

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה
ISRAEL SOCIETY FOR PHYSIOLOGY AND PHARMACOLOGY

הכנס השנתי Annual Meeting

9/10/2002

Ma'ale Hachamisha

PROGRAM & ABSTRACTS

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

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

**The Israel Society for Physiology and Pharmacology wishes to acknowledge the following
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MEETING PROGRAM

Wednesday morning, October 9, 2002

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

Session A: New pharmacological targets in atherosclerosis

Chairpersons: **A. Levy**, *Technion, Haifa*
 R. Levy, *Ben-Gurion University, Beer-Sheva*

- 9:30 A1 **A. Levy**, *Technion, Haifa*
Haptoglobin and cardiovascular disease
- 9:55 A2 **E. Ben-Assayag**, *Tel-Aviv University*
 β -fibrinogen gene polymorphism (-455g/a) mediates inflammation degree and is associated with asymptomatic carotid artery atherosclerosis
- 10:20 A3 **B. Fuhrman**, *Technion, Haifa*
Cellular oxidative stress increases in monocytes during their differentiation to macrophages
- 10:45 A4 **R. Levy**, *Ben-Gurion University, Beer-Sheva*
NADPH oxidase and oxidative stress

Session B: Higher Brain Functions

Chairpersons: **S. Hochstein**, *Hebrew University, Jerusalem*
 Y. Dudai, *Weizmann Institute, Rehovot*

- 9:30 B1 **S. Hochstein**, *Hebrew University of Jerusalem*
What perceptual learning tells us about conscious perception itself
- 9:55 B2 **E. Barkai**, *Ben Gurion University*
Enhanced neuronal excitability underlies rule learning in rats
- 10:20 B3 **Y. Dudai**, *Weizmann Institute*
Learning the new and learning anew: dissociations in the molecular machinery of learning, relearning and extinction
- 10:45 B4 **G. Richter-Levin**, *Haifa University*
The amygdala and evaluation processes: Stimulus and response complexity as an organizing factor

Session C: Normal and Abnormal Effects of High and Low Oxygen Tension

Chairpersons: **R. Arieli**, *Israel Naval Medical Institute, IDF*
Y. Grossman, *Ben Gurion University, Beer-Sheva*

- 9:30 C1 **R. Arieli**, *I.N.M.I.*
Modeling pulmonary and CNS O₂ toxicity and estimation of parameters for humans
- 9:55 C2 **M. Horowitz**, *Hebrew University of Jerusalem*
The pathway to heat acclimation involves O₂ sensing signaling
- 10:20 C3 **M. Einan**, *I.N.M.I.*
Heat acclimation prolongs the time to CNS oxygen toxicity in the rat. Possible involvement of HSP72.
- 10:45 C4 **H. Bitterman**, *Technion*
Hypoxia inhibits macrophage iNOS activity by disruption of its interaction with α -actinin4.

11:30 - 12:00
Business Meeting

12:00 - 14:30
Poster Session
Lunch

14:30 - 15:30
Plenary Lecture

Prof. Zvi Selinger
Hebrew University, Jerusalem

**"Substrate Assisted Catalysis:
From Concept to Novel Target for Anti-Cancer Drugs"**

(continued)

Session D: Pharmacogenomics: Application to Cancer and Other Therapies

- Chairpersons: **H. Soreq**, *Hebrew University, Jerusalem*
Y. Caraco, *Hebrew University, Jerusalem*
- 15:30 D1 **Y. Caraco**, *Hebrew University, Jerusalem*
Introduction
- 15:40 D2 **Y. Assaraf**, *Technion*
Loss of transcription factor binding to the human reduced folate carrier promoter as a novel mechanism of anticancer drug resistance in leukemia cells
- 16:05 D3 **G. Rechavi**, *Tel Aviv University*
Functional genomics of cancer
- 16:30 D4 **B-S Kerem**, *Hebrew University, Jerusalem*
Mutation specific therapies for cystic fibrosis
- 16:55 D5 **A. Miller**, *Technion*
"Fit for the benefit": implementation of pharmacogenetics towards the development of 'personalised medicine' in multiple sclerosis
- 17:20 D6 **H. Soreq**, *Hebrew University, Jerusalem*
The pharmacogenomic basis of cholinergic drug responses

Session E: Neurotransmitter Receptors; Implications and Applications

- Chairpersons: **Z. Vogel**, *Weizmann Institute, Rehovot*
B. A. Weissman, *I.I.B.R. Ness-Ziona*
- 15:30 E1 **S.Fuchs**, *Weizmann Institute*
Neurotransmitter receptors in blood lymphocytes as possible peripheral markers for schizophrenia.
- 15:55 E2 **Y. Sarne**, *Tel Aviv University*
Multiple cellular pathways mediate opioid receptor down regulation.
- 16:20 E3 **E. Fride**, *College of Judea and Samaria in Ariel*
Role of cannabinoid CB1 and "CB3" receptors in milk ingestion
- 16:45 E4 **M. Gavish**, *Technion*
Peripheral type benzodiazepine receptors – a new role in cell proliferation and relevance to cancer and survival immediately after birth

Session F: Applied Physiology and Pharmacology

- Chairpersons: **G. Fink**, *Pharmos*
N. Darvish, *Pitango*
- 15:30 F1 **Y. Mika**, *Impulse dynamics Ltd*
Non-excitatory electrical signals, new concept in treatment of heart failure
- 15:55 F2 **T. Bar**, *Digopharm Ltd*
New class of drugs based on 19-Norbufalin regulating the sodium/potassium ATPase to treat congestive heart failure and other diseases
- 16:20 F3 **A. Garzon**, *Q.B.I. Ltd.*
The chemistry of drug design and lead optimization
- 16:45 F4 **A. Avraham**, *Pharmos Ltd*
Gene expression and cellular pathway profiling of anti inflammatory drug candidates

תקצירי
הרצאות מוזמנות

**ABSTRACTS OF
INVITED PRESENTATIONS**

HAPTOGLOBIN AND CARDIOVASCULAR DISEASE

Andrew P. Levy,
Faculty of Medicine and Rappaport Institute, Technion, Haifa

Haptoglobin is a hemoglobin binding protein which is present in two common allelic forms in man. The biochemical and biophysical properties of the protein products of the two different allelic forms of haptoglobin differ dramatically. We will present data demonstrating that the haptoglobin genotype is a newly identified risk factor for cardiovascular disease most prominently in the diabetic state. Data from multiple independent prospective and cross sectional population studies from the USA, Germany and Israel will be presented supporting this contention. These data point towards the widespread use of haptoglobin genotyping to be used in the risk stratification and treatment algorithm of patients at risk for cardiovascular disease.

The molecular mechanisms mediating the differential susceptibility of individuals with the different haptoglobin alleles is not clear. We have demonstrated functional differences in the antioxidant and immunomodulatory properties of the haptoglobin allelic protein products. A specific haptoglobin receptor on monocyte/macrophages will be discussed which mediates the uptake of haptoglobin hemoglobin complexes in a haptoglobin type dependent fashion. The functional implications of this interaction in regards to macrophage activation and increased oxidative stress within the macrophage will be discussed.

The haptoglobin polymorphism exists only in man. We have developed a novel transgenic model that will allow for the testing of the role of the haptoglobin polymorphism in the development of atherosclerotic disease in vivo. Furthermore, these animals will be used as a platform technology in which we can test the ability of novel therapeutic agents that modulate the biochemical activity of haptoglobin in vitro to block the atherosclerotic process in vivo.

β-FIBRINOGEN GENE POLYMORPHISM (-455G/A) MEDIATES INFLAMMATION DEGREE AND IS ASSOCIATED WITH ASYMPTOMATIC CAROTID ARTERY ATHEROSCLEROSIS

Einor Ben-Assayag¹, Irena Bova², David Zeltser³, Shlomo Berliner³, Mali Levkovski³, Itzhak Shapira³, Natan M Bornstein²

¹Department of Human Genetics and Molecular Medicine, Sackler Faculty of Medicine, Tel-Aviv University, Departments of ²Neurology and ³Internal “D”, Tel-Aviv Sourasky Medical Center, Tel-Aviv University, Israel.

Background: The -455G/A polymorphism at the promoter region of the β-fibrinogen gene has been related to plasma fibrinogen concentration and to the severity of coronary artery disease, the progression of atheroma, thrombosis risk and was associated with stroke in Japanese. Since fibrinogen is an acute-phase protein, an increased plasma fibrinogen level may reflect the inflammatory state of the vascular wall. Inflammatory processes may facilitate the transition of clinically stable to unstable atherosclerotic plaques.

Methods: We investigated the relation of this polymorphism with carotid atherosclerosis and inflammation in 162 neurologically asymptomatic individuals. Atherosclerosis was quantified as intima-media thickness (IMT) measured in the common carotid artery and degree of stenosis measured in the internal and external carotid arteries by high-resolution ultrasonography. Inflammation examined using common inflammatory markers and erythrocyte aggregation test by a simple slide technique and image analysis.

Results: The β-fibrinogen -455G/A polymorphism was significantly associated with elevated inflammatory degree and atherosclerosis: elevated plasma fibrinogen level (p=0.033), high-sensitive CRP (p=0.011), WBC (p=0.004), erythrocyte sedimentation rate (ESR), plasma interleukin-6 and increased degree of stenosis in the right carotid artery and slightly increase in mean IMT in the right common carotid artery. Individuals homozygous for the A allele had very high correlation between plasma fibrinogen and ESR (r=0.91, p=0.001) versus individuals homozygous to the G allele (r=0.62, p=0.000). The homozygous AA individuals presented also very high correlation between their other inflammatory markers, erythrocyte aggregation and with their IMT.

Conclusions: These data suggest that the A⁻⁴⁵⁵ allele of the β-fibrinogen promoter is associated with elevated inflammation and increased susceptibility for atherosclerosis in the carotid artery. The data suggest that their fibrinogen is more adhesive, and therefore they have such high correlation between their fibrinogen and ESR. Due to their baseline inflammatory degree, individuals homozygous for the fibrinogen – 455A allele may be at particular risk for thrombotic event following an acute phase stimulus. Once identified, they may benefit from risk factor reduction and early therapy with statins or anti-inflammatory agents.

CELLULAR OXIDATIVE STRESS INCREASES IN MONOCYTES DURING THEIR DIFFERENTIATION TO MACROPHAGES.

Bianca Fuhrman, Nina Volkova, Michael Aviram
Lipid Research Laboratory, Technion Faculty of Medicine and Rambam
Medical Center, Haifa, Israel 31096.

Upon adherence to endothelial cells, blood monocytes enter the arterial wall intima, undergo differentiation into macrophages, and later on convert to macrophage-foam cells, the hallmark of early atherogenesis. In the present study we examined changes over time in cellular oxidative stress, including cell capacity to oxidize LDL, in differentiating monocytes. THP-1 cells were used as a monocyte model system, and were induced to differentiate to macrophages by the addition of PMA (100ng/ml). Monocyte differentiation into macrophages was characterized by: 1) a progressive increase in cellular size, density and granulation; 2) an increased expression of the surface markers CD14 and CD36; 3) a gradual increase in NaF-resistant non-specific esterase activity. The intracellular oxidative stress, measured by a cytofluorimetric assay with 2'7'-dichlorofluorescein-diacetate with a flow cytometer scan, increased progressively during the transformation of monocytes to macrophages by up to 4 fold in the relative fluorescence intensity and by 7 fold in the relative percent of fluorescent cells. HPLC analysis of cellular cholesteryl-linoleate hydroperoxide revealed an increment by up to 3 fold in mature macrophages in comparison to monocytes. In parallel, as monocytes matured into macrophages, their capacity to oxidize LDL increased progressively by up to 2 fold in mature macrophages in comparison to monocytes.

We thus conclude that cellular oxidative stress increases progressively during monocyte differentiation into macrophages, and this effect may be related to enhanced macrophage-foam cell formation and atherogenesis.

NADPH OXIDASE IN PHAGOCYtic CELLS AND ITS REGULATION BY CYTOSOLIC PHOSPHOLIPASE A₂.

Rachel Levy,
Ben-Gurion University in the Negev, Beersheva

The production of superoxides by NADPH oxidase and proinflammatory lipid mediators phospholipase A₂ is one of the most important functions for host defense. However, during altered physiological states, reactive oxygen products may promote inflammatory reactions and participate in processes that lead to tissue injury. Understanding the biochemical processes that regulate this function may provide a means of more effectively controlling the activity of the cells during infection and inflammation. Recently, we have created in the human phagocyte myeloid cell line, PLB-985, a p85 cPLA₂-deficient model cell and demonstrated an essential requirement for arachidonic acid (AA) in activation of the assembled phagocyte NADPH oxidase, in addition to its role as the major enzyme in the formation of pro-inflammatory lipid mediators on nuclear membranes. In order to elucidate the molecular mechanism for the regulation of the oxidase by cPLA₂, we demonstrated the requirement of cPLA₂ for the oxidase-associated H⁺ channel and for the oxidase-associated diaphorase activity. Association between cPLA₂ and the NADPH oxidase was detected only in the plasma membrane of activated neutrophils and in differentiated granulocyte-like PLB-985 cells by coimmunoprecipitation experiments and double-labeling immune fluorescence analysis. The unique presence of cPLA₂ in the plasma membranes is mediated by the NADPH oxidase. The binding these two enzymes and their presence in the same compartment during activation provides a basis for AA-mediated regulation of oxidase activity. Subcellular localization of cPLA₂ to the plasma membranes and to the nuclei at different times after stimulation regulates its participation in different processes in the same cell.

