

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

ISRAEL SOCIETY FOR PHYSIOLOGY
AND PHARMACOLOGY



הכנס השנתי **Annual Meeting**

29/9/2005

Ma'ale Hachamisha

PROGRAM & ABSTRACTS

הפקולטה למדעי החיים, אוניברסיטת בר-אילן, רמת-גן 52900
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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 כנס זה

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



The Recetor of Bar-Ilan University donated the prize for the student
competition in the sum of \$750. The award will be presented to the best student
lecture and is intended to support active participation in international meetings.

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

13:10-14:00 Lunch

14:00-15:00 Posters

14:45-15:00 Business meeting

15:00-16:00 Plenary lecture

Prof. Michel Revel

Department of Molecular Genetics, The Weizmann Institute
Role of IL-6 signaling in differentiation of nerve
myelinating cells: potential for treatment of
neuropathies and for transplantation of ES cell-
derived oligodendrocytes.

16:00-18:00 Student Lecture Competition

Meeting Program

9:30-11:10 - MORNING SESSION

A - New Developments in the Study of Channels and Transporters
Chairpersons: Bernard Attali (TAU) and David Lichtstein (HUJI)
Hall 1

- 9:30 Haim Garty and Steven J.D. Karlish
Dept. of Biological Chemistry, The Weizmann Institute, Rehovot
Regulation of the Na, K ATPase by FXD proteins
- 9:55 Ioav Z. Cabantchik, Hava Glickstein, Maya Shvartsman, Rinat Ben El, Taly Cohen and William Breuer
Department of Biological Chemistry, Institute of Life Sciences, Hebrew University, Jerusalem
A panoramic journey into the labile iron centers of the cell
- 10:20 Ofer Yifrach
Department of Life Sciences the Ben Gurion University
Conserved gating hinge in ligand- and voltage-dependent K⁺ channels
- 10:45 Alon Korngreen¹, M. Helmstaedter², B. Sakmann² and A.T. Schaefer²
¹Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel
²Dept Zellphysiologie, MPI f. med. Forschung, Heidelberg, Germany
High conductance densities of voltage-gated K⁺ channels in cortical pyramidal neurons

B - Drug Delivery and Targeting
Chairperson: Gershon Golomb (HUJI)
Hall 2

- 9:30 Abraham Rubinstein
School of Pharmacy, Faculty of Medicine, HUJI
Colonic mucosa targets: can they be approached by the oral route?
- 9:55 Gershon Golomb
School of Pharmacy, Faculty of Medicine, HUJI
Nanoparticles as Trojan horses for vascular therapy"
- 10:20 Alberto Gabizon
Shaare Zedek Medical Center
Pros and cons of the liposome platform in cancer drug targeting

10:45 Amnon Sintov
Faculty of Health Sciences, Ben-Gurion University
New methods of dermal drug delivery

C - Signal Transduction
Chairpersons: Jeremy Don (BIU), Danielle Melloul (HUJI)
Hall 3

9:30 Jeremy Don
Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan
CREB/ATF transcription factors in spermatogenesis and fertilization

9:55 Uri Nir
Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan
Balancing between signaling and anti-signaling cascades: a Fer play or a sexy trick?

10:20 Oded Meyuhas
Department of Biochemistry, The Hebrew University-Hadassah Medical School, Jerusalem
Phosphorylation of ribosomal protein S6 is a determinant of cell size and glucose homeostasis

10:45 Sigalit Boura-Halfon, Avia Herschkovitz, Diana Gurevitch-Rabinovitch and Yehiel Zick
Department of Molecular Cell Biology, Weizmann Institute, Rehovot
Phosphorylation- based uncoupling of insulin signaling: a molecular basis for insulin resistance

11:10-11:30 Coffee break

11:30-13:10 - AFTERNOON SESSION

D - Cure for the Brain
Chairpersons: Eran Gilat (IIBR), Hagai Bergman (HUJI)
Hall 1

11:30 Eugenia Bloch-Shilderman, Tamar Kadar, Aharon Levy, Rita Sahar, Ishai Rabinovitz and Eran Gilat
Department of Pharmacology, Israel Institute for Biological Research, Ness Ziona
Organophosphate poisoning induced subcellular alterations of protein kinase C isozymes in the rat brain

11:55 Hermona Soreq
The Hebrew University of Jerusalem, Department of Biological Chemistry, Safra Campus-Givat Ram
Alternative splicing as a neuroprotection strategy

12:20 Michal Schwartz
Department of Neurobiology, The Weizmann Institute of Science, Rehovot

T-cell-based vaccination for maintenance of plasticity, neurogenesis, and repair in the CNS in health and disease

12:45 Hagai Bergman
The Hebrew University, Jerusalem

The basal ganglia and the pathophysiology of Parkinson's disease

E - Cardiovascular System

Chairpersons: Binah Ofer (Technion), Herzel Shwalb (HUJI)

Hall 2

11:30: Ofer Binah¹, Katya Dolnikov¹, Mark Shilkrot^{1,2}, Naama Zeevi-Levin¹, Michal Amit^{1,3}, Asaf Danon¹, Joseph Itskovitz-Eldor^{1,3}

¹Rappaport Faculty of Medicine, Technion, Haifa

Departments of ²Oncology and ³Obstetrics and Gynecology, Rambam Medical Center, Haifa

The excitation contraction coupling of human embryonic stem cells-derived cardiomyocytes.

11:55 Zaid Abassi
Dept. of Physiology and Biophysics, Faculty of Medicine Technion, Haifa

Heart failure, neurohormonal activation, and new pharmacological therapies

12:20 Michael Arad
Heart Institute, Sheba Medical Center, Tel Hashomer

Genetic and metabolic causes of inherited cardiomyopathies.

12:45 Meir Preis, Belly Koren, Tzafra Cohen, Mira Israeli Amit, Yael Sarnatzki, Dana Levin, Rona Shofti, Basil S. Lewis and Moshe Y. Flugelman

Department of Cardiovascular Medicine, Lady Davis Carmel Medical Center, Bruce Rappaport Faculty of Medicine, Technion IIT, Haifa

Induction of angiogenesis by genetically modified venous endothelial and smooth muscle cells.

F - Cell Death

Chairpersons: Chaya Lorberboum-Galski (HUJI), Atan Gross (Weizmann)

Hall 3

- 11:30 Iris Kamer, Rachel Sarig, Yehudit Zaltsman, Hagit Niv, Galia Oberkovitz, Limor Regev, Gal Haimovich, Yaniv Lerenthal and Atan Gross
Department of Biological Regulation, Weizmann Institute, Rehovot
Pro-apoptotic bid is an ATM effector in the DNA damage response
- 11:55 Osnat Alsheich¹, Shin'chi Sito², Ettore Appella² and Ygal Haupt¹
¹*The Lautenberg Center for General and Tumor Immunology, The Hebrew University Hadassah Medical School, Jerusalem*
²*The Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD*
A role for PML in the regulation of p53 by CK1
- 12:20 Tamar Kleinberger, Rakefet Sharf, Haggit Ben-Israel, Tsofnat Maoz, Roni Koren and Eliya Bitman
Unit of Microbiology, Faculty of Medicine, Technion, Haifa
Induction of cancer cell-specific apoptosis by the adenovirus E4orf4 protein
- 12:45 Ruth Belototsky, Michal Lichtenstein, Ofra Sabag, Inna Grodzovsky, Ronen Eaveri and Haya Lorberboum-Galski
Department of Cellular Biochemistry and Human Genetics, Hebrew University-Hadassah Medical School, Jerusalem.
Pro-apoptotic-proteins as novel therapeutic tools For targeted human therapy
- 13:10-14:00 Lunch
- 14:00-15:00 Posters
- 14:30-14:45 Dr. Yona Geffen, BioLineRx (Hall 3)
BioLineRx: a drug development company advancing a first in class antipsychotic for the treatment for schizophrenia
- 14:45-15:00 Business meeting (Hall 3)
- 15:00-16:00 **Plenary lecture Prof. Michel Revel: Role of IL-6 signaling in differentiation of nerve myelinating cells: potential for treatment of neuropathies and for transplantation of ES cell-derived oligodendrocytes. (Hall 1)**
- 16:00-18:00 **Student Lecture Competition**

Program at a glance

Session A (Hall 1): Developments in the Study of Channels and Transporters		Session B (Hall 2): Drug Delivery and Targeting		Session C (Hall 3): Signal Transduction	
Chairpersons: Bernard Attali and David Lichtstein		Chairperson: Gershon Golomb		Chairpersons: Jeremy Don, Danielle Melloul	
9:30	Steven J.D. Karlish Regulation of the Na, K ATPase by FXYP proteins	9:30	Abraham Rubinstein Colonic mucosa targets: can they be approached by the oral route?	9:30	Jeremy Don CREB/ATF transcription factors in spermatogenesis and fertilization
9:55	Ioav Z. Cabantchik A panoramic journey into the labile iron centers of the cell	9:55	Gershon Golomb Nanoparticles as Trojan horses for vascular therapy	9:55	Uri Nir Balancing between signaling and anti-signaling cascades: a Fer play or a sexy trick?
10:20	Ofer Yifrach Conserved gating hinge in ligand- and voltage-dependent K ⁺ channels	10:20	Alberto Gabizon Pros and cons of the liposome platform in cancer drug targeting	10:20	Oded Meyuhas Phosphorylation of ribosomal protein S6 is a determinant of cell size and glucose homeostasis
10:45	Alon Korngreen High conductance densities of voltage-gated K ⁺ channels in cortical pyramidal neurons	10:45	Amnon Sintov New methods of dermal drug delivery	10:45	Yehiel Zick Phosphorylation- based uncoupling of insulin signaling: a molecular basis for insulin resistance

Session D (Hall 1): Cure for the Brain		Session E (Hall 2): Cardiovascular System		Session F (Hall 3): Cell Death	
Chairpersons: Eran Gilat, Hagai Bergman		Chairpersons: Binah Ofer, Herzel Shwalb		Chairpersons: Chaya Lorberboum-Galski, Atan Gross	
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11:55	Hermona Soreq Alternative splicing as a neuroprotection strategy	11:55	Zaid Abassi Heart failure, neurohormonal activation, and new pharmacological therapies	11:55	Ygal Haupt A role for PML in the regulation of p53 by CK1
12:20	Michal Schwartz T-cell-based vaccination for maintenance of plasticity, neurogenesis, and repair in the CNS in health and disease	12:20	Michael Arad Genetic and metabolic causes of inherited cardiomyopathies.	12:20	Tamar Kleinberger Induction of cancer cell-specific apoptosis by the adenovirus E4orf4 protein
12:45	Hagai Bergman The basal ganglia and the pathophysiology of Parkinson's disease	12:45	Meir Preis Induction of angiogenesis by genetically modified venous endothelial and smooth muscle cells	12:45	Haya Lorberboum-Galski Pro-apoptotic-proteins as novel therapeutic tools For targeted human therapy

Abstracts of invited presentations

Regulation of the Na, K ATPase by FXYD proteins

HAIM GARTY and STEVEN J.D. KARLISH. Dept. of Biological Chemistry.
The
Weizmann Institute of Science, Rehovot 76100.

The FXYD proteins are a family of single span transmembrane proteins with unique tissue distributions and cellular regulations. Six members of this group have been shown to interact with the Na, K ATPase and alter its kinetic properties, thus acting as physiological modulators. CHIF and γ are two FXYD proteins, which are preferentially expressed in the kidney and have non-overlapping distributions along the nephron. They have opposite effects on the apparent affinity of the Na, K ATPase for cytoplasmic Na^+ and thereby provide a convenient means to adjust pumping rates to the unique requirements of different nephron segments. Recent studies have characterized structural and functional interactions between these FXYD proteins and the $\alpha 1\beta 1$ subunits of the pump in transfected HeLa cells and in purified renal Na,K-ATPase. We have identified domains and residues participating in the structural and functional interaction of these proteins with the $\alpha\beta$ complex. A model of interaction between the trans-membrane segments and cytoplasmic sequences of the γ subunit and a homology model of the $\alpha 1$ subunit has been proposed, and is being tested. These studies demonstrate a similar overall disposition of the two FXYD proteins with respect to trans-membrane segments of α and β .

A panoramic journey into the labile iron centers of the cell

Cabantchik Z. Ioav, Hava Glickstein, Maya Shvartsman, Rinat Ben El, Taly Cohen and William Breuer. Department of Biological Chemistry, Institute of Life Sciences, Hebrew University, Jerusalem

Iron is an essential component of life. In animals, iron levels are tightly regulated over the entire life span of the organism. Most of the bioiron resides in protein stations and only a minute fraction is mobilized between those stations via labile forms that serve as transitory metabolic pools and/or as indicators of cellular iron status. The labile iron forms represent *labile iron pools (LIP)*, which can be of *cellular (cLIP)* or *extracellular (eCLIP)* nature. *Pharmacologically*, those pools are characterized in terms of their propensity to engage in redox-cycling in an oxygenated environment and/or following pro-oxidant challenges. *Biochemically*, cLIP is the cross-roads of cell iron metabolism that is translationally regulated primarily by expression of transferrin receptors and ferritin molecules under a variety of physiological conditions; *Diagnostically*, eLIP appears in human plasma and other extracellular fluids as non-transferrin bound iron (NTBI) which comprises a redox-active and chelatable component (termed *labile plasma iron, LPI*) that serves as an indicator of impending iron overload in a variety of pathological conditions (thalassemia, MDS and hereditary hemochromatosis) and *Therapeutically*, the LIPs are the immediate targets of chelators designed to reduce iron load in the organism (both preventively and correctively), with emphasis on organs of accumulation such as liver, endocrine glands and heart. With the aim of obtaining quantitative and real time information about the localization and dynamics of the labile metal in various biological compartments we designed fluorescence-responsive metallo-sensors and redox-sensors of cLIPs and combined them with organelle targeting techniques. In this presentation we will review the principles of metalosensor probe design and their applications in solution, cells and the whole organism, from single organelle microscopic analysis to HT screening methods.

The versatility of the novel methodologies for assessing cLIP and eCLIP, have opened the road for exploring basic mechanisms of cell iron regulation and for early diagnosis of abnormal iron metabolism in various pathological conditions. Following a review of the various applications of the novel methodologies we will focus on intracellular iron traffic using microscopy (laser scanning and epifluorescence) and fluorescent metal sensors (free probes and/or coupled to dendrimers and nanoparticles) targeted to different cell organelles. The distribution of iron acquired by cells from Tf and various iron(II) and (III) iron sources is fluorescence traced among the major organelles in real time. This used for assessing intracellular and intra-organellar traffic of labile iron, and the mode by which expression of specific transporters or pharmacologically important chelators affect their cellular distribution. Our results suggest that iron traffic from plasma membrane to organelles like mitochondria largely proceeds as diffusion of non-labile iron, in association with specific high-affinity cytosolic carriers. That putative chaperon seemingly shields transitory iron, thereby reducing its involvement in ROS formation and its susceptibility to cytosolic chelation. Thus intracellular transfer of potentially toxic metals is seemingly tuned to evade production of ROS as well as to ensure safe delivery to cellular factories such as mitochondria, nucleus and possibly other components.

Conserved Gating Hinge in Ligand- and Voltage-Dependent K⁺ Channels

Ofer Yifrach

Department of Life Sciences and the Zlotowski Center for Neurosciences, the Ben Gurion University of the Negev, Israel

Ion channels open and close their pore in a process called gating. Based on crystal structures of two voltage-independent K⁺ channels, KcsA and MthK, a conformational change for gating has been proposed whereby the inner helix bends at a glycine hinge point (gating hinge) to open the pore and straightens to close it. Here we ask if a similar gating hinge conformational change underlies the mechanics of pore opening of two eukaryotic voltage-dependent K⁺ channels, *Shaker* and BK channels. In the *Shaker* channel, substitution of the gating hinge glycine with alanine and several other amino acids prevents pore opening, but the ability to open is recovered if a secondary glycine is introduced at an adjacent position. A proline at the gating hinge favors the open state of the *Shaker* channel as if by preventing inner helix straightening. In BK channels, which have two adjacent glycine residues, opening is significantly hindered in a graded manner with single and double mutations to alanine. Our results imply an important role of the conserved glycine gating hinge for pore-opening in both ligand and voltage-dependent potassium channels.

High conductance densities of voltage-gated K⁺ channels in cortical pyramidal neurons

A. Korngreen^{1*}; M. Helmstaedter²; B. Sakmann²; A.T. Schaefer²

1. Dept Life Sci, Bar-Ilan Univ, Ramat-Gan, Israel 2. Dept Zellphysiologie, MPI f. med. Forschung, Heidelberg, Germany

Electrophysiological properties of single neurons are crucially determined by the number and types of ionic channels expressed. Thus, for understanding single neuron function it is essential to determine exact distribution and kinetics of the relevant ionic conductances. So far the lack of space clamp has strongly limited the options to obtain correct conductance estimates and kinetic details. Here, we present an approach that retrieves accurate K⁺- and Ca²⁺- conductance properties from voltage-clamp measurements in non-space-clamped structures. Essentially, we have implemented successive one-parameter fitting routines in NEURON to repeat previously performed experiments in a detailed compartmental model of the recorded cell. Using recorded currents as a template, this paradigm extracts correct conductance values over a wide range of experimental conditions. In particular, it is capable of retrieving complete Hodgkin-Huxley-style K⁺-channel parameters (activation, inactivation steady-state and inactivation kinetics) with high spatial resolution. Additionally, Ca²⁺ channel parameters can be extracted with noise levels of up to 70pA r.m.s. Complementarily to the simulation results, we have corrected voltage-clamp experiments from dendrites of cortical layer-5 pyramidal cells (n=3), illustrating the feasibility of our approach. Finally, we investigated the physiological role of the increased accuracy in channel parameter measurements in full compartmental neuronal models. Inactivation and activation parameters of K⁺ channels were varied and their influence on physiological measures such as size and shape of backpropagating APs was determined. The described approach will allow the fast determination of channel distributions in individual neurons and might contribute to a more complete understanding of the biophysical basis of neuronal function.

Colonic mucosa targets: can they be approached by the oral route?

Abraham Rubinstein

The Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy

Numerous experimental techniques for targeting the large bowel via the oral route have been reported in the pharmaceutical literature for the past 15 years. The majority of these techniques did not reach clinical phase. The main reasons for the failure to move from promise to reality are: **(a)** the lack of appealing medical opportunities to justify complicated developments, **(b)** non-realistic assumptions regarding the therapeutic advantage of targeting the colon with molecules that probably would have worked after systemic administration (in other words: the lack of accompanying in depth pharmacological research) and **(c)** the ongoing difficulty to launch oral protein products (“biologics”), which impacts on the development of colonic platforms for this complicated group of molecules. Still, there are some intriguing reports that inspire further efforts in the research of colonic drug delivery. Colonocyte targeting is one example. However, existing methods of colon drug delivery are appropriate for ferrying large drug doses only and their ability to “target” is certainly not acceptable. It is unlikely that a new biologic aimed at topical treatment of colon inflammation at the cellular level, or a new anti cancer drug, designed to anchor the cell membrane, can arrive intact to its site of action if left in the large bowel lumen. Thus, it is about time to explore realistic modes of safe colonocytes drug disembarkation.

Nanoparticles As Trojan Horses For Vascular Therapy

Gershon Golomb

Dept. of Pharmaceutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel (golomb@md.huji.ac.il)

Intimal hyperplasia is a universal response of the arterial wall to mechanical injury and it is a major cause of restenosis following percutaneous coronary interventions (PCI). The pathophysiology of restenosis is multifactorial; emerging experimental and clinical data indicate that the innate immunity and inflammation are of major importance in vascular repair, and to outcome of PCI.

