXNip expression affect glucose uptake in differentiated L6 myotubes. We are in process of shedding some light on the mechanisms by which TXNip inhibits glucose uptake and improving glucose uptake through intracellular TXNip silencing. As any impairment in skeletal muscle glucose uptake disturbs the whole body glucose homeostasis, new means to interfere with the inhibitory effect of TXNip would have a major therapeutic potential in the prevention of type 2 diabetes.

ZOOMING OUT ON AMPK SUBSTRATES: A PHOSPHOPROTEOMIC APPROACH

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AMP-activated protein kinase (AMPK) is the downstream component of a kinase cascade that senses cellular energy status. AMPK modulates multiple metabolic pathways and is activated by a large variety of cellular stresses, such as hypoxia and muscle contraction, or by adipokines and anti-diabetic drugs. In addition to its role in maintaining energy homeostasis, AMPK function is now recognized to extend to non-metabolic processes for example the control of cell structure and cell polarity. Elucidating the functional components of these complex signalling networks is a challenging prospect. Most of the *bona fide* AMPK substrates have been found by zooming in on candidate target proteins and validating them by classical biochemical approaches. As a complementary approach, phosphoproteomics can be used to identify new AMPK targets and identify their phosphorylation sites. As a proof of principle, this approach is being applied to full extracts from isolated hepatocytes treated with AICA riboside to activate AMPK. Tryptic peptides are first separated by the use of Hydrophilic-Interaction Liquid Chromatography (HILIC). Phosphopeptides are then selectively enriched on TiO_2 beads and analyzed by on-line reverse phase separation coupled to mass spectrometry. This will hopefully allow a comprehensive list of phosphorylation sites to be compiled and identify new AMPK targets. Phosphorylation of purified recombinant protein substrates by AMPK will be validated *in vitro* and phosphorylation of the targets *in vivo* will be confirmed after obtaining phosphorylation site-specific antibodies. Once validated, new areas of control by AMPK will be investigated.

CAN WE REVEAL AN AGONIST-INDUCED Ca^{2+} ENTRY HIDDEN BY THE UBIQUITOUS STORE-OPERATED Ca^{2+} ENTRY IN ENDOTHELIAL CELLS?

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During physiological cell stimulation, many processes are engaged that might eventually lead to Ca^{2+} entry. One can roughly separate the different Ca^{2+} entries as 1) activated by a second messenger produced by an agonist (receptor-activated Ca^{2+} entry, RACE) and 2) linked to the depletion of the ER Ca^{2+} store, the SOCE (store-operated Ca^{2+} entry). The latter is well characterized and involved the proteins STIM1 (the ER Ca^{2+} sensor) and Orai1 (the Ca^{2+} entry channel). Regarding the RACE pathway, it appears that a diversity of activation mechanisms as well as ionic channels is involved. As agonist stimulation produced both second messengers and a depletion of the ER, it is difficult to separate both types of influx. In this study, our aim was to differentiate between RACE and SOCE pathways during agonist-induced cell stimulation, using electrophysiological and imaging approaches on a human endothelial cell line (EA.hy926).

In Ca^{2+} containing medium, histamine induced an ER Ca^{2+} depletion of about 15% of what was achieved by thapsigargin (TG), while in the absence of Ca^{2+}, histamine fully depleted the ER. This pointed to a modest involvement of the SOCE upon histamine stimulation. In whole-cell perforated patch, histamine and TG activated both an inwardly rectified current, when 10 mM Ba^{2+} (and 2 mM Ca^{2+}) was present in the bath. The current shared characteristics with the I_{CRAC}, like the inward rectification, the block by La^{3+} and the behavior in divalent free medium. Surprisingly, in presence of 10 mM Ca^{2+} in the bath (without Ba^{2+}) the currents activated by TG and histamine were clearly different. In particular, histamine activated an outwardly rectified current, blocked by Ba^{2+}, while TG activated a similar, but smaller current as in Ba^{2+} containing medium. We also showed that during voltage clamp recordings, the ER gets more depleted in 10 mM Ba^{2+} compared to 10 mM Ca^{2+} medium, explaining the activation of a CRAC-like current (due to store depletion) upon histamine in Ba^{2+} medium. In Ca^{2+} medium, the outward current activated by histamine was increased upon Na^{+} removal and inhibited by Ni^{2+} and KB-R7943, arguing in favor of the Na^{+}/Ca^{2+} exchanger (NCX). Ni^{2+} and KB-R7943 also inhibited histamine-, while not TG-induced Ca^{2+} entry, measured by fura-2. These data led us conclude that under physiological conditions, part of the RACE was due to the NCX, working in the reverse mode, that accomplishes sufficient ER refilling to largely prevent the activation of SOCE.

ENDOPLASMIC RETICULUM-MITOCHONDRIA CROSSTALK AND CELLULAR STRESS SIGNALING

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Mitochondria couple cellular metabolic state with Ca^{2+} transport processes. They therefore control not only their own intra-organelle [Ca^{2+}], but they also influence the entire cellular network of cellular Ca^{2+} signalling, including the endoplasmic reticulum (ER), the plasma membrane and the nucleus. Through the detailed study of mitochondrial roles in Ca^{2+} signalling has emerged a remarkable picture of inter-organelle communication. The talk will give an overview of this picture and focus on data showing (i) preferential Ca^{2+} transfer from the ER to the mitochondrial matrix through a macromolecular complex associated with the both mitochondrial and ER membranes (ii) Ca^{2+} dependent regulation of mitochondrial movements and cellular distribution through the mitochondrial Rho GTPase Miro.

MITOCHONDRIAL Ca^{2+} UPTAKE IS DIFFERENTIALLY DETERMINED BY THE Ca^{2+} SOURCE AND THE EXPRESSION-LEVEL OF THE NOVEL UNCOUPLING PROTEINS UCP2 AND UCP3

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Mitochondrial Ca^{2+} sequestration is an important physiological process that is linked to many different cellular responses such diverse as metabolic stimulation and energy deprivation or cell proliferation and cell death. This diversity of physiological consequences for a cell triggered by mitochondrial Ca^{2+} loads is controlled by the spatial and temporal patterns as well as by the intensity of mitochondrial Ca^{2+} signals. Mitochondrial Ca^{2+} uptake under physiological conditions is thought to be mainly accomplished by the so called mitochondrial Ca^{2+} uniporter (MCU) albeit alternative pathways such as a reversed mode of the mitochondrial $3Na^{+}$/ Ca^{2+}-exchanger (NCX_{mito}) have been recently introduced, too. The MCU was characterized as a highly Ca^{2+} selective inward rectifying ion channel at the inner mitochondrial membrane with a quite low Ca^{2+} affinity. We have recently demonstrated that the novel uncoupling proteins UCP2 and UCP3 are elementary for mitochondrial Ca^{2+} uniport albeit a definite molecular identification of the MCU has not been completed yet.
Currently, we integrate these findings to a novel concept that describes distinct characteristics of mitochondrial Ca^{2+} uptake in intact cells depending on the source and mode of cytosolic Ca^{2+} elevation. Using siRNA induced knock-down of UCP2 and UCP3 it emerges that mitochondrial Ca^{2+} uptake upon IP_3-induced Ca^{2+} release is mainly under the control of the UCP2/3 dependent MCU, whereas the transfer of entering Ca^{2+} into mitochondria is accomplished via an UCP2/3 independent pathway. However, increased levels of these UCPs dramatically enforced the mitochondrial Ca^{2+} uptake of entering Ca^{2+}, whereas an expression of mutated UCPs reduced exclusively mitochondrial Ca^{2+} signals that were fueled by Ca^{2+} entry. These studies demonstrate the importance of UCP expression levels as a determinant of the magnitude and velocity of mitochondrial Ca^{2+} sequestration pending on the Ca^{2+} source and point to the existence of molecularly distinct mitochondrial Ca^{2+} uptake sites facing either sites of ER Ca^{2+} release or Ca^{2+} entry. Although we are only at the beginning to characterize and understand the functioning of different molecular components of distinct sites of mitochondrial Ca^{2+} uptake, it occurs that these different mitochondrial Ca^{2+} uptake sites match the different functional properties of local and global Ca^{2+} events at sites of ER Ca^{2+} release and those of Ca^{2+} entry in order to properly integrate different Ca^{2+} signals into many vitally cellular processes.

SPATIAL Ca^{2+} SIGNALING IN CARDIAC MYOCYTES

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Local increases in Ca^{2+} concentration ($[Ca^{2+}]$) in the cytoplasm and nucleus of cardiac myocytes are key to excitation-contraction and excitation-transcription coupling, respectively. Ca^{2+} increases in these compartments are brought about by Ca^{2+} release from internal stores, i.e. the sarcoplasmic reticulum (SR) and the nuclear envelope (NE). Dysregulation of Ca^{2+} release from these stores may elicit arrhythmias and cellular remodeling.

In atrial myocytes – due to a lack of T-tubules – SR Ca^{2+} release during excitation-contraction coupling is spatially and temporally inhomogenous. Depolarization first triggers Ca^{2+} release from the subsarcolemmal SR, from where it spreads centripetally via active Ca^{2+}-induced Ca^{2+} release from the central SR to induce contraction. SR Ca^{2+} release during excitation-contraction coupling occurs mainly via functional units of ryanodine receptors (RyRs), which are distributed in a sarcomeric, grid-like pattern distanced ~2µm apart. SR Ca^{2+} release is regulated by the microenvironment of RyRs. Local disturbances of RyR-mediated Ca^{2+} release (e.g. by inhibition of glycolysis) may result in spatially confined, subcellular Ca^{2+} alternans and generation of arrhythmogenic $[Ca^{2+}]$ waves. In addition to RyRs, atrial myocytes also contain IP_3 receptors (IP_3Rs), albeit at much lower density. Activation of subsarcolemmal IP_3Rs, however, through IP_3 generated from ET receptors coupling to phospholipase C, induces spontaneous diastolic Ca^{2+} release and arrhythmic extra-contractions.

The nucleus is separated from the cytoplasm by the NE. Due to the presence of nuclear pores, however, cytoplasmic Ca^{2+} may also diffuse into the nucleoplasm. Thus, during excitation-contraction coupling $[Ca^{2+}]$ also increases passively in the nucleus, but with a considerable delay as compared to the cytoplasmic $[Ca^{2+}]$ transient. The NE is a functional Ca^{2+} store directly connected to the SR. It forms tubular structures traversing the nucleoplasm, i.e. the nucleoplasmic reticulum, and contains RyRs and IP_3Rs as Ca^{2+} release channels. Thus, the NE has the potential to regulate actively local Ca^{2+} in the nucleoplasm and, thereby, to modulate transcription (excitation-transcription coupling). Activation of nuclear IP_3Rs causes a selective increase in the nuclear $[Ca^{2+}]$ transient, thus demonstrating directly that nuclear Ca^{2+} may be regulated independently from cytoplasmic Ca^{2+}.

In conclusion, spatial Ca^{2+} signaling in cardiac myocytes depends on the subcellular distribution, local regulation and activation of RyRs and IP_3Rs. It is essential for physiological processes such as excitation-contraction and excitation-transcription coupling but may also be involved in pathological processes such as arrhythmogenesis and the development of hypertrophy.

SUNDAY, 15. NOVEMBER 2009:

SESSION X

PATTERNS OF CARDIOVASCULAR CONTROL DURING REPEATED TESTS OF ORTHOSTATIC LOADING

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To investigate patterns of cardiovascular control, a protocol of head up tilt (HUT) followed by lower body negative pressure (LBNP), which represents a significant cardiovascular control challenge, was employed. Linear regression of beat-to-beat heart rate and mean blood pressure data collected over repeated tests was used to analyze control response during the LBNP phase of the combined HUT-LBNP protocol. Four runs for each of 10 healthy young males reaching presyncope were analyzed. Subjects were classified into 2 groups based on the consistency of mean blood pressure (MBP) regulation in response to central hypovolemia induced by LBNP. The consistent group tended to exhibit consistent heart rate slope (rate of change of HR over time as calculated by linear regression) whereas subjects in the inconsistent group could not be easily classified. Subjects with consistent MBP maintenance exhibited patterns suggesting a consistency of response in cardiovascular control whereas subjects less successful in maintaining MBP exhibited less clearly defined patterns over four runs.

MULTIPARAMETRIC STRUCTURAL ANALYSIS OF RETINAL VESSELS INDUCED BY PATHOLOGICAL STATES: POSSIBLE SPACE FLIGHT APPLICATIONS?

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Background: Fluorescein angiography (FLAG) became a standard and essential diagnostic tool, especially in diabetic retinopathy (DR). Our computer program, developed for semi-automatic analysis (Dendron Pro Ret) of vascular ramification, enabled us to define a set of structural parameters resulting in a more powerful diagnosis of DR.
Materials and Method: FLAG was performed on 12 DR patiens of different age and gender (TOPCON TRC-50IX – 5 ml of 10 % Na-fluorescein i.v.). Series of pictures were taken from the 5th to the 30th sec of session and fed into a PC. First step of offline computerized analysis distincts the silhouette of vessels from the background. On the separated arterial and venous profiles, the contour- and midline of all the well presented branches are generated. An abundant set of geometrical parameters are then calculated for the branching areas and internode/terminal sections: i, general thickness, local strictures, and courseness of intersections, ii, angles, symmetry and related mouths thicknesses of branching points.
Results: Variable set of the above structural parameters apparently promote the diagnosis and follow up of DR and objective judgement of therapic interventions. Similarly, it can enhance the relevance of clinical pharmacologic trials. Refined classification of the different forms and stages of the illness can also be resulted.
Conclusions: Multiparametric structural analysis of retinal vessels appears to be a useful clinical diagnostic tool. As spaceflight induced microgravity changes result in cephalad fluid shifts, retinal angiography might provide insight into accompanying cerebral blood flow changes.

CONTINUOUS EPINEPHRINE INFUSION MODULATES PASSIVE HEAD-UP TILT INDUCED CARDIOVASCULAR RESPONSES

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Neurohumoral effects of head-up tilt (HUT) induced hemodynamic responses, with or without pharmacological blockades, are well known. However, little is known about the modulation of theses cardiovascular responses during catecholamine hormone infusions. We investigated how continuous epinephrine infusion modulates HUT induced cardiovascular responses using two passive 5 min 70° HUT trials: A control run (without infusion) and a supplemented run (with prior epinephrine infusion, titrated to a dose which increased supine mean arterial pressure by 20% above resting values). This randomized study was carried out in eight healthy male volunteers and each trial was separated by two weeks. Compared to the control run, epinephrine infusion increased basal heart rate by 17 bpm, systolic pressure by 19 mm Hg and stroke volume index by 12.6 ml/m^2, whereas diastolic pressure and thoracic impedance stayed unchanged. Despite similar HUT induced thoracic fluid shifts, reflected by the thoracic impedance changes, the epinephrine infused group showed no significant changes in heart rate and stroke volume index compared to the controls. An increase in total peripheral resistance during HUT in epinephrine treated subjects elevated the diastolic blood pressure, an effect not observed in the controls. This suggests that during epinephrine infusion supine blood pressure is maintained primarily by changes in cardiac functions, whereas during HUT vascular resistance appear to play a more important role.

PREDICTING PULSATILE VARIATIONS IN FINGER ARTERIAL PRESSURE USING A NOVEL CARDIOVASCULAR SYSTEM MODEL

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Cardiovascular modeling has been used to study the behavior of blood pressures in the peripheral and systemic compartments, cardiac output, ventricular elastance and contractility in the human circulatory system under various conditions such as constant workload and orthostatic stress. In this study we investigated, modified, and combined two existing cardiovascular models: a non-pulsatile global model (Kappel) and a simplified pulsatile left heart model (Olufsen). The non-pulsatile global model incorporates all the essential subsystems such as systemic and pulmonary circulation, left and right ventricles, baroreceptor loop, etc. This model considered the mean values of quantities over one heart cycle instead of the instantaneous values. The pulsatile left heart model utilizes a minimal cardiovascular structure to close the circulatory loop. The first goal was to integrate the pulsatile left heart model with the Kappel global model. The main objective of this study was to develop a global pulsatile lumped compartment model that predicts the pressures in the systemic and peripheral circulation and specifically the pulsatile pressures in the finger arteries where real-time measurements can be obtained. A finger artery compartment was included to reflect measurements of pulsatile pressures. Linking the average flow model with a pulsatile flow was the main difficulty. Modifications were made in the ventricular elastance to model the stiffness of heart muscles under stress or exercise state. A sigmoidal function, which is dependent on the heart rate, was used to characterize the maximum elastance of the left ventricle. Preliminary simulation results show pulsatile pressures in the systemic and peripheral compartments, including the systemic aorta and finger arteries compartment. The model parameters were estimated to obtain an average normal finger arterial pressure of 120/90 mmHg. In the simulations, a decrease in pulsatility range was observed in the arterial systemic compartment. Also, pulsatility was almost negligible in venous pulmonary and arterial pulmonary compartments. Moreover, increasing the heart rate increased the pulsatility and the blood pressures in the compartments. Our results indicate that a pulsatile cardiovascular model could be developed without excess complexity so that pulsatile information would be available and which could be incorporated into the baroreflex control loop.

CARDIO-POSTURAL INTERACTIONS: WAVELET ANALYSIS OF GASTROCNEMIUS ELECTROMYOGRAPHIC ACTIVITY AND BLOOD PRESSURE VARIATION WITH RESPECT TO POSTURAL SWAY DURING QUIET STANCE

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Purpose: A relationship has been recently demonstrated between blood pressure (BP) variation and postural sway during quiet stance, and a physiological model proposed for an adaptive compensatory cardio-postural interaction as part of an integrated orthostatic reflex control system (Blaber A.P. et al., Respir. Physiol. Neurobiol. (2009), doi:10.1016/j.resp.2009.04.007). Methods and Study Design: To further validate this model, we investigated simultaneous changes of medio-lateral center of pressure coordinates (M/L COP), filtered electromyographic activity (EMG) of muscles essential to postural stance along with continuous non-invasive BP data. The study was approved by the Office of Research and Ethics for Simon Fraser University. Five young males between the ages of 19 to 26 volunteered to participate. Participants were seated for 20 minutes and then asked to stand with eyes closed (blindfolded) in a quiet stance for 5 minutes. Postural sway was characterized by the time-varying position of the centre-of-pressure (COP). Surface EMG (transdermal differential recording), BP (photoplethysmography), and COP (force platform) were recorded with a sampling rate of 1000 Hz for the entire period of the test. Data from last 4 minutes was used for analysis purposes. Results: The data was resampled at 15Hz and Discrete Wavelet Transform (DWT) was applied using the Daubechies 5 (db5) wavelet to decompose the signals (EMG, BP and COP) at the 7^{th} and 8^{th} scale to extract the approximation signal in the low frequency range (~ 0.11 Hz). The approximation signal revealed several regions with significant correlations ($r>0.5$, $P<0.0001$) between the EMG and BP and EMG and COP in the last 4 minutes of quiet stance. Conclusions and Significance of Findings: The presence of correlation in the low frequency (~0.11Hz) region indicates a possible adaptive functional relationship between M/L COP sway, postural muscle EMG activity and BP variations during quiet stance.

EFFECTS OF MENTAL CHALLENGE APPLIED BEFORE PASSIVE HEAD UP TILT ON ORTHOSTATIC NEUROHORMONAL RESPONSES

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Mental challenge (MC) applied during orthostatic challenge (OC) increases cardiovascular responses. Passive head up tilt (HUT) and mental arithmetic (MA) are laboratory-based models for providing OC and MC, respectively. We investigated whether MC applied before OC elicits synergergistic responses in orthostatic heart rate, heart rate variability and arterial blood pressure. 15 healthy young males were subjected to two randomized protocols: a) OC and b) MC before OC, with sessions randomized and ≥2 weeks apart. Beat to beat continuous hemodynamic variables were measured and saliva samples taken for hormonal assay. OC alone increased heart rate from 59 ± 7 (baseline) to 80 ± 10 bpm (mean ±SD) and mean arterial blood pressure from 88 ± 10 to 91 ± 14 mmHg. MC applied before OC resulted in greater, but not significant, increases in heart rate and cardiac output. Mental challenge applied preceding HUT induced increases in orthostatic cardiovascular responses that are, however, not different from those compared with HUT alone. While central drive induced by mental challenge adds to physiologically mediated cardiovascular reflexes, beneficial effects of mental challenge on orthostatic cardiovascular responses are not present when mental challenge is applied before orthostatic challenge.

THE LYSOSOMAL PATHWAY OF APOPTOSIS: A COMPLEX BIOLOGICAL NETWORK

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For a long time, proteases were mainly considered as protein degrading enzymes. However, the current view focuses on proteases as signalling molecules [1]. This principle, is clearly exemplified on apoptosis, one of the major mechanisms by which eukaryotic organisms eliminate potentially dangerous, superfluous and damaged cells. Eventhough in this process the caspases were considered to play a central role, more recently, other proteases, in particular the cysteine cathepsins, have also been shown as being important in apoptosis [2]. Initially, the nuclei and the mitochondria were reported to be the key organelles participating in this process. However, more recent data, showed that the lysosomes also play an important role [3].
In order to get an integrative view of the lysosomal pathway of apoptosis, we attempted to build a signalling network using several 'omics' tools.
At the level-1 network, 286 proteins lead to 7729 non-redundant protein-protein interactions. Interestingly, several signalling modules were identified on 243 significant complexes, thus having different molecular functions.

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THE NON-GASTRIC H^+/K^+-ATPASE ATP12A EXERTS AN ANTI-APOPTOTIC EFFECT ON BUTYRATE-TREATED MYELOMONOCYTIC HL60 CELLS

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Myelomonocytic HL60 cells are a proper model for multidirectional (monocytic, eosinophilic and granulocytic) differentiation. In this work we investigate the relationship between induction of differentiation and of apoptosis in HL60 cells. Treatment of the cells with 10 mM butyrate induces apoptosis within 48 h as assessed by flow cytometry (7AAD nuclear staining/phosphatidylserine exposure; activation of caspases) whereas a lower concentration of butyrate (1 mM) elicits the expression of CD86 within 48-72 h, indicating differentiation mainly towards the monocytic lineage. In cells treated with 1mM butyrate the number of apoptotic cells is not different compared to untreated cells, whereas inhibition of the non-gastric H^+/K^+-ATPase ATP12A by 100 µM SCH28080 in 1mM butyrate-treated HL60 cells is followed by a significantly accelerated apoptosis within 48-72 h. Similar apoptotic volume decrease (AVD) in 1 mM butyrate-treated HL60 cells is significantly more pronounced in the presence of the inhibitor. In addition HL60 cells show a significant up-regulation of ATP12A mRNA within 48 h of butyrate treatment (1 mM) as examined by quantitative PCR. Thus inhibition of ATP12A by SCH28080 fosters apoptosis in cells differentiated by 1 mM butyrate. In conclusion these data suggest an anti-apoptotic function of the non-gastric H^+/K^+-ATPase ATP12A in HL60 cells.

THE POSSIBLE EFFECTS OF TOPICALLY ADMINISTIRED CARVACROL ON PHYSIOLOGICAL MECHANISMS IN WOUND HEALING

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In this study; we aimed to investigate the effects of carvacrol, obtained from the plant "origanum onites", on the physiological mechanisms of wound healing. The physiological mechanism of these effects and the role of cytokines (TNF-α, IL-1β and TGF-β1) in these mechanisms were investigated.
Thirty-two, 6 to 8 months old wistar albino rats weigthing 350 to 450 g were used. Twelve of them were used in the first and 20 in the second stage of the study. At the first stage, punch biopsy was performed under anesthesia to create wounds, spaced 1cm from each other, on the dorsal midline skin of the animals. Preliminary results gave the amount of required carvacrol dose for affecting wound healing. In the second stage animals were divided into two groups as experimental (n=10) and control (n=10).
Carvacrol dose (amount defined at the first stage) was applied to the experimental group rat wound tissues for 5 days while in the control group sunflower oil was applied.
On the third, eighth, twelfth and fiifteenth days of the wound healing tissue samples were taken. The tissue samples were evaluated in terms of wound depth and granulation tissue thickness (with light microscope), wound tissue healing measurement ratios (with photographic images), TNF-α, IL-1β and TGF-β1 levels (with ELISA method).
In the statistical analysis of the data repeated measures variance analysis were used to asses the effect of group and time and their interaction. Parameters of the study and control groups belonging to the same day were evaluated using Mann Whitney U test. The wound tissue thickness versus cytokine levels (tissue surface maesurement) and wound depth versus granulation healing ratios were assessed by Pearson twotailed correlation test. Significance was set at $p< 0.05$.
Results showed that TNF-α levels of the experimental group on the third day, were significantly higher ($p< 0.001$) than that of the control group. IL-1β levels of the group on the eighth day were significantly higher ($p=0.034$) than that of the control group. TGF-β1 levels of the experimental group on the eighth day were significantly lower than the control group ($p=0.009$).
TGF-β1 levels of the experimental group on the 12 th day, were significantly lower than that of the control group ($p=0.003$). The wound depth of the experimental group on the eighth day were significantly higher ($p=0.002$) than that of the control group. Granulation tissue thickness of the experimental group on the eighth day were significantly lower than the control group ($p=0.008$). Wound tissue surface measurement healing ratios of the experimental group on the 12 th day were significantly higher ($p=0.014$) than the control group. On the third day, in the experimental group a statistically significant negative correlation between TGF-β1 levels and wound depth were determined ($r= -0.768$, $p=0.009$). On the other hand in the

control group statistically significant negative correlation was established between the TGF-β1 levels and tissue thickness on the 12 th day (r= -0.712, p=0.031).
As a conclusion the solution of carvakrol in the %12.5 concentration helps to fasten covering of the wound tissue and to leave fewer scar tissue and may be used for curing full depth skin wounds. If we take in to account the interactions between mediators taking role in wound healing, carvacrol's possible effects on the growth factors and cytokines are open to research.

Key Words: Carvacrol, Wound healing, TGF-β1, IL-1β, TNF-α.

APOPTOSIS WITHOUT KARYORRHEXIS IN MICROGLIA

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Apoptosis is characterized by morphological features, among the most prominent are chromatin condensation, shrinking of cell and nucleus, and the fragmentation of the nucleus into membrane-enclosed bodies (karyorrhexis). After UV irradiation, we found in mouse cell line (BV-2) microglia an apoptotic phenotype in which, after chromatin condensation, the nucleus does not fragmentize, but shrinks due to production of vesicles from the nuclear envelope and release of chromatin into the cytoplasm [1]. DNA laddering revealed internucleosomal fragmentation already 1 h after irradiation, in contrast to the later onset of structural features of apoptosis (e.g. chromatin condensation). By conducting an immunogold labelling with anti-histone H3 for transmission electron microscopy, we found that histone H3 was not only located in the cytoplasm, but also at different areas of the plasma membrane region.
Recently, we were able to make similar observations in primary murine microglia. Primary apoptotic microglia also showed condensed chromatin in the nuclear periphery, a strongly dilated nuclear envelope with numerous vesicles in its immediate vicinity, and cytoplasmic chromatin, although the process of chromatin release from the nucleus is less pronounced than in BV-2 microglia.
Our study demonstrated that apoptotic microglia cells release chromatin into their cytoplasm and, presumably, accumulate histones in their plasma membrane. Since nuclear components are known to be potent immunoreactive agents, this mechanism could play a role in inflammatory processes.

