

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

Israel Society for Physiology and Pharmacology



Annual Meeting **הכנס השנתי**

December 10th 2009

Ma'ale Hachamisha

PROGRAM & ABSTRACTS

תכנית ותקצירי

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

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The Annual Meeting of the ISPP Ma'ale Hachamisha Thursday December 10, 2009

PROGRAM OUTLINE

8:30 - 9:30	Registration and Refreshments (posters hang up)
9:30-11:10	Morning Sessions A and B
11:10-11:30	Coffee Break
11:30-12:45	Student Presentation Competition
12:45-13:30	Poster Session
13:30-14:15	Lunch
14:15-14:30	Business Meeting
14:30-16:10	Afternoon Sessions C and D
16:10-16:30	Coffee Break
16:30-16:45	Ceremony Awarding the Winners of the Poster and Student Lecture Competitions

PROGRAM

9:30-11:10 Morning Sessions A and B

Session A: "Mechanisms of Aging and Alzheimer's Disease" (Hall A)

Chairs: Danny Michaelson (Tel Aviv University) and Haim Cohen (Bar-Ilan University)

- 9:30-9:50 Haim Cohen** (Bar-Ilan University): Would MOSES (mice overexpressing exogenous SIRT6) reach 120? And if so, How?
- 9:50-10:10 Sivan Korenblit** (Bar-Ilan University): Insulin/IGF-1 signaling alters the ER stress response to affect longevity.
- 10:10-10:30 Abraham Fisher** (Israel Institute for Biological Research, IIBR): Small molecules therapeutics and disease modification in Alzheimer's disease.
- 10:30-10:50 Danny Michaelson** (Tel Aviv University): The mechanisms underlying the pathological effects of apoE4 in Alzheimer's disease.
- 10:50-11:10 Hanna Rosenmann** (Hadassah Hebrew University Hospital): Tau mediated Neurodegeneration in Alzheimer's disease: Animal model studies and therapeutic approaches.

Session B: "When Environmental Physiology Meets Molecules: Digging into the Regulation of Physiological Processes " (Hall B)

Chair: Michal Horowitz (Hebrew University) Yoram Epstein (Sheba Medical Center)

- 9:30-9:50 Yehuda Arieli** (Israel Naval Medical Institute, Haifa): Preconditioning to HBO may provide protection against CNS oxygen toxicity in the rat: beating the "devil's triangle".
- 9:50-10:10 Rotem Cohen** (Tel Aviv University): From brain to behavior.
- 10:10-10:30 Noam Meiri** (Volcani Center): Epigenetics: mechanism of environmental influence on gene expression.
- 10:30-10:50 Michal Horowitz** (Hebrew University): Does heat acclimation have a (cytoprotective) memory? Why can't we tolerate heat? Is it an epigenetic mechanism?
- 10:50-11:10** Round table discussion.

11:10-11:30 Coffee Break

11:30-12:45 Student Presentation Competition

Chairs: Bernard Attali (Tel Aviv University) and Yoav Paas (Bar-Ilan University)

11:30-11:42 Nir Fluman (Weizmann Institute of Science): A promiscuous conformational switch in the secondary multidrug transporter MdfA.

11:42-11:54 Nurit Keinan (Ben-Gurion University): Apoptotic stimuli trigger VDAC oligomerization as monitored in live cells using bioluminescence resonance energy transfer (BRET2).

11:54-12:06 Tal Laviv (Tel Aviv University): Endogenous GABA regulates GABAB receptor conformation and release probability at individual hippocampal synapses.

12:06-12:18 Shimon Lecht (The Hebrew University of Jerusalem): Differential Temporal Activation of Erk, PI3K/Akt and PLC γ Signaling Pathways Induced by Nerve Growth Factor in Endothelial Cells.

12:18-12:30 Moran Rathaus (Bar-Ilan University): The non-histone targets of the longevity gene SIRT1, a proteomic study.

12:30-12:42 Moran Yadid (Technion): Sarcomere velocity regulates the cross-bridge cycling rate in cardiac muscle: A novel theory for the muscle molecular motor.

12:45-13:30 Poster Session

13:30-14:15 Lunch

14:15-14:30 Business Meeting

14:30-16:10 Afternoon Sessions

14:30-16:10 Afternoon Sessions C and D

Session C: “Sensory Neurons Under Stress: From Injury to Neuropathic Pain” (hall A)

Chairs: Marshall Devor (Hebrew University) and Mike Fainzilber (Weizmann Institute)

- 14:30-14:40** **Mike Fainzilber** (Weizmann Institute): A brief introduction to the session.
- 14:40-15:02** **Izhak Michaelievski** (Weizmann Institute): Retrograde signaling and proteomics of injury response.
- 15:02-15:24** **Oded Behar** (Hebrew University): Correction of axon guidance errors results in neuronal cell loss: a possible predisposition for neuropathies.
- 15:24-15:46** **Noam Zilberberg** (Ben Gurion University): Regulation of K2P2.1, a K⁺ channel involved in pain perception.
- 15:46-16:08** **Marshall Devor** (Hebrew University): How does injury response yield hyperexcitability and neuropathic pain ?

Session D: “Ion Transporters: Mechanistic and Structural Insights” (Hall B)

Chair: Baruch Kanner and Shimon Schuldiner (Hebrew University)

- 14:30-14:50** **Israel Sekler** (Ben Gurion University): Not just ATP, The identification and physiological role of the mitochondrial Na⁺/Ca²⁺ exchanger in cellular signaling.
- 14:50-15:10** **Christopher Grewer** (Binghamton University, USA): Cation binding sites in Na⁺-coupled amino acid transporters.
- 15:10-15:30** **Baruch Kanner** (Hebrew University): What is chloride doing to the sodium-coupled neurotransmitter transporters?
- 15:30-15:50** **Shimon Schuldiner** (Hebrew University): Ion-coupled transporters: evolution forwards and evolution backwards.
- 15:50-16:10** **Yael Stern-Bach** (Hebrew University): Assembly of AMPA-type glutamate receptors.
- 16:10-16:30** **Coffee Break**
- 16:30-16:45** **Ceremony Awarding the Winners of Poster and Student Lecture Competitions**

*Abstracts of Invited
Speakers
(Sessions A & B)*

Session A
“Mechanisms of Aging and
Alzheimer's Disease”
(Hall A)

**Would MOSES (mice over-expressing exogenous SIRT6) reach 120?
If so, how?**

Haim Cohen, Yariv Kanfi, Yosi Gozlan, Victoria Peshti, Reuven Gil, Asaf Gertler, Liat Nachum, Shoshana Naiman and Batya Lerrer

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

Several theories have been proposed in an attempt to explain the regulation of lifespan, including the "disposable soma", "snow ball" and "free radical" models; all these theories share the basic claim that defects in cellular maintenance and proper metabolism will result in changes characteristic of the aging process. One family of proteins that have been implicated in aging, metabolism and genome stability are the sirtuins. Sirtuins are highly conserved enzyme homologues of yeast Sir2, with NAD⁺ dependant deacetylase and/or mono ADP ribosyltransferase activity. The SIRT6 deacetylase is one of the seven human sirtuins, SIRT1 to 7. Mice deficient for SIRT6 develop premature aging phenotypes including metabolic defects, exhibit defects in base excision repair and are hypersensitive to oxidative stress. To explore the role of SIRT6 in metabolism, genome stability and aging, we generated a new transgenic model of mice over expressing exogenous SIRT6 (MOSES). Here we will describe our recent findings demonstrating the molecular mechanisms by which SIRT6 protects against the physiological damages caused by diet-induced obesity and against DNA lesions typically repaired by the BER pathway. The implications of these findings to the aging process and to the development of new therapeutic approaches for treating age related diseases will be discussed in depth.

Reducing insulin/IGF-1 signaling reprograms ER-stress response proteins to promote longevity

Sivan Henis-Korenblit and Cynthia Kenyon

The Mina & Everard Faculty of Life Sciences, Bar-Ilan University, Israel.

Reduced insulin/IGF-1 signaling is thought to extend lifespan by catalyzing a physiological shift towards pathways that promote cell maintenance and protection. Thus it was not surprising to find that mutants with reduced insulin/IGF-1 signaling also exhibited increased ER stress resistance and that loss of the ER stress response genes *ire-1* or *xbp-1* shortened their lifespan substantially. However, unlike most genes encoding proteins predicted to increase environmental stress resistance, that are expressed at higher levels when insulin/IGF-1 signaling is reduced, the level of spliced *xbp-1* mRNA and expression of XBP-1's normal target genes is not increased when insulin/IGF-1 signaling is reduced. Instead, we find that in insulin/IGF-1-pathway mutants, XBP-1 collaborates with DAF-16, a FOXO-transcription factor that is activated in these mutants, to enhance ER stress resistance and to activate new genes that promote longevity.

Small molecules – the link between cognitive therapy and disease modification in Alzheimer’s disease (AD)

Abraham Fisher

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Due to an elusive etiology of AD, future treatments should address all AD hallmarks [*e.g.* β -amyloid ($A\beta$ and tau pathologies, the cholinergic hypofunction and cognitive impairments], regardless of the prime etiological culprit. Among such potential treatments, low molecular weight (MW) compounds ("small molecules", preferred by pharma industries) are at various stages of R&D and include, *inter alia*: cholinergic modulators [cholinesterase inhibitors (AChE-Is), M1 muscarinic agonists, nicotinic agonists], BACE1 inhibitors, γ -secretase inhibitors (GSI) or modulators (GSM), inhibitors of tau proteins hyperphosphorylation and GSK-3 β inhibitors. While some of these compounds have a defined target, others exhibit mixed mechanism of actions ('dirty drugs'). This overview is an attempt to evaluate to what extent such low MW drugs can provide a comprehensive therapy in AD. Some of the advantages and drawbacks of the listed treatments and their respective target(s) are compared, when possible, with select M1 selective agonists (*e.g.* AF267B, AF292). Notably the brain M1 muscarinic receptor (M1AChR), preserved in AD, was identified as a pivotal therapeutic target that links major hallmarks of AD, *e.g.* cholinergic and cognitive deficits, $A\beta$ and tau pathologies. AF267B, a functionally selective M1 orthosteric agonist, is a cognitive enhancer and a potential disease modifier in AD. AF292, its active metabolite, is a selective orthosteric partial muscarinic agonist with unprecedented M1 agonistic selectivity and with no M2-M5 agonistic profile, both *in vitro* and *in vivo*. Both AF267B and AF292 have: excellent PK profile and high oral plasma & brain bioavailability; wide safety margin in cognitive tests. AF267B *via* M1AChR-modulation of PKC-TACE and PKC-GSK-3 β pathways, respectively – i) elevated α APPs and decreased $A\beta$ levels; and ii) decreased $A\beta$ -induced neurotoxicity and tau hyperphosphorylation. In 3xTgAD mice, AF267B rescued cognitive deficits and decreased $A\beta_{42}$ and tau pathologies in the cortex and hippocampus, *via* M1AChR-activation of TACE and M1AChR-decrease of BACE1 and GSK-3 β levels. In fact, AF267B can be branded as the best TACE activator / BACE1 & GSK-3 β inhibitor, *in vivo*, free from adverse effects of activators of α -secretase, GSI, & GSK-3 β inhibitors and poor brain penetration of most BACE1 inhibitors. Conclusions: A plethora of "small molecules" are currently evaluated both for treatment and DM in AD. Some, like selective M1 agonists, can bridge both treatments of cognitive impairments with disease modification. Others are approaches that target only part of these problems and may not meet the stringent acceptance criteria for a comprehensive treatment. In such situations multipharmaceuticals may be required. How many of the reviewed therapies will eventually be prescribed in AD patients cannot be predicted, however the pace of current research raises the exciting perspective that slowing the progression of AD will soon be possible.

Cross talk and synergistic interactions between molecular and genetic risk factors of alzheimer's diseases

Danny Michaelson

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

This study is directed at unraveling the intracellular targets, which mediate the synergistic pathological effects of A β and apoE4 in vivo and at an investigation of the possible role of A β oligomerization in this process. Activation of the amyloid cascade in vivo by inhibition of the A β degrading enzyme neprilysin resulted in the specific accumulation of A β and oligomerized A β in CA1 hippocampal neurons of apoE4 targeted replacement mice. This was associated with lysosomal activation and with the occurrence of enlarged lysosomes which contained A β and oligomerized A β and whose time course of accumulation paralleled that of the loss of CA1 neurons. The mitochondria of the CA1 neurons were also affected isoform specifically by apoE4 following activation of the amyloid cascade. This included an increase in COX-I immunoreactivity, which reached a plateau prior to neuronal loss and which was also associated with subsequent ultrastructural morphological aberrations. The accumulated A β and oligomerized A β also co-localized with the mitochondria. This affect however was smaller than that observed with the activated lysosomes.

