

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה  
ISRAEL SOCIETY FOR PHYSIOLOGY AND PHARMACOLOGY

הכנס השנתי Annual Meeting

23/9/2004

Ma'ale Hachamisha

## PROGRAM & ABSTRACTS

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הפקולטה לרפואה ע"ש רפפורט, טכניון, ת.ד. 9649 חיפה 31096  
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**האגודה הישראלית לפיזיולוגיה ופרמקולוגיה**  
**ISRAEL SOCIETY FOR PHYSIOLOGY AND PHARMACOLOGY**

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**האגודה הישראלית לפיזיולוגיה ופרמקולוגיה מודה לגופים הבאים שתמיכתם  
הנדיבה אפשרה קיום כינוס זה**

**The Israel Society for Physiology and Pharmacology wishes to acknowledge the following  
sponsors whose generous support has made this meeting possible**

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# Program Outline

*8:30-9:30 Registration and Refreshments*

**9:30-11:10 Parallel Sessions A-C**

*11:10 - 11:30 Coffee Break*

**11:30 - 13:10 Parallel Sessions D-F**

*13:10 - 13:30 Business Meeting  
All ISPP members are invited*

*13:30 – 14:30 Lunch*

**13:30 – 15:00 Poster session**

**15:00 – 16:00 Plenary lecture:**

“New directions in mechanisms and treatment of neurodegenerative diseases”

**Professor Peter Jenner,**

Dept of Biomedical Sciences, King’s College London, University of London

**16:00 – 18:00 Student Lecture Competition**

Six abstracts from research students

# MEETING PROGRAM

## Thursday morning, September 23, 2004

8:30 - 9:30      *Registration and refreshments*  
9:30 – 11:10    *Sessions A-C*

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### **Session A: Physiological-Molecular Linkage in Adaptation to Stressful Environment** *Dedicated to the memory of Professor Amiram Shkolnik*

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Chairperson:    **Michal Horowitz** *Hebrew University Jerusalem*

- 9:30      A1    **Noga Kronfeld-Schor** *Department of Zoology, Tel Aviv University*  
Circadian rhythms of golden spiny mice: do overt responses reflect entrainment?
- 9:55      A2    **Yosef Yarom**, *Faculty of Life Sciences, Hebrew University Jerusalem.*  
GABA, chloride and circadian rhythm.
- 10:20     A3    **Yehuda Arieli**, *Israel Naval Medical Institute, IDF Medical Corps*  
Combined acclimation to heat and slightly elevated atmospheric pressure prevents the protection against CNS oxygen toxicity induced by heat acclimation alone.
- 10:45     A4    **Michal Horowitz**, *Faculty of Dental Medicine, Hebrew University Jerusalem*  
Genes and molecules shape the heat acclimated phenotype: geno-physiological linkage

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### **Session B: Molecular and Pharmacological Aspects of Inflammation**

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Chairperson:    **Yoel Kloog**, *Tel Aviv University.*

- 9:30      B1    **Yoel Kloog**, *George S. Wise Faculty of Life Sciences, Tel Aviv University.*  
Prenyl binding domains in Ras binding partners: targets for Ras inhibitors.
- 9:55      B2    **Francesca Levi-Schaffer**, *School of Pharmacy, Hebrew University.*  
Mast cells and eosinophils: New roles for 'old' cells.
- 10:20     B3    **Joab Chapman**, *Sackler Faculty of Medicine, Tel Aviv University and Department of Neurology The Sheba Medical Center*  
Thrombin as a factor in neuroinflammation.
- 10:45     B4    **Jacob George**, *Department of Cardiology and Cardiovascular Research, Tel Aviv Sourasky Medical Center.*  
Immunity and autoimmunity in arteriosclerosis.

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**Session C: Proteomics of Neurodegeneration and Neuroprotection**

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Chairpersons: **Moussa Youdim**, *Technion*

**Ephraim Yavin**, *Weizmann Institute of Science*

- 9:30 C1 **Moussa Youdim**, *Rappaport Faculty of Medicine, Technion*  
Genomic and proteomic profiling of MPTP neurotoxicity and its prevention by the monoamine oxidase B inhibitor, rasagiline
- 9:55 C2 **Ephraim Yavin**, *Department of Neurobiology, Weizmann Institute of Science*  
Omega-3 fatty acid nutritional deficiency and ontogeny of neurotransmitter receptors: gene clusters and gene products
- 10:20 C3 **Marta Weinstock**, *School of Pharmacy, Hebrew University Jerusalem*  
Changes in hippocampal gene expression, neural plasticity and behaviour induced by prenatal stress in rats
- 10:45 C4 **Daniel Offen**, *Felsenstein Medical Research Center, Tel Aviv University*  
Spinal cord mRNA profile in patients with ALS: comparison with transgenic mice expressing the human SOD-1 mutant

**11:10 – 11:30**

**Coffee break**

11:30 – 13:10 *Sessions D-F*

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**Session D: Cardiovascular Protection**

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Chairperson: **Asher Shainberg**, *Bar-Ilan University*

- 11:30 D1 **Asher Shainberg**, *Faculty of Life Sciences, Bar-Ilan University*  
Activation of Adenosine A1 and A3 receptors protects cardiomyocytes in hypoxia by different mechanism.
- 11:55 D2 **Edith Hochhauser** *Felsenstein Medical Research Center, Tel Aviv University*  
Bax ablation protects against myocardial infarction in transgenic mice.
- 12:20 D3 **Herzl Schwalb** *Joseph Lunenfeld Cardiac Surgery Research Center, Hadassah University Hospital, Jerusalem.*  
From preconditioning to protection of the ischemic heart.
- 12:45 D4 **Gania Kessler-Icekson**, *Felsenstein Medical Research Center, Tel Aviv University*  
Protecting the heart through exercise training: tips from the genechips

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**Session E: Cellular and Subcellular Motion**

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Chairpersons: **Yoram Oron**, *Tel Aviv University*.  
**Shoshana Ravid**, *Hebrew University, Jerusalem*

- 11:30 E1 **Yoram Oron**, *Sackler School of Medicine, Tel Aviv University*.  
New ways of looking at cellular aggregation - Putative involvement of PARs
- 11:55 E2 **Shoshana Ravid**, *Faculty of Medicine, Hebrew University Jerusalem*  
The cross-talk between the signaling and the cytoskeletal systems that leads to directed cell motility
- 12:20 E3 **Ilan Tsarfaty**, *Sackler Faculty of Medicine, Tel Aviv University*  
HGF-SF and MET signaling in cell motility and metastasis
- 12:45 E4 **Rafi Korenstein**, *Sackler Faculty of Medicine, Tel Aviv University*  
Characteristics and regulation of nanoscale fluctuations of the cell surface

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**Session F: Secondary Transporters**

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Chairpersons: **Eitan Bibi**, *Weizmann Institute of Science*.  
**Eitan Reuveny**, *Weizmann Institute of Science*

- 11:30 F1 **Bernard Attali**, *Sackler School of Medicine, Tel Aviv University*  
Permeation and Gating Operations of Cardiac KCNQ1 Potassium Channels
- 11:55 F2 **Israel Sekler**, *Faculty of Health Sciences, Ben Gurion University of the Negev*  
Lithium-calcium exchange is mediated by a distinct potassium-independent sodium-calcium exchanger
- 12:20 F3 **Nathan Nelson**, *George S. Wise Faculty of Life Sciences, Tel Aviv University*  
Biochemistry and electrophysiology of imperfection – slips in metal ion transporters
- 12:45 F4 **Oded Lewinson**, *Department of Biological Chemistry, Weizmann Institute of Science*  
Alkalitolerance: a novel biological function for a multidrug transporter in pH homeostasis

### Scientific Program at a Glance

<i>Session A:</i> <b>Physiological-Molecular Linkage in Adaptation to Stressful Environment</b>		<i>Session B:</i> <b>Molecular and Pharmacological Aspects of Inflammation</b>		<i>Session C:</i> <b>Proteomics of Neurodegeneration and Neuroprotection</b>	
Chairperson: <b>Michal Horowitz,</b>		Chairperson: <b>Yoel Kloog,</b>		Chairpersons: <b>Moussa Youdim</b>	
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10:45	<b>Michal Horowitz,</b> Genes and molecules shape the heat acclimated phenotype: genophysiological linkage	10:45	<b>Jacob George,</b> Immunity and autoimmunity in arteriosclerosis.	10:45	<b>Daniel Offen,</b> Spinal cord mRNA profile in patients with ALS: comparison with transgenic mice expressing the human SOD-1 mutant

Coffee Break 11:10 – 11:30

<i>Session D:</i> <b>Cardiovascular Protection</b>		<i>Session E:</i> <b>Cellular and Subcellular Motion</b>		<i>Session F:</i> <b>Secondary Transporters</b>	
Chairpersons: <b>Asher Shainberg</b>		Chairpersons: <b>Yoram Oron,</b> <b>Shoshana Ravid,</b>		Chairpersons: <b>Eitan Bibi,</b> <b>Eitan Reuveny</b>	
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תקצירי  
הרצאות מוזמנות

**ABSTRACTS OF  
INVITED PRESENTATIONS**

## **CIRCADIAN RHYTHMS OF GOLDEN SPINY MICE: DO OVERT RESPONSES REFLECT ENTRAINMENT?**

Ofir Levy, Tamar Dayan, and **Noga Kronfeld-Schor**

Department of Zoology, Tel Aviv University, Tel Aviv 69978, Israel.

Nocturnal common spiny mice (*Acomys cahirinus*) and diurnally active golden spiny mice (*A. russatus*) coexist in a rocky desert near the Dead Sea. In absence of *A. cahirinus*, *A. russatus* are also active during the night, suggesting that they are competitively displaced into diurnal activity. We monitored the effect of *A. cahirinus* and of food availability on body temperature (Tb) and activity (Ac) rhythms of *A. russatus* in four 1000 sq m experimental field enclosures: two controls (both species) and two experimental (*A. russatus*). We implanted the mice with abdominal radio-transmitters, and monitored Tb and Ac with excess food added to the enclosures, and then with no food added. We found no differences in Tb and Ac patterns of *A. russatus* between control and experimental enclosures. When food was added, *A. russatus* were active during both day and night in all enclosures. When no food was added, *A. russatus* turned entirely diurnal both in the control and in the experimental enclosures, suggesting that resource level rather than interference is the proximate cue for temporal partitioning in this system. Next, we trapped all *A. russatus* individuals, and transferred them to the laboratory, where they were held under constant conditions (27°C, DD). Tb of all individuals revert within one day, showing high Tb during the subjective night, suggesting that diurnal activity of *A. russatus* in the field reflects a masking effect, or a difference in the way the clock entrains Tb (and Ac) rhythms, rather than the process of entrainment. The mechanisms determining nocturnal and diurnal activity are largely unknown, and most of what we know about mammals' circadian physiology comes from studies on nocturnal mammals. We now use *Acomys russatus* as an alternative animal model for the study of the neural mechanisms determining nocturnal and diurnal activity in Mammals.

**GABA, CHLORIDE AND CIRCADIAN RHYTHM.**

**Yosef Yarom**

Dept of Neurobiology, Life Sciences Hebrew University.

While substantial progress has been made in locating and identifying the molecular basis of the circadian clock, the mechanisms by which it is translated into cyclic firing activity are poorly understood. Here we propose that the GABA transporter, GAT-1, participates in the process of transferring information from the clock to the cell membrane. Using in vitro brain slices, we demonstrate that: a) Due to changes in intracellular chloride concentration, GABA has a dual effect on SCN neurons. b) The regulation of chloride concentration is under circadian control. c) The efficacy of GAT-1 undergoes circadian modulation. d) GAT-1 participates in the regulation of intracellular chloride concentration. These observations indicate that the circadian modulation of GAT-1 activity induces circadian changes in the chloride concentration thereby modulating the GABAergic synapses. The resultant excitatory effect of GABA during the subjective day increases the firing rate of SCN neurons.