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ROLE OF CELL SWELLING-INDUCED PEPTIDE SECRETION IN ISCHEMIA-REPERFUSION INJURY AND PRECONDITIONING

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Swelling-induced peptide secretion represents cellular reaction when material stored in secretory vesicles is expulsed from various types of cells. The exocytosis induced by cell swelling is a broad unspecific phenomenon affecting many hormones, enzymes and bioactive peptides after exposure of cells to relative hyposmolarity or treatment with permeant agents. Dynamics of secretion is indistinguishable from that induced by specific secretagogue. Cell swelling represents alternative way of stimulation of secretion since its signaling pathway bypasses Ca^{2+} involving steps and conventional intracellular signal mediators and is resistant to physiological inhibitors. Both 5 mmol/l N-ethylamaleimide and 10 μmol/l $ZnCl_2$ (inhibitor of protein tyrosine phosphatases), which block disassembly of SNARE complexes and their further participation in exocytosis, increased basal insulin secretion. In contrast to glucose, already high insulin secretion was further increased after cell swelling. In contrast to conventional stimulation an extra pool of secretory granules is available for swelling-induced insulin exocytosis. Cell swelling could be important mediator of changes, which take place at pathophysiological conditions; shift to anaerobic glycolysis and production of metabolites in ischemia increase intracellular osmolarity, thus increasing transmembrane osmotic pressure differences and producing cell swelling. Products released after swelling could participate in the development of ischemia-reperfusion injury, but could be also mediators of local or remote preconditioning when factors released at the place of ischemia have protective effect. Perfusion of isolated rat heart with hyperosmolar high glucose medium followed by washout with isosmolar medium (relative hyposmotic stress) prior to ischemia substantially decreased postischemic contractile dysfunction, size of myocardial infarction and the severity of reperfusion-induced arrhythmias. Sustained perfusion with hyperosmolar high glucose medium without washout period with isosmolar medium did not have any protective effect on ischemia-reperfusion injury thus indicating important role of cell swelling. PI3K/Akt inhibitor wortmannin (100 nM) completely abolished improved contractile function recovery and increased the size of infarction, but failed to reverse lower severity of arrhythmias. In conclusion: Factors released during relative hyposmotic stress (washout period) most likely participate in the mechanism of preconditioning.

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NICOTINE PROTECTS AGAINST KAINIC ACID INDUCED HIPPOCAMPAL DAMAGE

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Using the histochemical analysis (Fluoro-Jade B and bis-benzimide 33342 Hoechst), the influence of nicotine on kainic acid (KA) induced hippocampal damage in 35-day-old Wistar male rats was studied. Kainic acid administration is a well established and relevant chemical model of temporal lobe epilepsy and from previous experiments it is known that nicotine is capable to diminish the severity of pathological phenomenons, accompanying KA administration. Unfortunately the protective dose of nicotine (1mg/kg) had significant side-effects (lost of posture control etc.), preventing the reasonable use of nicotine as a neuroprotectant. In this study we tried to administer nicotine in low doses (0.25 mg/kg) repeatedly (six times). According to the results obtained from histological analysis (using semi-quantitative grading scale), the fractionized administration of nicotine was able to decline the KA-induced damage significantly, moreover the nicotine side-effects were robustly decreased. Hence the repeated low-doses of nicotine seem to be more beneficial compared to the undivided high-dose administration.

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DISTINGUISHING BETWEEN THE SUB- AND SUPERTHRESHOLD REGIME OF NEURONAL FIRING

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Most neurons and sensory cells respond to input signals with a spike train. They encode the input information into the rate of the spike discharge and/or into timings of individual spikes. In the case of spike codes the regularity of spike discharge becomes a major issue. The main sources of the discharge irregularity are the input signal stochasticity, the intrinsic neuronal noise and the intensity of the input signal. Additive noise has relatively little effect on the spike discharge if the input stimulus is superthreshold. For subthreshold stimuli, however, the neuron might be still able to fire spikes because of the noise that randomly pushes the membrane voltage above threshold. The resulting irregularity of spike discharge in this fluctuation driven regime is high and the interspike interval distribution broad. The distinction between sub- and superthreshold regime has therefore important consequences for the firing and coding behavior of neurons in the presence of noise (Gerstner and Kistler, 2002).
We present a method for establishing whether an output of a neuron is signal or noise driven. The method is based on a measurement of the spike latency in response to a test stimulus, and the time interval between the stimulus onset and the last spike preceding it. We assume that the cell is active in the absence of the test stimulus and that the latency of the response decreases the closer the membrane potential is to the firing threshold. In the superthreshold regime the average membrane potential increases throughout the whole interval between two spikes. The average latency therefore monotonically decreases with increasing distance of the test stimulus from the "prestimulus" spike. In the subthreshold regime, however, the average membrane potential settles at a plateau under the threshold. At some distance away from the "prestimulus" spike the latency therefore does not decrease anymore but reaches a constant value. This hypothesis was tested and confirmed with a computational simulation of the experiment, using standard "leaky integrate-and-fire" and Hodgkin-Huxley type model neurons. The method was then applied to the firebug (*Pyrrhocoris apterus*) filiform sensilla. These sensilla exhibit an ongoing spike discharge in the absence of a stimulus. By using the method we showed that the resting activity of type T_1 sensilla is signal driven, whereas that of type T_2 is noise driven.

Gerstner W. and Kistler W.M. (2002). Spiking neuron models. Cambridge University Press, UK.

MATURATION AND FUNCTIONAL PLASTICITY OF CELLULAR RESPIRATORY APPARATUS IN FLIES EYES

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In insect photoreceptor cells there is a close relationship between the phototransduction cascade in the rhabdomere and the oxidative phosphorylation in the mitochondria. On one hand the light-induced depolarization and the Ca^{2+} entry into the cytosol present a load on mitochondria, while on the other the blocking of oxidative phosphorylation can cause the opening of normally phototransduction cascade-activated channels TRP and TRPL. The functional status of both systems during animal's life determines the strength and the nature of this relationship. We assessed the status and the plasticity of the photoreceptor cellular respiratory apparatus during the course of blowflies' adult lives. This was done by non-invasively measuring the changes of individual respiratory pigments' redox states upon exposure to anoxia and upon illumination. Measurements were performed in situ using dynamic differential absorption spectroscopy and the individual respiratory pigments redox states were extracted using PCA-based spectral deconvolution. While the responses to anoxia gave us the overall redox state dynamic range of individual pigments, their responses to 10 second illuminations told us how responsive the mitochondria are to sudden changes in metabolic loads. In animals kept on a 12h/12h day/night cycle there was a 3.4 (haem b) to 5.6 (haem c) times increase in the respiratory pigment redox state dynamic range in three weeks post-eclosion, while in animals kept in darkness the increase is 2.0 (haem a_3) to 3.1 (haem c) times in the same time period. The dynamic range plasticity is retained even in adult life. If the culturing regimes changed after two weeks (light/dark to dark and vice versa), the dynamic ranges changed correspondingly. The observed values however never reached the levels of the other group. The responses to 10 s illumination periods also varied with age. While most of this variability is due to smaller dynamic range reflecting the cytochrome content, some features especially the shapes of the reduction (oxidation) time courses did change substantially – most notably in haems c, b and a_3. Taking into consideration the close relationship between the phototransduction and mitochondria our results demonstrate the need for caution in interpreting the electrophysiological data from freshly hatched animals, with immature biochemical apparatuses, normally used in preparation of isolated ommatidia for patch-clamp experiments.

IMPROVING EFFECTS OF CHRONIC MELATONIN TREATMENT ON 5-HT NEUROTRANSMISSION AND SPATIAL MEMORY TASK IN OLD RATS

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Pineal hormone melatonin has an important role in the ageing process as potential drug to relieve oxidative damage, a likely cause of age-associated brain dysfunction. As age advances, the nocturnal production of melatonin decreases suggesting physiological alterations in elderly. As serotonin (5-HT) plays a role in adult memory processing, present experiments were performed to study in vivo the effects of exogenously administered melatonin (1 mg/kg/day, diluted in drinking water, 4 weeks) on central serotonin (5-HT) and working memory task in aged rats (20 months, n=6). Animals were maintained under controlled conditions (22ºC, 70% humidity, 12/12LD). The accumulation of 5-hydroxytryptophan (5-HTP) after decarboxylase inhibition was used as a measure of tryptophan hydroxylation rate in the brain *in vivo*. Also, spatial memory in 8-arm radial-maze test was tested. Modulation of tryptophan hydroxylation and working memory test were assessed in male Wistar young (3 months, n=6) and old (20 months, n=6) control rats for comparisons. After treatments, to test radial maze memory task, trials were judged complete when rats had chosen all 8 baited arms or spent 20 minutes in the trial. After that, rats were sacrificed by decapitation and hippocampus and striatum samples analyzed by HPLC with electrochemical detection to measure 5-HTP, 5-HT and the metabolite 5-HIAA. Tryptophan hydroxylation decreased significantly in the hippocampus (48%) and striatum (44%) of aged rats indicating an impairment in serotonin synthesis with age. However, when aged rats were repeatedly treated with melatonin, an important increase in tryptophan hydroxylation in hippocampus (53%) and striatum (83%) was observed. Similar results were obtained for 5-HT and 5-HIAA metabolite. All these neurochemical observations correlated well with an impairment of task performance in radial maze in aged controls and an improvement in the rats treated with melatonin. In conclusion these in vivo findings suggest that melatonin chronic treatment is able to improve 5-HT cerebral neurotransmission in aged rats, which might aid to improve the cognitive deficit that normally occurs as a consequence of aging.

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SUNDAY, 15. NOVEMBER 2009:

SESSION XI

GRAVITY, THE HYDROSTATIC INDIFFERENCE CONCEPT, AND THE HEART

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Gravitational and/or accelerational forces cause hydrostatic pressure gradients within a body's fluid-filled compartments. The hydrostatic pressure difference (Δp) between two given points within such a compartment depends on the fluid's density (D), the magnitude of the force imposed onto the system (f), the distance (d) between the points of interest, and the direction of the gravitational field / accelerational force relative to the line connecting those points. If we take α as the angle between the latter two, than the pressure effect is proportional to $\cos \alpha$, and $\Delta p = D \times f \times d \times \cos \alpha$.

This is relevant for the cardiovascular system because there is a direct link to orthostatic resilience and the syncope problem: With alteration of body posture, the heart has to deal with changed preload and afterload. Unfortunately, the hydrostatic indifference concept (Gauer & Thron, Handbook of Physiology, Sect.2, Vol. III, 1965) all but disappeared from modern textbooks of physiology - despite its obvious significance in terms of basic science (cardiovascular physiology) as well as clinical application (cardiology).

Upon reorientation of a body which is subject to gravitational attraction (on Earth, 1G) or when accelerational forces act upon this body, hydrostatic pressures within fluid filled compartments change, except one location that is 'hydrostatically indifferent', i.e. it does not experience pressure change. This is, *per definitionem,* the Hydrostatic Indifference Point (HIP).

Various experiments have shown that (1) for any postural change there is a certain specific HIP, (2) the referring HIP has a different location for any fluid-filled compartment, (3) the HIP location depends on the physiological state of the referring compartment, and is influenced by the actual filling volume, vessel compliance etc. For instance, the venous HIP lies below the diaphragm when a movement from supine to upright is considered, but in the atrial region for a movement from supine to head down; the arterial and venous HIPs are different; and with increasing blood volume, the venous HIP moves footward.

Typically, the right heart has to cope with a significant drop in diastolic filling pressure (preload) when a person stands up. This causes stroke volume to decrease by up to 40% and cardiac output by about 30%. The carotid baroceptor is much more influenced by this postural change than the aortic receptor area, and is in a privileged position to regulate blood pressure and cerebral perfusion with orthostatic stress.

CARDIOVASCULAR RESPONSES TO THE UPRIGHT POSTURE

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Although the upright position seems natural for humans, it is unique in the animal kingdom. For animals the heart is positioned at the level of the main part of the circulation, while for upright humans about 80% of the blood volume is positioned below the level of the heart. Accordingly, cardiac output is reduced in upright humans and the heart is operating at the ascending part of the Starling curve. A reduced cardiac output is of consequence for regional flow and may be surprisingly also for cerebral blood flow (CBF). That is the case although blood pressure at the level of the brain is virtually similar to the value in the supine position since blood pressure at the level of the heart increases when upright. It has been considered whether a reduction in the arterial carbon dioxide tension can explain the reduction in cerebral perfusion when upright. The end-tidal carbon dioxide tension is reduced markedly when humans are upright since the matsh been pulmonary perfusion and ventilation is enhanced. On the other hand, the reduction in the arterial carbon dioxide tension is comparatively modest and, at the most, has only a transient influence on CBF in upright humans. Thus, as demonstrated during physical exercise, both experimentally and in different patient populations, CBF is influenced not only by cerebral autoregulation and the arterial carbon dioxide tension but also by the ability to increase cardiac output. When upright, cerebral perfusion is affected by the reduction in cardiac output and only if the muscle pump provides sufficient venous return to the heart can cerebral perfusion and oxygenation be maintained.

CARDIOVASCULAR CONTROL, EXERCISE AND GRAVITY

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The transition from rest to exercise includes a marked increase of muscle blood flow, a reduction of total peripheral resistance, but nevertheless increases in arterial blood pressure. This review will address to what extent these readjustments are altered after long-term inactivity, such as bedrest, or long-term absence of hydrostatic gradients in the head-to-feet direction, such as during and after space flight.

Rest: Resting subjects frequently show impaired orthostatic tolerance after bedrest and spaceflight. Although small but significant changes of the carotid-cardiac-chronotropic baroreflex sensitivity has been observed under such circumstances, the tachycardic response to orthostatic stress seems to be intact during stand tests. Rather, present data suggests that is vascular arm of the baroreflex that is impaired in non-finishers during stand tests after spaceflight.

Exercise: The cardiovascular response to isometric muscle activity is a commonly used model to study the effects of central command and peripheral muscle receptors during exercise. Using such a model, bedrest was found to be followed by marked decrements of heart rate (HR) and blood-pressure (BP) responses to isometric lower arm exercise (ISO). After spaceflight, however, only a modest impairment were seen of the HR response to ISO.

The ability to maintain BP during orthostatic challenges in subjects performing dynamic leg exercise, has been studied by our group: Blood pressure swings in response to sudden up- and down tilts during exercise were exaggerated after long-term bedrest. This was associated with reduced amplitudes of tilt-induced compensatory HR responses. Over-all, the impaired buffering of BP in exercising humans after bedrest is likely caused by a higher background sympathetic drive and reduced stroke volumes. In turn, reduced stroke volumes are likely caused by combination of hypovolemia and cardiac atrophy.

NEUROENDOCRINE RESPONSES TO ORTHOSTASIS COMBINED WITH OTHER STRESS STIMULI

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Among the models of mental stress, the most intensive neuroendocrine activation has been observed in tests based on public speech. Frequently, the speech task is done standing in upright position following a period of sitting during which the subject has been preparing his performance. We tested whether simulation of postural changes, which occur during public speech task, activates neuroendocrine systems that could interfere with the effect of psychosocial stress (1). Plasma cortisol, ACTH and adrenaline increased during public speech performed in standing but not in response to postural changes only. The simulation of postural changes was associated with increases in the hear rate, blood pressure, plasma noradrenaline, aldosterone and renin activity, which were similar to those observed during the complete public speech task though the alterations were of much smaller magnitude. Thus, changing the sitting and standing position seems to interfere with neuroendocrine effects of the mental component of public speech tasks. The results of a public speech task study performed under real-life conditions, namely the first oral presentation of young neuroscientists at a scientific meeting also showed a contribution of changes in body posture. Moreover, we observed clear gender differences. In another group of healthy volunteers of both genders, a combination of a 30 min orthostatic stress and a mental stressor (Stroop test) failed to modify salivary cortisol. However, salivary aldosterone showed a significant increase in response to these two stressor, particularly in women in the luteal phase of the menstrual cycle. Our results show that daily stress situations or laboratory models combining mental stressors and changes in body posture have to be interpreted as complex stress stimuli.

(1) Mlynarik M., Makatsori A., Dicko I., Hinghofer-Szalkay H.G., Jezova D.: J Physiol. Pharmacol. 58, 95-103, 2007.

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ION AND WATER CHANELS INVOLVED IN CELL VOLUME CONTROL: REGULATION AND PHYSIOLOGICAL ROLES

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Ion and water transport mechanisms are critically involved in the regulation of cell proliferation and programmed cell death. In Ehrlich ascites tumor cells (EATC) we find, that the volume regulated anion channel (VRAC), the volume sensitive potassium channel TASK-2, and the water channel AQP5 all play essential roles in cell volume control as well as in cell proliferation and programmed cell death (PCD). In NIH3T3 cells there is an additional role for the Ca^{2+} permeable channel TRPC1 probably functionally coupled to Ca^{2+} activated K^+ channels. The molecular identity of VRAC is undefined and the mechanisms involved in VRAC activation are poorly understood, although several players have been identified. We find e.g that RhoA although not the volume sensor per se is an important upstream modulator of VRAC in NIH3T3 cells. The roles of VRAC in PCD and in cell proliferation were studied by measurement of VRAC currents, Cl^- movements and membrane potential changes during the cell cycle and during apoptotic volume decrease (AVD), and by using a high affinity anion channel inhibitor, the acidic di-aryl-urea NS3728. Moreover, we have cloned and expressed some members of the TMEM16 family of putative anion channels and using miRNA mediated knockdown and over-expression, respectively, we have investigated the possible involvement of the TMEM16 proteins in volume regulation, proliferation and apoptosis. The role of TASK-2 was studied by over-expression of TASK-2 and by inhibitor studies using clofilium. Tyrosin kinases are involved in the RVD response, but the specific mechanism is not known. An eventual tyrosine phosphorylation of TASK-2 during swelling was thus studied after immunoprecipitation of the channel from HEK cell in which we had over-expressed TASK-2. Using western blotting and the phospho-tyrosin antibody PY100 we found, that there is a large biphasic increase in the tyrosine phosphorylation of the TASK-2 channel after hypotonic cell swelling. EATC express mRNA transcript for AQP5, AQP3 and AQP9, with the expression of AQP5 being 50 times higher than the expression of the others. In multidrug resistant EATC we find, that the expression of AQP5 is strongly downregulated. We have generated stable EATC cell lines with constitutive miRNA mediated knockdown (miR-AQP5) or over-expression (AQP5ex) of AQP5, respectively, and studied them with regard to water permeability, cell volume regulation, growth rates and drug-induced PCD.

THE HYPERTONICITY-INDUCED CATION CHANNEL (HICC) IN HUMAN HEPATOCYTES: ROLE IN PROLIFERATION VS. APOPTOSIS AND MOLECULAR CHARACTERIZATION

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The molecular correlate of hypertonicity-induced cation channels (HICCs) and their role in proliferation vs. apoptosis is a matter of debate. We report here that, in whole-cell patch-clamp recordings, hypertonic stress (340 → 450 mosM) reversibly increased the Na^+ conductance of HepG2 cells from 0.8 to 5.8 nS. The effect was dose-dependently inhibited by flufenamate and amiloride, known blockers of HICCs, with some 50% efficiency at 300 μM. In parallel, both drugs decreased HepG2 cell proliferation (in MTT assays and with automatic cell counting). siRNA silencing of the α-subunit of the epithelial Na^+ channel (ENaC) reduced hypertonicity-induced Na^+ currents to 60% whereas the rate of HepG2 cell proliferation was approx. half of that of control. Moreover, α-ENaC siRNA inhibited the regulatory volume increase (RVI) of HepG2 cells (measured with scanning acoustic microscopy) by 60%. In FACS measurements, silencing of α-ENaC led to a significant decrease in the G1 and an increase in the G2/M phase of the cell cycle whereas the S phase was not changing. Finally (determined by a caspase 3/7 assay), HICC inhibition by flufenamate and Gd^{3+} as well as siRNA silencing of α-ENaC increased the rate of apoptosis in HepG2 cells. These data strongly suggest that α-ENaC is one of the functional elements of the HICC in HepG2 cells and that this channel subunit is an important mediator of cell proliferation; likewise, HICC blockage shifts the system from a proliferative into an apoptotic one.
The molecular partners of α-ENaC in completing the architecture of the HICC are currently determined by use of the split-ubiquitin yeast two-hybrid membrane system. As a further approach, a high-throughput screening of siRNA libraries is performed employing scanning acoustic microscopy, thus functionally relating the process of regulatory volume increase to various ion transporters, channels and putative regulators.

KINETICS OF FORCE-INDUCED CELL REORGANIZATION DEPENDS ON MICROTUBULES AND ACTIN

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The cytoskeleton is one important factor in the functional and structural adaption of cells to mechanical forces. We studied the kinetics of force-induced actin and microtubules reorganization to reveal a possible communication of the two networks at uniaxial cyclic stretching of NIH 3T3 fibroblasts. As reported in the past, cells responded by a reorientation perpendicular to the stretch direction. We showed that inhibiting or enhancing actin polymerization, respectively, or blocking myosin II activity abolished the stretch-induced perpendicular cell alignment. The maximum degree in cell reorganization was independent of functional microtubules, as previously reported; however, we demonstrated that the kinetics of reorientation was microtubule-dependent. The time of cellular reorientation was reduced upon microtubule-disruption and increased upon microtubule-stabilization. We contribute this to a sterical interaction of microtubules and actin cytoskeleton where microtubules impede actin reorganization. That finding is supported by our observation of a stretch-enhanced local co-alignment of microtubules and actin stress fibers. Furthermore, we reveal that a decreased migration for myosin II-inhibited cells at non-stretched control conditions could be rescued by cyclic stretch application.

We concluded that the kinetics of the force-induced cell reorientation was influenced by an interaction of MTs and actin, whereas the final degree of orientation was MT-independent.

OVERVIEW OF THE PATHOPHYSIOLOGY OF PURINERGIC SIGNALLING

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After a brief initial description of some of the important steps in the establishment of purinergic signalling, the talk will focus on some of the exciting developments relating to purinergic pathophysiology and potential therapeutic applications. There will be discussion of the long-term trophic effects of purines and pyrimidines in blood vessel remodelling in restenosis; plasticity of purinergic cotransmission of diseased urinary bladder and hypertensive rats; sperm motility in IVF; purinergic mechanosensory transduction in visceral pain and the role of spinal microglial purinoceptors in neuropathic pain; the potential for $P2X_7$ receptor antagonists in osteoporosis and kidney failure; the growing literature about the roles of purinergic signalling of disorders of the central nervous system; and the role of ATP in the treatment of cancer. Finally, a novel hypothesis will be presented for the involvement of purinergic signalling in acupuncture.

ATP-MEDIATED SIGNALLING IN TRIGEMINAL NEURONS IN A MIGRAINE ANIMAL MODEL

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Migraine attacks are characterised by strong headache with persistent duration that originates from long-lasting sensitisation of nerve terminals of trigeminal ganglion neurons that innervate meninges. Despite complex and multiple mechanisms remain poorly understood, current theories propose that endogenous substances, like Calcitonin gene related peptide (CGRP) and Nerve growth factor (NGF), released during a migraine attack from the trigeminal-vascular system sensitize trigeminal neurons to transmit nociceptive signals to the brainstem. Recently CGRP receptor antagonists have been proposed and they are currently under clinical trials on migraine patients. Recent studies indicate that acute, long-lasting sensitization of trigeminal nociceptive neurons occurs via distinct processes involving enhanced expression and function of ATP-gated P2X3 receptors known to play a role in chronic pain. We demonstrated that in trigeminal neurons, CGRP induces in a slowly-developing up-regulation of the ionic currents mediated by P2X3 receptors by enhancing receptor trafficking to the neuronal membrane and activating their gene transcription. Such up-regulated receptors acquire the ability to respond repeatedly to extracellular ATP, thus enabling long-lasting signalling of painful stimuli. In contrast, NGF induces rapid, reversible up-regulation of P2X3 receptor function via protein kinase C phosphorylation, an effect counteracted by in vivo NGF neutralisation. Furthermore studying the functional role of P2X3 phosphorylation, we demonstrated that these receptors are controlled by tyrosine kinase Csk, that operate a new direct important negative modulatory action on sensory neuron exitability. Using a genetic animal model of migraine pain, we are currently studying novel aspects of neuronal signalling transduction mechanisms in trigeminal neurons. The diverse intracellular elements used by CGRP and NGF show that sensitization of P2X3 receptor function depends from the complex integrated action of multiple cellular pathways. Our findings imply that combinatorial strategies to inhibit a chronic migraine pain attack might be most efficient approaches, and their efficacy might highly depend on the time of administration.

NUCLEOTIDE RECEPTORS AT THE NEURO-VASCULAR INTERFACE

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At least 25 years ago, ATP had been widely recognized as co-transmitter to noradrenaline in sympathetically innervated tissues, in general, and in blood vessels, in particular. Meanwhile, seven subunits of ionotropic P2X receptors and eight different G protein-coupled P2Y receptors have been identified. Several of these P2 receptors have been reported to mediate vasocontraction and/or vasorelaxation. In the smooth muscle cells, ionotropic $P2X_1$ receptors mediate fast and transient neurogenic vasoconstriction. Activation of mainly $P2Y_{12}$, but also of $P2Y_{1,2,4,6}$, mediates long lasting vasoconstriction. In addition, activation of endothelial P2Y receptors (mainly $P2Y_1$) has been shown to mediate vasodilatation via NO, prostacyclin, and endothelium-derived hyperpolarizing factor. Concerning the classical sympathetic transmitter noradrenaline, it is well known that presynaptic inhibitory α_2 and facilitatory β_2 receptors are responsible for the fine-tuning of the neuro-vascular transmission. For nucleotides, evidence has been obtained that presynaptic $P2Y_{12}$ receptors mediate an inhibition, while presynaptic $P2X_2$ and $P2Y_1$ receptors mediate a facilitation of sympathetic transmitter release. Further complexity is added to the regulatory system of nucleotide receptors at the neuro-vascular interface by direct interactions between different P2Y receptors and between P2Y receptors and ectonucleotidases. Together, the data summarized here show that the co-transmitter ATP is much more versatile than the original neuro-vascular transmitter noradrenaline.

