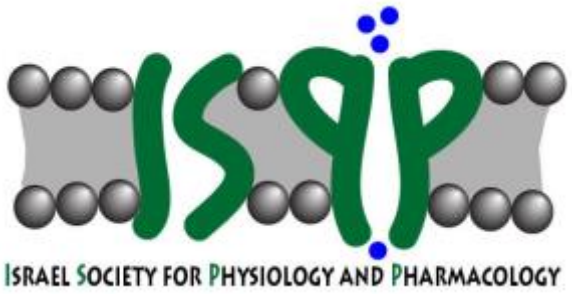

The ISPP 2016 Meeting

Feb 18th, 2016 in Tel-Aviv University

Dan David building, Auditorium 003 and rooms 110 and 106.

Poster Abstracts

EPIGENETIC TRANSGENERATIONAL MECHANISM UNDERLYING HIGH FAT DIET

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Parental obesity can affect the children's likelihood to overeat and develop obesity. Changes in epigenetic programming have been implicated as one of the mechanisms underlying this phenomenon. Using a rat model, we designed a study in which we exposed only the first generation to chronic high fat diet (HFD) and followed the effect on two consecutive generations of standard fed offspring. We focused on the promoter of the hypothalamic neuropeptide Pomc, which is crucially involved in control of food intake. HFD consumption by non-mated female rats (F0) significantly increased body weight and plasma leptin levels and attenuated Pomc mRNA expression. This was associated with hypermethylation of the Pomc promoter. As expected, high leptin levels that were presented in the HFD group, increased the expression of the transcription factor Sp1. Nevertheless, the binding of Sp1 to the hypermethylated Pomc promoter was significantly reduced. Furthermore, perinatal exposure to maternal HFD lead to long term acquired alteration in DNA methylation patterns and posttranslational modifications of histone H3 lysine 9 (H3K9) that affect Pomc transcription in the F1 and F2 offspring. As a potential tagging of the nucleosome at the Pomc promoter for histone post-translation modification, we describe the binding of methyl binding domain 1 (MBD1) to the methylated Pomc promoter, interacting with SETDB1 methyltransferase to promote the formation of methylation of H3K9. This combined DNA and histone methylation produces a repressor complex potentially attenuating the expression of Pomc. These findings contribute to our understanding of the mechanisms through which environmental cues are translated into stable changes in the Pomc gene, leading to obesity.

THE CARDIOPROTECTIVE EFFICACY OF TVP1022 AGAINST ISCHEMIA/REPERFUSION INJURY AND CARDIAC REMODELING IN RATS

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Background: Following acute myocardial infarction (MI), early and successful reperfusion is the most effective strategy for reducing infarct size and improving the clinical outcome. However, immediate restoration of blood flow to the ischemic zone can result in myocardial damage, often termed "reperfusion-injury". TVP1022 (the S-isomer of rasagiline, FDA-approved anti-Parkinson drug) was found to exert a significant anti-apoptotic and cyto-protective efficacy in cultures of neuronal cells and cardiomyocytes, as well as cardioprotective activities against various cardiac insults such as chronic heart failure and ischemia/reperfusion in rat models. Therefore, we tested the hypothesis that TVP1022 will provide cardioprotection against ischemia/reperfusion (I/R) injury and post-MI remodeling in rats.

Methods: To simulate the clinical settings of MI followed by reperfusion therapy, we employed a rat model of left anterior descending artery occlusion for 30 min followed by reperfusion and a follow-up for 14 days. TVP1022 was initially administered post-occlusion-pre-reperfusion, followed by chronic daily administrations. Cardiac performance and remodeling were evaluated using customary and advanced echocardiographic methods, hemodynamic measurements by Millar Mikro-Tip® catheter and histopathological techniques.

Results: TVP1022 administration decreased the remodeling process. Specifically, TVP1022 attenuated post-MI left ventricular enlargement, cardiac hypertrophy (both at the whole heart and cellular level), cardiac fibrosis and reduced ventricular BNP levels. Functionally, we found that TVP1022 treatment attenuated the decline and even preserved cardiac function. Specifically, the echocardiographic measurements and most of the direct hemodynamic measurements were markedly improved by the TVP1022 treatment.

Conclusion: Collectively, these findings indicate that TVP1022 provides prominent cardioprotection against I/R injury and post-MI remodeling in this I/R rat model.

THE REGULATORY ROLE OF ZINC ON ION TRANSPORT IN DIARRHEA

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The effectiveness of zinc, Zn^{2+}, as a complement to oral rehydration in attenuation of the duration and severity of diarrhea is well established. In addition, a role of Zn^{2+}, activating a specific membrane receptor ZnR/GPR39 in the colon has been demonstrated. However, the mechanisms by which Zn^{2+} affects diarrhea are, unknown.