**COMBINED ACCLIMATION TO HEAT AND SLIGHTLY  
ELEVATED ATMOSPHERIC PRESSURE PREVENTS THE  
PROTECTION AGAINST CNS OXYGEN TOXICITY INDUCED BY  
HEAT ACCLIMATION ALONE.**

Mirit Eynan<sup>1</sup>, Yehuda Arieli<sup>1</sup>, Ofir Ertracht<sup>1</sup>, Hanan Gancz<sup>2</sup>, Ran Arieli<sup>1</sup>,  
and Yechezkel Kashi<sup>2</sup>

<sup>1</sup>Israel Naval Medical Institute, IDF Medical Corps, P.O.B. 8040, Haifa 31080, and <sup>2</sup>The Faculty of Food Engineering and Biotechnology, Technion, Haifa, Israel.

**Background:** The medical benefits of the Dead Sea are well known. We have previously shown that heat acclimation provides protection against central nervous system oxygen toxicity (CNS-OT). This protection was well correlated with increased levels of heat shock protein 72 (HSP72). **Hypothesis:** In the current study we tested the hypothesis that acclimation to the heat and slightly elevated atmospheric pressure prevailing in the Dead Sea region, will further improve the protection against CNS-OT induced by heat acclimation alone. **Methods:** Four groups of male Sprague Dawley rats were exposed for four weeks to one of the following treatments: a) 32 ° C; b) ambient pressure of 106 kPa; c) a combination of a' and b', and d) controls. All groups were exposed to oxygen at 608 kPa at the end of the acclimation period. EEG was recorded continuously until the appearance of the first electrical discharge (FED). Brain samples were taken from each group at the end of the acclimation period, after compression-decompression. The levels of the enzymes catalase, CuZnSOD, MnSOD, and glutathione-peroxidase, as well as the level of HSP72, were quantified using Western blot. **Results:** In heat-acclimated rats the time to the FED was significantly longer, and was associated with significantly higher levels of HSP72 and CuZnSOD compared with control rats. However, these changes were prevented in rats acclimated to combination of heat and slightly elevated pressure. The levels of the other enzymes under examination were not significantly affected by either treatment. **Conclusions:** We conclude that a slight increase in barometric pressure prevents the protection induced by heat acclimation alone, most probably by affecting the expression of HSP72 and CuZnSOD in the rat brain.

## GENES AND MOLECULES SHAPE THE HEAT ACCLIMATED PHENOTYPE: GENO-PHYSIOLOGICAL LINKAGE

**Michal Horowitz**

Laboratory of Environmental Physiology, Faculty of Dental medicine,  
The Hebrew University, Jerusalem, Israel. [horowitz@cc.huji.ac.il](mailto:horowitz@cc.huji.ac.il)

Acclimation to environmental heat involves a transient perturbed phase followed by a long lasting period during which acclimatory homeostasis is developed. Conceptually, acclimation can be delineated as a *transition from an early transient, “inefficient”, phase where accelerated autonomic excitability alleviate the developed strain to an “efficient” state* when acclimatory homeostasis has been reached. At that period, physiological mechanisms are ‘translated’ into an expanded dynamic thermoregulatory range, due to decreased heat production, reduced temperature thresholds for the activation of heat-dissipation mechanisms and an elevated temperature threshold for the development of thermal injury. The final shaping of the heat acclimated phenotype depends on a cross talk between transcriptional and posttranslational processes. The broad-scale genomic approach and the development of gene chip array technology allow us now the study of batteries of genes of different functions. Stress associated gene profiles during the course of heat acclimation indicate a two-tier defense strategy. Whereas the immediate transient response is associated with the maintenance of DNA and cellular integrity during the strain developed at the onset of acclimation, the sustained response is correlated with slowly developed, long-lasting cytoprotective signaling networks involving genes encoding proteins that are essential for the heat-shock response, anti-apoptosis and antioxidation, collectively leading to delayed thermal injury.  $\beta$  Adreno- blockade during the acclimation regimen implies that invocation of at least some of these protective networks is associated with the sympathetic nervous system. Such adaptive plasticity correlates with our perception of the physiological acclimatory response. It is notable, however, that changes in gene profile is tissue specific and up or downregulated genes in specific tissues are not from the same functional groups. These changes and neurohumoral triggering factors will be discussed. An inseparable outcome of acclimation is that adjusting to one environmental stressor can, in addition to evolving primary adaptations, add to the amount of adjustment to additional stressors. Such cross-reinforcement raises the possibility of inducing adaptation to a stressor without prior exposure to that particular stressor (exaptation). An important beneficial effect of heat acclimation observed in our laboratory is the development of “cross-tolerance” against O<sub>2</sub> supply/ O<sub>2</sub> demand mismatching and its consequences. Genomic responses provide us with cues to the understanding of this cross-tolerance phenomenon. Although gene activation is truly stress-specific, faster activation/suppression of signaling pathways shared by heat stress and the O<sub>2</sub> supply/O<sub>2</sub> demand mismatch insults probably contribute to this exaptation. Our data reconfirms the universality of HSP 70 as stress-responding genes. Likewise, reinforcement of cyto-protective gene-networks (which are possibly multipotential in their utilization) or different rates of response of various genes enables the adaptation to a stressor without prior exposure. In line with our consistent finding of acclimation mediated enhanced metabolic efficiency, the role of HIF-1, the master regulator of oxygen sensing, essential factor for both acclimation and cross tolerance will be discussed.

**PRENYL BINDING DOMAINS IN RAS BINDING PARTNERS: TARGETS FOR RAS INHIBITORS**

**Yoel Kloog**, Barak Rotblat and Hagit Niv

Department of Neurobiochemistry, The George S. Wise Faculty of Life Sciences,  
Tel-Aviv University, 69978 Tel-Aviv, Israel

Ras and RhoGTPases are prominent participants in malignant transformation. They possess an essential prenyl group (farnesyl or geranylgeranyl) that endows them with membrane-tethering ability and functional specificity. Accumulating evidence suggests that farnesyl group of Ras proteins is involved primarily in lipid-protein interactions, and recent experiments point to prenyl-binding hydrophobic pockets in proteins regulating Ras in normal cells and cancer cells. We showed previously that Ras and galectin-1, a protein that like Ras is associated with human malignancies, are binding partners. The transforming activity of Ras necessitates membrane anchorage that depends on the Ras farnesyl moiety and is strengthened by Ras/galectin-1 interactions. We now identified a hydrophobic pocket in galectin-1, analogous to the Cdc42 geranylgeranyl-binding cavity in RhoGDI, possessing homologous isoprenoid-binding residues, including the critical L11, whose RhoGDI L77 homologue changes dramatically upon Cdc42 binding. To explore the role of the computed farnesyl-binding pocket in galectin-1 we substituted the critical L11 in the hydrophobic pocket of galectin-1 and obtained a dominant interfering mutant, galectin-1(L11A). Galectin-1 (L11A) possessed normal carbohydrate-binding capacity, but inhibited H-Ras GTP-loading and ERK activation, dislodged H-Ras(G12V) from the cell membrane, and attenuated H-Ras(G12V) fibroblast transformation and PC12-cell neurite outgrowth. Unlike galectin-1 that co-operates with Ras, galectin-1(L11A) extricates oncogenic H-Ras from the membrane and inhibits Ras transforming activity. Thus, the prenyl-binding domain in galectin-1 and in other binding partners of Ras-like GTPases are significant players in the control of the Ras family of proteins supporting the emerging concept of prenyl-binding domains as potential targets for Ras inhibitors and anti-cancer drugs. Such findings hold promise that specific inhibitors of prenyl-binding domains will yield selective and efficacious cancer treatment.

**NEW INSIGHTS IN THE MAST CELL-EOSINOPHIL FIBROBLAST  
CROSSTALK IN ALLERGY AND FIBROSIS**

**Francesca Levi-Schaffer**

Department of Pharmacology, School of Pharmacy, The Hebrew University of  
Jerusalem, Jerusalem, Israel

Mast cells and eosinophils are the recognized key cells in allergic inflammatory reactions and the role of mast cells in the early phase reactions as well as the mechanisms by which eosinophils penetrate and damage the inflamed tissues have been extensively studied.

In addition, the presence of mast cells and/or eosinophils in increased numbers in several inflammatory conditions that result in either physiological or pathological repair processes has long been described. Nevertheless, the possibility that activated mast cells still play an important role in later/chronic stages of allergy especially by their interaction with the eosinophils and that both mast cells and eosinophils can influence repair/fibrosis by modulating fibroblast properties and vice versa has not been thoroughly evaluated.

In my lecture, I will review our most recent finding showing that: 1) Mast cells can activate eosinophils in a non IgE-dependent fashion and the role of fibroblast derived SCF in this process; 2) Eosinophils and mast cells influence *in vitro* and *in vivo* repair/fibrosis and angiogenesis.

Altogether our new data further demonstrate that multi-faceted interactions between mast cells, eosinophils and fibroblasts exist and can be of importance both in allergy and in other chronic inflammatory conditions.

## THROMBIN AS A FACTOR FOR NEUROINFLAMMATION

Orit Beilin<sup>1</sup>, Constantin Sylantiev<sup>2</sup>, Amos D. Korczyn<sup>1,2,3</sup>, Ramona Aronovich<sup>1</sup>, Vivian E Drory<sup>2</sup>, David Gurwitz<sup>4</sup>, Rachel Bar-Shavit<sup>5</sup>,  
**Joab Chapman**<sup>1,2</sup>.

<sup>1</sup>Department of Physiology and Pharmacology, <sup>2</sup>Department of Neurology, <sup>3</sup>Sieratzki Chair of Neurology, <sup>4</sup>Department of Human Genetics and Molecular Medicine, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978. <sup>5</sup> Department of Oncology, Hadassah- Hebrew University Hospital, Jerusalem, Israel.

**OBJECTIVE:** To examine the electrophysiological effects of thrombin applied directly to peripheral nerve

**BACKGROUND:** Thrombin is shown to induce neural tissue damage and activation of the coagulation pathway is associated with CNS demyelination. Thrombin mediates many of its effects through specific protease activated receptors (PAR).

**METHODS:** Thrombin 10-200U/ml, thrombin receptor activating peptide (TRAP) 150-300  $\mu$ M, trypsin 180-360U/ml, and thrombin with its specific antagonist PPACK 500  $\mu$ M were applied to exposed rat sciatic nerve. The nerve was stimulated proximately to the application site and plantar muscle electrical response was measured. The expression and activation of PAR-1 in rat sciatic nerve were assessed by RT-PCR and ERK phosphorylation immunoblot respectively.

**RESULTS:** Thrombin and TRAP produced a conduction block within 30 minutes of application and the effects were maintained for at least 1 hour. The conduction block was reversed significantly by washing. When thrombin was applied simultaneously with its antagonist, no significant reduction of muscle action potential amplitude was shown. Trypsin also led no effect. Expression of PAR-1 was detected in the sciatic nerve and its activation by TRAP caused a 3-fold increase in phosphorylated ERK

**CONCLUSIONS:** Thrombin has the potential to cause conduction block by a receptor-mediated pathway. This may have significant relevance for neuropathies in which conduction block is a major factor.

## **IMMUNITY AND AUTOIMMUNITY IN ATHEROSCLEROSIS**

**Jacob George**

The Department of Cardiology, Tel Aviv Sourasky Medical Center

Considerable data gathered in recent years implicates active involvement of the immune system in atherosclerosis. Evidence arises from human and experimental studies indicating the presence of immune effectors within the atherosclerotic plaque. We and others have pursued the idea that autoimmune reactions are operable in atherosclerosis. Employing studies in transgenic mice with a proatherogenic phenotype, we have shown that immunization against a panel of given antigens such as the heat shock protein 65 (Hsp65), oxidized low density lipoprotein and beta-2 glycoprotein I (B2GPI) resulted in significant enhancement in atherosclerotic lesion initiation and progression. We next sought to elucidate the mediators of the proatherogenic effects and found, by active, adoptive transfer studies, that transfer of antigen specific lymphocytes reactive to HSP65 and B2GPI, were capable of accelerating lesion progression irrespective of the lipid profile in these animals. We have also shown that costimulatory antigens are present in atherosclerotic plaques and colocalize with the antigens involved in the local autoimmune process. Ablation of some costimulatory pathways was however proatherogenic indicative of the complex regulation of the local immune system within the plaque.