WHAT PERCEPTUAL LEARNING TELLS US ABOUT CONSCIOUS PERCEPTION ITSELF

Shaul Hochstein & Merav Ahissar,
Departments of Neurobiology and Psychology and Center for Neural
Computation, Hebrew University of Jerusalem

Using a battery of perceptual tests we show that the cortical site of modifications underlying perceptual learning is at first at high levels of the cortical visual hierarchy and proceeds gradually to its lower levels. We now propose that explicit vision itself may also advance in this reverse hierarchical direction. This new view of visual perception distinguishes between the flow of implicit and explicit mechanisms, in the following way: Processing along the well-studied feedforward hierarchy may be automatic and implicit, while conscious explicit perception begins at the top of the hierarchy and gradually works its way back down. This explains why our initial conscious percept is of a high-level, generalized and categorical interpretation of the visual scene. We suggest that just as in perceptual learning, later explicit mechanisms may cascade in reverse direction along the same hierarchy, incorporating into conscious perception detailed information available at lower levels. According to this Reverse Hierarchy Theory (RHT), rapid *vision at a glance* identifies "the forest before the trees" and brief presentations suffice to identify object categories. Subsequently, *vision with scrutiny* uses reverse hierarchy routines focusing attention to specific, active, low-level units. This proposal of late reentry to low-level cortex is consistent with delayed top-down effects recently reported for neurons of primary visual cortex.

ENHANCED NEURONAL EXCITABILITY UNDERLIES RULE LEARNING IN RATS

Edi Barkai and Drorit Saar
Center for Brain and Behavior, Haifa University
and Faculty of Health Sciences, Ben-Gurion University

Learning-induced enhancement of neuronal excitability is apparent in piriform (olfactory) cortex pyramidal neurons in rats following olfactory-discrimination training. This enhanced excitability is manifested in reduced spike frequency adaptation. Accordingly, the amplitude of the post-burst after-hyperpolarization (AHP) is significantly smaller in neurons from trained rats, compared to neurons from pseudo-trained and naives. The AHP's amplitude returns back to its initial value within days when training is suspended. This recovery is not accompanied by memory loss, but the enhanced ability to acquire new memories rapidly and efficiently (rule learning) is strongly affected.

The cholinergic agonist carbachol reduces the AHP amplitude in neurons from controls, but not in neurons from trained rats. Similar results are obtained by applying the calcium chelator BAPTA or by PKC activators.

At the behavioral level, application of the muscarinic blocker, scopolamine, prior to each training session, delays rule learning, but has no effect on further acquisition of odor memory.

We suggest that AHP reduction sets a time window in which pyramidal neurons are more excitable and thus activity-dependent synaptic modifications are more likely to occur. Training-induced reduction in AHP results from reduction in an ACh-sensitive calcium dependent potassium current, and maintained by activation of PKC.

**LEARNING THE NEW AND LEARNING ANEW: DISSOCIATIONS
IN THE MOLECULAR MACHINERY OF LEARNING, RELEARNING
AND EXTINCTION**

Yadin Dudai, Diego Berman and Amir Bahar
Department of Neurobiology, The Weizmann Institute of Science
Rehovot 76100

Occasionally we encounter information that is entirely novel and surprising, but often, when we learn, we only modify the information that we already have. Does the brain use the same circuits and molecular devices to encode both types of information? Studies of the cellular and molecular mechanisms of conditioned taste aversion in the rat cortex and amygdala show that this is not the case; the brain distinguishes, already at the molecular level, between learning the new and learning anew.

THE AMYGDALA AND EVALUATION PROCESSES: STIMULUS AND RESPONSE COMPLEXITY AS AN ORGANIZING FACTOR

Yaniv Dan¹, Vouimba Rose-Marie², Diamond David², Aline Desmedt³,
Robert Jaffard⁴ and Richter-Levin Gal¹.

¹Department of Psychology, University of Haifa, Israel, ²Department of Psychology and Neuroscience Program, University of South Florida, USA, ³Department of Neurobiology, The Weizmann Institute, Israel, and ⁴University of Bordeaux, France.

In spite of the fact that the amygdala has been implicated in a variety of functions, ranging from attention to memory to emotion, prevailing theories of amygdala function had derived their principles mainly from its role in conditioned fear. In such theories, the lateral nucleus of the amygdala (LA) is the primary, even unique, interface for incoming conditioned sensory stimuli while the central nucleus is the major output station. However, recent studies indicate that amygdala *output* pathways may be dissociated as a function of the type of conditioned fear behavior. Based on behavioral, electrophysiological and anatomical evidence, the present discussion proposes a modification of the traditional model of *input* pathways to the amygdala such that the LA activation as a sensory interface is limited to relatively simple, unimodal conditioned stimulus features whereas the basal amygdaloid nucleus may serve as an amygdaloid sensory interface for complex, polymodal conditioned stimulus information. We further argue that the partition of amygdalar nuclei according to a complexity dimension appears to correspond both for input and output pathways and thus constitutes a *common* organizing factor in the functional neuroanatomy of the amygdala. The extensive intra-amygdala wiring is assumed to underlie the computations necessary to perform behavioral decisions of various levels of complexity. Compelling new evidence point to a role of the amygdala also in signaling the *prospective* outcomes of emotional responses, thus extending the evaluative roles of the amygdala. Collectively, these results endow the amygdala with a more sophisticated role in guiding behavior by way of emotional-cognitive interactions.

**MODELING PULMONARY AND CNS O₂ TOXICITY AND
ESTIMATION OF PARAMETERS FOR HUMANS**

R. Arieli

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The power expression for cumulative oxygen toxicity for either measurable damage or for the all-or-none phenomenon and the exponential recovery were successfully applied to various features of oxygen toxicity. The parameters of the power equation were solved using non-linear regression for the reduction in vital capacity in humans: $|\% \Delta VC| = 0.0082 \times t^2 (PO_2/101.3)^{4.57}$, where t is the time in hours and PO_2 is expressed in kPa. The recovery of lung volume is: $|\Delta VC_t| = |\Delta VC_e| \times e^{-(0.42 + 0.00379PO_2)t}$, where ΔVC_t is the value at time t of the recovery, ΔVC_e is the value at the end of the hyperbaric oxygen (HBO) exposure and PO_2 is the pre-recovery oxygen pressure. Data from different experiments on CNS oxygen toxicity in humans ($n=661$) in the hyperbaric chamber and data from actual closed-circuit oxygen diving ($n=2039$) were analyzed using a maximum likelihood method. The power equation for active diving: $K = t^2 (PO_2/101.3)^{6.8}$, where t is the time in min, and when K reaches a value of 2.31×10^8 , CNS oxygen toxicity may be expected. It is suggested that the risk of CNS oxygen toxicity in diving can be derived from the calculated parameter of the normal distribution: $Z = [\ln(t) - 9.63 + 3.38 \times \ln(PO_2/101.3)] / 2.02$. The recovery equation: $K_t = K_e \times e^{-0.26 t}$, where K_t and K_e are the values of K at time t of the recovery process and at the end of the HBO exposure, respectively, and t is the recovery time in min. The effect of metabolic rate and CO_2 was quantified.

THE PATHWAY TO HEAT ACCLIMATION INVOLVES O₂ SENSING SIGNALING

Michal Horowitz, Alina Maloyan, Mirit Eynan and Millet Treinin.
Dept. of Physiology, The Hebrew University, Jerusalem

Heat acclimation is a biphasic process comprising earlier, acute transient responses aimed at alleviating the initial heat strain. This is followed by long acting processes, which are delayed in their appearance, but lead to enhanced energy-metabolism efficiency. In the heart, for example, this is demonstrated by rightward shifts of the O₂/Cardiac work relations compared with pre-acclimation. Challenging the heart of heat acclimated animals by ischemic or hypoxic stress reveals that greater ischemic endurance is associated with larger glycogen reserves and greater glycolytic capacity, but at a slower rate. Hence, on heat acclimation, impaired oxygen supply/oxygen demand ratio is compensated for by altered anaerobic pathways. The underlying mechanisms mediating these biochemical modalities have remained obscure. Altered glucose metabolisms, as well as glycolytic enzymes, particularly during oxygen shortage, implicated a role for HIF-1 α transcription factor in these processes. Surprisingly, Western immunoblot analysis showed upregulation of this transcription factor under normoxic conditions in the heat acclimated hearts. This suggested to us that chronic environmental heat switches on oxygen-deprivation signal pathways to achieve heat acclimation homeostasis. To further validate this hypothesis the *C. elegans* genetic model was employed. We found that HIF-1 loss of function mutants were unable to heat acclimate. The similarity between the mammalian species and the nematode *C. elegans* in the demand for HIF-1 mediation upon acclimation implies that the requirements for HIF-1 signaling is evolutionary conserved.

HEAT ACCLIMATION PROLONGS THE TIME TO CNS OXYGEN TOXICITY IN THE RAT. POSSIBLE INVOLVEMENT OF HSP 72

Yehuda Arieli,¹ Mirit Eynan,¹ Hanan Gancz,² Ran Arieli¹ and
Yechezkel Kashi²

¹Israel Naval Medical Institute, IDF Medical Corps, and ²The
Faculty of Food Engineering and Biotechnology, The Technion,
Haifa, Israel.

Oxygen toxicity of the central nervous system (CNS-OT) can occur during diving with oxygen-enriched gas mixtures, or during hyperbaric medical treatment. CNS-OT is characterized by convulsions and sudden loss of consciousness, which may be fatal in diving. Heat acclimation is known to provide cross-tolerance to various forms of stress in different organs, including the brain. We hypothesized that heat acclimation may delay the onset of CNS-OT in the rat. Male Sprague-Dawley rats were acclimated to an ambient temperature of 32°C for four weeks. Rats in the control group were kept at 24°C. Both groups were exposed to oxygen at 608 kPa. EEG was recorded continuously until the appearance of the first electrical discharge preceding clinical convulsions. CO₂ production was measured simultaneously with the EEG. Latency to CNS-OT was measured and brain samples were taken for evaluation of heat shock protein 72 (HSP72) levels by Western blot analysis at the end of the acclimation period and during four weeks of deacclimation. Latency to CNS-OT was twice as long in the heat-acclimated rat, with insignificant changes in CO₂ production. This prolongation continued for two weeks during deacclimation. There was a significant increase in the level of HSP72 following heat acclimation, with a subsequent decrease during deacclimation. During deacclimation there was a linear relationship between latency to CNS oxygen toxicity and the level of HSP72. We conclude that heat acclimation prolongs latency to CNS-OT in a way that does not involve changes in metabolic rate.

HYPOXIA INHIBITS MACROPHAGE iNOS ACTIVITY BY DISRUPTION OF ITS INTERACTION WITH α -ACTININ 4.

H. Bitterman, S. Daniliuc, M.A. Rahat, and N. Lahat. Carmel Medical Center. The Bruce Rappaport Faculty of Medicine, Technion, Haifa 34362, Israel.

Macrophages are key players in inflammatory responses accompanying ischemia and reperfusion (I/R). They express inducible nitric oxide synthase (iNOS), and allegedly secrete high toxic amounts of nitric oxide (NO). We simulated I/R by exposure to hypoxia and reoxygenation (H/R) to evaluate their effects on iNOS expression and activity in macrophages. Mouse monocytic RAW 264.7 cells were subjected to either normoxia (21% O₂, 5% CO₂, 74% N₂) or hypoxia (<0.3% O₂, 5% CO₂, 95% N₂), with or without triggering (100U/ml IFN γ , 1 μ g/ml LPS or their combination) for 24h, or further exposed to reoxygenation for 2h or 24h. iNOS protein was determined by western blots. Supernatants were assayed by Griess reaction for nitrites. Dimers and monomers of iNOS were separated by gel filtration. Protein-protein interactions with iNOS were appraised by immunoprecipitation. Precipitated proteins were identified by mass spectroscopy. IFN γ alone or combined stimulation induced similar high levels of iNOS in normoxia (56.5 \pm 8-fold from control). H/R did not change iNOS protein expression. NO accumulated during prolonged normoxia with IFN γ or both stimulators (156 \pm 33 μ M), but was significantly reduced by hypoxia (13.6 \pm 2 μ M, p<0.01), and only partially restored by prolonged reoxygenation (85 \pm 21 μ M). Hypoxia did not change the ratio of iNOS dimers/monomers, ruling out the possibility that it inhibits iNOS activity by interruption of its dimerization. Hypoxia disrupted the protein-protein interaction between iNOS and α -actinin 4, which was observed in normoxia. The results indicate that hypoxia inhibits the activity of iNOS, but not its expression. iNOS is a cytosolic protein whose localization is not yet known. We suggest that in normoxia iNOS is anchored to the cytoskeleton by interaction with α -actinin 4, previously shown to anchor proteins to the cytoskeleton, and that hypoxia inhibits iNOS activity by disruption of this interaction.