These findings show that the synergistic pathological effect of apoE4 and A β following activation of the amyloid cascade in vivo is associated with the intracellular accumulation of A β and oligomerized A β in CA1 neurons and suggest that they are mediated via direct interactions of A β and aggregated A β with neuronal lysosomes and mitochondria. The possibility that apoE4 also targets non-neuronal targets such as the vasculature will be discussed.

Tau-mediated neurodegeneration in Alzheimer's disease: animal model studies and therapeutic approaches

Hanna Rosenmann

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Neurofibrillary tangles (NFTs), composed of phosphorylated microtubule-associated tau proteins, are a major brain pathology in Alzheimer's disease (AD), and correlate best with clinical dementia. While in AD the tangles appear together with amyloid plaques, in other tauopathies (such as frontotemporal dementia, Pick's disease etc.) the tangles appear without plaques. While for years the amyloid pathology was considered the key pathology of AD and the NFTs as only a secondary phenomenon, it is now clear that the tangle pathology is a direct cause for neurodegeneration and can develop independently of amyloid plaques. The development of rodent animal models presenting NFTs in the brain was a breakthrough in the research of AD and of tauopathies in general. We generated animal models for the NFTs using a few strategies: generating tg mice expressing the mutant tau gene under the regulation of the authentic tau promoter in order to obtain a physiological mode of regulation of the tau protein. We also used an auto-immune approach model by immunizing wild-type mice with full length tau protein. These approaches led to the generation of NFT-mouse models. We also used an environmental approach by exposing the NFT-tg mice to environmental risk factors related to AD, based on epidemiological studies. This study revealed that CNS-inflammation, low ovarian hormones, oxidative stress and poor intellectual environment accelerate the formation of NFTs, leading to the generation of combined genetic-environmental models for NFTs. Subsequently, we used these models for the development of therapeutic approaches. We detected that statin treatment reduced NFTs accompanied by a decrease in microglial burden, and that an enriched environment reduced tangles *via* inactivation of GSK- β . We also investigated the therapeutic potential of immunotherapy against NFTs by addressing both the efficacy and the safety of our vaccine, and our results showed promising results as an anti-NFT approach.

Session B

”When Environmental Physiology
Meets Molecules:
Digging into the Regulation of
Physiological Processes”
(Hall B)

Preconditioning to HBO may provide protection against CNS oxygen toxicity in the rat: beating the "devil's triangle"

Doron Kotler^{1,2}, Ayala Hochman², Mirit Eynan¹, and Yehuda Arieli¹

¹Israel Naval Medical Institute, P.O. Box 8040, Haifa 31080; and ²The Faculty of Life Sciences, Department of Biochemistry, Tel-Aviv University, Israel.

Background: Previously we demonstrated the protective effect of heat acclimation on CNS oxygen toxicity. In this investigation we tested the hypothesis that repeated hyperbaric O₂ preconditioning (HBO-PC) may have a protective effect against CNS oxygen toxicity (CNS-OT) in the rat.

Methods: The rats in control group 1 (C1) were kept in normobaric air together with five sham rats. Rats in the experimental group and control group 2 (C2) were exposed to HBO at 202 kPa for 1 h as preconditioning once every other day for a total of three sessions. Twenty-four hours after preconditioning, the rats in both the experimental and C1 groups were exposed to 608 kPa. We measured the latency to CNS-OT, after which all of the animals were sacrificed and tissues were harvested from the hippocampus and frontal cortex for biochemical examination.

Results: Time to CNS-OT increased significantly following preconditioning. There was a 20%, statistically significant increase in the activity of glutathione-S-transferase (GST) and glutathione-peroxidase (GP) in the cortex of the preconditioned rats. Nitrotyrosine levels in the cortex did not demonstrate any significant trend. In the hippocampus of the preconditioned rats, a significant decrease was found in the activity of glutathione-reductase (GR) and G6PD, whereas there was a significant increase in the activity of GP. Nitrotyrosine levels in the hippocampus demonstrated the same trend in all five marked proteins. The highest levels were found in the C1 group, whereas the lowest levels were found in the experimental group.

Conclusions: This study demonstrates that under well-defined conditions, repeated exposure to HBO may have a preconditioning effect, providing protection against CNS-OT. The protective mechanism involves alterations in the enzymatic activity of ROS scavengers induced by HBO exposure, mainly in the hippocampus. These alterations result in lower levels of distractive RNS (and probably ROS) and prolonged latency.

Masking and temporal niche switches in spiny mice

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Activity patterns are the product of interactions between an internal circadian clock and direct responses to photic and non-photoc features of the environment that are said to “mask” the influence of that clock. Evolutionary transitions between nocturnality and diurnality involve changes in mechanisms underlying both of these processes. Here, we examined how masking influences activity patterns of golden spiny mice (*Acomys russatus*), which can be either nocturnal or diurnal, and common spiny mice (*A. cahirinus*), which are strictly nocturnal. Animals kept on a 12:12 light:dark (LD) cycle were exposed to 3 h dark pulses starting at zeitgeber time (ZT) 2, light pulses of varying intensities (50, 100, 700 or 1500 lux) at ZT 14 and to a 3.5:3.5 h LD cycle. In common spiny mice activity increased by 379% during the dark pulse and decreased during light pulses to 23% of baseline levels. Golden spiny mice also increased their activity in response to the dark pulse (by 345%), but there was extreme inter- and intra-individual variability and no significant response to light pulses at night. In the 3.5:3.5 LD cycle common spiny mice showed a preference for the dark phase with 86 ± 0.01 % of activity occurring then, whereas golden spiny mice showed a pronounced circadian rhythm but no evidence of masking. Masking responses to light and dark were thus unsurprising in common spiny mice but were highly unusual in golden spiny mice. Patterns seen in the latter species may reflect mechanisms enabling these animals to occupy either a diurnal or a nocturnal niche in their natural habitat.

Epigenetics: mechanism of environmental influence on gene expression

Noam Meiri, Tatiana Kisliouk, Maya Yossifoff

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As with other sensory mechanisms, determination of thermal-control set point is refined during a critical period of development by alterations in cellular properties in the frontal hypothalamus. These alterations in hypothalamic plasticity are achieved by renewal of the protein repertoire via activation or silencing of gene transcription, both of which are regulated by chromatin remodeling.

Here we demonstrate increase of global histone H3 di-methylation at lysine 27 during thermal-control establishment in general and at the initiation of the BDNF coding region in particular. Furthermore, antisense “knockdown” of the H3K27-specific methyltransferase, EZH2, which was induced in correlation with the methylation of H3K27, disrupted the thermal set-point and inhibited *Bdnf* mRNA expression.

In a second stage of epigenetic regulation of thermal control establishment we demonstrate alteration in the pattern of CpG methylation in the promoter area of BDNF. During heat conditioning there was a transient induction of methylation of two CpG positions (The first and the third CpG location downstream from the BDNF-ATG) and a reduction of methylation of one CpG position (1000bp downstream from the BDNF-ATG), while the other CpG positions did not show significant change. Furthermore, our data demonstrate a significant difference between the levels of methylation at the *Bdnf* promoter of conditioned compared to non-conditioned chicks during a thermal challenge a week after conditioning, indicating long-term epigenetic regulation.

Taken together, these results correlate epigenetic chromatin methylation with thermal- adaptation-related hypothalamic plasticity.

Supported by ISF

*Abstracts of Student
Lecture Competition*

A promiscuous conformational switch in the secondary multidrug transporter MdfA

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Multidrug (Mdr) transporters are membrane proteins that actively export structurally dissimilar drugs from the cell, thereby rendering the cell resistant to toxic compounds. Similar to substrate-specific transporters, Mdr transporters also undergo substrate-induced conformational changes. However, the mechanism by which a variety of dissimilar substrates are able to induce similar transport-compatible conformational responses in a single transporter remains unclear. To address this major aspect of Mdr transport, we studied the conformational behavior of the *E. coli* Mdr transporter MdfA. Our results show that indeed, different substrates induce similar conformational changes in the transporter. Intriguingly, in addition, we observed that compounds other than substrates are able to confer similar conformational changes when covalently attached at the putative Mdr recognition pocket of MdfA. Taken together, the results suggest that the Mdr binding pocket of MdfA is conformationally sensitive. We speculate that the same conformational switch that usually drives active transport is triggered promiscuously by merely occupying the Mdr binding site.

(will also be presented as poster # 30)

Apoptotic stimuli trigger VDAC oligomerization as monitored in live cells using bioluminescence resonance energy transfer (BRET2)

Nurit Keinan, Dalia Toymkin, Varda Shoshan-Barmatz

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Beer-Sheva, Israel.

Mitochondria are essential for cell survival, providing sources of cellular energy, as well as lying at the heart of apoptotic regulation. Mitochondria-mediated apoptosis results in the efflux of a number of potential apoptotic regulators, such as Cytochrome *c* (Cyto *c*), to the cytosol, triggering the caspases cascade and cell destruction. Accumulating evidence implicates the voltage-dependent anion channel (VDAC) in mitochondria-mediated apoptosis, and as a critical player in the release of apoptogenic proteins from mitochondria in mammalian cells (e.g Cyto *c*). The precise mechanisms regulating Cyto *c* release remain unknown, as does the molecular architecture of the Cyto *c*-conducting channel.

Here, the relationship between VDAC oligomerization and apoptosis induction was examined. We demonstrate that apoptosis induction by various stimuli, acting through different mechanisms, all involving mitochondria, is accompanied by an up to 20-fold increase in VDAC oligomerization, as revealed by chemical cross-linking. In addition, VDAC1 oligomeric state was directly monitored in living cells using BRET (Bioluminescence Resonance Energy Transfer) in cells expressing rVDAC1-GFP2 and rVDAC1-Luciferase. The BRET signal, indicating VDAC oligomerization, shows a dramatic increase upon cells exposure to apoptotic stimuli. Conversely, the apoptosis inhibitor, DIDS, inhibits staurosporine-induced VDAC oligomerization and apoptosis. We propose that VDAC oligomerization is a key step in mitochondrial-mediated apoptosis representing a general mechanism common to numerous apoptogens acting via different initiating cascades. Targeting the VDAC oligomeric status, and hence apoptosis, offers therapeutic strategies for combating cancers and neurodegenerative diseases.

(will also be presented as poster # 31)

Endogenous GABA regulates GABA_B receptor conformation and release probability at individual hippocampal synapses

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Probability of neurotransmitter release is a key determinant of synaptic efficacy in neuronal connections. The presynaptic GABA_B receptors (GABA_BRs), signaling via G_{i/o} proteins, are well-known to mediate baclofen-induced inhibition of basal transmitter release at central synapses. However, the patterns of neuronal activity that activate presynaptic GABA_BRs by endogenously released GABA remain controversial. To explore the GABA_BR activation at individual presynaptic boutons, we integrated optical tools for simultaneous monitoring protein-protein interactions and synaptic vesicle recycling. Fluorescence resonance energy transfer (FRET) spectroscopy has been utilized to estimate inter-molecular associations between CFP/YFP-tagged subunits of the GB_{1a}/GB₂ receptor heterodimer, whereas activity-dependent FM styryl dyes have been used to estimate release probability at single boutons of cultured pyramidal hippocampal neurons. We found that quantal GABA release induced highly variable conformational changes in GB_{1a}/GB₂ associations across presynaptic boutons. Antagonist prevented the observed GB_{1a}/GB₂ conformational changes, relieving vesicle release from a tonic block imposed by endogenous GABA. Block of SNARE-mediated vesicle exocytosis or receptor binding site reduced variability of GB_{1a}/GB₂ conformational states, indicating that local extracellular GABA concentration is the major determinant of variability in the GABA_{B1a}R tone under quantal synaptic transmission. Notably, the degree of GB_{1a}/GB₂ association negatively correlated to release probability at the single bouton level. Altogether, our results imply a potential role for GABA_BR conformational dynamics in setting release probability at single hippocampal synapses.

This work was supported by Binational Science Foundation (I.S. and P.S.) and Israel Science Foundation (I.S.).

(will also be presented as poster # 32)

Differential temporal activation of Erk, PI3K/Akt and PLC γ signaling pathways induced by nerve growth factor in endothelial cells

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Nerve growth factor (NGF) is a neurotrophin promoting survival and differentiation in the embryonic and adult nervous systems. Recently, NGF was characterized as a novel angiogenic factor promoting neovascularization and capillary sprouting. These findings motivated us to investigate in endothelial cells (ECs) NGF-induced angiogenic effects mediated by TrkA receptors and the emerging signaling.