PURINERGIC SIGNALING IN THE PULMONARY NEUROEPITHELIAL BODY MICROENVIRONMENT UNRAVELED BY LIVE CELL IMAGING

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Pulmonary neuroepithelial bodies (NEBs) occur in the airway epithelium as densely innervated groups of neuroendocrine cells that are largely shielded from the airway lumen by a specialized type of Clara cell, the so-called Clara-like cells. In *ex vivo* mouse lung slices, NEB cells respond with a rise in intracellular calcium ($[Ca^{2+}]_i$) upon membrane depolarisation with high K^+, typically followed by a delayed $[Ca^{2+}]_i$ increase in the surrounding Clara-like cells, suggestive of an indirect activation. Aim of the present study was to explore the mechanism of this potential interaction between NEBs and Clara-like cells. We employed confocal live cell imaging microscopy and novel electrophysiological techniques in the *ex vivo* lung slice model and focused on a possible purinergic signalling pathway.
Quinacrine histochemistry indicated high amounts of vesicular ATP in NEB cells. Using a 'reporter-patching' method adapted to create a uniquely sensitive and selective biosensor for the direct detection of ATP release from NEBs *ex vivo*, we demonstrated quantal ATP release from NEBs following their depolarisation with high K^+. Use of enhanced extracellular enzymatic ATP hydrolysis or the P2 receptor blocker suramin confirmed the central role of ATP in the paracrine interactions between NEBs and Clara-like cells. Combined calcium imaging, pharmacology and immunohistochemistry showed that ligand-binding to functional $P2Y_2$ receptors underpins the activation of Clara-like cells.
Hence, NEB cells communicate with their cellular neighbours in the NEB microenvironment by releasing ATP, which rapidly evokes purinergic activation of surrounding Clara-like cells. Besides ATP acting on the $P2X_3$ receptor expressing vagal sensory nerve terminals that we reported earlier between NEB cells, local paracrine purinergic signalling within this potential stem cell niche may be important to both normal airway function, airway epithelial regeneration after injury, and/or the pathogenesis of small cell lung carcinomas.

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EXTRACELLULAR ATP AND P2 RECEPTORS IN NEURODEGENERATIVE DISEASES: P2X7 IS AN OBLIGATE COMPONENT OF MICROGLIA RESPONSE TO AMYLOID BETA

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Extracellular ATP is a mediator of intercellular communication and a danger signal. Release of this nucleotide activate the $P2X_7$ receptor subtype which stands out for its pro-inflammatory activity. Furthermore, the $P2X_7$ receptor is up-regulated in a transgenic model of Alzheimer's disease and in brains from Alzheimer's patients. In this study we show that amyloid β (Aβ) triggers increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$), ATP release, IL-1β secretion and plasma membrane permeabilization in microglia from wild type but not from $P2X_7$-deleted mice. Likewise, intra hippocampal injection of Aβ causes a large accumulation of IL-1β in wild-type but not in $P2X_7^{-/-}$ mice. These observations suggest that Aβ activates a purinergic autocrine/paracrine stimulatory loop of which the $P2X_7$ receptor is an obligate component. Identification of the $P2X_7$ receptor as a non-dispensable factor of Aβ-mediated microglia stimulation may open new avenues for the treatment of Alzheimer's disease.

TRANSCRIPTIONAL REGULATION OF TYPE-2 METABOTROPIC GLUTAMATE RECEPTORS: A POTENTIAL STRATEGY FOR CHRONIC PAIN TREATMET

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All current analgesic drugs target functional proteins (such as ion channels, G-protein coupled receptors, membrane transporters, and intracellular enzymes) that are widespread and undergo plastic modifications in response to exogenous ligands. As a result, tolerance and serious adverse effects are frequently encountered with analgesic drugs. In addition, there are types of pain, such as neuropathic pain, that may be refractory to multiple classes of analgesics, and are difficult to treat.
The study of group-II metabotropic glutamate receptors (mGlu2 and mGlu3 receptors) has disclosed a new strategy for the treatment of chronic pain. mGlu2 and mGlu3 receptors are coupled to Gi proteins and are preferentially localized on axon terminals, where they negatively regulate neurotransmitter release. Activation of mGlu2/3 receptors inhibits pain transmission at the synapses between primary afferent fibres and neurons in the dorsal horn of the spinal cord. As expected, mGlu2/3 receptor agonists produce analgesia in models of inflammatory and neuropathic pain but their use is limited by the development of tolerance.
A new therapeutic strategy stems from the finding that the acetylating agent, L-acetylcarnitine (LAC), that has been shown to have analgesic activity, enhances the expression of mGlu2 receptors in the dorsal root ganglia (DRG), dorsal horn of the spinal cord., and cerebral cortex. Interestingly, LAC-induced analgesia is abrogated by a single injection of the mGlu2/3 antagonist, LY341495, suggesting that LAC relieves pain through the induction of mGlu2 receptors.
LAC up-regulates mGlu2 receptors by acetylating the transcription factor p65/RelA of the nuclear factor-κB (NFκB) family. Acetylation/deacetylation processes of p65/RelA are mediated by histone acetyl transferases and histone deacetylases (HDACs), respectively. We have shown that HDAC-1 and -2 are expressed in DRG neurons and that the HDAC inhibitors, SAHA and MS-275, up-regulate mGlu2 receptors in the DRG and spinal cord, and relieve pain in mice subjected to the formalin test.
These findings suggest that the epigenetic control of mGlu2 receptors may be targeted by novel analgesic drugs and that "epigenetic" drugs could optimize the analgesic activity of receptor agonists by increasing the receptor reserve of mGlu2 receptors along the pain neuraxis, thereby limiting the development of tolerance in response to receptor activation.

GABAb IMPAIRMENT IN THE DORSAL HORN OF NEUROPATHIC RATS: POSSIBLE ROLES FOR ASSOCIATED PROTEINS

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Aim of Investigation:
Pathological states of neuropathic pain are related to sensitization of deep dorsal horn neurons that are likely to amplify nociceptive transmission, causing allodynia and hyperalgesia. The efficiency of GABAb agonists in the treatment of chronic pain is controversial. The present project aims to investigate possible dysfunction of GABAb inhibitory control of pain sensitization in the spinal cord of animal models.
Our working hypothesis is that functional alteration of heterodimeric GABAb receptor depends on its dissociation, possibly triggered by intracellular partner proteins such as 14.3.3.
Methods:
We investigated the co-distribution of GABAb1 and 14.3.3 with microscopy techniques. The physical interactions between the various partner proteins have been studied with FRET/FLIM imaging and with co-immunoprecipitation approaches. The functional outcome of these interactions have been assessed in vitro with patch-clamp techniques, and in vivo with behavioural tests.
Results:
Our results showed that in a rat neuropathic pain model, 14.3.3 expression is up-regulated in the dorsal horn. GABAb receptor and 14.3.3 are codistributed in the same spinal neurons. The relative distribution of GABAb and 14.3.3 in spinal cord, investigated by electron microscopy, showed that the subcellular colocalization between GABAb1 and 14-3-3 is increased after spinal nerve ligation.
Moreover, in cultures of spinal neuron, 14.3.3 overexpression resulted in a partial loss of colocalization between GABAb1 and b2 subunits, suggesting that 14.3.3 induced the dissociation of the GABAb receptor. A FRET-based imaging approach further confirmed the dynamic of 14.3.3-GABAb1 interactions in neurons.
In COS cell culture, immunoprecipitation experiments demonstrated the physical association between 14.3.3 and GABAb1 subunit. Furthermore, it ensured the effects of 14.3.3 overexpression in disrupting the GABAb heterodimer.
To reverse GABAb dysfunction in the dorsal horn, we designed different strategies based on siRNA-mediated 14.3.3 knock-down and on the use of synthetic peptides that block 14.3.3 binding site on GABAb1 subunit. The behavioural assessment of mechanical allodynia revealed that both strategies are efficient in potentiating the inhibitory effect of intrathecally-injected Baclofen in neuropathic rats.
Conclusions:
By targeting associated proteins, it may thus be possible to improve the pharmacological efficiency of GABAb agonists in the treatment of neuropathic pain.

METABOTROPIC GABA-B RECEPTOR-MEDIATED EFFECTS IN NOCICEPTION

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The metabotropic GABA-B receptors are present in the nervous system where they play important roles also in the nociceptive processing. The GABA-B specific agonist baclofen, in fact, proved to be anti-nociceptive in models of acute and chronic pain. In this context, the study of GABA-B1$^{-/-}$ knockout mice revealed a hyperalgesic status, supporting the contribution of GABA-B receptors in the central nociceptive processing. A tonic GABA-B receptor activation, therefore, appears to contribute to the establishment of the nociceptive threshold. Interestingly, GABA-B receptors are expressed in the peripheral nervous system (PNS), mainly in the Schwann cells where they participate in the control of cell proliferation and myelination. Recent studies in GABA-B1$^{-/-}$ mice suggested the peripheral contribution of these receptors also to the nociceptive processing. These mice presented a thermal hyperalgesia, a higher mechanical threshold to Von Frey filament without signs of allodynia, and typical gait alterations. GABA-B1$^{-/-}$ mice also show morphological and molecular changes in peripheral nerves, including an increased number of small myelinated fibers and small neurons of the lumbar dorsal root ganglia. These fibers were supposed to be Adelta nociceptive fibers, suggesting that GABA-B receptors are involved both in the PNS nociception and in the myelination processes. Our recent studies in conditional mice specifically lacking GABA-B1 receptor in Schwann cells were aimed at clarifying the specific role of GABA-B receptors in peripheral pain sensitivity. These conditional mice showed a hyperalgesic status, which seem to correlate with an increase in unmyelinated fibers. Although the GABA-B1 conditional mice show several myelin abnormalities, however, the morphological features of these mice were different from those previously observed in total null GABA-B1$^{-/-}$ mice. Altogether, our observations suggest a putative Schwann cell autonomous nociceptive phenotype, which might be of therapeutic interest for the treatment of pain.

DEVELOPMENT OF POTENTIAL ANTIHYPERALGESIC DRUGS TARGETING GROUP III MGLURS

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Glutamate is the main excitatory neurotransmitter of the mammalian central nervous system. As a result, glutamate neurotransmission is involved in many physiological and pathophysiological processes. Glutamate exerts its action through activation of two broad classes of receptors: ionotropic and metabotropic G-protein coupled receptors.
Metabotropic glutamate receptors (mGluRs) have been shown to regulate nociceptive signalling at different levels of the nervous system. While the role of group I and II mGluRs in pain is now well documented, less is known on group III mGluRs due in part to the lack of specific pharmacology for these receptors.
Recently, we and others have shown that spinal group-III metabotropic glutamate receptors activation reduced hyperalgesia in different animal models of neuropathy and inflammation, while acute pain perception remained unchanged in healthy animals. Agonists of these receptors thus appear as promising new therapeutic agents to treat neuropathic or inflammatory pain.
This talk will present our efforts to improve the pharmacological toolbox of these receptors by the development of new ligands in order to reinforce the evidences of group III mGluRs involvement in pain modulation and ideally validate these receptors as therapeutic target to treat chronic pain.

FRIDAY, 13. NOVEMBER 2009:

SESSION IV – POSTER SESSION

MEMBRANE DYNAMICS OF 3T3-L1 ADIPOCYTES AND THE EFFECT OF ROSIGLITAZONE

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White adipocytes constitute one of the most important target cells for insulin action to lower blood glucose level in the body. Thiazolidinediones (TZD), a class of new oral hypoglycemic agents, mediate their effect through the peroxisome proliferator-activated receptor γ (PPARγ), which is highly abundant in adipose tissue. Rosiglitazone is a potent agonist of PPARγ and could significantly improve the adipocyte differentiation in 3T3-L1 cells. Application of insulin induces an increase in membrane capacitance (C_m) of single white adipocytes. We wanted to determine: (I) whether the treatment of 3T3-L1 cells by rosiglitazone affects the rate of differentiation and the size of single 3T3-L1 adipocytes, (II) whether changes in C_m of insulin-treated single 3T3-L1 adipocytes exhibit an increases in PM area as shown previously for primary adipocytes, (III) whether activation of PPARγ by rosiglitazone significantly alters the specific insulin induced changes in membrane surface area of single adipocytes. We differentiated 3T3-L1 fibroblasts into 3T3-L1 adipocytes and used them for monitoring net changes in PM surface area by the electrophysiological measurements of membrane capacitance (C_m), a parameter linearly proportional to the PM surface area. The presence of rosiglitazone increased the fraction of differentiated 3T3-L1 adipocytes in cell culture vs. controls and significantly reduced the size of single 3T3-L1 adipocytes. Insulin increased the rate of C_m, which was significantly different from controls. Pretreatment of cells with rosiglitazone prior to the treatment with insulin resulted in attenuated rate of Cm increase. In rosiglitazone treated cells, insulin stimulates a rapid increase in exocytosis that is associated with a similar increase in the rate of endocytosis, since both processes appear to be controlled by insulin. Thus secretory function and/or transporter density regulation would be preserved under such condition, while the insulin-induced increase in C_m is attenuated. The current study suggests that activation of PPARγ by a competitive PPARγ receptor agonist, rosiglitazone, significantly attenuates the specific insulin-induced time-dependent changes in membrane area of single adipocytes.

CONNEXIN 43 ENHANCES MIGRATION OF HELA CELLS IN A GAP JUNCTION INDEPENDENT MANNER

Juliane Behrens, Ulrich Pohl, Kristin Pogoda

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Background: Connexin 43 (Cx43) is one of the most widely expressed connexins in the body. Beside its channel forming capacity, Cx43 has also an important role as an adapter protein with binding sites for various proteins at its cytoplasmic C-terminal part. Since connexin expression is often altered during processes with enhanced cell migration such as wound healing, angiogenesis and arteriosclerosis, we analyzed whether Cx43 affects migration in a gap junction dependent or independent manner.
Methods: HeLa cells, which as wild type do not express any connexins, were stably transfected with cDNAs encoding either full length Cx43 (Cx43fl, aa 1-382) or a C-terminus truncated Cx43 mutant, defined here as the "N-terminal part" (Cx43NT-GFP; aa 1-258). Alternatively, a cDNA encoding only the cytoplasmic C-terminal tail was transfected (Cx43CT-GFP; aa 258-382). Both mutants were coupled with the green fluorescent protein (GFP). HeLa cells expressing GFP only (HeLa-GFP) were used as additional controls.
Results: Confocal microscopy as performed in confluent HeLa cells showed cytosolic and mainly membranous localization of Cx43NT-GFP, while Cx43CT-GFP and GFP alone were found to be expressed in the cytosol only. Likewise, immunofluorescence stainings of HeLa 43fl cells with Cx43 antibody showed punctated expression of Cx43 at cell-cell-contact regions and also in the cytoplasm. To analyze cell coupling, the transfer of a gap junction permeable dye (Calcein-AM) between two differently stained HeLa populations, coincubated for 3 hours, was measured. FACS analysis (n = 4) indicated functional cell coupling between cells expressing Cx43fl (64 ± 16%) and to a lesser but still significant extent in HeLa-Cx43NT-GFP cells (41 ± 9%). In contrast, no coupling was observed in HeLa-GFP cells as well as in cells expressing Cx43CT-GFP. Cell migration was studied optically by time lapse microscopy for 24 hours using wound assay chambers (Ibidi) in which the cells migrate into a defined gap (n = 5). HeLa-Cx43CT-GFP cells showed significantly increased migration distances (accumulated distance 212 ± 16 µm) compared to HeLa cells expressing Cx43NT-GFP (accumulated distance 157 ± 17 µm). These results suggest that Cx43 enhances cell migration in a gap junction independent manner. In fact its C-terminal part seems to be essential for this function, which does not require membrane localization of the molecule.

ROLE OF CALCIUM AND MEMBRANE CHOLESTEROL IN PECULIAR SECRETORY RESPONSE OF INS-1E CELLS TO CELL SWELLING

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An important role of plasma membrane is to maintain flexible barrier between intracellular and extracellular space. Cells undergo volume changes associated with secretion of material stored in secretory vesicles. Swelling of secretory vesicles precedes exocytosis, so we hypothesized, that cell swelling stimulated exocytosis is induced and mediated by direct biophysical effect of hypotonicity. Our previous results showed, that in contrast to natural ß-cells and INS-1 cell line, INS-1E cells do not release insulin in response to cell swelling in spite of good response to glucose stimulation. One of possible explanations of this phenomenon is a special role of calcium; surprisingly, perifusion with Ca^{2+}-depleted medium showed distinct secretory response of INS-1E cells to hypotonicity while that of INS-1 cells was partially inhibited. To analyze the mechanism, we tested the effect of blockers of Ca^{2+} channels on hypotonicity-induced insulin secretion: they did not prevent stimulation of secretion from isolated pancreatic islets or INS-1 cells, secretion from INS-1E cells appeared. Tetanus toxin, a metalloprotease inactivating soluble SNARE proteins, in presence of Ca^{2+} blocks hypotonicity-induced secretion from pancreatic islets. Unexpectedly, tetanus toxin in presence of calcium and Ca^{2+} channel blockers (nifedipine, ω-agatoxin and mibefradil) did not prevent swelling-induced insulin secretion from INS-1E cells. Another participating mechanism of special behavior of INS-1E cells may be a change of membrane fluidity dependent on cholesterol content; INS-1E cells have significantly higher cholesterol content than INS-1 cells at basal condition. To define the role of membrane cholesterol in glucose- and swelling-induced insulin secretion from isolated Langerhans islets and insulinoma cell lines INS-1 and INS-1E we added 2-hydroxypropyl-ß-cyclodextrin or carboxymethyl-ß-cyclodextrin (agents removing membrane cholesterol) to preincubation medium (2 hours). Glucose- and hypotonicity-induced insulin secretion from freshly isolated rat Langerhans islets was prevented by preincubation with ß-cyclodextrins (2% w/v). Both glucose and swelling stimulated insulin secretion were not changed in insulinoma cell lines at this concentration. Higher concentration of ß-cyclodextrins inhibited swelling-induced insulin secretion from INS-1, but stimulated secretion from INS-1E cells. These data demonstrated different sensitivity of secretory mechanism to changes of cholesterol content in two tumor cell lines and pancreatic islets. Conclusion: Mechanism of exocytosis from INS-1E seems to differ from that in pancreatic islets or INS-1 cells. Ca^{2+} and membrane cholesterol participate in the mechanism of this difference.

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ROLE OF CALCIUM, PROTEIN KINASE C (PKC), PROTEIN TYROSINE PHOSPHATASES (PTP1B) AND SNARE PROTEINS IN ETHANOL- INDUCED INSULIN SECRETION FROM TUMOR BETA CELL LINES

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Secretion of insulin could be stimulated by several ways. Comparison of glucose- and swelling induced mechanisms in pancreatic islets revealed the involvement of a novel signal transduction pathway with specific features of osmotically stimulated peptide hormone release including Ca^{2+} independence and resistance to noradrenalin inhibition. Cell swelling can be induced by hypotonicity or small permeant molecules (e.g. ethanol, urea). Objective of this study was characterization of ethanol-induced insulin secretion. Signaling of ethanol- and glucose-induced insulin release from INS-1 and INS-1E cells was compared. Both cell lines responded similarly to all experimental interventions. In contrast to glucose, ethanol-induced insulin secretion was not hindered in calcium depleted medium or by addition of 10 μmol/l BAPTA/AM (intracellular chelator). Inhibitor of protein kinase C Bisindolylmaleimide (3 μmol/l) in contrast to glucose stimulation did not inhibit ethanol-induced insulin secretion. Tetanus toxin (20 nmol/l), inhibitor of SNARE proteins complex formation, blocked ethanol-induced insulin secretion. Both 5 mmol/l N-ethylamaleimide and 10 μmol/l $ZnCl_2$ (inhibitor of protein tyrosine phosphatases), which block disassembly of SNARE complexes and their further participation in exocytosis, increased basal insulin secretion. In contrast to glucose, already high insulin secretion was further increased after ethanol stimulation in either treatment. Conclusion: Signaling of ethanol-induced insulin secretion from INS-1 and INS-1E cell lines bypasses calcium and PKC involving steps, is sensitive to tetanus toxin but resistant to N-ethymaleimide and $ZnCl_2$. An extra pool of secretory vesicles not available for glucose is exploited for exocytosis after ethanol stimulation.

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MEASUREMENTS OF THE MEMBRANE CAPACITANCE IN RAT LACTOTROPHS AT SPONTANEOUS AND STIMULATED CONDITIONS

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Hormones and neurotransmitters are released from cells by being passed through a fusion pore that forms following the fusion of the vesicle and the plasma membrane. The fusion pore can be a rate limiting step since it can either expand fully leading to the release of the entire vesicle content (full fusion exocytosis), or reversibly closes retaining vesicle integrity and some of the residual cargo ("kiss-and-run" exocytosis). By measuring changes in membrane capacitance, which is proportional with the membrane surface area, we can directly monitor fusion and fission events of secretory vesicles. We therefore used the cell-attached patch-clamp technique to directly monitor discrete changes in membrane capacitance in isolated rat lactotrophs at spontaneous conditions and after stimulation with cAMP-raising agents. Our results confirm that discrete changes in membrane capacitance very likely represent fusion/fission of prolactin-containing secretory vesicles with the plasma membrane. Furthermore, transient fusion events ("kiss-and-run" exocytosis) appear to be the predominant mode of exocytosis in rat lactotrophs at spontaneous and stimulated conditions. Finally, stimulation increased the frequency of these events events, whereas the frequency of full fusion exocytotic events was not affected by the cAMP-enhancing agents.

IMPAIRED SEROTONYLATION IN PANCREATIC β-CELLS CAUSES DIABETES MELLITUS

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Diabetes mellitus is one of the most important metabolic disorders and is characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. However, many aspects of insulin secretion remain elusive for the complex machinery involved, in particular the role of serotonin (5-HT) in endocrine pancreatic beta-cells. It is known for more than three decades that 5-HT is stored together with insulin in secretory beta-granules and it is co-released when pancreatic islets are stimulated with glucose. Nevertheless, the function of 5-HT in this context was not identified so far. 5-HT is a neurotransmitter of major importance as well as a peripheral hormone with multiple actions in primary haemostasis, the cardiovascular and immune system. It is synthesized independently by two rate-limiting tryptophan hydroxylase isoenzymes in peripheral tissues (TPH1) and neurons (TPH2). In addition to the classic signalling between cells via 5-HT surface receptors, the generation of *Tph1*-/- mice has recently allowed us to identify a receptor-independent signalling mechanism of 5-HT within thrombocytes, which we have termed "serotonylation". This transglutaminase-dependent mechanism bases on the constitutive activation of small GTPases by covalent binding of 5-HT to a highly conserved glutamine residue within their catalytic core, thereby triggering the exocytosis of alpha-granules of the thrombocytes.
In our present study we show that 5-HT regulates insulin secretion by serotonylation of GTPases within endocrine pancreatic beta-cells. These data suggest that intracellular 5-HT acts in various 5-HT containing tissues via this signalling mechanism.

THE ROLE OF SEROTONIN IN AETIOLOGY AND COMPLICATIONS OF DIABETES

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Recently, we have shown that serotonin (5-HT) regulates the insulin secretion of pancreatic beta-cells. As a consequence, mice lacking 5-HT in peripheral tissues (*Tph1*-/-) have an impaired insulin secretion and are diabetic. Diabetes mellitus, if left untreated, significantly curtails life expectancy, because chronic hyperglycaemia damages blood vessels and shortens energy supply of organs. Therefore we expected a curtailed life span of the diabetic *Tph1*-/- mice. Unexpectedly, these mice showed none of the typical diabetes-associated complications like retinopathy, nephropathy, or neuropathy and lived as long as their wild type littermates. We have previously demonstrated that *Tph1*-/- mice are less prone to succumb to vessel occlusion in experimental thromboembolism and thrombosis due to a reduced primary haemostatic response. Combining these findings, therapeutic peripheral 5-HT reduction specifically in thrombocytes, together with blood glucose management, might ameliorate vascular disease and its complications in diabetic patients.

To verify this hypothesis, we are generating an inducible animal model for type 1 diabetes, as most of the currently applied models have some major drawbacks concerning reproducibility, disease incidence and time of onset. Our model bases on the b-cell specific expression of the innoxious *E. coli* nitroreductase (NTR) which is able to convert the inactive protoxin 5-aziridin-1-yl-2,4-dinitrobenzamide (CB1954) to a potent cytotoxin. Targeted cells are thus specifically killed upon CB1954 administration. Instead of the wild type gene we used a version with several silent mutations (*ntro*) to adapt the prokaryotic gene to the codon usage in mammalian cells, which largely enhances protoxin sensitivity. The reduction of 5-HT levels in the *ntro*-mice should clarify its impact on healthiness in diabetes.

INVOLVEMENT OF ATP DEPENDENT K CHANNELS TO PROLIFERATIVE EFFECT OF ERYTHROPOIETIN

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Aim and scope: The haematopoietic factor erythropoietin (EPO) has recently been recognized to play a physiological role in cellular survival. In vitro studies have shown that EPO has direct effects on proliferation and cell death in proximal tubular epithelial cells. Recently, it has been reported that mitochondrial K-ATP channel openers have an effect on myocardial protection via a pharmacological preconditioning action with EPO. The studies related to role of K-ATP channels in renal ischemia pathophysiology and they have conflicting results. In this study we aimed to evaluate effect of K-ATP channels blocker glybenclamide and opener diazoxide in hypoxia and EPO treatment.
Methods and material: In this study; human renal proximal tubular cell line CRL-2830 incubated with EPO (20 iu/ml), glybenclamide (100 μM) and diazoxide (100 μM) at the 2-24-48th hours. Apoptotic activity, (caspase-3 levels), cellular proliferation (MTT) and expression of Kir6X channels was evaluated with western blot.
Results: EPO induced prominent proliferation in renal tubuler cells. Erythropoietin attenuates hypoxia-induced apoptosis of tubular cells in a dose-time dependent manner. Diazoxide has similar effect with EPO. Glybenclamide decrased cellular proliferation, induced caspase-3 activity, apoptosis and cell death. Glybenclamide blocked protective role of EPO. EPO treatment increased Kir 6.1 and 6.2 expression while glybenclamide decreased.
Conclusions: EPO and diazoxide treatment are found protective against hypoxia induced cytotoxicity. K-ATP channels are important for regulation of metabolic and acid-base balance of the cells, especially for renal tubular functions. Glybenclamide is currently used as an antidiabetic and renal pathologies are very common in progress of diabetes. Our results might be informative for regulation of treatment protocols of diabetic patients.

This study is supported by TUBITAK (SBAG-108S248).

PROTECTIVE EFFECT OF ERYTHROPOIETIN IN TESTICULAR TORSION

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Aims and scope: testis torsion is an acute condition which is characterized by circulation disturbances in testis tissue. It was shown that testis torsion has similar pathophysiological mechanisms. Our previous studies showed that erythropoietin (EPO) is protective in testicular torsion of rats. Hypoxia inducible factor (HIF-1) expression increases in hypoxic conditions and accepted as protective against apoptosis. This study was designed to investigate the effects of EPO treatment after unilateral testicular torsion.
Material-Method: Fifty male Sprague-Dawley rats were divided into five groups. Group 1 underwent a sham operation of the right testis under general anesthesia. Group 2 was same as sham, and EPO (3,000 IU/kg) infused i.p., group 3 underwent a similar operation but the right testis was rotated 720° clockwise for 1 h, maintained by fixing the testis to the scrotum, and saline infused during the procedure. Group 4 underwent similar torsion but EPO was infused half an hour before the detorsion procedure, and in group 5, EPO was infused after detorsion procedure. Four hours after detorsion, ipsilateral and contralateral testes were taken out for evaluation. HIF-1 α and TNF- α mRNA expressios are evaluated with RT-PCR and apoptosis via measurement of caspase-3 activity by spectrophotometric method.
Results: Treatment with EPO improved testicular tissue and germ cell apoptosis in both testes following testicular IR. HIF-1 α mRNA expression was increased, TNF- α expression was decreased after EPO treatment. Tissue caspase-3 activity was negatively correlated with tissue HIF-1 mRNA expression levels.
Conclusion: We concluded that testicular I/R causes an increase in germ cell apoptosis both in the ipsilateral and contralateral testes. Eryhropoietin has antiapoptotic and anti-inflammatory effects following testicular torsion by inducing most important hypoxia regulated gene HIF- α.