Having confirmed the active involvement of immunity and autoimmunity in atherosclerosis, we were able to prove that induction of T cell tolerance to the proposed antigens attenuated lesion formation. Moreover, functional inhibition of Ras signaling by employment of farnesylthiosalicylic acid (FTS), led down regulation of Ras GTP expression in the lesions and suppressed atherogenesis in transgenic mice.

In conclusion, immune and autoimmune reactions are operable in atherosclerosis and can be harnessed for future therapeutic purposes.

**GENOMIC AND PROTEOMIC PROFILING OF THE MPTP  
NEUROTOXICITY AND ITS PREVENTION BY MONOAMINE  
OXIDASE B INHIBITOR, RASAGILINE**

Sagi Y., Mandel S. and **Youdim M.B.H.**

Eve Topf and NPF Centers of Excellence for Neurodegenerative Diseases  
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Rasagiline is a monoamine oxidase (MAO) B inhibitor antiParkinson's disease (PD) drug. Its neuroprotective properties have been examined in many cell culture as well as In Vivo models, among them the MPTP model of PD in mice and monkeys. These studies and cDNA microarray gene expression have indicated involvement of Bcl-2 family proteins and other anti apoptotic genes. In order to reveal these mechanisms we adopted two novel methods: genomic and proteomic profiling systems, in the mouse model of MPTP. Both RNA, as well as whole lysate were produced from midbrains of mice, cDNA was synthesized and was hybridized to microarray gene chip (Clontech, USA), containing ~2000 genes, and were analyzed using a software (Atlasimage, Clontech, USA), while protein samples were detected and analyzed in Powerblot Proteomic array system (BD, CA, USA) containing over 1000 mAbs. Gene analysis confirmed that MPTP treatment involves proapoptotic mechanism and proteomics suggested that the JNK pathway is recruited by MPTP, in addition to Fas ligand involvement. Furthermore, MPTP involved altered expression of n-nitric oxide synthase (nNOS), glutamate receptor, and phosphorylated form of Glycogen Synthase Kinase-3b (GSK3). Proteomic analysis confirmed many of the gene expression profile related to MPTP neurotoxicity. On the contrary, rasagiline reversed the pro apoptotic action of MPTP, enhanced anti apoptotic, growth factor and in particular  $\beta$ -NGF, its downstream transcription factors: EGR1 (NGF-I A binding protein) and EGR3 (early growth response protein-3) gene expressions. Rasagiline's ability to up regulate the protein levels of pro-survival signaling of Ras, PI3K, and AKT, while down regulating FAS, indicates that part of its neuroprotective mechanism of action is mediated via  $\beta$ -NGF receptor, TRK-A and FAS ligand.

## OMEGA-3 FATTY ACID NUTRITIONAL DEFICIENCY AND ONTOGENY OF NEUROTRANSMITTER RECEPTORS: ON GENE CLUSTERS AND GENE PRODUCTS

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$\omega$ -3 Docosahexaenoic acid ( $\omega$ -3DHA) is a most ubiquitous polyunsaturated fatty acid (FA) compound in brain. Experimental studies with animals have demonstrated that depriving the  $\omega$ -3FA precursor linolenic acid ( $\omega$ -3LNA) from the diet, reduces markedly the DHA content of cerebral membrane lipids, a process that is accompanied by impairment in behavior, learning ability, sensory motor activity, motivational processes and vision.  $\omega$ -3LNA deficiency induces abnormal functioning of the mesolimbic and mesocortical dopaminergic pathways by reducing levels of the available dopamine in the synaptic cleft. To evaluate possible molecular mechanisms responsible for altering these brain functions we have examined gene profiles that may be associated with these biochemical and functional changes. Cross subtracted libraries prepared from total RNA extracts of hippocampus and cortex regions from two-weeks old rats subjected to an intrauterine and early postnatal  $\omega$ -3FA dietary deficiency were used for identification and profiling of specific genes. A commercial array was used and hybridization of the labeled cDNAs probes revealed approx. 46 known up-regulated genes. The results were verified by both relative and quantitative PCR. Among the over-expressed genes, a group of neurotransmitter receptors of the dopaminergic system were most prominent. In addition several receptors for the excitatory neurotransmitters molecules acetylcholine and glutamate, and the inhibitory GABA-ergic and serotonergic receptors, were elevated. Immunohistochemical visualization using specific antibodies for the above neurotransmitter receptors are under way to determine the relative levels of some of these receptors in discrete sections of the postnatal brain. Emerging experimental evidence suggest that the overall increase in receptor gene expression levels may constitute an adaptive mechanism induced by the  $\omega$ -3 deprivation to possibly cope with a decrease in the neurotransmitter apparatus at this stage of the developing brain. *Supported by a grant from the Gulton Foundation, NY.*

## CHANGES IN HIPPOCAMPAL GENE EXPRESSION, NEURAL PLASTICITY AND BEHAVIOUR INDUCED BY PRENATAL STRESS IN RATS.

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Prenatal stress in primates and rodents influences the development and function of the nervous system, resulting in signs of mild cognitive impairment, hyperanxiety and depressive-like behaviour in adulthood. This study examined the influence of maternal stress during the last week of gestation, a period critical for synaptogenesis, on gene expression in the hippocampus of rats. We used the Affymetrix microarray (8 samples x 19,000 genes each) on extracts of hippocampal tissues from control and prenatally stressed male offspring aged 23 days. Prenatal stress caused a significant down-regulation of the expression of about 2.4% of the genes. The most prevalent of these involved components of neurogenesis and synapse formation. They included a) 14 genes of the myelin components of newly developed axons, e.g. myelin basic protein, MAL - a proteolipid that has been implicated in myelin biogenesis and/or function, and other genes involved in the formation, stabilization and maintenance of glycosphingolipid-enriched membranes; b) Several genes associated with synaptic activity and trafficking; i) complexin 1 and 2, key genes in protein-protein interactions that are altered in hippocampal spatial learning; ii) endophilin-1, a protein that optimizes the coupling of exocytosis and endocytosis; iii) PSD-95/SAP90-associated protein-3, a scaffolding protein that is part of the cytoskeletal mesh and is involved in the construction of post-synaptic density structures. The reduction of these genes implied suppression of synaptogenesis. These findings show for the first time that prenatal stress can alter programming of the fetal brain and suppress key genes involved in myelination, synaptogenesis and neurogenesis. A reduction in myelination can reduce the rate of impulse transmission and long-term potentiation. The gene changes provide a plausible explanation for the occurrence of spatial learning deficits and depressive-like behaviour in adulthood. Similar changes have also been reported in the brains of human subjects with major depressive disorder.

**SPINAL CORD mRNA PROFILE IN PATIENTS WITH ALS:  
COMPARISON WITH TRANSGENIC MICE EXPRESSING THE  
HUMAN SOD-1 MUTANT**

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Amyotrophic lateral sclerosis (ALS) is a progressive, neurodegenerative disease characterized by loss of motor neurons in the cerebral cortex, brain stem, and spinal cord. The cause of sporadic ALS is unknown. A minority of ALS patients (5-10%) has a familial form (FALS) and 20% of these demonstrate mutations in the Cu/Zn SOD1 gene. Mice expressing the human mutant SOD1 gene develop age-dependent ALS-like neurological symptoms. We studied the mRNA expression profile in three post-mortem spinal cord sections from sporadic ALS patients and three matched controls using cDNA microarray. In spinal cord of SALS patients we found seventy genes with various physiological functions that are differently expressed. In the present study we focused on three genes that were markedly overexpressed in ALS patient's spinal cords. We found an increase in mRNA of cathepsin B (200%) and cathepsin D (230%), cysteine proteases that mediate intracellular protein turnover in the lysosome. The mRNA levels of apolipoprotein E, which is closely associated with the pathogenesis of neurodegenerative diseases, were also markedly increased (420%). Further analysis with specific probes revealed that the expression of these genes also increases in hSOD1-G93A transgenic mice, show enhancement with disease progression and peak at end stage. Our data in ALS patients, supported by findings from the transgenic mouse model, indicates a crucial role of apolipoprotein-E and cathepsin B/D in the pathogenesis of sporadic and familial forms of ALS.

**PROTECTIVE EFFECTS OF ADENOSINE A<sub>1</sub> AND A<sub>3</sub> RECEPTOR  
ACTIVATION AGAINST HYPOXIA IN RAT CARDIOMYOCYTE  
CULTURES**

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Ischemic preconditioning (IPC) is a well-known phenomenon in which brief episodes of ischemia, paradoxically protect the myocardium from damage induced by subsequent prolonged ischemia. The precise mechanism of IPC remains elusive. Exposure of the heart to adenosine, instead of to ischemia, can also induce a protective effect against subsequent ischemia-induced damage (the pharmacological preconditioning). The released adenosine interacts with sarcolemmal membrane adenosine receptors (ARs). Although activation of both the A<sub>1</sub> and the A<sub>3</sub> subtype of the AR can mimic the cardioprotective effect of ischemic preconditioning, emerging evidence suggests that the two receptors mediate distinct cardioprotective functions. Therefore, the **aim** of this study is to define the signal transduction pathways by which activation of A<sub>1</sub> adenosine receptor (A<sub>1</sub>R) and A<sub>3</sub> adenosine receptor (A<sub>3</sub>R) exert their protective cascade against hypoxic damage on isolated cardiomyocytes. Primary cardiac myocyte cultures were subjected to hypoxia in glucose-free media. The A<sub>1</sub>R and A<sub>3</sub>R selective agonists (CCPA and Cl-IB-MECA, respectively) significantly decreased myocytes damage under hypoxic conditions. Activation of both A<sub>1</sub>R and A<sub>3</sub>R together (100 nM), was more efficient in protection against hypoxia than by each one alone, suggesting distinct signalling for A<sub>1</sub>R and for A<sub>3</sub>R. The role of A<sub>1</sub>R and A<sub>3</sub>R activation on functional tolerance after inhibition of the terminal link of the mitochondrial respiratory chain with sodium azide was tested. Treatment with sodium azide causes a rapid depletion of ATP, leading to K<sub>ATP</sub> channel activation. Thus, under our conditions the K<sub>ATP</sub> channels are already opened and diazoxide or adenosine receptor activation could not act through modulating this channel activity. It was found that stimulation of A<sub>3</sub>R activates the sarcoplasmic reticulum (SR) Ca<sup>2+</sup> uptake and may prevent Ca<sup>2+</sup> overloading. We presume that opening of mitochondrial K<sub>ATP</sub> channels may not exclusively be the final mediator of cardioprotection. **In conclusion**, our data establish that adenosine can mediate myocardial protection against hypoxia by acting on A<sub>1</sub> and A<sub>3</sub> adenosine receptors. However, the cascades of events involved in cardioprotection appear to be distinct for A<sub>1</sub> and A<sub>3</sub> receptors.

## **BAX ABLATION PROTECTS AGAINST MYOCARDIAL ISCHEMIA/REPERFUSION (I/R) INJURY IN TRANSGENIC MICE**

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The role of the proapoptotic bax gene in I/R injury was tested in vitro and in vivo in two experimental groups: homozygotic knockout mice lacking the bax gene Bax (-/-) and wild type Bax (+/+). In the first stage of this study, isolated hearts were subjected to ischemia (30 min, 37°C) then to 120 min of reperfusion. Left ventricular developed force of contraction that decreased to  $37\pm 3\%$  vs.  $27\pm 5\%$  of baseline in Bax (-/-) and Bax (+/+), respectively ( $p<0.05$ ). This protection was accompanied by a decrease in creatine kinase release ( $110 \pm 10$ ;  $270 \pm 45$  units/L, respectively) and infarct size as seen by TTC staining ( $22.3 \pm 6\%$  and  $43.6 \pm 12\%$  respectively,  $p=0.001$ ). Cardiomyocyte death due to apoptosis, and caspase 3 activity were lower in Bax (-/-) compared to the Bax (+/+) group. Electron microscopy evaluation revealed reduced damage to the mitochondria and to the chromatin structure of nucleus in bax deficient mice.