The NGF receptor TrkA, was detected by RT-PCR and Western blotting. NGF stimulated TrkA phosphorylation that resulted in transient Erk1/2, Akt and PLC γ phosphorylation. The transient kinetics of signal transduction were characterized by a strong phosphorylation of signaling molecules 10 min after stimulation. Sixty min after exposure to NGF a fast reduction in the levels of these phosphoproteins to basal levels was measured. These effects were inhibited by K252a, a selective antagonist of TrkA receptor, by PD98059 (MEK inhibitor) and by LY294002 (PI3K inhibitor). NGF induced in vitro proliferation and migration of ECs was blocked by K252a. In the ex vivo rat aorta rings and quail embryonic chorioallantoic membrane models in which NGF induced significant angiogenesis, K252a was found as a potent inhibitor of NGF actions.

These results cumulatively support the hypothesis that NGF, besides its well known neurotrophic effects, may play a role in the cardiovascular system. The angiogenic properties of NGF may be beneficial for developing novel anti-angiogenesis therapies for cancer.

Acknowledgment

SL is supported by "Eshkol" fellowship from the Israeli Ministry of Science and Technology.

(will also be presented as poster # 33)

The non-histone targets of the longevity gene SIRT1, a proteomic study

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Calorie restriction (CR) diet was shown to extend the life span of every organism tested, but its mechanism is poorly understood. Sirtuins are NAD⁺ dependent deacetylases that were implicated in the regulation longevity and CR response. The mammalian sirtuin SIRT1, which is conserved throughout all eukaryotes, was shown to mediate the beneficial effects of CR on cell survival and to regulate the development of many age related diseases such as cancer, diabetes, neurodegenerative diseases, etc. However, despite the wide range of physiological pathways that are regulated by SIRT1, to date only a limited number of proteins are known as substrates for the deacetylation enzymatic activity of SIRT1. Finding novel direct or indirect substrates for SIRT1 is a major challenge, since SIRT1 is an essential gene, and mice deficient for Sirt1 gene (*Sirt1*^{-/-}) rarely survive postnatally. Therefore, to understand the downstream effects of SIRT1, we used a stable isotope labeling with amino acids in cell culture (SILAC) proteomics analysis, In order to compare the acetylaome of SIRT1^{-/-} and wild type mouse embryonic fibroblasts (MEFs). We found that SIRT1 targets hundreds of proteins with over a thousand specific sites. SIRT1 regulates all major biological processes in the cell including chromosome and ribosome biogenesis, glycolysis and metabolism. A major family of SIRT1 targets is ribosomal proteins, both in the large and in the small subunits. We have further verified SIRT1 regulatory role on translation efficiency. This is of special interest, since it expands the traditional role of SIRT1 as a transcription regulator catalyzing histone deacetylation (HDAC) also to be a general lysine deacetylase (KDAC) and particularly regulating translation by the Ribosome. Taken together, we identified hundreds of novel non-histone targets for SIRT1 and established the role of SIRT1 as a master regulator of cell metabolism. These findings have great implication on the role of SIRT1 in regulating longevity and mediating the CR response.

(will also be presented as poster # 34)

Sarcomere velocity regulates the cross-bridge cycling rate in cardiac muscle: A novel theory for the muscle molecular motor

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Background: The mechanisms regulating cross-bridges (XBs) cycling during stretch and shortening are controversial. We hypothesize that XB strong to weak transition (weakening) rate increases during shortening and decreases during lengthening in an identical velocity dependent manner. Our hypothesis reproduces the muscle basic properties as the force-velocity relationship and regulation of energy consumption. The study investigates this unifying hypothesis during lengthening and shortening.

Methods: Trabeculae were isolated from rat right ventricles ($n = 9$). Sarcomere length was measured by laser diffraction. The number of strong XB (N_{XB}) was evaluated by measuring the dynamic stiffness. Stretches ($n = 42$) and releases ($n = 48$) at different velocities and instants were imposed on sarcomere isometric contractions.

Results: Faster stretches yielded larger forces. An overt identical linear correlation between force and N_{XB} development was obtained for any stretch velocity ($0-2.17 \mu\text{m/s}$), implying that the force increased due to the increase in N_{XB} , whereas the unitary force per XB (F_{XB}) was constant. The stiffness development rate linearly depended on the lengthening velocity with a proportion coefficient of 6.9 ± 0.46 . Shortening yielded both a decrease in N_{XB} and F_{XB} . Interestingly, the stiffness decline rate depended linearly on the shortening velocity ($0-6.27 \mu\text{m/s}$) with similar proportion coefficient of 6.08 ± 2.45 . When identical perturbation (lengthening or shortening) was imposed at different instants during the twitches, similar rate of change in the stiffness and force development were observed. Thus, the phenomena are not dominated by N_{XB} but relate to an inherent property of the single strong XB.

Conclusions: The independence of XB weakening rate on the perturbation onset time and the identical dependence on the velocity during shortening and lengthening strongly support the hypothesis that XB dynamics is dominated by a single velocity dependent kinetics.

(will also be presented as poster # 35)

*Abstracts of Invited
Speakers
(Sessions C & D)*

Session C

“Sensory Neurons Under Stress:
From Injury to Neuropathic Pain”
(Hall A)

Retrograde signaling and proteomics of injury response

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Investigations of the molecular mechanisms underlying responses to nerve injury have highlighted the importance of the retrograde transport system in axons. Previous studies from our groups have revealed that a protein complex containing importin β 1, vimentin and p-ERK 1/2 associates with dynein motors to signal retrogradely from an axonal lesion site to the neuronal cell body. In order to obtain a comprehensive view of the protein complexes associated with retrograde injury signaling, we have now applied proteomics approaches to characterize the effects of injury on composition of the retrograde signaling complex, and the extent and role of protein phosphorylation in nerve injury signaling. We used nerve ligatures to concentrate proteins transported in both anterograde and retrograde directions with and without crush injury. Protein quantitation and identification was done by LC/MS/MS analysis of iTRAQ labeled peptides generated from the axoplasm extracted from the ligatured nerves. The data was further subjected to principal component analysis (PCA) and factor analysis with subsequent clustering to determine the most prominent injury-related transported proteins. In parallel, TiO₂-based affinity enrichment was used to characterize the retrograde injury phosphoproteome. Bioinformatic tools were then employed to link these data-sets to microarray data-sets generated from dorsal root ganglia under similar lesion paradigms. Overall, these diverse approaches identified 879 proteins and 2465 phosphorylation sites involved in retrograde injury signaling. Juxtaposition of the axonal phosphoproteome with the cell body transcriptome identified 342 signaling networks that can influence the injury response of peripheral sensory neurons. These signaling networks may comprise a highly redundant system, providing robustness to the injury response. Analysis of the networks revealed a list of proteins, hub proteins, standing on cross-roads of multiple pathways leading to gene expression in response to the axonal injury. *Ex vivo* functional assay confirmed that most of the hub proteins, e.g. ABL, AKT, PKC, p38, affect ability of neurons to recover neurite outgrowth.

Correction of axon guidance errors results in neuronal cell loss: a possible predisposition for neuropathies

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Mice embryos lacking the guidance molecule Semaphorin 3A (Sema3A) exhibit numerous sensory axon guidance errors, but surprisingly, most errors are absent a few days later of development. During the period of error correction significant increase in apoptosis is detected. Blocking naturally occurring cell death by using BAX null mice, delay this error correction, nevertheless correction still occurs, suggesting another form of correction is compensating for the lack of apoptosis. Consistent with this idea we see an induction in the levels of Autophagy marker LC3BII when apoptosis is blocked (in BAX/Sema3A double null mice as compared to other littermates). Moreover, in newborn mice lacking both Sema3A and BAX the number of neurons in the DRG is significantly reduced compared to BAX only null mice. Furthermore, in Sema3A null mice the number of neurons is about 40% lower than in wild type littermates. Thus, our results strongly support the idea that guidance errors are eliminated by BAX-dependent, as well as BAX-independent, cell death. As a result of these axon error corrections, neuron survival is reduced, making the organism predisposed for neuropathology as a result of reduced neuron numbers.

Regulation of K_{2p}2.1, a K⁺ Channel Involved in Pain Perception

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Pain is a physiological state promoting protective responses to harmful episodes. However, pain can become pathophysiological and become a chronic disruptive condition, damaging quality of life. The mammalian K_{2p}2.1 (KCNK2, TREK-1) channel, expressed in sensory neurons of the dorsal root ganglia, was previously identified as a polymodal molecular sensor involved in pain perception. K_{2p}2.1 is a member of the two-pore domain potassium channels family that carry leak or 'background' currents that are mostly time- and voltage-independent. Such channels are essential for neurophysiological function as they suppress excitability through the maintenance of a resting membrane potential below the threshold for action potential firing. Here, we report that two pain-associated signals, external acidosis and lysophosphatidic acid (LPA), known to rise during injury, inflammation and cancer, profoundly down-modulate human K_{2p}2.1 activity. The pH regulatory effect was mediated by activation of proton-sensitive G-protein coupled receptors and phospholipase C. Physiological concentrations of LPA overcame the effects of known K_{2p}2.1 activators, such as arachidonic acid, lysophosphatidylcholine and temperature, by activating cell-surface receptors stimulating the G_q pathway. Furthermore, we identified three K_{2p}2.1 carboxy-terminal residues that mediate both pH and LPA regulatory effects. Our results highlight the important role of K_{2p}2.1 channels as receptors for mediators known to cause nociception.

How does injury response yield hyperexcitability and neuropathic pain ?

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Accumulating evidence implicates ectopic discharge originating in the peripheral nervous system as both: 1) the primary nociceptive signal underlying spontaneous neuropathic pain, and 2) a factor that triggers and dynamically maintains central sensitization, and hence tactile hypersensitivity. But how does nerve injury induce ectopic discharge and pain ? Examination of the phenotype of primary sensory neurons in experimental models of neuropathic pain using oligonucleotide expression arrays reveals massive changes. Expression in >2000 genes is significantly regulated in dorsal root ganglion (DRG) neurons within 3 days of nerve section. This number can be reduced by identifying transcripts whose regulation correlates with pain behavior across mouse strains. However, it remains large. There are many consequent functional changes. For example, many neurons become intrinsically hyperexcitable as a result of emergent membrane resonance, reflected in enhanced depolarizing afterpotentials and subthreshold oscillations of the membrane potential. Numerical simulations suggest that a delayed (~20 msec) Na⁺ conductance component may play a particularly important role in the enhancement of electrogenesis. Functional changes also occur in sensory signaling. For example, DRG neurons with A-beta axons that normally evoke light touch sensation begin to express neurotransmitters normally associated with C-nociceptors. As a result they become capable of activating ascending pain signaling pathways. Novel strategies are needed to identify which of the numerous changes triggered by nerve injury are the key contributors to neuropathic pain.

Session D

”Ion Transporters: Mechanistic and
Structural Insights”
(Hall B)

Not just ATP: the identification and physiological role of the mitochondrial Na⁺/Ca²⁺ exchanger

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Mitochondrial Ca²⁺ transport is linked to numerous cellular activities and pathophysiological processes. Although it has been established for more than 30 years that a Na⁺-dependent mechanism mediates mitochondrial Ca²⁺ efflux, the molecular identity of this transporter has remained elusive. We found that the Na⁺/Ca²⁺ exchanger, NCLX is enriched in the cristae of the mitochondria. Employing fluorescent-imaging, we demonstrate that mitochondrial Na⁺-dependent Ca²⁺ efflux is enhanced upon overexpression of NCLX, is reduced by silencing of NCLX expression by siRNA, but can be fully rescued by the concomitant expression of heterologous NCLX. NCLX-mediated mitochondrial Ca²⁺ transport was inhibited by the inhibitor CGP-37157 and exhibited Li⁺-dependence, both hallmarks of the mitochondrial Na⁺-dependent Ca²⁺ exchange. NCLX-mediated mitochondrial Ca²⁺ exchange was blocked in cells expressing a catalytically inactive NCLX mutant indicating that NCLX is essential and sufficient for mediating mitochondrial Ca²⁺ transport. Finally we show that expression of NCLX modulates Ca²⁺ influx mediated by the plasma membrane Ca²⁺ store operated channels. Taken together, our results converge to the conclusion that NCLX is the long-sought mitochondrial Na⁺/Ca²⁺ exchanger which by mediating mitochondrial Ca²⁺ efflux also shapes global cellular Ca²⁺ homeostasis.

Cation binding sites in Na⁺-coupled amino acid transporters

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Many mammalian amino acid transporters that catalyze the uphill uptake of amino acids into cells, are energetically driven by the cotransport of Na⁺ ions down their transmembrane concentration gradient. Recent advances in the structure determination of several prokaryotic amino acid transporters, as well as functional studies, have revealed important information on the localization of Na⁺ binding sites in these bacterial transporters. However, cation binding is understood less well in their mammalian counterparts. Here, I discuss the mechanism of cation interaction with two amino acid transporters belonging to different protein superfamilies, the EAATs (excitatory amino acid transporters) and SNATs (sodium coupled neutral amino acid transporters). Our data suggest the existence of a Na⁺ binding site in SNATs that is conserved among a number of prokaryotic and eukaryotic amino acid transporter families that do not have significant similarity in their primary amino acid sequence. For EAATs, a Na⁺ binding site is proposed based on mutagenesis studies, which is localized deep within the transmembrane domain, and which is not observed in the crystal structure of the bacterial homologue GltPh. The results are validated with an empirical valence screening method, allowing the identification of suitable cation binding sites. The results are discussed in terms of the potential transport mechanisms of the two transporter families.