D2

**LOSS OF TRANSCRIPTION FACTOR BINDING TO THE HUMAN
REDUCED FOLATE CARRIER PROMOTER AS A NOVEL
MECHANISM OF ANTICANCER DRUG RESISTANCE IN
LEUKEMIA CELLS**

Prof. Yehuda G. Assaraf, Department of Biology, The Technion, Haifa 32000,
Israel

D3

FUNCTIONAL GENOMICS OF CANCER

G. Rechavi, Tel Aviv University

THE ROLE OF SPLICING REGULATION AS A GENETIC MODIFIER- CYSTIC FIBROSIS AS A MODEL

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A wide variability in disease expression is found among cystic fibrosis patients carrying the same mutations. Among patients carrying splicing mutations, a wide variability in the level of the correctly spliced pre mRNA is found. This variability correlates with disease severity such that higher levels of correctly spliced RNA are associated with a milder phenotype. We thus hypothesize that splicing regulation is a genetic modifier of disease severity in patients carrying splicing mutations. Thus, promotion of higher levels of correctly spliced transcripts is expected to lead to correction of protein function. To test this hypothesis we established epithelial cells lines from polyp samples of two unrelated CF patients carrying the CFTR splicing mutation 3849+10kb C->T, which leads to the insertion of a new 84bp cryptic exon containing an in-frame stop codon between exon 19 and exon 20. The CFTR gene is expressed in these cells, and both correctly and aberrantly spliced CFTR transcripts are generated. The effect of overexpression of factors, known to be involved in regulation of alternative splicing, on the splicing pattern in these cells was studied. This included the cellular splicing factors ASF/SF2, SRp20, SC35, hnRNP A1, Htra2-B1 and the adeno viral splicing factors E4-ORF3, and E4-ORF6. The splicing pattern was analyzed by RT-PCR, and revealed factors that promoted the inclusion of the 84bp cryptic exon and others which promoted the skipping of this exon. The latter led to higher levels of correctly spliced transcripts. We then analyzed the effect of the splicing modulation on the function of the CFTR protein by measuring chloride efflux in the cells using the MQAE fluorescent indicator. We found that in both cell lines, carrying the 3849+10kb C->T mutations, there is no CFTR chloride efflux, similar to the findings in cells from patients homozygous for the severe F508 mutation. Upon overexpression of several splicing factors a significant chloride efflux was observed, indicating activation of the CFTR channel and restoration of its function. Importantly, in cells homozygous for the F508 mutation no activation was detected. Currently, we analyzed the effect of sodium butyrate, known to increase the level of splicing factors, on the CFTR function. Our preliminary results suggest that upon administration of sodium butyrate restoration of the CFTR channel activity can be achieved. Our results indicate that modulation of the splicing pattern of CFTR allele carrying splicing mutations can lead to restoration of the CFTR chloride efflux and thus open new avenues for therapies of patients carrying splicing mutations.

**FIT FOR THE BENEFIT: IMPLEMENTATION OF PHARMACOGENETICS
TOWARDS THE DEVELOPMENT OF 'PERSONALIZED MEDICINE' IN MULTIPLE
SCLEROSIS**

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The goal of pharmacogenetics is to identify “genetic fingerprints” that may predict a patient’s response to pharmaceutical treatment and to provide data that would enable replacement of the “trial and error” strategy which governs much of our clinical decision making regarding treatment allocation in current medical practice, with therapy tailor-made to the individual. A pharmacogenetic research model which intends to implement high-throughput single nucleotide polymorphisms (SNPs) technology to determine the genetic polymorphisms of Copaxone-treated Multiple Sclerosis (MS) patients, subdivided into “good-”, “poor-” or “adverse-responders”, based on correlation between drug-responsiveness and SNPs in specific candidate genes, will be reviewed. Implementation of similar pharmacogenetic approaches may promote the development of ‘personalized medicine’ in MS as well as other diseases.

THE PHARMACOGENOMIC BASIS OF CHOLINERGIC DRUG RESPONSES

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Over 5% of the Israeli population, but only 0.5% of the US population, carry a dominant activating polymorphism in the ACHE promoter. This is associated with AChE overexpression and with hypersensitivity to both anticholinesterases and muscarinic antagonists, inducing symptoms reminiscent of post-traumatic stress disorder (PTSD). AChE's enzymatic activity, the complexity of ACHE gene regulation and the "non-classical" AChE activities call for exploring its relationship with cholinergic drug responses. There are three 3' splicing of AChE variant, with different C-terminal peptides, capacity for multimeric assembly and potentially distinct non-classical function(s). Exposure to stressful stimuli and AChE inhibitors elevate transcription and shift alternative splicing toward overproduction of the normally rare "readthrough" variant, AChE-R. AChE-R mRNA, usually located in neural cell bodies, then translocates to dendritic processes in a rapid, yet long-lasting, manner. This may be beneficial for suppressing the initial insult, but detrimental under long-term conditions. Partially 2'-oxymethylated oligonucleotides inducing AChE-R mRNA destruction can be used to prove the involvement of AChE-R in such detrimental consequences. Antisense agents display high degree of variant selectivity, which enables maintenance of cholinergic neurotransmission via the synaptic AChE, while preventing the stress-induced imbalance among AChE variants. Their therapeutic potential is currently tested in clinical trials.

**NEUROTRANSMITTER RECEPTORS IN BLOOD
LYMPHOCYTES: POSSIBLE PERIPHERAL MARKERS FOR
SCHIZOPHRENIA**

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Schizophrenia is a neuropsychiatric disorder that afflicts about one percent of the population. To date, there is no reliable peripheral marker for identifying schizophrenia. Thus, there is a crucial need for developing some convenient assays for the diagnosis, evaluation and follow-up of schizophrenia during treatment. We attempt to develop reliable markers for schizophrenia by analyzing the correlation between schizophrenia and levels of dopamine receptors and of neuronal acetylcholine receptors (AChR) in peripheral blood lymphocytes (PBLs). We have reported recently on a significant elevation of 2-6 fold in the levels of mRNA that encodes for the D3 dopamine receptor, in PBLs of schizophrenic patients, when compared with healthy controls. In view of recent studies suggesting that neuronal $\alpha 7$ nicotinic AChR ($\alpha 7$ AChR) play a role in the pathogenesis of schizophrenia, we also investigate the levels of mRNA for $\alpha 7$ AChR in PBLs, as another potential biological marker for schizophrenia. In a preliminary study, we have observed a significant decrease (20-95%) of $\alpha 7$ mRNA levels in PBLs of schizophrenic patients, compared with controls. The availability of two different biological markers (D3 and $\alpha 7$) that can both be tested in PBLs, makes the evaluation of schizophrenic patients by biochemical objective tests rather promising.

MULTIPLE CELLULAR PATHWAYS MEDIATE OPIOID RECEPTOR DOWN-REGULATION

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Most G protein-coupled receptors (GRs) undergo down-regulation upon prolonged exposure to agonists. It is well accepted (following the study by Lefkowitz et al. on β -adrenergic receptors [β -AR]) that GR down-regulation involves phosphorylation of the receptor by β -AR kinase (also known as GRK), internalization through clathrin-coated pits, and degradation within the lysosomes. Nevertheless, the literature concerning opioid receptors is inconsistent in regard to the mechanism underlying their down-regulation. In the present study we show that, even within the same cell, several different mechanisms take part in the elimination of functional receptors from the cell surface following chronic exposure to an opioid agonist. Phosphorylation of the receptor can be carried out by any of several kinases, and degradation involves both lysosomes and proteosomes in parallel pathways. Moreover, membrane-delimited proteolysis contributes to down-regulation of opioid receptors under certain experimental conditions.

ROLE OF CB1 AND “CB3” RECEPTORS IN NEWBORN FOOD INTAKE AND SURVIVAL

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It is well established that the *Cannabis sativa* plant (marijuana) stimulates appetite and food intake. This property has been exploited to benefit AIDS and cancer patients by administering marijuana's major active ingredient, Δ^9 -tetrahydrocannabinol (THC). Endogenous cannabinoids (“endocannabinoids”) are found in the brain, peripheral organs, and in maternal milk. We have recently shown that endocannabinoids are critical for milk ingestion in newborn mice, and hence for survival, by administering the CB1 blocker SR141716A.¹ In further experiments, we investigated whether CB1 receptors are solely responsible for the effects of SR141716A in newborns. To this end, we compared CB1 receptor knockout (CB^{-/-}) to C57BL6/J wild type mice. Milk intake, weight gain, and developmental landmarks were recorded. Wild type pups were affected by SR141716A injections at least as potently as Sabra mice: all pups had died by day 4. In CB^{-/-} knockout mice, pup survival was significantly lower compared to wild type, although litter sizes were similar; milk was absent in CB^{-/-} newborns. The CB1 receptor antagonist partially affected milk ingestion and survival in CB^{-/-} pups. We conclude that the initiation of milk ingestion by newborn mice is entirely dependent on the presence of cannabinoid receptors. A major role is played by CB1 receptors, while the partial effect of SR141716A in CB^{-/-} pups supports evidence for the existence of an additional (“CB3”) receptor. *This work was supported by the Danone Research Fund in Israel.*

¹Fride et al., *Eur. J. Pharmacol.* 2001; 419: 207-214.

**PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS: A
NEW ROLE IN CELL PROLIFERATION AND RELEVANCE
TO CANCER**

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The peripheral benzodiazepine receptor (PBR) is localized mainly on the mitochondrial outer membrane. Many cellular functions have been suggested for the PBR; however, its main role in cell biology remains an enigma. We and others have demonstrated a correlation between cell proliferation and PBR expression in some cell types. This led us to ask the question whether PBR dysregulation is involved in diseases of cell proliferation, such as cancer. Our observations on studying PBR binding in different primary human cancers include a 3- to 5-fold increase in PBR ligand binding in ovarian cancer and a similar increase in colon adenocarcinoma. Interestingly, up to a 20-fold increase in PBR density was observed in glioma and astrocytoma. Parallel increases in PBR binding were observed for cell lines derived from the same tissues. On genetically manipulating PBR expression, we found that overexpression in C6 glioma cells reduced cell proliferation, while antisense knockout of a key subunit of PBR in MA-10 Leydig cells made these cells more tumorigenic *in vitro*. It seems that the data we have so far point to a basic function for PBR that, upon disruption, can lead to disease(s) of cell proliferation.

**NON-EXCITATORY ELECTRICAL SIGNALS, NEW
CONCEPT IN TREATMENT OF HEART FAILURE**

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Yehuda Snir, B.Sc. and Shlomo Ben-Haim¹, M.D. Ph.D. Impulse
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Myocardial contractility can be altered using voltage clamp techniques by modifying transmembrane action potential duration. We previously showed that extracellular electrical currents enhance contractility of rabbit papillary muscle and human trabeculae obtained from explanted failing hearts at the time of cardiac transplantation. Along with the increase in contractile force, intracellular recordings have shown that these non-excitatory electrical Cardiac Contractility Modulation (CCM) signals increased the duration of the action potentials. We have also shown that the mechanism by which CCM signals enhanced contractility involved increasing intracellular systolic calcium without change in diastolic calcium and that the sarcoplasmic reticulum is involved in mediating the CCM effect, which was shown to be additive to pharmacologic inotropic effects.

Further studies were performed and showed that CCM signal application during the absolute refractory period of the cardiac cycle augment cardiac contractility in animal models of failing heart and, acutely, in heart failure (HF) patients. Our recent study evaluated the long-term effect of CCM signals on patients with Class III HF (Ejection Fraction (EF) <35%).

The results show that chronic CCM signal treatment to the HF patients significantly improves cardiac contractility and quality of life. A large controlled study is currently being initiated to further determine the long-term effect of this promising therapy.

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NEW CLASS OF DRUGS BASED ON 19-NORBUFALIN REGULATING THE SODIUM/POTASSIUM ATPASE TO TREAT CONGESTIVE HEART FAILURE AND OTHER DISEASES

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¹Digopharm Ltd, Jerusalem, ²Physiology Dept, Medical Faculty, Hebrew University of Jerusalem.

19-Norbufalin is a natural endogenous steroid isolated from human lenses. 19-norbufalin analogs and their synthetic derivatives were found to modulate the activity of the Na⁺,K⁺-ATPase.

A family of 19-norbufalin-based molecules was synthesized, which differ from known cardenolides in some key structure entities, the main difference being the absence of a methyl group at the C-10 site (19-nor steroids).

In-vivo trials performed on rabbits showed a significant increase in $\pm dP/dt$ and in pulse pressure while no arrhythmia events and no clinically important decrease in regional or global left ventricular function were observed.

In vitro trials performed on papillary muscles further supported these findings, where significant increase in tension and in $\pm dT/dt$ were observed following the administration of the 19-norbufalin derivatives.

Both *in vitro* and *in vivo* results are in contrast to results obtained using Digitalis glycosides, the main drugs used nowadays to achieve positive inotropic effect. With digoxin, severe arrhythmias were observed, leading to animal death.

The 19-norbufalin family could open a new era for drugs affecting and regulating the cellular Na⁺,K⁺-ATPase pump, and serve as a basis for the development of drugs to treat various diseases such as those of the autonomic nervous system, renal diseases, cancer and inflammatory diseases.

THE CHEMISTRY OF DRUG DESIGN AND LEAD OPTIMIZATION

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The cost of discovering new drugs has increased dramatically in recent years due mainly to competition and higher level of regulation. On the other hand, genomics and proteomics have offered the opportunity to explore new pharmacological targets.

The medical chemist is thus faced with the urgent need to accelerate the drug discovery process. Different strategies used in the pharmaceutical industry for that purpose will be discussed with examples. The judicious (drug-like properties) screening of libraries (domestic and commercial) of synthetic and natural products combined with HTS has spurred the discovery of new leads. Combinatorial chemistry has reinvented itself in just the past few years from the synthesis and screening of large mixtures to parallel synthesis of individual compounds in an attempt to streamline the discovery and optimization of new leads. The indispensable intuition of the medical chemist is reinforced by modern computational tools (virtual docking) and artificial intelligence. Cheminformatics is pivotal in helping sample management, storing and retrieval of biological data. Early ADMET and PK cassettes are the most recent strategies for providing lead compounds for pharmaceutical development and clinical trial.