The second stage of our study examined the effect of the bax gene knockout in vivo following ligation of the left anterior descending coronary artery (LAD). Echocardiography was performed before surgery and after the induction of infarction. Left ventricular end diastolic diameter (LVEDd), end systolic diameter (LVESd) and fractional shortening (FS) were measured. The progressive increase in LVEDd and LVESd in Bax (-/-) was significantly smaller when compared to the wild type 28 days after MI ( $p<0.03$ ). Concomitantly, FS was higher in Bax (-/-), ( $35\pm 4.1\%$  and  $27\pm 2.5\%$ ,  $p<0.001$ ). Infarct size was smaller in the Bax (-/-) compared to the wild type, 28 days after MI ( $24 \pm 3.7\%$  and  $37 \pm 3.3\%$ ,  $p<0.001$ ).

In conclusion, the superior tolerance of Bax knockout hearts, demonstrating smaller infarct size and better myocardial function to I/R injury both in vivo and in vitro, recommends this gene as a potential target for therapeutic intervention.

## FROM PRECONDITIONING TO PROTECTION OF THE ISCHEMIC HEART

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Background: Ischemic and pharmacological preconditioning are considered powerful tools for protecting of the ischemic heart. In this study we compared preconditioning to protection of the ischemic heart in different models. Preconditioning was tested in the isolated perfused rat heart model. The efficacy of myocardial preconditioning by cyclosporin A (CSA) and protection by insulin was tested in human right atrial myocardial slices subjected to ischemia and reoxygenation (I/R).

Methods and Results Study I: Isolated rat hearts were perfused for 38 min, followed by 25 min no flow global ischemia, and then 60 min reperfusion. Hearts were preconditioned by 2 episodes of 3 min global ischemia followed by 2 min of reperfusion (IP); or by 10 min perfusion with 50  $\mu$ M nicorandil (Nic) followed by 10 min washout. Following isolation of cardiac mitochondria, the respiratory control ratio (RCR) was calculated from State 3 and State 4 respiration. IP and Nic significantly ( $P<0.05$ ) improved post-ischemic hemodynamic function. Improvement was abolished by bracketing the protocols with 200  $\mu$ M 5-hydroxydecanoate (5HD) or 300  $\mu$ M N-(2-mercapto-propionyl)-glycine (MPG). RCR was depressed to 64% from preischemic values in control hearts. Both IP and Nic significantly ( $P<0.05$ ) improved post-ischemic RCR. The protective effects of IP and Nic were partially abolished by bracketing with 5HD or MPG. Furthermore, mitochondria from ischemic hearts had significantly ( $P<0.05$ ) less ability to resist swelling upon  $Ca^{+2}$  loading, which was improved by both IP and Nic. Using an immunoblot technique, carbonyl content of multiple mitochondrial proteins was observed to be elevated after 25 min ischemia; and still elevated by the end of 60 min reperfusion. Both IP and Nic attenuated the increased protein oxidation observed at the end of ischemia. The protective effect of IP was almost completely abolished by MPG and partially by 5HD, which also partially abolished the protective effect of Nic. Conclusion Study I: This study supports the conclusion that one mechanism for enhanced post-ischemic function in the preconditioned heart is improved mitochondrial function as a result of decreased oxidation of mitochondrial proteins.

Methods and Results Study II: Slices of right atrial trabeculae were obtained from patients undergoing elective cardiac surgery. Trabeculae were superfused with oxygenated glucose containing phosphate buffered saline ( $O_2$ , G-PBS). Following 30 min of stabilization the sections were exposed to 90 min ischemia ( $N_2$ , PBS without glucose) followed by 90 min reoxygenation ( $O_2$ , G-PBS). CSA (0.2  $\mu$ M) or insulin (5 mU/ml) were added during the stabilization period prior the ischemia. Cell viability was measured by using 3-[4,5 dimethylthiazol 2-yl]-2,5-diphenyltetrazolium bromide (MTT), which is cleaved by active mitochondrial dehydrogenases of living cells. The viability of untreated slices (control) was  $30.45\pm 2.5\%$  vs  $52.65\pm 4.4\%$  in the CSA treated slices,  $p<0.001$ . The extent of protection by CSA was affected by oral antiglycemic drugs (glibenclamide). The effect obtained by CSA was inhibited by 5-hydroxydecanoate (5HD), a specific blocker of mitochondrial  $K_{ATP}$  channels. Protection of the myocardial slices with insulin appears to be superior and not affected by the medication before surgery. This protection was maximal when insulin was present during both preischemic equilibration and reoxygenation periods ( $68.9\pm 9.3\%$  viability with insulin vs  $33.2\pm 6.9\%$  in the control,  $p<0.001$ ).

Conclusion Study II: Protection of right atrial trabeculae slices with insulin is superior to that obtained with CSA, and is independent of preoperative medication.

**PROTECTING THE HEART THROUGH EXERCISE TRAINING:  
TIPS FROM THE GENECHIPS**

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We have previously shown that prior swimming exercise training improves the outcome of acute myocardial infarction (MI) as manifested by better heart function, reduced scar size and increased arteriole density four weeks after MI. To elucidate molecular mechanisms underlying this outcome we conducted high throughput expression analysis in prior exercised hearts subjected to surgical induction of acute MI and taken for analysis at different time points following MI. RNA extracted from non infarcted hearts and from the surviving myocardium of infarcted hearts in both prior exercised and corresponding sedentary controls was profiled by wide range DNA-microarrays to provide the transcriptome at each post MI time point. Global analysis of the 3,686 detected transcripts indicated that early after MI the impact of infarction on the genes expressed is stronger than that of training. At 4 weeks, however, the prior-exercised hearts differed markedly from their non-exercised counterparts suggesting altered remodeling in these hearts. Coupled two-way clustering analysis of 1500 genes showing the highest inter-group variance highlighted over 20 clusters and sub-clusters of genes grouped due to similar expression pattern. Regulators of transcription and translation as well as catabolic enzymes were enhanced in both the exercised and non-exercised hearts 4 hours after MI. By contrast, the load dependent atrial natriuretic peptide and genes involved in interstitial fibrosis were moderately elevated in the exercised hearts 2 days and 4 weeks after MI whereas in the sedentary hearts they exhibited a marked enhancement at 4 weeks, supporting the notion of attenuated remodeling in the exercised hearts. It is concluded that swimming exercise training conducted prior to acute MI reprograms the heart for better tolerance of MI injury leading to diminished remodeling and improved heart function.

**NEW WAY OF LOOKING AT CELLULAR AGGREGATION –  
PUTATIVE INVOLVEMENT OF PARS**

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Human pancreatic ductal carcinoma cells (PANC-1) aggregate and express markers of differentiated islet cells when transferred to serum-free medium. PANC-1 cells express proteinase-activated receptors (PAR-1 and PAR-2), which couple to the phosphoinositides-phospholipase C-calcium pathway. Activation of PAR-1 by thrombin or specific tethered receptor agonist peptide accelerates the first morphological step in the differentiation process, resulting in a larger number of smaller aggregates. Time-lapse microscopy reveals that PANC-1 aggregation proceeds by extension of extremely long filopodia, which form bridges between groups of PANC-1 cells. The resulting two-dimensional network of micro-aggregates proceeds to form larger aggregates, either by active constriction or passive tension of the filopodia. We have developed a novel algorithm for analysis of cell and aggregate movement, which allows a quantitative description of this phenomenon.

**THE CROSS TALK BETWEEN THE SIGNALING AND  
CYTOSKELETAL SYSTEMS THAT LEADS TO DIRECTED CELL  
MOTILITY**

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Many signaling pathways regulate the function of the cellular cytoskeleton, however we know very little about the identity of the proteins involved in the cross talk between the signaling and cytoskeletal systems. PAK and Rock are the effectors of Rac and Rho, small GTPase proteins that play a key role in the regulation of cytoskeletal organization. Non-muscle myosin II is an important component of the cytoskeleton, which has been shown to play a major part in cell motility and chemotaxis. We investigated the role of PAK and Rock in the regulation of myosin II in prostate cancer cells in response to EGF stimulation. We found that both PAK and Rock affect EGF-dependent myosin II phosphorylation, localization and chemotaxis, in an opposite manner. We found that myosin II resides in a complex with PAK and aPKCz and that the interaction between these proteins is EGF-dependent. We showed that aPKCz phosphorylates myosin II directly and specifically. Myosin II also resides in a complex with Rock and protein phosphatase type 1 (PP1) that dephosphorylates myosin II in response to EGF stimulation. The signaling pathway involving PAK1, aPKCz, Rock, PP1 and myosin II that provides the link between the signaling and the cytoskeletal systems leading to chemotaxis will be discussed.

**MET-HGF/SF IN MOTILITY AND METASTASIS**

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HGF/SF and its receptor Met play an important role in normal cellular processes as well as in tumorigenicity and metastasis. Molecular imaging of Met could shed a light on the early transforming events. We constructed GFP-Met and DsRed-HGF/SF chimeric proteins and generated transient and stable transfected cells, as well as transgenic mice to perform direct molecular imaging of Met. FRAP was used to monitor the Met receptor basal mobility in the plasma membrane, HGF/SF induced changes in Met lateral mobility and the effects of actin organization inhibitors on Met lateral mobility. Additionally, we characterized and analyzed HGF/SF induced membrane ruffling. We demonstrate that the Met receptor accumulates in ruffling areas, and that its mobility rate in the plasma membrane is dependent on HGF/SF activation and integrity of the actin cytoskeleton. To study the subcellular localization of Met activity we utilized a YFP-CFP fluorescence resonance energy transfer (FRET) based phosphorylation sensitive chimeric molecule. Both GFP-Met and DsRed-HGF/SF and the YFP-CFP chimeric protein retained their biological activity. Intravital imaging of live transgenic mice showed expression in epithelial and endothelial tissues. Enhanced fluorescence was mainly observed in sebaceous glands. LSM 510 with a Meta detector was used to characterize the spatial distribution and association of Met and HGF/SF. High resolution intravital analysis revealed GFP-Met membrane subcellular localization. At the age of 4-6 months, male transgenic mice developed angiosarcomas and/or sebaceous gland adenocarcinomas in their lower abdominal area. GFP-Met was highly expressed in the tumors. Intravital FRAP analysis revealed attenuated GFP-Met trafficking in sebaceous glands, degradation of the receptor was evident 1 minute following exposure to HGF/SF. Functional molecular imaging of the tumors using ultrasound contrast media was performed and increased contrast media signal intensity upon HGF/SF treatment was observed. Here we demonstrate that direct molecular imaging of a tyrosine kinase growth factor receptor in living cells and mice can provide a new insight into the molecular mechanisms of their activity. These novel techniques could help identify alterations in protein expression, isolate cells that undergo the early events of transformation and metastasis *in vivo*, and reveal the mechanisms of Met-HGF/SF-induced transformation and metastasis.

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**NANOSCALE MECHANICAL FLUCTUATIONS OF THE CELL  
MEMBRANE**

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Nanoscale mechanical fluctuations of the cell membrane were found to be a property common to many different cells. They are best manifested in human erythrocytes where they have been extensively studied. These membrane fluctuations were shown to be modulated by ligand-receptor interactions including ligands such as  $\beta$ -adrenergic agonists, atrial natriuretic peptide or oxygen. Studies of red blood cell ghosts suggest that these fluctuations are driven by a metabolic driving force in addition to a thermal one. Time series of cell membrane fluctuations, recorded by laser scanning phase contrast microscopy, were subjected to nonlinear time series analysis. It was found that membrane fluctuations of living cells possess characteristic features of chaotic dynamics.

Cell membrane fluctuations reflect changes in the elastic properties of the membrane-cytoskeleton complex. It has been demonstrated that there exists a linear correlation between cell membrane fluctuations and filterability of erythrocytes through pores narrower than their largest dimension. Changes of cell elasticity were also found in T-lymphocytes where a metastatic clone was shown to possess higher amplitude of fluctuations than that of its non-metastatic clone.