This study is supported by TUBITAK project no: SBAG-AYD-487.

SYNERGIC CYTOTOXIC EFFECT OF IMATINIB MESYLATE AND GLIBENCLAMIDE IN HUMAN GLIOBLASTOMA CELL LINE

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Aims and scopes: Imatinib Mesylate, a novel tyrosine kinase inhibitor, has recently been shown to be efficacious in preclinical trials for glioblastoma. It is an ATP analogue, potently and selectively inhibits several protein tyrosine kinase receptors for PDGF, including bcr-abl, and c-kit by interacting with their ATP-binding site. ATP dependent K channels (K-ATP) have important roles in cell metabolism, survival. Glibenclamide, an antidiabetic agent, has been shown to effect drug resistance site SUR subgroups and specific K-ATP channel blocker. Despite these results, the potential modulatory effect of glibenclamide or combined efficacy of imanitib against brain tumours have not been evaluated experimentally. In this study we investigated whether glibenclamide has a synergistic or an antagonistic effect on imatinib-induced cytotoxicity.

Material-method: The T98G human glioma chemoresistant experimental brain tumour cell line, that is notoriously difficult to treat with combination chemotherapy, was used in monolayer cultures. We treated T98G glioma cells with a combination of glibenclamide and imatinib at a concentration of 100 and 10 μM respectively at 24^{th} and 72^{th} h. Following treatment, we evaluated cell proliferation (MTT), apoptosis (caspase-3 activity), midkine (ELISA) and multidrug resistance protein-MRP (western blot) levels.

Results: Glibenclamide and imatinib induced apoptosis and cytotoxicity in gliablastoma cells. Combined usage of two drug has synergic effect on cytotoxicity. Glibenclamide decreased drug resistance protein levels at 24 and 72^{th} h whereas gliablastoma cells develop resistance to imatinib at 72^{th} h. Both drugs have decreasing effect on midkine levels which is a hypoxia induced growth factor; takes part in cell proliferation, migration, angiogenesis and inflammation.

Conclusions: Our data shows that a combination of imatinib and glibenclamide resulted in enhanced *in vitro* antitumour activity against human T98G glioma cells in monolayer cultures. Although imatinib's antiproliferative effect has been shown in several cancer cell lines, including human myelogenous leukemia, breast tumor, and melanoma, this is the first study to report glibenclamide's inhibitory effects against a glioma cell line, as well as demonstrate anti-glioma synergy with imatinib and its ability to induce apoptosis. Glibenclamide might be a potent chemotherapic agent to reverse multidrug resistance to cancer chemotherapy in several tumor cell lines *in vitro*.

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LIVER ISCHEMIA-REPERFUSION INJURY AND ITS EFFECT ON THE ACTIVITY OF NEURONS IN SELECTED BRAIN AREAS

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Liver ischemia-reperfusion injury (LIRI) is associated with an increase in alanine (ALT) and aspartate (AST) aminotransferases levels in the blood, production of proinflammatory cytokines like TNF or IL-1 by immune cells, and reduction in the bile flow. These information are transferred to the brain by vagal and spinal cord visceral afferents. In this study, an attempt was made to reveal the responses of liver related brain structures to 1) the ligation of the hepatic artery for 30 min and 2) the combined ligation of the portal triade (the hepatic artery, portal vein, and bile duct) for 15 min in male Wistar rats. Serum levels of ALT, AST, TNF, and IL-1α were measured and cell activities analysed in the subfornical organ (SFO), suprachiasmatic (SCH), paraventricular (PVN), supraoptic (SON), arcuate (Arc) and ventromedial (VMN) hypothalamic nuclei, parabrachial nucleus (PBN), locus coeruleus (LC), nucleus of the solitary tract (NTS) and A1/C1 catecholaminergic cell groups by counting Fos nuclear immunoreactivity, as a general marker of neuronal activity, 90 min, 5 h and 24 h after the liver reperfusion. Ligation of the portal triade elevated ALT serum level after 90 min and 24 h. Increased AST level was observed only after 24 h in all operated groups of rats. IL-1α serum level was increased only 90 min after ligation of the portal triade. TNF level did not change in any group of rats studied. Ninety minutes after both types of surgeries Fos immunoreactivity was markedly elevated in comparison with sham-operated animals. Five hours after surgeries Fos increased in the PBN and NTS and after 24 hours decreased in all the experimental groups. Obtained data indicate that increased neuronal activity after both types of LIRIs is a primary consequence of the liver damage, but an effect of partial impact of certain non-specific factors can not be also excluded. Anatomical distribution of Fos protein detected after LIRIs gives an opportunity to perform more detailed study, i.e. in the future to identify the specific neuronal phenotypes activated by LIRIs.

This work was supported by grant of the Ministry of Health of the Slovak Republic under project „Stimulation of the vagus nerve as a new method for prevention of ischemia-reperfusion injury of transplanted organs“.

STRUCTURAL AND COMPUTATIONAL CLUES TO SENSORY ADAPTATION AND DIRECTIONAL SENSITIVITY OF FILIFORM SENSILLA IN THE FIREBUG (*PYRRHOCORIS APTERUS*, HETEROPTERA)

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Insect filiform sensilla are simple sensory organs. They consist of a thin cuticular hair and a single sensory cell. Despite the simplicity they can encode a surprising amount of information, such as stimulus intensity, velocity and direction, and are therefore an interesting case study of mechanosensory systems. The hair of the sensillum is a lever that transforms movement into force that acts upon the sensory dendrite. The sensory cell encodes the time course of the force, first into a receptor potential and then into a spike train. During this process mechanical components of the sensory apparatus and the sensory adaptation play a crucial role. In the first part of this poster we show that in firebug filiform sensilla the adaptation has multiple sources, and in the second part we explore the basis of their directional sensitivity. The firebug filiform sensilla are classified into three types. Types T1 and T2 have a phasic-tonic response and T3 a phasic response. During response to a ramp stimulus all units exhibit a complex sensory adaptation that decreases spike frequency already during the dynamic part of the stimulus. During the static part it has at least two phases ($\tau 1 \approx 10$ ms and $\tau 2 \approx 1$ s), which differ between the types. A negative correlation between neighboring interspike-intervals during resting activity indicates that the adaptation takes place on the level of the spike generator. To test this hypothesis we modeled the response of type T1 receptor with a mathematical model of sensory adaptation (Benda, 2003) that assumes adaptation on the level of the spike generator. The simulation was successful for slow stimuli, however, it failed at higher velocities. Another mechanism, activating at higher velocities and adapting the signal prior to the generator, can therefore be anticipated. Out of all three types only T1 is directionally sensitive. We investigated the stimulus transmitting apparatus of types T1 and T3 with standard methods of light and electron microscopy to link its structure to sensilla directionality. Our preliminary results show that the dendrite is on one side attached to a fibrous lamina, which appears to be the main stimulus transmitting component. However, only the dendrite of type T1 is pronouncedly bent in the excitatory direction. This distinction between the types could explain the directional sensitivity as well as the differences in their excitatory dynamics.

Benda J., Herz A. 2003. A universal model for spike-frequency adaptation. Neural Comput, 15:2523-2564.

AGE-RELATED INFLAMMATORY PROCESSES IN HIPPOCAMPUS OF MALE RATS. BENEFICIAL ACTIONS OF MELATONIN AND GROWTH HORMONE

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During normal aging levels of melatonin and growth hormone (GH) decreases and brain suffers both morphological and functional modifications. Markers of chronic inflammation and oxidative damage increase in CNS with age.

Twenty-four male Wistar rats of 22 months of age were divided into three groups. One group remained untreated and acted as the control group. The second was treated with growth hormone (GH) (2 mg/kg/d sc) and the third was submitted to treatment with 1 mg/kg/d of melatonin in the drinking water. A group of 2-months-old male rats was used as young controls. After 10 wk of treatment the rats were killed by decapitation and hippocampus was collected. The level of TNFα, IL1β, IL6 and HSP70 were analysed by ELISA. The protein expressions of TNFα, IL1β, nNOS, iNOS, HO-1 and BVR were study by Western blot. The expression of TNFα, IL1β, nNOS, iNOS, HO-1, NFkB genes were detected by RT-PCR.

The level of TNFα, IL-1β and IL-6 and expressions of mRNA pro-inflammatory cytokines and NFkB were significantly elevated during ageing and these results were correlated with increasing also of proteins expression. The level of HSP 70 significantly decreased in hippocampus of old males as compared to young controls. Aging induced also a significant elevation in the expression of HO-1 and iNOS proteins and genes. On the contrary nNOS was decreased. Melatonin decreased the level all pro-inflammatory cytokines whereas, GH significantly decreased TNFα and IL6 level, but did not affect IL-1β level. Administration of melatonin decreased the mRNA and protein expression of HO-1 and increased protein expression of BVR, but this was not the case in those treated with GH. However, a significant increase in expression of nNOS and decreased iNOS was observed after both treatments. HSP70 was increased after hormonal treatments and GH was shown more marked effect.

These results suggest that aging in hippocampus is associated with an increase in pro-inflammatory substances, increased expression of NFkB genes and decreased of nNOS. Melatonin and GH treatments are capable to reducing inflammatory markers.

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THE EFFECT OF SUBDIAPHRAGMATIC VAGOTOMY AND CHEMICAL SYMPATHECTOMY ON PROGRESSION OF YOSHIDA AH 130 ASCITIC CELLS IN RATS

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Both divisions of autonomic nervous system, sympathetic and parasympathetic, participate in the regulation of innate immune responses during various physiological as well as pathological conditions including cancer. Sympathetic nervous system influences cancer genesis and progression by direct innervation of the immune organs, regulating their activities by release of norepinephrine and by hormones released by adrenal medulla. The role of parasympathetic nervous system, particularly the vagal nerve, in regulation of immune functions is perused well too. Recently a lot of attention has been paid to the influence of cholinergic anti-inflammatory pathway (efferent arm of the vagal nerve) on the cancer genesis and growth. The cholinergic anti-inflammatory pathway represents highly effective mechanism which participates in the inhibition of elevated levels of anti-inflammatory cytokines (e.g. TNF, IL-1β, IL-6) and it might create basis for efficient suppressor of cancer growth accompanied by inflammation. In this study we investigated influence of subdiaphragmatic vagotomy and chemical sympathectomy on survival of tumor-bearing animals. We used male Wistar rats in which were performed subdiaphragmatic vagotomy or chemical sympathectomy by administration of 6-hydroxydopamine (100mg/1kg BW). One week after the chemical sympathectomy each animal was intraperitoneally injected with Yoshida AH 130 cancer cells dispersed in phosphate buffer. In case of subdiaphragmatic vagotomy, after recovery rats were injected with Yoshida AH 130 cancer cells dispersed in phosphate buffer too. We determined body weight, food and water intake and survival period of animals. We found that survival of animals undergone chemical sympathectomy was significantly shortened (mean survival 15.2 ± 0.9 days) in comparison with control animals injected only with cancer cells (mean survival 18.7 ± 0.6 days). Subdiaphragmatic vagotomy only slightly shortened rats survival (mean survival 18.3 ± 0.8 days) compared to sham operated animals injected with cancer cells (mean survival 21.4 ± 0.8 days). Our results suggest that sympathetic and parasympathetic nervous systems attenuate tumor progression of Yoshida AH 130 ascitic cells in Wistar rats. These data indicate that the brain might exert modulatory influence on peripheral tumor growth via autonomic nervous system.

METAL DEPOSITION AND FUNCTIONAL ALTERATIONS IN THE CNS OF RATS EXPOSED BY MANGANESE-CONTAINING NANOPARTICLES

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Metal-containing airborne particles represent an important occupational and environmental health hazard including nervous system effects. In this work, adult male Wistar rats were treated with MnO_2 nanoparticles of ca. 23 nm nominal particle diameter, instilled into the trachea for 3, 6 and 9 weeks in daily doses of 2.63 and 5.26 mg Mn/kg. The animals' body weight was checked weekly. At the end of treatment, the rats' spontaneous motility was tested in an open field box. Then, spontaneous and stimulus-evoked cortical activity and action potential of the tail nerve were recorded in urethane anesthesia. The rats were finally dissected, organs weights were measured, and Mn level in blood and brain samples was determined by ICP-MS.

Control rats had normal weight gain but the body weights of the treated rats showed no growth from the 6^{th} week on. In the electrocorticogram of the treated rats, high-frequency activity increased and low frequency, decreased. The latency of the evoked potentials was lengthened, and the conduction velocity of the tail nerve decreased. In the open field these rats showed less ambulation and rearing, and more local activity and immobility. These changes developed in a dose- and time dependent way.

Mn level in the treated rats' blood and brain samples was significantly higher than in the controls and there was some parallelism between the behavioral and electrophysiological alterations and the amount of Mn deposited in the brain.

According to these results the Mn content of instilled nanoparticles had access from the airways to the brain and affected functions at several levels. Such experiments may thus be suitable for modeling the neurological damage seen in humans exposed to airborne Mn.

THE SYNAPTIC VESICLE PROTEIN OTOFERLIN IS ESSENTIAL FOR EFFICIENT VESICLE REPLENISHMENT IN INNER HAIR CELLS

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The absence of the multi-C2 domain protein otoferlin causes profound deafness probably due to failure of Ca^{2+}-triggered neurotransmitter release from inner hair cells (IHCs). We now studied deafness in *pachanga* mice that carry a missense mutation in the *Otof* gene (*Otof*$^{Pga/Pga}$). This mutation leads to a 70% reduction of otoferlin protein in IHCs, quantified by immunohistochemistry. Membrane capacitance measurements in mutant IHCs revealed unimpaired exocytosis of the readily releasable pool of vesicles (RRP) when sufficient time for RRP recovery is provided. However, the sustained transmitter release and re-supply of vesicles to the RRP were strongly diminished. The presence of normal multivesicular synaptic transmitter release was confirmed by recordings from postsynaptic afferent boutons of *Otof*$^{Pga/Pga}$ mice. *In vivo*, sound-evoked single neuron activity in the region of the cochlear nucleus was very low and observed only at very high sound pressure levels. Onset coding and rates of the sound-evoked spiking both improved after longer pauses (providing a longer period for recovery). Electron microscopy of IHC synapses of *Otof*$^{Pga/Pga}$ mice revealed a normal number of docked vesicles and immunohistochemistry showed only a minor reduction in the number of ribbon-containing synapses. To conclude, in this work we identify a specific function of otoferlin in the early step of exocytosis at the hair cell active zones and demonstrate the significance of efficient vesicle replenishment at the hair cell synapse for hearing.

MOLECULAR NEUROSCIENCE OF MOTONEURON DISEASE: INSIGHTS INTO SPINAL NETWORK ACTIVITY INVESTIGATED IN VITRO IN A GENETIC MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease in which motoneurons (MN) in the nervous system die. The study of ALS, namely the main MN disease in the adulthood, has been hampered by the absence, for a long time, of available animal models. The discovery of mutation in the gene for the cytosolic Cu/Zn super-oxide dismutase in 20% of familiar ALS patients led to an animal model in which the human mutant SOD1 is over expressed in mice (G93A). These transgenic mice develop limb paralysis associated with loss of spinal motor neurons (Gurney et al 1994). The development of new in vitro models of SOD1 mutations is instrumental to investigate the mechanisms of MN death associated with this gene defect. We investigated the interactions between mutations associated to ALS and the spinal micro-environment, focusing on early functional markers of disease. In particular we monitored functional aspects, such as spinal network synaptic activity in cultured models. We employed the long term spinal cord organotypic culture developed from G93A embryonic mice and their wild type (WT) littermates. We previously reported in such cultures (Avossa et al., 2006) an increase susceptibility of MN to excitotoxic stimuli and the presence of a discrete synaptic rearrangement in G93A ventral cord during the second week in vitro (Avossa et al., 2006). Based on these results, we decided to characterize the spinal network activity, via interneuron patch clamp recordings (voltage clamp) at several time points of in vitro growth, of both WT and G93A. During the first week in vitro, both WT and G93A recorded interneurons, displayed spontaneous bursting. At later stages of in vitro growth, spontaneous synaptic activity was characterised by a large increase in PSCs frequency. In the majority of ventral interneurons we did not detect spontaneous population bursts similar to those recorded at one week, although PSCs temporal summation often led to the appearance of clusters of synaptic currents. In the presence of CNQX (10 μM) inhibitory post synaptic currents (IPSC) and mIPSC (during co-application of CNQX and TTX, 1 μM) displayed a decrease in frequency in the G93A samples when compared to WT ones. Within the temporal window of 2 and 3 weeks in culture, glycinergic PSC underwent a different development profile in G93A interneurons, displaying a mean decay time constant (τ) which was significantly faster when compared to that detected in control WT neurons at similar age in vitro.

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PECULIARITIES OF AUTONOMIC NERVOUS SYSTEM FUNCTION IN MIGRAINE PATIENTS WITH INCREASED STRESS REACTIVITY IN HEADACHE FREE PERIOD

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There is lack of data about autonomic nervous system (ANS) function in migraine patients with increased stress reactivity. Migraine patients (female, mean age 29.3 ± 4.8 years, n=22) in headache free period and age and gender matched healthy controls (n=10) were tested at physical rest, during 10 s. precontraction period (mental stress), isometric contraction and recovery period. Heart rate (HR) and baroreflex sensitivity (BRS) were analyzed.

At rest 40 % of migraine patients (group M1) had increased HR comparing to controls (78.8 ± 8.4 vs. 77.1 ± 4.9 beats/min; NS). The rest of migraine patients (group M2) had significantly decreased HR comparing to control group (61.9 ± 5.6 beats/min; P=0.002). M1 group had statistically significant tendency to decreased BRS at rest comparing to control group (9.5 ± 2.3 vs. 15.0 ± 3.4 ms/mmHg) and statistically significant (P=0.004) difference comparing to M2 group 29.7 ± 10.1 ms/mmHg. In 10 s. precontraction period to M2 group HR statistically significant difference were stated comparing to other analyzed groups (70.7 ± 6.3 vs. 89.0 ± 3.6 vs. 84.2 ± 8.8 beats/min P=0.005). HR in M2 group during recovery period was significantly decreased comparing to controls and M1 group (61.6 ±5.9 vs. 76.0 ± 6.2 vs. 74.5 ± 9.5 beats/min; P=0.018).

It was concluded that M1 group had decreased parasympathetic activity. But patients of M2 group – increased parasympathetic activity. M1 group patients would be suitable for biofeedback trainings. Further investigation will be needed.

ACUTE AND SUBACUTE FUNCTIONAL NEUROTOXICITY OF MANGANESE IN RATS

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Nervous system disorders are among the leading symptoms of human manganese exposure of occupational or environmental origin. A number of alterations in various forms of nervous activity have been reported in humans and experimental animals but the underlying mechanisms and the relationship of effects at different organization levels are not yet fully elucidated.

In the acute experiments, cortical evoked potentials obtained with electrical stimulation of the whisker pad and the tail base, and compound action potential of the tail nerve, were recorded from adult male Wistar rats anesthetized with urethane (1000 mg/kg b.w.). After three control stimulation series (trains of 50 stimuli) in 30 min intervals, 50 mg/kg b.w. in form of $MnCl_2$ solution was injected ip. and further records were taken until the general deterioration of the animal. It was found that the changes in the amplitude and latency of the cortical responses evoked from the two stimulation sites were in good correlation (R^2>0.7) but had no correlation to the changes of the tail nerve action potential. Consequently, the changes induced by acute Mn administration have taken place most likely within the CNS.

In the subacute experiments, a novel method was applied: simultaneous recording of electrocorticogram and motor behaviour in awake rats. The rats were equipped with a connector "crown" - allowing ECoG lead-off while the animal's motor activity was recorded in an open field cage - and had 30 min session weekly. After two control sessions, the rats were orally exposed by Mn through the drinking water (2.5 mg/ml Mn) for 4-8 weeks with continued weekly recordings. In the treatment period, the rats' total ambulation distance and time was significantly less than before Mn exposure. Also, the decrease of locomotion during a 30 min session was increased by Mn. The changes of the ECoG were less characteristic but a correlation between higher total cortical activity and lower motility was observed.

First of all the "crown" technique is promising for further neurotoxicological and neuropharmacological studies.

EFFECTS OF SOME NEUROPEPTIDES AND CALCIUM ON DETRUSOR STRIPS FROM RAT URINARY BLADDER

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Background: Many neuropeptides, like angiotensines and arginine-vasopressin, are involved in the regulation of bladder contractile activity. They activate the inositol-triphosphate way and increase intracellular calcium concentration.
Aim: To establish the effect of ghrelin and calcium chloride over amplitude and force of angiotensin and vasopressin-mediated contractions of detrusor organ strips from rat urinary bladder.
Materials and methods: Urinary bladders from Wistar rats, weighting 200-250 g were used. Obtained preparations were induced by angiotensin and vasopressin, independently and in combination with $CaCl_2$ or ghrelin.
Results: Angiotensin II (Ang II) and arginine-vasopressin (AVP) provoked contractile response with amplitudes 1.9 ± 0.2 g and 1.75 ± 0.12 g and maximal integral force (AUC)- 115.6 ± 20.7 gs and 761.29 ±113.3 gs, respectively. The presence of additional 10 mmol calcium in the enviroment led to increased amplitudes of nevropeptide-mediated contractions: for Ang II -2.18 ± 0.22 g and AVP-2.47 ± 0.4 g. Meanwhile the force of developed contraction was increased and maximum was reached with AVP-1237.14 ±163.8 gs. Added 30 minutes in advance, ghrelin reduced the development of Ang II-mediated contractions to 0.87 ±0.2 g. Ghrelin did not affect over the amplitude of AVP-mediated contraction, but significantly lowered its integral force to 299.08 ±44.95 gs.
Conclusion: Our experimental data indicated that the increase of calcium in extracellular fluid has a synergistic effect over the neuropeptide mediated contraction`s force development in detrusor smooth muscle strips. Ghrelin antagonizes the contractile effect of Ang II and partially – of AVP.

THE EFFECT OF STRONTIUM RANELATE TREATMENT ON SKIN BIOMECHANIC AND BIOCHEMICAL PROPERTIES IN EXPERIMENTAL OSTEOPOROSIS MODEL

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Osteoporosis is a bone disease characterized by reduced bone strength, which predisposes to increased risk of bone fractures. Strontium ranelate is a newly licensed drug, which has shown great promise in the treatment of osteoporosis and has been approved by the European Union on September 2004. In the present study, the effect of strontium ranelate treatment on skin biomechanical and biochemical properties were investigated. Twenty-one adult albino female Wistar rats weighing 200-250g were used in the study. Fourteen animals were ovariectomized by ventral incisions. The ovariectomized rats were randomly assigned into two groups. These groups were designed as ovariectomized group and treatment group. Ovariectomized group: Animals in this group (n=7) were administered placebo (saline) via oral gavage. Treatment group: Animals in this group (n=7) were treated with strontium ranelate (500 mg/kg/day p.o.) for 120 days, starting 90 days after ovariectomy. Other seven animals were named as control group: Animals in this group were administered placebo (saline) via oral gavage. The skin samples were excised and they were used for biomechanical and biochemical experiments. We measured stress, strain, toughness, malondialdehyde (MDA) concentration and antioxidant enzyme activities. In ovariectomized and treatment groups, stress and toughness values significantly reduced compared to the control group. In the ovariectomized group, strain value was significantly lower than control group. No statistical significance was found when treatment group was compared to ovariectomized and control groups. Skin MDA level increased almost two times in ovariectomized group, three times in treatment group compared to control group. Although catalase activity of skin decreased in treatment group compared to control, there were no significant differences for catalase activity between ovariectomized and control group. Skin SOD activity decreased in ovariectomized and treatment groups compared to control group. The results of this study show that the treatment of strontium ranelate has a negative effect on ovariectomized rats' skin.

INVOLUNTARY ATTENTION SWITCHING IS MODULATED BY SELECTIVE ATTENTION IN HUMANS: AN EVOKED POTENTIAL STUDY

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Scalp-recorded event-related potentials (ERPs) are among fundamental research tools for cognitive brain activity at a high temporal resolution. Orienting to new, unexpected or unpredictable stimuli is an involuntary shift of attention that is a way of alerting to potentially significant environmental events. The novelty paradigm in cognitive electrophysiology is an experimental design, in which the effects of novel stimuli on brain electrical activity are measured using unexpected and ever-changing novel non-target stimuli interspersed in the set of standards and targets in the classical oddball paradigm. The typical ERP component obtained during the novelty paradigm is an N2-P3a complex occurring in response to novel non-targets, where the P3a is a positive wave around 300 ms after the stimulus and has a more centro-frontal topography in contrast to the parietal P3b of the target responses. The P3b potential is related to selective or voluntary attention and memory updating processes, while P3a potential is assumed to reflect passive, involuntary switching of attention or orienting. The aim of this study is to investigate the effects of selective attention on the involuntary attention switching in humans. Twenty-one healthy volunteers (ages between 19 and 24 years) participated in the study. ERPs were recorded with 30 electrodes (international 10/20 system) using an auditory novelty paradigm. Intervals between target and novel stimuli were randomly changed at 4, 6, 10, and 14 s. Obtained ERP trials from the responses to novel stimuli were separately averaged in each interval period. The amplitudes and latencies of N2 and P3a responses were measured for each interval period. The differences among the four intervals were statistically tested with repeated measures analyses of variance (ANOVA). Statistical analyses indicate that the amplitudes of N2 and peak-to-peak amplitudes of P3a were significantly higher in the ERP trials with shorter target-novel intervals ($p<0.005$ and $p<0.001$, respectively). However, this interaction was not observed for the latencies of N2 and P3a potentials ($p>0.05$). Our results suggest that selective attention modulates involuntary attention switching in humans.