We validated the hypothesis that systemic and transient depletion of monocytes and macrophages inhibits balloon-injured as well as in-stent neointimal formation in the normal and hypercholesterolemic rabbits. Monocytes/macrophage depletion was achieved with a systemic injection of nanoparticulated dosage forms (PLGA-based nanoparticles and liposomes) containing bisphosphonates (BP) formulated for effective phagocytosis. Following phagocytosis the vesicles discharge their encapsulated drug like a Trojan Horses, inactivating the cell with no effect on non-phagocytic cells.

We investigated the effect of different bisphosphonates (alendronate and clodronate), polymeric NP type, liposomal size (0.1-0.8nm), and phospholipids type on the formulation properties, and on the bioactivity in cell cultures of RAW 264 macrophages and primary SMC of rat, rabbit and human. Inhibition of blood monocytes was measured by flow cytometry (FACS) following IV injection of different formulations, and in vivo therapeutic efficacy was studied in rat and rabbit restenosis models. Monocytes count was reduced by 70%, 24-48 hrs. after a single injection of the alendronate liposomal formulation, at a dose 10-fold lower than that of clodronate. Monocyte blood levels returned to basal values after 7 days. A significant reduction of stenosis (>70%) was achieved in both rat and hypercholesterolemic-rabbit models of restenosis, without side effects.

It is concluded that a single liposomal bisphosphonate injection, concurrent with injury, reduces in-stent neointimal formation and arterial stenosis in rat and rabbits, and highlight the role of circulating monocytes in vascular injury and repair.

Pros and Cons of the Liposome Platform in Cancer Drug Targeting

Alberto A. Gabizon

Shaare Zedek MC and Hebrew University-Faculty of Medicine, Jerusalem

Incorporation in the liposome bilayer of a small fraction of polyethylene-glycol (PEG)-derivatized lipids results in inhibition of liposome uptake by the reticulo-endothelial system and significant prolongation of liposome residence time in the blood stream. Parallel developments in drug loading technology have improved the efficiency and stability of drug entrapment in liposomes, particularly with regard to cationic amphiphiles such as anthracyclines. An example of this new generation of liposomes is a formulation of PEG-coated liposomal doxorubicin known as DOXIL, whose clinical pharmacokinetic profile is characterized by slow plasma clearance and small volume of distribution. A hallmark of these long-circulating liposomal drug carriers is their enhanced accumulation in tumors. The mechanism underlying this passive targeting effect is the phenomenon known as enhanced permeability and retention (EPR) which has been described in a broad variety of experimental tumor types. It is still unclear how prevalent is the EPR phenomenon in human cancer, and particularly in metastatic cancer. At a clinical level, DOXIL confers an improved therapeutic index to doxorubicin, but this is mostly accounted for by a reduction of toxicity. Further to the passive targeting effect, the liposome drug delivery platform offers the possibility of grafting tumor-specific ligands on the liposome membrane for active targeting to tumor cells, and potentially intracellular drug delivery. Ligand-specific targeting may enhance tumor drug accumulation and reduce the toxicity of liposome-delivered drugs in comparison to passively targeted systems. The pros and cons of the liposome platform in cancer targeting will be discussed vis-à-vis non-targeted drugs and drug-ligand conjugates, using as an example a liposome drug delivery system targeted to the folate receptor.

New Methods of Dermal Drug Delivery

Amnon Sintov

Department of Pharmacology and School of Pharmacy, Ben Gurion University of the Negev, Beer Sheva

The fundamentals of a successful pharmaceutical formulation are to enable delivery of the active substance to the target organ at therapeutically relevant levels, with negligible discomfort and adverse effects to the patients. In this respect, the route of administration is of major influence in achievement of this goal. Topical and transdermal administrations offer several attractions compared to the oral and parenteral routes. Transdermal drug delivery to the systemic circulation excels itself by avoidance of hepatic first-pass metabolism, potential of long-term controlled release with avoidance of the typical peak-trough plasma profiles associated with frequent dosage regimens, ease of administration and possibility of immediate withdrawal of the treatment.

Obviously, it is not an easy task because the natural function of the skin, mainly the *stratum corneum*, is to protect the body against the loss of endogenous substances and against diffusion of exogenous pollutants/chemicals. Although the transdermal delivery of drugs and the risk prevention of dermal exposure to toxic chemicals are two different topics opposing each other, the skin's barrier function is important for both goals. The most prevalent method describing dermal absorption of small organic compounds is based on a solubility-diffusion process. Thus, the rate of dermal absorption depends on partitioning between skin and the topically applied chemical as well as the chemical's effective diffusivity through the skin barrier. Therefore, various methods and strategies have emerged to increase the partitioning/diffusivity of drugs. Some methods are based on chemical enhancers and various vehicle formulations. Other methods rely on physical techniques, such as microneedle technologies, iontophoresis, electroporation and ultrasound. Recently, various combinations of enhancing methods have been tested and some of them have indeed resulted in improved skin penetration.

Recently, we have developed several effective methods to deliver hydrophilic drugs through the skin barrier. The potential of nano-sized emulsion system ("microemulsion") was tested for the topical and the transdermal delivery of polar and ionized drugs. Although the mechanism is not thoroughly elucidated, a proper combination of the new nano-emulsion system with iontophoresis has shown to be more advantageous than iontophoresis treatment alone. Another method using propylene glycol-fatty acid conjugates has also demonstrated to provide a safe skin penetration enhancement of active molecules. In addition, prodrugs of vitamin D₃ being conjugated to polyunsaturated fatty acids have been presented as a more effective alternative to vitamin D₃-based therapy of psoriasis.

CREB/ATF Transcription Factors in Spermatogenesis and Fertilization

Rami (Jeremy) Don and Gil Stelzer

Faculty of Life Sciences, Bar-Ilan University Ramat Gan 52900, Israel.

The importance of the CREB/ATF family of transcription factors during mouse spermatogenesis is exemplified by two members of this family, CREB and CREM. Both operate at various stages of spermatogenesis, with several alternatively spliced forms each, and homologous genes were found to play similar roles in various mammals. CREB plays a crucial role in determining germ cell viability mainly by regulating expression of Sertoli-derived factors. CREM on the other hand, seems to determine haploid germ cell processes since its ablation in knockout mice causes complete arrest of spermatogenesis after completion of meiosis, preventing spermiogenesis. We have recently identified a new member of the CREB family, ATCE1, which exhibits unique cell type and spermatogenic stage specificity as well as DNA binding specificity. ATCE1 accumulates in late round and in elongating spermatids, corresponding to developmental stages considered transcriptionally silent. ATCE1 accumulation is acrosome specific, and persists up to mature epididymal cells, at which stage the protein remained associated with the inner acrosome membrane even after acrosomal reaction. ATCE1 binds NF- κ B enhancer sequences rather than the expected CRE elements and it efficiently activated transcription of a reporter gene through a directional 5 tandem repeat of the NF- κ B binding element, establishing it as a potent transcriptional activator. This raises the question of why would a transcription factor be anchored to the acrosome inner membrane? The hypothesis that ATCE1 is a paternally delivered transcription factor that is needed for zygotic genome activation will be discussed.

Balancing Between Signaling and Anti-Signaling Cascades: A Fer Play or a Sexy Trick?

Uri Nir, Orel Pasder, Sally Shpungin, Yaniv Salem, Shlomit Vilchick,
Shulamit Michaeli and Hana Malovani.

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Fer is a nuclear and cytoplasmic tyrosine kinase whose levels are increased in malignant tumors. Herein we show that Fer is required for malignant cell-cycle progression *in-vitro* and for tumor progression *in-vivo*. Decreasing the level of Fer using the RNA interference (RNAi) approach, impeded the proliferation of prostate and breast carcinoma cells and led to their arrest at the G0/G1 phase. At the molecular level, knock-down of Fer resulted in a profound hypo-phosphorylation of the retinoblastoma protein (pRB) on both CDK4 and CDK2 phosphorylation sites. De-phosphorylation of pRB was not seen upon the direct targeting of either CDK4 or CDK2 expression, and was only partially achieved by the simultaneous depletion of these two kinases. Amino acids sequence analysis revealed two protein phosphatase 1 (PP1) binding motifs in the kinase domain of Fer and the association of Fer with PP1 α *in-vivo* was verified using co-immunoprecipitation analysis. Down-regulation of Fer potentiated the de-phosphorylation of pRB by PP1 α and over-expression of Fer decreased the phosphatase activity of PP1 α . Thus, knock-down of Fer subverts redundant G1-S promoting activities and induces cell-cycle arrest in malignant cells.

Our findings portray Fer as a novel modulator of the balance between signaling and anti-signaling cascades in malignant cells. The presumed physiological significance of the interaction between the tyrosine kinase Fer and PP1 serine/threonine phosphatases in malignant and in normal cells will be discussed.

Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis

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The regulated phosphorylation of ribosomal protein (rp) S6 has attracted much attention since its discovery in 1974, yet its physiological role has remained obscure. To directly address this issue we have established viable and fertile knockin mice, whose rpS6 contains alanine substitutions at all five phosphorylatable serine residues (rpS6^{P^{-/-}}). Here we show that contrary to the widely accepted model, this mutation does not affect the translational control of TOP mRNAs. rpS6^{P^{-/-}} mouse embryo fibroblasts (MEFs) display an increased rate of protein synthesis, accelerated cell division, and they are significantly smaller than rpS6^{P^{+/+}} MEFs. This small size reflects a growth defect, rather than a byproduct of their faster cell division. Moreover, the size of rpS6^{P^{-/-}} MEFs, unlike wild type MEFs, is not further decreased upon rapamycin treatment, implying that the rpS6 is a critical downstream effector of mTOR in regulation of cell size. The small cell phenotype is not confined to embryonal cells, as it also selectively characterizes pancreatic β -cells in adult rpS6^{P^{-/-}} mice. These mice suffer from diminished level of pancreatic insulin, hypoinsulinemia and impaired glucose tolerance.

Phosphorylation Based Uncoupling of Insulin Signalling: A Molecular Basis for Insulin Resistance

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Obesity-induced insulin resistance is associated with the development of type 2 diabetes, an uprising epidemic of the 21st century. Our studies revealed that agents prevalent in obesity, such as TNF-alpha and free fatty acids, as well as chronic insulin treatment, induce Ser/Thr phosphorylation of insulin receptor substrate (IRS) proteins. Such phosphorylation inhibits complex formation between the insulin receptor the IRS proteins (IRS-1 and IRS-2) and impairs the ability of the IRS proteins to undergo insulin-stimulated Tyr phosphorylation. Failure to undergo Tyr phosphorylation impedes the ability of the IRS proteins to recruit effector molecules, such as PI3K, that further propagate insulin signalling, thus leading to the induction of an insulin resistance state. We could further demonstrate that PKC-zeta and IKK-beta function as IRS kinases, triggered by inducers of insulin resistance or by chronic insulin treatment.

To identify potential Ser residues that could undergo phosphorylation, seven Ser residues, located at putative PKC/IKK phosphorylation sites, within the P-Tyr binding (PTB) domain of IRS proteins, which is a major interaction domain with the insulin receptor, were mutated to Ala. Five Ser residues at the corresponding sites of IRS-2 were mutated as well. The mutant proteins, termed IRS-1(7A) and IRS-2(5A), underwent rapid Tyr phosphorylation in response to acute insulin treatment. However, there was no reduction in their P-Tyr content, otherwise observed upon chronic insulin treatment of wild-type IRS proteins. Similarly, IRS-1(7A) and IRS-2(5A) were protected from the action of selected inducers of insulin resistance. These findings suggest that the mutated Ser residues are targets for IRS kinases that negatively regulate IRS proteins function. As such they serve as points of convergence, where feed back control mechanisms, triggered by IRS kinases following chronic insulin treatment, overlap with IRS kinases triggered by inducers of insulin resistance that phosphorylate the very same sites and terminate insulin signalling.

Organophosphate Poisoning Induced Subcellular Alterations of Protein Kinase C Isozymes in the Rat Brain

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Organophosphate (OP) poisoning, which irreversibly inhibits cholinesterase activity, leads to severe cholinergic symptoms, producing robust convulsions and subsequent brain damage. Effective and quick management of these symptoms is considered critical to the clinical outcome. PKC signaling pathway has been associated with modulation of NMDA receptor activity, motor behavior, learning and memory, all of which are severely impaired in OP intoxication. Nevertheless, the role of PKC in OP intoxication is largely unknown. The present study characterized alterations in the immunoreactivity levels of PKC isozymes expressed in different brain areas in the rat following exposure to the nerve agent sarin (1xLD₅₀), which were markedly reduced with the treatment of the anticonvulsant midazolam (0.5 mg/kg). Also, possible neuroprotective effect of selective PKC regulating peptide after such insult was evaluated. The observations suggest a role for both conventional and atypical PKC isozymes in OP-induced neuropathy in the rat and further support their involvement in cell death.

Alternative splicing as a neuroprotection strategy

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Mammalian stress responses and neurodegenerative diseases involve numerous neurotransmission pathways. Because of the modulatory role of acetylcholine, we study the potential contributions of acetylcholinesterase (AChE) to these processes. The *ACHE* gene is subject to stress-induced modifications in promoter usage and alternative splicing.^{1,2} Human genotype-phenotype analyses further point to alternative splicing of AChE mRNA as causally associated with anxiety and stress-associated disease risk. Specifically, polymorphism(s) in the adjacent *PON1* and *ACHE* genes interactively affect the expression levels of AChE splice variants and reflect the variations in human anxiety parameters.³ Polymorphisms which debilitate PON activity constitutively impair AChE over-production under anticholinesterase exposure and were jointly over-represented in Parkinsonian patients from agriculturally exposed areas in Israel.⁴ In transgenic mice, failure to adapt the alternative splicing configuration of AChE mRNA splice variants to a changing environment impairs brain diffusion and blood-brain barrier functioning.⁵ Moreover, while exposure to the neurotoxin MPTP notably destroys dopaminergic neurons in mice, transgenic mice representing robust splicing shift of AChE mRNA showed neuroprotection. Microarray analyses of neuronal gene expression, using both discrete and continuous bioinformatics approaches,⁶ point at an organismal capacity to confront damaging insults by re-adjusting the spliceosomal configuration as a neuroprotection strategy. Our findings demonstrate the relevance of this concept for AChE gene expression and suggest that the capacity for neuroprotective alternative splicing may be exhausted once a threshold of multiple insults has been surpassed.

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T-cell-based vaccination for maintenance of plasticity, neurogenesis, and repair in the CNS in health and disease

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The brain is now known to be endowed with morphological and functional plasticity throughout life, which however cannot compensate for losses resulting from acute insult, chronic degeneration, or aging. Our studies over the last few years have shown that the brain needs its resident immune cells (microglia), assisted by the peripheral immune system (PNS), for its maintenance, repair, and renewal. Having the microglia without the PNS can be likened to having an engine without an on/off switch. We discovered that the PNS, in the form of T cells directed against central nervous system (CNS) self-antigens residing in damaged CNS sites, is needed for dialog with the resident microglia, yielding cytokines and growth factors vital for fighting off cytotoxic agents, supporting neuronal survival, and promoting neurogenesis and oligodendrogenesis from endogenous or exogenous stem cells. This unexpected finding shows that T cells in the periphery are essential for brain plasticity in health and disease. It also explains why aging of the immune system affects the brain, and suggests new ways to bridge disease-related or age-related gaps between the potential for plasticity and the actual compensatory ability of the brain. Development of a T cell-based vaccination for the aging brain and for brains threatened by acute chemical or mechanical insults or by degenerative diseases is discussed.

The Basal Ganglia and the Pathophysiology of Parkinson's disease

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Early physiological studies have reported changes in the activity of neurons in the subthalamic nucleus (STN) and in the internal and external segments of the globus pallidus (GPi and GPe) in Parkinsonian MPTP-treated monkeys compared to healthy ones. Firing rates in the GPe are reduced, whereas in GPi and STN cells display increased firing rates. Subsequent findings showed that inactivation of STN and GPi can improve the motor symptoms in Parkinsonian animals and human patients. Finally, mounting evidence has demonstrated reversed trends of pallidal discharge rates in response to dopamine replacement therapy (DRT) in both human patients and primates.

Nevertheless, recent works have challenged the basic tenets of the basal ganglia rate model. Several primate studies have failed to find the expected significant changes of firing rates in MPTP monkeys. Similarly, biochemical and metabolic studies indicate the GPe activity does not change in Parkinsonism. Whereas the rate model strongly predicts that the enhanced GPi inhibitory output in Parkinsonism should reduce thalamic and motor cortex firing rates, several works in dopamine-depleted primates have shown no change in spontaneous thalamic and motor cortical firing rates. Finally, the rate model fails to explain the success of pallidal and thalamic inactivation in the treatment of dyskinesia.

The inconsistencies with the rate model have put more emphasis on the potential role of other aspects of neuronal activity such as firing patterns and neuronal synchronization, in the pathophysiology of Parkinson's disease (PD). Recent studies have shown increase in both oscillatory activity and in neuronal correlation of pallidal cells in MPTP primates and Parkinsonian patients. This increase in pallidal synchronization has been shown to decrease in response to dopamine replacement treatment. However recent human studies have found oscillatory neuronal correlation only in tremulous patients and raised the question whether the increased neuronal synchronization in Parkinsonism is not merely an epi-phenomenon of the tremor or of independent oscillators with similar frequency.

Human studies are limited by constraints related to recording duration, selected anatomical targets and clinical state of the patients (e.g most patients are after many years of DRT and have already developed dyskinesia). We therefore investigated the role of oscillatory activity and of neuronal correlation throughout the different clinical states of PD in the MPTP primate models of this disease. The tremulous vervet monkey and the rigid-akinetic rhesus monkey were selected to imitate tremulous and non-tremulous subtypes of human patients. We combined multi-electrode recordings with a newly improved tool for spectral analysis of both single cells discharge and interneuron relations and distinguished between neuronal correlations of oscillatory nature and non-oscillatory correlations. We found that a major fraction of pallidal cells develop both oscillatory and non-oscillatory synchronization, following the induction of PD, and that this abnormal synchronized activity can't be solely attributed to the tremor phenomena. We therefore suggest that abnormal synchronization of basal ganglia activity play a major role in the pathophysiology of PD.

Functional properties of human embryonic stem cells-derived cardiomyocytes

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Since cardiac transplantation is limited by the small availability of donor organs, regeneration of the diseased myocardium by cell transplantation is an attractive therapeutic modality. To determine the compatibility of human embryonic stem cells-derived cardiomyocytes (7-55 days old) with the myocardium, we investigated their functional properties respecting intracellular Ca^{2+} handling and the role of the sarcoplasmic reticulum in the contraction. The functional properties of hESC-CM were investigated by recording simultaneously $[\text{Ca}^{2+}]_i$ transients and contraction. Additionally, we performed Western blot analysis of the Ca^{2+} -handling proteins SERCA2, calsequestrin, phospholamban and $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX). Our major findings are: (1) hESC-CM displayed temporally-related $[\text{Ca}^{2+}]_i$ transients and contraction, negative force-frequency relations and lack of post-rest potentiation. (2) Ryanodine, thapsigargin and caffeine did not affect the $[\text{Ca}^{2+}]_i$ transient and contraction, indicating that at this developmental stage, contraction depends on transsarcolemmal Ca^{2+} influx rather than on sarcoplasmic reticulum Ca^{2+} release. (3) In agreement with the notion that a voltage-dependent Ca^{2+} current is present in hESC-CM and to the mechanical function, verapamil completely blocked contraction. (4) While hESC-CM expressed SERCA2 and NCX at levels comparable to those of the adult porcine myocardium, calsequestrin and phospholamban were not expressed. Our study shows for the first time that functional properties of hESC-CM differ markedly from mature myocardium, mainly due to non-functional sarcoplasmic reticulum function.