**PERMEATION AND GATING OPERATIONS OF CARDIAC KCNQ1 POTASSIUM CHANNELS**

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In the post-crystallographic era of bacterial potassium channels, it becomes important to investigate how the various mammalian voltage-gated  $K^+$  channels differ from one another, both structurally and functionally. The KCNQ family of voltage-gated  $K^+$  channels plays a major role in cardiac and brain excitability. The cardiac KCNQ1 channels can interact with various KCNE auxiliary subunits to form  $K^+$  channels with very different gating behaviours going from instantaneous voltage-independent  $K^+$  currents to very slow voltage-dependent  $K^+$  currents. To characterize the nature of the promiscuous gating of KCNQ1 channels, we performed a tryptophan-scanning mutagenesis of the voltage sensor and analyzed the mutation-induced perturbations in gating free energy. Mutations of specific S4 residues mimic the gating phenotypes produced by co-assembly of KCNQ1 with KCNE auxiliary subunits. The high energetic impact on mutated S4 residues indicates that the voltage sensor lies at a protein-protein interface. The data suggest that the interactions between KCNQ1 and KCNE subunits are allosteric in nature and that the auxiliary subunits act to lower the energetic cost for triggering the channel to intrinsic gating modes. The physical constraints imposed on S4 movement are probably very different compared to other voltage-gated  $K^+$  channels. We also used external barium to investigate the permeation characteristics of KCNQ1 channels. The results indicate that  $Ba^{2+}$  exerts a series of complex effects, including gating alterations as well as a voltage-dependent pore blockade. Barium interacts with the permeation pathway of KCNQ1 at two discrete and non-sequential sites. Compared to *Shaker*-like  $K^+$  channels, barium uniquely affects KCNQ1 channel gating, especially inactivation. In all, our data suggest that despite the high degree of homology of the pore and sensor regions among the various  $K^+$  channels, KCNQ1 channels display significant structural and functional uniqueness.

**LITHIUM-CALCIUM EXCHANGE IS MEDIATED BY A DISTINCT POTASSIUM-INDEPENDENT SODIUM-CALCIUM EXCHANGER**

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Sodium calcium exchangers have long been considered inert with respect to monovalent cations such as lithium, choline and NMG. A key question that has remained unsolved is how despite this, Li<sup>+</sup> catalyzes calcium exchange in mammalian tissues. Here we report that a Na<sup>+</sup>/Ca<sup>2+</sup> exchanger, NCLX cloned from human cells (known as FLJ22233), is distinct from both known forms of the exchanger, NCX and NCKX in structure and kinetics. Surprisingly, NCLX catalyzes active Li<sup>+</sup>/Ca<sup>2+</sup> exchange, thereby explaining the exchange of these ions in mammalian tissues. The NCLX protein, detected as both 70KDa and 55KDa polypeptides, is highly expressed in rat pancreas, skeletal muscle and stomach. We demonstrate, moreover, that NCLX is a K<sup>+</sup>-independent exchanger which catalyzes Ca<sup>2+</sup> flux at a rate comparable to NCX1 but without promoting Na<sup>+</sup>/Ba<sup>2+</sup> exchange. Activity of NCLX is strongly inhibited by zinc though it does not transport this cation. NCLX activity is only partially inhibited by the NCX inhibitor, KB-R7943. Our results provide a cogent explanation for a fundamental question: How can Li<sup>+</sup> promote Ca<sup>2+</sup> exchange while the known exchangers are inert to Li<sup>+</sup> ions? Identification of this novel member of the Na<sup>+</sup>/Ca<sup>2+</sup> superfamily, with distinct characteristics, including the ability to transport Li<sup>+</sup>, may provide an explanation for this phenomenon.

**BIOCHEMISTRY AND ELECTROPHYSIOLOGY OF  
IMPERFECTION – SLIPS IN METAL ION TRANSPORTERS**

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Metal-ion transport by DCT1, a family member of NRANP, is loosely coupled to the proton driving force. Consequently, the stoichiometry of proton to metal-ion is variable and under optimal transport conditions more than ten protons are co-transported with a single metal-ion. To better understand this phenomenon we used site-directed mutagenesis of DCT1 and analyzed the mutants by complementation of yeast SMF-null mutants and electrophysiology with *Xenopus* oocytes. The mutation F227I resulted in up to fourteen-fold increase in the coupling between protons to metal-ion transported. This observation suggests that the loose coupling property of DCT1 was not a necessity resulting from the transport mechanism in which positively charged protons are driving two positive charges of the metal-ion to the same direction. It supports the idea that the proton slippage has physiological advantage and the proton slip was positively selected during the evolution of DCT1.

## ALKALITOLERANCE: A NOVEL BIOLOGICAL FUNCTION FOR A MULTIDRUG TRANSPORTER IN PH HOMEOSTASIS

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MdfA is an *Escherichia (E.) coli* multidrug (Mdr) transporter. Cells expressing MdfA from a multicopy plasmid exhibit multidrug resistance against a diverse group of toxic compounds. In the present report we show that in addition to its role in multidrug resistance, MdfA confers alkaline pH resistance and allows growth of transformed cells under conditions close to those normally used by alkalophiles (up to pH 10). *mdfA* deleted *E. coli* cells are sensitive even to mild alkaline pH conditions, and the phenotype is fully restored by MdfA expressed from a plasmid. Internal pH measurements demonstrate that cells expressing MdfA maintain a constant intracellular pH even under conditions of extreme pH. This novel activity of MdfA requires Na<sup>+</sup> or K<sup>+</sup>. Fluorescent studies with inverted membrane vesicles demonstrate that MdfA catalyzes Na<sup>+</sup> or K<sup>+</sup> dependent dissipation of ΔpH, and experiments with reconstituted proteoliposomes confirm that MdfA is solely responsible for this phenomenon. Studies with Mdr-defective MdfA mutants, and competitive transport assays suggest that the two activities of MdfA are related. Taken together the results demonstrate an unprecedented capacity of a single protein to turn *E. coli* from an obligatory neutrophile into a facultative alkalophile, and suggest a novel physiological role for MdfA in pH homeostasis.

הרצאת אורח

**PLENARY LECTURE**

## **NEW DIRECTIONS IN MECHANISMS AND TREATMENT OF PARKINSON'S DISEASE.**

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The current treatment of Parkinson's disease is based mainly on dopamine replacement therapy and although this is initially highly effective a loss of drug efficacy occurs with disease progression accompanied by the onset of motor complications. In addition, many components of Parkinson's disease do not respond to current dopaminergic medications and require novel forms of therapeutic intervention. All treatments for Parkinson's disease are currently aimed at controlling the symptoms of the illness and there are no established neuroprotective or neurorestorative therapies that can stop or slow disease progression.

Current research is aimed at producing novel symptomatic treatments for Parkinson's disease that act through non-dopaminergic mechanisms. A range of approaches are being employed that target adenosine A2a receptors,  $\alpha$ -2 adrenergic receptors, 5HT1A receptors and many others. The advantage of this approach is that symptomatic relief may be obtained without evoking the same profile of unwanted side effects as occurs with dopaminergic drugs. For example, it may be possible to improve motor performance without provoking dyskinesia and without a loss of drug efficacy during the course of the illness.

In the search for neuroprotective strategies, there has been considerable disappointment in the failure of a number of different approaches in clinical investigations that had looked promising from the pre-clinical studies undertaken. However, by focusing on aspects of the pathogenic process thought to underly Parkinson's disease it may be possible to develop new models, which are more predictive of drug action in man. For example, Lewy body formation in Parkinson's disease appears to be related to a failure of the ubiquitin-proteasome system and this may now be a means of modeling nigral cell death in experimental models and also in intervening in the cell death cascade. In addition, glial cell activation and inflammatory change may well contribute to the progression of nigral pathology in Parkinson's disease and using models of glial mediated cell death it may be possible to slow down the course of the illness. For example, LPS induced glial cell activation in substantia nigra leads to the destruction of dopaminergic neurons that appears to be prevented by selective inhibitors of iNOS and by anti-inflammatory drugs.

The treatment of Parkinson's disease is likely to change markedly in the coming years due to a multitude of new pharmacological approaches and also to the use of agents that affect the disease process. A clearer understanding of why Parkinson's disease occurs and the mechanisms involved is leading to highly innovative changes in its treatment which will improve and enhance current therapeutic options.

תקצירי  
פוסטרים

**ABSTRACTS OF  
POSTERS**

## **GENDER INDUCED DIFFERENCES IN RENAL HANDLING OF PHOSPHATE.**

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Phosphate (Ph) is an essential element for bone formation and pregnancy, both of which are gender-dependent. Thus we hypothesized that renal handling of phosphate is affected by gender. To test this hypothesis four groups of rats were studied: 1) Young (12 weeks) male rats, 2) Female rats age and weight matched to the males. 3) Female rats 14 weeks after ovariectomy (OVX) and 4) Aged matched intact females. Renal function was similar in all groups. Plasma Ph was significantly higher in the males and the OVX rats than in the female rats. The Ph clearance was significantly higher in the female rats than in male and OVX rats. Accordingly, the fractional reabsorption of Ph ( $1 - C_{Ph}/C_{Cr}$ ) was significantly lower in females than in males and OVX. The gene (mRNA) and protein expression of the sodium-dependent phosphate co-transporter (NaPi2) were determined in renal cortex in all subgroups. In male rats, mRNA and protein abundance of NaPi2 were significantly higher than in both age and weight-matched females. In OVX females NaPi2 mRNA and protein abundance were also markedly higher than in control age-matched animals with intact ovaries. These data pointed, for the first time, to gender-dependent differences in the regulation of Ph balance, with lower Ph plasma levels, decreased tubular reabsorption and diminished NaPi2 gene and protein expression in females. These variations are modified by ovariectomized state. This study suggests that the gender related variations in renal Ph handling and NaPi2 expression may be estrogen-dependent.

## POSSIBLE INVOLVEMENT OF ENDOGENOUS DIGITALIS-LIKE COMPOUNDS IN DEPRESSION

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Digitalis-like compounds (DLC) are newly identified steroid hormones synthesized by and released from the adrenal gland. These compounds that resemble the structure of plant cardiac glycosides bind to and inhibit the activity of the Na<sup>+</sup>, K<sup>+</sup>-ATPase. Two groups of steroids have been identified, cardenolides, like ouabain and digoxin (OLC), and bufadienolides, like 19-norbufalin and proscillaridin A (BLC). Numerous studies have shown that Na<sup>+</sup>, K<sup>+</sup>-ATPase activity is reduced in depressed and manic depressed patients as compared to normal or euthymic subjects. We raised the hypothesis that this reduction is due to increased levels of DLC and suggested a role for these compounds in the etiology of depression. This hypothesis was addressed by determining DLC levels in samples of human brains as well as in Lipopolysaccharide (LPS)-treated rats, an experimental model for depression. DLC levels were determined using specific and sensitive ELISA in samples of human frontal cortex of four groups: bipolar (manic depressive disorder), schizophrenic, depressed patients and normal subjects. OLC levels were increased in bipolar patients and were significantly higher than in the samples from depressed patients ( $18.05 \pm 4.94$  vs  $5.67 \pm 1.15$  pmol/g tissue). Injection of LPS (2 $\mu$ g/kg) to rats elicited a depression-like symptoms manifested in reduced social exploration, line crossing, and rearing. These symptoms were significantly attenuated by a pre-injection of ouabain-antibodies. Adrenal OLC levels increased following LPS injection ( $17.59 \pm 7.41$  vs  $82.44 \pm 28.4$  pmol/mg) and this increase was inhibited in the ouabain-antibodies-treated rats. These results are in accord with the hypothesis that the endogenous DLC are involved in depression.

## METABOLIC ACTIVATION OF ZEBULARINE, A NOVEL DNA METHYLATION INHIBITOR, IN HUMAN BLADDER CARCINOMA CELLS.

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Gene silencing by abnormal over-methylation of the promoter regions of regulatory genes is commonly associated with many cancers. Thus, inhibition or control of DNA hypermethylation is an attractive strategy for cancer chemotherapy. Zebularine (2(1H)-pyrimidinone riboside, Zeb), a synthetic analogue of cytidine that is a potent inhibitor of cytidine deaminase, has been recently identified as a general inhibitor of DNA methylation. Zeb is an attractive drug candidate because of its low toxicity, excellent hydrolytic stability and apparent oral bioavailability. Among the model systems in which Zeb has exhibited an ability to inhibit DNA methyltransferases (DNMT) and reactivate silenced genes are T-24 human bladder carcinoma cells grown *in vitro* and as tumors in BALB/c *nu/nu* mice. In the latter case, a silenced *p16* gene could even be reactivated by orally administered drug.