We aimed to examine if extracellular Zn^{2+}, acting via ZnR/GPR39, initiates signaling that regulate Cl^- transport during health and cholera toxin (CT) induced diarrhea and thereby restores ion homeostasis.

We conducted *in vivo* experiments on WT and ZnR/GPR39 KO mice where we induced cholera toxin (CT) dependent diarrhea and compared the intestinal length and secretion. We also conducted *in vitro* experiments on Caco-2 cells and colon tissues from WT and ZnR/GPR39 KO mice to test if Zn^{2+} regulates K^+ and Cl^- ion transport via the K^+/Cl^- cotransporters, NKCC/KCC, applying the NH_4^+ prepulse paradigm using fluorescence imaging.

We show that ZnR/GPR39 KO mice had longer intestine with larger volume of secretion after CT induced diarrhea compared to WT mice. To determine the mechanism underlying the effect of Zn^{2+}, we pretreated the colonocytes and colon tissue from WT and ZnR/GPR39 KO mice with Zn^{2+} and monitored NH_4^+ transport. These experiments indicated that ZnR/GPR39 activation upregulated cotransporters activity which was reversed by an inhibitor of the ZnR pathway. Pretreating the cells with a KCC inhibitor, DIOA, attenuated the Zn^{2+}-dependent ion transport whereas the NKCC inhibitor, bumetanide, did not.

Our results suggest that Zn^{2+}, acting via ZnR/GPR39, upregulates KCC transport in colonocytes. Moreover, ZnR/GPR39 KO mice developed enhanced symptoms of diarrhea following CT exposure. Hence, our results suggest that upon activation, the ZnR/GPR39 initiates signaling pathways that upregulate KCC-dependent ion transport and reverse the impaired ionic transport during diarrhea. Thus, ZnR/GPR39 restores solute and water balance in WT but not KO mice.

COLLABORATIVE CROSS MICE IN A GENETIC ASSOCIATION STUDY REVEAL NEW CANDIDATE GENES FOR BONE MICROARCHITECTURE.

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Background: The microstructure of trabecular bone is a composite trait governed by a complex interaction of multiple genetic determinants. Mapping of their full spectrum can drastically improve our ability to predict of osteoporosis and associated risks. The use of collaborative cross mice (CC), the latest recombinant inbred mouse reference panel, in association studies enables reproducible genetic mapping at a resolution under one mega base-pairs, with a relatively small cohort size.

We utilized 31 CC lines of both sexes to perform haplotype mapping across 77,808 genome-wide SNPs. Haplotype scans were refined by merging the complete catalogue of sequence variation segregating in the CC to pinpoint candidate genes. Trabecular traits were obtained following microtomographic analysis, performed on 10-µm resolution scans of the femoral distal metaphysis. We measured the trabecular bone volume fraction (BV/TV), number (Tb.N), thickness (Tb.Th), and connectivity density (Conn.D).

Results: Heritability ranged from 0.6 to 0.7. There was a significant sex effect in all traits except Tb.Th. Our haplotype scans yielded five quantitative trait loci (QTL); BV/TV produced two loci on chromosome 2 and each other trait yielded one locus on chromosomes 8, 7, and 14. Using merge analysis we located with high confidence several genes with previously-reported functions in bone biology as well as candidate genes for which a role in bone biology has yet to be assigned. Genes ranked particularly high, and already have a documented skeletal role, are *AVP*, *OXT*, *B2m*, Cnot7, *Rb1*, and *Cpb2*.

Conclusion: These data demonstrate for the first time genome-wide significant association between several genetic entities and trabecular microstructural parameters for genes with previously reported experimental observations, as well as proposes a role for new candidate genes with no characterized skeleton function. Our findings thus validate our approach and its ability to reveal new determinants of bone microarchitecture.

EVIDENCE FOR EXTRACELLULAR SECRETION OF CYCLOOXYGENASE-2

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Cyclooxygenase (COX) is the rate limiting enzyme that generates prostanoids from arachidonic acid (AA). This enzyme has two main isoforms: COX-1, which is the constitutive form of the enzyme, and COX-2, whose expression is rapidly and transiently upregulated by a variety of pathological signals. As opposed to COX-1, which resides mostly in the membrane of the endoplasmic reticulum (ER) due to a strong ER retention sequence, COX-2 is also located in the Golgi apparatus. Given this observation we raised the hypothesis that besides its localization inside the cell, certain signals may cause the secretion of COX-2 outside the cell. To test this, we expressed COX-2 in HEK 293, in the absence or presence of AA for 7h, after which the cells were washed, media was replaced with normal media and the cells were allowed to grow overnight. On the next day, we isolated COX-2 from the cells and their overlaying media.