Heart failure, neurohormonal activation, and new pharmacological therapies

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Vasoconstrictor neuro-humoral systems play a key role in the pathophysiology of congestive heart failure (CHF), a growing medical problem in the western world with significant morbidity and mortality. Understanding the pathophysiologic mechanisms of CHF and its complications is therefore imperative for the development of new therapies to improve the outcome of the disease. While the role of the renin angiotensin aldosterone system (RAAS) and the sympathetic nervous system (SNS) in the pathogenesis of CHF is well established and angiotensin-converting enzyme and β -blockers are central to the treatment of patients with CHF, the contribution of the endothelin (ET) system and antidiuretic hormone (ADH) has not been thoroughly studied. Endothelin and ADH systems are concomitantly activated in CHF. Although it is well documented that ET and ADH play essential role in the development of vasoconstriction and hyponatremia in advanced CHF, respectively, their relative contribution to the cardiovascular and renal dysfunction in CHF in earlier stages of the disease is less understood compared with the other vasoconstrictor systems. In part, this was due to the lack of effective pharmacologic blockers of ADH. The recent development of highly selective non-peptide ET and ADH-receptor antagonists provided the scientific and clinical communities with the opportunity to evaluate the role of ET and ADH in CHF. The biological effects of endothelin-1 on its target organs are mediated by two receptors: ETA and ETB. It is widely accepted that the vascular, cardiac, and renal adverse effects of ET-1 are mediated by ETA, while activation of ETB receptors leads to beneficial effects such as: attenuating the vascular and cardiac hypertrophic effects of ET-1 as well as the vasodilatory action of this peptide. Although several experimental and clinical studies have revealed that ET-1 antagonists are clinically beneficial therapeutic agents for the treatment of several cardiovascular diseases, including CHF, recent studies demonstrated no benefits in patients treated with bosentan (ETA/ETB antagonist). Actually the mortality rate was even higher in patients treated with bosentan compared with those treated with placebo.

Several ADH antagonists have been utilized in experimental and clinical CHF, and were found to produce hemodynamic improvement with transient decrease in systemic vascular resistance, increase cardiac output, and to improve water diuresis. Thus novel non-peptide ADH antagonists may have important therapeutic implications, given that mortality in CHF remains high despite the use of effective ACE blockers and β -receptor antagonists.

Heritable Cardiomyopathies in Heart Failure Clinics

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Familial cardiomyopathies account for ~ 30% of idiopathic dilated cardiomyopathy (DCM) in clinical practice and for ~ 50% of referrals to cardiac transplantation. Diversity of clinical presentations and variability in penetrance lead to under-recognition of this entity as an inherited disorder. Appreciation of DCM as an inherited disease might avoid unnecessary diagnostic effort, thereby directing appropriate attention to the timely detection of sub-clinically affected family members.

Our Heart Failure Unit opened a clinic dedicated to cardiomyopathies and other inherited heart diseases. Our main objective is to identify familial heart disease among patients presenting with heart failure, provide genetic counseling, and conduct genetic analyses of the molecular causes of inherited heart diseases.

During 2004 we investigated 20 families with a high prevalence of heart failure of non-ischemic etiology (2-12 affected/family). Ninety percent of the index cases were either treated in the advanced heart failure clinic or referred for heart transplantation. Among them, 16 families had familial DCM, 1 family had restrictive cardiomyopathy and 3 families had hypertrophic cardiomyopathy with either a high prevalence of early death or progression to the "burned-out" stage. When kindred size allowed us to determine the inheritance pattern, it appeared to be autosomal dominant.

Clinical evaluation of an Arab clan with familial DCM, (carried out as part of an effort to identify the disease gene locus) identified 3 asymptomatic subjects showing early echocardiographic signs of cardiac dysfunction. Genetic analysis of familial hypertrophic cardiomyopathy with a malignant phenotype, identified a β -myosin heavy chain Arg719Trp mutation in a heart transplant recipient and in his 17 year old daughter.

Clinical management of the patient and his family requires understanding disease heritability and will benefit from establishing molecular diagnosis and providing genetic (and prenatal) counseling. Introducing preventive therapy in asymptomatic family members showing early signs of cardiac dysfunction, will improve heart failure management in Israel.

Induction of angiogenesis by genetically modified venous endothelial and smooth muscle cells

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Angiogenesis and arteriogenesis are complex processes that involve multiple genes, transcription factors and cells. The process involves growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor, angiopoietins and inflammatory cytokines, as well as cells such as endothelial cells, smooth muscle cells and macrophages. Angiogenesis occurs in healthy adults as part of healing wounds, restoring blood flow to tissues after injury, or in women during the monthly reproductive cycle. When blood flow to the heart or peripheral organs is impaired, the body responds by angiogenesis and arteriogenesis, forming collateral blood vessels that bypass the site of arterial narrowing or occlusion. In many serious disease states the body's natural neo-vascularization mechanism is insufficient; the blood vessels that develop are inadequate and circulation is not properly restored, leading to tissue damage or death.

Our group developed an integrated cell and gene therapy approach to achieve therapeutic angiogenesis. We employ autologous venous endothelial and smooth muscle cells to induce angiogenesis. The cells are activated by VEGF₁₆₅ and Angiopoietin-1 gene transfer, and are injected intra-arterially. We tested our integrated approach in a rabbit model of hind limb ischemia. Superficial femoral artery occlusion was induced surgically and the activated autologous cells were injected intra-arterially to the collateral dependent circulation feeding the femoral artery distally to the occlusion site.

Intra-arterial injection of genetically modified endothelial cells expressing Ang-1 and smooth muscle cells expressing VEGF₁₆₅ was shown to be efficacious in a hind limb ischemia model of both miniature pigs and rabbits. Blood flow and tissue perfusion to the ischemic limb were significantly increased when compared to the control animals. The increased flow and perfusion were measured by Doppler-based techniques and newly remodeled arteries were demonstrated using multislice CT angiography.

PRO-APOPTOTIC BID IS AN ATM EFFECTOR IN THE DNA DAMAGE RESPONSE

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The “BH3-only” pro-apoptotic BCL-2 family members are sentinels of intracellular damage. We have recently demonstrated that the BH3-only BID protein partially localizes to the nucleus in healthy cells, is important for apoptosis induced by DNA damage, and is phosphorylated following induction of double-strand breaks in DNA. We also found that BID phosphorylation is mediated by the ATM kinase, and occurs in mouse BID on two ATM consensus sites. Interestingly, *BID*^{-/-} cells failed to accumulate in the S phase of the cell cycle following treatment with the topoisomerase II poison etoposide; reintroducing wild-type BID restored accumulation. In contrast, introducing a non-phosphorylatable BID mutant did not restore accumulation in the S phase, and resulted in an increase in cellular sensitivity to etoposide-induced apoptosis. These results implicate BID as an ATM effector, and raise the possibility that pro-apoptotic BID may also play a pro-survival role important for S phase arrest.

A role for PML in the regulation of p53 by CK1

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The p53 protein is critical for the suppression of cancer. P53 integrates stress signals into growth restrictive cellular responses. As a determinant of cell fate, p53 has to be activated properly, efficiently and temporally. The p53 protein is kept labile under normal conditions, which is largely governed by its major negative regulator, Mdm2. In response to stress however, p53 accumulates and becomes activated. For this to occur, the inhibitory effects of Mdm2 have to be neutralized. Several mechanisms have been demonstrated to explain the protection of p53 from Mdm2 upon exposure of cell to stress, such as DNA damage. A dominant mechanism involves post-translational modifications of both p53 and Mdm2. Of special importance are modifications that modulate the p53/Mdm2 interaction. We have previously described the effect of Ser20 phosphorylation on the protection of p53 from Mdm2. This modification is enhanced by the action of PML, which recruits both p53 and Chk2 into the PML nuclear bodies in response to DNA damage. We found that PML is critical for the accumulation of p53 in response to DNA damage under physiological conditions. PML protects p53 from Mdm2-mediated ubiquitination and degradation and from inhibition of apoptosis. Phosphorylation of p53 on a nearby site, Thr18, has been proposed to be even more critical in the modulation of the p53/Mdm2 interaction. We therefore examined whether PML regulates this modification in response to stress. We report here that PML enhances the phosphorylation of p53 by CK1, and this phosphorylation is important for the protection of p53 by PML in cells exposed to DNA damage. We will describe the interplay between PML and CK1 in the regulation of p53 in response to stress.

INDUCTION OF CANCER CELL-SPECIFIC APOPTOSIS BY THE ADENOVIRUS E4orf4 PROTEIN

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Studies in mammalian tissue culture cells indicate that the adenovirus E4orf4 protein induces a non-classical (caspase-independent), cancer cell-specific apoptosis, which requires an interaction between the viral protein and an active protein phosphatase 2A (PP2A). Our laboratory is using various genetic model systems to investigate the mechanisms underlying induction of apoptosis by E4orf4. In *S. cerevisiae*, we showed that E4orf4 induces PP2A-dependent mitotic arrest and functionally interacts with cell cycle regulators. A genetic screen in yeast revealed an additional E4orf4-associating protein, Ynd1, which is required for E4orf4-induced toxicity. Ynd1 genetically interacts with PP2A, and epistasis analysis indicates that Ynd1 and PP2A share a common downstream target. This target is likely to be essential for E4orf4-induced growth arrest, and experiments to identify it are under way. A mammalian homologue of Ynd1 interacts with E4orf4 in human cells and its contribution to E4orf4-induced apoptosis is under investigation. We also utilize *Drosophila melanogaster* as a model to investigate E4orf4 function in the context of a whole multicellular organism. Experiments testing the sensitivity of *Drosophila* cancer cells to E4orf4-induced cell death are underway and will be discussed.

Pro-Apoptotic-Proteins As Novel Therapeutic Tools For Targeted Human Therapy

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In recent years chimeric proteins have been developed by gene fusion techniques, to selectively recognize and kill cell populations expressing specific surface molecules.

Generally these molecules carry a bacterial or plant toxin as their killing moiety. Specificity is then added to the toxin by fusion, at the DNA level, with protein-recognition elements, which can direct the chimera to the selected target cells. Immunogenicity and non-specific toxicity of bacterial or plant toxins remain as the most serious withdrawn in the clinical application of those proteins.

In our effort to overcome the above-mentioned shortcomings, we developed a new prototype of chimeric proteins, taking advantage of apoptosis-inducing proteins of the Bcl-2 family, DNases and Caspases as novel human cell killing components. A number of new chimeric proteins were constructed using different target moieties (Fce for targeting mast cells, GnRH for targeting adenocarcinoma cells and IL-2 for targeting activated T-cells). The new chimeric proteins were expressed in *E. coli* and two of them were highly purified.

Treatment with the newly developed chimeric proteins increased the population of apoptotic cells in their specified target cell lines. The different chimeric proteins were highly specific in their activity, as they showed no effect on cells not carrying the targeted receptor. We have also shown that the ability of the novel chimeric proteins to affect the intracellular apoptotic machinery within the target cells, and to force the cells to die, is similar to that induced following a normal apoptotic signal. Moreover, preliminary *in vivo* results with one of the new chimeric proteins showed that treatment of colon adenocarcinoma xenografts in nude mice caused a reduction in tumor weight. Apoptosis-inducing chimeric proteins are novel potential therapeutic tools for human targeted therapy.

BioLineRx: a drug development company advancing a first in class antipsychotic for the treatment for schizophrenia

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BioLineRx founded in 2003 by leaders in the Israeli life-sciences sector, BioLineRx is building a robust pipeline of promising therapeutics for unmet medical needs. BioLineRx is focused on advancing projects from early stage discovery and lead generation to advanced clinical trials, regulatory approval, and marketing. Partnering with researchers, universities and biotech companies to further the commercialization of promising compounds, BioLineRx enriches the pipeline of large pharmaceutical companies seeking their next blockbuster drugs.

BL-1020 is a novel antipsychotic drug consisting of a typical antipsychotic drug covalently bonded to GABA, which transports GABA across the BBB and releases free exogenous GABA in the brain. It is designed to target simultaneously the overactivity of dopamine and the hypoactivity of γ -aminobutyric acid (GABA) that have been implicated in schizophrenia. In addition, it targets the GABA deficiency that has been described in antipsychotic-induced extrapyramidal symptoms (EPS). Pharmacokinetic studies demonstrate that BL-1020 provides effective transport of GABA to the CNS, a therapeutic approach that has been challenging due to the inability of exogenously administered GABA to cross the blood-brain barrier (BBB). In animal models of schizophrenia, orally administered BL-1020 shows significant antipsychotic efficacy with minimal induction of EPS. BL-1020 presents a promising first-in-class molecule, targeting the dopamine and GABA pathways for the treatment of schizophrenia. Its therapeutic effect provides additional support for the role of GABA hypoactivity in schizophrenia and in the development of EPS.

ROLE OF IL-6 SIGNALING IN DIFFERENTIATION OF NERVE MYELINATING CELLS: POTENTIAL FOR TREATMENT OF NEUROPATHIES AND FOR TRANSPLANTATION OF ES CELL-DERIVED OLIGODENDROCYTES.

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Myelin sheaths around axons are not only necessary for neural conduction but also protect axonal integrity. Myelin is made by membranes of oligodendrocytes in the brain and spinal cord, and of Schwann cells [SC] in peripheral nerves. We found that a potent agonist of the IL-6 receptor system (IL6RIL6) promotes differentiation of myelinating Schwann cells from embryonic dorsal root ganglia (DRG) precursor cells. The cytokine activates the transcription activity of myelin genes, in particular the Myelin Protein Zero (Po) gene, and we identified several promoter elements and protein ligands involved. In particular, we found that Zinc Binding Protein ZBP-99 binds a CACC-box element, forms a complex with transcription factor Sox10 and plays an essential role in the activity of the Po gene promoter. Knock-down of ZBP-99 expression by siRNA leads to a loss of the myelin Po protein from Schwann cells. The importance of IL-6 signaling for myelination was demonstrated in vivo. Thus, protection of neuronal functions and of myelin integrity by IL-6 injections was documented in several rat models of Peripheral Neuropathies, caused by diabetes or secondary to chemotherapy. Furthermore, IL6RIL6 was found to enhance markedly the development and the maturation of myelinating oligodendrocytes from pluripotent Embryonic Stem (ES) cell lines. In particular, IL6RIL6 causes the expansion of the network of branches and the formation of myelin membranes where Myelin basic protein (MBP) accumulates. By gene expression profiling, we identified Stathmin-like 2 (SCG10, a regulator of microtubule dynamics) as induced by IL6RIL6 in oligodendrocytes and its knock-down shows that it is required for the development of the branches network. The capacity of transplanted oligodendrocyte precursors to myelinate brain tissue was much higher following IL6RIL6 treatment. Remyelination in brain slices organotypic cultures from the dysmyelinated shiverer mouse (lacking the MBP gene) was recently obtained by transplanting oligodendrocyte precursors similarly differentiated from human ES cell lines (in collaboration with J. Itskovitz-Eldor and M. Amit, Technion). The protocols for ES cell differentiation with the use of IL6RIL6 or IL-6, holds promises for transplantation into brain and spinal cord to promote in situ remyelination and nerve repair.

Abstracts of Student Lecture Competition

**The Proteomatrix, a novel tool for cellular protein expression profiling:
application to proteins involved in resistance to chemotherapy**

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We have developed the Proteomatrix, a tool for identifying the protein profile of cells. The system is an ELISA-based matrix assay that identifies and quantitates a specific subset of the proteins expressed in any biological sample. The principle of the Proteomatrix is to produce a complete proteolytic digest of the sample and, using the human genome database, to design “*in silico*” a proteolysis peptide map for any protein of interest. Peptides that are likely to be unique and specific can then be selected by appropriate use of an existing database, synthesized, and antibodies against them prepared. The peptide-antibody pairs are used in a competition ELISA or other immunoassay to identify and quantitate these proteins in a proteolytic digest of the tissue sample

As an initial application of the system, three MDR (multidrug resistance)- related proteins were selected as targets: Pgp170, the first MDR protein; the multidrug resistance-associated protein, MRP; and the mitoxantrone resistance protein, MXR. An additional target protein, Na/K ATPase, present in almost every animal cell, is used as “load control”. *In silico* peptides from the above proteins were checked by BLAST for uniqueness, synthesized, and antibodies against them prepared. Optimization steps involved finding the optimal conditions for the competition between attached and free peptides, calibration of the amount of attached peptide and the concentration of first and second antibodies, and the time of reaction. The specificity of the reaction was confirmed using different, unrelated peptides in the competition reaction and using preimmune serum.

The presence of MDR, MRP and MXR proteins was assessed in several drug-resistant cultured cell lines and on cells obtained from leukemia patients, and the data normalized to the Na/K ATPase control. Our values compare well to those obtained by independent assays (western blotting, FACS using anti-whole protein antibodies and Rhodamine efflux, and Pgp ATPase activity assay), showing a high degree of correlation between the values obtained by the Proteomatrix and other assays. The Proteomatrix provides a rapid (3-4 hours) and quantitative means of determining the level of expression of any set of proteins in cell lines or cell samples from patients. No dedicated instrumentation is required beyond that in any laboratory. Since we compete against known amounts of synthetic peptide, we can accurately quantify the amount of the protein in our sample.

Neuroprotective Anti-Apoptosis Effect Of Hyperbaric Oxygen Treatment In Secondary Brain Damage

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Traumatic brain injury (TBI) is a major health problem in all developed countries, with cerebral contusions been the most common consequence of TBI. Recent evidence has clearly demonstrated, that TBI, may give rise to the development of the delayed secondary brain damage and that the apoptotic cell death is involved in the secondary brain damage.

The goal of the present study is to evaluate the expression of apoptosis-related proteins of bcl-2 family (bcl-2, bcl-xL and bax) in the traumatic penumbra area in correlation with the extent of apoptosis in the rat model of dynamic cortical deformation (DCD), treated by HBOT. four groups of 5 Sprague-Dawley rats each were included in this study. The study protocol was as follows: group 1-DCD, group 2-DCD and HBOT; group 3-DCD and perioperative hypoxia ; group 4-DCD, perioperative hypoxia and HBOT. The bcl-2 family of proto-oncogenes was revealed by Immunohistochemical staining for bcl-2, bcl-xL and bax. The expression of bcl-2 in the penumbra area was lower in the animals, which underwent hypoxemia before the treatment, than in non-hypoxemic rats. The decrease in the expression of bcl-2 includes both the intensity of staining and its extent (the area). After the HBOT we observed statistically significant increase in the intensity and the extent of bcl-2 expression in both groups of animals (hypoxemic and non-hypoxemic) with hypoxemic animals showing still lower expression, but the difference was not significant.

The changes in the expression of bcl-xL were generally parallel to those of bcl-2, but differences between the groups were not statistically significant.

Bax protein expression, increase insignificantly after posttraumatic hypoxemia. After the HBOT there was some decrease in bax staining intensity and extent, but the measurement revealed marked variability of staining pattern and the differences between the groups were statistically significant ($p>0.1$).