Inhibition of DNMT is mechanism-based and is hypothesized to result from formation of a covalent complex between the enzyme and Zeb-substituted DNA. Metabolic activation of Zeb thus requires that it be phosphorylated and incorporated into DNA. Accordingly, we have quantitatively assessed the phosphorylation and DNA incorporation of Zeb in T-24 cells using 2-[<sup>14</sup>C]-Zeb in conjunction with gradient anion-exchange HPLC and selected enzymatic and spectroscopic analyses. Incorporation of 2-[<sup>14</sup>C]-Zeb into both cellular DNA and RNA following drug exposures up to 72 h has been measured using the Tri-reagent® procedure. Male BALB/c *nu/nu* mice inoculated s.c. with the EJ6 variant of T-24 bladder carcinoma have been treated i.p. with 500 mg/kg 2-[<sup>14</sup>C]-Zeb, and the 24-h Zeb phosphorylation profile has been determined in both tumor and in normal muscle. *In vitro*, Zeb is readily phosphorylated to form the corresponding 5'-mono-, di- and triphosphates in a dose- (1-500 :M) and time-dependent (0-24 h) manner. Two additional metabolites containing the intact Zeb base are also observed. Selective enzymatic experiments and a double-label study using [<sup>3</sup>H]-choline and [<sup>3</sup>H]-ethanolamine have allowed tentative identification of these conjugates as diphosphocholine (Zeb-DP-Chol) and diphosphoethanolamine (Zeb-DP-EA) adducts. Intracellular concentrations of Zeb-TP and Zeb-DP-Chol are comparable and greatly exceed those of the other metabolites. DNA incorporation occurs but is surpassed by that of RNA by at least 7-fold. *In vivo*, the phosphorylation pattern of Zeb in EJ6-inoculated tumor is similar to that observed *in vitro*. However, only low concentrations of Zeb metabolites are detectable in normal, straight muscle. The complex metabolism of Zeb and its limited DNA incorporation suggests that this is the reason why it is less potent than either 5-azacytidine or 5-aza-2'-deoxycytidine and requires higher doses for equivalent inhibition of DNMT.

**HSV THYMIDINE KINASE GENE TRANSDUCTION ENHANCES  
TUMOR GROWTH RATE, NEOVASCULARIZATION AND  
CYCLOOXYGENASE-2 EXPRESSION IN MURINE COLON CANCER  
XENOGRAFTS**

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Transduction of tumor cells with Herpes Simplex virus type I thymidine kinase (HSV-tk) gene and subsequent treatment with the prodrug ganciclovir (GCV) is the most common system studied for “suicide” gene therapy of cancer. However, the HSV-tk/GCV strategy has several limitations that, to some extent, plague all the cancer gene therapy strategies. Limitations include low transduction efficiency, vector cytotoxicity, high immunogenicity of transduced cells and resistance or loss of GCV sensitivity. In the present report we show that HSV-tk gene transduction significantly augments tumor growth rate ( $1.63 \pm 0.15$  fold increase in mean tumor volume) and neovascularization ( $1.83 \pm 0.32$  increase in mean intratumor microvessel density) in xenografts of murine colon cancer cells. Transduction of tumor cells with control vector carrying the neomycin resistance gene alone did not increase tumor growth rate but even decreased it, indicating that the observed phenomenon is related to the presence of the HSV-tk sequence insert in the retroviral vector used for HSV-tk gene delivery. Additionally, we demonstrate herein that HSV-tk gene transduction significantly enhances cyclooxygenase-2 (COX-2) expression and leads to  $2.5 \pm 0.17$  fold increase in prostaglandin  $E_2$  release in tumors *ex vivo*. Tumor growth rate of HSV-tk transduced murine colon cancer xenografts was significantly inhibited by treatment with the selective COX-2 inhibitor nimesulide. This observation may suggest a causal relationship between COX-2 overexpression and the enhanced growth rate of tumors expressing HSV-tk gene. It is further shown that the enhanced tumor growth rate observed in HSV-tk transduced murine colon cancer xenografts persists upon the development of GCV resistance, characterized by decreased susceptibility of resistant cells to the antiproliferative effect of GCV. Thus, one may expect the prognosis of HSV-tk transduced tumors to seriously deteriorate when they become resistant to GCV since tumor growth rate is enhanced, without a benefit of a prodrug to accomplish the suicide mission of this treatment. Taken together, these results may provide an insight to further understanding of the direct effect of HSV-tk gene transduction on tumor cell biology as well as to potential harmful effects of “suicide” gene therapy using retroviral vectors.

## **EFFECT OF SELECTED ANTIDEPRESSANTS ON INFLAMMATORY PROCESSES ASSOCIATED WITH MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) IN MICE**

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Multiple sclerosis (MS) is a chronic disabling autoimmune neurological disorder targeting the white matter of the central nervous system (CNS). MS symptoms result from inflammatory damage to the myelin sheath and involve activation of TH1 cells and secretion of pro-inflammatory TH1 cytokines. Our aim was to determine the potential immunomodulatory effect of some antidepressants on developed motor deficits in experimental autoimmune encephalitis (EAE) model in mice and on the secretion of pro-inflammatory cytokines from activated splenocytes *in-vitro*. EAE was induced in C57/bl mice by immunization with the rat myelin oligodendrocyte glycoprotein (MOG). Animals were divided into 4 groups (10/each) receiving saline/MOG alone or MOG and the antidepressants paroxetine, fluoxetine and clomipramine (15mg/kg x3/week i.p.). Animals were followed daily for 24 days and the clinical manifestations of EAE were scored (scale of 1-6). MOG animals developed paralysis manifestation during reaching the mean score of 1.65, all 3 antidepressants treated groups were significantly protected against the induced neurological symptoms (score of 0.35, 0.6 and 0.72 respectively for paroxetine, clomipramine and fluoxetine). In a 2<sup>nd</sup> experiment 4 groups of animals were treated with saline/MOG alone and MOG and paroxetine or sertraline at lower dose (5mg/kg x3/week i.p.) or dexamethasone (1mg/kg x3/week). Results showed that after 22 days of therapy dexamethasone completely inhibited the manifestations of neurological signs (0 score compared to 0.9 of MOG alone). Sertraline induced a delay in paralysis manifestation appearances as well as inhibition of the total score (0.45 vs 0.9), while paroxetine did not protect the animals (total score of 0.81). *In-vitro* experiments revealed that all antidepressants caused a marked inhibition of Concanavaline-A or MOG-induced splenocyte proliferation. Moreover, the antidepressants significantly suppressed the secretion of the proinflammatory cytokines IL2, INF $\gamma$  and TNF $\alpha$  resembling the effect of dexamethasone. Conclusions, our results suggest that some antidepressants could be of value for the treatment of MS mainly during initial stages.

**ASSOCIATION BETWEEN ONSET OF BEHAVIORAL EFFECT OF  
ANTIDEPRESSANT TREATMENT AND SEROTONERGIC  
REGULATION OF ACCUMBAL DOPAMINE RELEASE IN AN  
ANIMAL MODEL OF DEPRESSION**

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The aim of the current study was to determine the pharmacological mechanism of alterations in serotonin-induced accumbal dopamine release in an animal model of depression, Flinder sensitive line (FSL) rats. We previously found that FSL rats are characterized by absence of accumbal serotonin-dopamine interaction and antidepressant-induced restoration of this interaction directly correlated with improvement of their depressive-like behavior. In the current study, using in-vivo microdialysis, we found that: (a) 5HT<sub>2C</sub> receptor blocking resulted in an increase in accumbal dopamine levels, which was significantly higher in FSL than in control rats; (b) 5HT<sub>3</sub> receptor agonist administration resulted in elevation of accumbal dopamine levels, which was significantly lower in FSL than in control rats; (c) antidepressant treatment of FSL rats resulted in a decrease in 5HT<sub>2C</sub> antagonist-induced and 5HT<sub>3</sub> agonist-induced dopamine release; (d) the onset time for each antidepressant treatment for normalizing 5HT<sub>2C</sub>- and 5HT<sub>3</sub>-mediated dopamine release was equal to that needed to improve depressive-like behavior of FSL rats. We concluded that: (a) the absence of accumbal serotonin-dopamine interaction in FSL rats can be explained by increased inhibitory-like activity of 5HT<sub>2C</sub>- and decreased excitatory-like activity of 5HT<sub>3</sub> receptors; (b) repetitive antidepressants decrease 5HT<sub>2C</sub>- and increase 5HT<sub>3</sub> receptor activity; (c) antidepressants with more rapid effect on 5HT<sub>2C</sub> and 5HT<sub>3</sub> receptor activity are characterized by a faster onset of behavioral effect of the treatment.

**PRS-211,375, A NOVEL SELECTIVE CB2 RECEPTOR AGONIST,  
DEMONSTRATES ANALGESIC ACTIVITY IN SEVERAL ANIMAL  
MODELS.**

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Cannabinoid receptor agonists inhibit responses to painful stimuli by activation of the CB1 receptor, located mainly in the CNS, and the CB2 receptor, expressed mainly by inflammatory and immune cells. PRS-211,375, a selective CB2 receptor agonist (CB2 affinity  $IC_{50} \sim 1$  nM) was tested for its analgesic effect using 3 experimental animal pain models. The models were: (i) the tail-flick in mice for noxious pain; (ii) carrageenan-induced thermal and mechanical hyperalgesia in the rat hind paw for inflammatory pain; (iii) IP acetic acid induced visceral pain in the mice. PRS-211,375 showed relatively little analgesia in the tail flick test. However, PRS-211,375 administered either IP or PO showed significant dose-dependent analgesia (a decrease of more than 50%;  $p < 0.05$ ) in the carrageenan-induced inflammatory paw test (both in the thermal and mechanical stimuli). The compound also reduced paw edema volume in a dose-dependent manner (30 mg/kg PO reduced the edema by 50%). Pre-treatment with PRS-211,375 significantly reduced visceral pain as assessed by a reduction in writhing counted during 5 minutes ( $0.1 \pm 0.1$  compared with  $25.7 \pm 1.3$  in vehicle-treated control animals;  $p < 0.01$ ). PRS-211,375 proved to be much more potent than NSAIDs tested in the visceral pain model (Ketoprofen, Diclofenac and Celecoxib). Administration of a CB2, but not CB1 antagonist significantly reversed the effect of PRS-211,375 in both the inflammatory and visceral pain models suggesting that the analgesic activity of PRS-211,375 is mediated by the CB2 receptor. These data indicate that PRS-211,375, a CB2 selective agonist may be a potent analgesic compound for treatment of inflammatory related pain.

## EFFECT OF THE CELLULAR REDOX STATE ON THE ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR (AhR) BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD)

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The AhR is an intracellular protein that is activated by environmental pollutants (polycyclic aromatic hydrocarbons and dioxins) into a functional transcription factor that regulates the elimination of both endogenous and exogenous compounds. Binding of a ligand to the inactive receptor initiates a multistep process leading to the activation and nuclear translocation of AhR and formation of an AhR/Arnt complex. This heterodimer binds to specific DNA sequences, enhancing expression of genes encoding for xenobiotic metabolizing enzymes.

Redox-based modulation of gene expression has emerged as a fundamental regulatory mechanism in cell biology. It has been shown that DNA binding of a purified AhR/Arnt complex decreases under oxidizing conditions, while a reducing environment antagonizes this effect. It is not known whether the activation of AhR could be also regulated by redox-modifying agents.

Cultured HeLa cells were transfected with green fluorescent protein (GFP)-tagged AhR constructs and exposed to oxidants or antioxidants, in the absence or presence of TCDD, a “classical” AhR ligand. The intracellular localization of AhR was assessed using fluorescence microscopy.