Mechanistic insights in ion-coupled glutamate transport

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Glutamate transporters maintain low synaptic concentrations of neurotransmitter by coupling uptake to flux of other ions. Their transport cycle consists of two separate translocation steps, namely cotransport of glutamic acid with three Na⁺ followed by countertransport of K⁺. Two TI⁺ binding sites, presumed to serve as sodium sites, were observed in the crystal structure of a related archeal homologue. Our recent studies indicate that the conserved aspartate is required for transporter-cation interactions in each of the two separate translocation steps and likely participates in an overlapping sodium and potassium binding site. Available crystal structures of an archeal homologue of these transporters, GltPh, resemble an extracellular-facing state, in which the bound substrate is occluded only by a small helical hairpin segment called HP2. However, a pathway to the cytoplasmic side of the membrane is not clearly apparent. In GltPh, we identified two distinct sets of inverted-topology repeats, and used these repeats to model an inward-facing conformation of the protein. In this conformation, the core of the protein, containing the binding sites and two hairpins HP1 and HP2, has moved toward the cytoplasm relative to the rest of the protein so that the extracellular hairpin becomes buried and the other hairpin is free to open to the cytoplasm. To test this model, we introduced pairs of cysteines into the neuronal glutamate transporter EAAC1, at positions that are >27 Å apart in the crystal structures of GltPh, but ~10 Å apart in the inward-facing model. Subsequent treatment with abolished this activation. The inhibition of transport of these mutants by the oxidizing agent copper(II)(1,10-phenantroline)₃ was potentiated under conditions thought to promote the inward-facing conformation of the transporter. By contrast, the inhibition was reduced in the presence of the non-transportable analogue D,L-threo-β-benzyloxyaspartate, which favors the outward-facing conformation. Other conformation-sensitive accessibility measurements are also accommodated by our inward-facing model.

Ion-coupled transporters: evolution forwards and evolution backwards

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Topology and accurate design of protein-protein interaction are considered essential for function of oligomeric proteins and large multicomponent complexes. In the case of homo-oligomeric membrane proteins, the topology determinants are identical in each protomer and they must dictate a similar mode of insertion resulting in a parallel arrangement. In our laboratory we use the small multidrug transporter EmrE from *Escherichia coli* to show a case of promiscuity where two identical subunits can interact either parallel or antiparallel to each other. In addition, we show promiscuity in the topology of parallel dimers that can be inserted into the cytoplasmic membrane with the N- and C- terminal domains facing the cell inside or its outside and retain activity. We suggest that in the case of EmrE the simplicity of the mechanism of coupling ion and substrate transport and the rather few requirements for multiple substrate recognition provide the robustness necessary to tolerate the promiscuity of interaction of the subunits and the ambiguity in the dimer topology. We suggest that EmrE is a “living fossil” that provides a model for the evolution of structure of transport proteins (Schuldiner, 2009, Schuldiner, 2007).

The mammalian vesicular neurotransmitter transporter VMAT2 is responsible for the transport of monoamines into synaptic and storage vesicles. VMAT2 is thought to have evolved from bacterial multidrug transporters. Expression of rVMAT2 in *Saccharomyces cerevisiae* confers resistance to the parkinsonian toxin 1-methyl-4-phenylpyridinium (MPP⁺) by its removal into the yeast vacuole. The use of directed evolution has allowed identification of three mutations that when combined generates a VMAT2 mutant that lost most of the properties of the neurotransmitter transporter but displayed enhanced resistance to the above toxicants. The results show that, in the case of rVMAT2, loss of traits acquired in evolution of function (such as serotonin transport and inhibitor binding) brings about an improvement in older functions such as resistance to toxic compounds. A process that has taken millions of years of evolution can be reversed by three mutations (Gros and Schuldiner, 2009).

Schuldiner, S. (2007) When biochemistry meets structural biology: the cautionary tale of EmrE. *Trends Biochem.Sci.*, 32, 252-258.

Schuldiner, S. (2009) EmrE, a model for studying evolution and mechanism of ion-coupled transporters. *Biochim Biophys Acta*, 1794, 748-762.

Gros, Y. and Schuldiner, S. (2009) Directed evolution provides a glimpse of hidden properties of VMAT, a neurotransmitter transporter, submitted.

Assembly of AMPA-type glutamate receptors

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AMPA-type glutamate receptors (AMPA receptors) mediate the majority of fast excitatory synaptic transmission in the brain. AMPARs are tetrameric ion-channels that assemble from a pool of four homologous subunits: GluA1-4. AMPAR subunit composition determines channel conductance properties and gating kinetics as well as receptor traffic to and from synaptic sites, and is thus critical for synaptic function and plasticity. Recent studies indicate that the majority of AMPARs in the hippocampus are heteromers of GluA2 with either GluA1 or GluA3, whereas homomeric assemblies or heteromers of GluA1 with GluA3 are rare. Since the two principal assemblies serve different functions it is important to understand the mechanism for this preferential AMPAR subunit composition. Using electrophysiological and biochemical assays to quantify heteromeric assembly of recombinant receptors expressed in *Xenopus* oocytes we found that GluA2 is preferentially incorporated in heteromeric assemblies with either GluA1 or GluA3, and these complexes better traffic to the cell surface compared to homomeric assemblies. Conversely, GluA1/A3 assemblies are less favored than the counterpart homomers, and these complexes are retained in the ER. Using chimeric subunits and deletion mutants we further found that the subunit amino-terminal domain (particularly lobe-2) constitutes the major determinant for the apparent preferred AMPAR subunit composition.

*Abstracts of Posters**

**Arranged alphabetically according to the family name of the first author*

Poster # 1

Popeye domain containing-1 (Popdc1) is a protein of the caveolae and plays a role in the heart response to ischemia-reperfusion

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The muscle-restricted Popdc gene family encodes novel highly-conserved developmentally-regulated membrane proteins (Popdc 1-3), of which Popdc1 has emerged as a putative adhesion protein involved in cell-cell contact and Rho signaling. Despite a decade of efforts, the exact function and detailed cellular localization of Popdc1 have not been determined. Caveolae are detergent-insoluble cholesterol-rich plasma membrane microdomains that function in signal transduction. Of the three caveolae signature proteins, caveolin 1-3, caveolin3 (Cav3) has been shown to be important in cardioprotection.

We hypothesized that Popdc1 is present in the caveolae of cardiomyocytes and is important for the response to ischemia-reperfusion (I/R) injury. Using confocal laser microscopy, we localized Popdc1 to the sarcolemma, intercalated discs (ID) and cytoplasm of mouse heart myocytes. Co-immunolabeling demonstrated co-localization of Popdc1 with Cav3, the gap junction ID protein connexin43 (Cx43), and the costameric protein vinculin that points to the association of Popdc1 with the caveolae, ID and costameres, respectively. Popdc1 co-sedimented with Cav3 in sucrose density gradients and, similar to Cav3, was redistributed following cholesterol removal (methyl- β -cyclodextrin), both in density gradients and in confocal images, further substantiating Popdc1 as a protein of the caveolae. Importantly, Popdc1-null hearts have demonstrated alterations in the membrane distribution of Cav3 and in the pattern of Cav3 density sedimentation. Induction of I/R in isolated hearts (Langendorff, 30min ischemia/90min reperfusion) markedly reduced Popdc1 in the sarcolemma, ID and costameres, and diminished Popdc1 co-localization with Cav3 and Cx43. When compared to wild type (wt), the Popdc1-null hearts displayed inferior functional recovery ($41.7\pm 7.9\%$ vs $62.1\pm 18\%$, left ventricular developed pressure at 30min reperfusion in null vs wt, respectively, mean \pm SD, N=12/group, $p=0.002$) and greater infarct size ($30.0\pm 13.9\%$ vs $14.5\pm 6.7\%$, null vs wt, n=5/group, $p=0.029$) at 90 min reperfusion.

In conclusion, this study presents novel findings on the importance of Popdc1 in the heart structure and function, namely (i) Popdc1 localization and association with the caveolae, (ii) Popdc1 sensitivity to I/R, and (iii) increased vulnerability to I/R in hearts lacking Popdc1. Together, these findings allude to a link between Popdc1 in the caveolae and Popdc1 function during I/R representing significant progress in solving the Popdc1 puzzle.

IFN- γ and NGF-responsive neuronal progenitors from human umbilical cord blood

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Cell therapy based approaches for new treatments of neurological disorders require reliable sources of human neuronal cells, which may be generated from neuronal progenitors. Since the availability of human neuronal stem cells derived from early embryos is extremely limited, progenitors of other origins, such as human umbilical cord blood (HUCB), an established source of hematopoietic stem cells for allogeneic transplantation in hematological disorders, are being considered.

For this purpose we selected HUCB neuronal progenitors (HUCBNP) based on the neuronal prerequisite for adherence to collagen. Populations of collagen-adherent, nestin-positive (94.8 \pm 2.9%) progenitors expressing α 1/2 integrin receptors, were isolated from the mononuclear fraction and survived for more than 14 days. *In vitro* differentiation of the HUCBNP was achieved by treatment with 10% human SH-SY5Y neuroblastoma cell-conditioning media (CM) supplemented with 10ng/ml nerve growth factor (NGF). Neuronal differentiation was characterized by analysis of neurite outgrowths, MAPK kinases (ERK and p38) activation and the expression of the neuronal markers microtubule-associated protein 2 (MAP-2), neurotrophin receptor (TrkA), neurofilament-160 (NF-160), β -tubulin III and neuron specific enolase (NSE) (1). The result point to operationally defined conditions for activating neuronal differentiation of HUCBNP and emphasize the role of neuronal CM and NGF in this process.

Nevertheless, since the growth factors requirement for neuronal differentiation *in vitro* is poorly understood, we aimed to clarify the possibility that interferon- γ (IFN- γ) contributes to the HUCBNP differentiation and demonstrated IFN- γ -induced neuronal differentiation alone and in a cooperative manner with NGF. IFN- γ was detected by Western blot and ELISA in the CM and a neutralizing antibody of IFN- γ significantly inhibited CM induced-differentiation. Transcriptome analysis of CM-differentiated HUCBNP, using Affimatrix microarray technology, identified 79 genes highly up-regulated, among them 26 genes were interferon-induced. Treatment of HUCBNP with human recombinant IFN- γ , resulted in inhibition of cell proliferation and induction of neuronal differentiation, manifested by neurite outgrowths and neuronal markers expression and was significantly inhibited by an addition of neutralizing antibody. These findings propose IFN- γ and NGF use in future protocols of neuronal differentiation of HUCB-derived progenitors (2).

The neuroprotective potential of HUCBNP was evaluated using a neuronal ischemic *in vitro* model. In this model, HUCBNP conferred ~30% neuroprotection towards apoptotic and necrotic neuronal cell death. HUCBNP decreased by 95% the level of free radicals in the insulted-neuron, in correlation with the appearance of antioxidants in the medium. An increased level of NGF, VEGF and FGF-2 protein and mRNA modulation were temporally correlated with the neuroprotection effect. These findings indicate a "bystander" effect of HUCBNP-induced neuroprotection involving antioxidant(s) and neurotrophic factors, which, by paracrine and/or autocrine interactions between the insulted-neuron and the HUCBNP, conferred neuroprotection (3). Altogether, these studies contribute a novel understanding on human umbilical cord blood-derived neuronal progenitors for future cell therapy approaches.

(1) Arien-Zakay et al., *J Mol Neurosci* 2007;32(3):179-91; (2) Arien-Zakay et al., *Leukemia*.in press; (3) Arien-Zakay et al., *Exp Neurol* 216:83-94, 2009.