Our results provide more evidence of the importance of apoptotic mechanisms in delayed cell death in traumatic penumbra area of brain injury. We also demonstrate the role of posttraumatic hypoxemia in aggravation of apoptosis, probably through the decrease of bcl-2 and bcl-xL expression, which normally repress apoptosis. We suppose, that the beneficial effect of HBOT in traumatic brain injury may have the same mechanism, as in the brain ischemia and that the important deal of this effect is related to increased antiapoptotic proteins expression following the treatment, with the appropriate decrease in the extent of apoptosis.

Specific Pkc Isoforms Determine The Fate Of multipotential Neural Precursor Cells

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Multipotential neural precursor cells (MNPC) exhibit self-renewal potential and are capable of differentiating along different lineages of the CNS. In this study we explored the role of specific protein kinase C (PKC) isoforms in the differentiation of MNPC. MNP neurospheres expressed PKC α , β 2, δ , ϵ , ζ and μ and their differentiation was followed by increased expression of PKC β 2 and PKC ζ , a slower electrophoretic mobility of PKC α and PKC ϵ and an increased phosphorylation of PKC ϵ on serine 729. Overexpression of PKC γ selectively increased the differentiation and migration of neurons from the neurospheres and a PKC γ KD mutant inhibited neuronal differentiation induced by BMP4. In contrast, overexpression of PKC ϵ selectively increased the generation of GFAP⁺ cells, whereas PKC ϵ KD mutant increased the generation of oligodendrocytes. The phosphorylation of serine 729 was essential for the effect of PKC ϵ and a PKC ϵ S729A mutant inhibited the astrocytic differentiation of MNPC. PKC α and PKC δ did not play a significant role in the proliferation or differentiation of MNPCs. Our results implicate PKC as a major signaling pathway in the function of MNPCs and suggest that different PKC isoforms can selectively direct the fate of MNPCs and affect the generation of neurons, astrocytes and oligodendrocytes from multipotential precursor cells.

UTP PROTECTS CARDIOMYOCYTES FROM HYPOXIC STRESS VIA ACTIVATION OF P2Y RECEPTORS

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Extracellular pyrimidine and purine nucleotides are released from the heart during hypoxia and activate P2 purinoceptors, classified as P2X or P2Y. P2X receptors are ligand-gated intrinsic ion channels, and P2Y receptors are G protein-coupled receptors. The aim of this study is to investigate the role of UTP in protecting cardiomyocytes subjected to hypoxia, as well as, the involvement of P2Y receptors in the protection signaling pathway. This study reveals that UTP, but not UDP or uridine, induced early and late preconditioning effect on rat neonatal cardiomyocytes. The presence of UTP receptors, P2Y₄ and P2Y₂, in cardiomyocytes was demonstrated by immuno-fluorescence staining and Western immuno-blot assay.

UTP cardioprotective effect was reduced in the presence of the P2 antagonist suramin. In addition, UTP caused a transient increase of $[Ca^{2+}]_i$ in cardiomyocytes. PPADS or RB-2, other antagonists of P2 receptors, abolished the $[Ca^{2+}]_i$ elevation caused by UTP. We used various inhibitors of the Ca^{2+} signaling pathway to show that UTP elevates levels of $[Ca^{2+}]_i$, originating from intracellular sources, via activation of PLC and the IP₃ receptor. Interestingly, these inhibitors of the Ca^{2+} signaling pathway did not prevent the immediate protective effect caused by UTP. Although mK_{ATP} channels are involved in other preconditioning mediator pathways, the involvement of these channels in the cardioprotective effect induced by UTP was ruled out, because 5-HD, a specific inhibitor of these channels, does not prevent protection.

In conclusion, UTP nucleotide protects cardiomyocytes against hypoxic damage via nucleotide receptor(s). Although UTP caused a transient increase in $[Ca^{2+}]_i$ level in cardiomyocytes, the protection obtained by UTP was Ca^{2+} independent.

KCNQ1 long QT mutations impair calmodulin binding and channel assembly and stabilize inactivation gating

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Voltage-gated potassium channels are key regulators of cellular excitability. The slow I_{KS} K^+ channel plays a major role in repolarizing the cardiac action potential and consists of the assembly of KCNQ1 and KCNE1 α and β subunits, respectively. Mutations in either KCNQ1 or KCNE1 genes produce the long QT syndrome, which increases the risk of life-threatening ventricular arrhythmia. Here we show that long QT mutations located in the proximal KCNQ1 C-terminus impair the binding of calmodulin. These mutations dramatically decrease the current density and stabilize voltage-dependent inactivation gating. Generation of a soluble KCNQ1 C-terminus necessitates coexpression of calmodulin. Compared to wild type KCNQ1 and I_{KS} , the R366W mutant current is more sensitive to the disruptive effect of the calmodulin antagonist W7 and to the stimulating action of internal Ca^{2+} . KCNE1 forms a ternary complex with KCNQ1 and Ca^{2+} -CaM and could prevent the voltage-dependent inactivation in several mutants. These data suggest that proper calmodulin binding to KCNQ1 C-terminus is necessary for correct channel assembly and for triggering a gating module that boosts Ca^{2+} -dependent channel activity and prevents inactivation gating.

ABSTRACTS OF POSTERS

The Role Of Adenosine A₁ & A₃ Receptor Activation During Ischemia Reperfusion (I/R) In Normal And Hypertrophied Heart

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Background and objectives: The increased susceptibility of hypertrophied hearts to ischemic injury has long been recognized. The purpose of this study is to investigate the effects of pharmacological preconditioning (PC) with adenosine A₁ and A₃ receptors activation on the recovery of the isolated myocardium after cardioplegic ischemia and the role of p38 MAPK. Two different modes of PC in WKY and SHR hearts were studied: In the perfusion mode (P), isolated rat hearts were perfused with A₁ receptor agonist (CCPA) or A₃ CI-IB-MECA (10nM) for 20 minutes, followed by 30 minutes of warm cardioplegic ischemia and 30 minutes of reperfusion. In the injection mode (I) CCPA or CI-IB-MECA (100 µg/kg), were administered 24 hours before the experiment. Phosphorylated p38 MAPK was examined using Western blot analysis.

Results: CCPA improved recovery of the hearts in both modes of treatment in terms of mechanical left ventricular developed pressure (LVP) (p<0.0005), ATP levels (p<0.05), and infarct size. CI-IBMECA was partially beneficial (see table). Both agonists mediated activation p38 MAPK in both modes of treatment. This protection was completely abolished by prior treatment with their antagonists, which had no effect on its own.

Conclusions: CCPA in both modes of treatment and CI-IB-MECA especially in the injected mode were beneficial in protecting the perfused isolated rat heart subjected to normothermic cardioplegic ischemia (1st and 2nd window of PC). This protection was partially related to the increased phosphorylation of p38 MAPK before and during ischemia.

The recovery of LVP (%) and ATP (nmole/mg protein) after at 30 min post I/R:

	LVP WKY	LVP SHR	ATP WKY	ATP SHR
Control	57.6± 4.1	38.5±3.7	3.1±0.5	3.3±1.1
CCPA (P)	78.3±2.9*	60.1±5.9*	15.4±0.1*	12.4±0.3*
CCPA (I)	75.2±4.0*	64.2±8.1*	15.8±0.2*	14.9±2.8*
CI-IBMECA (P)	55.6±3.3*	40.9±6.2*	13.2±0.1*	11.1±1.6*
CI-IBMECA (I)	77.7±3.4*	61±5.7*	15.7±1.5*	15.7±1.2*

Real-time Activation of Avian Heat Shock Factors

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Living organisms respond to heat exposure by selectively expressing heat shock proteins (HSPs). Transcriptional induction of heat shock genes in response to temperature elevation and other stresses is mediated by heat shock transcription factors (HSFs). Avian cells express two redundant heat-shock responsive factors, HSF1 and HSF3, which differ in their activation kinetics and threshold induction temperature. Unlike the ubiquitous activation of HSF1, the DNA-binding activity of HSF3 has been reported only from undifferentiated cells and embryonic tissues.

Recently, we have shown by intraspecific, real time, *in vivo* measurements of genetically diverse fowl strains that thermoresistance in endotherms is characterized by a delayed activation of HSF. Herein we report on a non-redundant and tissue-specific activity of avian HSF1 and HSF3 *in vivo*, and discuss the physiological and developmental significance of this phenomenon.

Involvement Of Na⁺, K⁺-Atpase And Endogenous Digitalis-Like Compounds In Depressive Disorders

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The sodium and potassium-activated adenosine triphosphatase (Na⁺, K⁺-ATPase) is an enzyme, present in the plasma membrane of all mammalian cells. The enzyme hydrolyzes ATP and uses the free energy to drive the transport of potassium into the cell and sodium out of the cell, against their electrochemical gradients. Na⁺, K⁺-ATPase is the major determinant of cytoplasmic Na⁺. Digitalis-like compounds (DLC) are 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 Na⁺, K⁺-ATPase. Numerous studies have shown that Na⁺, K⁺-ATPase activity is reduced in major depressed and bipolar mood disorder 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. In the present study, this hypothesis was examined by the determination of Na⁺, K⁺-ATPase/DLC system in parietal cortex of patients with mood disorders (depressed, bipolar), another mental disease (schizophrenia) and normal individuals and in Forced Swimming Test, an animal model of depression. ³H-ouabain binding to synaptosomes prepared from brains of bipolar patients was significantly lower than in those from major depressed and schizophrenic patients. Three α isoforms (α 1, α 2, and α 3) of Na⁺, K⁺-ATPase, quantified by western blot, were not significantly different in the four groups studied. DLC levels, determined using specific and sensitive ELISA, were significantly higher in the brain of bipolar mood disorder patients than in normal individuals and major depressed patients. Injection of ouabain (0.8 pg, i.c.v) elicited anti-depressant behavior manifested in decreased immobility and increased swimming periods. Surprisingly, injection of ouabain-antibodies (50 μ g, i.c.v.) also resulted in anti-depressant behavior as seen by decreased immobility and increased swimming and climbing periods. These results are in accord with the hypothesis that Na⁺, K⁺-ATPase/DLC system is involved in depressive disorders.

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Cardiac Protection by 3 Weeks Swimming Exercise Training Prior to AMI: Echocardiographic Assessment of a Rat Model.

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Exercise training prior to acute myocardial infarction (AMI) can attenuate cardiac remodeling by reducing abnormal left ventricular (LV) hypertrophy, LV free wall thinning and dilatation, thus leading to preservation of LV dimensions, stroke volume and cardiac output. **Objective:** To investigate the primary “protective effect” of 3 weeks (3w) swimming exercise training on cardiac morphology and function using echocardiography. **Methods:** SD rats (n=48) underwent 3w of swimming exercise training (Ex) (90 min, 5 days/wk), or remained sedentary (Sed). At the end of the training period, all rats were subjected to AMI induced by surgical ligation of the left coronary artery. Following closure of the chest, rats were allowed a 4-week (4w) recovery period with no exercise training (in both groups). Trans-thoracic echocardiography was performed in each group at the end of the exercise/sedentary period (Pre-AMI), 24 hours (24h) after AMI (24h-Post-AMI) and following 4w of a recovery period (4w-Post-AMI).

	Pre-AMI		24h-Post-AMI		4w-Post-AMI	
	Ex	Sed	Ex	Sed	Ex	Sed
LVEDD	0.76 ±0.05	0.75 ±0.10	0.78 ±0.06	0.80 ±0.04	0.90 ±0.12 ^{³³ψψ}	0.95 ±0.06 ^{³³ψψ}
LVEDS	0.40 ±0.07	0.41 ±0.11 ^Δ	0.48 ±0.07 [#]	0.61 ±0.07 ^{*###}	0.56 ±0.07 ^{³ψ}	0.74 ±0.10 ^{*³³ψψ}
SF(%)	47.2 ±6.1	45.7 ±9.03 ^Δ	38.4 ±12.5 [#]	23.7 ±7.4 ^{*###}	39.11 ±12.5 ^ψ	22.6 ±7.9 ^{*³ψψ}
FAC%	57.8 ±4.3	56.0 ±7.3	46.9 ±11.7 ^{##}	38.3 ±12.4 [#]	48.1 ±14.6 ^ψ	37.6 ±8.8 ^{ψψ}
LVEDA	0.48 ±0.04	0.42 ±0.08	0.48 ±0.07	0.48 ±0.04	0.65 ±0.13 ^{³³ψψ}	0.75 ±0.10 ^{³ψψ}
LVESA	0.20 ±0.03	0.19 ±0.06	0.26 ±0.09 [#]	0.29 ±0.06 [#]	0.33 ±0.17 ^{³ψ}	0.41 ±0.13 ^{³ψψ}

LV End-Diastolic and End-Systolic Diameters (LVEDD, LVEDS) (mm); Percent shortening-fraction (SF%); Fraction Area Contraction (FAC%); Short axis LV End-Diastolic and End-Systolic area (LVEDA, LVESA) mean values (mm).

*P<0.05, **p<0.01(Ex vs. Sed); #p<0.05, ###p<0.005 (24h-Post-AMI vs. Pre-AMI); ^ψp<0.05, ^{ψψ}p<0.005(4w-Post-AMI vs. Pre-AMI); ^³p<0.05, ^{³³}p<0.005 (4w-Post-AMI vs. 24h-Post-AMI); changes in Ex vs. Sed: ^Δp<0.05 (Pre-AMI vs. 24h-Post-AMI and 4w-Post-AMI);

Conclusions: Three weeks of swimming exercise training significantly attenuated LV remodeling 4w post MI and was associated with better LV function despite no changes in cardiac parameters at the end of the exercise session. The data imply that exercise training may induce genotypic changes that may result in phenotypic adaptation during severe insult (AMI).

Neuronal Stem Cell Progenitors From Human Umbilical Cord Blood (Ucb): Development Of A Pharmacological Approach Towards Induction Of Neural Differentiation

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Neuronal stem technology provides a novel approach for cell therapy of neurodegenerative diseases. Stem cells derived from embryonic and adult tissue are known to differentiate into neural-like cells. A few recent reports also suggest that umbilical cord blood (UCB) progenitors can differentiate into neuronal-like cells under certain operationally defined conditions. Our goal was to develop technologies for future use of neuronal progenitors of UCB origin for therapy of central nervous system disorders in Israeli patients.

In the present study we describe a protocol for isolation and differentiation of human umbilical cord blood neuronal-like progenitors (UCBNLPs) on type I collagen-coated tissue culture plates, thus selecting progenitors expressing specific integrin receptors, allowing their adhesion to the collagen extracellular matrix. During 14 days of *in vitro* tissue culture, using unique neuronal conditioning media and treatment with nerve growth factor (NGF), we found populations of progenitors that survive and generate a neuronal-like morphological phenotype expressed by “neurite” outgrowths of different length. These progenitors were found to express neuronal markers such as microtubule-associated protein 2 (MAP-2), neurotrophin receptor (TrkA), neurofilament-160 (NF-160), β -tubulin III, and neuron specific enolase (NSE) as revealed by immunofluorescence and polymerase chain reaction (PCR) methods. These markers were detected in the neuron-like differentiated phenotype but not in the control undifferentiated cells. The house-keeping control, β -actin, was present in both differentiated and undifferentiated cells. Further characterization, enrichment and propagation of UCBNLPs is in progress.

The present study contributes to the proof of the concept that UCBNLP are present in the UCB and may represent a reliable and feasible source of neuronal progenitors for use in cell therapy of neurodegenerative disorders.

Nerve growth factor (ngf)-dependent expression of trka receptors in human umbilical cord blood neural-like progenitos (hucbnlp) grown in vitro.

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Recent findings on the presence and ability of human umbilical cord blood neuronal-like progenitors (HUCBNLP) to differentiate into neuronal phenotypes *in vitro* and *in vivo* represent a most promising technology for future cellular and genetic therapies for central nervous system disorders. Since the NGF-trkA receptors are expressed very early in the embryonic neuronal development and are responsible for the differentiation of sympathetic and cholinergic neurons, we further characterize their role in HUCBNLPs.

Non-hematopoietic precursors, from mononuclear cells (MNC) of umbilical cord blood (UCB), were cultured on type I collagen coated plates for a period of two weeks. NGF treatment (10 ng/ml) was found to induce morphological differentiation as expressed by neurite outgrowth, as well as neuronal markers expression. The progenitors were found to express NGF/TrkA receptors on the first day of culture, but were not detected thereafter and up to 14 days of culture, whereas in the presence of NGF the progenitors retain their ability to express the trkA receptors for the entire period of culture *in vitro*. Kinetic experiments evaluated by PCR revealed high expression of NGF/TrkA receptors mRNA 48 hours after culturing in the presence of NGF, while in its absence the receptors were completely down regulated. The pan-neurotrophin receptor p75NTR, which is responsible for neuronal elimination during embryonic development, by cell death signals, was not detected by PCR method. The effect of NGF (10ng/ml) on HUCBNLPs gene expression after three days of culture was analyzed, using Affimatrix human DNA chip technology. NGF was found to increase the expression of various genes encoding proteins participating in neural differentiation. 28% of known increased genes were related to neural differentiation as opposite to only 2% of known decreased genes, supporting the conclusion that NGF induces neuronal differentiation in HUCBNLPs.

These results suggest an important role for NGF in regulating HUCBNLPs growth, neurotrophin trkA receptors stable expression and neuronal differentiation.

An *In Vitro* Blood Brain Barrier Model using Endothelial Cells of Different Origins and Glial Cells

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The blood brain barrier (BBB), composed of specialized endothelial cells, basement membrane and juxtaposed glial cells, is the anatomical structure, which controls the entry of drugs and chemicals into the brain.

Our aim is to use pharmacological approaches for creating a high-fidelity *in vitro* BBB model, which will be useful for facilitating the discovery and testing of novel neuropharmacological agents.

Towards this goal, we recreated the anatomical structure of the BBB on polyester terphthalate cell culture inserts. Endothelial cells, derived from brain capillaries and from peripheral blood vessels, were cultured on the apical side of the filter, while rodent glial cells were cultured on the baso-lateral side. Endothelial monolayer formation was confirmed by light microscopy and assessing endothelial cytoskeletal organization. Scanning electron micrographs of the engineered BBB indicate glial foot projections contacting the endothelial cells on the apical side. Physiological functions of the BBB model was evaluated by measuring: a) the transendothelial electrical resistance (TEER) using an EndOhm™ electrode and b) permeability of fluorescent markers of different molecular weights (300-60,000 Dalton).

Ten different endothelial cell types were evaluated for TEER values. Co-culturing of endothelial cells with glial cells resulted in synergistically increased TEER, as compared to the resistance of each monolayer alone. A linear relationship between fluorescent paracellular-markers permeabilities and their molecular weight was observed.

Future studies will focus on the effects of the tissue-specific extracellular matrix and cAMP-elevators on the physiological properties and permeability characteristics of our *in vitro* BBB model.