**GENE EXPRESSION AND CELLULAR PATHWAY PROFILING OF
ANTI INFLAMMATORY DRUG CANDIDATES**

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Inflammation is a beneficial defense reaction to tissue damage or injury. However, under some pathological conditions, such as autoimmune diseases, brain ischemia or neurodegeneration, inflammation enhances tissue damage and thereby aggravates the disorder. During the inflammatory response, immune-cells migrate to the site of injury, are activated and secrete pro-inflammatory agents such as cytokines, chemokines and eicosanoids – notably the prostaglandins. These processes involve ligand stimulation of cell surface receptors that in turn trigger intracellular cascades that affect gene transcription, metabolism and cell motility. Targeting key molecules that play a role in these signaling networks has been a major goal for the discovery of anti-inflammatory drugs. These points are illustrated by the results of our studies on the mode of action of Pharmos' lead neuroprotective compounds in an animal model for brain ischemia. Analysis of ischemic brains revealed profound changes in expression of genes that encode inflammatory mediators, some of which are modulated by our neuroprotective compounds. Parallel studies in cell culture identified specific transcription factors that regulate these genes and are inhibited by our compounds. Further studies including DNA arrays are in progress to enhance our understanding of drug action in moderating central neuroinflammation.

תקצירי
פוסטרים

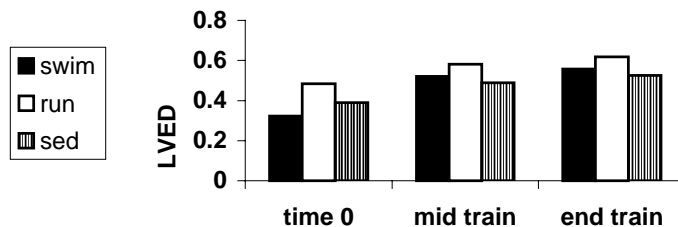
**ABSTRACTS OF
POSTERS**

ECHOCARDIOGRAPHIC ASSESSMENT OF MYOCARDIAL STRUCTURE AND FUNCTION IN SWIMMING-TRAINED AND RUNNING-TRAINED RATS

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Institute; The Heart Institute, Sheba Medical Center; Tel Aviv University.

Background: Recently, a new mode of exercise training for rodents was developed, which include activity wheel that enables to quantify the activity performed in kilometers per time unit. **Purpose:** To investigate the chronic effect of running and swimming exercise training on myocardial structure and function. **Methods:** Sprague Dawley male rats weighing 250-280 grams underwent 1) swimming exercise training for 90 minutes/session, 6 days/ weeks for 7 weeks, n=6; 2) voluntary running activity (Lafayette Instruments) for 7 weeks, n=4, or 3) sedentary, aged-matched animals (n=6). Echocardiography was performed using a 12 MHz transducer. Two-dimensional and M-mode imaging of the left ventricle (LV) were obtained. LV end-diastolic and end-systolic internal diameters (cm) and areas (cm²) were measured, and percent shortening fraction (SF%) and fractional area change (%) and LV mass (gram) were calculated. Measurements were acquired at baseline (time 0), during mid-training, and at the end of the training. **Results:** Running group accomplished 24±16 km/ week. No major differences were observed between the 3 groups in all parameters.

LVED - area



LVED - left ventricular end diastolic area.

Conclusion: Despite the impact of the running-trained group who completed 168 kms/ 7 weeks, echocardiographic analysis of myocardial structure and function showed no differences with the swimming-trained or their sedentary counterparts. Further analyses are on going in order to assess the impact of the various training modes on histological and molecular markers.

CALCIUM CHANNEL BLOCKERS PREVENT ZINC TOXICITY IN MICROGLIA

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Zinc is the second most abundant transition metal in the body after iron. It is essential for normal cellular function and likely serves an additional signaling role in the CNS. The CNS contains high levels of chelatable Zn^{+2} , which is largely localized into synaptic vesicles of excitatory nerve terminals. On its release, Zn^{+2} alters the behavior of several ion channels and receptors, including NMDA and AMPA receptors. Zinc is released from nerve terminals into the extracellular environment after transient global ischemia, sustained seizures and head trauma and may attain concentration of hundreds of micromolar. Such concentrations of Zn^{+2} in the extracellular space may become cytotoxic, thus contributing to the pathogenesis of glial cell-loss. A key step in Zn^{+2} -induced glial death appears to be excessive influx across the plasma membrane, probably through L-type calcium channels (voltage gated). Despite the great importance of such mechanisms, the pathways of zinc entry in glia cells are unknown. Glial cells were cultured from 1 day-old rat brains and grown in culture for 2 weeks. The calorimetric WST-1 viability test was used to determine the extent of microglia death in the presence different concentrations of Zn^{+2} with and without various channel blockers agents. The rate Zn^{+2} influx across the plasma membrane was determined utilizing a single cell fluorescent imaging system. Toxicity was apparent when zinc concentrations exceeded 130 μ M. Specific L-type Ca-channel blockers lowered the Zn influx through these channels and inhibit its cytotoxicity. Taken together we have determined that the L-Type Ca^{+2} channels are a major route for the entry of toxic zinc into microglia.

**ENHANCED PROSTAGLANDIN E₂ RELEASE IN HSV-TK
TRANSFECTED MURINE COLON CANCER CELLS IS NOT
RELATED TO HSV-TK ACTIVITY**

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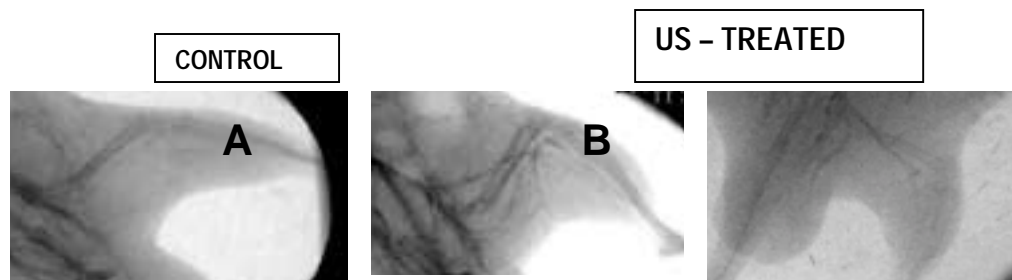
Herpes Simplex virus thymidine kinase (HSV-tk) gene transfection and subsequent treatment with the prodrug ganciclovir (GCV) is a widely used technique for “suicide” gene therapy of cancer. Numerous studies have suggested that production of prostaglandins (PGs) is upregulated in various cancers. We previously showed that HSV-tk gene transfection of tumor cells enhances the release of prostaglandin E₂ (PGE₂) and expression of inducible cyclooxygenase-2 (COX-2), a key enzyme in the biosynthesis of PGs. In this study we aimed to investigate whether the enhanced PGE₂ production is related to HSV-tk activity. Murine colon cancer cells (MC38) were transfected with HSV-tk gene and made resistant to GCV by continuous (6-8 weeks) culturing with increasing concentrations of GCV (0.1-10 μM). GCV resistant cells (MC38/HSV-tk/GCV-R) showed diminished GCV phosphorylation rate as a result of loss or significant decrease of HSV-tk enzymatic activity. However, PGE₂ release in both MC38/HSV-tk and MC38/HSV-tk/GCV-R cells was 5.8-fold higher than in non-transfected MC38 cells. To further clarify the role of HSV-tk activity in PGE₂ release, we utilized Ro 32-2313, a highly potent and selective HSV-tk inhibitor. Treatment of GCV sensitive MC38/HSV-tk cells, which express active HSV thymidine kinase, with 1 μM of Ro 32-2313 completely abolished HSV-tk enzymatic activity, as confirmed by absence of GCV phosphorylation. However, incubation of MC38/HSV-tk cells in the presence of Ro 32-2313 did not alter the enhanced PGE₂ release observed in MC38/HSV-tk non-treated cells. Thus, our findings suggest that PGE₂ upregulation in MC38/HSV-tk cells is unrelated to the actual HSV-tk activity.

EFFECT OF ULTRASOUND IRRADIATION ON THE RECOVERY FOLLOWING SMALL AND LARGE LIMB ISCHEMIA

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Background: Recent studies have demonstrated the capability of ultrasound (US) energy to stimulate angiogenic growth factors in vitro. **Purpose:** To investigate the effect of US irradiation on tissue blood flow following small and large acute limb ischemia in vivo. **Methods:** Sprague Dawley male rats underwent surgical ligation of either the femoral artery (small ischemia 'A', n=24) or the iliac artery (large ischemia 'B', n=12). Half of the animals from each group were exposed to US irradiation using a 2 MHz transducer beginning on the day of surgery, and 1 and 3 days thereafter, for 5 minutes each irradiation. Three weeks post occlusion angiographic images were taken from all rats. Two independent investigators evaluated the images using scoring method of 0 (no blood vessels apparent) to 3 (large number of blood vessels). **Results:** US irradiation significantly improved blood flow of the femoral occlusion animals (from 1.43 ± 0.94 in controls to 2.5 ± 0.59 in US treated, $p=0.02$) but not in the iliac occlusion animals (1.0 ± 1.65).



A – femoral occlusion; B – iliac occlusion

Conclusion: Ultrasound irradiation of small but not large limb ischemia significantly improve collateral formation as evident angiographically. Further studies are necessary to understand the underlying mechanisms associated with such effect.

SELEGILINE AND BOSENTAN AND SURVIVAL IN CONFINED ATMOSPHERE

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Hypoxia develops in confined atmospheres, such as sealed rooms, and sunken submarines. Survival in these conditions depends on O₂ consumption, volume of the enclosed space, and temperature. Previous studies tested the effect of ambient conditions on survival in confined atmospheres, i.e., terminal inspired O₂ pressure (PIO_{2t}) and survival time. The present study tested the effect of two pharmaceuticals in swine: 1) Selegiline, an excitotoxicity protector (n=6); 2) Bosentan, an endothelin receptor antagonist, which inhibits hypoxic pulmonary vasoconstriction (n=5). The control group consisted of eight pigs. On the day preceding the experiment, each immature pig was implanted with EEG and ECG electrodes, a thermistor was located close to the carotid artery and cannulae were inserted into the carotid and pulmonary arteries and jugular vein. On the experimental day the animal was placed in the a sealed chamber while consuming oxygen. Physiological data were monitored until death. The action of Selegiline (10 mg/kg) given in normoxia was tested by its effect on monoamino-oxidase B activity in brain samples. The efficiency of Bosentan (10 mg/kg) given at a PIO₂ of 60 torr was tested by its effect on the pulmonary circulation. Developing hypoxia changed physiological parameters in all groups, although the PIO_{2t} weren't different: 35±11, 37±3 and 30±5 torr for control, Bosentan and Selegiline, respectively. Selegiline failed to reduce PIO_{2t} or extend survival and its sympathomimetic effect induced elevation of breathing frequency and CO₂ output. In the Bosentan group, survival time increased and pulmonary vascular resistance was lowered, but hemoglobin levels were elevated, and stroke volume, pulmonary and systemic vascular resistance were reduced, probably due to pre-experimental lung inflammation and subsequent hypoxic acclimation. This study documents physiological changes during developing hypoxia. Failure to prolong survival by pharmacological protection of these two critical systems warrants a similar investigation of the cardiovascular system.

LACK OF FOOD INTAKE AND REDUCED GROWTH AND DEVELOPMENT IN CB1 RECEPTOR KNOCKOUT MICE: ATTEMPT AT REVERSAL

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The ability of cannabis to stimulate appetite has been used to benefit AIDS and cancer patients suffering from cachexia. Endogenous cannabinoids (“endocannabinoids”) are found in brain, peripheral organs, and maternal milk and activate cannabinoid (CB1, CB2) receptors. We have recently shown that CB1 receptors are critical for milk ingestion in newborn Sabra mice and, hence, for survival and growth.¹

In the present study, we investigated 1) the generality of the critical requirement for CB1 receptors, by measuring the effects of the CB1 receptor antagonist SR141716A in newborn mice of a different strain (C57/BL6), 2) the specific requirement for CB1 receptors by following the development and milk intake in CB1 receptor-knockout (CB^{-/-}) mice. 3) the potential of several cannabinoids to improve growth in CB^{-/-} pups.

Milk intake, weight gain, and development were recorded. C57/BL6 pups were injected with SR141716A on day 1 of life. C57BL/6 pups were affected by SR141716A injections as severely as Sabra pups. In CB^{-/-} mice, pup survival was significantly lower compared to wild types; the neonates did not ingest milk the first day of life. SR141716A partially affected milk ingestion, growth, and survival in CB^{-/-} knockout pups.

Effects of treatment of CB^{-/-} pups with endocannabinoids, WIN55,212-2 or cannabidiol (CBD), a cannabinoid which does not bind CB receptors, will be presented.

We conclude that milk ingestion by newborn mice is entirely dependent on the presence of an intact endocannabinoid-receptor system. A major role is played by the CB1 receptors, while the partial effect of SR141716A in CB^{-/-} pups supports evidence for the existence of an additional (“CB3”) receptor.

¹Fride et al., *Eur. J. Pharmacol.* 2001; 419: 207-214.