MODEL OF SKIN BLOOD FLOW DURING COLD EXPOSURE BASED ON THERMOSENSITIVE NEURONS AND NEUROPHYSIOLOCIAL PATHWAYS

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Background: In humans skin blood flow plays a major role in body heat loss. Therefore the accuracy of models of human thermoregulation depends for a great deal on their ability to predict skin blood flow. Various models predicting perfusion response have in common that they require an explicit set-point. Although from an engineering perspective the meaning of a set-point might be clear, application of the concept in human physiology is still under debate. As pointed out by Mekjavic and Eiken (2006), using input from thermosensitive neurons and implementing neuro-physiological pathways of excitation and inhibition voids the necessity to explicitly declare a set-point. In this study a mathematical model for skin blood flow was developed based on current available knowledge on neural thermo-sensitivity and neural pathways. The model was fitted on human experimental data.
Measurements: Skin temperature (wireless thermistors), core temperature (telemetric pill) and perfusion (laser Doppler flowmetry) in glabrous and non-glabrous skin were measured on 8 males and 8 females during a 15 minute baseline period followed by 15 minutes of whole body cooling (water perfused suit).
Validation: Mean sum of squared residuals (MSSR) were assessed through *k*-fold cross validation. For comparison the same test was conducted for an existing model of skin perfusion (Fiala, 2001).
Results: The model adequately explains the variance of the measurements. r^2-statistics of fit on all subjects for dorsal hand (males: r^2=0.908 and females: r^2=0.696); and ventral hand (males: r^2=0.946 and females: r^2=0.977). *k*-fold cross validation yields MSSR dorsal hand (males Neural: 1.068 vs. Fiala: 5.066; females Neural: 1.899 vs. Fiala: 3.292); and ventral hand (males Neural: 1.994 vs. Fiala: 3.844; females Neural: 1.300 vs. Fiala: 1.487).
Conclusion: Compared with an existing model for vasoconstriction the currently developed model yields significantly less error, especially in non-glabrous skin. In conclusion an explicit declaration of a set-point is not needed to accurately model skin blood flow during cold exposure.

FAMILIAL HYPOCALCIURIC HYPERCALCEMIA: REDUCED AFFINITY OF CALCIUM SENSING-RECEPTOR HETERODIMERS UNDERLIES IMPAIRED RECEPTOR FUNCTION

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Heterozygous loss-of-function mutation of the calcium sensing-receptor (CaSR), causes familial hypocalciuric hypercalcemia (FHH), a typically benign condition characterized by mild hypercalcemia. In contrast, homozygous mutation of this dimer-forming G-protein coupled receptor manifests as the lethal neonatal severe hyperparathyroidism (NSHPT). To investigate the mechanisms by which CaSR mutations lead to these distinct disease states, we engineered wild-type (WT) and disease-causing CaSR mutations into the cDNA, and transfected expression constructs into *human* embryonic kidney (HEK) cells. WT protein was primarily membrane-expressed whereas the mutant CaSR protein was distributed throughout the cytoplasm. Co-expression of WT CaSR directed mutant CaSR to the cell membrane. In assays of CaSR function at physiological extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$), increases in $[Ca^{2+}]_o$ increased intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in cells expressing wild-type CaSR while the response was moderately reduced in cells transfected with mutant and WT receptor. Untransfected cells and those expressing mutant receptor alone, both showed minimal but equivalent responses when stimulated with increased $[Ca^{2+}]_o$. Both the $[Ca^{2+}]_i$ response and the CaSR membrane density were linearly related to the plasmid DNA concentration used for transfection. Equimolar transfection of WT and mutant CaSR resulted in protein membrane levels that were predicted by random association. Immunoprecipitation experiments confirmed that these mutant and wild-type CaSR associate in a complex. Taken together these data indicate CaSR self-associates to form a dimer, and that mutant receptor heterodimerization with WT receptor completely rescues the trafficking defect by directing it to the cell surface. Function of the WT and mutant homodimeric receptors and the heterodimeric receptors were then explored under conditions designed to minimize desensitization. To determine the function of the heterodimeric receptor, and affinity for $[Ca^{2+}]_o$, we subtracted the WT homodimer response (25%) expected for the random assortment of hetero- and homodimers following equal co-expression. The maximal response to $[Ca^{2+}]_o$ was dependent on CaSR membrane level and not whether the receptors were homo- or heterodimers. The affinity of the WT CaSR was three times greater than that of the heterodimer (EC_{50} of 2.5 ± 0.1 mM versus 7.2 ± 0.7 mM). In summary, these results suggest that heterodimerization of WT and mutant CaSR receptors, rescues the trafficking defect of half the mutant receptors and also results in a lower affinity for Ca^{2+} by the WT-mutant heterodimer. In contrast the homozygous mutant does not produce functional receptors on cell membrane. These data indicate major differences in the mechanisms producing FHH and NSHPT.

SATURDAY, 14. NOVEMBER 2009:

SESSION VIII – POSTER SESSION

CHARACTERIZATION OF AMP-ACTIVATED PROTEIN KINASE (AMPK) IN GOAT OVARY

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AMPK is a serine/threonine kinase considered as a key energy sensor. It regulates lipid and glucose metabolism. AMPK also controls reproductive functions such as gonadotropin secretion and steroids production in rodents. Here, we characterized AMPK in goat ovary, a small ruminant species. We also investigated the effect of metformin, a pharmacological activator of AMPK, on the Thr172 phosphorylation of AMPK (pAMPK) and progesterone secretion by cultured goat granulosa cells. Since polyunsaturated fatty acids (PUFAs) are known to modulate AMPK in different cell types, we finally studied the *in vivo* effect of two diets enriched in PUFAs on the AMPK subunits and pAMPK in goat ovary. Using RT-PCR and western-blot, we identified the messenger and the protein of the different AMPK subunits in adult goat ovary, small follicles (< 4 mm), large follicles (> 4 mm), granulosa cells, corpus luteum, oocyte, cumulus cells and muscle (as positive control). Interestingly, the AMPKβ1 subunit was not expressed in the oocyte and the AMPKγ3 subunit was only detected in muscle. Metformin (10 mM) increased by 2 to 3 fold pAMPK in granulosa cells from small follicles, from 5 to 120 minutes of stimulation ($p<0.05$). Metformin (10 mM, 48 heures) also inhibited progesterone secretion in both basal state by 30 % and under IGF-1 (10^{-8} M) or FSH ($5x10^{-8}$ M) stimulation by 47 to 55 % ($p<0.05$). Finally, we studied the effect of two diets on the expression of AMPK subunits and pAMPK in goat ovary. One diet was enriched in α-linolenic acid (ALA, diet 1) and the other one was enriched in ALA, eicosapentaenoic acid and docosahexaenoic acid (diet 2). After slaughter, we dissected small and large ovarian follicles. The protein level of AMPKα1 and AMPKβ1/β2 was not affected by these two diets. In contrast, protein level of AMPKγ1 was increased by 1.5 fold and AMPKγ2 was inhibited by 75 % by the diet 2 only in small follicles ($p<0.05$). pAMPK was inhibited by 28 to 56 % by the two diets in small follicles and was activated by 1.5 fold by the diet 1 in large follicles ($p<0.05$). So, the AMPK subunits, except for γ3, are expressed in the different cell types of the goat ovary. Moreover, in cultured goat granulosa cells, metformin activated AMPK and reduced progesterone secretion. A diet enriched in PUFAs can modulate *in vivo* the expression of the AMPK subunits and pAMPK in goat ovarian follicles.

POTENTIAL INVOLVEMENT OF ADIPONECTIN AND RESISTIN (ADIPOKINES) IN GRANULOSA CELL STEROIDOGENESIS AND OVOCYTE MATURATION IN COW

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Adiponectin and resistin are two hormones mainly produced by adipose tissue. These hormones, also called adipokines, play a key role in lipid metabolism and glucose homeostasis. Moreover, they have recently been shown to be involved in the control of female reproduction in different species. Nevertheless, few data are available about the involvement of these adipokines in bovine fertility, which is known to be decreased since several decades, especially for dairy cows. Thus, the objectives of this work were to study in cow: 1) the protein expression of adiponectin, its receptors (AdipoR1, AdipoR2) and resistin in the reproductive axis, and 2) the effects of these adipokines on the oocyte maturation, early embryo development and/or granulosa cell (GC) proliferation and steroidogenesis.

In vitro maturation (22h) of bovine cumulus-oocyte complexes (COCs) (from 3-6mm follicles) was performed with or without human recombinant adiponectin (10µg/mL). Nuclear maturation of oocytes was determined by chromatin DAPI-staining. Cleavage and blastocyst rates were assessed 48h and 8 days after *in vitro* fertilization of COCs, respectively. Bovine GC were incubated with or without human recombinant resistin (667ng/mL) or adiponectin (10µg/mL) in presence or absence of IGF1 (10^{-8}M) and insulin (10^{-8}M). Cell proliferation (24h) and progesterone secretion in the culture medium (48h) were measured by [3H] thymidine incorporation and RIA assay, respectively. The protein detection of adiponectin, its receptors and resistin and the phosphorylation of ERK1/2, p38, AMPKα and AKT1/2/3 were performed by immunohistochemistry and/or Western Blotting.

Adiponectin, its receptors and resistin were detected in ovary (small and large follicles, corpus luteum, oocytes), pituitary and hypothalamus.

Nuclear maturation, cleavage and blastocyst rates were unchanged by adiponectin.

In GC, basal or insulin-stimulated cell proliferation was not modified by adiponectin or resistin. Conversely, IGF-1- induced cell proliferation was increased by adiponectin treatment only (+18%, $P<0.05$). Resistin has decreased IGF-1-stimulated progesterone secretion (-24%, $P<0.05$), whereas it did not affect basal or insulin-induced secretion (*in progress for adiponectin*). A transient increase of ERK1/2 phosphorylation was observed after 1min of adiponectin or resistin stimulation (+54% and +36%, respectively, $P<0.05$). The other cell signalling pathways studied did not seem to be modified.

In conclusion, the results of our study suggest a role of adiponectin and resistin in bovine ovarian functions and especially on cell proliferation or progesterone secretion of GC. The role of these hormones on the whole reproductive axis has to be investigated, in the presence or in the absence of various adipokines or growth factors.

OXYTOCIN AGONISTS AND ANTAGONISTS AS EXPERIMENTAL TOOLS FOR THE STUDY OF BEHAVIORAL EFFECTS OF OXYTOCIN IN THE CNS

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Neuropeptides vasopressin (AVP) and oxytocin (OXY) have not only typical physiological peripheral effects but they also have significant central regulatory actions as neuromodulators and neurotransmitters. Recent studies have shown that these neuropeptides modulate a variety of neurophysiological phenomena including behavior, learning, memory and they also modify stress responses. Very important are findings showing that OXY is responsible for some psychic disorders, and therefore new therapeutic approaches are searched among analogs of OXY. The aim of this study was investigation of the effect of OXY receptor antagonists on spontaneous behavior of rats influenced by peripheral application of oxytocin or its long lasting analog carbetocin; for evaluation of spontaneous behavior of rats was selected open-field device. We used male Wistar rats that were injected i.p. by agonists and/or antagonists 60 min before the start of behavioral test in the open-field. Spontaneous behavior of rats (horizontal) was video-monitored by automated activity monitoring system (AnyMaze, Stoelting, USA) in a circular arena with diameter of 150 cm. Rearing and grooming (face washing, body and genital grooming, body and paw licking and scratching) were recorded by the experimenter. As OXY receptor antagonists we used a nonpeptide antagonist L-368,899 (Tocris, UK), as classical antagonist we applied clinically used atosiban (Polypeptide Labs, Sweden), and as highly selective OXY antagonist we synthesized cpmProp-D-Tyr-Ile-Thr-Asn-Cys-Pro-Orn-NH2, originally prepared by Manning et al. (1). We found that all three oxytocin antagonists (non-peptide as well as peptide) revealed very similar effects that are characterized by complete blocking of grooming while other behavioral parameters, like horizontal and vertical exploratory activities, were antagonized only partially. It is possible to consider that this diversity in their antagonistic actions on oxytocin induced behavioral parameters and grooming can be explained by differences in receptor localization and/or involvement of different signaling pathways.

(1) Manning et al. Int. J. Pep. Protein Res. 46:244-252, 1995.

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MAXIMAL OXYGEN CONSUMPTION IN UPRIGHT AND SUPINE POSTURE AT THE END OF PROLONGED BED REST IN HUMANS

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To test the hypothesis that bed rest leads to larger decrease in maximal oxygen consumption (VO_{2max}) upright (U) than supine (S), because of adequate cardiovascular response in S, but not in U, VO_{2max} and maximal systemic oxygen delivery (QaO_2) were determined during graded exercise on a cycle ergometer on 10 healthy subjects. At each power, VO_2 was determined breath-by-breath and VO_{2max} was established from the plateau in the relationship between steady state VO_2 and power. Blood pressure and heart rate (f_H) were recorded beat-by-beat. Stroke volume (Qst) was determined by Modelflow from pulse pressure profiles. Cardiac output (Q) was obtained as f_H times Qst. Arterial oxygen concentration (CaO_2) was computed from haemoglobin and arterial oxygen saturation measurements. QaO_2 was obtained as Q times CaO_2. Before bed rest,VO_{2max} was the same in U and S. After bed rest, it was reduced in both U and S (- 38.6% and -17.0%, respectively), resulting 30.8% higher in S than in U. Maximal f_H was the same in all conditions. Maximal Qst was reduced after bed rest in U (- 44.3 %), but unchanged in S (+ 3.7 %), resulting 98.9 % higher in S than in U. Maximal Q was equal in U and S before bed rest. After bed rest, it decreased in U (- 45.1%), but not in S (+ 9.0 %), resulting higher in S than in U (+ 98.4 %). QaO_2 was reduced after bed rest in U (- 37.8%), but not in S (+ 14.8 %), being higher (+ 74.8%) in S than in U. We conclude that i) the VO_{2max} decrease after bed rest in S is not due to cardiovascular alterations, and thus it depends on peripheral oxygen transfer limitation only; ii) the lower VO_{2max} after bed rest in U than in S is due to cardiovascular response in U; iii) the VO_{2max} decrease after bed rest in U is the result of the interaction of impaired cardiovascular response and limited peripheral oxygen transfer. The cardiovascular system is preserved in microgravity, but upon gravity resumption, it provides inadequate response to exercise in U.

INVOLVEMENT OF MIDKINE IN CADMIUM INDUCED LIVER, HEART AND KIDNEY DAMAGE

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Accumulation of the wide spread environmental toxin cadmium in tissues results in toxicity. Cadmium (Cd) exposure induces inflammation and apoptosis in the effected tissues. Midkine (MK) is a heparin-binding growth factor. Its expression is induced in tissues as a result of stress. After cadmium toxicity, MK expression is increased in liver cells. The present study was focused to evaluate the MK expression differences in liver, kidney and heart in a chronic Cd toxicity model and their relationships between tissue damage stage relevance and apoptosis.

Male Wistar rats were exposed to Cd at the dose of 15 ppm for 8 weeks. MK levels were measured in kidney, heart and liver tissue. The relationship between tissue midkine levels and apoptosis was evaluated with tissue caspase-3 levels. Tissue MK levels were measured by ELISA and confirmed by MK mRNA expressions. Highest Cd level was in the liver. A significant increase in caspase-3 tissue levels was seen after Cd toxicity, this was accompanied with a significant increase in the MK mRNA expressions. Tissue midkine levels were significantly increased in Cd group. Apoptosis was more prominent in liver tissues than kidney and heart. MK levels increased 3, 1.7, 1.3X folds in liver, kidney and heart respectively.

Chronic Cd administration induces inflammation and apoptosis in rat liver, kidney and hearts. MK might be important for the internal tissue prevention mechanisms against Cd toxicity.

ROLE OF HIF-1 AND PROINFLAMMATORY CYTOKINES IN CHRONIC CADMIUM TOXICITY

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Background: Accumulation of the wide spread environmental toxin cadmium in tissues results in toxicity. Cadmium (Cd) exposure induces inflammation and apoptosis in the effected tissue. It is shown that, all mRNAs of various genes, which are known to be upregulated by HIF-1 activation under hypoxia, were suppressed by Cd in a dose-dependent manner in hypoxic Hep3B cells.
Aim: The present study was focused to evaluate the hypoxia inducible factor-1 (HIF-1) mRNA expression in kidney in a chronic Cd toxicity model and their relationships between tissue inflammation stage , apoptosis.
Methods: Male Wistar rats were exposed to Cd at the dose of 15 ppm for 8 weeks. TNF-α, IL-6 levels were measured by ELISA in renal tissue. Apoptosis was evaluated with tissue caspase-3 levels. HIF-1 mRNA expressions were analyzed by RT-PCR.
Results: A significant increase in caspase-3, TNF-α, IL-6 tissue levels was seen after Cd toxicity ($p<0.001$), this was accompanied with a significant decrease in the HIF-1 mRNA expressions. Chronic Cd administration significantly suppressed HIF-1 mRNA expression in renal tissue. This decrease was correlated with TNF-α and caspase-3 levels ($p<0.001$).
Conclusion: Chronic Cd administration induces, inflammation, apoptosis in rat kidney. HIF-1 is proposed to be an important molecule in anti-apoptotic responses. Our results showed that Cd is one of regulator of HIF-1 and hypoxia inducible genes. Cd, induces toxicity not only by proinflammatory mechanism but also blocking endogenous protective mechanism of the body, HIF-1 mRNA expression.

PARASYMPATHETIC POSTGANGLIONIC NEURONS DO NOT MODIFY THE TRAINING-INDUCED CHANGES ON MYOCARDIAL HETEROGENEITY

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It has been reported that chronic physical exercise protects against cardiac sudden death. Several authors have attributed this protective effect to the increase of vagal parasympathetic tone produced by the trained state. Nevertheless, the exact underlying mechanisms are not completely clear. On the other hand, several authors have pointed out that physical training decreases ventricular heterogeneity, which is a very important factor in the initiation and maintenance of ventricular fibrillation (VF). However, the role of ventricular parasympathetic postganglionic neurons on the modifications of electrophysiological heterogeneity by training are not known. Our aim was to investigate the role of these neurons on the training-induced changes of ventricular heterogeneity in isolated rabbit heart.

For this purpose, 25 New Zealand rabbits were divided in two groups: trained group (n=8) and control group (n=17). Animals in trained group were submitted to 6 weeks of chronic physical exercise on treadmill, while control group was housed in the animal quarter during the same period. After these 6 weeks, rabbits were anaesthetized, killed and their hearts excised and isolated in a Langendorff system. A pacing electrode and a plaque with 256 recording electrodes were positioned on left ventricle. VF was induced by pacing at increasing frequencies ($2 Hz \cdot min^{-1}$), and maintained without interrupting perfusion, before and after the infusion of atropine (1μM). In each experiment, dominant frequency (DF) of VF was determined (Welch´s method) in multiple points of ventricular myocardium. Mean DF and its standard deviation were used in order to calculate the coefficient of variation (CV). CV was compared (control vs trained group) before cholinergic blockade to evaluate the training effect on ventricular heterogeneity with an ANOVA test (repeated measures). We also used an ANOVA test (repeated measures) to compare CV in trained group (before vs after atropine) for analyzing the role of cholinergic postganglionic neurons on this training-induced modification.

CV significantly decreased ($p<0.05$) in trained group (8.8±4% vs 13.6±5% at 30 seconds, 14.03±6% vs 10.02±2% at 60 seconds, 14.5±6% vs 9.7±3% at 90 seconds and 13.1±5% vs 10.1±3% at 120 seconds). Parasympathetic blockade did not modify the decrease of CV observed in trained group.

In conclusion, since parasympathetic postganglionic neurons do not seem to play any role in the decrease of ventricular heterogeneity, this training-induced modification could be intrinsic in nature.

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TRAINING-INDUCED CHANGES IN ATRIOVENTRICULAR CONDUCTION SYSTEM PROPERTIES: ROLE OF PARASYMPATHETIC POSTGANGLIONIC NEURONS

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It is well-known that aerobic endurance training modifies the balance between sympathetic and parasympathetic nervous system, increasing basal parasympathetic tone. This nervous mechanism produces modifications on the electrophysiological properties of the myocardium, such as a decrease in resting heart rate and a depression of atrioventricular (AV) conduction. However, the influence of the intrinsic cardiac nervous system on the depression of AV dromotropism is not well-known. Thus, our aim is to determine the role of postganglionic parasympathetic neurons in the depression of atrioventricular conduction produced by physical training in isolated rabbit heart.

The study was carried out in 28 New Zealand rabbits which were assigned to control (n=9), sham operated (n=10) and trained (n=9) group. The trained group was submitted to 6 weeks of chronic physical exercise on treadmill, while the other groups were housed in the animal quarter during the same period. After these 6 weeks, rabbits were anaesthetized, killed and their hearts excised and isolated in a Langendorff system. Pacing and recording electrodes were positioned on left ventricle. We evaluated Wenckebach cycle length (WCL) and atrioventricular functional refractory period (AVFRP), a parameter closely related to AV conduction, before and after the infusion of atropine (1μM). WCL was determined by pacing at increasing frequencies. To determine AVFRP, we used several extrastimuli tests at four different pacing cycle lengths (10% less than the basal cycle length, 250, 200 and 150ms). An ANOVA test (repeated measures) was applied to analyze the effects of parasympathetic blockade on atrioventricular refractoriness. A Student-t test was used to evaluate changes in WCL. In sham operated group, time and other manoeuvers as stimulation protocol did not modify WCL and AVFRP. WCL was longer ($p<0.05$) in trained animals (135.1±13 versus 122.4±10ms). AVFRP was also longer ($p<0.05$) in trained animals than controls (i.e. 160.3±14 versus 144.7±6ms at 250ms of pacing cycle length). There were no differences in WCL and AVFRP after atropine administration within both groups.

In conclusion, the depression of atrioventricular conduction produced by physical training does not seem to be dependent on parasympathetic postganglionic neurons activity.

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INTERMITTENT DYSSYNCHRONY PROTECTS THE HEART AGAINST ISCHEMIA REPERFUSION INJURY IN WITHOUT ANY GENDER DISCRIMINATION

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Purpose: We previously showed that intermittent dyssynchrony, induced by ventricular pacing immediately upon reperfusion, induces cardioprotection (pacing-postconditioning, (PPC). We hypothesized that stretch, rather than ischemic stimuli, triggers cellular pathways involved in PPC in male and female hearts.
Methods: Isolated ejecting male and female rabbit hearts were subjected to 30 minutes coronary occlusion and 2 hours reperfusion. PPC consisted of 10 cycles of 30-sec intervals of ventricular pacing alternated with atrial pacing during early reperfusion. For gender differences the following groups were studied: (n=5-7/group): control (males and females), PPC (males and females). For pathways study only female rabbits were used in the following groups, control, PPC in combination with selective inhibitors of the adenosine receptor (8-SPT), AT1 receptor (Candesartan), mitochondrial K^{+}_{ATP} channel (5HD), PKC (Chelerythrine), and PI3K (Wortmannin) and the microtubule depolymerizer (Colchicine). In addition we increased preload by raising the level of the atrial reservoir (stretch, no pacing). Infarct size and area at risk were determined using TTC and blue dye. Lactate release was determined in the coronary effluent. In 5 control and 5 PPC hearts fluorescent microspheres were injected during early reperfusion to measure myocardial blood flow (MBF) in the reperfused region during both ventricular and atrial pacing.
Results: Infarct size (IS) following PPC, normalized to area at risk, was significantly smaller in male and female hearts (23.5±4% and 26.0±5%, respectively) compared to controls (47.3±3% and 48.7±2% $p<0.0001$). In female hearts following PPC or stretch, IS is significantly smaller (26.0±5% and 22.6±2%, respectively) than in control hearts (47.3±3% $p<0.0001$). In the early reperfusion phase, ventricular pacing did not change lactate release or MBF, indicating that PPC does not cause graded reperfusion. 8-SPT and Candesartan did not affect the protection gained from pacing (24.6±7% and 20±2% respectively $p<0.001$). Colchicine abrogated the protection by PPC, indicating a role for microtubule in PPC. Furthermore, 5HD, Chelerythrine and Wortmannin abrogated the protection provided by PPC (infarct sizes: 49.0±5%, 45.0±4%, 49.6±4%, and 52.5±2% respectively).
Conclusions: These results support the idea that PPC protects male and female hearts through a stretch mediated trigger, not involving graded reperfusion or adenosine and angiotensin receptors. Although upstream signaling differs PPC shares downstream signaling pathways with ischemic postconditioning.

ZINC RELEASE MODULATES CARDIAC RYANODINE RECEPTORS

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Ca^{2+} release from intracellular stores plays an important role in the regulation of muscle contraction and electrical signals that determine the heart rhythm. In the heart, Ca^{2+} released from sarcoplasmic reticulum (SR), is the principal determinant of cardiac contractility. SR Ca^{2+} release is controlled by dedicated molecular machinery, composed of the cardiac ryanodine receptor (RyR2). Several Ca^{2+} binding proteins, including calmodulin and calsequestrin, bind Zn^{2+} suggesting that Zn^{2+} can compete with Ca^{2+} and can modulate the structure and function of many proteins involved in cardiac EC-coupling. We demonstrated for the first time the variations in intracellular free Zn^{2+} level ($[Zn^{2+}]_i$) and its important role in cardiac EC-coupling by using confocal microscopy in cardiomyocytes (loaded with a Zn^{2+}-specific dye, FluoZin-3) isolated from normal and diabetic rats. In addition our data for the first time demonstrated that not only $[Zn^{2+}]_i$ played important role in the hyperphosphorylation of CaMKII, which is a key regulatory enzyme of RyR2, but also it induced activation of PKC and hyperphosphorylation of ERK-1 and NF-κB due to oxidized protein thiol level. Activation of ERK-1 induced the serine phosphorylation and participated diabetes-induced heart dysfunction via an inhibition in insulin-signaling pathway. Therefore, our data showed the important role of $[Zn^{2+}]_i$ in regulation of heart function as well as in alteration of transcription and gene expression. $[Zn^{2+}]_i$ is not only a secondary actor in EC-coupling but also it is a second messenger in cardiomyocytes which indicate its importance in the fine control of diseased heart.

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THE EFFECT OF BRADYKININ POTENTIATING PEPTIDE C ON ISOLATED RAT HEARTS

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Bradykinin potentiating peptide C (BPPC) inhibits angiotensin-converting enzyme and bradykinin-destroying plasma kinases. It is possible that BPPC contributes to the regulation of cardiovascular functions during hypertension. The effect of BPPC on cardiovascular functions has not been investigated. Therefore, we studied the probable effects of this peptide on coronary perfusion pressure, heart rate and contractile force in rat hearts. Isolated hearts were perfused under a constant flow with modified Krebs-Hanseleit solution and 1, 10 and 100 nM BPPC infused to the hearts. Coronary perfusion pressure, heart rate and left ventricular developed pressure, an index of myocardial contractile force, were measured. All the doses of BPPC caused a significant reduction in perfusion pressure and developed pressure ($p<0.001$ for all). One and 10 nM BPPC increased the heart rate ($p<0.05$ and $p<0.01$, respectively) but, 100 nM decreased significantly ($p<0.001$). From our study, there is sufficient evidence that BPPC possesses vasodilatory efficacy with a modest negative inotropic action. Low doses of BPPC may show tachycardic effect, whereas higher dose may exert bradycardic action.