We show here that: 1) H<sub>2</sub>O<sub>2</sub> inhibited the TCDD-induced nuclear translocation of full-length AhR in a dose-dependent fashion. 2) Addition of *N*-acetylcysteine (NAC) or Tempol to the cell cultures, after 2 h of exposure to H<sub>2</sub>O<sub>2</sub>, restored about 60% of the TCDD-induced stimulation of AhR nuclear translocation. 3) NAC and Tempol increased the nuclear content of AhR (in a dose-dependent fashion) also in the absence of TCDD. 4) NAC increased the nuclear content of AhR also in cells expressing a GFP-tagged mutated AhR that lacks the minimal ligand-binding domain. 5) NAC and bilirubin (an endogenous antioxidant) act synergistically in stimulating nuclear localization of AhR.

These results suggest that redox modulation may be a novel mechanism for regulating the activation of AhR.

## INVOLVEMENT OF URACIL NUCLEOTIDES IN A PROTECTION OF CARDIOMYOCYTES FROM HYPOXIC STRESS

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**Background:** Cardiomyocytes express one or more subtypes of receptors for extracellular nucleotides. P1 receptors, which are activated by adenosine, have four subtypes ( $A_1$ ,  $A_{2A}$ ,  $A_{2B}$ ,  $A_3$ ). P2 purinoceptors which are activated by nucleotides, classified into P2X and P2Y, where P2X are ligand-gated intrinsic ion channels and P2Y are G protein-coupled receptors.

Extracellular pyrimidine and purine nucleotides are released from the heart during hypoxia. While the cardioprotective effects of purines acting via purinoceptors were studied thoroughly, the physiological role of uracil nucleotide-responsive P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub> and P2Y<sub>14</sub> receptors is yet unclear especially in the cardiovascular system.

**Goals:** The aim of this study is to examine the physiological effect of UTP on cultured rat cardiomyocytes, during hypoxic condition, and the UTP signaling pathway.

**Results:** We found that UTP significantly reduced cardiomyocyte death induced by hypoxia. This effect of UTP was not observed with its derivative, UDP. Even incubation (1 hour) with UTP, 24 hours before exposing the cells to hypoxic conditions, protected the cells. The cardioprotective effect of UTP was reduced in the presence of the non selective P2 antagonist - suramin. In addition, UTP caused a transient increase of  $[Ca^{2+}]_i$  level in cardiomyocytes. PPADS or RB<sub>2</sub>, other antagonists of P2 receptors, abolished  $[Ca^{2+}]_i$  elevation caused by UTP. Using various inhibitors of the  $Ca^{2+}$  signaling pathway, we have shown that UTP originating from intracellular sources, elevated the  $[Ca^{2+}]_i$  level via PLC and the IP<sub>3</sub> receptor. Interestingly, BAPTA-AM, a  $[Ca^{2+}]_i$  chelator, and other inhibitors of the  $Ca^{2+}$  signaling pathway, did not prevent the protective effect caused by UTP.

**Conclusion:** This study describes the cellular protective role of UTP nucleotide on cardiomyocytes against hypoxic damage. This effect is apparently mediated via nucleotide receptor(s). Although UTP caused a transient increase in  $[Ca^{2+}]_i$  level in cardiomyocytes, the protection obtained by UTP is probably  $Ca^{2+}$ -independent.

## **INDUCTION OF CARDIOMYOCYTE DEATH BY ENDOTOXIC FACTORS - NEW APPROCHES FOR PROTECTION**

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Endotoxemia is a major cause of morbidity and mortality as a result of multiple organ dysfunction syndrome. Suitable medical treatment is needed to prevent sepsis and cardiac dysfunction. After bacterial infection, the host defense mechanisms trigger antimicrobial activity of macrophages and neutrophils, resulting in production of pro-inflammatory cytokines and reactive species such as NO and H<sub>2</sub>O<sub>2</sub>. The role of endotoxic factors in myocardial dysfunction remains to be determined. We aim to examine whether lipopolysaccharide (LPS) and the NO donor- sodium nitroprusside (SNP) induce cultured cardiomyocyte death in distinct paths.

Cultured cardiomyocytes from newborn rats were treated with 0.01-1mg/ml LPS or 0.5-20mM SNP for 24 hours. Cell viability was assessed by the propidium iodide (PI)-Hoechst assay. Morphological assessment was done with  $\alpha$ -sarcomeric actin staining. The role of cardiac mitochondria in modulation of cell death was studied by elucidation of the mitochondrial membrane potential, activity of respiratory enzymes and measurements of ATP content. Apoptotic gene activity was done by Western analysis. Cardiomyocytes treated with LPS displayed a characteristic phenotype of apoptotic cells: condensation and margination of nuclear chromatin, nuclear and cytoplasmic fragmentation and production of apoptotic bodies. Treatment with SNP for 24 hours induced a loss of cell viability, identified by PI staining, and oncotic damage consistent with necrosis. LPS induced redistribution of mitochondrial patterns while maintaining energetic parameters, whereas SNP induced mitochondrial destruction. These data indicate that LPS and SNP induce different signaling cascades leading to distinct forms of cardiomyocyte death and therefore require different therapeutic approaches. We studied the protective effects of 1,4-naphthoquinone derivatives which may restore damaged mitochondrial respiratory chains. The obtained data showed favorable effects of vitamin K derivatives against pathophysiological conditions leading to mitochondrial disruption.

## THE VOLTAGE-DEPENDENT ANION CHANNEL MODULATES APOPTOTIC CELL DEATH

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The voltage-dependent anion channel (VDAC) provides passage for adenine nucleotides,  $\text{Ca}^{2+}$  and other metabolites into and out of the mitochondria. VDAC also plays a central role in the release of apoptogenic factors including cytochrome *c*. Recently, we have shown that both ruthenium red (RuR) and hexokinase-I (HK-I) induce VDAC channel closure in a time-dependent manner, and stabilize the channel in a completely closed state. Both HK-I and RuR inhibited opening of the mitochondrial permeability transition pore, cytochrome *c* release and apoptotic cell death. However, the mechanism(s) underlying the protective action of RuR against apoptosis remains unknown.

The role of the VDAC in cell death was investigated upon over-expression of native and mutated murine VDAC (mVDAC) in the U-937 cell line or yeast, and using VDAC channel inhibitors. Binding of HK-I to mitochondria expressing E72Q-mutated mVDAC (in which glutamate 72 was replaced by glutamine) was decreased by ~70% and rendered insensitive to DCCD. This is in contrast to DCCD-sensitive HK-I binding to mitochondria harboring native or E87Q, D88N-mVDAC. Moreover, HK-I reduced the conductance of native VDAC reconstituted into a planar lipid bilayer (PLB), but not of E72Q-mVDAC. RuR, known to interact with  $\text{Ca}^{2+}$  binding proteins, was able to inhibit channel activity of recombinant native VDAC1 reconstituted into PLB, but had no effect on E72Q-mVDAC channel activity. Over-expression of murine or rat VDAC1 in U-937 induced a 3-5-fold increase in apoptotic cell death (from 12% to >70%). This cell death was prevented by pre-incubation with RuR or by co-transfection of the cells with pcDNA3.1-HK-I. As with native mVDAC, E72Q-mVDAC enhanced apoptotic cell death when expressed in U-937 cells. However, this cell death was not prevented by RuR or HK-I.

These findings indicate that a single amino acid mutation in VDAC prevents HK-I or RuR-mediated protection against apoptosis and provide the first direct evidence for VDAC regulation of mitochondria-mediated apoptotic pathways as well as showing that the protective anti-apoptotic effect of RuR is mediated via its direct interaction with VDAC.

## TPEN PRESERVES HOMEOSTASIS OF Ca IN CARDIAC CELLS BY Na/Ca EXCHANGER ACTIVATION

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TPEN, a transition-metal chelator, was found to protect against myocardial ischemia-reperfusion injury, but the mechanism of its function is not completely understood. Previously we have shown that TPEN prevented the increase in intracellular calcium ( $[Ca^{2+}]_i$ ) in cardiocytes during hypoxia. The prevention of Ca overload could explain the protection obtained by TPEN. The **goal** of this study was to investigate the mechanism by which TPEN reduces  $[Ca^{2+}]_i$  in cardiomyocytes.

**Methods:** Cardiomyocytes from 2-3 days old rat hearts were cultured and after 4-5 days in-vitro were used.  $[Ca^{2+}]_i$  level was measured by indo-1 fluorescence ratiometric method.

**Results:** To find out where Ca was sequestered upon TPEN treatment, the cells were treated with thapsigargin to inhibit  $Ca^{2+}$  uptake into the sarcoplasmic reticulum (SR). In this conditions  $[Ca^{2+}]_i$  was elevated and TPEN reduced it to the basal level, indicating that the drug did not sequestered  $Ca^{2+}$  into the SR. However, TPEN didn't reduce Ca overload in the Na-free medium in which Na/Ca exchanger was inhibited. This result shows that TPEN extrude  $Ca^{2+}$  through Na/Ca exchanger.

**Conclusion:** TPEN activates sarcolemmal Na/Ca exchanger to reduce  $[Ca^{2+}]_i$  to basal level (100 nM). This activation can explain the beneficial effect of TPEN in cardiocytes subjected to hypoxia.

## **CARDIOPROTECTION OF EPIRUBICIN-INDUCED ACUTE HEART-TOXICITY BY URIDINE TRIPHOSPHATE RECEPTOR ACTIVATION**

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The clinical use of the anticancer anthracyclines doxorubicin and epirubicin is limited by the development of cardiomyopathy and congestive heart failure. Due to the well-known resemblance between doxorubicin and epirubicin, we tried to use substances that were proven to protect against doxorubicin cardiotoxicity in cultured cardiomyocytes. (V. Shneyvays et al. *J. mol. Cell. Cardiol.* **34**; 493-507; 2002). These experiments failed to find an evident cardioprotection against epirubicin. The aim of this study was to explore the protective efficacy of uridine triphosphate (UTP) receptors activation against cardiac cells damage induced by epirubicin. This was tested on heart cultures which are obtained from 2-3 days old rats. The cell cultures were exposed to 2 $\mu$ M or 5 $\mu$ M of epirubicin for 24h. before damage assessment. Cardiomyocyte damage was expressed in:

1. Significant increase in lactate dehydrogenase (LDH) release to the surrounding medium.
2. Morphological changes expressed in increasing oncotic and necrotic characterized in: vacuolization, swelling and disintegration of the nucleus, condensation of the cytoplasm and demolition of the myofibril's cross-striated structure.
3. A significant increase in intracellular Ca<sup>2+</sup> level.

Pretreatment of cardiomyocyte cultures with UTP (50 $\mu$ M), leads to a significant protection expressed in low levels of LDH release, negligible morphological alterations and prevention of Ca<sup>2+</sup> overload. To our knowledge, this is the first study showing protective role of P2Y purinreceptors on cultured cardiomyocytes against the toxicity induced by an effective anti cancer drug - epirubicin. This suggests that UTP is potentially effective as a cardiac protection in clinical use

תקצירי הרצאות  
תחרות סטודנטים

**LECTURE ABSTRACTS  
STUDENT COMPETITION**

## THE ROLE OF STRESS AND SURVIVAL SIGNALING PATHWAYS IN THE PROTECTIVE EFFECT OF CALCITRIOL FROM STRESS-INDUCED APOPTOSIS IN KERATINOCYTES

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**Introduction:** Calcitriol, the hormonal form of vitamin D, is produced and metabolized in keratinocytes. Calcitriol protects keratinocytes from UV and chemotherapy-induced damage. The major survival signaling pathways in keratinocytes are mediated by EGF receptor (EGFR), ERK1/2, PI-3 kinase and Src, whereas p38 and JNK are key elements in stress-induced signaling pathways leading to cell death. We have previously shown that calcitriol enhanced signaling via the EGFR, resulting in enhanced ERK1/2 activation, and on the other hand inhibited the activation of JNK due to various stresses. This work aims to investigate the mechanism of the protective effect of calcitriol against apoptosis induced by various environmental and pathophysiological stresses.

**Methodology:** Our experimental model was autonomously proliferating non-tumorigenic human HaCaT keratinocytes. Apoptosis was assessed by determination of executioner caspases (caspases 3 and 7), assayed by cleavage of the fluorogenic substrate ac-DEVD-AMC and the endogenous substrate, PARP. Protein levels were assessed by immunoblotting and mRNA levels by quantitative real time PCR.