Results: our data show that COX-2 is present outside the cell and that AA treatment increases its presence in the extracellular media. Similar observations were obtained with a cell line that expresses COX-2 endogenously. Furthermore, application of COX-2 conditioned media on to cells that do not express COX-2 endogenously showed its presence 24h post application, suggesting that COX-2 may be transferred through the extracellular media.

Conclusion: our findings propose the existence of a novel COX-2 secretory pathway. We are currently engaged in studying the mechanism that underlies this phenomenon.

CHARACTERIZATION OF THE SPECIFICITY DETERMINANTS OF RGS2 TOWARD HETEROTRIMERIC G PROTEINS.

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Background: Regulator of G protein signaling (RGS) proteins turn off heterotrimeric G proteins and thereby determining the timing of G protein signaling. RGS2, an RGS protein involved in numerous signaling cascades and human pathologies, was shown to be unique in its selectivity for G proteins of the G_q subfamily. Previous studies identified three amino acids that contribute to this specificity, but the full complement of structural features within RGS2 that determine its selectivity for G_q over $G_{i/o}$ subfamily members has not been identified.

Purpose and methods: We aim to decipher which amino acids encode RGS2 specificity towards G proteins using an integrated computational and experimental approach.

Results: We identified unconserved and previously uncharacterized positions in RGS2 as candidates for determining its specificity. We further analyzed the 3D structures of RGS2 with G proteins to understand the mechanistic basis that underlies this selective recognition between RGS2 and its G protein partners.

Conclusion: Our results enable to better understand how the mechanisms that determine interaction specificity between G proteins and their negative regulator RGS2. These results will be used to redesign RGS2 with different specificities towards G proteins, in order to provide insights into these signaling pathways and for eventually manipulating them with drugs.

BONE LOSS IN C57BL/6J-OLAHSD MICE, A SUBSTRAIN OF C57BL/6J CARRYING MUTATED ALPHA-SYNUCLEIN AND MULTIMERIN-1 GENES

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The inbred mouse strain C57BL/6 is commonly used for the generation of transgenic and knockout mice and is a well-established strain in bone research. Different vendors supply different sub-strains of C57BL/6J as wild-type animals when genetic drift did not incur any noticeable phenotype. However, we sporadically observed drastic differences in t bone phenotype of WT C57BL/6J controls originating from different labs and speculated that these variations are attributable, at least in part, to the variation between sub-strains. C57BL/6J-OlaHsd is a commonly used sub-strain that despite a deletion in the alpha-synuclein (Snca) and multimerin-1 (Mmrn1) genes, was reported to display no obvious phenotype and is commonly used as WT. We compared the bone phenotype of C57BL/6J-OlaHsd (6J-Ola) to C57BL/6J-RccHsd (6J-Rcc), a strain lacking the deletion and genetically close to the original 6J sub-strain developed by the Jackson labs. Both sub-strains are supplied by Harlan Laboratories. Using micro-CT analysis, we found that 6J-Ola mice display a significantly lower trabecular BV/TV, Conn.D, Tb.N, and Tb.Th. 6J-Ola mice also demonstrate reduced cortical thickness. PCR analysis revealed that both the Snca and Mmrn1 genes are detectable in RNA derived from bone marrow cells and bone tissue of 6J-Rcc animals but not of 6J-Ola mutants. Up to date, there is no data in the literature linking Snca and/or Mmrn1 to bone remodeling but our data implies that either or both genes may play a role. Our findings not only highlight the potential new role of these genes in bone biology but they further emphasize the critical importance of sub-strain unity. The use of littermate controls may also be questioned in the case of cross breeding between different sub-strains. Our data may help elucidating some of the unexplained differences in basal bone measurements between different research centers and reiterate the need of specifying the exact sub-strain type.