THE ROLE OF CYTOKINES IN THE OBESITY AND METABOLIC SYNDROME

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Objective: The metabolic syndrome (MS) is a complex of symptoms that resulted from as a reply to inner and outer effects that influence the organism. Main components of MS are the obesity related to insulin resistance, dyslipidemia, hypertension, and hyperinsulinemia for compensation of hyperglycemia. It is considered that resistin and interleukin (IL)-6 are as effective on developing of insulin resistance and inflammation processes and there are a little studies on this subject.
Material and Methods: In our study, WC ≤88 cm 22 women; ≤102 cm 22 men; WC > 88 cm 20 women and > 102 cm 22 men were choosen from 86 subjects totally. According to ATP III criteria, results of mean cystolic and diastolic blood pressure, levels of fasting plasma glucose, fasting plasma insulin, total cholesterol, very low density lipoprotein cholesterol, triglyceride, plasma uric acide, resistin and IL-6 were compared each other statistically.
Results and Conclusion: In the group MS (+) mean systolic blood pressures ($p < 0.001$), mean diastolic blood pressures ($p < 0.001$), levels of mean fasting plasma glucose ($p < 0.05$), mean fasting plasma insulin ($p < 0.05$), total cholesterol ($p < 0.05$), low density lipoprotein-cholesterol ($p < 0.05$), very low density lipoprotein-cholesterol ($p < 0.001$), triglyceride ($p < 0.001$), plasma uric acid were found high ($p < 0.001$) compared with MS (-) group. Values of high density lipoprotein-cholesterol were found less than those.
In MS (+) group, hypertension, insulin resistance progress, dyslipidemia, hyperurisemia were found high by comparing with MS (-) group. Levels of plasma resistin and IL-6 were not statistically significant ($p > 0,05$), compared with each others; however levels of IL-6 were found correlated with WC.

Key Words: metabolic syndrome, waist circumference, lipid profile, resistin, IL-6

THE EFFECTS OF DILTIAZEM AND ATROPIN ON SELENIUM-INDUCED CONTRACTILE RESPONSE IN ISOLATED RAT ILEUM

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Selenium is known to play an important role in the physiology of many cell types and extracellular application of high concentration (1 mM and higher) of sodium selenite causes cellular dysfunction in different types of mammalian tissues. In the present study, we aimed to investigate the effects of sodium selenite (1 mM) application on the contractile activity of isolated rat ileum. We also investigated the effect of diltiazem and atropin application on these contractions.

Adult Wistar rats (200-250 g) were used. Isolated ileum contractility was measured by using convential organ bath system with Tyrode perfusion solution. All contractions were given as percentage changes. Sodium selenite application (15 min) caused a very significant ($p<0.001$) incrase (230±55%) in the ileal contraction. Both diltiazem and atropin enhanced this contraction significantly ($p<0.001$) in the same order (280±34% and 275±49%, respectively). On the other hand, acetylcholine did inhibite this sodium selenite-induced contraction in the order of 43±14%.

Selenium, to be a multifunctional trace element, can show different types of effects mostly due to tissue, dose, or time dependency. This hypothesis is supported with many studies even including our studies. İn smooth muscle preparations such as ileum, it caused enhancement of contractile response. On the other hand, enhancement mechanisms of the ileal sodium selenite-induced responses with both diltiazem and atropin are not clear yet and this subject should be under further investigation.

OSTREOLYSIN, A PROTEIN FROM THE OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*), FORMS PORES IN PLANAR LIPID BILAYERS AND BIOLOGICAL MEMBRANES

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Ostreolysin is a 15 kDa thermolabile protein isolated from the edible oyster mushroom *Pleurotus ostreatus*. Previous studies show that nanomolar concentrations of ostreolysin exerts cytolytic effects on erythrocytes and tumor cells. Moreover, it is also toxic to rodents when intravenously injected (LD_{50} = 1170 μg/kg), leading to death by cardiorespiratory arrest. These effects are assumed to result from ostreolysin-induced pores in plasma membrane. Therefore, we have investigated the ability of ostreolysin to form pores when incorporated into both planar lipid bilayers and plasma membranes of CHO cells, using electrophysiological techniques and have characterized their conductance and ionic selectivity.

Ostreolysin was able to form pores in cholesterol-enriched planar lipid phosphatidylcholine bilayers. The amplitude of the resulting transmembrane current varied linearly with the applied membrane potential with a mean conductance of 700 pS at 20 mM KCl. According to this result, the pore-forming activity was investigated in cells. Addition of ostreolysin to the CHO cells resulted in formation of pores with wide size distribution. The toxin induced a current that reversed at about 0 mV. The current reversal potential was not markedly modified when Na^+ and Cl^- concentrations were changed in the extracellular medium, indicating that the corresponding pores are not selective for one of these ion species.

These results suggest that the toxicity of ostreolysin on mammalian cells may be explained by its ability to induce non-selective ion-conducting pores.

cAMP MODULATES THE Ca^{2+} SENSITIVITY MAINLY THROUGH PKA IN PRIMARY BETA-CELLS

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It is generally accepted that cAMP regulates Ca^{2+}-dependent exocytosis in many secretory cells but the precise molecular mechanism of this phenomenon remains unexplained. Two separate cAMP-modulated pathways were described to control Ca^{2+}-dependent exocytosis, through either PKA or Epac2. The aim of our study was to characterize the role of these two cAMP-dependent pathways on the kinetics of regulated exocytosis in mouse pancreatic beta-cells. The effects on different exocytotic components were studied using whole-cell patch-clamp based capacitance measurements (C_m). Exocytotic activity was stimulated using slow photo-release of Ca^{2+} bound to NP-EGTA producing a ramp-like increase in cytosolic Ca^{2+} $[Ca^{2+}]_i$. In control cells when $[Ca^{2+}]_i$ reached threshold level, typically a biphasic increase in C_m has been triggered. The first phase of C_m change (exocytotic burst) reached its maximal amplitude within the first second (amp1) and the second phase reaching maximal amplitude (amp2) within approximately next 5 seconds. In addition, we observed that the Ca^{2+}-dependency of the rate of the C_m follows the saturation kinetics with high cooperativity and half-maximal rate of the C_m change (EC_{50}) at 2.6 ± 0.2 μM. To assess the effect of cAMP we first performed the intracellular washout of ATP that should result in reduced cAMP production. This manipulation pushed the Ca^{2+}-dependency of the first C_m phase towards higher $[Ca^{2+}]_i$ but did not influence the C_m amplitude. On the other hand the amplitude of the second phase was strongly reduced. In the following experiments, we clamped the cytosol at saturating 200 μM cAMP. This manipulation pushed the Ca^{2+}-dependency of the exocytotic burst to significantly lower $[Ca^{2+}]_i$ in comparison to the controls, while again there were no differences in the amplitudes of C_m change. To address the question, whether cAMP acts through PKA- or Epac2-sensitive mechanism we included 100 μM 6-Phe-cAMP (selective PKA agonist), 500 μM Rp-cAMPs (a competitive antagonist of cAMP-binding to PKA) and 100 μM 8-pCPT-2'-Me-cAMP (specific Epac activator) into the pipette solution. PKA activation or inhibition in primary beta-cells significantly shifted the EC_{50} of the exocytotic burst in the opposite directions (1.6 ± 0.3 μM and 3 ± 0.1 μM in cells treated with 6-Phe-cAMP and Rp-cAMPs, respectively), while specific activation of Epac2 did not change the Ca^{2+} sensitivity. Our findings suggest that cAMP modulates the rate of exocytosis in primary beta-cells mainly through PKA-dependent mechanism by sensitizing the insulin releasing machinery to $[Ca^{2+}]_i$.

THE ADAPTIVE RESPONSE OF LEYDIG CELLS TO IMMOBILIZATION STRESS: PKG-I "OVERDRIVE OPERATION" ON StAR PROTEIN

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Growing body of evidence identify nitric oxide (NO) as one of endogenous mediators elicited by stress exposure, but precise identification of downstream signaling pathway molecules are still missing. In this study, we examined the effects of acute (2h) and repeated (2 or 10 days, 2 hours daily) immobilization stress (IMO) on NO-cGMP signaling pathway in adult rat Leydig cells. Results showed that only repeated IMO was accompanied with a decrease in levels of eNOS and iNOS transcript and protein, followed by declined NO production. In parallel, TLDA analysis revealed that again only repeated IMO lowered expression of all cGMP specific and almost all dual specific PDEs in Leydig cells. Furthermore, PDE5 protein level was reduced by repeated IMO, suggesting that Leydig cells PDEs system is trying to preserve cGMP. In this scenario, increased level of PKG-I transcript and protein was observed. Moreover, repeated IMO increased level of StAR and PKG-I immunoprecipitated complex, further enhanced with *ex vivo* sGC stimulation, but reduced with sGC inhibition. Additionally, *ex vivo* inhibition of PKG-I abolished increased level of mature StAR protein in Leydig cells obtained from rats repeatedly exposed to IMO. The physiological significance of the presented results was suggested by the recovery of androgen production, reduced by acute IMO, but starts to recover after repeated IMO. Increased expressions of PKG-I and StAR complex after repeated IMO, accompanied with decreased PDE5 protein, may suggest the possible role of cGMP signaling in the adaptive response of Leydig cells to stress-impaired steroidogenesis. Taking together, presented results showed that repeated IMO affects cGMP signaling pathway localized in Leydig cells and that this signaling scenario exhibits significant impact on recovery of stress-impaired testicular steroidogenesis, mainly trough "overdrive" interaction between PKG-I and StAR protein.

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ROLE OF PROTEIN KINASE A AND G IN RECOVERY OF STRESS-REDUCED LEYDIG CELL STEROIDOGENESIS

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The ability of immobilization stress (IMO) to decrease Leydig cell steroidogenesis and serum androgen level is well documented, but the possible mechanism(s) involved in the adaptation to prolonged or repeated stress have not been identified. In this study we investigate impact of two sister kinases, protein kinase A (PKA) and protein kinase G-I (PKG-I) in stress adaptive responses of Leydig cells obtained from adult rats subjected to acute (2h) and repeated IMO (2 or 10 days, 2 hours daily). Results showed reduced cAMP and unchanged cGMP production of Leydig cells isolated from animals exposed to both acute and repeated IMO. Despite the reduced cAMP production, immunoblot analysis revealed increased level of PKA as well as level and phosphorylation of mature steroidogenic acute regulatory (StAR) protein. In this scenario, increased level of PKG-I transcript and protein was observed. Also, repeated IMO increased level of StAR and PKG-I immunoprecipitated complex, and this complex was further enhanced with *ex vivo* soluble guanylate cyclase (sGC) stimulation, but reduced with sGC inhibition. Aditionaly, *ex vivo* treatment of Leydig cells isolated from control and IMO rats with specific PKA and PKG inhibitor (H89 and KT5823 respectivily) showed that repeated IMO-induced increase in StAR immunoreactivity was decreased by inhibition of PKG and PKA. Moreover, it looks like two inhibitors have an additive effect on decreasing of StAR protein level, as well as androgen production. Results presented here suggest significant impact of both PKA and PKG-I cascade dependent activation of StAR protein**,** as adaptive mechanism, in recovering of stress-disturbed Leydig cells androgen production.

ESTROGEN RECEPTOR ALPHA (ERα) IN LEYDIG CELLS OF HYPOGONADAL RATS IS DOWN-REGULATED AND CAN NOT BE RECOVER BY TESTOSTERONE TREATEMENT

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Estrogens exert a variety of regulatory functions on growth development and differentiation in both the female and male. The estrogen actions are mediated by the estrogen receptors (ERs), members of the nuclear receptor (NR) superfamily, which are encoded by two distinct genes, ERα and ERβ. Recently, it has been suggest that estrogens, acting through the ERα, have been shown to repress expression of the gene encoding insulin-like 3, a small peptide produced by testicular Leydig cells that is essential for normal testis descent and that ERα is required for male fertility. In this study we investigated the effects of triptorelin (the long-acting gonadotropin releasing hormon analog able to induce hypogonadism) and/or testosterone treatement on the expression of ERα and aromatase in Leydig cells of adult male rats. In addition, levels of testosterone and estradiol produced by Leydig cells from control and treated rats were followed. The results of real time PCR analysis showed that triptorelin treatement dramaticaly decreased the expression of both, aromatase and ERα. Moreover, testosterone treatment of triptorelin-induced hypogonadal rats was not able to recover decreased aromatase and ERα expression, while testosterone alone induced the same inhibitory effect as triptorelin. In the same cells, production of both, aromatase substrate testosterone, as well as estradiol was significantly decreased in all treated groups. Collectively, these results sugest that triptorelin and testosterone treatement, alone or in commbination, strongly inhibited the estrogenic mashinery in Leydig cells and that model of hypogonadal rats maybe the good model to extend knowledge on the role ERα in male reproductive functions.

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SHEAR STRESS INDUCED INTEGRIN $\alpha_V\beta_3$ ACTIVATION IS DEPENDENT ON THE ACTIVITY OF AN ENDOTHELIAL ELASTASE

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Vascular remodelling can be often observed as consequence of chronic changes in blood flow. Whereas formation and modification of collaterals also known as arteriogenesis is induced by elevated flow, a decrease in flow may result in plaque formation or atherosclerosis. Both processes are tightly controlled not only by specific growth factors and proteases but also by their individual inhibitors. We have previously shown that porcine aortic endothelial cells (PAEC) when exposed to shear stress (16 dyn/cm²) actively release FGF-2, a growth factor, which is known for its proangiogenic activity. The release mechanism was dependent on the activation of the integrin $\alpha_v\beta_3$, because its inhibition reduced shear stress induced FGF-2 release significantly. The aim of this study was to investigate the process of shear stress induced integrin activation and to identify key steps of the corresponding signal cascades.
PAEC exposed to shear stress showed a significant higher elastolytic activity in their supernatants compared to corresponding static control cells (static 0.0619 U/ml ± 0.0206, shear 0.2036 U/ml ± 0.0577). Inhibition of the integrin $\alpha_v\beta_3$ (Abciximab, 1 µg/ml) had no influence on the shear stress induced elastase release, indicating that although activation of $\alpha_v\beta_3$ is a necessary step for shear stress mediated FGF-2 release it is not relevant for the release of elastase. Moreover, in parallel experiments we were able to show that this shear stress induced elastase activity led to an activation of integrin $\alpha_v\beta_3$. Likewise, as proof of concept, the exogenous application of elastase activated $\alpha_v\beta_3$ and induced the release of FGF-2 similar as shear stress did. To further analyze elastase mediated $\alpha_v\beta_3$ activation, we performed coimmunoprecipitation experiments with integrin $\alpha_v\beta_3$ and its adaptor protein Shc, indicating an active integrin clustering. After 10 minutes of elastase stimulation a significant higher amount of all three Shc isoforms was complexed with the integrin (44 kDa: 2.7 fold more, 52 kDa: 1.7 fold more, 66 kDa: 3.3 fold more). By the use of specific elastase inhibitors (MeOSuc-Ala-Ala-Pro-Val-chloromethylketone, 50 µM) it could be verified that also shear stress induced activation of integrin $\alpha_v\beta_3$ was elastase mediated.
Our results demonstrate that shear stress induced FGF-2 release is proteolytically controlled by an elastase dependent activation of integrin $\alpha_v\beta_3$.

THE ACTIVITY OF PROTEIN C INHIBITOR (PCI) IS MODIFIED BY PHOSPHOINOSITIDES AND OTHER GLYCEROPHOSPHOLIPIDS

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PCI (SERPINA5, PAI3) is a non-specific, secreted serine protease inhibitor (serpin) with affinity for heparin (review: Geiger 2007, Suzuki 2008). First described as an inhibitor of activated protein C (aPC) an anticoagulant serine protease in human plasma, it has been shown that PCI inactivates a variety of other proteases and has a wide tissue distribution.

As PCI can be internalized by cells and translocated to the nucleus, the PCI sequence was inspected for sequences homologous to the nuclear localizing sequence (NLS). 2 homologous sequences could be identified, located on the A^+ and on the H-helix. Further investigations showed that the NLS homologous sequence localized in the H-Helix could be functional.

Phosphatidylserine (PS), oxidized PS (OxPS) or oxidized phosphatidylethanolamine (OxPE) interact with PCI and stimulate its activity towards aPC in a heparin-like manner (Malleier et al., 2007; Nishioka et al., 1998). The head group of PE fits into the helix A gap and the acyl chains in the hydrophobic D' and H' channels of PCI. In addition, the internalization of PCI by cells is supported by PE, and internalized PCI promotes phagocytosis of bacteria (Baumgärtner et al. 2007).

The interaction of PCI with various glycerophospholipids in particular phosphoinositides and derived second messengers was analyzed, focusing on differences in fatty acid composition and headgroup phosphorylation.

Binding was studied by protein overlay assays (dot blot analysis) and native PAGE. Stimulation of PCI activity was analyzed by modulation of aPC-inhibition.

Inositol-1,4,5-trisphosphate and inositol-1,3,4,5-tetrakisphosphate did not interact with PCI. Mono- and diacylglycerols showed interaction in dot blot analysis but no stimulatory activity on aPC-inhibition. Among the different phosphatidic-acids (PAs), oxidized PA exhibited the lowest binding to PCI, but highest stimulatory activity, while saturated or lyso-PA did not stimulate aPC inhibition. From all studied lipids phosphatidylglycerol (PG) had the strongest stimulatory effect on aPC-inhibition. Oxidation of PG led to a decrease and saturation to a complete loss in stimulatory activity. Cardiolipin, PG and oxidized PA showed the highest stimulatory effect on aPC inhibition, similar to heparin.

Among the PI-monophosphates, PI(5)P showed the strongest binding to PCI. A mobility shift of PCI antigen was observed when PCI was incubated with phosphatidylinositol-3,5-diphosphate and phosphatidylinositol-4,5-diphosphate ($PI(4,5)P_2$).

Therefore we conclude that phosphoinositides and other glycerophospholipids may function as additional intracellular interaction partners of PCI.

PARTITIONING OF PULMONARY VASCULAR RESISTANCE IN RATS BY ARTERIAL AND VENOUS OCCLUSIONS

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In order to partition pulmonary vascular resistance in its arterial (Ra), venous (Rv) and capillary (Rcap) components, we applied the method of arterial and venous occlusions (Hakim et al., J Appl Physiol 52: 710-715, 1982) in 7 anesthetized (Chloralose 50 mg/ 100g i.p.), tracheotomized, positive pressure ventilated rats. The thorax was opened, and two catheters were inserted into the pulmonary artery and left atrium through two small incisions performed on the right and left ventricular walls respectively. The catheters were firmly kept in place by a single binding passed in the atria-ventricula solcum. Hydraulic resistance of the catheters (Req) was separately measured and subtracted from results, which hence represent intrinsic values. The lungs were perfused with eparinized Emagel at a fixed flow rate of 5 ml/min, and pulmonary artery pressure was monitored and recorded on paper. Left atrium pressure was mantained near athmospheric. Three to five arterial and venous occlusions were performed for each rat, and the pressure drops due to arterial and venous resistances were measured. The resistance of the highly distensible middle component, which most likely includes capillaries, and small arterioles and venules, was quantified by subtraction of Ra and Rv from total vascular resistance (R). Pulmonary vascular compliance (C) was also measured analyzing the rise of intravascular pressure during venous occlusion and maintained perfusion (see Hakim et al.). Results were as follows:

a) the mean values of R, Ra, Rv, Rcap resulted 5.7±0.8, 2.05±0.4, 1.26±0.16, 2.4±0.9 $cmH_2O/ml\ sec^{-1}$ respectively, while C resulted 0.048 ml/ cm H_2O.

b) Ra, Rv, Rcap represent about 36%, 22% and 42% of R respectively.

R values resulted rather high, probably because of the low flow we used in order to avoid the risk of pulmonary edema, and of the low lung volume. Some degree of hypoxic vasoconstriction also may not be excluded.

These data are rather similar to those previously published (Rubini, Europ J Appl Physiol 93: 435-439, 2005), and show that the higher resistance in the pulmonary vascular tree is attributable to the middle segment. This is different from what previusly observed in tha isolated dog lung lobe.

AN INVESTIGATION OF BLOOD PRESSURE CHANGE WITH POSTURAL MUSCLE ACTIVATION: A WAVELET APPROACH

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Purpose: The present investigative study to find the relation between the blood pressure (BP) and postural sway is based on a model for adaptive compensatory cardio-postural interactions which has been proposed as a part of an integrated orthostatic reflex control system. (Blaber AP et al. Respir. Physiol. Neurobiol., 2009: in press). Methods: To further validate this model, we investigated the simultaneous changes of medio-lateral center of pressure coordinates (M/L COP), filtered electromyographic activity (EMG) of muscles essential to postural stance along with continuous non-invasive BP data. Five young males between the ages of 19 to 26 volunteered to participate. Participants were seated for 20 minutes and then asked to stand with eyes closed (blindfolded) in a quiet stance for 5 minutes. All data were acquired at a sampling rate of 1000 Hz and resampled at 15 Hz. Discrete Wavelet Transform (DWT) was applied using the Daubechies 5 (db5) wavelet to decompose the signals at the 8^{th} scale and extract the approximation signal in the low frequency range (~ 0.11 Hz). Results: The approximation signals of individual leg EMG activity and BP showed regions of significant correlation ($r>0.5$, $p<0.0001$) during last 4 minutes of quiet stance. Analysis of the correlation plots in the time domain revealed regions with opposing correlation patterns between left and right legs indicating alternating contraction of each leg in accordance to the ML posture sway. Regions of high correlation in the same direction were also observed indicating the co-contraction of both legs to regain the postural stability. Conclusion: The presence of cardio-postural interactions in healthy individuals guides our way to applications for rehabilitation of returning astronauts and reducing fall proneness in the elderly.

Keywords: Electromyography; orthostatic reflex; Center of Pressure; Postural Sway

ELECTROPHYSIOLOGICAL DETERMINANTS FOR ARRHYTHMOGENESIS FOLLOWING PREMATURE EXCITATION IN MURINE HEARTS

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Background: Circus type re-entry is classically associated with reduced action potential (AP) conduction velocity through partially refractory tissue resulting in unidirectional conduction block. We assessed the extent to which premature extrasystolic APs under such conditions resulted in ventricular arrhythmogenesis in isolated Langendorff-perfused murine hearts.
Methods and Results: A novel programmed electrical stimulation (PES) protocol applied trains of 8 S1 stimuli at 100 ms intervals followed by extrasystolic S2 stimuli at successively decreasing S1S2 intervals. S2 stimulus strengths required to overcome refractoriness, reduce ventricular effective refractory period (VERP) and thereby elicit extrasystolic APs, increased with shortened S1S2 intervals, despite constant durations at 90% recovery (APD_{90}) of the preceding APs. Critical interval, CI, the difference APD_{90}-VERP, consequently increased with stimulus strength. The corresponding latencies and peak amplitudes of the extrasystolic APs consequently sharply increased and decreased respectively with CI thereby potentially replicating necessary conditions for re-entrant, circus-type, arrhythmia. The dependence of CI upon stimulus strength tended to consistent limiting values expected from approaches to absolute refractory periods. These values were greater in arrhythmogenic (mean CI 18.9±0.55 ms, *n*=4) than in non-arrhythmogenic hearts (mean CI 15.1±0.37, *n*=4; P=0.001, ANOVA), despite their statistically indistinguishable APD_{90} (arrhythmogenic hearts: 40.9±2.23 ms, n=4 vs non-arrhythmogenic hearts: 36.5±2.61ms, n=4; p>0.05, ANOVA) or VERP values (arrhythmogenic hearts: 22.5±2.66 ms, n=4 vs non-arrhythmogenic hearts: 21.8±2.53 ms, n=4; p>0.05, ANOVA).
Conclusions: These findings suggest existence of a specific CI (CI*) in turn corresponding to specific conditions of latency and action potential amplitude that would be sufficient to result in arrhythmogenesis.

PHYSIOLOGICAL CONSEQUENCES OF AGE AND SEX FOR SINOATRIAL AND ATRIOVENTRICULAR FUNCTION IN MURINE *SCN5A*$^{+/-}$ HEARTS

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The generation and propagation of the cardiac impulse is dependent upon the function of the cardiac isoform of the voltage gated, Na^+ channel, encoded by the *SCN5A* gene. Haploinsufficiencies in this gene have been associated with cardiac disorders that include the Brugada (BrS) and Lev-Lenegre syndromes. Recent studies suggest that the *SCN5A* mutation is not directly causal to a Brugada Syndrome (BrS) like ECG pattern, suggesting further background complexity for the condition. Such a finding is consistent with the possibility that BrS-like electrocardiographic (ECG) patterns may vary with both age and sex. In the present study we validate our model through patch-clamp studies in single cells. We then investigate for *interacting* effects of age and sex on ECG features of Scn5a$^{+/-}$ mice used to model BrS. Action potential measured by patch-clamp showed reduced rate of rise of action potential in Scn5a$^{+/-}$ mice confirming loss of voltage-gated sodium channel function. In in-vivo ECG we observed that: (1) Sinoatrial function: RR intervals in old male Scn5a$^{+/-}$ hearts were greater than old female Scn5a$^{+/-}$. (2) Atrial function: P-wave durations were indistinguishable between fully separated groups. (3) Atrioventricular function: *Scn5a*$^{+/-}$ mutation prolonged PR intervals only in older males compared to *Scn5a*$^{+/-}$ older females. PR intervals were in general longer in young female compared to young male regardless of genotype. However, age dependent prolongation of PR interval was observed in older male *Scn5a*$^{+/-}$ mice but not in older male and female WT and older female *Scn5a*$^{+/-}$. (4) Ventricular activity: T-wave durations were greater in old male *Scn5a*$^{+/-}$ compared to old male WT. QRS durations and QTc intervals were indistinguishable between fully separated groups. We demonstrate for the first time *interacting* effects of age and sex on the Scn5a$^{+/-}$ phenotype resulting in alterations particularly in pacemaker and atrioventricular conduction greatest in ageing males. This establishes that a complex interplay of age and sex on cardiac electrophysiological changes may have a profound effect on the pathophysiology of conditions related to *Scn5a* mutations.

EFFECT OF ACUTE HYPERGLYCEMIA ON ENDOTHELIUM-DEPENDENT, NO-MEDIATED VASODILATATION IN RAT AORTA

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Diabetes is known to induce endothelial dysfunction and thus an impairment of endothelium-dependent vasodilatation of micro- and macrocirculation in different animal species as well as in humans. It has been shown that even transient increases in plasma glucose concentrations impair vascular function. One of the proposed mechanisms involves reduced bioavailability of nitric oxide (NO) due to increased scavenging by reactive oxygen species (ROS). Moreover, hyperglycemia is reported to induce endothelial nitric oxide synthase (eNOS) uncoupling, leading to increased production of superoxide instead of NO by eNOS and thus aggravating endothelial dysfunction. eNOS uncoupling is among others the result of enhanced oxydation of tetrahydrobiopterin (BH4), an essential cofactor of eNOS, by ROS that cause cofactor insufficiency. The aim of our study was to assess the effect of acute hyperglycemia on endothelium-dependent, NO-mediated vasodilatation in rat aorta. Further, we wanted to investigate whether the potential impairment of NO-mediated vasodilatation could be reversed either by the application of sepiapterin, a precursor of BH4, or by preatretment of aortic rings by antioxidants.