**Results:** We exposed HaCaT keratinocytes to various stresses to which the skin is routinely exposed: heat shock (45°C, 30-90 minutes), hyperosmotic shock representing dehydration (sorbitol 0.1-0.5 M, 1 hour), oxidative stress (H<sub>2</sub>O<sub>2</sub> 0.2-0.8 mM, 1 hour) and the inflammatory cytokine TNF (5-20 ng/ml, 24 hours). Exposure to these stresses brought about activation of executioner caspases that was markedly inhibited by 24 hours pretreatment with calcitriol (100 nM). Blocking of EGFR signaling through inhibition of its tyrosine kinase by AG1478 resulted in activation of caspases and synergistically enhanced sorbitol- and H<sub>2</sub>O<sub>2</sub>-induced caspase activation, yet it did not reduce the protective effect of calcitriol. Similar results were obtained by inhibition of Src kinase with PP1 or by inhibition of PI-3 kinase with Wortmannin. The inhibitory effect of calcitriol on caspase activation was manifested also in the presence of the ERK inhibitor U0126. The inhibitory effect of calcitriol on the activation of JNK and p38 was mimicked by the pharmacological inhibitors SP600125 and SB203580, respectively. This inhibition resulted in markedly reduced stress-induced caspase activation, indicating that JNK and p38 activation is involved in the protective effect of calcitriol. It is established that pro- and anti-apoptotic members of the Bcl2 family play a role in determination of the cell fate. Pretreatment of HaCaT keratinocytes with calcitriol increased protein and mRNA levels of the antiapoptotic protein, Bcl-2 but did not affect the levels of the pro-apoptotic proteins, Bak, or Bax or Bcl-x<sub>s</sub>, or the level of the anti-apoptotic Bcl-x<sub>L</sub>.

**Conclusions:** We conclude that calcitriol protects keratinocytes from apoptosis induced by various stresses and that this effect is at least partially due to inhibition of the stress activated JNK and p38 pathways, and to enhanced genes expression of the anti-apoptotic protein, Bcl2

THE EXCITATION-CONTRACTION COUPLING IN HUMAN EMBRYONIC STEM  
CELLS-DERIVED CARDIOMYOCYTES

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Since cardiac transplantation - the treatment of choice for end-stage heart failure, is limited by the small availability of donor organs, regeneration of the diseased myocardium by cell transplantation is an attractive therapeutic modality. Yet, because the transplanted cardiomyocytes (CM) should functionally integrate within the diseased myocardium, their properties must resemble those of the host. To determine the functional compatibility of 45-60 day old human embryonic stem cells-derived CM (hESC-CM) we investigated in these cells the excitation-contraction coupling (ECC) and its responsiveness to a variety of common physiological stimuli. The major findings were: **(1)** hESC-CM contraction was highly dependent on  $[Ca^{2+}]_o$ ; at 4 mM contraction amplitude was  $145 \pm 13\%$  ( $n=3$ ) of that at 2 mM. **(2)** hESC-CM contraction was blocked by verapamil and increased by the DHPR channel opener BayK. Both findings indicate that hESC-CM contraction depends to a large extent on the L-type  $Ca^{2+}$  channel. Based on these findings, we investigated the contribution of the sarcoplasmic reticulum (SR)  $Ca^{2+}$  stores to contraction. Surprisingly, depleting SR of its  $Ca^{2+}$  stores with thapsigargin ( $10^{-7}$  M) or ryanodine ( $10^{-8}$  M) did not affect the  $[Ca^{2+}]_i$  transients (measured by Fura-2 fluorescence) or contraction. Moreover,  $10^{-5}$  M ryanodine which blocks SR  $Ca^{2+}$  release, did not affect  $[Ca^{2+}]_i$  transients or contraction. Collectively, these results indicate that at this developmental stage the contraction is largely dependent on  $[Ca^{2+}]_o$  rather than on SR  $Ca^{2+}$  stores. **(3)** As increasing heart rate is a fundamental mean to enhance muscle contraction, we constructed the force-frequency relations of hESC-CM, at 0.5, 1, 1.5, 2 and 2.5 Hz. In contrast to the positive staircase characterizing the majority of cardiac preparations, hESC-CM exhibited a negative staircase, possibly due to the insignificant contribution of SR  $Ca^{2+}$  stores to contraction. Our results show that the excitation contraction coupling properties of 45-60 day old hESC-CM differ from adult CM, and therefore should be further developed to attain functional compatibility with the adult myocardium.

## **ANTIDEPRESSANT EFFECT OF THE THYROID GLAND HORMONE T3 ( TRIIODOTHYRONINE) AS DEMONSTRATED IN THE FST (FORCED SWIM TEST) BEHAVIORAL PARADIGM.**

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The forced swim test (FST) is a well established behavioral animal model for depression. This test is also considered as an efficient screening test for antidepressant properties of drugs including those of the SSRI group. The FST quantifies the behavior of an animal swimming in a water tank for a total of 5 minutes. The behavioral modes examined include inactivity versus different activities (swimming, climbing and diving). An antidepressant effect in this test is defined by a significant increase in the time spent in the different activity modes at the expense of the time spent in inactivity.

The thyroid hormone T3 (triiodothyronine) has been used in the treatment of depression for decades, either as a sole agent or as a supplement to antidepressant drugs, in order to augment or accelerate their activity. In our experiments, we have studied the antidepressant effect demonstrated in FST of T3 and fluoxetine, given either alone or in combination. In order to further differentiate the treatment effect on both genders, we used male and female Sabra rats.

Our experimental findings can be summarized as follows:

- 1) Low doses of T3 or fluoxetine administered for 2 weeks failed to generate an antidepressant effect in the FST in both genders.
- 2) High doses of fluoxetine administered for 7 days in male rats produced an antidepressant effect in the FST, while high doses of T3 given for the same period to males failed to produce such an effect.
- 3) High doses of T3 administered to females produced an antidepressant effect in the FST only 72 hours after the last injection of the hormone.
- 4) Over all, marked differences were found between the genders in their responses to the different treatments as demonstrated in the FST.

## NOVEL CANNABINOIDS AND THEIR ROLE AS VASCULAR MODULATORS

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Cannabinoids exert a wide range of biological activities including the well-known psychotropic and hypotensive effects. Previous attempts to separate the psychotropic activity from the cardiovascular effects of cannabinoids have been only partially successful as the compounds still kept some behavioural activity. We have synthesized a series of derivatives of non-psychotropic cannabinoids and tested their activity *in vivo* and in isolated blood vessels. This investigation aimed to determine the vascular activity of the synthesized compounds and the underlying mechanism involved.

Male Sabra rats of 300 g average body weight were used. For the *in vivo* tests the rats were anaesthetized with Pentobarbital sodium 6% 60 mg/kg and were cannulated for i.v. injections, a catheter was connected to a pressure transducer and blood pressure was measured before and after the administration of different cannabinoids.

Rats' thoracic aortic rings of 2-3 mm length were used. Both intact rings and endothelium denuded were used and compared. The rings were placed in a 10-ml organ bath containing Krebs' solution gassed with 95%O<sub>2</sub>/5%CO<sub>2</sub>, and maintained at 37 °C. The rings were stretched until an optimal basal tension of 1.0 g was achieved and allowed to equilibrate for 60 minutes. The segment was then stimulated with phenylephrine (1 µM) leading to force generation and in turn vasoconstriction. Addition of two of the novel cannabinoids in different concentrations led to relaxation of the contracted segments, even in the presence of cannabinoid antagonists (CB<sub>1</sub> and CB<sub>2</sub>). The levels of relaxation were then measured. Dose response curves were established.

A 60 mM final concentration of Cannabigerol-Dimethyl Heptyl (CBG-DMH), which induced blood pressure fall in the intact animal (5 mg/kg), resulted in 63 ± 2% ring relaxation. 120 mM concentration didn't induce further marked relaxation. 100 µM final concentration of Arachidonoyl-serine induced 74± 6% vasorelaxation. No significant vasorelaxation was observed in endothelium-denuded vessels.

These results demonstrate a significant vascular effect of CBG-DMH and of the brain constituent Arachidonoyl-Serine, *ex vivo*, which is endothelium dependent and not related to the known cannabinoid receptors.

## NEURORESCUE EFFECT OF RASAGILINE IN THE MPTP MOUSE MODEL OF PD

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Rasagiline (N-propargyl-1R-aminoindan) is a novel highly potent irreversible monoamine oxidase B inhibitor, recently approved by the FDA for the treatment of Parkinson's disease (PD). In vivo and in vitro evidence suggest that rasagiline not only halts cell death (neuroprotection), but actually promotes the recovery and survival or even rescue from neuronal cell death (neurorescue).

In this study, we have employed the 1-Methyl-4-Phenyl- Tetrahydropyridine (MPTP) mouse model of Parkinson's disease (PD), to examine putative neurorescue properties of rasagiline, in-vivo. The experimental paradigm consisted of 4 consecutive injections of MPTP (24mg/kg/ day, i.p). 4 days after last injection, rasagiline (0.05mg/kg/ day) was orally administrated for 10 days. As expected, MPTP reduced both striatal dopamine content and tyrosine hydroxylase (TH) activity. To our surprise, although rasagiline was given after MPTP, it induced a significant recovery in all these parameters. Striatal DA content was increased by a 1.74 fold, and TH activity was increased by a 1.5 fold comparing to MPTP ( $p < 0.05$ ) To get an insight into the molecular mechanism of the neurorescue effect of the drug, gene and protein expression analysis were conducted in the midbrain, the major affected area in PD. Our study shows that, rasagiline induced a particular increase in the H-Ras small GTPase gene and Ras protein, which operates as a molecular switch in signal transduction pathways downstream to tyrosine kinase receptors (TRK). In line with this, rasagiline up-regulated the TRK adaptors, CRK, Sos1 and ShcC, and the TRK-A substrate beta-nerve growth factor (NGF) level. The activation of TRK-Ras pathway led to a specific elevation in PI-3 kinase (PI-3K) protein, a downstream effector of Ras. Confirmatory immunohistochemical studies demonstrated that this PI-3K elevation resulted in an increase of both the phosphorylated, active substrate, Akt and the phosphorylated ,inactive effectors, GSK-3b and Raf1 which are downstream to Akt. The distinctive in vivo selection pressure of rasagiline to one Ras effector pathway, namely, the PI-3K/Akt, may thus account for its potent neurorescue attributes.

## SGK IS NOT THE ONLY PROTEIN KINASE PHOSPHORYLATING Nedd4-2 AND REGULATING ENaC.

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The Epithelial Sodium Channel (ENaC) mediates active sodium reabsorption in kidney collecting duct, distal colon, lung and exocrine glands. A major pathway controlling ENaC's cell surface expression (and activity) involves its interaction with the ubiquitin ligase Nedd4-2. The WW domains of Nedd4-2 bind to the proline-rich PY motifs on the COOH termini of  $\beta$ - and  $\gamma$ -ENaC, leading to channel ubiquitination, internalization and degradation. SGK is an aldosterone-regulated protein kinase that strongly stimulates ENaC cell-surface expression and function when expressed in *Xenopus* oocytes. Recently, it was reported that SGK phosphorylates Nedd4-2 on several residues and in particular on Ser328. Phosphorylated Nedd4-2 fails to interact with ENaC leading to an increased cell surface expression of the channel and an elevated rate of transepithelial sodium absorption. We have used an *in vitro* phosphorylation assay to identify protein kinases other than SGK that phosphorylate Nedd4-2. Ser328 was found to be specifically phosphorylated by several kinase-enriched cytosolic fractions derived from HEK293T cells. The phosphorylation observed was not mediated neither by SGK nor by AKT/PKB known to phosphorylate the same consensus sequence. The Ser328 phosphorylating kinases have been found to specifically and tightly interact with the 2<sup>nd</sup> and 3<sup>rd</sup> WW domains of Nedd4-2 and the minimal sequence essential for high affinity interaction includes S328. Affinity based purification and mass spectroscopical analysis are being used to identify the S328 phosphorylating protein kinases. Our results indicate that Ser328 on Nedd4-2 is a converging point of several signalling pathways and protein kinases, regulating ENaC activity.