Poster # 3

'Mini' Cys-loop receptors reveal the minimal structural requirements for functional coupling of neurotransmitter binding to channel gating

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Eukaryotic cysteine (Cys)-loop receptors are pentameric ligand-gated ion channels that convert neurotransmitter binding to opening of an intrinsic ion channel, a process that enables flow of ions down their electro-chemical gradient. Previous attempts to crystallize a full-length eukaryotic Cys-loop acetylcholine receptor (AChR) resulted in crystals that were not sufficiently ordered to provide diffraction at high resolution. Based on electron microscopy studies, which revealed the pattern of receptor packing in these crystals, we inferred that the intracellular loop that connects the transmembrane segments 3 and 4 (M3-M4 loop) interrupts the organization of the receptor molecules in the crystals. To ease the process of crystallization, we generated nine constructs encoding 'mini' ACh-receptor chimeras that are lacking the M3-M4 loop as well as N-terminal segments suspected to be noncrucial for receptor-channel activity. Ligand-binding assays performed on live cells expressing the various receptor mutants indicate that, unlike N-terminus truncated receptors, mini-receptors lacking the M3-M4 loop can readily bind ^3H - α -Bungarotoxin (a competitive antagonist) and nicotine (an agonist). These mini-receptors were visualized on the cell surface by confocal microscopy using rhodaminylated α -Bungarotoxin and specific antibodies, and were also found to be fully functional in terms of channel activity. Taken together, coupling of agonist binding to channel gating requires intact ligand-binding domain but not the intracellular M3-M4 loop.

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VDAC inhibitors prevent apoptosis and VDAC oligomerization

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Mitochondria-mediated apoptosis can be triggered by both external and internal stimuli and results in the release of a large number of apoptogenic proteins from the intermembrane space to the cytosol, including Cytochrome *c* (Cyto *c*). A key protein in mitochondria-mediated apoptosis is the voltage-dependent anion channel (VDAC), an outer mitochondrial membrane protein. In a previous study we demonstrated that apoptosis induction is associated with VDAC oligomerization, and suggested that VDAC oligomer forms a mega pore mediating the release of Cyto *c*. In order to further support the involvement of VDAC oligomerization in apoptosis, we tested the effects of known VDAC inhibitors as well as other anion channels inhibitors on VDAC oligomerization and apoptosis. VDAC oligomerization was revealed by chemical cross-linking using EGS and immunoblotting using anti-VDAC antibodies, and apoptotic cell death was determined by FACS analysis. The effects of DIDS, SITS, H₂DIDS and DPC on VDAC oligomerization and apoptosis as induced in HeLa cells by the apoptosis inducer selenite show that the reagents inhibited VDAC oligomerization and apoptosis, with the overall potency: H₂DIDS>DPC>SITS>DIDS. These findings clearly support the suggestion that VDAC oligomerization is coupled to apoptosis induction. Targeting the VDAC oligomeric status, and hence apoptosis, thus offers a therapeutic strategy for neurodegenerative diseases such as Alzheimer or Parkinson's disease.

Novel selective serotonin reuptake inhibitor as an inhibitor of platelets aggregation

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In the present study we describe the synthesis and the pharmacological characterization of a new selective serotonin reuptake inhibitor, N-methyl citalopram (NMC), with periphery restricted effect due to its inability to cross the blood brain barrier (BBB). Addition of a methyl group to the tertiary amine in citalopram converted it to a quaternary drug positively charged incapable of entering the brain due to its permanent charge. NMC recognized the human platelets serotonin transporter and rat brain serotonin transporter with similar affinity to that of citalopram as was evident from competition binding studies with [³H]citalopram and uptake studies with [³H]5HT. Both citalopram and NMC were unable to inhibit dopamine and norepinephrine uptake in rat brain synaptosomes at 10⁻⁷M. A comparison of mice brain radioactivity following intraperitoneal injections of labeled NMC and citalopram showed that NMC did not penetrate the brain. Taking together, all these results suggest that N-methyl citalopram is a new selective serotonin reuptake inhibitor that does not or minimally penetrate the mouse brain. Several studies suggested that SSRI could have beneficial effects on cardiovascular system by reducing platelet serotonin and thus reducing platelet aggregation. Our preliminary experiments show that the new drug (NMC) inhibits human platelet aggregation in vitro and that chronic NMC administration is at least as effective as aspirin in preventing collagen-induced thromboembolism in mice. Our new drug could be a promising new anti platelet drug that does not cross the BBB and therefore devoid of the adverse CNS effects of citalopram.

Poster # 6

Differential regulation of A β 40 and A β 42 levels by ongoing neuronal activity

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Accumulation of cerebral amyloid β (A β) peptide is critical for developing synaptic and cognitive deficits in Alzheimer's disease (AD). However, the mechanisms regulating extracellular A β concentration ([A β]_o) and, consequently, synaptic function, remain elusive. Recent studies demonstrate that neural and synaptic activity rapidly and directly regulate [A β]_o. Given that the rate and temporal pattern of ongoing spikes may have a profound influence on synaptic plasticity and memory encoding, we explored relationships between ongoing neuronal activity and isoform composition of A β in the extracellular fluid. Our results show that A β 40 and A β 42 isoforms are differentially affected by stimulation frequency in hippocampal neurons grown in culture. Higher [A β 40]_o were detected during high-frequency bursts than during low-frequency stimulation, suggesting facilitation of A β 40 release by bursts. Furthermore, [A β 40]_o correlated with dynamics of synaptic vesicle release. In contrast, [A β 42]_o displayed weaker dependency on both neuronal and synaptic activity. Utilizing pharmacological manipulations of NMDAR-mediated Ca²⁺ flux and neuronal activity, we were able to control metaplasticity of hippocampal network. Notably, burst-evoked facilitation of [A β 40]_o highly correlated to short-term facilitation and long-term potentiation of neurotransmitter release in hippocampal synapses. Understanding the mechanisms regulating [A β]_o and its isoforms should contribute to the elucidation of physiological A β functions and to identify endogenous mechanisms that trigger primary synaptic deficits at very early stages of AD.

Neuronal rescue by Rasagiline (MAO-B inhibitor) in thiamine deficiency

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Thiamine deficiency (TD) is a model of chronic impairment of oxidative metabolism leading to neuronal loss. Rats are fed a thiamine-deficient diet accompanied by injections of the central thiamine antagonist, pyriethamine. They exhibit neurological impairments, cognitive deficits and neuropathological lesions. Neurodegeneration develops over a period of 12 to 14 days and can be reversed by thiamine administration. Using this model can help elucidate mechanisms of neuronal degeneration as well as important paths of neuronal rescue. Furthermore, since TD is partially reversible after thiamine administration, the effect of neuroprotective drugs on recovery can also be studied. In this study we investigated the neuroprotective effects of rasagiline, a MAO-B inhibitor, in TD rats, using neurobehavioral tests and histopathology. Magnetic resonance imaging (MRI) was used to study the extent and location of brain lesions in the rasagiline-treated and untreated-TD rats. Magnetic resonance spectroscopy (MRS) was used to measure brain metabolites. Rasagiline significantly delayed the induction of the TD symptoms and decreased the severity of the associated neuropathology. Rasagiline improved cognition in the Morris water maze. The severity of both the cognitive and histopathological changes was significantly less in rasagiline - TD rats. T₂ maps revealed severe time-dependent damages, mainly in the thalami and the inferior colliculi which were diminished with rasagiline treatment. We found that rasagiline both ameliorated and delayed neuronal injury. MRS showed an increase in the lactate peak in the thalamus of untreated-TD rats as compared to the rasagiline-TD group. Our results demonstrated significant neuroprotection by rasagiline in this model which could have implications for clinical neurodegenerative disorders.

Poster # 8

Involvement of endogenous digitalis-like compounds in the regulation of neuronal cell viability

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Digitalis like compounds (DLC) are a family of steroids synthesized in and released from the adrenal gland. These steroids interact with the Na⁺, K⁺-ATPase, a major plasma membrane transporter. The interaction of DLC with Na⁺, K⁺-ATPase inhibits the enzymatic activity and induces cell specific activation of several signaling pathways. Among the various signal transduction cascades activated by DLC, is the pathway affecting cell growth. Numerous studies have shown that ouabain induces proliferation of several cell types. Nevertheless, its effect on neuronal cell viability and growth has been scarcely studied even though DLC are present in animal brain tissues and cerebrospinal fluid. We raised the hypothesis that endogenous DLC have a role in the regulation of neuronal cells viability and proliferation. This hypothesis was addressed by investigating the effect of digitalis compounds on neuron/endocrine cell lines, PC12 and NT2. Low concentration of ouabain (10⁻⁹M) stimulated cell growth determined by the MTT and the thymidine incorporation assays. Surprisingly, the addition of antibodies against ouabain to NT2 cells growing in complete media (10% FBS) resulted in a significant dose-dependent reduction in cell viability as compared to cells treated with normal rabbit IgG. Furthermore, the addition of 10 nM ouabain to the anti-ouabain-antibodies-treated cells attenuated the effect of the antibodies on cell viability. These effects were not seen in PC12 cells. We observed that ouabain-induced growth stimulation in NT2 cells was associated with stimulation of ERK1/2 phosphorylation. Furthermore, both, growth and ERK activation were blocked by the MEK inhibitor (U0126, 1μM). We conclude that low concentrations of ouabain stimulate cell growth by an ERK kinase-dependent signaling pathway and that the endogenous DLC exert constitutive stimulatory effect on neuronal cells viability.

Poster # 9

The role of NGF and its receptors p75, TrkA and $\alpha 9\beta 1$ integrin on skeletal muscle regeneration

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Disruption of membrane-associated proteins, including dystrophin, and merosin result in Duchene and congenital muscular dystrophies. Following muscle plasma membrane (sarcolemma) damage, muscle injury produces dysregulation of a broad spectrum of structural and regulatory proteins, which accompany muscle fiber death. NGF transgenic knock out mice and anti-NGF transgenic mice present with severe skeletal muscle damage resembling myopathy and muscular dystrophy, implying that NGF plays an important role in muscle physiology. Our aim in this project is to evaluate the role of NGF and its receptors on muscle proliferation, differentiation and regeneration. The pharmacological models employed include C₂C₁₂ skeletal muscle cell cultures as well as wild type and homozygote dystrophic *dy^{2J}* (merosin deficient congenital muscular dystrophy) mice muscles. We characterized mRNA expression of NGF and its receptors p75^{NTR} and $\alpha 9$ integrin in the C₂C₁₂ cell line and both WT and *dy^{2J}* mice. In C₂C₁₂ cell cultures a similar expression of mRNA for NGF and p75^{NTR}/ $\alpha 9$ integrin was found in parallel to the expression of TGF β 1 and its receptor TGF β RI. Serum starvation induced C₂C₁₂ differentiation reflected by increased expression of the myogenic markers myoD and myogenin. TGF β 1 inhibited the morphological differentiation of the cultures. In undifferentiated C₂C₁₂ cultures, the level of β NGF secreted to the media was about 60pg/ml/24h compared to no secretion by differentiated cultures. The NGF tyrosine kinase receptor TrkA was not detected in undifferentiated or differentiated muscle cultures or in the mice muscles. The effect of NGF on C₂C₁₂ proliferation and differentiation is under investigation. The potential output of this research is the clarification of the role of NGF and its receptors in the muscle physiology and under pathologic conditions. Clarification of the role of NGF in muscular dystrophy models will provide novel targets for drug development in the treatment of these devastating diseases.

Reduction of KCNQ2 currents by syntaxin 1A is not associated with changes in channel surface expression levels

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M-channels are very slowly activating, noninactivating, voltage-dependent potassium channels. Heteromeric assembly of subunits encoded by two members of the KCNQ gene family, namely KCNQ2 and KCNQ3, recapitulate the functional properties of the M-current. KCNQ2 and KCNQ3 are coexpressed on the cell bodies and dendrites of many hippocampal and cortical neurons. Importantly, KCNQ2, but not KNCQ3, is expressed on neuronal axons where it might regulate action potential propagation or neurotransmitter release.

Previously we showed that syntaxin 1A interacts with homomeric KCNQ2 physically in brain synaptosomes and in *Xenopus* oocytes. In oocytes, its interaction results in a reduction in the current amplitudes and slowing of the activation kinetics of the channel.

In this study, we sought to determine if the reduction of amplitudes in oocytes coexpressing syntaxin 1A arises from effects on channel surface expression or macroscopic conductance; the latter reflects changes in channel gating.

To this end, we studied KCNQ2 cell surface expression versus current amplitude reduction in oocytes expressing the channels with and without syntaxin 1A, using three experimental approaches. In the first two approaches, surface expression levels were measured by confocal imaging of either KCNQ2-HA or KCNQ-YFP channels. The third approach was a coimmunoprecipitation analysis of manually dissected plasma membranes. All methods provided very similar assessment of surface expression, indicating that the reduction of currents amplitudes is not associated with channel surface expression.