In isolated, precontracted, endothelium-intact rat aortic rings, incubated with diclofenac (0.01 mM) 30 minutes as well as two hours exposure to high glucose concentration (30 mM) caused a rightward shift in the concentration-relaxation curve to acetylcholine (ACh), as compared to 5 mM -glucose solution or 30 mM-mannitol solution. The effect of hyperglycemia was more pronounced after 30 minutes ($pD2=7.16\pm0.07$ for 5mM glucose and 6.61 ± 0.14 for 30 mM glucose solution, $p\leq0.01$, one-way ANOVA) than after two hours exposure to hyperglycemia ($pD2=7.46\pm0.12$ for 5mM glucose and 7.11 ± 0.13 for 30 mM glucose solution, $p\leq0.05$). The effect of hyperglycemia was significantly attenuated when rings were preincubated with sepiapterin (10 μM) for one hour. On the contrary, incubation of rings with the antioxidant vitamin C (100 μM) did not affect the glucose-induced impairment of NO-mediated relaxation. However, coincubation of rings with vitamin C and EDTA (26 μM) did abolish the effect of high glucose.

The results show that acute hyperglycemia diminishes the endothelium-dependent, NO-mediated relaxation in rat aorta. There is indirect evidence that diminished relaxation might be the consequence of eNOS uncoupling and increased superoxide production as sepiapterin, precursor of BH4, as well as vitamin C complemented by EDTA did restore the reduced vasodilatation. We may implicate that administration of antioxidants and/or precursors of BH4 might reverse the detrimental effects of hyperglycemia on endothelial function.

β-ADRENOCEPTOR STIMULATION POTENTIATES INSULIN-STIMULATED PKB PHOSPHORYLATION IN RAT CARDIOMYOCYTES VIA cAMP AND PKA

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Genetic approaches have shown that protein kinase B (PKB or Akt) is an important regulator of normal heart functions and dysregulation causes cardiac disease. Overexpression of constitutively activated PKB increases heart size and causes dilated myopathy. Insulin is a powerful activator of PKB whereas adrenaline is not considered a major physiological regulator of PKB in heart. However, we recently reported that adrenaline strongly potentiated insulin-stimulated PKB activation in skeletal muscles without having effect in the absence of insulin. The aim of the present study was to investigate the effect of isoprenaline on insulin-stimulated PKB phosphorylation in heart cells from adult rats. Isoprenaline increased insulin-stimulated PKB Ser^{473} and Thr^{308} phosphorylation > 3-fold in cardiomyocytes from adult rats. Isoprenaline alone did not increase PKB phosphorylation. Isoprenaline also increased insulin-stimulated GSK-3β Ser^{9} phosphorylation ≈ 2-fold. PKB phosphorylates GSK-3β at Ser^{9}; our data, therefore, support that isoprenaline increases insulin-stimulated PKB activity. Dobutamine (β_1-agonist) increased insulin-stimulated PKB phosphorylation as effectively as isoprenaline (> 3-fold) whereas salbutamol (β_2-agonist) only potentiated insulin-stimulated PKB phosphorylation by ≈ 80 %. Dobutamine, but not salbutamol, increased phospholamban Ser^{16} phosphorylation and glycogen phosphorylase activation (PKA-mediated effects). Furthermore, the cAMP analogues that activate PKA (dibutyryl-cAMP and N^6-benzoyl-cAMP) increased insulin-stimulated PKB phosphorylation by > 3-fold without effect alone. The Epac specific activator 8-(4-chlorophenylthio)-2`-O-methyl-cAMP (007) increased insulin-stimulated PKB phosphorylation by ≈ 50 %. Db-cAMP and N^6-benzoyl-cAMP, but not 007, increased phospholamban Ser^{16} phosphorylation. In conclusion, β-adrenoceptors are strong regulators of PKB phosphorylation via cAMP and PKA when insulin is present. We hypothesise that PKB mediates important signalling in the heart during β-adrenergic receptors stimulation.

SELENIUM MEDIATED CARDIOPROTECTION AGANIST ADRIAMYCIN INDUCED MITOCHONDRIAL DAMAGE

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Cardiotoxicity associated with adriamycin (ADR) treatment limits the therapeutic efficiency of this drug aganist cancer. ADR causes morphological and functional alterations in mitochondrial, nuclear and fibrous protein structures in myoblasts. The study's aim was to determine whether the protection effect of selenium (Se) on ADR-induced cardiomyocyte toxicity.Rats were injected with (1) saline i.p for 21 days, (2) 2 mg/kg i.p ADR every alternate day (four times), (3) 0.15 mg/kg i.p Se for 21 days, (4) Se and ADR co-administration i.p. Left ventricular functions, ECG parameters and blood pressures were assessed by invasive techniques at the end of injection period. Mitochondrial membran potential (MMP, Cambrex Kit), ATP level (Cayman Kit) and cytosolic thioredoxin reductase activity (TrxR, Cayman Kit) were determined. Cytosolic, intramitochondrial and plasma total antioxidant (TAS) and oxidant statuses (TAS) were measured with Erol's method.

Left ventricular data demonstrate a decrease in left ventricular systolic pressure (LVSP), left ventricular developed pressure (LVDP), and an increase in left ventricular end diastolic pressure (LVeDP) in ADR treated animals, compared to the control and Se groups. ADR increased ST interval and decreased systolic blood pressure, compared to the control and Se groups. ADR decreased membran potential and ATP level in myocite mitochondria. TrxR decreased in ADR group, compared to Se group. Cytosolic and mitochondrial TAS decreased and mitochondrial and plasma TOS increased in ADR group, compared to the control. ECG, blood pressure and left ventricular function changes by ADR recovered to control levels with co-administration Se with ADR. Se improved MMP and ATP level by decreasing oxidant and increasing antioxidant statues.

These data suggest that selenium reduces oxidative stress, prevents mitochondrial damage and protects cardiac myocytes against ADR toxicity.

SUNDAY, 15. NOVEMBER 2009:

SESSION XII – POSTER SESSION

DIAMAGNETIC LEVITATION AFFECTS MORPHOLOGY, CYTOSKELETON AND INDUCES APOPTOSIS OF BONE CELLS

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With the development of superconducting technology, the superconducting magnet, which can produce a stable large gradient high magnetic field (LG-HMF) environment to make diamagnetic levitation, such as biological macromolecules, cells, tissues and model animals, has become available. In this study, a superconducting magnet which can generate a magnetic force field [B (dB/dz)] of -1360 T2/m, 0 T2/m and 1312 T2/m in a 51 mm diameter room temperature bore was employed. Three magnetic force fields correspond to three apparent gravity levels (0, 1, and 2 g) and three magnetic induction intensities (12, 16, and 12 T), respectively. The superconducting magnet therefore can simulate gravitational environment from microgravity (0-g) to hypergravity (2-g). The effects of LG-HMF on bone cells, including osteoblast, osteocyte and bone mesenchymal stem cells (BMSC) were investigated.
The results showed that diamagnetic levitation affected morphology and cytoskeleton architecture of osteoblast, osteocyte and BMSC. Under diamagnetic levitation conditions, the BMSC and osteocyte presented obviously apoptosis, and the process number and cell area of ostecyte dramatically decreased. The cytoskeleton of osteoblast, osteocyte and BMSC reorganized and the expression and distribution of cytoskeleton-related proteins, including vinculin, talin, paxillin was also clearly changed. The cell cycle, the secretory function and adhesive ability of bone cells were also changed by diamagnetic levitation. These findings will enhance our understanding on the biological effects of the high gradient static magnetic field.

Key words: diamagnetic levitation, cytoskeleton, morphology, apoptosis, bone cell
Acknowledgments: This work was supported by National Natural Foundation of China (No. 30670520) and the National High-Tech R&D Program (863) of China (Key Program, 2008AA12A220).

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PERIPHERAL INJECTION OF METYRAPONE IMPAIRS MEMORY RETRIEVAL IN RATS

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Previous studies have indicated that memory retrieval is impaired under high levels of blood glucocorticoids as seem in severe stress. In this study, we investigated the effect of peripheral injection of metyrapone (an inhibitor of glucocorticoid synthesis) on memory retrieval in rats.
Forty young rats were trained in a passive avoidance task. Retention test was done 48hr after training. Metyrapone (25, 50 and 100 mg/kg) or vehicle was injection IP 90 min before of retention test. Memory retention of each animal was measured as latency time to enter the dark chamber of the task.
The results showed that administration of metyrapone at doses of 25 and 100 mg significantly impaired memory retrieval as compared with control group. No significant effect was found with intermediate dose of metyrapone (50 mg/kg).
These findings provide evidence that inhibition of glucocorticoid synthesis by metyrapone impair memory retrieval in a U shape manner. Further study are required the specificity of observed effects of metyrapone.

Key words: glucocorticoids, metyrapone, memory retrieval, passive avoidance task

EVALUATION THE INTERACTION OF NITRIC OXIDE AND CORTICOSTERONE ON NEUROGENIC AND INFLAMMATORY PAINS IN MICE

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Many evidence indicated that glucocorticoids have modulatory effects on pain and probably one of the mechanism that mediate these effects is nitric oxide. The aim of this study was determine of interaction between Corticosterone and Nitric oxide (NO) system on acute (Neurogenic) and chronic (Inflammatory) pain in Formalin Test (FT) model in mice.
In this experimental study we used 70 male albino mice (25-30 gr) in 7 groups. Also we used of Formalin test for evaluation of acute and chronic pain. The criteria for evaluation of pain was measure of all time that animal reaction to pain responses after of injection of formalin (5%) in sub-plantar area of the right hind paw in each of 5 min, during 40 min. All animals received two IP injection, L-Name (10 mg/kg) as a NO synthesis inhibitor or L-Arginin (20 mg/kg), as a NO precursor 60 min and different doses of Corticosterone (1and 3 mg/kg) or Vehicle (Propylene glycol 40% + Saline in 6 ml) were injected 30 min before of FT.
Analysis of data indicated that Corticosterone at doses of 1 and 3 mg significantly reduced pain reaction in mice. Also pretreatment of L-Name enhanced the analgesic effects of Corticosterone in FT, while injection of L-Arginin, as a NO precursor had not significantly effects.
The findings indicated that glucocorticoids induce analgesic effects through modulation of NO system.

Keyword: Pain, Glucocorticoids, Formalin Test, Nitric oxide, L-NAME, L-Arginin, Mice

ROLE OF CHOLINERGIC SYSTEM IN THE EFFECTS OF GLUCOCORTICOIDS ON MEMORY CONSOLIDATION AND RECONSOLIDATION IN MICE

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Glucocorticoids have important role in cognitive functions. Evidences indicated that modulating effects of Glucocorticoids on memory, at least in part; mediate via cholinergic system, but the underlying mechanism(s) of these effects on consolidation and reconsolidation are not clear. The aim of this study was to determine the role of cholinergic system in glucocorticoids-induced modulate of memory consolidation and reconsolidation memory in mice.

Experiments were performed on 100 male albino mice (about 30 gr). In consolidation experiments, the animals were trained in an inhibitory avoidance task (0.5 mA shock for 3 seconds). Immediately after training, the animals received of corticosterone (0.3 mg/kg). Then, effects of corticosterone were examined in the presence of absence of atropine (an antagonist of muscarinic receptors, 0.5 and 2 mg/kg) or mecamylamine (an antagonist of nicotinic receptors, 0.5 and 2 mg/kg). In reconsolidation experiments, mice were trained in a passive avoidance task as above. For memory reactivation, mice were returned to the chamber 48 h later. Immediately after reactivation, mice were injected with corticosterone (3 mg/kg) in the presence or absence of atropine (0.5 or 2 mg/kg) or mecamylamine (0.5 or 2 mg/kg). Two, four, and seven days after memory reactivation, rats were returned to the context for 10 min, and step-through latency was recorded.

Our findings revealed that corticosterone significantly improved memory consolidation. This effect was blocked by atropine but not mecamylamine. Conversely, corticosterone impaired memory reconsolidation and this effect was not blocked neither with atropine nor mecamylamine.

It is concluded that glucocorticoids has opposing effects on memory consolidation and reconsolidation. Further, cholinergic system may mediate glucocorticoids on memory consolidation, but not reconsolidation.

Keywords: Atropine, Mecamylamine, Glucocorticoids, Memory consolidation and reconsolidation, Mice

PECULIARITIES OF THE ELECTRICAL CONDUCTIVITY AND TEMPERATURE OF THE SKIN IN ACUPUNCTURE POINTS

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Acupuncture points require detailed study because they are the main and well known component of the meridian system of the traditional Chinese medicine that participate in the acupuncture treatment mechanisms, the therapeutic effect of which is unclear today.
The aim of the investigation was the study of both: electrical conductivity and temperature (contact and radiant) of the skin in acupuncture points. They are P9, MC7, C7, iG5, TR4, Gi5, RP3, F3, R3, V64, VB40 and E42 points, which also named representative acupoints of the meridians (by Nakatani).
Results of the study show that parameters of the skin electrical conductivity in acupuncture points were characterized by high variability. They could be above or below temperature of nearby skin sites and correlate with functional activity of correspondent organs and systems. Changes of the skin electric conductivity of acupuncture points in reply to their irritation specifies in high reactance of point tissues. Stimulation of acupuncture points by superhigh-frequency electromagnetic field (54-75 GHz), chosen according to canons of east medicine, was accompanied by increase initially low and decrease in initially overestimated parameters of the skin electric conductivity in representative points to average physiological level. Some findings confirming existence of the meridian system and functional ties between the meridians were noted. The contact skin temperature in acupuncture points was differ in comparison with beside acupoint one. The radiant skin temperature as a rule was above contact temperature and could change quickly on 2-3 degrees during the short time interval. Certain interdependence between skin electrical conductivity and temperature parameters was noted.
The hypothesis of acupuncture mechanisms was offered. It assumes that meridians and acupoints are important components of the whole regulatory system that phylogenetically take priority of the nervous and endocrine systems and can induce the genetically fixed adaptive and compensatory mechanisms for realization its therapeutic effect by specific bioactive substances.
Results of the present study show that skin electric conductivity and skin temperature in area of acupuncture point differed variability, and their parameters are caused by a functional condition of organs and systems. Level and dynamics of the changes of radiant temperature in acupuncture points assume presence in them of the generators of infra-red radiation.

INVOLVEMENT OF THE INTRAPULMONARY RENIN ANGIOTENSIN SYSTEM IN THE DEVELOPMENT OF ALLERGIC LUNG DISEASE

Irina Luciana Dumitriu[1], Bogdan Gurzu[1], Marcel Costuleanu[2], Beatrice Mihaela Gurzu[2], Simona Mihaela Slătineanu[1], Petru Plămădeală[3], Gheorghe Petrescu[1]

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Recent studies have demonstrated the involvement of intrapulmonary activation of the renin-angiotensin system (RAS) in the progression of various pulmonary diseases. We analyzed to what extent the pulmonary RAS could modulate the development of allergic lung disease. Therefore, valsartan (a blocker of angiotensin II type 1 receptors; 0.5 mM) or pepstatin A (a renin inhibitor; 0.5 mM) were nebulized during the last week of sensitization protocol. The bronchial reactivity, total number of cells from bronchoalveolar lavage fluid (BALF) and inflammatory cell infiltration were assessed. Valsartan treatment reduced allergen - induced bronchoconstriction by more than 20%, and (within 1300 characters including spaces, decreased Emax of contraction induced by acetylcholine (ACh) (with 16.86%). Both, valsartan and pepstatin A, prevented the challenge-induced alteration of the bronchorelaxant effect of terbutaline and decreased the total number of cells from BALF. Even more, valsartan treatment decreased infiltration of inflammatory cells on the walls of lobar and segmental bronchi and in peribronchiolar space from this level. These results emphasize the RAS roles on such pathophysiological circumstances and suggest that blocking of pulmonary RAS could partially prevent the effects of allergen sensitization/ challenge.

Keywords: airway, allergy, angiotensin

THE ANGIOTENSINS VASOMOTOR EFFECTS ON RAT RENAL ARTERIES

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Published data revealed that Ang II could have either vasoconstrictor or vasodilatator effects depending on the species or experimental conditions. The present study examined the vasomotor effects of angiotensinogen (Aogen), Ang I and Ang II on rat renal artery rings (RAR) using two experimental models. In the first one, the different doses of Aogen, Ang I and Ang II was administered before cumulative administration of phenylephrine (10nM - 10microM, Phe). In the second experimental model, the RAR were precontracted with Phe (10microM) and the relaxant responses to cumulative concentration of Aogen, Ang I and Ang II (1nM – 10microM) were subsequently studied. The experiments were carrying out in the absence and in the presence of losartan (10microM, LOS) or PD123319 (10microM). The pretreatment of RAR with Ang I (100nM) or Ang II (10nM) potentiated the Phe – induced contractile effects, by an AT2 – mediated mechanism. High doses of Ang II (1 or 10 microM) reduced the Emax of Phe – induced contractions with more than 20%; this Ang II effects were blocked by LOS pretreatment. All studied angiotensins had relaxing effects. The order of potency was: Ang II > Ang I >> Aogen. The relaxant effects of Ang I and Ang II were decreased by PD123319 pretreatment but were not significantly modified by endothelial removement. In conclusion, the angiotensins could have either AT1 – mediated contractile effects or AT2 – dependent but endothelium – independent relaxing effects.

Key words: angiotensinogen, angiotensin I, angiotensin II, renal artery

NITRIC OXIDE INVOLVEMENT ON APELIN EFFECTS ON BRONCHIAL REACTIVITY

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Although the apelin (AP) and its specific receptor APJ are highly expressed in the lung, biological functions of AP/APJ system are still under investigation. We studied the effects of AP on bronchial reactivity to acetylcholine (ACh) and terbutaline induced by allergen sensitization and challenge. Experiments were performed on bronchial rings obtained from normal (NR) or ovalbumin sensitized rat (OSR). Because recent studies have demonstrated that involvement of nitric oxide (NO) on AP effects, the bronchial tone and the NO production were simultaneously assessed. On OSR, the 10 uM API 3 pretreatment reduced the Emax of ACh-induced bronhoconstriction with more than 20% and shifted to the left the terbutaline dose-response curve. AP 13 effects were totally prevented by inhibition of all NO synthases (NOS) with 0.1 mM N(G)-nitroL-arginine methyl ester and only reduced by 0.1 mM aminoguanidine (inhibitor of inducible NOS). The simultaneously monitoring of NO releasing from bronchial rings confirmed the NO involvement on AP 13 effects. These results suggest that AP could reduced airway hyperreactivity by increasing bronchial constitutive NOS activity and provides a very interesting target for developing suitable therapeutic approaches.

Keywords: bronchus, nitric oxide, peptid

EFFECTS OF GLUTATHIONE DEPLETION ON MALE FERTILITY (A PROSPECTIVE *STUDY IN MALE SPRAGUE DAWLEY RATS)*

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The objective of this study was to investigate the role of glutathione depletion in fertility in rats. Forty male Sprague Dawley rats were divided into two groups. Twenty rats administered paracetamol (glutathione depletory) and the rest (the control) received 0.9% normalized saline for 90 days. At day 15, 30, 60, and 94 rats from each group were assessed for semen quality, sperm motility, morphology, glutathione concentration and histology of testis, liver and epididymis. The quality of semen was highly affected in the study group with semen homogeneity disappearing and liquefaction happening as early as 30 seconds, sperm motility, progressiveness and glutathione levels dropped, sperm abnormalities rose. Glutathione depletion affects male fertility especially on the quality of sperms affecting ability of the sperm to fertilize the egg. Abnormal mid-piece and tail were the most affected. 90 days post treatment, libido; mounting and intromission were also significantly affected.

A BRIDGE BETWEEN PHYSIOLOGY AND GENETICS: PRENATAL DIAGNOSIS

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The rapid and great advance in molecular biologic and genetic techniques had led to investigate many physiological conditions in living organisms. One of the results of this situation is the enhanced development and application of prenatal diagnosis techniques. In fact the physiologists are generally concerned with the systems and geneticians with the components of the genes and the genetic code that indicates the phenotype. On the other hand , one of the main mechanisims in genetics is ‘ gene conversion’ which is defined as a physiological interaction between to alleles of a gene. Also, the methods for the molecular analysis of DNA have been introduced into physiology at such an increasing scale in the last two decades that the gulf between physiology and genetics is now being bridged. One of the main fields in this area is prenatal diagnosis including cell culture and chromosome analysis in order to define any physiological disorder in human beings and also in animals.
In the present study the results of the patients who had underwent prenatal diagnosis were evaluated and discussed from this point of view.

IDENTIFICATION AND TISSUE DISTRIBUTION OF A NOVEL ALTERNATIVELY SPLICED MRNA OF HUMAN SULFONLYUREA RECEPTOR 1 (SUR1Δ2) CODING FOR A TRUNCATED K_{ATP}-CHANNEL OPENING PEPTIDE

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A novel splice variant of sulfonylurea receptor 1 (SUR1), a regulatory subunit of K_{ATP}-channels, is described. SUR1 mRNA lacking exon 2 (SUR1Δ2) was discovered in human heart using a transcript scanning approach and 5'RACE-PCR. The omission of exon 2 causes a frame shift and an immediate stop codon in Exon 3 leading to a "walking stick"-like peptide composed of the N-terminal extracellular domain and the first transmembrane helix of SUR1 containing the most prominent sulfonylurea-receptor-family signature sequence. Tissue distribution experiments reveal detectable SUR1Δ2 in human heart and skeletal muscle, brain and spinal cord, testis and uterus, as well as pancreas. In some tissues, SUR1Δ2 is expressed concurrently with SUR1 and their ratio differs between individuals. Whole cell patch clamp experiments with Rin5mF cells expressing SUR1 containing K_{ATP}-channels show, that current is strongly increased in SUR1Δ2 transfected cells at 2 mM intracellular ATP. We conclude that SUR1Δ2 is a novel endogenous K_{ATP}-channel opening peptide.

POLYELECTROLYE NANOASEMBLED MICROCAPSULES FOR BIOSENSING OF GLUCOSE IN HUMAN SWEAT

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The new biocompatible and anti allergic approach to detect selected human sweat ingredients as glucose, important for evaluation of human physiological and metabolical conditions was implemented by using suitable enzymes be able to transfer selected sweat substrates to optically detectable submicron products.
Today the glucose determination apparatus are widely used in common life, medicine and pharmacology. Commercially available sensors are frequently used for detection and determination of glucose concentration in macroscopic range. Our aim was to develop and assemble submicrometer polyelectrolyte microcapsule sensor that will increase the lower sensitivity for glucose and will be capable to measure glucose concentration above 200 mM with linear response. The enzyme's optimal substrate activity was studied in the polyelectrolyte LbL (Layer-by-layer) assembled microcapsules for being able to exchange analyte with outer measuring environment, i.e. human sweat. For that purpose GOX and HRP were encapsulated and immobilised in microcapsules with suitable enzyme substrate, o-dianisidine or guaiacol. The efficacy of encapsulated enzymes was determined by fluorescence spectra measurements using partially labeled enzymes with fluorescence dyes, while the glucose or lactate detection was evaluated by UV/Vis photospectroscopy for various reaction times (0 to 11 minutes) at 35 ^{o}C and depending on the concentration of glucose and volume of administrated samples. Diffusion of glucose and lactose from the bulk sweet solution through semipermeable polyelectrolyte microcapsule wall into inner microcapsule enzyme solution was taken into account. Small ions are distributed between inner and outer solution according to well known Donnan distribution due to their concentration and presence of big nonpermeable macroions. The variation in pH and salt concentrations in human sweet depends also on different skin glands secretion on specific parts of body.

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EFFECTS OF ENVIRONMENTAL POLLUTANTS ON AUDITORY FUNCTION IN ADULT RATS AFTER EXPOSURE DURING DEVELOPMENT

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Various environmental compounds have been shown to affect the development of organisms leading to long-lasting alterations of adult function. Polychlorinated biphenyls (PCBs) are a group of industrial compounds which were used in numerous applications. Due to their persistence and accumulation in food chains, elevated levels of PCBs are still found in humans and animals. Developing organisms exhibit a particular sensitivity to PCBs. PCB-induced effects on thyroid hormone and retinoids are among the most pronounced effects. Since thyroid hormones and retinoids regulate the development of the auditory system, we examined brainstem auditory evoked potentials (BAEPs) in rats after perinatal exposure to PCBs. Rat dams were orally treated with purified PCB180 or PCB52 during gestation and/or after delivery. There were six dose levels, ranging from 0 to 1000 mg/kg (PCB180, total dose) or 0 to 3000 mg/kg (PCB52, total dose). These PCB congeners were highly purified to exclude effects of dioxin-like contaminants. BAEPs were recorded in male and female offspring at the age of approximately 7 months. Animals were sedated with ketamine and xylazine during the measurements. Clicks and tone pips, ranging from 0.5 to 16 kHz, were used to elicit BAEPs. Sound pressure levels (SPL) were varied to examine BAEP thresholds. Also, latencies of wave II and IV were determined. Results were analyzed with general linear models (GLM) to determine significant dose-response relations. There were slight to moderate effects of purified PCB180 on BAEPs of female offspring in the low frequency range. Significant linear dose-response relations were found for thresholds at 0.5 kHz and 4 kHz and for latency of wave IV at 0.5 kHz ($p<0.05$). Alterations in males were not significant ($p>0.05$), with the exception of a small prolongation of wave II latency after stimulation with clicks at 70 dB. In contrast, PCB52 resulted in threshold elevations in the frequency range from 0.5 to 8 kHz ($p<0.05$) which were particularly pronounced in male offspring. In addition, there marked elevations of wave II latency at 0.5 to 2 kHz in males ($p<0.05$). These results show that exposure to highly purified PCBs during development impairs auditory function in adulthood and that these effects cannot be attributed to dioxin-like contaminants.
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ENDOCANNABINOIDS POTENTIATE SYNAPTIC TRANSMISSION THROUGH ASTROCYTE STIMULATION

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We investigated the effects of the endocannabinoid-mediated neuron-astrocyte signalling on synaptic transmission in mouse hippocampal slices.
We recorded from CA1 pyramidal neurons and monitored astrocyte Ca^{2+} levels. We stimulated Schaffer collateral single synapses. Endocannabinoids (ECBs) were released by neuron depolarization (ND) while EPSCs were monitored in an adjacent neuron. We found:
ND transiently increased the probability of transmitter release (Pr) in 40% of the synapses, without affecting EPSC amplitudes, which resulted in a short-term synaptic potentiation (e-SP). These effects were abolished by AM251, and were absent in $CB1R^{-/-}$ mice.
In 27% of the synapses, ND transiently decreased Pr without changing EPSC amplitudes. This was blocked by AM251 and was absent in $CB1R^{-/-}$ mice, like depolarization-induced suppression of excitation (DSE).
ND elevated Ca^{2+} in astrocytes, which was abolished by AM251 and were absent in $CB1R^{-/-}$ mice.
ND-evoked ECB-mediated e-SP and astrocyte Ca^{2+} signal were unaffected by pertussis toxin, but were blocked by phospholipase-C antagonists and thapsigargin, indicating that they were not mediated by $G_{i/o}$ proteins, but by $G_{q/11}$ proteins and phospholipase-C-mediated Ca^{2+} mobilization from internal stores.
After loading astrocytes with BAPTA, DSE was unaffected, but ND failed to induce e-STP.
The ND-evoked e-SP was abolished by the type I mGluR antagonists.
Synapses that showed ND-evoked synaptic potentiation were depressed by CB1R agonists.
When pairing the ND-evoked ECB-mediated astrocytic Ca^{2+} signal with a mild postsynaptic depolarization, the transient potentiation became persistent.
We conclude that endocannabinoids potentiate excitatory synaptic transmission through stimulation of Ca^{2+}-dependent release of glutamate from astrocytes.