**ALTERED EXPRESSION OF COLLAGEN SPECIES IN THE
REMODELED MYOCARDIUM OF *PRIOR* EXERCISE-
TRAINED RATS**

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Background: We have previously shown that swimming exercise training prior to acute myocardial infarction (MI) improves heart function and reduces scar area in the remodeled myocardium. To get an insight into mechanisms underlying this favorable outcome, a high throughput analysis of gene expression was carried out. **Methods:** Rats underwent 7 weeks of swimming regime (Ex) or remained sedentary (Sed). At 7 weeks, animals from both groups were subjected to acute MI (ExMI, SedMI) and were sacrificed after 4 hours, 2 days, or 4 weeks with no additional exercise (ExMI4h, ExMI2d, ExMI4w, SedMI4h, SedMI2d, SedMI4w). RNA extracted from the viable left ventricle (LV) was hybridized to Affymetrix microarrays containing 20,000 rat specific oligoprobes. **Results:** 3,686 expressed genes were detected, 976 of which were regulated 1.5-fold or more in at least one experimental group. Genes associated with fibrosis including collagens type I, III & V, as well as procollagen C-proteinase enhancer (PCPE) were down regulated with exercise and enhanced at 4h after MI in ExMI rats. By contrast, in SedMI, expression of these genes was unchanged early after MI but was markedly enhanced during remodeling (2d & 4w). Collagen XII, a fiber associated low molecular weight collagen, appeared in SedMI hearts in a cluster of genes regulated by hemodynamic load, including ANP and eNOS whose expression increased steeply during remodeling. This cluster was not evident in ExMI: while collagen XII was enhanced by exercise and unchanged during remodeling, ANP and eNOS were unchanged with exercise and increased up to 2d post MI. **Conclusions:** Swimming exercise training down regulates fibrillar collagens, upregulates a tension-associated collagen and lessens their induction following acute MI. These changes may be of significant clinical importance due to their protective effect following AMI.

CENTRAL AND PERIPHERAL EFFECTS OF THE CANNABINOID CANNABIDIOL: A NOVEL RECEPTOR?

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Delta-9-tetrahydrocannabinol (THC) is the major psychoactive constituent of the cannabis plant. THC has additional effects, including antiinflammatory and intestinal, mediated peripherally. The existence of two receptors (CB1, CB2) explains most of the effects of THC. Cannabidiol (CBD) is the major non-psychoactive cannabinoid in cannabis, does not bind CB receptors, but exerts several “cannabinoid-like” effects. To date, no receptor for CBD has been found.

The natural form of CBD is the (-) isomer. We have synthesized a series of (+) and (-) CBD derivatives. None of the (-), but all of the (+) derivatives bind CB receptors.

Presently, we investigated, whether *in vivo* central and peripheral activities of these CBD isomers correspond to their CB binding properties. Mice were injected with CBD, CBD-DMH (dimethyl heptyl), 7-OH-CBD, 7-OH-CBD-DMH, COOH-CBD and COOH-CBD-DMH of both the (-) and (+) series and tested in a series of 4 assays which reflect central cannabimimetic activity and for intestinal motility (to reflect peripheral activity).

(+)7-OH-CBD-DMH induced highly significant central activity, (+)CBD-DMH had very weak central effects, all of which were reversed by the CB1 receptor antagonist (SR141716A). None of the other compounds (mainly –COOH-acids) were centrally active. In contrast, all (-) and (+) derivatives inhibited intestinal activity, which was partially reversed by SR141716A, but not at all by the CB2 antagonist SR144528.

We conclude that 1) the CBD-“acids” do not cross the blood brain barrier, 2) an unknown receptor for (-) and (+) CBD derivatives, mediates their effects on intestinal hypomotility. 3) CBD-derivatives devoid of central effects have therapeutic potential for gastrointestinal and inflammatory conditions.

**DEFINING THE NATURE AND SITES OF INTERACTION
BETWEEN FXYD PROTEINS AND THE Na,K ATPase**

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The FXYD family of one trans-membrane segment proteins appear to act as tissue-specific regulators of Na,K-ATPase. We are looking at interactions of γ , CHIF and phospholemman (PLM) with the α subunit in kidney (γ , CHIF), colon (CHIF) and heart (PLM) membranes. Specific co-immunoprecipitation of α with γ , CHIF or PLM has been demonstrated and characterized. Co-immunoprecipitation is optimal after solubilization with the non-ionic detergent C₁₂E₁₀ in media containing Rb/ouabain or Na/oligomycin. These conditions maintain the protein structure intact as judged by Rb or Na occlusion and Fe-catalysed cleavages. The order of sensitivity to the conditions is CHIF> γ >PLM. For kidney membranes the experiments demonstrate the existence of $\alpha/\beta/\gamma_a$, $\alpha/\beta/\gamma_b$ and α/β /CHIF complexes and exclude mixed complexes such as $\alpha/\beta/\gamma_a/\gamma_b$ and $\alpha/\beta/\gamma$ /CHIF. As a second approach covalent cross-linking of γ and α/β is being studied. We have observed specific cross-links between γ and α , and γ and β in pig kidney Na,K-ATPase. The α - γ cross-link is optimal in conditions stabilizing E₁ conformations (Na, AMPPNP). Using digested renal Na,K-ATPase, "19kDa-membranes", the γ - β cross-link can be located to more than one site including the extracellular domain.

MODULATION OF VDAC AND THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE BY GLUTAMATE

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Voltage-Dependent Anion Channel (VDAC) is an outer mitochondrial membrane and is proposed to be a core component of the permeability transition pore (PTP). Recently (Gincel *et al.*, 2001, *Biochem J.* **358**, 147-155), we reported that purified VDAC reconstituted into lipid bilayers or liposomes is highly permeable to Ca^{2+} and possesses Ca^{2+} binding sites. Ruthenium Red, Ru360 and La^{3+} , known to interact with Ca^{2+} binding sites inhibited the VDAC channel activity. In this study we demonstrate and characterized the interaction of glutamate with VDAC and PTP and modulation of their activities. At physiological concentration, glutamate modified VDAC channel activity by eliminating the bell shape voltage-dependence channel conductance. At low voltages ($\pm 10\text{mV}$), in the presence of 1M NaCl, glutamate (1-20mM) induced rapid fluctuations of the channel between the fully open, low conducting, and the closed states. The PTP activation, as reflected in mitochondria swelling or Ca^{2+} efflux from preloaded rat liver mitochondria, were also inhibited by the same glutamate concentrations. The effects of glutamate on both channel activity and PTP opening are specific since they were not obtained by aspartate, GABA or glutamine. These results suggest the modulation of PTP by glutamate via its interaction with VDAC.

THE RELATION BETWEEN NGF AND BDNF IS DISTINCT IN BRAIN REGIONS AND MICE STRAINS

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The neurotrophic factors, nerve growth factor (NGF) and brain-derived growth factor (BDNF) are involved in neuronal survival, chemoattractant and morphogenesis during brain development. In the adult brain, they also modulate synaptic strength. Both factors are differentially regulated and their levels are varied in different brain regions. We used ELISA to examine NGF and BDNF levels in different brain areas of adult Balb/c and JBC-57 mice. BDNF levels were higher than NGF in all examined areas. In Balb/c mice, BDNF/NGF ratio was 10:1 in the cerebellum and resembling relation was found in the hippocampus and cerebral cortex. However, in the thalamus, NGF levels were constant 1.47 ± 0.77 pg/ug protein (n=8) and independent of BDNF levels. Nevertheless, in JBC-57 mice, both thalamus and cerebellum exhibit constant levels of NGF (1.7 ± 0.61 pg/ug protein, n=12 and 1.9 ± 0.6 pg/ug protein, n=14, respectively). The cortex of these mice showed BDNF/NGF linear relation similar to those observed in Balb/c mice. These observations emphasize the distinct role these factors play in the different brain areas, and indicate that in some brain regions they may act in a synergistic manner. The differences in NGF/BDNF observed between mice strains may suggest a mechanism behind the known behavioral differences between these strains.

**ACTIVATION OF NF- κ B BY HTLV-I Tax PROTEIN AND ITS
ABROGATION BY A NEGATIVE TRANSDOMINANT Tax MUTANT:
POTENTIAL IMPLICATION FOR GENE THERAPY OF HTLV-I
RELATED CLINICAL DISORDERS.**

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HTLV-I has been originally implicated with adult T-cell leukemia (ATL), which is a fatal malignancy with survival time of 6 to 24 months. Later it has been associated with a chronic progressive neurological syndrome called tropical spastic paraparesis/ HTLV-I associated myelopathy (TSP/HAM) and with certain other clinical disorders. None of these diseases is curable by any presently available medication. Therefore, gene therapy might be very helpful in this concern. The pathogenic mechanism of HTLV-I is not completely clear yet, but it is widely agreed that the viral transactivator Tax protein is a key factor in this mechanism and is therefore an attractive target for anti HTLV-I gene therapy. A major effect of Tax involves activation of transcription factors of the NF- κ B family, which regulate the expression of large number of cellular genes. In this study we show that Tax activates a reporter luciferase expressing vector driven by a promoter controlled by NF- κ B [(NF- κ B)Luc]. Moreover, we demonstrate that Tax acts both, at the cell cytoplasm, where it releases NF- κ B from its inhibitor I κ B, and in the nucleus, where it increases the affinity of the free NF- κ B to its binding target in this promoter. We also show that the nuclear Tax activity is essential for NF- κ B full transcriptional effect. We generated a Tax mutant that cannot release NF- κ B from its inhibitor in the cytoplasm (TaxM148). PKC is one of the factors that can induce this release. We found that activation of PKC by the phorbol ester TPA had a moderate stimulatory effect on (NF- κ B)Luc expression (3-4 fold above background). The effect of TaxM148 on this vector was marginal. But the combination of TPA and TaxM148 stimulated (NF- κ B)Luc by 13 fold above background. We also generated a truncated Tax mutant that cannot translocate to the nucleus (Tax Δ 58) but can dimerize with w.t. Tax and trap it in the cytoplasm. This mutant could dissociate NF- κ B from its inhibitor but had a moderate stimulatory effect on (NF- κ B)Luc. It also conferred a moderate (NF- κ B)Luc activation ability on the w.t. Tax. Finally we inserted the M148 mutation to Tax Δ 58 (Tax Δ 58M148). This mutant had no effect on (NF- κ B)Luc and strongly abrogated the effect of the w.t. Tax on this reporter construct,

indicating that Tax Δ 58M148 can strongly block NF- κ B activation by the viral Tax protein.

**CARDIAC STEROIDS INDUCE SPECIFIC CHANGES IN
MEMBRANE TRAFFICKING IN HUMAN NT2 CELLS**

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Cardiac steroids (CS) and CS-like compounds are present in human tissues but their physiological role has not been elucidated. Treatment of human neuronal NT2 cells with nanomolar concentration of CS caused the accumulation of 'mega vesicle-like structures' (MVS) adjacent to the nucleus. Apoptosis but not MVS accumulation was detected when NT2 cells were treated with etoposide indicating that the MVS accumulation is a CS-specific effect. Experiments using FM1-43 showed that the MVS are product of endocytosis. The effects of CS on FM1-43 accumulation were dose- and time-dependent and reversible. CS-induced FM1-43 accumulation was observed also in astrocytoma SF676, TE671 neuroblastoma and the kidney epithelial 293T cells, indicating the generality of this phenomenon. Partial inhibition (50%) of Na⁺, K⁺-ATPase activity by reducing extracellular K⁺ concentration in the growth medium did not affect FM1-43 accumulation while the treatment with bufalin which caused a similar inhibition of Na⁺, K⁺-ATPase activity caused a striking increase in the accumulation of FM1-43 within the cells. The results demonstrate that CS, at physiological concentrations induces changes in membrane trafficking, independent of inhibition of Na⁺, K⁺-ATPase activity. This underlines a novel role for the Na⁺, K⁺-ATPase protein and the endogenous CS as physiological regulators of membrane trafficking.

**CARDIOVASCULAR EFFECTS OF MAO INHIBITORS:
MODIFICATION OF BARORECEPTOR ACTIVITY IN
CONSCIOUS RATS BY CLORGYLINE BUT NOT SELEGILINE.**

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Clinical studies have pointed to a depressor effect of selegiline on blood pressure in Parkinsonian patients, possibly mediated via an effect on the baroreceptor reflex system. Baroreceptor activity was estimated in conscious rats treated daily for 7 days with selegiline (1 or 5 mg/kg p.o.), clorgyline (2 mg/kg p.o.) or saline by determination of blood pressure (BP) and heart rate (HR) responses to i.v injections of a vasoconstrictor (phenylephrine 2-20 μ g/kg) and a vasodilator (sodium nitroprusside 1-40 μ g/kg). The BP-HR responses were fitted individually to sigmoidal equations. The values of the HR plateaus and HR range were not significantly different between the 4 treatment groups. The average gain of the HR/BP curve in selegiline-treated rats was not different from the control group, but the gain value was approximately twice as high in the clorgyline-treated rats. This finding correlates with our earlier reports of reduced sympathetic activity following acute clorgyline treatment in anesthetised rats, but does not indicate an effect of selegiline on baroreceptor activity in the rat. On the other hand, we find that combined treatment of conscious rats with selegiline (5 mg/kg daily for 8 days) and L-DOPA/carbidopa (50/12.5 mg/kg twice daily) causes a small but significant fall in mean blood pressure.