Poster # 11

On the mechanisms regulating synaptic vesicle release by endogenous Amyloid β peptides

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Accumulation of amyloid β peptides ($A\beta$) is central to Alzheimer's disease pathogenesis. However, physiological functions of $A\beta$, a normal product of neuronal metabolism, remain largely unknown. Furthermore, the primary mechanisms by which endogenous $A\beta$ initiates synaptic and cognitive impairments have not been identified. Our recent study demonstrates that endogenous $A\beta$ peptides positively modulate release probability on a rapid timescale in hippocampal synapses (Abramov et al., 2009). To identify the cellular and molecular mechanisms underlying $A\beta$ -mediated increase in release probability, we combine optical imaging of vesicle recycling by FM dyes, fluorescence resonance energy transfer (FRET) spectroscopy, electrophysiology, and molecular biology using cultured hippocampal neurons. Our preliminary results show that both pertussis-toxin-sensitive Gi/o protein and amyloid precursor protein (APP) are essential for $A\beta$ -induced presynaptic potentiation. Moreover, we observed a close association between CFP/YFP tagged APP and G α -subunit of G-protein heterotrimer in presynaptic boutons. We are currently examining the hypothesis whether direct interaction of $A\beta$ with its own precursor induces signal transduction leading to enhancement of release probability. Elucidating the mechanisms underlying $A\beta$ effects on synaptic function may help to understand physiological $A\beta$ signaling and to identify primary pathological events initiating synaptic dysfunction in Alzheimer's disease.

Efrat Abramov*, Iftach Dolev*, Hilla Fogel, Giuseppe D. Ciccotosto, Eyal Ruff, and Inna Slutsky. Amyloid- β as a positive endogenous regulator of release probability at hippocampal synapses (*Nature Neuroscience, in Press*).

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Na⁺, K⁺-ATPase as a target for drugs for the treatment of depressive disorders

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Depression is a mental state characterized by a pessimistic sense of inadequacy and a despondent lack of activity. The treatment of depressive disorders is limited due to the lack of appropriate drugs and the side effects of the existing ones. In light of the high prevalence of this disorder and its high impact on the society, is a genuine need for the development of new antidepressants. Na⁺, K⁺-ATPase is a major plasma membrane transporter for sodium and potassium. Digitalis-like compounds (DLC) are a family of steroid hormones synthesized in and released from the adrenal gland. The binding of DLC to the Na⁺, K⁺-ATPase inhibit the ion transport leading to several cell specific signaling pathways. Our recent studies demonstrated the association between Na⁺, K⁺-ATPase α isoforms and bipolar disorders and provided evidence for the involvement of this enzyme and the endogenous DLC in the etiology of depressive disorders (Biol. Psychiatry. 60:491-499, 2006; Biol. Psychiatry, 65:985-991, 2009). The goal of the present study was to test the possible involvement of DLC in depressive behavior using normal Sprague-Dawley (SD) and the Flinder Sensitive Line (FSL) rats, a genetic model of depression. Depressive-like behavior was determined by the Forced Swimming Test (FST). Intra-cerebro-ventricle (i.c.v) injection of anti-ouabain antibodies (50 μ g) to SD rats elicited anti-depressive effect manifested by a significant increase in the mobility time in the FST, but not when injected with non-specific rabbit IgG. Furthermore, chronic i.c.v administration of anti-ouabain antibodies (14 days, 15 μ g/day) to FSL rats, also significantly increased the mobility time in comparison to rats receiving non-specific rabbit-IgG. These results suggested that the reduction in DLC activity in the brain ensued by the treatment with anti-ouabain antibodies results in an anti-depressive response. We have recently synthesized a new DLC derivative 4-(3' α -15' β -dihydroxy-5' β -estran-17' β -yl) furan-2-methyl alcohol (termed here Compound 16). This compound showed antagonistic properties to DLC in several experimental systems. Injecting it (intraperitoneal 6 mg/kg) to SD rats significantly increased the mobility time in the FST, indicating an anti-depressant properties. Similar results were obtained in FSL rats treated chronically for 14 day with compound 16 (1-2 mg/kg/day). These results are in accordance to the proposition that mal functioning of the Na⁺, K⁺-ATPase/DLC system may be involved in manifestation of depressive disorders and identify our new compound as potential drug for the treatment of this maladies.

Identification of the binding site of the anthelmintic drug ivermectin in glutamate-gated Cl⁻ channels of *C. elegans*

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Ivermectin (IVM) is an anti-parasitic drug widely administered to cattle against intestinal parasitic worms. It is also used to treat human parasitic diseases such as Onchocerciasis (river blindness). At nanomolar concentrations, IVM irreversibly activates the invertebrate pentameric glutamate (Glu)-gated Cl⁻ channels (GluCl receptors; GluClRs). By that, IVM induces continuous influx of Cl⁻ ions into peripheral motor neurons, leading to sustain hyperpolarization, suppression of electric transmission, paralysis and death of the worm. At micromolar concentrations, IVM is able to activate and/or potentiate mammalian pentameric ionic channels that are naturally activated by neurotransmitters (NTs) like γ -aminobutyric acid (GABA), glycine or acetylcholine (ACh). Native GluClRs consist of α and β subunits, but can be expressed as homomers. The homomeric GluCl β R responds to Glu but not to IVM, while the homomeric GluCl α R responds to IVM but not to Glu, indicating that in the native GluCl α/β R the α subunit is responsible for the sensitivity to IVM. Yet, it is not known where IVM binds.

Here, the effect of IVM was first examined on the chimeric $\alpha 7$ -GluCl β R, a homopentamer having the ligand-binding domain of a vertebrate $\alpha 7$ nicotinic ACh receptor ($\alpha 7$ -nAChR) and the pore domain of the GluCl β R. Since the activity of the $\alpha 7$ -nAChR is potentiated by IVM and the homopentameric GluCl β R is not sensitive to IVM, we anticipated that the activity of the chimeric $\alpha 7$ -GluCl β R would be potentiated by IVM. Surprisingly, IVM strongly inhibited the chimeric $\alpha 7$ -GluCl β R. Since the major structural differences between the aforementioned IVM-activated/potentiated receptors and the IVM-inhibited $\alpha 7$ -GluCl β chimera reside at the interface between the neurotransmitter-binding domain and the pore domain, we hypothesize that IVM binds at this interface. In order to further locate the IVM binding site and its docking mode onto the GluCl α/β R, we replaced the $\beta 8\beta 9$ and Cys loops of the GluCl β subunit by the homologous loops of the α subunit, one at a time and concomitantly. This replacement was done because: (i) in all pentameric NT-activated channels, these loops reach the interface between the neurotransmitter-binding domain and the pore domain (an interface suspected by us to be involved in IVM accommodation), and (ii) we expected to see an improvement of the activation by IVM, if these loops were to be involved in IVM binding. Our results show that a GluCl α/β mutant receptor that consists of a WT- α subunit and a mutated β subunit that has both the $\beta 8\beta 9$ and Cys loops of the α subunit, becomes much more sensitive to IVM than the WT GluCl α/β R. We suggest that IVM binds at the interface between the ligand-binding domain and the channel domain, in a crevice between the $\beta 8\beta 9$ loop and the Cys loop of two adjacent subunits.

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Molecular mapping of an I_{KS} channel opener reveals crucial interactions between KCNE1 and the Kv7.1 voltage sensor paddle

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Voltage-gated K^+ channels co-assemble with accessory subunits to form macromolecular complexes. In heart, assembly of Kv7.1 pore-forming subunits with KCNE1 auxiliary subunits generates the repolarizing K^+ current I_{KS} . We and others, recently suggested a strategic location of KCNE1 wedged close to helices S1 and S4 of two adjacent Kv7.1 voltage sensing domains (VSD) and nearby helix S6 of another Kv7.1 subunit. Here we show that the I_{KS} channel opener, diisothiocyanostilbene-2',2'-disulfonic acid (DIDS) acts on I_{KS} as a gating-modifier, thereby converting the time- and voltage-dependent channels into nearly voltage- and time-independent currents. While DIDS activates Kv7.1, it does not affect Kv7.2. The two isothiocyanate functionalities are crucial for the potent activating effect of DIDS on I_{KS} , since 4'-acetamido-4'-isothiocyanostilbene-2',2'-disulfonic acid (SITS) that has only one of these groups and 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS), which lacks isothiocyanate groups and thus cannot form covalent bonds with amino acids, do not activate I_{KS} currents. Mutagenesis and modeling data indicate that DIDS activates I_{KS} by docking to an externally-accessible pocket, formed at the interface between the superficial N-terminal boundary of the KCNE1 transmembrane segment and the VSD paddle motif of Kv7.1. DIDS does not activate the channel complex formed by co-expression of KCNE1 and a chimeric Kv7.1 endowed with a Kv7.2 VSD paddle. DIDS binding at the Kv7.1 VSD-KCNE1 interface reveals that two lysine residues, K41 in KCNE1 and K218 in Kv7.1 S3-S4 linker are distant to about 10 Å. Thus, KCNE1 affects Kv7.1 channel gating by closely interacting with the VSD paddle motif.

Targeting the voltage sensor of Kv7.2 channels with a new gating-modifier: novel approaches to cure hyperexcitability disorders

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The pore and gate regions of voltage-gated cation channels have been often targeted with drugs acting as channel modulators. In contrast, the voltage sensing domain (VSD) was practically not exploited for therapeutic purposes, though it is the target of various toxins. We recently designed novel diphenylamine carboxylates that are powerful Kv7.2 channel openers or blockers. Here we show that a new Kv7.2 channel opener, NH29, acts as a non-toxin gating modifier. NH29 increases Kv7.2 currents, thereby producing a hyperpolarizing shift of the activation curve and slowing both activation and deactivation kinetics. In neurons, the opener depresses evoked spike discharges. NH29 dampens hippocampal glutamate and GABA release, thereby inhibiting excitatory and inhibitory post-synaptic currents. Mutagenesis and modeling data suggest that in Kv7.2, NH29 docks to the external groove formed by the interface of helices S1, S2 and S4 in a way which stabilizes the interaction between two conserved charged residues in S2 and S4, known to interact electrostatically, in the open state of Kv channels. Results indicate that NH29 may operate via a voltage-sensor trapping mechanism similar to that suggested for scorpion and sea anemone toxins. Reflecting the promiscuous nature of the VSD, NH29 is also a potent blocker of TRPV1 channels, a feature similar to that of tarantula toxins. Interestingly, we found that mutations in the TRPV1 VSD markedly affect the activity of the modulator, suggesting that the VSD of both Kv7.2 and TRPV1 is promiscuous and share some common structural and biophysical features. Our data provide a structural framework for designing novel gating-modifiers targeted to the VSD of voltage-gated cation channels and used for the treatment of hyper-excitability disorders.

The cannabinoids, THC and CBD, inhibit the LPS activation of BV-2 microglial cells by different combination of intracellular pathways

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Cannabinoids have been shown to exert anti-inflammatory activities in various *in vivo* and *in vitro* experimental models of inflammatory CNS degenerative diseases. However, the mechanisms of these effects are currently unknown. Using the BV-2 mouse microglial cell line and lipopolysaccharide (LPS) to induce inflammatory response, we revealed the signaling pathways engaged in the anti-inflammatory effects of cannabinoids. We found that the two major cannabinoids present in marijuana, Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) at 10 μ M, decrease the release of interleukin (IL)-1 β and interferon (IFN) β proinflammatory cytokines from LPS activated microglial cells. However, they seem to differ in their mechanisms of action.

NF κ B transcription factor is a primary modulator of the inflammatory response. It is known that LPS leads to the degradation of I κ B (inhibitor of NF κ B) and activates NF κ B dependent transcription. We found that CBD, but less so THC, reversed the LPS-induced I κ B degradation.

STAT transcription factors are main mediators of IFN β signaling. Both CBD and THC decrease the activation of the LPS induced STAT1 transcription factor, a key player in IFN β dependent proinflammatory processes. However, CBD but not THC, upregulates the LPS-induced activation of the STAT3 member of transcription factors.

In summary, we found that THC and CBD anti-inflammatory activity in BV-2 microglia occurs through different, although partially overlapping, mechanisms.

Anxiolytic effect of a novel adaptogenic treatment

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Anxiety disorders are among the ten most important public health concerns, which in recent years reached epidemic proportions. Current treatments are of limited efficacy and are associated with a wide variety of side-effects. Therefore, the need for further examination of alternative pharmacological agents is evident.

One source may be traditional eastern herbal medicine. Thus far, hardly enough empirical research using western scientific methodology has been conducted for the evaluation of eastern herbal drugs that are currently used by practitioners around the world. The present study was aimed to fill this void and investigate a novel treatment for anxiety disorders based on natural herbs - adaptogens.