Neuroprotective Effect of 4-Hydroxy-TEMPO in the *in vitro* Parkinson's Model of NGF-differentiated Pheochromocytoma PC12 Cells Exposed to MPP⁺ Toxin

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Parkinson's disease (PD), the most frequent movement disorder in adults, is caused by dopamine neurotransmitter deficiency in the striatum, due to selective loss of the dopaminergic neurons in the substantia nigra pars compacta. The etiology of PD is still unknown and the treatment is symptomatic. We used nerve growth factor (NGF)-differentiated pheochromocytoma PC12 cells exposed to 1-methyl-4-phenylpyridinium (MPP⁺) toxin – common dopaminergic cell line as an *in vitro* pharmacological PD model. The MPP⁺ induced neuronal cell death was assessed by lactate dehydrogenase (LDH) release and caspase-3 enzyme activity. Kinetic and dose response experiments indicate activation of both isoforms of extracellular signal-regulated kinase (ERK1 and ERK2) which was observed 72 hr after exposure of NGF-differentiated PC12 cells to MPP⁺ (approximately 40-fold activation of ERK). Since oxidative stress plays important role in degeneration of dopaminergic neurons in PD, we have examined the neuroprotective effects of 4-Hydroxy-TEMPO (Tempol), a novel free radical scavenger. Treatment of the cells with Tempol reduced the release of LDH (50-60%) as well as caspase-3 activity and increased the mitochondrial function by 35% and 30% respectively. Tempol also attenuated the activation of ERK enzyme by 70%. The present study supports the contribution of apoptosis and ERK enzymes activation to the neurodegenerative process in PD and suggests a novel neuroprotective role of Tempol in PD by a mechanism involving attenuation of mitogen activated protein kinases.

Increase in maximal P_o of $Ca_v1.2$ channel by β_{2A} subunit depends on the long N-terminus of α_{1C} .

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An auxiliary β_{2A} subunit modulates the functionality of voltage-dependent Ca^{2+} in several ways. At least four effects are known: an increase in surface expression of the main α_{1C} subunit; a leftward shift in conductance-voltage curve; changes in voltage-dependent inactivation (VDI); an increase in maximal open probability, $P_{o,max}$, of a single channel. Using the *Xenopus* oocyte expression system, we asked whether these effects are determined by different molecular mechanisms and thus separable. Although the oocytes possess measurable levels of an endogenous β_{3x0} subunit, we show that all four functions of expressed β_{2A} are observed with high reproducibility. The dose-response analysis of the effects of the β_{2A} on channel conductivity allows us to propose that the increase in $P_{o,max}$ occurs at higher levels of than other effects. We show that the increase in $P_{o,max}$ depends on the presence of the initial N-terminal (NT) segment of α_{1C} subunit. This is an inhibitory regulatory element, exerting a tonic inhibition upon the channel gate; its deletion significantly increases macroscopic Ca^{2+} currents in heterologous expression systems. We show that the coexpression of β_{2A} elevates $P_{o,max}$ only in the long-NT isoform of α_{1C} , that has a 46-aa initial segment encoded by exon 1a. On the contrary, the β_{2A} does not increase $P_{o,max}$ of the short-NT isoform of α_{1C} , with a 16-aa initial NT segment encoded by exon 1. Deletion mutagenesis maps the crucial gating element to aa 6-20 of the long NT. It turns out that amino acid sequence of long-NT inhibitory element is unique and crucial for greater maximal conductance of the channel co-expressed with β_{2A} . The other two functions of β_{2A} are not affected by any of the NT deletions (leftward shift in I-V curve, increase in surface expression). We propose that the molecular mechanism underlying the increase in $P_{o,max}$ (removal of a tonic inhibitory effect present in long-NT isoform of α_{1C}) is different and separable from other effects of β_{2A} which do not depend on NT of α_{1C} .

Mitochondrial NADH and microcirculatory blood flow in brain and small intestine exposed to changes in oxygen delivery.

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Introduction: The pathophysiological events of emergency clinical situations mostly involve changes in oxygen delivery. Mitochondrial activity is often disrupted leading to major energy failure in cells, tissue and organs. These conditions are mostly involved with sympathetic pathways activation, resulting in redistribution of blood flow towards vital organs (brain) on the expense of blood supply non-vital organs (small intestine).

Aim: To examine the responses of metabolic and hemodynamic parameters monitored simultaneously in the brain and small intestine to various oxygenation conditions.

Materials and Methods: Adult male Wistar rats (250-300g) were anesthetized with Equithesine solution. Brain and intestinal serosa were exposed and monitored using the multi-site multi-parametric monitoring system (MSMP). Mitochondrial NADH was monitored by the fluorometry technique and tissue blood flow (TBF) was monitored using Laser Doppler flowmetry (Kraut et al 2004). Four groups of rats (n=9) were used. **Anoxia-** rats were exposed to pure N₂ until respiratory arrest. Additionally, three groups of rats were exposed to different mixtures of gases for 20 minutes. **Hypoxia-** 12% O₂ + 87% N₂ + 1% CO₂, **Hypercapnia** 10% CO₂ in air, **Hyperoxia** 100% O₂.

Results: Anoxia induced a decrease of 40±12% in intestinal TBF and an increase in CBF to 315±53%, however, both were coupled with 50% increase in NADH. Similar tendency was observed under hypoxia although the kinetic of responses were different. Hyperoxia induced no changes in TBF although NADH was oxidized in both organs by approximately 5%. Hypercapnia led to an increase in CBF to the level of 133±10% and NADH was oxidized by 10%, with no significant changes in intestinal parameters.

Conclusion: Our results demonstrated that the MSMP monitoring system enables identify hemodynamic and mitochondrial activity changes in non-vital and vital organs simultaneously, during different oxygenation conditions. This information may be applied in various critical conditions involved with deterioration of oxygen balance.

Rtvp-1 Is A Novel Diagnostic Marker And A Therapeutic Target In Astrocytic Tumors

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Gliomas are the most frequent primary brain tumors, accounting for more than 50% of all brain tumors. Current treatment options include surgery, radiation therapy and chemotherapy. Unfortunately, the prognosis of patients with glioblastomas remains extremely poor and the median survival of 12 months from the time of diagnosis has not significantly changed during the last decades. Limitations to therapy include the infiltrative nature and enhanced angiogenesis of these tumors. In addition, glioma cells are resistant to current modalities of irradiation, chemotherapy and immunotherapy. Therefore, innovative approaches are essential for the treatment of patients with gliomas, especially since the occurrence of gliomas and the high-grade glioblastomas is increasing. In this study we examined the expression and function of RTVP-1 in glioma cells. RTVP-1 was expressed in high levels in glioblastomas, whereas its expression in low-grade astrocytomas and normal brains was very low. Infection of glioma cells with siRNAs targeting RTVP-1 decreased cell proliferation in all the cell lines examined and induced cell apoptosis in some of them. Overexpression of RTVP-1 increased glioma cell proliferation and the anchorage-independent growth of the cells. In addition, overexpression of RTVP-1 rendered the cells more resistant to the apoptotic effect of TRAIL and serum-deprivation. To delineate the molecular mechanisms involved in the apoptosis induced by RTVP-1, we examined the expression and phosphorylation of various apoptosis-related proteins. We found that overexpression of RTVP-1 increased the expression of Bcl2 and decreased the phosphorylation of JNK, whereas the expression of Bax and the expression and phosphorylation of AKT were not altered. Finally, we found that RTVP-1 regulated the migration and invasion of glioma cells as was evident by their enhanced migration through Matrigel and their increased invasiveness in a brain spheroids confrontation assay. The increased invasive potential of the RTVP-1 overexpressors was followed by increased activity of MMP2. Our results suggest that the expression of RTVP-1 is correlated with the degree of malignancy of astrocytic tumors and that RTVP-1 is involved in the regulation of the growth, survival and invasion of glioma cells. Collectively, these findings suggest that RTVP-1 presents a novel diagnostic marker and potential therapeutic target in gliomas.

Adenosine and TNF- α Cause Similar Effects in Cardiomyocyte Cultures

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Myocardial tumor necrosis factor (TNF)- α and adenosine levels increase in stress conditions. Most studies report that adenosine as being therapeutic and cardioprotective, whereas TNF- α as proinflammatory cytokine induces cardiac dysfunction. Recently, a few studies reported that TNF- α is involved in ischemic tolerance and in ischemic preconditioning mediated cardioprotection. We studied TNF- α and adenosine treatments in heart cultures and in H9c2 cardiomyocytes, in order to compare their effects.

Adenosine and TNF- α stimulated ⁸⁶Rb efflux during 10-15 minutes of incubation. Glibenclamide – a blocker of ATP sensitive potassium ($K_{(ATP)}$) channels attenuated this effect. R-PIA – an A₁ adenosine receptor agonist, reduced ⁴⁵Ca uptake following 10-15 minutes of incubation. Isoproterenol – a β adrenoceptor agonist, doubled ⁴⁵Ca uptake, while R-PIA restricted this phenomenon by 80%. Similar effects were obtained by TNF α , which reversed the increase of ⁴⁵Ca uptake levels following isoproterenol treatment.

Treatment for 2 or 22 hours with adenosine or R-PIA reduced ³H-deoxyglucose uptake by 30-40%. However, isoproterenol accelerated ³H-deoxyglucose uptake by 90% and 35% following 2 and 22 hours of treatment, respectively. TNF- α attenuated the stimulatory effect of isoproterenol on ³H-deoxyglucose uptake. Similar results were obtained for adenosine. TNF- α decreased 66% and 42% LDH release, from heart cultures, after 75 min hypoxia and 150 min reoxygenation, respectively. Similar results obtained in hypoxic cardiomyocytes after glibenclamide or 5HD (5-hydroxydecanoic acid- a specific mitochondrial $K_{(ATP)}$ channel blocker) and TNF- α treatment. MTT measurement after 80 min hypoxia and 90 min reoxygenation increased 48% cell viability in hypoxic TNF- α treated cultures and similar results obtained in cardiomyocytes after glibenclamide or 5HD and TNF- α treatment.

Conclusions: Adenosine and TNF- α caused similar effects in cardiomyocytes in culture:

- 1) Stimulation of $K_{(ATP)}$ channels and attenuation of calcium and glucose uptake.
- 2) Inhibition of the stimulatory effect of isoproterenol on calcium and glucose uptake.
- 3) Restricted LDH release from hypoxic cultures and increased cardiomyocytes viability after reoxygenation. Our findings further suggest that TNF α , as well as adenosine mediate an adaptive effect in the heart, in order to restore homeostasis.

Two discrete inactivation states with distinct pore properties coexist in a long QT mutant of KCNQ1 K⁺ channels

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The KCNQ channels are members of the voltage-gated K⁺ channel superfamily and their inherited mutations in humans cause cardiovascular and neurological disorders. The KCNQ1 pore-forming subunit can interact with various KCNE auxiliary subunits to form K⁺ channels with very different gating behaviors. Inactivation of wild-type (WT) KCNQ1 channels is invisible macroscopically (hidden) but can be revealed by hooked tail currents which reflect recovery from inactivation. Here, we show that in a long QT mutant of KCNQ1, L273F, residing in the S5 segment of the pore region, two distinct inactivation states coexist: a hidden inactivation similar to WT KCNQ1 and a macroscopic inactivation whose time course and recovery are much slower compared to that of WT channels. A kinetic model of the LQT channel mutant faithfully describes the coexistence of two distinct inactivation states. External barium ions discriminate between the two discrete inactivation states by suppressing the hidden and barely affecting the macroscopic one. When L273F channels undergo the macroscopic inactivation process, they considerably delay the exit of barium ions from the deep pore (more than 6-fold at 0 mV). Interestingly, a tryptophan mutation at the same location (L273W) does not produce macroscopic inactivation and does not trap barium bound to the pore. In contrast, the mutation V310G residing at the base of the pore helix causes a macroscopic inactivation and markedly prevents barium exit from its deep pore site. Homology modeling of KCNQ1 with the bacterial KvAP crystal structure reveals that macroscopic inactivation arises from simultaneous hydrophobic interactions between the phenyl ring side chain of F273 in the S5 segment with two residues located in the pore helix, the C α of valine 307 and the side chain of valine 310. We suggest that these short-distance interactions produce conformational changes that propagate to the selectivity filter and the external vestibule in such a way that barium becomes trapped in the deep pore.

Anti-tumoral activity of the immunomodulator AS101 in Multiple Myeloma: Mechanism of action and clinical applications

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Multiple Myeloma (MM) is a clonal B-malignancy affecting both the immune and the skeletal systems, which accounts for 10% of all haematological cancers. The immunomodulating compound AS101 has shown to have direct antitumoral properties in several tumor models. We therefore examine here its antitumoral activity in three MM cell lines and in a mice model. The mechanism of action and signal transduction was also explored. Treatment of MM cells with increased concentrations of AS101 showed a significant inhibition of cell proliferation which was also reflected in cell viability reduction. We found that AS101 induces G2/M arrest, an effect associated with an increase of the double-stranded RNA activated protein kinase R (PKR) mRNA levels. Longer incubation of MM cells with AS101 resulted in a rise in the early apoptotic cells population. More important, mice transplanted with 5T33MM cells showed prolonged survival followed AS101 treatment versus untreated mice. We next examined the signal transduction involved in AS101 activation and found that AS101 reduces IL-6 mRNA levels in MM cells, which is considered the main growth and survival factor for these cells. Suppression of MM cell growth and apoptosis induction followed AS101 treatment, resulted in downregulation of pAkt and survivin protein expression levels. Our results indicate that AS101 may be candidate in clinical applications as an alternative or additional approach in treatment of MM patients.

Novel Involvement of the Tyrosine Kinase Fer in IL-10 Signaling and Down Regulation of this Pathway by AS101

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Interleukin-10 (IL-10) is a dominant immunosuppressive cytokine found in many pathologies, as well as cancer cells environment and is essential for tumor cell proliferation. In this study we intended to verify whether in human peripheral blood mononuclear cells (PBMC) there is an obvious correlation between IL-10 secretion levels and the cellular levels of Fer, as well as the influence of IL-10 levels on the association between Fer and pStat3. Fer tyrosine kinase was found to be essential for the proliferation of malignant cell-lines. We show that IL-10 increases the cellular level of Fer, while on the other hand, IL-10 inhibition by the AS101 compound treatment, results in down regulation of Fer cellular levels. Up and down regulation of Fer was paralleled by the activation and subsequent deactivation of Stat3 which is a potent oncogene and a putative substrate of Fer. Moreover, IL-10 induces the association of Fer with the phosphorylated form of Stat3 (pStat3), while AS101 reduces this connection. These results indicate that IL-10 plays an important role in the up and down regulation of Fer. In addition we showed that the association of Fer and pStat3 is influenced by IL-10. This association could be either for the phosphorylation of Stat3 by Fer or for inhibition of Stat3 by Fer. Therefore, anti-IL-10 treatment with compounds such as AS101, which down regulates the oncogene Stat3 activation probably via Fer down regulation, may be effective in the treatment of certain malignancies and other pathologies.

* These authors contributed equally to this work.

Regulation Of Glioma Cell Apoptosis By Pkc Delta

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Gliomas are characterized by deregulation of cell apoptosis and hence by increased resistance to apoptotic stimuli. Studies in our lab indicate that the expression PKC δ is inversely correlated with the malignancy of gliomas and with their proliferative response. Moreover, PKC δ plays a role in the apoptosis of glioma cells in a stimulus-dependent manner; however the molecular mechanisms underlying its diverse effects are not understood.

In this study, we examined the role of PKC δ in the apoptosis of glioma cells stimulated with etoposide and TRAIL. Using PKC δ WT, PKC δ KD mutant and siRNAs targeting the PKC δ mRNA, we found that PKC δ played opposite effects in the apoptosis induced by etoposide and TRAIL; overexpression of PKC δ enhanced the apoptotic effect of etoposide, whereas it significantly reduced the apoptosis induced by TRAIL. Similarly, inhibition or knockdown of PKC δ attenuated the apoptotic effect of etoposide and increased that of TRAIL. Both etoposide and TRAIL induced tyrosine phosphorylation of PKC δ albeit on different residues. Etoposide induced phosphorylation on tyrosine 64 and 187, whereas TRAIL induced phosphorylation on tyrosine 155. Etoposide and TRAIL also induced differential translocation of PKC δ ; etoposide to the nucleus and TRAIL to the ER. Both stimuli induced cleavage of PKC δ and accumulation of the catalytic fragment however the cleavage of PKC δ by etoposide mediated the apoptotic effect of etoposide, whereas the cleavage of PKC δ induced by TRAIL was essential for the protective effect of PKC δ . The phosphorylation of PKC δ preceded its translocation and cleavage in response to the two stimuli. Using PKC δ single tyrosine mutants, we found that the phosphorylation of PKC δ on tyrosines 64 and 187 was not required for the nuclear translocation of PKC δ in response to etoposide, but the phosphorylation on tyrosine 155 was essential for the translocation of PKC δ to the ER in response to TRAIL. Tyrosine phosphorylation of PKC δ was necessary for the cleavage of PKC δ by caspase 3 in both etoposide and TRAIL-treated cells.

Our results suggest that PKC δ plays an opposite role in the apoptosis induced by etoposide and TRAIL and implicate the tyrosine phosphorylation of PKC δ as an important mediator of the diverse effects of this isoform. Phosphorylation of PKC δ on distinct tyrosine residues can regulate the subcellular localization of PKC δ , its caspase-dependent cleavage and its pro and anti-apoptotic function. These studies may lead to the identification of novel approaches for altering the sensitivity of glioma cells to specific therapeutic modalities.

KCNQ1 long QT mutations impair calmodulin binding and channel assembly and stabilize inactivation gating

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Voltage-gated potassium channels are key regulators of cellular excitability. The slow I_{KS} K^+ channel plays a major role in repolarizing the cardiac action potential and consists of the assembly of KCNQ1 and KCNE1 α and β subunits, respectively. Mutations in either KCNQ1 or KCNE1 genes produce the long QT syndrome, which increases the risk of life-threatening ventricular arrhythmia. Here we show that long QT mutations located in the proximal KCNQ1 C-terminus impair the binding of calmodulin. These mutations dramatically decrease the current density and stabilize voltage-dependent inactivation gating. Generation of a soluble KCNQ1 C-terminus necessitates coexpression of calmodulin. Compared to wild type KCNQ1 and I_{KS} , the R366W mutant current is more sensitive to the disruptive effect of the calmodulin antagonist W7 and to the stimulating action of internal Ca^{2+} . KCNE1 forms a ternary complex with KCNQ1 and Ca^{2+} -CaM and could prevent the voltage-dependent inactivation in several mutants. These data suggest that proper calmodulin binding to KCNQ1 C-terminus is necessary for correct channel assembly and for triggering a gating module that boosts Ca^{2+} -dependent channel activity and prevents inactivation gating.

UTP Protects Cardiomyocytes From Hypoxic Stress Via Activation Of P2y Receptors

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Extracellular pyrimidine and purine nucleotides are released from the heart during hypoxia and activate P2 purinoceptors, classified as P2X or P2Y. P2X receptors are ligand-gated intrinsic ion channels, and P2Y receptors are G protein-coupled receptors. The aim of this study is to investigate the role of UTP in protecting cardiomyocytes subjected to hypoxia, as well as, the involvement of P2Y receptors in the protection signaling pathway. This study reveals that UTP, but not UDP or uridine, induced early and late preconditioning effect on rat neonatal cardiomyocytes. The presence of UTP receptors, P2Y₄ and P2Y₂, in cardiomyocytes was demonstrated by immuno-fluorescence staining and Western immuno-blot assay.

UTP cardioprotective effect was reduced in the presence of the P2 antagonist suramin. In addition, UTP caused a transient increase of $[Ca^{2+}]_i$ in cardiomyocytes. PPADS or RB-2, other antagonists of P2 receptors, abolished the $[Ca^{2+}]_i$ elevation caused by UTP. We used various inhibitors of the Ca^{2+} signaling pathway to show that UTP elevates levels of $[Ca^{2+}]_i$, originating from intracellular sources, via activation of PLC and the IP₃ receptor. Interestingly, these inhibitors of the Ca^{2+} signaling pathway did not prevent the immediate protective effect caused by UTP. Although mK_{ATP} channels are involved in other preconditioning mediator pathways, the involvement of these channels in the cardioprotective effect induced by UTP was ruled out, because 5-HD, a specific inhibitor of these channels, does not prevent protection.