**UPREGULATION OF CYCLOOXYGENASE-2 EXPRESSION AND
ACTIVITY IN HUMAN AND MURINE TUMOR CELLS
TRANSFECTED WITH HSV THYMIDINE KINASE GENE**

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Transfection of tumor cells with the Herpes Simplex virus thymidine kinase (HSV-tk) gene and subsequent treatment with the prodrug ganciclovir (GCV) is the most common system utilized to date for “suicide” gene therapy of cancer. It is well established that cyclooxygenase-2 (COX-2), a key enzyme in the biosynthesis of prostaglandins (PGs), is overexpressed in various cancers. We previously reported that transfection of murine colon cancer cells (MC38) with HSV-tk gene augments the release of prostaglandin E₂ (PGE₂). The objective of this study was to further evaluate the mechanism and specificity of HSV-tk gene transfection on upregulation of PGE₂ production. The release of PGE₂ was examined in rat gliosarcoma (9L) and human urinary bladder cancer cells (T24) transfected with HSV-tk gene. PGE₂ release was more than 5-fold higher in HSV-tk transfected cells, compared to parental, non-transfected cells. COX-2 expression, as determined by Western blot, was significantly enhanced after HSV-tk transfection in these tumor cell lines. Furthermore, PGE₂ release, measured in MC38 tumor model *ex-vivo*, was 2.5-3.5-fold higher for HSV-tk transfected tumors compared to non-transfected tumors. To elucidate the specificity of the effect of HSV-tk gene transfection on PGE₂ release and COX-2 expression, MC38 cells were transfected with either neomycin resistance (NeoR) or HSV-tk gene using LNL6 and STK vectors, respectively. LNL6 vector, which encodes for neomycin phosphotransferase II, only differs from STK vector by lacking HSV-tk sequence insert. Interestingly, NeoR gene transfection of MC38 cells resulted in a significant decline in COX-2 expression and PGE₂ release. Our data suggest that HSV-tk gene transfection augments PGE₂ release both in tumor cells and in an *ex-vivo* tumor model by enhancing COX-2 expression. Moreover, these results show that increased COX-2 expression and activity in HSV-tk transfected cells is correlated with the presence of HSV-tk sequence insert in the vector used for HSV-tk gene delivery.

CARDIAC RESISTANCE TO DOXORUBICIN TOXICITY ACHIEVED BY ADENOSINE SIGNALLING

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The anthracycline antibiotic Doxorubicin (DOX), also called Adriamycin, have been used for more than 30 years for the treatment of a variety of malignances. However, the clinical use of DOX is limited because of its serious cumulative dose-dependent cardiotoxicity, which leads to irreversible degenerative cardiomyopathy. Pharmacological methods of interrupting the side effects of DOX have involved numerous antioxidants, Ca channel blockers and other compounds. Unfortunately, none of these compounds has been proven to be effectively cardioprotective. Adenosine (ADO), the purine nucleoside, is a well-known regulator of a variety of physiological functions in the heart. Recently we have shown that stimulation of the A_3 but not the A_1 adenosine receptor (A_3R and A_1R) with the highly selective agonist Cl-IB-MECA, reduced cardiomyocyte damage induced by doxorubicin and prevented intracellular calcium overloading. In this work we aimed to investigate the signaling leading to cardiac protection after DOX treatment. The kinetics of mitochondrial membrane potential were monitored by cytospectrofluorimetry of DASPMI fluorescence using succinate for mitochondrial energy hyperpolarization and FCCP for its depolarization. It was found that when cardiac cells grown in culture were treated with 0.5 μ M DOX in the presence of A_3R agonist, the intensity of DASPMI fluorescence was similar to the control, whereas DOX alone caused a decrease in the intensity of mitochondrial fluorescence. The present study identifies a novel role for A_3R agonist Cl-IB-MECA and suggests its importance in regulating cardiac cellular function. Understanding the mechanism by which DOX exerts its damaging effects on the heart and its attenuation by A_3R activation might instruct us how to utilize DOX in the most efficient way for cancer treatment.

EVIDENCE FOR THE EXISTENCE OF HUMORAL PHOSPHATE SIGNAL EMANATING FROM THE DIGESTIVE SYSTEM

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A diet deficient in phosphate led to suppression of growth in juvenile rats. Addition of phosphate to drinking water of those rats restored growth without a concomitant increase in serum phosphate concentration (Landsman et al Brit. J. Nutrition 86: 217, 2001). Similarly, the level of phosphate in the diet affects renal handling of phosphate independent of the changes in plasma phosphate concentration. Thus, the existence of phosphate signals that emanate from the digestive system is suggested. In order to test the possibility that effects of dietary phosphate on kidney are mediated by humoral factors in plasma, OK cells were incubated in media, containing 10% of plasma of rats exposed to low, normal or high phosphate intake. The phosphate concentration in the culture media was always the same. After 24 hours of incubation the activity of the sodium-phosphate transporters in these cells was determined. The results showed that the sodium-driven phosphate transport in OK cells, cultured in a medium containing 10% of plasma of rats exposed to high phosphate was suppressed, compared to cells cultured with plasma of rats that were phosphate restricted. Thus, at least part of the dietary Pi effect on the kidney is mediated by a humoral factor(s).

ROLE OF ADENOSINE A₁ & A₃ RECEPTORS IN REGULATION OF CARDIOMYOCYTE HOMEOSTASIS AFTER MITOCHONDRIAL RESPIRATORY CHAIN INJURY

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Activation of either A₁ adenosine receptor (A₁R) or A₃ adenosine receptor (A₃R) elicits protection against infarction, ischemia or hypoxia. Now it is evident that cardiomyocytes protection may be mediated via mitochondrial rather than sarcolemmal K_{ATP} channels. Mitochondrial contribution in the progression of cardiomyocytes injury is well known, however the protective efficiency of adenosine receptor activation and mitochondrial K_{ATP} channel opening in cardiac cells with the respiratory chain deficiency needs to be elucidated. The aim of our study was to further define the role of A₁ and A₃ receptor activation on functional tolerance against inhibition the terminal link of the mitochondrial respiratory chain with sodium azide, in a state of normoxia or hypoxia, and to compare those with effects of the mitochondrial K_{ATP} channel opener diazoxide. It was shown in this study that protective effect of the A₁ adenosine agonist against irreversible damage was more noticeable when intact cardiomyocytes were exposed to hypoxia. Treatment with 10 mM sodium azide for 2h in normoxic conditions caused a considerable decrease in the total level of ATP. Activation of adenosine receptors showed a protective action, but in this case the effect of A₃ receptor activation was more pronounced than that of A₁ receptors. Diazoxide (100 μM) was less effective. After treatment of cultured cardiomyocytes with hypoxia in the presence of 1mM sodium azide, the A₁R agonist CCPA was ineffective but the A₃R agonist Cl-IB-MECA significantly restricted the decrease in ATP level. Adenosine A₁ agonist CCPA and A₃ agonist Cl-IB-MECA were effective in retarding a decrease in DASPMI fluorescence and, hence, dissipation in mitochondrial membrane potential during 60min of hypoxia. The protective activity of Cl-IB-MECA was more evident. In conclusion, the cascades of events involved in cardioprotection appear to be distinct for A₁ and A₃ receptor signaling. This may be important for the development of effective pharmacological agents when mitochondrial dysfunction is a leading factor in the pathophysiological cascade of heart disease.

**REGULATION OF CROSS BRIDGE RECRUITMENT IN THE RAT
CARDIAC MUSCLE.**

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The study tests the hypothesis that XB recruitment is regulated by a cooperativity mechanism whereby the number of strong XBs determines the affinity of troponin for calcium. The hypothesis predicts that there is no unique force-length relation at constant activation and the force depends on a short term history of contraction. The study examined the force responses to slow and small (40 ± 11.02 nm) sarcomere length (SL) oscillations, at constant free Ca^{2+} concentration, during steady tetanus contractions. Eight trabeculae were obtained from rat right ventricles and were tetanized using cyclopiazonic acid. SL was measured by laser diffraction techniques. The number of strong XBs was evaluated by measuring the stiffness, utilizing high-frequency (50 Hz) oscillations. The force response lagged the SL oscillations at frequencies smaller than 4Hz (111.87 ± 40.98 msec at 1 Hz) and a counter clockwise hysteresis was obtained between the force and the SL, in accordance with the prediction. At higher frequency (>4 Hz) the force preceded the SL. The phase shift in the stiffness relative to the SL resembled the phase shift in the force. The study establishes the existence of a cooperativity mechanism that regulates XB recruitment. This mechanism can explain Frank Starling Law and the dependence of sarcomere energy consumption on the prevailing loading conditions.

**SPECIFIC INTERACTIONS BETWEEN CHIF AND THE
NA, K-ATPase**

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CHIF is a member of the FXYD family which also include the γ subunit of Na,K-ATPase. Both proteins have been shown to regulate pump function. We have characterized physical interaction between CHIF and α in CHIF transfected HeLa cells, expressing the $\alpha 1$ subunit of the rat Na, K ATPase. Like γ , CHIF could be immunoprecipitated from the transfected cells solubilized in $C_{12}E_{10}$ by an anti- α antibody. Stability of the CHIF/ α complex in $C_{12}E_{10}$ was considerably lower than that of the γ/α complex. FXYD domains involved in the interaction with α have been identified by studying CHIF- γ chimeras. Chimera having the extracellular N-termini of γa or γb and transmembrane (TM) of CHIF failed to translate protein even though, FXYD mRNA levels were comparable to those of CHIF. Other chimeras were immunoprecipitated by the anti- α antibody. Chimeras having the TM domain of CHIF formed complexes with low stability in $C_{12}E_{10}$ while those with the TM domain of γ formed complexes with stability comparable to that of γ . Furthermore, mutations in the transmembrane domain of CHIF increased complex stability. Thus, the TM domain of FXYD proteins plays a key role in their association with α .

DIGOXIN DISPOSITION DURING PREGNANCY IN THE MOUSE

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P-glycoprotein (Pgp), a multispecific efflux protein, is the main constituent of most barriers in the body, namely, the blood-brain barrier, placental barrier, intestinal and testicular barriers. Progesterone (Prg) was shown to inhibit Pgp in several cell lines. During pregnancy the levels of Prg increases significantly. In the present study we investigated the integrity of barriers during pregnancy in mice, following the administration of digoxin, as a substrate of Pgp. [³H]-Digoxin was administered intravenously (0.05 mg/kg) to virgin and pregnant mice (at the end of 1st, 2nd and 3rd trimester). Cyclosporine A (CsA) was administered intravenously to an additional group of pregnant mice, 1 hour prior to digoxin administration. The mice were sacrificed at 4 and 24 hours after digoxin administration. Digoxin content was determined in selected tissues (after tissue solubilization) by liquid scintillation counting. Prg levels in plasma were determined by RIA. The results were analysed by ANOVA or t-test. Most of the digoxin was found in the gastrointestinal tract, both at 4 and 24 hours (20-50% and 0.3-2% of the dose/g tissue, respectively). There was increase in the brain, kidney and liver content of digoxin in pregnant mice (all stages) compared to non-pregnant mice at 4 and 24 hours after dosing. No correlation was found between Prg plasma concentration and digoxin distribution in pregnant mice. In CsA-treated pregnant mice the levels of digoxin at 4 and 24 hours were significantly higher in all tissues examined, except for the levels in gastrointestinal tract, which at 4 hours were significantly lower compared with pregnant mice at the same stage. It is concluded that during pregnancy the activity of Pgp is slightly impeded, but not completely inhibited as in CsA-treated mice.

PROTECTION OF SKELETAL MUSCLE FROM HYPOXIC DAMAGE BY ACTIVATION OF ADENOSINE RECEPTORS

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In cardiac muscle, activation of adenosine receptors has been found to correlate with improved heart survival after ischemia-reperfusion injury. Comparable studies in skeletal muscle are rare. It is widely accepted that adenosine is released within tissues under hypoxic conditions. This adenosine, by activating adenosine receptors, protects against ischemic damage by a mechanism not completely known. The aim of the present study was to test the working hypothesis that stimulation of the A₁ and A₂ adenosine receptors (A₁R and A₂R) with the highly selective agonists CCPA and C-141 would reduce skeletal muscle damage after hypoxia. The hypoxic conditions were simulated by exposure of cultured rat skeletal muscle (6-7 days in vitro) to an atmosphere of N₂ (99.9%) in glucose-free medium for 1.5 hr. The hypoxic damage was analyzed by measuring creatine kinase activity released to the medium. Our results show that A₁R agonist CCPA and A₂R agonist C-141 can protect cultured muscle against hypoxia-induced injury. A₂R agonist C-141 after 1.5 hr hypoxia was more effective, but activation of both A₁R and A₂R together gives better protection against hypoxia than by each one alone. Western blot analysis of α -sarcomeric actin and desmin demonstrated that the level of these proteins were markedly enhanced with agonist A₁R and A₂R treatment a during hypoxia. The ATP and glycogen levels also show protection of A₁R and A₂R in muscle culture after 1.5 hr hypoxia. When intracellular Ca²⁺ was analyzed in skeletal muscle subjected to hypoxia, it was found that [Ca²⁺]_i was gradually elevated. However pretreatment with A₁R or A₂R agonists prevented [Ca²⁺]_i elevation. In conclusion, our data establish that adenosine can mediate muscle protection by acting via A₁R and A₂R adenosine receptors. However, research must be continued in order to elucidate the mechanisms of A₁ and A₂ receptor signaling, which appears to be distinct.

CHARACTERIZATION OF A NOVEL $\text{Na}^+/\text{Zn}^{2+}$ EXCHANGER

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Zinc influx driven by steep inward transmembrane zinc gradients, occurs in excitatory and secretory cells, playing a fundamental role in zinc signalling and in pathophysiological conditions linked to accumulation of toxic zinc in cells. Yet, the cellular transport mechanisms that actively generate or maintain the zinc transmembrane gradients are unknown. Zinc transport was monitored in HEK 293 cells utilizing a microscope based single cell fluorimetric system. Pre-treatment of the cells with CaPO_4 precipitates induced the activity of $\text{Na}^+/\text{Zn}^{2+}$ exchange. The exchanger catalysed Na^+ dependent Zn^{2+} extrusion against a 1000 fold transmembrane zinc gradient. Reversal of cellular Na^+ gradient triggered net Zn^{2+} influx. The stoichiometry of the exchanger was determined to be $3\text{Na}^+/\text{Zn}^{2+}$ ions. Depolarization by exposure to high K^+ containing medium led to increase in zinc efflux which may indicate allosteric effect of potassium on the exchanger. To our knowledge the $\text{Na}^+/\text{Zn}^{2+}$ exchanger is the first example of a mammalian transporter capable of Na^+ dependent active extrusion of zinc. Such system may play a key physiological role, not only in generating the transmembrane zinc gradient, but also in protecting cells from the potentially toxic permeation of this ion.