CARDIAC STEROIDS AND Na^+, K^+ -ATPASE IN THE MANIC PHASE OF BIPOLAR DISORDER

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Bipolar disorder (BD) is a devastating mental illness characterized by cycling episodes of mania and depression. The etiology of BD is not completely clear. Cardiac steroids (CS) and their only established receptor, Na^+, K^+-ATPase, are considered to be involved in mood disorders. We have shown that reduction of CS in the brain has an anti-depressive effect in rats. In the present study we tested the effects of reduction or inhibition of brain CS in mice in an amphetamine-induced hyperactivity model for mania. Here we show that ICV administration of anti-ouabain antibodies in BALB/c mice reduces CS levels in the brain, thereby abolishing the increase in mobility caused by amphetamine. A similar anti-manic effect of anti-ouabain antibodies was received in black Swiss mice, a strain of mice that manifest manic-like behavior. The anti-manic effect was reversed by pre-incubating the antibodies with ouabain, indicating that the effect of the antibodies is indeed attributable to the reduction in endogenous ouabain. The behavioral changes were accompanied by molecular changes in the brain: whereas amphetamine treatment induced an increase in CS immunoreactivity, the levels were significantly lower in animals treated with anti-ouabain antibodies. Furthermore, amphetamine treatment induced an increase in the levels of phospho-Erk and phospho-Akt in the frontal cortex, which were reduced by anti-ouabain antibodies. We also examined the effect of a synthetic 3αOH 19-nor androsterone derivative on animal behavior. This compound, termed "Compound 16", resembles CS in structure, but does not inhibit Na^+, K^+-ATPase ion transport activity, thus acting as a functional CS antagonist. This steroid attenuated the amphetamine-induced hyperactivity in mice. Cumulatively, our results strengthen the hypothesis that the CS- Na^+, K^+-ATPase system is involved in mania, and suggest that this system may be regarded as a novel target for the development of drugs for the treatment of BD.

Intracellular regulation of the slc26A9 Cl^- channel and inhibition by salicylic acid

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Cl^- is the major anion in most living cells, which maintain the concentration of intracellular Cl^- (Cl^-_{in}) above electrochemical equilibrium. Importantly, aberrant Cl^--dependent fluid and ion secretion is associated with many high morbidity, high mortality diseases such as hypertension, cystic fibrosis (CF), pancreatitis, and more. One of the major chloride channels that have been recently characterized is SLC26A9. However, functional and regulatory aspects of slc26a9 Cl^- transport activity are still poorly understood.

Interest in the SLC26A9 Cl^- channel dramatically increased when a link between CF and SLC26A9 was established by genome wide association study with marked upregulation of SLC26A9 in CF. Moreover, slc26a9 regulates arterial blood pressure since measurements in slc26a9$^{-/-}$ mice indicate that they are hypertensive. Other members of the slc26 family of transporters as well as the Cl^- channel CFTR have been shown to be regulated by the 'master regulator' protein, IRBIT.

Here, we show that IRBIT plays a major role in slc26a9 regulation. Moreover, we show that Slc26a9- mediated Cl^- transport is inhibited by salicylic acid in a dose dependent manner.

Our findings identify slc26a9 regulation pathway as a potential therapeutic target for future treatment of Cl^- channel pathologies.

Regulation of succinate transport and homeostasis

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Succinate is a tricarboxylic acid cycle intermediate that is gaining attention as a universal metabolic marker of ischemia and a pivotal signaling molecule. High-glucose-induced elevation and secretion of intracellular succinate stimulates the succinate-sensing receptor (GPR91), a G_q-coupled receptor that is expressed in several tissues, most notably, in kidney epithelia and in blood vessels. Importantly, since succinate is a charged molecule, succinate transport across membranes is mediated by transport proteins. The major succinate transporters are NaDC-1 and the organic anion transporters (OAT1 and 3) expressed on the apical and basolateral membranes of epithelia, respectively. However the regulation of transepithelial succinate transport via these transporters and how they maintain succinate homeostasis is not well understood. We have previously reported that NaDC-1 forms a complex with slc26a6, a Cl^-/oxalate and Cl^-/HCO_3^- exchanger, to regulate succinate transport through interaction. Therefore, we hypothesize that succinate homeostasis is regulated by crosstalk between G-coupled receptors signaling and succinate transport via apical NaDC-1-slc26a6 complex and basolateral OATs. We first asked how the function of the apical and basolateral succinate transport proteins is regulated by the ubiquitous intracellular 'master regulator' protein, IRBIT. To address this question, we utilized electrophysiological measurements to monitor succinate transport following IRBIT expression. Furthermore, we have utilized biochemical assays and fluorescent imaging to study the interaction of the succinate transporters with IRBIT in live cells. Our results indicate that IRBIT regulates both apical and basolateral succinate transporters. Moreover, our results indicate that stimulation of the succinate receptor GPR91 increases IRBIT interaction with NaDC-1 attesting for IRBIT mediated regulation of succinate transport via receptor stimulation.