In conclusion, UTP nucleotide protects cardiomyocytes against hypoxic damage via nucleotide receptor(s). Although UTP caused a transient increase in $[Ca^{2+}]_i$ level in cardiomyocytes, the protection obtained by UTP was Ca^{2+} -independent.

KCNQ1 long QT mutations impair calmodulin binding and channel assembly and stabilize inactivation gating

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Voltage-gated potassium channels are key regulators of cellular excitability. The slow I_{KS} K^+ channel plays a major role in repolarizing the cardiac action potential and consists of the assembly of KCNQ1 and KCNE1 α and β subunits, respectively. Mutations in either KCNQ1 or KCNE1 genes produce the long QT syndrome, which increases the risk of life-threatening ventricular arrhythmia. Here we show that long QT mutations located in the proximal KCNQ1 C-terminus impair the binding of calmodulin. These mutations dramatically decrease the current density and stabilize voltage-dependent inactivation gating. Generation of a soluble KCNQ1 C-terminus necessitates coexpression of calmodulin. Compared to wild type KCNQ1 and I_{KS} , the R366W mutant current is more sensitive to the disruptive effect of the calmodulin antagonist W7 and to the stimulating action of internal Ca^{2+} . KCNE1 forms a ternary complex with KCNQ1 and Ca^{2+} -CaM and could prevent the voltage-dependent inactivation in several mutants. These data suggest that proper calmodulin binding to KCNQ1 C-terminus is necessary for correct channel assembly and for triggering a gating module that boosts Ca^{2+} -dependent channel activity and prevents inactivation gating.

Identification and Characterization of a Novel α_{1C} Isoform

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The voltage-dependent, Dihydropyridine (DHP)- sensitive, L-type calcium channel (L-VDCC) is the main calcium channel in the heart, where it plays a role in cardiac excitability and excitation-contraction coupling by contributing to the action potential plateau. In smooth muscle (SM) this channel also plays a role in contraction and regulation of tonus.

L-DVCC are multisubunit protein complexes containing several subunits. α_1 , is the pore forming subunit. The α_1 protein found in cardiac and SM tissue and also present in the brain, is α_{1C} ($Ca_v1.2$), encoded in humans by CACNL1A1 gene located on chromosome 12 (12p13). α_1 contains four homologous domains, I–IV, and 5 large intracellular segments: N-terminus (NT), C-terminus (CT), and four intracellular linkers LI-II, LII-III and LIII-IV.

The cytosolic parts of α_{1C} are most relevant for modulations by protein kinases. An especially notable variant from rabbit lung, α_{1Cb} , has a short NT (encoded by exon 1A) but has an insertion in linker LI-II. A similar insertion was found in some rat and mouse α_{1C} cDNAs but *not in any known human α_{1C} isoforms*.

By screening human genome with the cDNA sequence of rabbit LI-II insertion, we have identified a putative exon 9A on chromosome 12, between exons 9 and 10, situated downstream of the β -binding domain (AID). The deduced a.a. sequence is highly homologous to that of rabbit. The presence of RNA transcripts containing the LI-II insertion was identified by RT-PCR of human heart, bladder, aorta and brain RNA followed by sequencing of PCR products.

We have constructed two α_{1C} chimeras containing the LI-II insertion: a long- and a short-NT one, in order to study the effects of this insertion on the biophysical properties along with PKC modulation of α_{1C} .

The activation and inactivation properties of the four constructs studied were not significantly altered. However, modulation by PKC was significantly enhanced in exon9A-containing α_{1C} isoforms. The long-NT isoform is known to be enhanced by PKC activation, while only a decrease in I_{Ca} is observed with the short-NT one. Nevertheless, I_{Ca} was enhanced in the short-NT isoform containing exon 9A, as compared to the wt, short-NT channel.

The physiological response of SM cells to PKC is well established, demonstrating an increase in I_{Ca} . This modulation was never successfully reconstituted in heterologous expression systems, probably due to poorly-selected α_{1C} isoform for expression. Modulation of this unique exon 9A-containing isoforms by kinases provides further information regarding the isoform diversity expressed in different tissues, and may offer insight to the response of this channel to various hormones and transmitters.

Regulation of inactivation of L-type Ca²⁺ channels by Ca²⁺-binding proteins and cytosolic segments of the α_{1C} subunit.

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L-type voltage-dependant Ca²⁺ channels (L-VDCCs) are important for cardiac and neuronal activity. During depolarization, Ca²⁺ entry is prevented by channel inactivation, a process triggered by two elements: voltage (voltage-dependent inactivation, VDI) and/or calcium (calcium-dependent inactivation, CDI). These two mechanisms may use the same set of protein interactions. Two Ca²⁺ binding-proteins, calmodulin (CaM) and the novel Ca²⁺-binding protein-1 (CaBP-1) interact with the pore forming subunit, α_{1C} , and have opposing effects on inactivation of Ca²⁺ current through the channel. CaM enhances CDI, while CaBP-1 totally prevents the process. Surprisingly, we discovered that CaBP-1 enhances the VDI (of Ba²⁺ currents), and also shifts the IV curve of calcium and barium currents to the right. These effects also take place when the inhibitory module, the first 46 amino acids of the long N-terminus (NT) isoform, is removed, as well as in a short NT isoform. CaBP-1 and CaM are known to bind the C-terminus (CT) of the channel through the IQ domain. Removal of the first half of NT is known to abolish the CaBP-1 effect on CDI. Mapping of the binding sites of CaM and CaBP-1 to the NT revealed that the deleted part was a CaM binding site, while the CaBP-1 binding site remained untouched. Apart from the possible mutual role of CaM and CaBP-1 in VDI and CDI, the contribution of the different cytosolic segments of α_{1C} seems crucial. We demonstrate novel interactions between various cytosolic parts of α_{1C} that participate in CaM or CaBP1 binding and/or inactivation processes. The interactions of different Ca²⁺ binding proteins with cytosolic segments of the channel as well as interactions between these cytosolic segments might be the key to an in-depth understanding of the inactivation mechanism.

G α_{GDP} primes GIRK for activation by G $\beta\gamma$: an alternative scheme of regulation of GIRK activity by G α and G $\beta\gamma$.

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GIRK (Kir3) channels are activated by neurotransmitters coupled to G-protein, by direct binding of G $\beta\gamma$. The role of G α subunits in GIRK gating appears to become increasingly significant. Previously, we presented evidence that G α_i is not only a donor of G $\beta\gamma$ but also regulates GIRK gating (Peleg et al, 2002). When overexpressed in *Xenopus* oocyte, GIRK channels show extreme basal activity and poor activation by agonist or G $\beta\gamma$. We proposed that at high GIRK expression levels a shortage in G α causes an excessive basal current, and further addition of G $\beta\gamma$ evokes little response. Indeed, coexpression of G α_{i3} or G α_{i1} restores the correct gating parameters: it reduces the basal current and "primes" the channel for activation coexpressed G $\beta\gamma$ (in whole cells) or purified G $\beta\gamma$ (in excised patches), leaving the total G $\beta\gamma$ -evoked current intact. In contrast, when phosducin is used to reduce the basal activity to a similar extent, it reduces both basal activity of the channels, and the total G $\beta\gamma$ -evoked current very significantly. G α_i seems to relocate to PM together with GIRK and G $\beta\gamma$ (Rishal et al, 2005), as seen in confocal images (tested with antibody to G α_{i3}) and also suggested by an excessive basal current at very high GIRK densities we propose that G $\alpha\beta\gamma$ is normally permanently complexes with GIRK.

Here we propose that G α_i acts in its GDP bound form, most likely as a part of G $\alpha\beta\gamma$ heterotrimer. We verify this hypothesis by using two G α_{i3} mutants: "constitutively active" (QL) G α_{i3} Q205L and "constitutively inactive" (GA) G α_{i3} G204A. The latter mimics the wild-type (WT) G α_{i3} in reducing the basal current and priming GIRK for activation by G $\beta\gamma$. *In vitro* binding experiments indicate that G $\beta\gamma$ enhances the binding of channel to G α_{i3} -GDP. The extent of "priming" (by either WT or G α_{i3} G204A) correlates linearly with the extent of reducing in GIRK basal current. We interpret these data to suggest that binding of G α_{GDP} removes activation induced by the constitutively bound G $\beta\gamma$. However, the G $\alpha\beta\gamma$ complex does not move away but stays with associated the channel; in this configuration, either G $\beta\gamma$ released from the heterotrimer associated with the channel, or G $\beta\gamma$ added from an external source, activate GIRK with high potency.

ZnT-1, A Missing Link In Tachycardia- Induced Electrical Remodeling

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BACKGROUND: Tachycardia induced electrical remodeling is an important factor in the pathogenesis of atrial fibrillation (AF) as well as other cardiac pathologies. Down regulation of L-type Ca²⁺ channels (LTCC) activity is the hallmark of atrial electrical remodeling, and probably an important contributor to the initiation and perpetuation of AF, but its underlying molecular mechanisms are not fully understood. ZnT-1 is a ubiquitous membrane protein protecting against zinc toxicity, presumably through inhibition of cations influx, yet its role in cardiac electrophysiology is unknown. We have studied ZnT-1 effects on ionic fluxes through the LTCC in cardiomyocytes and rat atria in a quest for a missing link in the mechanism of tachycardia induced electrical remodeling.

METHODS AND RESULTS: The expression of ZnT-1 was manipulated in cultured cardiomyocytes by transfection with ZnT-1, or siRNA designed to inhibit ZnT-1 expression. Ba²⁺ (substituting for Ca²⁺) influx through LTCC was assessed in cardiomyocytes loaded with fura-2. Cardiomyocytes were rapidly paced at double threshold intensity by electrodes inserted in the culture plate. Transfection lead to an increased in ZnT-1 expression to 464±55 %, while siRNA transfection reduced the expression to 60.6±3.6 % (P<0.05 for both, n=5, n=4, respectively). Nifedipine sensitive Ba²⁺ influx was reduced in ZnT-1 transfected cells to 51.0±2.5 % of controls (P<0.01, n=4), while siRNA increased the rate of influx to 167.0±4.25 % (P<0.01, n=4). Rapid (RP) pacing of cardiomyocytes increased ZnT-1 expression to 175±18 % of controls (p<0.01, n=4) and reduced Ba²⁺ influx to 48.0±1.8 %, p<0.01, n=4). This was without changes in expression of LTCC α_1 C subunit.

Using a novel model of bipolar epicardial right atrial pacing in anesthetized and ventilated adult rats, the atrial effective refractory period (AERP) was measured with a drive cycle length of 150 ms before and after 1, 2, and 4 hours of rapid atrial pacing at cycle length of 50 ms (n=4). Following RP the animals were scarified and ZnT-1 expression was assessed by western blot analysis. Controls were 5 Sham without RP, and 5 un-anesthetized rats. Rapid pacing led to 166.5±15.3 % increased ZnT-1 expression (p<0.05), and a reduction of 54.3±5.2 % in the AERP (P<0.05).

CONCLUSIONS: These findings indicate ZnT-1 as a major physiological modulator of the cardiac LTCC. Furthermore, this is the first observation linking ZnT-1 over-expression to rapid electrical pacing and atrial electrical remodeling. The observed changes in ZnT-1 expression in parallel with the inhibitory effect on the Ba²⁺ influx through LTCC (in-vitro), and the reduction in AERP (in-vivo) mark ZnT-1 as a missing link mediating down regulation of LTCC activity in tachycardia- induced cardiac electrical remodeling.

Reversal of GCV resistance in Tumor cells transduced with HSV-tk gene by Zebularine, a novel DNA hypomethylation agent

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One of the main obstacles of HSV-thymidine kinase (HSV-tk)/ganciclovir (GCV) approach for suicide gene therapy of cancer is the development of GCV resistance. Although the exact mechanism of GCV resistance remains controversial, one of the proposed mechanisms is silencing of HSV-tk expression due to hypermethylation of HSV-tk gene. Therefore, we assumed that inhibitors of DNA methyltransferase (DNMT) would be able to reactivate silenced HSV-tk gene in HSV-tk-transduced cells resistant to GCV. In order to test this hypothesis, we examined the ability of Zebularine (Zeb), a novel DNMT inhibitor, in reverting GCV resistance. GCV resistance was developed in human bladder carcinoma cells (T24) transduced with HSV-tk gene (T24/HSV-tk) by two different methods. Slow GCV resistant cells (T24/HSV-tk/SR) were generated by continuous (6 weeks) exposure to increasing concentrations of GCV (0.02-10 μ M), while rapid resistant cells (T24/HSV-tk/RR) were obtained by treatment with high GCV concentration (10 μ M) for 48 hrs. Thereafter, we assessed GCV cytotoxicity and phosphorylation in GCV sensitive T24/HSV-tk, T24/HSV-tk/SR and T24/HSV-tk/RR cells before and after treatment with Zeb, 100 μ M for 5 days. It has been found that GCV IC₅₀ on cell proliferation of T24/HSV-tk/RR cells was significantly decreased by 9-fold in Zeb treated cells. In contrast, Zeb failed to reverse GCV resistance in T24/HSV-tk/SR cells. Moreover, Zeb treatment resulted in a significant increase (3-fold) in the level of GCV-TP, the active metabolite of GCV, in T24/HSV/RR cells, while it failed to affect GCV-TP level in T24/HSV-tk/SR cells. Our data may suggest that reversal of GCV resistance in T24/HSV/RR cells occurred following possible reactivation of the silenced HSV-tk gene by Zeb. More it seems that HSV-tk hypermethylation play a role in the development of GCV resistance and the novel hypomethylation agents Zeb may have a potential use for the reversal of GCV resistance.

Activity and Significance of the Brain-ZnR

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Dynamic changes in zinc play a key role in regulating synaptic transmission, but are also a leading factor in neuronal death following excitotoxic syndromes. We hypothesized that an extracellular zinc sensing receptor, ZnR, is mediating intracellular signaling following changes in extracellular zinc concentrations. Following the application of zinc in acute hippocampal slices, loaded with Fura-2, a fluorescence rise that was observed in the CA3 and neocortical regions. TPEN, a membrane permeable zinc chelator, did not significantly affect this fluorescence rise indicating that the signal is indeed related mostly to Ca^{2+} , and not induced by Zn^{2+} permeation. The zinc-dependent Ca^{2+} response is inhibited by the Gq inhibitor YM-245180, the PLC inhibitor, U73122, and emptying of the intracellular Ca^{2+} stores using thapsigargin. Thus, our results indicate that the IP_3 pathway is mediating brain-ZnR activity. ZnR activity was induced along a wide range of zinc concentrations, from nanomolar to micromolar, indicating that the ZnR may be responding to normal synaptic zinc release as well as massive excitotoxic released zinc. Interestingly, ZnR activity was induced following electrical stimulation of the mossy fibers and was blocked in the presence of the extracellular zinc chelator, CaEDTA. Thus, our results indicate that endogenous released zinc at the CA3 region may activate the ZnR. Our work therefore suggests a role for zinc acting as a neurotransmitter via a specific zinc sensing receptor.

Preconditioning by heat acclimation increases Akt-1 phosphorylation and decreases pro-inflammatory cytokine expression after closed head injury in mice

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Introduction: Long term exposure to moderately high ambient temperature (heat acclimation, HA) has been shown to lead to the induction of cross tolerance toward a variety of subsequently applied stressors. We have recently reported that HA leads to improved motor and cognitive outcome in a mouse model of closed head injury (CHI), indicating that the exposure to the sub-lethal heat serves as a means of preconditioning in this model. Our previous report also showed that HA increases the expression of Hypoxia inducible factor 1 α (HIF-1 α) and in turn, of the erythropoietin receptor (EpoR). Erythropoietin (Epo) has been shown to foster neuroprotection in a variety of brain injury models. These protective effects have been suggested to involve the activation of anti-apoptotic signalling pathways by the phosphorylation of Akt-1 following Epo binding to EpoR. However, the overall beneficial effect of HA on CHI outcome may involve both the activation of protective pathways and the attenuation of deleterious ones. The aim of the current study was three-fold: 1) to examine the possible involvement of Akt-1 phosphorylation in HA induced neuroprotection, 2) to investigate the effect of HA on the expression of tumor necrosis factor α (TNF α) and interleukin 1 β (IL1 β), proinflammatory cytokines known for their early detrimental effects in CHI pathophysiology, 3) to compare the extent of protection which is offered by HA, Epo treatment and a combination of both.

Methods: Two groups of adult male Sabra mice were studied: HA, held in a climatic chamber (34 \pm 1 $^{\circ}$ for 30 days) and normothermic (NT) controls. Mice were subjected to CHI induced by a weight drop device and injected 1h later with either 5000 units/Kg of Epo or saline. Cognitive function was evaluated 3 and 7 days post-injury using an object preference test (object recognition test, ORT). Akt-1 phosphorylation was assessed 4h following CHI by western blotting and the levels of TNF α and IL1 β were examined at thirty minute intervals during the initial 2 hours post-CHI, using semi-quantitative RT-PCR. **Results:** HA and Epo treatment led to a similar, but not additive improvement in cognitive function. Akt-1 phosphorylation levels were similar in HA and NT sham mice, yet a far more pronounced increase occurred in the HA group 4h following injury than in NT animals (p<0.05). The increase in TNF α following trauma was far less pronounced in HA mice post injury (p<0.001) and NT levels continued to be higher up to 2h post-injury. IL1 β levels were also much higher in NT mice as compared to HA at 30 min following injury (p<0.01). **Discussion:** HA and Epo provide a similar extent of protection. The increase in Akt-1 phosphorylation following HA suggests that activation of EpoR could play a role in HA- as well as in Epo-induced neuroprotection. Our findings also indicate that the beneficial effect of HA on CHI probably involves both the enhancement of survival promoting pathways and a reduction in the effects of the detrimental proinflammatory factors. The involvement of Akt-1 in both HA- and Epo-induced neuroprotection raises the possibility of the existence of common protective pathways, shared by distinct neuroprotective protocols.

HU-331 – A Novel Antiangiogenic and Anticancer Quinone

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INTRODUCTION: Anthracyclines, a large group of quinonoid compounds produced by different strains of streptomyces, exert antibiotic and antineoplastic effects. They are used to treat some forms of cancer. The development of quinonoid compounds that display antineoplastic and/or anti-angiogenic activity, but are more selective and less toxic is a major therapeutic goal. HU-331 (cannabidiol-hydroxyquinone) was synthesized from cannabidiol. HU-331 shows very high effectivity against human cancer cell lines *in-vitro* and also against *in-vivo* tumor grafts in nude mice. At 35 days after cancer cell injection, the tumors in the treated group were half the size of the tumors in the controls, a difference that was highly significant.