**PATHWAY OF ZINC PERMEATION INTO PANCREATIC
β CELLS**

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Pancreatic islet β cells contain a substantial amount of chelatable zinc (Zn^{2+}), where it acts as a structural component of insulin packaging. Specifically, Zn^{2+} in β cells is involved in the formation of insoluble insulin hexameres in secretory granules, and is also co-secreted with insulin after stimulation with secretagogues. Little is known about the permeation pathway of zinc into β cells. In the present work, we have studied possible influx pathways of zinc into the insulinoma cell line, Min-6, using single cell fluorescent imaging. Depolarization of cells was followed by a massive influx of zinc. This was inhibited by the dihydropyridine compound, Nifedipine (0.5 μM), indicating that zinc permeates through L-type Ca channels (LTCC). Zinc influx persists in the presence of physiological concentrations of Ca^{2+} but in contrast to Ca^{2+} , was not pumped out. The dose response of zinc permeation through LTCC showed a $K_{0.5}$ close to the physiological level of zinc released in the islets. Therefore, our results indicate that LTCC is an important pathway for zinc permeation into β cells, and consequently, may contribute to zinc toxicity in these cells.

DETECTION OF PORCINE OLEIC ACID-INDUCED ACUTE LUNG INJURY USING PULMONARY ACOUSTICS

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To evaluate the utility of monitoring the sound filtering characteristics of the respiratory system in the assessment of acute lung injury, we injected a multi-frequency broadband sound signal into the airway of five anesthetized, intubated pigs, while recording transmitted sound over the trachea and on the chest wall. Oleic acid injections effected a severe lung injury predominately in the dependent lung regions, increasing venous admixture from 6 ± 1 to 54 ± 8 % ($p < 0.05$), and reducing dynamic respiratory system compliance from 19 ± 0 to 12 ± 2 ml/cmH₂O ($p < 0.05$). A two to fivefold increase in sound transfer function amplitude was seen in the dependent ($p < 0.05$) and lateral ($p < 0.05$) lung regions; no change occurred in the nondependent areas. High within-subject correlations were found between the changes in dependent lung sound transmission and venous admixture ($r = 0.82\pm 0.07$; range 0.74 – 0.90) and dynamic compliance ($r = -0.87\pm 0.05$; -0.80 – -0.93). Our results indicate that the acoustic changes associated with oleic acid-induced lung injury allow monitoring of its severity and distribution.

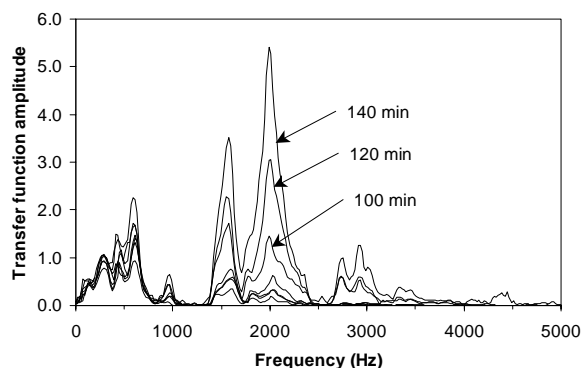


Figure: Multiple graphs of dependent lung Acoustic Transfer Function Amplitude during the worsening of Oleic Acid acute lung injury in one pig. Note the progressive prominence of the transmission peaks around 2 kHz. Arrows and times indicate duration from onset of Oleic Acid administration.

**POSSIBLE INTERACTION BETWEEN PGE2 AND CYCLIC
NUCLEOTIDE-GATED CHANNEL (CNG) IN BOVINE AORTIC
ENDOTHELIAL CELLS**

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Low concentrations of heavy metals that block ion channels such as barium, cadmium and cesium increased prostaglandin E2 (PGE2) binding to bovine aortic endothelial cells, while nickel decreased the binding. Verapamil, a calcium channel blocker, and glibenclamide, an inhibitor of ATP-sensitive K(+) channels, enhanced PGE2 binding. On the other hand, Bay K-86144, a calcium channel activator, and DIDS, a chloride channel inhibitor, decreased binding. Incubation of the intact cells with permeable analogs of cAMP, cGMP or with phosphodiesterase inhibitors resulted in attenuation of PGE2 binding to the cells. The reduction of PGE2 binding by cyclic nucleotides analogs was fast, completely reversible and unaffected by protein kinase inhibitors. These observations favor direct and reversible interaction of cGMP and cAMP with their cellular target. Permeable cGMP analogs that activate cyclic nucleotide gated channels (CNG) as Rp-8-Br-PET-cGMPS and Sp-8-Br-PET-cGMPS, reduced PGE2 binding. Pimozide, and diltiazem a calcium channel blockers and LY-83,583, a guanylate cyclase inhibitor that were reported to block CNG channels enhanced PGE2 binding. The presence of CNG channel-2 in bovine aortic endothelial cells was confirmed by western blot analysis. Permeable cAMP and cGMP were shown to cause a fast increase in the cellular calcium concentration. Overall, the data may suggest that the PGE2 binding site in bovine aortic endothelial cells is a part of, or is associated with, a CNG membrane ion channel.

**CONFORMATIONAL REARRANGEMENT ASSOCIATED WITH
THE GATING OF THE G PROTEIN COUPLED POTASSIUM
CHANNEL REVEALED BY FRET MICROSCOPY**

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G protein coupled potassium channels (GIRK/Kir3.x) are key determinants that translate inhibitory chemical neurotransmission into changes in cellular excitability. These channels are activated by the binding of the G $\beta\gamma$ subunits of G protein in concert with other intracellular components. To understand the mechanism of channel activation by G proteins it is necessary to define the structural rearrangements in the channel that result from interaction with G $\beta\gamma$ subunits. In this study we used a combination of fluorescence spectroscopy and through-the-objective total internal reflection microscopy to monitor the conformational rearrangements associated with the activation of GIRK channels. Conformational changes were assessed from changes in the efficiency of fluorescence resonance energy transfer (FRET) between CFP and YFP attached at various positions in the cytosolic domains. We detect activation-induced changes in FRET consistent with a rotation and expansion of the termini along the central axis of the channel. We propose that this rotation and expansion of the termini drives the channel to open by bending and possibly rotating the second transmembrane segment.

SUBJECTIVE FEELING, PERFORMANCE AND PHYSIOLOGICAL STRAIN WHILE DRIVING AFTER ALCOHOL INTOXICATION

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The aim of this study was to investigate the relationships among subjective feeling, performance and physiological strain, while driving under the influence of two blood alcohol concentrations (BAC), 0.05% and 0.1%. 12 subjects, 6 men and 6 female were asked to drive in a car simulator after drinking alcohol. Experimental protocol called for three separate sessions for each subject: 1. No alcohol consumed (control). 2. Alcohol consumed to reach a level of 0.05%. 3. Alcohol consumed to reach a level of 0.1%. Each session lasted 35 minutes: 5 minutes rest before drive (B), 20 minutes drive (D) and 10 minutes recovery (R). Before and after each session, subjects were asked to fill a "Fatigue Inventory-20" (Aahsberg 1998) questionnaire.

Four indicators of performance were used; of these, only the ability to maintain lane position was affected by both levels of alcohol. Root mean square (RMS) of steering deviation was different only at the high alcohol concentration, while RMS of linear speed and average speed were not affected at all. All subjects reported that driving was difficult only at the high alcohol level.

Alcohol increased heart rates, but no further increase was measured during the driving period compared to initial rest values. HRV and the HF band of the RR spectra decreased significantly at high alcohol levels. Ratios of Beta/Alpha waves of the EEG spectra showed that subjects were sleepier while driving at the high alcohol concentration.

In summary, subjective feelings and measurements of the autonomic nervous system activity seem to be a sensitive indicator of the stress imposed by alcohol, while performance measures showed inconsistent sensitivity to alcohol intoxication.

HTLV-I Tax-INDUCED INHIBITION OF NUCLEOTIDE EXCISION REPAIR AND ITS RESCUE BY NEGATIVE TRANS-DOMINANT Tax MUTANT: POTENTIAL IMPLICATION FOR PREVENTING ADULT T-CELL LEUKEMIA BY GENE THERAPY.

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HTLV-I is the etiologic agent of adult T-cell leukemia (ATL), an aggressive malignancy with short survival time which does not respond to any presently available anti cancer therapy. Therefore, new preventive or therapeutic approach is still needed for this malignancy, like gene therapy. The viral transactivator Tax protein is a key factor in initiating the process leading to ATL. Tax interferes with cellular mechanisms of DNA repair, thus rendering the infected cells accessible to mutagenesis and consequently genetically instable. In this study we investigated the effect of Tax on nucleotide excision repair (NER) using the plasmid repair assay. For this assay a reporter plasmid (MSV LTR- β -gal) was irradiated in vitro by UV and transfected into Jurkat cells. The level of β -gal activity in such cells correlates the extent of its repair by NER. When the irradiated plasmid was transfected alone it was fully repaired. However, when co-transfected with w.t. Tax its repair was strongly reduced. A truncated Tax mutant, Tax Δ 58 which cannot translocate to the nucleus, elicited only little effect on the repair level. However, Tax Δ 58 can dimerize with w.t. Tax and trap it in the cytoplasm. When this mutant was co-transfected with w.t. Tax the repair inhibition by w.t. Tax was diminished, indicating that the repair inhibition by Tax is exerted through its nuclear activity. Moreover, these data indicate that the truncated Tax interferes with this part of the w.t. Tax oncogenic activity. PCNA is an accessory of DNA polymerase δ and ϵ and is required for DNA synthesis and DNA repair. However, increased level of PCNA over Pol- δ interferes with NER. To pursue the nature of Tax nuclear activity mediating NER inhibition, we examined its effect on PCNA and found that it markedly elevated PCNA level. Tax Δ 58 had no effect on PCNA level, but when it was co-transfected with w.t. Tax PCNA elevating effect of w.t. Tax was abrogated. Notably, we found Tax to elevate also p21WAF-1. Elevated p21WAF-1 in the absence of Tax usually binds to PCNA and supports NER. However, we noted that Tax also binds to PCNA. Therefore we hypothesize that by this binding Tax prevents p21WAF-1 binding to PCNA.

CARDIOPROTECTION AGAINST HYPOXIA BY MENADIONE

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BACKGROUND: The function of vitamin K₃ (2-methyl-1,4-naphthoquinone) in cardiomyocyte toxicity and in induction of preconditioning is still not clear. Previous studies have shown that Vitamin K₃ acts as powerful prooxidant and may induce respiratory stress, but in certain conditions it may be a potent antioxidant. It was shown that menadione can protect stressed heart by causing mitochondria to produce ROS, may require mK(ATP) opening and p38 MAPK activation, induced by ROS. However, the actual order of these steps, remains a matter of current debate. The aim of our study was to elucidate the protective action of menadione against hypoxia in cardiomyocyte culture.

RESULTS: In this study cardiomyocytes from neonatal rat hearts were grown in culture. These cells were treated with various concentrations of menadione and subjected to hypoxia for 90 or 120 min. It was found that menadione (3 μ M) decreased the hypoxic damage as expressed by LDH release and propidium iodide staining. The results also demonstrated that menadione keeps mitochondrial activity (which is very sensitive to lack of oxygen) almost normal. Mitochondrial membrane potential and ATP level were significantly protected if the cells were pretreated with menadione before hypoxia. Finally, the result also show that menadione partially prevents $[Ca^{2+}]_i$ elevation as a result of hypoxia and keeps the contractile activity of the cardiomyocytes for the first hour of hypoxia.

CONCLUSION: Menadione (vitamin K₃) has a protective effect on cardiac myocytes exposed to hypoxia, probably due to preservation of mitochondrial function and by partially preventing $[Ca^{2+}]_i$ overload.

CLINICAL APPLICATIONS: Menadione is used for coagulation treatment. In recent clinical study it was demonstrated that in a child with a mitochondrial complex III respiratory chain deficiency, treatment with menadione caused a significant improvement. Our results support the possibility that this new vitamin K function might open a novel vitamin K clinical application.

ACOUSTIC CAVITATION IN PHACOEMULSIFICATION - PRINCIPLES AND MODES OF ACTION

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Phacoemulsification utilizes high-intensity ultrasound energy (HIUE) for fragmentation and emulsification of the cataractous lens. Damage to the unilayer corneal endothelium, attributed to mechanical effects of phacoemulsification, is a common, and sometimes vision-threatening, complication of this procedure. Since human corneal endothelial cells lack the ability to regenerate, severing the corneal endothelium may lead to permanent corneal damage and irreversible edema.

The objectives of this study were to evaluate the possible generation of FR and SL by acoustic cavitation in phacoemulsification, and their contribution to corneal damage. Generation of cavitation in the aqueous medium transforms and concentrates acoustic energy into electromagnetic, chemical, mechanical and thermal energies by many orders of magnitude. Cavitation was determined by detection of hydroxyl FR and SL. Threshold intensities for the generation of SL were established. Qualitative analyses of primary FR were performed.

It was concluded that generation of FR and SL by acoustic cavitation may play a significant role in corneal endothelial cell damage, and may be regarded an undesired byproduct of phacoemulsification. A new concept, the cavitation index (CI), is presented for standardizing medical ultrasound instrumentation employing HIUE. CI relates to the direct effects on tissue of FR and SL generated by the cavitation phenomenon.