The first experiment was conducted on BALB mice, a strain known to exhibit increased anxiety-like behavior. At the age of 4 weeks, mice were subjected to daily stress manipulations for the duration of 1 week. Afterwards, mice were randomly assigned into 3 groups of treatment: adaptogenic treatment (15 mg/kg), escitalopram (15 mg/kg) and a control group receiving only the vehicle. Drug administration was given through i.p injection. Following 3 weeks of treatment, mice were exposed to acute pharmacological stress (yohimbine i.p injection 1.25 mg/kg) and anxiety-like behaviors were assessed. Results from the first experiment demonstrate an anxiolytic effect of the adaptogenic mixture: In the Novel Open Field treated groups spent significantly more time inside the arena in comparison to the control group. The same effect was found in the Elevated-Plus Maze: Treated groups spent significantly more time in the open arms in comparison to the control group. In the second experiment, ICR mice were subjected to Maternal Separation paradigm during the postnatal period and to Unpredictable Chronic Mild Stress paradigm in adolescence. Anxiety-related behaviors were assessed using the Elevated-Plus Maze and indicated anxiogenic effect of the stress manipulation. During adulthood, mice were assigned to different groups of treatment: adaptogenic treatment (15, 30 mg/kg), escitalopram (15 mg/kg) and a control group, receiving only the vehicle. Following 3 weeks of treatment, behavioral assessments were performed. In addition, blood samples were collected following restraint stress and corticosterone levels were measure via RIA. In addition to attenuation of anxiety-like behavior, when confronted with acute stress, mice treated with the adaptogenic mixture exhibited a moderate hormonal reaction in comparison to the control group.

The results of those two experiments indicate an anxiolytic effect of the novel adaptogenic treatment, suggesting a further examination of this mixture as a candidate for treatment of anxiety disorders.

Osteoporosis-associated changes in bones, uterus and apoptosis: involvement of the mitochondrial protein VDAC

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To identifying new biochemical markers of osteoporosis, we established two animal models of osteoporosis. In the first model of bone re-absorption, the animals undergo thyro-parathyroidectomy to neutralize the parathyroid hormone (PTH) influence on Ca^{2+} homeostasis, and induce accelerated bone re-absorption by vitamin D_3 treatment that enhances calcium escape leak from the bone to the body fluids. In the second model, rats were ovariectomized (OVX) to cause postmenopausal-like bone loss due to estrogen deficiency. Histological analysis of bone indicates significant bone loss in the OVX group vs. mock operated group, and a profound decrease in uterine size, probably due to apoptosis. Since one of the key proteins in apoptosis is the mitochondrial protein, the voltage-dependent anion channel (VDAC), we analyzed its expression level in OVX animals. In mammals there are 3 VDAC isoforms (VDAC1, 2, 3) with a molecular mass of 32 to 36 kDa.

To assess changes in VDAC1 expression in uterus of OVX animals, we analyzed its expression in extracts of the uteri by immunoblotting using anti-VDAC antibodies.

The analysis showed approximately 2-fold decrease in the level of VDAC (31kDa) in the OVX group, and substantial increase in the levels of anti-VDAC antibody-reacted protein (24 kDa, P24). Surprisingly, the P24 expression levels in uterus of control or OVX animals were over 10-fold higher than in muscle, brain or liver obtained from the same animal. Interestingly, the P24 protein cross reacted with the N-terminal VDAC1 specific antibodies, but not with polyclonal antibodies directed against VDAC1 sequence of aa150 to aa250. These results indicate the presence of a VDAC1 variant in uterus it's the origin and function of which are not known yet. In addition, the relationship between the decrease in the expression level of VDAC in ovariectomized animal and ovariectomy-induced uterine apoptosis requires further research.

Physiological responses of lowering circulating ouabain in the rat

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Endogenous ouabain-like compounds are synthesized in and released from the adrenal gland. The binding of these hormones to the Na⁺, K⁺-ATPase elicits inhibition of ion transport and the activation of several signaling pathways in different cells. Although endogenous ouabain (EO) has been implicated in several pathological states such as hypertension and heart and kidney failure, its physiological roles in normal animal has not been elucidated. Recently, using passive immunization method, we have shown that EO, plausibly originating from the adrenal gland, participates in vascular tone homeostasis and sodium balance in the rat. The aim of the present study was to measure the physiological consequences of reduction in circulating EO by active immunization using ouabain-BSA conjugate.

An ouabain-BSA conjugate or BSA in complete Freund's adjuvant was injected s.c. into rats. The animals were rechallenged as above at 2 and 3 week intervals, with the same antigens. The rats were housed in metabolic cages for 7 days for measurement of water and food consumption and urine excretion. At the end of the experiment the thoracic aorta was isolated and used to study phenylephrine-induced contraction and atrial natriuretic peptide (ANP)-induced vasorelaxation.

The basal natriuresis of autoimmune rats against ouabain was significantly lower as compared to the control group. In addition, the response to ANP-induced vasorelaxation was significantly increased in aortic rings from autoimmune rats against ouabain. In addition, reduction of EO by passive immunization accomplished by the administration of anti-ouabain antibodies, resulted in a 25% increase in adrenal weight, as compared to rats receiving normal rabbit IgG. Histological analysis revealed that the increase in size took place in all cortical zones. Moreover, the adrenal size correlated with ouabain but not aldosterone or corticosterone plasma concentrations. Interestingly, the absolute and relative medulla mass did not differ between groups. These findings are in accord with the notion that circulatory EO originating in the adrenal cortex has physiological roles in controlling vasculature tone and sodium homeostasis in normal rats.

Chronic treatment of cultured hippocampal neurons with an M-channel blocker leads to a compensatory decreased excitability

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The neuronal M-current is a voltage-dependent potassium current that is encoded by the Kv7.(2-5) channel family genes. The M-channels have distinctive biophysical characteristics, including activation in the subthreshold voltage range, slow activation and deactivation kinetics, and no inactivation. Its unique biophysical properties lead to significant activation of the M-current near the action potential threshold but mainly during sustained neuronal activity. Thus, it acts as a brake for repetitive firing and plays a dominant role in regulating neuronal excitability. Recently, our laboratory showed that acute application of a novel M channel blocker, NH17, induces a hyperexcitability state in both central and peripheral neurons. In this study, our aim was to check the effects of chronic treatment of cultured hippocampal neurons with the M channel blocker NH17. Our results show that in contrast to the effect of acute application of NH17, chronic application of the M-channel blocker markedly reduced the spike firing frequency, decreased the average charge transfer of inhibitory postsynaptic currents (IPSCs), increased the average charge transfer of excitatory postsynaptic currents (EPSCs) and increased the expression of Kv7.2 subunits as revealed by Western blot of hippocampal cultures. Our results suggest that chronic treatment with the M-channel blocker NH17 may produce a compensatory negative feedback mechanism that involves upregulation of M-channels encoded in part by Kv7.2 subunits. This might represent a plasticity mechanism intended to counteract a hyperexcitability state of hippocampal neurons. In addition, the compensatory mechanism may involve a substantial depletion of neurotransmitter vesicles, subsequent to an intense period of network-driven release of neurotransmitters caused by persistent M-channel inhibition.

TRPM2 as a common target for anti-inflammatory and neuroprotective natural compounds

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The TRPM2 ion channel is activated by intracellular ADP-ribose which is produced downstream of various inflammatory and neurotoxic stimuli, such as hydrogen peroxide, alloxan and amyloid β a. It is critically involved in neuronal cell death and inflammation. Part of its physiological roles is chemokine production in monocytes and insulin release. A few pharmacological inhibitors of TRPM2 are known, e.g. econazole, flufenamic and N-(p-amylcinnamoyl)anthranilic acid. We tested several natural products with known anti-inflammatory and neuroprotective properties for their ability to inhibit ADP-ribose mediated currents in HEK-293 cells expressing TRPM2. We identified two structurally similar compounds which inhibit TRPM2 mediated currents, cannabidiol (a non-psychoactive cannabinoid) and caffeic acid phenethyl ester (isolated from propolis). Furthermore, incubating TRPM2 expressing HEK-293 with any of the compounds decreased the subsequent calcium influx caused by hydrogen peroxide. In addition we tested incensole acetate, a neuroprotective and anti-inflammatory substance isolated from Boswellia resin, for its ability to inhibit TRPM2. Although it was able to slightly reduce TRPM2 mediated currents it failed to inhibit calcium influx, indicating a specific effect of the former compounds and excluding a non-specific lipid mediated effect. Those findings identify a potential pharmacological target of those compounds and might help to further understand the physiological role of TRPM2 ion channels.

Cannabinoid CB1 receptor antagonist manipulation at birth and may be associated with ADHD

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Attention Deficit Hyperactivity Disorder (ADHD) is a condition that becomes apparent in some children in the preschool and early school years. It is estimated that between 3-5 percent of children have ADHD in USA, and 5-10 percent in the Israel. Attention Deficit Hyperactivity Disorder is characterized by inattention, impulsivity and hyperactivity. A familiar feature of ADHD is the response to psychostimulants such as methylphenidate (Ritalin) and D-amphetamine (Adderall). In ADHD patients, low doses of stimulants produce beneficial behavioral effects by reducing excess motor activity and enhancing concentration. Although ADHD has been known for over 80 years, the etiological and risk factors for ADHD are still unclear. Chadapin and colleagues (2005) found that low birth weight is one of the most important predictive factors of ADHD. Thus low birth weight infants are commonly found to have both cognitive and behavioral problems in childhood. In a series of studies performed in neonatal mice we have demonstrated that the cannabinoid CB1 receptor is critically important for the initiation of the suckling response (Fride et al., 2001; 2003; 2007). Non organic failure-to-thrive (NOFTT) is defined as an abnormally low weight and/or height for age without a known organic cause. Children which suffered from NOFTT are thought to display behavioral and cognitive dysfunctions in later years. Recent data from our laboratory suggest that an oral motor deficiency which is similar to symptoms of non organic failure to thrive in infants with NOFTT, develop cognitive and behavioral abnormalities at later stages of development. Now we propose that a deficient ECBR (Endocannabinoid-CB-receptor) system may comprise a risk factor for ADHD.

Methods: In order to investigate the effects of neonatal exposure to cannabinoid CB1 receptor blockade, male and female pups were administered a single injection of SR141617 (rimonabant, 5 or 10 mg/kg), within 24 hours of birth. At two months of age, mice were tested in an assay for pre-pulse inhibition of the acoustic startle response. At the age of 16 weeks the same mice were tested for motor activity in an open field, immobility on an elevated ring and for anxiety in the 'plus-maze' assay.

Results: We found a significant reduction in the acoustic startle response in the female mice treated with 10 mg/kg, and decreased performance in the PPI test at all doses. To the 5 mg/kg dose, mice responded in a sex-dependent manner: only male mice showed a decrease in PPI and increase in ASR while females only showed a trend. Both male and females displayed significant hyperactivity and decreased ring-immobility. In the plus maze, both males and females spend more time in the open arms than in the closed arms, suggesting decreased vulnerability to anxiety-provoking situations.

Conclusions: we have demonstrated here that neonatal manipulation of the cannabinoid CB1 receptor precipitates symptom of ADHD at adulthood. We conclude that at least a subgroup of ADHD may be caused by a developmental deficiency of the Endocannabinoid system.

Cannabidiol treatment modulates the expression of lipid related enzymes in a microglial cell line

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Microglia are resident macrophages that serve as early host defense against pathogens in the central nervous system. We found that treatment of the murine BV-2 microglial cell line with 10 μ M of the non-psychoactive plant cannabinoid, cannabidiol (CBD), increases the accumulation of the endocannabinoid *N*-arachidonoyl ethanolamine. In addition, CBD reduced cell viability, an effect that was reversed by lipid raft disruption. Here, we investigated the effects of CBD on gene expression of lipid related enzymes in the BV-2 microglial cell line. The cells were grown in DMEM with 5% FBS, expression profiling of lipid related enzymes was done using gene array analysis verified by quantitative RT-PCR analysis. BV-2 cells were tested in DMEM containing 5 % FBS.

Gene array analysis revealed a 3.6 fold increase in acetyl-coenzyme A acetyltransferase 2/sterol-o-acyl transferase (ACAT2/SOAT2), the gene product encoding the enzyme which is responsible for converting cholesterol to cholesteryl esters. In addition, we observed a 2.4 fold increase in gene expression of adipophilin (a protein known to enhance lipid accumulation and prevent lipid efflux from macrophages that were derived from differentiated human THP-1 cells). We are currently testing whether the gene expression profile, which suggests accumulation of cholesterol esters in the BV-2 microglial cells, is in line with the protein expression and lipid profile.

The external-work pressure-time integral relationships and the afterload dependence of frank starling mechanism

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Background: The mechanisms underlying the Frank-Starling Law of the heart are elusive. Despite the prevalent notion that it is afterload independent, isolated fiber studies reveal that the afterload affects the related force length relationship. The study explores the roles of the afterload, in situ.

Methods: The LV was exposed by left-thoracotomy in adult sheep (72.6 ± 8.2 Kg, $n=8$). Pressure transducers were inserted into the LV and aorta. Flowmeter was placed around the aortic root. LV volume was assessed by sonocrystals. Occluders around the aorta and the inferior vena-cava enabled to control the afterload and preload. Different afterloads were imposed by partial aortic occlusions. Transient inferior vena-cava occlusions (IVCOs) were performed at each steady afterload.