METHODS: For assaying the mechanisms of HU-331-mediated cancer cell death several standard methods were used, such as annexin V binding (for assaying apoptotic cell death), PI DNA staining (for cell cycle analysis), intracellular staining with anti-caspase-3 (for assaying caspases involvement), MTT (proliferation) test with HU-331 in presence of CB1/CB2 antagonists or ROS scavengers (for assaying free radicals and cannabinoid receptors involvement). The ability of HU-331 to inhibit topoisomerases was assayed on puc19 plasmid relaxation. The enantiomer of HU-331 was synthesized and tested alongside HU-331 itself. For evaluation of the anti-angiogenic action of HU-331 and some other cannabinoids, collagen-embedded rat aortic rings were incubated for 5-7 days with these compounds in the presence of FGF or VEGF (or with FGF/VEGF alone as positive controls). The ability of cannabinoids to inhibit endothelial cell proliferation was assayed as well. The cardiotoxicity of HU-331 compared to doxorubicin was assayed on mice by echo-cardiography.

RESULTS: HU-331 is highly selective. It does not cause cell cycle arrest, cell apoptosis and caspase activation. HU-331-caused cell death is partially mediated by ROS. (+)HU-331's potency in killing cells was not lower than that of (-)HU-331, which means that HU-331 does not act through binding to a receptor, but probably enters the cell and acts through its quinone moiety. HU-331 has limited influence on topoisomerase I action, but is able to inhibit topoisomerase II even in nanomolar concentrations. HU-331 is strongly anti-angiogenic. It partially inhibited aortic ring angiogenesis in concentrations as low as 0.1 µg/ml (300nM). The number of new vessels formed was not only lower, but even those that were formed were shorter. HU-331 also lowered endothelial cell proliferation. The tumors treated by HU-331 contained much less vessels than control tumors. In comparative assays HU-331 inhibited Jurkat (T cell lymphoma) cells growth more than some known anticancer drugs (doxorubicin, mitoxantrone and etoposide). In echo-cardiography tests HU-331 (7.5 mg/kg/week) is much less toxic than doxorubicin (1.5 mg/kg/week)

CONCLUSION: The cannabinoid quinone HU-331, which is more selective, more potent and less toxic than some known anticancer quinones, possesses high anti-angiogenic activity, specifically inhibits topoisomerase II and thus has a high potential as a new anticancer drug.

Modulation Of The Mitochondria As A Possible Mechanism Of Amiodarone Action In Cardiomyocytes

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The medical basis for heart arrhythmia treatment relies on knowledge of the mechanism of the drug action, its outcome, historical understanding of the treated arrhythmia and a clear pharmacological comprehension of the drug that is going to be used.

Amiodarone, a potent anti-arrhythmic drug, which is used for treating various abnormal rhythms in the heart muscle, has a very complicated pharmacological profile that is not clearly and completely understood.

The purpose of the present study was to establish the hypothesis that modulation of the mitochondria has crucial effects on the chronic pharmacological profile of amiodarone by lowering the energetic metabolism of cardiomyocyte.

So far, all the experiments were performed in cardiomyocyte cultures from newborns rat. Various mitochondrial parameters were analyzed following amiodarone treatment (3-30 μ M). A quantitative and qualitative decrease in the mitochondrial membrane potential was observed. Specific examination showed hindrance in complex 2 (succinate-coenzyme Q reductase) activity in the electron transport chain and lower cytosolic ATP level. An increase of the cytosolic Ca²⁺ level was observed as well as in the mitochondrial Ca²⁺ levels. There was no change of the Ca²⁺ level in the cytosol after treatment with caffeine, which indicates that the origin of such an increase comes from progressive continues depletion in the SR Ca²⁺ contents. We tested the hypothesis that deficiency of ATP for SR-Ca²⁺ ATPase activity is responsible for this phenomenon.

The results clearly exhibit that amiodarone causes a decrease in metabolic energy in the mitochondria. The damage reflects a decrease of mitochondrial function, which is correlated to dose and to the duration of the drug treatment. Thus, the research is focusing on the hypothesis that this energetic modulation, causes by amiodarone, is responsible for the decrease and elimination of the heart arrhythmia by inhibition some biochemical processes which depends on ATP.

Effect of extremely low electromagnetic field on ECG patterns of normal hearts of swine and rats

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Background: Several studies have shown that electromagnetic field (EMF) can stimulate growth and proliferation of various biological tissues, healing of skin lesions and angiogenesis. More recently, it has been shown that cardiac myocytes exposed to EMF have demonstrated increased Ca⁺ efflux which may be important following acute infarction. **Aim:** To investigate the effect of various low magnetic field on ECG pattern in vivo and the role of calcium efflux in vitro. **Methods:** We assessed the effect of extremely low EMF (10X10⁻⁶ - 10X10⁻¹² tesla, sinusoidal waves, 16Hz) on the ECG of anesthetized swine and rats, and cytosolic Ca⁺ flux of neonate cardiac myocytes. **Results:** In both swine and rats P wave, T wave and QRS complex were changed with EMF application (at magnetic field of 600pT). These changes were maintained for several hours even after the EMF was stopped (see table). In the isolated cardiac myocytes EMF reduced cytosolic Ca⁺ content by approximately 60%.

	PR (Sec)		QRS (Sec)		QT (Sec)	
	Base-line	600 pT	Base-line	600 pT	Base-line	600 pT
Rat 1	0.058	0.037	0.037	0.03	0.08	0.06
Rat 2	0.05	0.04	0.03	0.035	0.07	0.06
Rat 3	0.04	0.04	0.04	0.03	0.06	0.06
Rat 4	0.025	0.02	0.03	0.05	0.03	0.04

Conclusions: The preliminary data obtained on a small group of animals demonstrate that extremely low EMF induce ECG changes in vivo characterized by hyperpolarization probably due to the activation of K_{ATP} channels during repolarization, however, further studies are warranted to elucidate this hypothesis.

The mechanism of leak potassium channels regulation of by lysophospholipids

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Leak potassium channels (also called two-pore domain K⁺ channels or K2p) are background K⁺ selective channels that pass current across the entire physiological voltage range. Potassium leak currents exert control over cell excitability by shaping the duration, frequency and amplitude of action potentials, in part through their influence over the resting membrane potential. Increased potassium leak currents stabilize cells at hyperpolarized voltages, below the firing threshold of nerves and muscles, whereas leak suppression permits depolarization and excitation. Furthermore, K2p channels were found to play a major role in cell volume regulation, neuroprotection, heart function, anesthesia, cancer development and apoptosis. The activity of K2p channel family members is highly regulated by various cellular factors. We found that, when expressed in *Xenopus* oocytes and measured with the two electrode voltage clamp technique; application of 0.1-1.0% bovine serum significantly decreased currents through several K2p channels. This decrease was reproduced by lysophosphatidic acid (LPA) and not by lysophosphatidylcholine (LPC) and other lysophospholipid (LPLs), indicating that the former is the predominant LPL in the serum.

Lysophospholipides are a family of bioactive phospholipids controlling numerous cellular responses through activation of specific G protein coupled transmembrane receptors. Here we explore the mechanism by which LPA regulates K2p channels.

We found that LPA is not directly affecting the channel as it has no effect on a mutated channel (KCNK0Δ299-1001) lacking its carboxy terminus regulatory domain. LPA dose not operate by activating protein kinase A (PKA) as the K2p2.1 mutants, K2p2.1-S348A or D, are sensitive to LPA application. In the absence of serine residue at position 348, K2p2.1 channels are no longer phosphorylated by this kinase. Moreover, protein kinase C (PKC) inhibitors, staurosporine and bisindolylmaleimide I, did not prevent LPA induced current decrease. We thus conclude that LPA activity upon K2p channels is not mediated by either PKC or PKA.

We therefore believe that LPA regulates K2p channel activity via the phospholipase C (PLC) pathway and that current decrease is achieved by either reduction in membranal PIP2 concentration or by direct interaction with its hydrolysis products, DAG and IP₃. This hypothesis is now being tested.

Voltage-insensitive currents may efficiently modulate central pattern generator activity

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The pyloric network in the lobster *Homarus americanus* is a central pattern generator (CPG) that is involved in feeding. Its rhythmic activity is generated by a group of three electrically coupled pacemaker neurons. The rhythm depends on intact neuromodulation delivered to the CPG via the stomatogastric nerve (*stn*). When the *stn* nerve was cut, oscillations were abolished or dramatically slowed down. Using the dynamic clamp technique, we were able to restore the oscillations by artificially reducing potassium voltage-insensitive (leak) conductances in the pacemaker neurons. Similarly, when the *stn* nerve was intact, we were able to completely abolish the pyloric oscillations by artificially increasing potassium leak conductance. This manipulation, however, was accompanied by an unrealistic decrease in the input resistance of the cells. Previous studies suggested that leak currents in pyloric neurons are a mixture of potassium and sodium currents. We therefore repeated the dynamic clamp manipulation, modeling the leak conductance as a combination of potassium and sodium conductances. With this configuration, a modest increase and decrease in the potassium and sodium components of the leak conductance, respectively, was sufficient to completely abolish the oscillations, with no significant change in input resistance. These results raise the possibility that neuromodulatory inputs control the pattern of the pyloric rhythm by modifying the properties of leak currents in pyloric pacemaker neurons.

Hyperbaric Oxygen Therapy Reduces Neuroinflammation And Expression Of Matrix Metalloproteinase-9 In The Rat Model Of Traumatic Brain Injury

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The acute inflammatory response plays an important role in secondary brain damage after traumatic brain injury (TBI). Neutrophils provide the main source of matrix metalloproteinases (MMPs) which also play a deleterious role in TBI. Numerous preclinical studies have suggested that hyperbaric oxygen therapy (HBOT) may be beneficial in various non-cerebral and cerebral inflammatory diseases. The goal of this study was to evaluate the effects of HBOT on inflammatory infiltration and the expression of MMPs in correlation with secondary cell death in the rat model of dynamic cortical deformation (DCD). Twenty animals underwent DCD with subsequent HBOT (2.8 ATA, two sessions of 45 min each); 10 animals: DCD and normobaric oxygenation (1 ATA), 10 animals: not treated after DCD. Cell death was evaluated by TUNEL. Neutrophils were revealed by myeloperoxidase staining. Immunohistochemical staining for MMP-2 and -9 and TIMP-1 and -2 was also performed. In the animals treated by HBOT, a significant decrease in the number of TUNEL-positive cells and neutrophilic inflammatory infiltration was seen in comparison to non-treated animals and those treated by normobaric oxygen. The expression of MMP-9 was also significantly lower in the treated group. Staining for MMP-2 and TIMP-2 did not change significantly. Our results demonstrate that HBOT decreased the extent of secondary cell death and reactive neuroinflammation in the TBI model. The decline of MMP-9 expression after HBOT may also contribute to protection of brain tissue in the perilesional area. Further research should be centered on the evaluation of long-term functional and morphological results of HBOT.

Implications Of Hyperbaric Oxygen Therapy On Peripheral-Type Benzodiazepine Receptors In Traumatic Brain Injury

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Traumatic brain injuries (TBI) constitute a major health and socio-economic challenge in developed countries, representing the leading cause of death and disability among young adults. This formidable challenge has triggered an intense effort for the development of new therapeutic horizons, so far without success. Among the reasons often advocated for the failure of neuroprotective agents is the fact that they were over-targeted, neglecting apoptosis on one hand and, on the other hand, have focused on neuron survival without paying much attention to glial cells. Recently, increasing attention has been drawn to some astrocytic functions critical to neuron survival. Assessment of astrocyte functions has been recently characterized by evaluation of the density and activity of peripheral-type benzodiazepine receptors (PBR). Recent studies suggested that PBR activation may promote opening of the mitochondrial permeability transition pore and subsequently results in alteration of mitochondrial respiration and induction of apoptosis. In an animal model of cortical TBI, we showed that hyperbaric oxygen therapy reduced significantly the size of the traumatic lesion and markedly diminished apoptosis-related cell death. Assuming that PBR may be implicated in post-traumatic apoptosis, and considering the anti-apoptosis neuroprotective effect of HBOT, we hypothesized that HBOT may interfere with PBR activity. In order to investigate that, we assessed PBR expression in histological sections stained with anti-isoquinoline binding protein (IBP) – one of the three PBR components – and with glial acidic fibrillary protein (GFAP) in treated and non-treated injured animals. As expected from previous studies, both PBR and GFAP expression were increased in injured animals. In treated animals, however, reduction in apoptosis was associated with significantly decreased PBR density whereas GFAP expression remained unchanged. This study further supports the use of HBOT as a multifactorial drug for the treatment of TBI.

Improved myocardial performance after heat acclimation can be partially explained by hypothyroid dependent reduction in myocardial renin angiotensin response

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Background: Heat acclimation induces adaptive changes that improve the ability to cope with extreme environmental heat. Changes are seen in several body systems including the vascular system and the heart. We have shown that acclimated rats have lower plasma T4 and T3, which mediates acclimatory responses. Treating acclimated animals with L-thyroxin during the acclimation procedure reverts these responses. The possible connection between thyroxin hormone and the renin-angiotensin system (RAS) is known only partially. Angiotensin effects are mediated via receptors including AT₁ and AT₂. AT₁ is the main effector. **Aim:** To investigate the role of the renin angiotensin system in myocardial heat acclimation. **Methods:** Three groups of animals (n=27), (1) HA- heat acclimated- 4 weeks (AC) rats, (2) HA rats treated with L-thyroxin (AT) and (3) control animals (C) were used. The Langendorff perfusion system was employed to measure haemodynamic parameters (pulse pressure [PP] and coronary flow [CF]). The effect of Angiotensin as well as AT₁ and AT₂ receptor inhibitors (Losartan and PD123319, respectively) on pressure generation and coronary flow was measured. Additionally, protein levels of each receptor were measured using Western blotting. **Results** Administration of Angiotensin in the C group increased PP and decreased CF. In the AC group no effect was seen. Hearts from AT group responded similar to that of the C group, showing increased PP and decreased CF. When the AT₁ and AT₂ receptor inhibitors were injected through the langendorff apparatus, an increased PP was observed. Western blotting of the external membrane showed that AT₁ receptor protein level was significant decrease in AC group. In contrast, AT₂ receptor protein showed no significant differences among the groups. **Conclusion** : Myocardial heat acclimation is associated with a reduced response to angiotensin II. This effect can be reversed by thyroxin administration. The paradoxical response of the hearts to AT receptor antagonists is yet to be explained.

Role of the pro-apoptotic protein ARTS in 6-hydroxydopamine-induced neurotoxicity

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The protein ARTS is a member of the septin family of filament-forming proteins, and is formed by alternative splicing of the *H5/Sept4* gene locus. Over-expression of ARTS in cell lines such as COS leads to induction of apoptosis, by binding the apoptosis inhibitor protein XIAP. ARTS is highly expressed in the brain, especially in basal ganglia (Larisch et al, 2000). We have investigated the possible role of ARTS in 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in the rat. A bolus (1 μ l, 16 μ g) of 6-OHDA was injected into the left medial forebrain bundle of rats, under ketamine-xylazine anesthesia. The animals were sacrificed at various intervals subsequently (1-7 days) and the brains fixed with 4 % formaldehyde in vivo. Sections (30 μ) were cut on a cryotome and ARTS protein revealed by immunofluorescence using an antibody which recognizes the unique C-terminal 21 amino acids of ARTS. Double staining was carried out with TUNEL and antibody to cleaved caspase-3. Cells of the substantia nigra showed an increased level of ARTS expression, and ARTS co-localized with both TUNEL and caspase-3. Western blotting showed no change in the content of ARTS protein in the striatum despite a near absence of tyrosine hydroxylase on the ipsilateral side; the striatal content of ARTS is therefore not originating from dopaminergic neurons of the substantia nigra. ARTS may play a role in the apoptotic death of neurons of the substantia nigra following 6-OHDA administration, which may indicate a role of this protein in neurodegenerative conditions such as Parkinson's disease.

Larisch S, et al. (2000) ARTS, a novel mitochondrial septin-like protein, mediates apoptosis dependent on its P-loop motif. *Nature Cell Biology*, 2:915-921.

Static magnetic field improves survival of cerebellar granule cells in primary culture

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A number of reports in recent years have described actions of magnetic fields on living tissues and organisms, including analgesic effect, vasodilatation, and healing acceleration among others. Magnetic fields may affect cells in a variety of ways. Charged particles moving in a magnetic field will induce an electrical current, and charged macromolecules may attain altered configurations under the effect of magnetic field. Such effects can potentially alter ion shifts across cell membranes. We have examined the possibility that static magnetic field can affect neuronal survival in vitro. Cerebellar granule cells were dissociated from 7-day Sprague-Dawley rat pup brains with trypsin, plated in 30 mm dishes and maintained in culture in MEM-Eagle's medium containing 10% fetal calf serum and 25 mM potassium for 5 days. The dishes were placed either over disc-shaped permanent magnets at various distances from the surface, or on a shielded surface in the incubator with undetectable magnetic field. On the 6th day IV, serum was removed from the medium, and cells were placed either in medium containing 25 or 5 mM potassium without serum for 24 hours. Cells were then stained by exposure to propidium iodide (PI) or calcein-AM, and the numbers of fluorescent cells were counted using a UV microscope equipped with digital color camera (Nikon) and analytical software (Lucia). The number of damaged or dead cells (PI-positive) was expressed as a percentage of total cell number (PI + calcein-AM-positive). Cultures maintained in 25 mM potassium-containing medium and exposed to magnetic field (1000 to 400 G) had a reduced percentage of damaged or dead cells, but the increased cell death induced by reducing $[K^+]$ to 5 mM was not prevented. The mechanism whereby exposure to relatively low magnetic field strengths enhances cell survival is currently under investigation.

Natively unfolded tail domains of voltage-activated potassium channels encode an inter-molecular C-type ball-and-chain mechanism for channel tethering to scaffold proteins

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Natively unfolded protein domains refer to protein segments that are intrinsically disordered under physiological conditions. Important functional roles have been demonstrated for several unstructured protein segments, seemingly challenging the classic structure-function paradigm. Here we demonstrate, using bioinformatics analysis based on the mean charge-mean hydrophobicity content of the target sequences, that the C-terminal segments of voltage-activated potassium channels belong to this growing family of natively unfolded protein segments. Using phylogenetic analysis of the entire Kv channel family we identified a cluster of channel sequences belonging to three out of the four main Kv α subunits (Kv 1, 3 and 4 subfamilies) for which a clear correlation was demonstrated between the probability of the C-terminal channel domain being natively unfolded and the probability of having a consensus scaffold protein (PDZ) binding motif at the last three C-terminal amino acids. Our observations, combined with the analogy of the *intra*-molecular N-type ball-and-chain mechanism for K⁺ channel inactivation, suggest that the C-terminal natively unfolded segments of these voltage-activated potassium channel families encode an *inter*-molecular C-type ball-and-chain mechanism for ion channel binding to scaffold proteins. We found that other ion channel proteins, reported in the literature to interact with the PDZ domains of scaffold proteins, including, among others, voltage-gated sodium and calcium channels and NMDA receptors, are all predicted to have unstructured C-terminal domains. We suggest that this implies a general role of natively unfolded segments in mediating channel-scaffold protein interactions underlying processes such as synapse formation, maintenance and function.

The Protective Effects Of Menadione And Its Derivatives On Ischemic-Reperfusion Heart Damage

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Mitochondrial disorders are characteristic of many myocardial injuries including ischemia reperfusion (I/R) and myocardial infarction. Vitamin K₃ (menadione) and its newly synthesized derivative AK-135, are potent electron carriers between various mitochondrial electron-donating and electron-accepting enzyme complexes. Recently we have shown that cardiomyocytes were protected by menadione (M) and by AK-135 after hypoxia in culture*. The aim of this study was to test whether M or AK-135, will reduce cardiac damage after I/R of isolated rat hearts, *in vitro* mode, and myocardial infarction (MI), *in vivo* mode.