**PROGESTERONE INFLUENCE ON DIGOXIN TRANSFER IN
BeWo CELL LINE**

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The P-glycoprotein (Pgp) transporter functions as an energy– dependent, drug efflux pump with broad spectrum of compounds. It was previously shown that Pgp is expressed in primary trophoblast cells and BeWo cell line. Digoxin is a known substrate of Pgp transporter. Progesterone is highly expressed in trophoblast cell lines and is also a known Pgp modulator. In the present study we investigated the effect of P-glycoprotein modulators on the uptake of digoxin in BeWo cell line, following 3-4 days of culture. Transcellular transport of [H^3]-digoxin (10 μ M) across BeWo cells was investigated. In native BeWo cells the uptake of digoxin decreased with time, up to 1 hour. Following the treatment of BeWo cells with charcoal-fetal calf serum (to absorb endogenous progesterone), addition of progesterone (20 μ M) significantly increased the uptake of digoxin. The levels of progesterone in BeWo cells were determined by RIA. However, in the presence of progesterone (20 μ M) and verapamil (20 μ M) the uptake of digoxin was inhibited. The results may indicate that progesterone plays an important role in regulating the activity of Pgp in BeWo cells and that there is an interaction between progesterone and verapamil on binding to Pgp.

IDENTIFICATION OF THE CELLULAR CONTROL OF CROSS BRIDGE RECRUITMENT IN THE CARDIAC MUSCLE

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Recent studies have established that there is no unique force length relationship; The force response to sarcomere oscillation lags the length changes at slow frequencies ($<2\text{Hz}$) and precedes it at high frequencies. Theoretical studies suggest the existence of two feedback mechanisms: the cooperativity and the negative mechanical feedback. Cooperativity suggests that the number of force generating cross-bridges (XBs) determines the affinity of troponin for calcium. The mechanical feedback suggests that the sarcomere velocity determines the time over which the XBs generate force. The study simulates the above observations and defines the role of each feedback loop by analyzing the force response to sarcomere length (SL) oscillations, at constant calcium concentration. The model of the sarcomere, that couples calcium kinetics with XB dynamics, was built on Simulink. When only the cooperativity feedback exists the force lags the SL. When only the mechanical feedback loop exists the force precedes the SL. The cooperativity dominates at slow frequencies, when both feedbacks exist while the mechanical feedback dominates at higher frequencies, and the obtained force responses are in accordance with experimental observations. The study emphasizes the role of each feedback loop. Describing the intra cellular control of contraction is an important step toward understanding cardiac function.

DIVALENT CATIONS BINDING AND MODULATION OF VDAC

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Mitochondria play a central role in energy metabolism, Ca^{2+} signalling, aging and cell death. To control cytosolic or mitochondrial Ca^{2+} concentrations, mitochondria possess several Ca^{2+} transport systems across the inner membrane. Recently (Gincel *et al*, *Biochem J.* (2001) 358, 147-155), we reported that purified voltage-dependent anion channel (VDAC) reconstituted into lipid bilayers or liposomes is highly permeable to Ca^{2+} . In addition, Ruthenium Red, Ru360 and La^{3+} , completely inhibited VDAC channel activity, suggesting that VDAC possesses Ca^{2+} binding site(s). In this study we demonstrate and characterize the VDAC divalent cation-binding site(s). The interaction of the divalent cations with VDAC was characterized following its channel activity, nucleotide binding capacity and the mitochondrial permeability transition pore (PTP) activation. Mn^{2+} , Mg^{2+} , Ca^{2+} and Sr^{2+} , at micromolar ranges, inhibited the binding of the photoreactive ATP analog 3'-0-(4-benzoyl)benzoyl-5' adenosine triphosphate (BzATP) to purified VDAC with the following decreasing efficiency: $\text{Mn}^{2+} > \text{Mg}^{2+} > \text{Ca}^{2+} > \text{Sr}^{2+}$. VDAC channel activity was decreased by Mn^{2+} but not by Ca^{2+} . The divalent cations inhibited PTP opening when added to mitochondria after Ca^{2+} accumulation reaches a maximal level and before PTP is activated, as reflected in the release of Ca^{2+} and the accompanied mitochondrial swelling.

These results suggest that the divalent cations binding site(s) of VDAC are involved in the regulation of its activity and thereby of the PTP. Moreover, as a protein providing the pathway for transporting anions, cations, ATP, Ca^{2+} and other metabolites into and from the mitochondria, divalent cations regulation of VDAC has a very important role in the regulation of mitochondrial physiology.

**VOLTAGE-DEPENDENT ANION CHANNEL: CELLULAR AND
SUBCELLULAR LOCALIZATION IN CEREBELLUM AND ITS
INTERACTION WITH RYR AND IP3R**

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The voltage-dependent anion channel (VDAC) provides the passage for adenine nucleotides, Ca^{2+} and other metabolites movement to and from mitochondria. VDAC, thought to be located exclusively in the outer mitochondrial membrane, was recently localized to cell compartments other than mitochondria. It was found in the plasma membrane of various cells and in sarcoplasmic reticulum of skeletal muscles. The function of extra-mitochondrial located VDAC is yet unknown. Several lines of experiments suggest a cross talk between the ER and the neighboring mitochondria such as that IP3-mediated mobilization of intracellular Ca^{2+} stores resulted in large, rapid increase in mitochondrial matrix Ca^{2+} . In this study the localization and function of VDAC in the ER is demonstrated. VDAC immunostaining of rat cerebellum showed high labeling of the Purkinje neurons, both in their cell bodies and dendritic arbors in the molecular cell layer. Similar staining was obtained with anti-IP3 receptor antibodies. Immunogold labeling and electron microscopy analysis of cerebellar molecular layer showed that VDAC immunostaining is specific to mitochondrion outer membrane, and that VDAC is highly enriched in contact sites between mitochondria or mitochondria and associated ER. A membranal fraction, enriched in ER and mitochondria, was isolated from liver and characterized using membrane specific marker proteins. This fraction contains relatively high amount of VDAC and of IP3 receptor. We suggested that VDAC is involved in specialized intermembrane communication between the outer membranes of different mitochondria where VDAC in the connecting zones provide the pathway for electrical conductance and other compound movements. VDAC in ER and mitochondria contact region, as a Ca^{2+} transporting protein, may function in the tight association of mitochondria with intracellular Ca^{2+} release channels in ER.

ACTIVITY DEPENDENT TRANSLOCATION OF THE G-PROTEIN (DGq) IN DROSOPHILA PHOTORECEPTORS

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The covalent lipid modification of proteins plays a major role in targeting heterotrimeric (alpha-beta-gamma) G proteins to cellular membranes. In the case of the *Drosophila* visual system, palmitoylation of the cysteine residues at position 3 and 4 of an eye specific DGq-alpha is the sole lipid modification of the alpha subunit. Little is known, however, about the control of G α subunit localization within the natural endogenous environment of a specialized signaling cell. Here we show, using live *Drosophila* flies, that light causes massive and reversible translocation of the visual Gq α to the cytosol, associated with marked architectural changes in the signaling compartment. Molecular genetic dissection together with detailed kinetic analysis enabled us to characterize the translocation cycle and to unravel how signaling molecules that interact with Gq α affect this process. Using specific visual mutants our results indicate that the translocation is not influenced by phototransduction steps at the level of PLC or downstream of it and that Gq α is essential for efficient targeting of Gq β to the membrane. Together with analysis of a 3-dimensional model of Gq α , our in vivo results are mechanistically consistent with the 'two signal model' for membrane targeting. Immuno-electron microscopy revealed that both Gq α and the signaling compartment undergo dynamic and reversible light-dependent changes. These events give Gq α access to other cellular compartments and point to possible cross talk between sensory transduction and the cytoskeleton.

ELUCIDATING THE MECHANISM OF RAS GTPase USING SUBSTRATE DIRECTED SUPERIMPOSITION

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G-proteins are a multi-member family of molecular switches involved in a wide variety of essential cellular processes. Ras is a prominent member of this family due to its ubiquitous role in cell proliferation. G-proteins charged with GTP are in the 'on' state, capable of acting on their downstream effectors. Hydrolysis of the bound GTP (GTPase) switches the G-protein to the 'off' state, characterized by tightly bound GDP. Working out the details of Ras GTPase mechanism is crucial both for understanding its normal function and for revealing how interferes with the GTPase reaction causes diseases such as Cholera or Cancer.

Here we use a novel method, Substrate Directed SuperImposition (SDSI), to analyze the abundant structural data available for the active sites of Ras and other G-proteins in different activation states. We argue that this novel approach for comparative analysis of enzymatic mechanisms enables us to compare many structures simultaneously and without bias and to extract new and striking information about their function. Using SDSI and additional data, we propose a new model for the catalytic mechanism of Ras. We suggest that the rate-limiting step in the GTPase reaction is the correct positioning of the conserved glutamine and arginine, necessary for creating an electrostatic envelope around the substrate, preferentially stabilizing the transition state.

Using Substrate Assisted Catalysis, a unique enzymatic complementation approach, we obtained experimental evidence supporting our new model. We show the data supporting it and discuss the implications for enzymatic catalysis by Ras and other G-proteins and for future therapeutic approaches.

A NOVEL AMPHIPHILIC MOTIF IN THE N-TERMINAL HELIX OF HETEROTRIMERIC G-PROTEINS

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Heterotrimeric G-proteins relay signals between membrane-bound receptors and downstream effectors. The α subunits of this super-family are anchored to the membrane by one or more lipid modification at their N-termini. These modifications can be palmitoylation, myristoylation or both. As no sequence determinant for palmitoylation is apparent, we used systematic homology modeling of all different human G_α proteins to look for a three-dimensional structural determinant of palmitoylation, rather than a linear sequence motif.

Comparison of the N-termini of this super-family revealed that all α subunits modified only by palmitoylation contain a similar structural motif at their N-terminal helix. This motif is characterized by a prominent basic patch that extends a positive potential well beyond the molecular surface of the protein. Furthermore, this structural motif is oriented opposite to the face that interacts with the $\beta\gamma$ subunits. Hence, these positive patches are free to interact with the negatively charged inner surface of the plasma membrane.

Based on previous results, we suggest that efficient palmitoylation of G_α proteins requires prior targeting to the plasma membrane. The signal for this membrane localization can therefore be either myristoylation or the novel motif that we identified. This signal is cooperative with the interaction of the α subunit with the $\beta\gamma$ complex. The N-terminus of a G_α protein can therefore be described as amphiphilic, containing dual signals attracting it to the membrane and enabling it to undergo palmitoylation. As palmitoylation has been shown to modify a plethora of proteins extending beyond G-proteins, this motif could be widely applicable.

ASSAY OF GTP HYDROLYSIS BY RAS**Yael Litvak**, Mickey Kosloff and Zvi Selinger

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G-proteins are transducers of a wide range of cellular transactions, including transmembrane signaling, cell proliferation, intracellular transport and modulation of cytoskeletal organization. Despite their functional diversity, all members of the G-protein family share a common regulatory mechanism, the so-called regulatory GTPase cycle. The interaction of G-proteins with their downstream effectors is determined by the conformational state of the G-protein, which is influenced by the type of the guanine-nucleotide in the binding site. When a GTP (guanosine triphosphate) nucleotide is present in the binding site, the G-protein is in the 'on state' capable of affecting other proteins in the signaling cascade. Hydrolysis of the bound GTP to GDP (guanine diphosphate), causes the G-protein to lose this ability, and practically 'turns the protein off'. This transformation constitutes the G-protein's biochemical on/off switch.

Many GTPase assays have been designed. The challenge in developing such an assay is to provide a method that enables us to determine the net rate of GTP hydrolysis performed by the G-protein, without considering the rate of the bound GDP exchange for free GTP in the binding site of the G-protein. This is needed due to the fact that GTP hydrolysis is not necessarily the rate-limiting step of the GTPase cycle. The accurate measurements of GTP hydrolysis achieved by this assay enable us to investigate the effect of small molecules on the rate of GTP hydrolysis by G-proteins.

ניסויים בבעלי חיים: מצגת הסברתית המכוונת לבתי ספר תיכוניים

ג'והן פינברג

מח' לפרמקולוגיה, פק' לרפואה ע"ש רפפורט, טכניון, חיפה

מטרות:

בעקבות פעילותם הציבורית של המתנגדים לניסויים בבעלי חיים, נוצר רושם שלילי של דמות המדען במדעי החיים בקרב הנוער הישראלי. קיים צורך דחוף במתן הסברה נכונה המציגה את הניסויים בבעלי חיים כחלק הכרחי של המחקר ברפואה ובמדעי החיים.

בניית ההרצאה:

1. המרצה מציג את עצמו, מציג את הדיציפלינה של פיזיולוגיה/פרמקולוגיה, ומסביר את חלקם של הפיזיולוגיה/פרמקולוגיה ברפואה/מדע.
2. המרצה מסביר את ההשגים לאנושות שהתאפשרו בגלל המחקר בבעלי חיים, תוך כדי מתן מספר דוגמאות רלבנטיות (גילוי הפניצילין, אינסולין וסכרת, פיתוח חסון נגד שיתוק הילדים, קוצבי לב)
3. תאור ההתקדמות בנושא ספציפי אשר הביא לשיפור בטיפול מחלה (בדוגמה שלפנינו, מחלת הפרקינסון) ושניסויים בבעלי חיים שיחקו בו תפקיד מרכזי
4. שאלות

נקודות לציון:

1. מתן דוגמאות עם תמונות מרשימות
2. הצגת שאלות לקהל: "האם העובדה שצמיחת העובש בתרבית קטלה את החיידקים מבטיחה שמיצוי העובש יהיה שימושי כתרופה?"
3. ההרצאה הועברה בהצלחה לקהל של 60 ילדים בחטיבת הביניים