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CARDIOPROTECTION AT REPERFUSION: UNCOUPLING EFFECTS OF DIAZOXIDE AND CYCLOSPORINE A ON INFARCT SIZE AND FUNCTIONAL RECOVERY IN ISOLATED RAT HEARTS

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Mitochondrial ATP dependent potassium channels (mKATP) openers such as diazoxide (Dx) and the permeability transition pore (PTP) desensitizers such as cyclosporin A (CsA) have been constantly associated with cardioprotection at reperfusion both in vivo and in vitro experimental settings. The present study investigated the effects of Dx (100 microM/L), CsA (0.2 microM/L) and their association at reperfusion on infarct size and contractile function recovery in Langendorff perfused rat hearts subjected to 30 min of global ischemia (I) and 60 min of reperfusion (R). Postischemic recovery of left ventricular function was assessed by the rate pressure product (left ventricular developed pressure x heart rate) and by the maximal and minimal first derivatives of left ventricular pressure (dLVP/dtmax and dLVP/dtmin) as indices of contractility and relaxation, respectively. Infarct size was quantified by the 2.3.5-triphenyltetrazolium chloride staining and by the LDH leakage in the coronary effluent. At the end of reperfusion the cardioprotective drugs elicited uncoupling effects with respect to mechanical function and infarct size. A marked reduction in infarct size was obtained in Dx treated group (15 ± 3% vs. 41 ± 4% in control group, $p< 0.05$) without a significant increase in +dP/dtmax. Conversely, CsA lowered infarct size only by 26± 5% but induced a greater recovery of contractility. In the Dx+CsA group infarct size was also significantly (albeit not additively) reduced (20±4%, $p< 0.05$ vs. control) but the recovery of contractility was less important. In isolated rat hearts treated with Dx and CsA at reperfusion we report a disparity between infarct size and functional recovery. Further studies are required to elucidate the underlying mechanisms of these differential effects.

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THE EARLY EFFECTS OF MATERNAL TRANSIENT SYSTEMIC HYPOTENSION DURING PREGNANCY ON THE DEVELOPMENT OF FETAL BRAIN IN RATS

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Albeit apoptosis is observed in pathological conditions, a large number of cells which are involved in the development of central nervous system also undergo physiological apoptotic process in fetus. Hypotensive episodes in the course of pregnancy are frequent cause of circulatory failure in the fetus, thus it may represent a potential risk for hypoxic ischemic injury in fetal brain as well. Cellular injury after hypoxic-ischemic insult is caused by necrosis and/or apoptosis. Neuronal death depends on the severity and the site of the injury as well as the time after injury. This study was aimed to explore the early term (within 24 hours) effects of maternal transient systemic hypotension on the development of fetal brain in rats.

Thirty pregnant Sprague-Dawley rats (3-6 months old) were included in the study. On the 15th day of pregnancy, rats were subjected to either transient systemic hypotension (hypotension group, n=15) by blood withdrawal for 30 minutes or sham operation (control group, n=15). Two randomly selected fetuses were taken after cesarean section under ketamine/xylazine anesthesia in each rat both in hypotension and control groups after 6 hr or 12 hr or 24 hr (n=5 pregnant rats in each time points). Brain sections were stained with hematoxylin-eosine (HE). Apoptosis was evaluated in fetal brain sections both by TUNEL Method and caspase-3 staining. TUNEL (+) and caspase-3 (+) cells were counted and scored double-blindly.

HE staining did not reveal any morphological difference between hypotension and control groups. TUNEL (+) cells were more abundant in hypotension group at all three time points than in the corresponding control group (P<0.05). The most prominent increase was seen in the 12 hr group (P<0.05). In accord with our findings with TUNEL Method, caspase-3 (+) cells were more prominent in the hypotension group at all three time points when compared with the corresponding control group (P<0.05). The most prominent increase in caspase-3 (+) staining was detected in the 12 hr hypotension group when compared to other time points.

Maternal transient systemic hypotension caused a significant increase in apoptosis in fetal rat brain mainly at 12 hr, which may have a potential significance of the timing of neuroprotective agents against brain damage.

NON-INVASIVE OXIDATIVE STRESS MARKERS FOR LIVER FIBROSIS DEVELOPMENT IN TOXIC HEPATITIS EVOLUTION

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Introduction: Oxidative stress is related to the liver fibrosis. Hepatic stellate cells (HSC) play a key role in the pathogenesis of hepatic fibrosis by producing extracellular matrix proteins (ECM). The activation and the progression of activation of HSC depends on oxidative stress. It appears that oxidant stress anticipates HSC activation, being potential candidate for non-invasive quantification of fibrosis progression. Our aim was to correlate oxidative stress markers with the histological liver alterations in order to identify predictive, non-invasive parameters of fibrosis progression in toxic hepatitis evolution.
Material and Methods: The 50 animals (male Wistar rats, weight 200±10 gr) were randomly and equally divided into five experimental groups. In the four test groups, CCL_4 was administered intragastrically twice a week (1.2 ml/kg, CCL4 25% in sunflower oil). Control group received sunflower oil, same dose and way of administration. After 2, 3, 4 and 8 weeks of treatment, plasma levels of malondialdehyde (MDA), carbonylate proteins (CP), hydrogen donor ability (HD), sulfhydryl groups (SH), gluthation (GSH) and nitric oxide (NO) were measured, as well as the histological examination of the liver slices. Non-parametric Mann-Whitey U Test as well as receiver operating characteristic analysis with areas under curve (AUROC) determinations was used.
Results: Dynamic of histological disorders was assessed by the Knodell score (inflammation grade, fibrosis stage). Significant elevation of inflammation grade after only 2 weeks of experiment were obtained (p=0.001), while fibrosis alterations started to become significant (p=0.001) at 1 month of CCl4 administration. A good correlation between plasma MDA and liver fibrosis development was obtained (r=0.877, p= 0.050). Correlation between CP dynamics and liver alterations was marginally significant for inflammation grade(r=0.756, p=0.138). HD evolution revealed a decreasing trend. A marginally inverse correlation with inflammation grade was obtained for HD (r=-0.794, 0.108). However, no correlation with liver fibrosis development could be established(r=-0.187, p=0.762). No correlations could be established for the others parameters with either inflammation grade or fibrosis stage.
Conclusions: Our study shows that MDA elevation offers the best prediction potential for fibrosis, while marginal prediction fiability could be attributed to high levels of plasma CP and low levels of HD.

Keywords: oxidative stress, non-invasive markers, toxic hepatitis, fibrosis

OPSIN EVOLUTION AND SPECTRAL ADAPTATION OF VISION IN MYSID CRUSTACEANS

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Opsin genes and absorbance spectra of corresponding visual pigments from the genus *Mysis* were analyzed. Nucleotide sequences were obtained from 45 populations of 12 species from arctic marine waters, coastal littoral habitats, freshwater lakes and the Caspian Sea, representing a wide range of different separation histories and light conditions. All appear to have a single opsin encoded by a single gene. Extensive sequence variation was found, with a maximum 5.5% nucleotide sequence divergence within the genus and 12 % of amino-acid residues showing variation. The levels of intraspecies variation varied widely among the species but showed only weak correlation with previous estimates of mtDNA diversities. Likewise, the opsin gene phylogeny only partly corresponded with that from other data. In the main cluster of species, comprising seven continental and two coastal marine taxa, two main allelic lineages differing at several amino-acid sites were found. Of species from the *M. relicta* group, *M. salemaai* and *M. segerstralei* were represented in both. Statistical testing could not reject the null hypothesis that opsin evolution is neutral, showing no effects of selection. Yet it is almost self-evident that the opsin gene of visual animals inhabiting dim-light aquatic habitats is under a strong selection pressure favouring pigments that afford high visual sensitivity. Visual-pigment absorbance spectra were recorded and related to opsin amino-acid substitutions in 9 populations of 3 species of the *M. relicta* group from the Baltic Sea and Fennoscandian lakes with different spectral transmittance. The spectra of all lake populations were long-wavelength-shifted by 20-40 nm (8_{max} range 554-562 nm) compared with all sea populations (8_{max} range 521-535 nm), both within and between species. This distinction did not coincide with the allelic lineages, and the shifts were realized by partly different changes in the opsin genes. The results suggest that different but spectrally convergent lines of adaptive evolution have occurred repeatedly and sometimes rapidly on a statistically dominant background of neutral molecular divergence. Mechanisms apparently include loss of either of two ancestral alleles (I and II) as well as mutations within each of the alleles.

EFFECT OF *CALLUNA VULGARIS* ON SINGLE DOSE UVB-INDUCED OXIDATIVE STRESS IN SKH-1 MICE SKIN

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Skin carcinomas represent approximately 30% of the new cases of diagnosed cancer. Ultraviolet radiation (UV) from the sun is a major cause of non-melanoma skin cancer in humans. The prevention and mainly the photo chemoprevention with natural products represent a simple but very effective strategy in the management of cutaneous neoplasia.

Phenol compounds and triterpenes (ursolic acid and oleanoic acid) isolated from Calluna Vulgaris (Ericaceae family), had proven in vitro remarkable effects in prevention UV-induced photo carcinogenesis and toxicity.

In this study we have investigated the effects of topical application of Calluna Vulgaris (CV) extract in 70% ethanol to skin mice before one dose UVB irradiation on markers of oxidative stress and matrix metalloproteinase's activities (MMPs).

Forty-two 8-week-old female SKH-1 hairless mice were randomly divided into 6 groups: 1. group I (control) - no treatment, no irradiation; 2. group II - treated with 50 mg/cm^2 hydrogel vehicle, no irradiation; 3. group III – UVB-irradiated group; 4. group IV – vehicle and UVB irradiated; 5. group V – CV extract treated (4 mg polyphenols/30µl/cm^2), UVB irradiated; 6. group VI – CV extract incorporated in vehicle, UVB irradiated. UVB irradiation was performed after 30 minutes from topical application with 240 mJ/cm^2. The animals were sacrificed 24 hours after irradiation. Malondialdehyde (MDA) and glutathione (GSH) levels were determined in skin homogenates using fluorimetric methods. MMP-2 and MMP-9 activities were evaluated in skin with zymographic method.The results indicated that the treatment with CV extract inhibited UVB-induced oxidative stress especially when was incorporated in vehicle (MDA: 0,76±0,04 nmoles/mg protein vs. 2,81±0,94 nmoles/mg protein in irradiated group, $p<0,001$) or irradiated vehicle group (0,86±0,24 nmoles/mg protein). GSH level increased after irradiation (17,6±7,10 vs. control group 9,3±5,3 nmoles/mg protein) and decreased in animals treated with CV incorporated in vehicle (8,4±1.02 nmoles/mg protein, $p<0,05$).

MMP-9 and MMP-2 activities in skin slightly increased in vehicle and irradiation group comparatively with CV treated groups.

In conclusion our results demonstrated that the damage effects of one dose of UVB are mediated by generating of reactive oxygen species.

SWELLING INDUCED ACTIVATION OF THE TASK-2 POTASSIUM CHANNEL BY A TYROSINE PHOSPHORYLATION OF THE CHANNEL

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The TASK-2 channel belongs to the family of two-pore domain potassium channels, the so-called background or leak channels. TASK-2 has been implicated in cell volume regulation in several cell types among them the Ehrlich Ascites Tumor (EAT) cells. Withholding a constant cell volume is important for maintaining cell homeostasis. For that purpose the cell has several regulating mechanisms including regulatory volume increase and regulatory volume decrease (RVD) which both are seen in response to acute changes in cell volume. When experiencing an extracellular decrease or an intracellular increase in osmolarity, the cell swells and activates the RVD mechanisms, which includes efflux of KCl followed by osmotically obliged water through specific channels (see 1;2). The details of the activation of these channels has so far remained unknown, though it seems that tyrosine phosphorylation is somehow involved in the activation of TASK-2 in EAT cells, thus the purpose of this study was to investigate the role of tyrosine phosphorylation in the activation of the volume sensitive TASK-2 channel in EAT cells. When studying the effect of over-expressing the TASK-2 channel in HEK-293 cells we found that the over-expression resulted in an increased RVD (faster and with a greater ion loss) compared to what was seen in wild type cells. Furthermore we found that the tyrosine kinase inhibitor genistein impaired the RVD in response to swelling as if the channels remained closed and that the tyrosine phosphatase inhibitor mpV(pic) potentiated RVD as if the channel was kept open for a longer period of time. When precipitating the hypotonically stimulated channel (1, 5 and 10 min) and using western blotting together with antibodies against TASK-2 as well as against phospho-tyrosines we found that there was a significantly and time dependent increase in the tyrosine phosphorylation of the channel itself. Netphos prediction programme gave us 5 possible tyrosine phosphorylation sites, which are to be further analysed using mass spectrometry.

Hoffmann EK, Lambert IH and Pedersen SF(2009) *Physiol Rev* 89:193–277.
Okada Y (2004) *Cell Biochemistry and Biophysics* 41**:**233-258.

ASYMMETRIC DIMETHYLARGININE (ADMA) BY ACTIVATING THE ANGIOTENSIN II – NAD(P)H OXIDASE PATHWAY ELICITS SUPEROXIDE RELEASE, WHICH INTERFERES WITH NO-MEDIATED DILATIONS

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Increased level of asymmetric dimethylarginine (ADMA) contributes to the pathogenesis of cardiovascular diseases, but the underlying pathomechanisms have not yet been elucidated. Previously we have found that ADMA induced superoxide production, in isolated microvessels but the mechanisms remain unknown. We hypothesized that elevated level of exogenous ADMA, by activating vascular oxidases elicits increased superoxide production, which interferes with NO-mediated dilations. Isolated arterioles from rat gracilis muscle (~160 μm in diameter at 80 mmHg) were treated with indomethacin. Basal arteriolar diameter and flow-induced dilation were obtained in the presence of ADMA (10^{-4} mol/L). Incubation arterioles with ADMA elicited significant constrictions (from 162±4 μm to 143±4 μm) and eliminated the dilations to increases in intraluminal flow. In the presence of ADMA, superoxide dismutase (SOD) plus catalase (CAT) restored dilations to flow. Incubation of arterioles with the NAD(P)H oxidase inhibitor apocynin or the angiotensin-converting enzyme (ACE) inhibitor quinapril inhibited ADMA-induced constrictions. In addition, apocynin, quinapril or the angiotensin type 1 receptor blocker losartan restored flow-induced dilations reduced by ADMA. ADMA-induced increased production of superoxide - assessed by dihydroethidium fluorescence - was inhibited by apocynin, quinapril or losartan. ADMA or pyrogallol (known to generate superoxide) reduced arteriolar dilations to the NO donor, sodium nitroprusside (SNP), which was partially restored by SOD/CAT. Also, ADMA-induced constrictions were prevented by SOD/CAT and the NAD(P)H oxidase inhibitor apocynin, but not by the xanthine oxidase inhibitor oxypurinol.
In conclusion, we suggest that ADMA activates NAD(P)H oxidase via the renin-angiotensin system, and the produces superoxide reducing thereby the bioavailability of NO resulting in diminished NO mediated dilations. This pathophysiological mechanism may contribute to the development of cardiovascular diseases associated with elevated levels of ADMA.

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ISOINTEGRAL BODY SURFACE MAPS AND LEFT VENTRICULAR HYPERTROPHY IN PATIENTS WITH AN OLD MYOCARDIAL INFARCTION

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Ventricular remodeling causes left ventricular hypertrophy in myocardial infarction patients. We hypothesised that left ventricular hypertrophy can be evaluated using isointegral body surface maps.

32 patients with an old myocardial infarction underwent 64-electrodes body surface mapping (isointegral QRS, QRST, ST and STT maps) and 12-lead ECG. 16 of them (50%) had left ventricular hypertrophy according to 8 electrocardiographic criteria (Romhilt-Estes scoring system, Sokolow-Lyon voltage, Gubner-Ungerleider voltage, Cornell voltage index, Cornell product, the diagnostic criteria based on the Framingham Heart Study data, Perugia score and Mazzaro score) and 2D-echocardiography.

Isointegral maxima increased and the minima was more negative in patients with left ventricular hypertrophy, and the differences were statistical significant for: isointegral QRS maxima (35±16 versus 60±21 mV.ms, p=0.0085) and minima (25±15 versus 69±14 mV.ms, p=0.0067), isointegral maxima and minima in the second third of the QRS complex, isointegral QRST minima (34±9 versus 49±5 mV.ms, p=0.042) and isointegral ST minima (5±2 versus 10±4 mV.ms, p=0.0026). Isointegral multipolar maps prevalence was increased in patients with left ventricular hypertrophy (75% versus 50%). Isointegral QRS and QRST maxima correlated best with left ventricular mass (r=0.73 and 0.81).

Body surface mapping is a useful method for the evaluation of patients with left ventricular hypertrophy. The most sensitive parameters are: isointegral QRS maxima and minima, especially in the second third of the QRS complex, isointegral QRST maps (minima, maxima and multipolarity) and isointegral ST minima.

RESTORATIVE EFFECTS OF MELATONIN TREATMENT ON CEREBRAL DOPAMINE SYNTHESIS AND MOTOR COORDINATION

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During the normal process of aging, production of melatonin decreases as well as alterations in dopaminergic function are known. The aim of this study was to assess the effects of chronic treatment with melatonin on central dopamine (DA) neurotransmitter and motor ability in aged rats. Accumulation of 3,4-dihydroxyphenylalanine (DOPA) after decarboxylase inhibition was used as a measure of tyrosine hydroxylation rate *in vivo*. Male aged Wistar rats received melatonin (1 mg/kg/day in drinking water with 1% alcohol to increase melatonin solubility, 4 weeks, n=6). Control young (3 months, n=6) and aged rats (20 months, n=6) also received 1% alcohol in drinking water, and were maintained under controlled conditions (22ºC, 70% humidity, 12/12LD). Animals were evaluated for motor ability and balance by using rota-rod. The rats were given prior training sessions to acclimate them to apparatus. Rats were placed on the rotating rod and the length of time on the rod was taken as measure of competency. Each rat performed 5 separate trials and the results were averaged. After that, rats were sacrificed by decapitation and samples of striatum analyzed by HPLC with electrochemical detection to measure DOPA, DA and the metabolite homovanillic acid (HVA). Tyrosine hydroxylation decreased significantly in the striatum of aged rats (34%) respect to the young controls. In accordance, intraneuronal DA content and HVA metabolite were also reduced (55% and 33%, respectively), showing the impairment in dopamine neurotransmission with age. However, when aged rats were treated with melatonin, an important increase in tyrosine hydroxylation (44%), DA content (126%) and HVA (26%) were observed in the striatum. When control aged rats were tested using rota-rod impaired their skill and increased the number of falls compared to the young control group. However melatonin treatment resulted in significant improvement in fall off time as compared to control aged rats. In conclusion, the results indicate that repeated treatment with melatonin might aid to improve the descent in dopamine neurotransmission and motor ability that normally occurs as a consequence of aging.

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EXPRESSION OF TYROSINE HYDROXYLASE AND NEUROPEPTIDE Y IN THE HEARTS OF TWO RAT STRAINS: EFFECTS OF TWO TYPES OF RESTRAINT STRESS

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme of the synthesis of catecholamines (CA) in the catecholaminergic neurons and neuropeptide Y (NPY) is a cotransmitter of CA. Chronic stress leads to increased levels of plasma CA together with increased levels of TH and its gene expression in the adrenal medulla. Here, we investigated TH and preproNPY mRNA expressions in the hearts of two rat strains submitted to various stress protocols by real-time RT-PCR method. A restraint stressor (immobilization, IMO) and restraint stressor combined with partial immersion of rats into water (IMO+C) were applied for 1 hour to adult Lewis (Lew) rat, which shows a blunted hypothalamic-pituitary-adrenal (HPA) response, and to an comparator strain, Sprague Dawley (SD) rat. TH and preproNPY mRNA expressions were determined in four groups of animals after 1 and 3 hours after IMO and IMO+C (IMO1, IMO3, IMO+C1, IMO+C3) and compared to control animals. TH mRNA expression in the right atria of SD rats increased significantly (by 365% and 239%) after IMO3 and IMO+C3, respectively. An increase by 80% was also observed in the left atria after IMO+C3. In Lew rats, an increase by 73% was observed only in the left atria after IMO3. In SD rats, a significant decrease of preproNPY mRNA expression after IMO1 and IMO+C1 by 54% and 46%, respectively was observed in the right ventricles. A decline by 38% was also found in the left ventricle after IMO1. In Lew rats, preproNPY mRNA expressions have shown a decline in the right atria by 52% after IMO3 and by 58% in the left ventricles after IMO+C3. In conclusion, differential effects were observed between mRNA expressions for TH and preproNPY in all heart compartments in various stress protocols, however, a tendency for an increase in TH mRNA and a decline in preproNPY mRNA expressions were seen in both strains of animals. Our data show that increased TH mRNA expression in the heart in acute stress which may reflect increased synthesis of CA is not associated with increased expression of its main cotransmitter, NPY.

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APOPTOTIC VOLUME DECREASE AND MTMEM16F - A NOVEL CALCIUM ACTIVATED CHLORIDE CHANNEL

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A classical and stereotyped feature of apoptosis is cell shrinkage, also known as apoptotic volume decrease (AVD). AVD is driven by net loss of ions and osmotically obliged water and a prerequisite for caspase activation and execution of apoptosis. Here we show that cisplatin-induced AVD in Ehrlich ascites tumor cells (EATC) is a complex process consisting of at least 3 distinguishable stages defined by sequential efflux/influx of different ions, amino acids and water. These movements can be correlated to early and a late shrinkage stages and a transient cell volume recovery stage. While the shrinkage stages are associated with loss of K^+, Cl^-, Na^+ and amino acids, recovery is mediated by NaCl uptake. Cl^- efflux is a requisite component of AVD and, as expected, Cl^- channel inhibition in EATC alters AVD and NaCl movements, however in a unique manner in each AVD stage. To further investigate the importance and molecular mechanisms of Cl^- movements, we have cloned and expressed members of the recently identified TMEM16 family of Cl^- channels and analyzed their biophysical channel properties. When over expressed in HEK293 cells, mTMEM16F (mouse anoctamin 6) was found to induce an outwardly rectifying Ca^{2+}-activated chloride current ($I_{Cl,Ca}$). The current activates time-dependently at high positive potentials, has an anion selectivity sequence of $SCN^- > I^- > Br^- > Cl^-$ > Asp and is sensitive to tamoxifen > niflumic acid > NS3728 > DIDS. Mutation of R592, K616 and K636, the homelogue amino acids which in TMEM16A has been suggested to affect channel anion selectivity, to glutamic acid completely abolished the Ca^{2+} induced Cl^- current. In addition, we found that stable knock-down of mTMEM16F in EATC reduced $I_{Cl,Ca}$ significantly. In conclusion, Cl^- movements are important during different stages of AVD and for the proper execution of apoptosis. mTMEM16F appears to be a CaCC which may play important roles.

MENTAL CHALLENGE DURING SUPINE AND UPRIGHT POSITION

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Mental stress induces cardiovascular effects. The postural changes that arise during passive head up tilt (HUT) elicit complex cardiovascular and hormonal changes. We investigated the effects of posture on mental challenge by comapring mental challenge induced cardiovascular and salivary responses in supine and upright tilted participants. 18 healthy young males were subjected to two mental challenge, applied using mental arithmetic (MA), protocols: a) MA in supine position and b) MA during HUT (MA + HUT), with sessions randomized and ≥2 weeks apart. Beat to beat continuous hemodynamic variables were measured and saliva samples taken for hormonal assay. In relation to baseline, MA in supine position increased HR (+8.0±6.0 bpm), MAP (+10.0±6.5 mmHg), CO (+0.6±0.8 l/min), TPR (+27±134 dyne*s/cm^5) but decreased SV (-2.0±7.9 ml). However, MA + HUT increased HR (+28.8±8.4 bpm, $p< 0.001$), MAP (+11.2±11.6 mmHg) and TPR (+160±199 dyne*s/cm^5) but SV (-34.5±14.6 ml, $p< 0.001$)) and CO (-0.2±1.0 l/min, $p<0.01$) decreased. Mental challenge affects the cardiovascular responses dependent on the posture. Physiological alterations observed in models combining mental stressors and changes in body posture, such as in public speaking, must be interpreted with caution. The physiological responses to these combined stressors are not entirely due to the effects of mental challenge per se but may be modulated by the posture assumed by the subjects during the tests.

ALTERATIONS IN ZINC STATUS AND TISSUE STRUCTURES OF HEPARIN INDUCED OSTEOPOROTIC RABBITS

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Heparin can cause some complications in tissues when it is used for a long term therapy. Besides its beneficial effects, it may have some side effects.
In this study, we investigated the possible hematological and histopatholgical changes in rabbit tissues under long term (one month) and overdoses (1000 and 2000 IU/kg/day) heparin applications.
Group I was injected with serum physiologic and kept as control while Group II and Group III were injected with heparin as a dosage of 1000 IU/kg/day and 2000 IU/kg/day, respectively.
There was no significant differences between the average values of serum electrolytes and copper levels of two experimental groups and the control group while their serum zinc levels (4.54 ± 0.52 µg/ml and 4.72 ± 0.65 µg/ml) were significantly ($p<0.05$) lower than the controls (5.75 ± 0.72 µg/ml). The hemoglobin level of the Group III was significantly lower than both Group II and the control.
In this study, it is investigated the possible histopathological changes in rabbit kidneys under long term and overdoses heparin applications. In the light microscopic observations of kidney tissues of Group II and III were observed renal lesions in glomerular and tubular structures of cortex and medulla. Focal segmental necrotising glomerular lesions were seen. Cortex contained dilated distal tubules lined by flattened cells. Some of the proximal tubuler cells had no nuclei and their cytoplasm appeared pale stained. Some lesions observed to involve the cells of the medullary ray and outer stripe of the medulla. Interstitial spaces contained inflammatory infiltrated cells.
These results suggested that long term and overdose heparin application have degenerative side effects on renal tissues and on serum zinc level.

MONITORING ELEMENTARY FUSION EVENTS IN PITUITARY CELLS BY THE CELL-ATTACHED MEMBRANE CAPACITANCE TECHNIQUE

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Hormones and neurotransmitters are released from cells by being passed through an exocytotic pore that forms following the fusion of the vesicle and the plasma membrane. In a number of cell types exocytosis proceeds by two mechanisms: by the transient fusion also known as kiss-and-run, or by the full fusion. An elegant way to discern between these two mechanisms is to monitor fusion pore properties by the electrophysiological method of monitoring membrane capacitance (C_m), a parameter linearly related to the membrane surface area. In the past our focus was to study the release properties of pituitary cells at cellular level. However, to learn directly about the conductance and kinetics of the fusion pore, it is imperative to monitor the time-course of single fusion events in the cell-attached mode of recording. Our results show that by measuring discrete steps in C_m, we most likely monitored fusion-fission events of single vesicles. Moreover, the recorded reversible C_m steps most likely mirror transient (kiss-and-run) fusion events, while irreversible C_m steps most likely reflect full fusion events.

AUTHOR INDEX

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