Isolated rat hearts were perfused in the Langendorff mode and subjected to 30 min ischemia (I) followed by 30 min reperfusion (R). M and AK-135 (3 μ M) were perfused before and after I. Using the *in vivo* model, rats underwent left anterior descending coronary artery ligation for 30 min, were reperfused and were then sacrificed after one week. AK-135 was injected pre and post MI.

In the *in vitro* mode, AK-135 demonstrated the best myocardial protection, as seen by left ventricular pressure (LVP), $p < 0.05$, reduced enzyme leakage to the coronary effluent [lactate dehydrogenase ($p < 0.05$), creatine kinase ($p < 0.005$)] and triphenyltetrazolium chloride (TTC) staining compared to the M or I group. In the *in vivo* mode, AK-135 reduced infarct size, as seen by TTC staining ($16 \pm 4\%$ vs $7 \pm 2\%$ $p < 0.04$). Morphological staining with Hematoxylin Eosin, and cytochemical staining of respiratory enzymes (NADH-ubiquinone reductase, cytochrome C oxidase and succinate dehydrogenase), revealed that myocardial structure as well as mitochondrial enzymes were better preserved in this group compared to controls.

In conclusion, the superior tolerance of menadione and even better, AK-135 treated hearts, demonstrating reduction of infarct size and better myocardial function after I/R injury both *in vivo* and *in vitro*, recommends these drugs for therapeutic intervention.

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Transactivation of epidermal growth factor receptor by ouabain in sperm capacitation.

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Introduction: $\text{Na}^+\text{-K}^+\text{-ATPase}$ is a transmembrane enzyme active in transporting Na^+ and K^+ across the cell plasma membrane. Recent studies have demonstrated that low concentration of its specific inhibitor ouabain (0.1 mM) could transactivate multiple signal transduction pathways.

Methods: Specific antibodies for EGFR and p-EGFR were used for immunocytochemistry. Actin polymerization was measured by staining F-actin with Phalloidin FITC. Acrosome reacted cells were detected by Comassie-blue staining and protein tyrosine phosphorylation by western blotting.

Results: Sperm incubated with ouabain (10 μM) revealed a significant stimulation of hyperactivated motility, actin polymerization and protein tyrosine phosphorylation, three parameters known to occur during capacitation. EGF (1ng/ml) stimulated actin polymerization and hyperactivated motility but not protein tyrosine phosphorylation. EGF and ouabain induced EGFR phosphorylation and fast translocation of PKC from the cytosol to the plasma membrane, indicating its activation down stream to EGF. Hyperactivated motility and actin polymerization induced by ouabain were significantly inhibited by specific inhibitors of EGFR, PKC, Src or tyrosine kinases. On the other hand, the effect of ouabain on protein tyrosine phosphorylation was not observed when protein kinase A (PKA) was blocked by H89. Further, the stimulatory effects of ouabain was inhibited only upon cholesterol efflux from the plasma membrane, leading to bicarbonate influx and needed for soluble adenylyl cyclase /PKA activation.

Inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ by incubating the cells in low KCl (1 mM) medium revealed the same effects shown by 10 μM ouabain suggesting that EGFR transactivation occur in response to the $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibition.

Conclusions: The interaction of ouabain with $\text{Na}^+\text{-K}^+\text{-ATPase}$ might regulates sperm capacitation by transactivation of EGFR and PKA and PKC are involved in this process.

Optimal oxygen pressure and time for reduced bubble formation in the N₂-saturated decompressed prawn.

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Bubbles that grow during decompression are believed to originate from preexisting gas micronuclei. We showed in this study that pre-treatment of prawns with 203 kPa oxygen before nitrogen loading reduced the number of bubbles that evolved on decompression, presumably owing to the alteration or elimination of gas micronuclei. The present study examines the optimal pretreatment for this assumed crushing of gas micronuclei. Transparent prawns were subjected to various exposure times (0, 5, 10, 15, and 20 min) at an oxygen pressure of 203 kPa and to 5 min at different oxygen pressures (PO₂ values of 101, 151, 203, 405, 608, and 810 kPa), before nitrogen loading at 203 kPa followed by explosive decompression. After the decompression, bubble density and total gas volume were measured under a light microscope equipped with a video camera. Five minutes at a PO₂ of 405 kPa yielded maximal reduction of bubble density and total gas volume by 52 and 71%, respectively. In the past it was already reported that 2–3 h of hyperbaric oxygen at bottom pressure was required to protect saturation divers decompressed on oxygen against decompression sickness. If there is a shorter pretreatment that is applicable to humans, this will be of great advantage in diving and escape from submarines.

**The Effects of Sarcomere Lengthening Velocity on Cross-bridge Dynamics:
A novel theory for the linear muscle molecular motor.**

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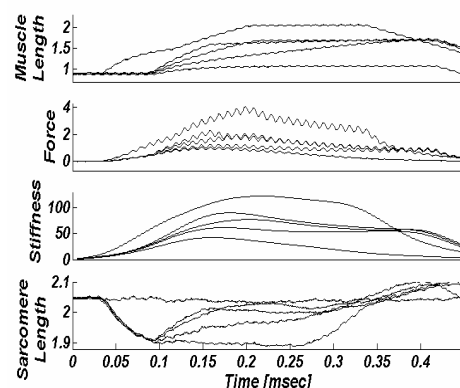
Introduction: The muscle's mechanical function is determined by actin-myosin cross-bridges (XBs). These motor units cycle between the force generating (strong) and the non-force generating (weak) conformations, due to nucleotide binding and release. There is a controversy regarding the mechanism which regulates the transition from strong to weak conformation. The classical prevalent theory suggests that this transition rate depends on the XB displacement (strain). This theory predicts that stretch increases the force by increasing the unitary force per XB while the number of strong XBs decreases. We have suggested an alternative theory of XB dynamics where the kinetic rate of XB turnover from strong to weak conformation is a linear function of the filament sliding velocity (strain rate) and not the displacement. This mechanical feedback, whereby the shortening velocity determines the biochemical rate of ADP dissociation, provides the analytical expression for the empirically established Hill's equation for the force velocity relationship, and for the observed linear relation between energy consumption and the generated contractile mechanical energy.

Objectives: To test the two hypotheses and to determine whether XB dynamics depends on the strain or the velocity. Specifically, the study will quantify the effect of the lengthening velocity on the number of strong cross-bridges and on the force per XB.

Methods: Trabeculae were isolated from the rat right ventricle. Sarcomere length is measured by laser diffraction technique, and is controlled by a fast servomotor. Isometric sarcomere contractions at two different sarcomere lengths (1.9 and 2.05 μm) are obtained by stretching the muscle. Two protocols were used: stretched at different velocities were imposed at the same instant during the twitch (Fig 1), and stretches with similar velocity were imposed at different instants during the twitch (Fig 2). The number of strong XB is derived from the measurement of the dynamic stiffness. The dynamic stiffness is measured by imposing fast (100Hz) and small (3-5nm) oscillations over the stretches. The instantaneous changes in the unitary force per XB were derived from the ratio of the force to the dynamic stiffness.

Stretches were associated with an increase in the force and the stiffness. The faster the lengthening was, the greater was the increase in the force and the stiffness. Tight linear correlation was obtained between the force and the stiffness, implying that the augmentation in the developed force is dominated by the increase in the number of strong XBs. For stretches imposed at different instances the force increased in a similar manner, implying that the phenomenon is independent of the activation level i.e. independent of the initial force at the time onset of lengthening. Hence, the observed effects of stretch on the force and stiffness reflect an inherent property of the individual XB. Since the measured changes in the unitary force per Xb have only a secondary effect on the force changes, the phenomenon is attributed to the effect of the sliding velocity on the rate of XB cycling.

The results validate the hypothesis that XB dynamics is determined by the filament sliding velocity. The results have significant scientific and clinical implications for muscle mechanics and energetics; it explains the observed decrease in ATP consumption during lengthening, skeletal muscle energetics during eccentric contractions and the interactions between normal and dyskinetic areas of the myocardium.



Single α -domain constructs of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, NCLX, oligomerize to form a functional exchanger.

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Two α -repeats are a hallmark of the mammalian $\text{Na}^+/\text{Ca}^{2+}$ exchanger superfamily. Yet several spliced isoforms, containing only a single α -repeat, have been identified although their activity and functional organization is controversial and poorly understood. We report here on the $\text{Na}^+/\text{Ca}^{2+}$ exchange mediated by single α -repeat constructs of a newly discovered member of the $\text{Na}^+/\text{Ca}^{2+}$ exchange family, NCLX. Using a calcium imaging system we monitored $\text{Na}^+/\text{Ca}^{2+}$ exchange in HEK293-T cells expressing either the $\alpha 1$ or $\alpha 2$ single-domain constructs. The sensitivity of the two constructs to the $\text{Na}^+/\text{Ca}^{2+}$ exchanger inhibitor, KB-R7943, however, was distinct, and only the $\alpha 1$ -domain exhibited partial sensitivity to this inhibitor. While both constructs were catalytically K^+ - independent, there was a slight activation effect of K^+ ions for the $\alpha 1$ construct. We then studied the functional oligomeric organization of the single α -domain constructs using a dominant negative approach. Co-expression of the $\alpha 1$ or $\alpha 2$ constructs with a mutated, non-functional $\alpha 2$ -S273T construct had a synergistic inhibitory effect on $\text{Na}^+/\text{Ca}^{2+}$ transport. Further dose dependence analysis of the inhibition of the $\alpha 2$ construct activity by the $\alpha 2$ -S273T mutant indicated that the functional unit is a dimer. Inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange was also monitored when the $\alpha 2$ -S273T mutant was co-expressed with the full length NCLX. Taken together, our data indicate that while single $\alpha 1$ or $\alpha 2$ -domain constructs are independently capable of $\text{Na}^+/\text{Ca}^{2+}$ exchange activity, oligomerization is required for their activity. Since single α -domain isoforms are co-expressed with full-length exchangers at the same tissues their oligomerization may give rise to transport activity of distinct kinetic parameters and physiological roles.

Adenosine A₃ receptor-mediated protection against doxorubicin-induced cardiac mitochondrial toxicity

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The cardiotoxicity associated with doxorubicin (DOX) therapy limits the therapeutic success of active anticancer chemotherapy. The objective of this study was to determine if the protection demonstrated by adenosine A₃ receptor activation (CI-IB-MECA) may prevent intramitochondrial DOX accumulation and Ca²⁺ overload and decrease DOX-induced cardiomyopathy in experiments *in-vivo*.

Methods: Changes in the DOX accumulation and mitochondrial membrane potential were determined with DASPMI probe, mitochondrial Ca²⁺ probe Rhod-2 and with indo-1 probe in newborn cultured cardiomyocytes. Echocardiography was performed in rats *in vivo* 3 weeks after DOX injection. Left ventricular end diastolic diameter, end systolic diameter and fractional shortening (LVEDd, LVESd, FS) were assessed.

Results: protection from damage was found after treatment with 0.5 μM DOX by incubation during the treatment with 100 nM A₃R agonist CI-IB-MECA but not with A₁R agonist. DOX decreased the signal of DASPMI and increased Ca²⁺ cytoplasmic and mitochondrial levels while CI-IB-MECA decreased these effects. Echocardiography data have shown that LVEDd and LVESd were higher and FS was significantly smaller in DOX treated animals compared to the control groups (p<0.01). These damages were significantly smaller in hearts treated with CI-IB-MECA. The stress test showed superior contractility dp/dt_{max} at each dose point in DOX and CI-IB-MECA treated hearts.

Conclusions: Activation of A₃ adenosine receptor promotes cardioprotection against DOX cardiotoxicity by regulation of intracellular Ca²⁺ level and prevention of mitochondrial Ca²⁺ overload.

A new multi-organ monitoring approach for tissue vitality evaluation in a newborn rat

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Pathophysiology of immature brain injury includes impaired water and electrolyte homeostasis, oxygen and nutrients supply and functional responses of major transmitters. In the last decade there has been some use of laser Doppler flowmetry technique (LDF) for cerebral blood flow evaluation mostly in animal experiments. Monitoring of mitochondrial NADH in newborns is rare in experimental animals and absent in clinical conditions.

The aim of the present study was to evaluate in real time newborns tissue metabolic state using LDF and mitochondrial NADH spectroscopy simultaneously, during various perturbations in newborn rat models. The combination of NADH monitoring with the LDF in the mature brain, was previously described. However the application of this unique approach to tissue metabolism monitoring in newborns was not described as yet.

Wistar pups were anesthetized by intra peritoneal (i.p.) injection (20 μ l/gm) of Equithesin solution. The skull was surgically exposed and thinned leaving the dura intact. The fiber optic probe was fixated in position. Several protocols included multi-organ tissue monitoring, in which the brain represented a “vital” organ and the footpad skin – a “less-vital” organ. This model was tested under anoxia and adrenaline injection (i.p.).

Anoxia caused reduction of cerebral blood flow (CBF) in all ages, but was followed by hyperemia only at ages 14 and 17 days. On the other hand, in the skin the kinetic of blood flow change was similar in all ages, namely the time needed for full recovery was the longest at age 8 days and shortened with maturation. Mitochondrial NADH and Reflectance levels increased in both organs, however the amplitudes of change were higher in the cerebral cortex comparing to those recorded from the skin. This phenomenon can be related to the differences in optical properties of these two kinds of tissues, and needs to be further and closer studied.

When epinephrine injection was performed in a 12 day-old pup, severe reduction in skin blood flow and a subsequent NADH elevation was observed. Although sympathetic stimulation is expected to increase CBF in mature rats, no such changes were observed in 12 days-old pups. This may indicate that the Central Nervous System at this stage is still not fully developed, especially its sympathetic branch.

Simultaneous monitoring of cerebral haemodynamics and tissue metabolism is feasible. Hopefully, this new approach will enable improved insight into the field of newborn monitoring in experimental stages and later in clinical practice.

Effects of Low Energy of Visible Light (LEVL) on cell physiology and function

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The effect of low energy visible light (LEVL) irradiation on cell function has recently been recognized. The mechanism of photobiostimulation is based on light absorption by endogenous photosensitizers such as cytochromes and flavoproteins. As these molecules have broad absorption spectra in the visible region, we used a low energy (about 40 mW/cm²) broadband visible light source (400-800 nm) and blue light (400-500 nm) to study cellular changes in sperm, cardiomyocytes and fibroblasts following irradiation. Using blue light, we found an increase in fibroblast proliferation 24 hours after illumination. In sperm cells, visible light illumination induced capacitation and acrosome reaction. These changes can be explained by the generation of reactive oxygen species (ROS) such as O₂^{•-}, ¹O₂ and •OH in cardiomyocytes, sperm and fibroblasts following illumination. The efficiency of ROS production was found to be dependent on cell number and type. By using fluorescence probes such as mito tracker red and dichlorofluorescein, we showed that LEVL increases ROS production in the plasma as well as in the mitochondria. In addition, we showed that illumination of isolated sperm plasma membrane can also produce •OH, which can be inhibited by the NADPH oxidase inhibitor, diphenyleneiodonium (DPI). As the NADPH oxidase system plays an important role in sperm capacitation, via ROS production, the improvement of sperm capacitation following LEVL illumination can be explained.

In addition, we found that light-induced ROS in cardiomyocytes are accompanied by a transient increase in intracellular calcium concentration ([Ca²⁺]_i), which probably affects cellular signalling. We have also found that illumination with broadband visible light increased mitochondrial membrane potential in cardiomyocytes and enhanced ATP production in sperm and cardiomyocyte cells.

We speculate that the mechanism by which LEVL induces biostimulation, includes physiological changes such as an increase in ATP, elevation of [Ca²⁺]_i and production of ROS which may alter cell function by various mechanisms.

Arcuate dopaminergic and endorphinic contribution to cocaine seeking behavior

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The mesolimbic axis that connects the ventral tegmental area to the nucleus accumbens is essential for feelings of well-being and hedonism. Dopamine is usually the neurotransmitter associated with hedonism and reward, but there is recent evidence that β -endorphin may also be involved. β -endorphin is an endogenous opioid peptide that acts as a neuromodulator and neurotransmitter in the central nervous system. The neurons in the brain that synthesize and release β -endorphin are predominantly present in the hypothalamic arcuate nucleus and project a dense innervation to the nucleus accumbens. There is an increase in β -endorphin release in the nucleus accumbens following experimenter-delivered and self-administered cocaine that is mediated by the local dopaminergic system.

In the present study, we used *in vivo* microdialysis to study the effect of a dopamine infusion into the arcuate nucleus on extracellular levels of β -endorphin in the arcuate nucleus and the nucleus accumbens. Dopamine stimulation of the arcuate nucleus increased extracellular levels of β -endorphin in the nucleus accumbens, but there were minimal changes in β -endorphin levels in the arcuate nucleus.

Cocaine injections (2mg/kg i.v.) increased β -endorphin levels in the nucleus accumbens (600%). Infusions of eticlopride HCL, a D₂ antagonist, into the arcuate nucleus during cocaine injection resulted in an attenuation of β -endorphin level increases in the nucleus accumbens. Furthermore, rats trained and maintained to self-administer cocaine displayed extinction behavior after D₂ dopamine receptors in the arcuate nucleus were blocked.

This study shed light on the interaction between dopaminergic and endorphinic systems in the arcuate nucleus that play an integral role in the release of endogenous opioids in the nucleus accumbens, and postulate its important role in the reward system.

Keywords: β -endorphin, dopamine, nucleus accumbens, arcuate nucleus, self administration, microdialysis

ACUTE, NON-GENOMIC EFFECT OF THYROID HORMONES IN PREVENTING CALCIUM OVERLOAD IN NEWBORN RAT CARDIOCYTES

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In this study we examined acute effects of the thyroid hormones T₃ and T₄, leading to improvement of myocardial function through activation of Ca²⁺ extrusion mechanisms and, consequently, prevention of intracellular calcium overload. Elevation of extracellular calcium from 1.8 to 3.8 mM caused an immediate increase in intracellular calcium level ([Ca²⁺]_i) in newborn cardiomyocyte cultures. Administration of 10 or 100 nM of either T₃ or T₄ rapidly (within 10 sec) decreased [Ca²⁺]_i to its control level. Similar results were obtained when [Ca²⁺]_i was elevated either by decreasing the extracellular Na⁺ concentration, which causes backward influx of Ca²⁺ through Na⁺/Ca²⁺ exchanger, or by administration of caffeine, which releases Ca²⁺ from the sarcoplasmic reticulum (SR). Under all these conditions, T₃ or T₄ decreased [Ca²⁺]_i. T₃ and T₄ also had protective effects during ischemia. The presence of T₃ or T₄ during hypoxia for 120 min in culture medium restricted the increase of [Ca²⁺]_i and prevented the pathological effects of its overload. An inhibitor of SR Ca²⁺-ATPase (SERCA2a), thapsigargin, increases [Ca²⁺]_i and in its presence neither T₃ nor T₄ had any effect on the [Ca²⁺]_i level. The reduction of [Ca²⁺]_i level by T₃ and T₄ was also blocked in the presence of H-89 (a PKA inhibitor), and by calmodulin inhibitors. The effect of thyroid hormones (TH) on the reduction of [Ca²⁺]_i was prevented by propranolol, indicating that the hormones exert their effect through interaction with adrenergic receptors. These results support our hypothesis that thyroid hormones prevent calcium overload in newborn rat cardiomyocytes, most likely by a direct, acute, and nongenomic effect on Ca²⁺ transport into the SR.