Results: A highly linear relationship was found between the external work (EW) and pressure-time integral (PTI) ($R^2=0.98 \pm 0.01$) during each transient IVCO ($n=48$). These EW-PTI relationships (WPTiRs) were preload independent since the preload had a proportional effect on the EW and PTI at constant afterload. Interestingly, the slope of the WPTiR was afterload dependant. The slope was 33.3 ± 4.1 mJ/(mmHg·s) at baselines and decreased by 1.0 ± 0.50 mJ/(mmHg·s) per 1 mmHg·min/L increase in the peripheral resistance. A unique WPTiR was obtained during both the occlusion and release phases of each IVCO, while two distinct EW-preload or PTI-preload relationships were observed. The same WPTiRs were also obtained for steady state conditions where the afterload was constant and the preload changes were only due to changes in lung ventilation and not an invasive IVCO.

Conclusions: The novel WPTiR ties the Frank (pressure development) and Starling (EW production) phenomena together. The dependence of the WPTiR on the afterload highlights the adaptive control of the Frank-Starling mechanisms to changes in the afterload. Since the WPTiR can be obtained in a minimally invasive manner, it also has the potential to be of clinical use.

The effect of chronic methylphenidate (Ritalin) treatment on presynaptic dopaminergic parameters in a rat model for ADHD

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Attention deficit/hyperactivity disorder (ADHD) is the most common behavioral, emotional, and cognitive disorder described in youth. ADHD is a prevalent disorder affecting 4% to 7% of children worldwide. The neurobiology of ADHD is not completely understood, although imbalances in dopaminergic and noradrenergic systems have been implicated in this disorder. Although several animal models of ADHD exist, the spontaneous hypertensive rats (SHR) is the most accepted and frequently used animal model for ADHD as it had displayed core behavioral symptoms and neurocognitive impairments similar to individuals with ADHD. More important, methylphenidate (Ritalin), the most widely prescribed medication for this disorder, counters this various ADHD - like symptoms in the SHR strain.

Our preliminary results indicate that there are differences in the expression of presynaptic dopaminergic parameters between rat model for ADHD (SHR rats) and its control (WKY rats). We found that the density of the presynaptic dopaminergic transporter (DAT) as well as the density of the vesicular monoamine transporter (VMAT2) were significantly lower (19%, $p < 0.005$ and 21%, $p < 0.01$ respectively) in the striatum of young SHR rats as compared to young WKY rats. [^3H]TBZOH affinity (K_d) to VMAT2 was significantly higher (22%, $p < 0.05$) in striatum of SHR rats compared to WKY rats. We have also demonstrated that the baseline [^3H]dopamine release from young SHR striatal brain slices was significantly lower than the release from WKY brain slices (30.5%, $p < 0.005$). Chronic methylphenidate (Ritalin) administration (21 days, 3mg/Kg) to 2 weeks old SHR rats reduced the density of the presynaptic dopaminergic transporter by 21% ($p < 0.05$) as compared to saline treated SHR rats. A significant decrease in baseline release of [^3H]dopamine was observed after the chronic methylphenidate treatment (25%, $p < 0.005$) although the potassium- and amphetamine- induced [^3H]dopamine release were significantly higher (29%, $p < 0.05$ and 29%, $p < 0.01$, respectively) after the chronic treatment.

From our results it appears that the widely used psychostimulant methylphenidate does not resolve the deficiency underlying ADHD for the long run, although it induces a short time compensatory mechanism in order to treat this disorder at the present state.

Modulation of voltage-gated calcium channel currents in pituitary GH₃ cells by lipophilic molecules

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In this study we examined the susceptibility of voltage-gated calcium channels (VGCC) in GH₃ cells to modulation by lipophilic molecules known as bilayer mechanical reagents. The lipophilic molecules used were; cone-shaped lysophospholipids (LPLs) and inverted cone-shaped poly unsaturated fatty acids (PUFAs). LPLs of different head group size and charge were used: lysophosphatidylcholine (LPC), lysophosphatidylserine (LPS) and lysophosphatidylethanolamine (LPE). PUFAs containing different numbers of double bonds were used: arachidonic acid (AA) and linoleic acid (LA).

We show that partition of LPC into the membrane of GH₃ cells suppressed L-type calcium channel currents (I_L). This suppression was slow in onset, reversible upon washout with BSA and associated with a depolarizing shift in activation (by ~ 8 mV), and in steady-state inactivation (by ~ 12 mV), of I_L . In contrast to these effects of LPC on I_L , LPS and LPE exerted minimal or insignificant effects. This difference may be attributed to the prominent conical shape of LPC compared to the shapes of LPS and LPE (which have smaller headgroups). Partition of AA and LA LPC into the membrane of pituitary cells also suppressed I_L . This suppression was slow in onset, reversible upon washout with BSA, but not associated with a depolarizing voltage shift in the activation of I_L .

Cone-shaped LPLs and inverted cone-shaped PUFAs are expected to exert opposite effects on bilayer curvature; LPLs promoting positive curvature whereas PUFAs promoting negative curvature. The similarity in the effects of LPC, AA and LA on I_L (except in voltage shift) suggests that their effects on bilayer curvature are not the major determinant in their action. Different physical parameters, such as bilayer elasticity or lateral pressure profile at the lipid-protein interface, may play a significant role in the mechanical perturbation of VGCC function in pituitary cells. The susceptibility of VGCC to these bilayer mechanical reagents fits well with the previous reports about osmo/mechanosensitivity of VGCC in these cells.

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What does it take to broaden the specificity of a secondary multidrug transporter from monovalent to divalent cationic substrates?

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The *Escherichia coli* proton/drug antiporter MdfA belongs to the Major Facilitator Superfamily (MFS) of secondary transporters. MdfA is a 410-amino-acid residue-long membrane protein with 12 transmembrane (TM) helices. Cells expressing MdfA from a multicopy plasmid exhibit multidrug resistance against a diverse group of structurally and electrically dissimilar drugs such as lipophilic monovalent cations, zwitterionic drugs, and uncharged compounds (e.g. Ethidium Bromide, Ciprofloxacin and Chloramphenicol, respectively). Previous studies have established the importance of acidic residues in substrate recognition by MFS secondary multidrug transporters, but their role in the transport mechanism remained unknown. The putative 12-TM helices of MdfA contain two membrane-embedded acidic residues, E26 and D34, in TM1. Previous work in our lab showed that E26 constitutes an important part of the drug recognition pocket in MdfA, although it is not essential for transport activity. Recent screens for MdfA mutants that confer resistance to a divalent cationic drug (methyl viologen) led to the isolation of an interesting mutant, I239T/G354E. Both mutations (I239T/G354E) are needed for resistance against methyl viologen. Regarding resistance to chloramphenicol (a neutral substrate) the single mutant MdfA-I239T has a wild-type phenotype whereas MdfA-G354E confers lower resistance compared to wild-type MdfA. The negatively charged acidic residue at position 354 (TM11) seems to be located inside the multidrug recognition pocket, according to the 3D structure model of MdfA. This assumption is supported by the findings that the I239T/G354E mutations are able to restore function of an MdfA mutant neutralized in any of its essential negative charges (E26 or D34). In addition, site directed mutagenesis suggests that residues 26 and 354 may be located close to one another in the folded conformation of the transporter. Although the mechanism of transport of methyl viologen by the double MdfA mutant is currently unknown, this work demonstrates how simple it is for Mdr transporters to acquire new properties by natural selection.

Non-L type calcium channel currents in rat anterior pituitary cells

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Electrical activity driven calcium influx through L-type channels underlies both basal and stimulated hormone secretion from normal anterior pituitary cells. Previous studies demonstrated the existence of high voltage activated (HVA) non L-type calcium channels in anterior pituitary tumor cell lines (GH₃ cells). In this study we examined whether or not these non L-type channels exist also in normal anterior pituitary cells.

Experiments were performed either on enriched or on mixed populations of anterior pituitary cells. Enriched populations of pituitary somatotrophs and lactotrophs were obtained by using density gradient centrifugation. Different types of calcium channels were identified by the use of specific toxin blockers, by immunocytochemistry and by RT-PCR. Toxins were delivered either by perfusion, or by direct application to the bath. Double blind experiments were performed to validate toxin versus control effects.

Our results demonstrate that ~30% of the sustained HVA calcium influx in pituitary somatotrophs and lactotrophs is carried through non-L type calcium channels. Our main findings may be summarized as follows: **1.** Saturating concentrations of Nifedipine (30 μ M) reduced sustained HVA currents by 67.8 \pm 5.5% (n=12). The residual currents, after nifedipine block, were completely blocked by cadmium (200 μ M). **2.** omega-Agatoxin IVA (250 nM), a specific blocker of P/Q-type channels, reduced sustained HVA currents by 18.3 \pm 3.7 % (n=16). **3.** omega-Conotoxin GVIA (2 μ M), a specific blocker of N-type channels, reduced sustained HVA currents by 10.6 \pm 1.4% (n=17). **4.** SNX-482 (30nM), a specific blocker of R-type channels, reduced sustained HVA currents by 6.0 \pm 0.5% (n=9). **5.** A cocktail containing the three toxins; omega-agatoxin IVA (250 nM), omega-Conotoxin GVIA (2 μ M) and SNX-482 (30nM), reduced sustained HVA currents in a mixed population of pituitary cells by 32.0 \pm 5.0% (n=8). **6.** Immunostaining, using antibodies against L-type and P/Q-type channels, demonstrated the existence of α 1C, α 1D and α 1A subunits of calcium channels in pituitary cells. **7.** RT-PCR demonstrated transcripts of α 1C, α 1D and α 1A subunits of calcium channels in pituitary cells.

In summary, our results unraveled the existence of non-L-type calcium channels; P/Q-type, N-type and R-type, in normal anterior pituitary cells. The known involvement of these channels in synaptic transmission suggests that they may also be involved in the secretion of pituitary hormones.

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Deactivation mechanism of a pentameric ligand-gated ion channel revealed by metal-ion trapping upon pore closure

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Eukaryotic pentameric ligand-gated ion channels (pLGICs) are receptors activated by neurotransmitters to rapidly transport ions across cell membranes, down their electrochemical gradient. Thereby, they modulate the propagation of electric signals throughout the nervous system. Recent crystal structures of two prokaryotic pLGICs revealed that the extracellular side of the transmembrane channel constricts to close the pore (Hilf and Dutzler, Nature, 2009). Here we used a eukaryotic chimeric pLGIC whose ligand-binding domain binds acetylcholine (ACh) to activate a channel domain of a serotonin receptor. The channel of this chimeric receptor was equipped with histidines in order to coordinate a metal ion within the pore, at its cytoplasmic side. While in a previous study the access of Zn^{2+} ions to the inserted histidines had been explored when the channel was either at rest (closed) or active (open) (Paas et al, PNAS, 2005), here the interactions of Zn^{2+} with the pore were probed during the transition of the receptor from the active conformation to the resting conformation (i.e., during deactivation). Application of Zn^{2+} onto neurotransmitter-bound receptors, which have an open channel, obstructed their pore and prevented ionic flow. Removing the neurotransmitter from its extracellular binding sites to induce deactivation while Zn^{2+} is still bound, led to tight trapping of Zn^{2+} within the pore. Together with single-channel recordings, made to explore single pore-blocking events, we show that receptor deactivation triggers the gate to shut on a Zn^{2+} ion that effectively acts as a ‘foot in the door’. Evidently, upon deactivation, the cytoplasmic side of the serotonin-receptor channel constricts to close the pore.

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Understanding the regulation of caloric-restriction diet benefits, to cancer and age-Related Diseases

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Understanding the mechanisms of various agents known to slow down aging, will promote novel treatments of cancer, as well as other age related systematic diseases (such as type-II diabetes, neurodegenerative and decline in the function of the immune system). The most widely accepted treatment to slow down aging is calorie-restriction (CR) diet, which is known to be effective in all eukaryotes in which it was tested (from yeast to primates). Furthermore, we found (Cohen et al. *Science* 2004), that the family of NAD⁺ dependent protein deacetylases Sirtuins mediates this effect, throughout various eukaryotes.

The study aims to explore the downstream effects of Sirtuins and CR. To date, such attempts to understand these effects at the system level were based on microarrays analysis. Yet, recent evidence from our lab suggests that other factors beside RNA levels also contribute to the changes in protein levels upon CR. For example, our lab recently showed that the levels of several Sirtuins as SIRT1 and SIRT6 increases upon CR without a parallel increase in their transcription (Kanfi et al. *FEBS lett.* 2008a,b). Thus we hypothesized that such regulation is a general phenomenon of CR, and hence we measure changes in protein levels directly, using a proteomic mass spectrometry and a library of tagged budding-yeast proteins. The study tracks the changes after various aging-related treatments, such as CR (defined as low glucose medium). We found key players, and established a notion by which CR can extend lifespan without up-regulating stress response.

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