DISTINCT DEVELOPMENTAL CHANGES IN THE EXPRESSION OF ZINC TRANSPORTERS (ZNT AND ZIP) IN WT AND ZNR/GPR39 KO MICE

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Zinc is one of the most copious microelements in the CNS regulating development and function and involved in neurodegenerative diseases. There are two main zinc transporter families, Zrt, Irt-like proteins (ZIP) which are responsible for uptake of zinc into the cytosol from the extracellular space or intracellular organelles and zinc transporters (ZnT) which function opposite to ZIPs. Both have a critical role in maintaining the cellular and systemic zinc homeostasis which is crucial for proper neuronal function and survival. It has been shown that synaptically released vesicular Zn^{2+} activates a metabotropic zinc sensing receptor mZnR/GPR39. Yet, how and when the Zn^{2+} homeostatic proteins are expressed in neurons is poorly understood. We sought to determine if the expression level of ZIPs and ZnTs changes during normal brain development and whether it is affected by Zn^{2+} signaling via the mZnR/GPR39 receptor. Hippocampal tissue was isolated from WT and GPR39 KO mice of different ages, and quantitative mRNA analysis was performed, followed by western blot to determine gene expression and protein levels of several zinc transporters. Our results point that a set of zinc transporters indeed shows developmental changes in their expression levels, which are based on the presence or absence of mZnR/GPR39. Further we will focus on understanding the regulation and relevance of these changes to physiological function.

QUANTIFICATION OF NEURITE LENGTH AND NEURONAL VIABILITY IN HEALTH AND DISEASE

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Amyotrophic lateral sclerosis (ALS) is a non-cell-autonomous neurodegenerative disease characterized by death of upper and lower motor neurons (MN). It involves interactions between the neuron and its diverse extracellular microenvironments via an unknown mechanism. Although the molecular basis for this neuronal dysfunction and death it is still poorly understood, it may be due to alterations in the nature and localization of extra- and intracellular signaling pathways. Here, we hypothesize that a stressful microenvironment in ALS can contribute to axon degeneration process by altering signaling factors that stabilize microtubules. To test this idea we examine cell viability and morphology of MN cultures under various manipulations using an unbiased automated imaging system. MN grown on rich media before stress-induction demonstrates longer axons compared to MN grown on rich media with no stress. Future work will further characterize the role of secreted factors and signaling pathways on microtubule stability and axon degeneration that take place in ALS.

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MOLECULAR MECHANISMS OF GDNF AND SEMAPHORIN3A ON AXON GROWTH AND DEGENERATION

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Axon growth or degeneration during development or in pathological situations such as Amyotrophic Lateral Sclerosis (ALS), is a non-cell-autonomous process that involves both intra- and extra-cellular communication mechanisms. These communication mechanisms and the nature of the axon elongation/disruption-modulating signals are still poorly understood.

In order to elucidate molecular mechanisms of axon elongation and degeneration, we will study the signaling effects of Glial cell-derived neurotrophic factor (GDNF), a neurotrophic factor that can elongate axon, and Semaphorins, chemorepellent factors that elevated in ALS and lead to axon degeneration. First, we will characterize the effect of GDNF or Sema3a/b/f on axon growth and degeneration on ALS models (SOD1, TDP43 and C9ORF72 models) and its control using live cell imaging techniques on an in vitro compartmental microfluidic platform with motor neuron cell bodies on one side and its axons on the other side. Then, we plan to perform differential proteomics screen of axons treated with these factors to identified downstream signaling factors. Altogether, successful completion of this project will highlight several key factors controlling axonal stability, which may serve as possible targets for therapeutic intervention in the axonal degeneration process.

RELEASE OF TITANIUM PARTICLE BY ULTRASONIC CLEANING OF DENTAL IMPLANTS MAY AGGRAVATE THE PERI-IMPLANT INFLAMMATORY RESPONSE

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Titanium alloys are widely used as dental implants due to their biocompatibility, mechanical strength, corrosion resistance and osseointegration. All-available implants undergo surface roughening in order to accelerate osseointegration. Peri-implantitis is a major clinical concern and main cause of long term implant failure. Triggered by oral bacteria, it consists of an inflammatory process that leads to bone resorption. Once the process starts it can hardly be controlled and often results in implant loss. Treatment includes mechanical cleaning of the surrounding oral flora by ultrasonic scaling (US). We hypothesize that the scaling process releases titanium particles into the implant microenvironment and that these particles trigger the inflammatory response. To test this hypothesis, we performed US of titanium discs with various surface types. The released particles were added to bone marrow-derived mouse macrophages (BMDM) cultures. Bacterial lipopolysaccharides (LPS) were added to parallel cultures as positive control and in addition to titanium particles to assess additive/synergistic effects in the RNA expression of inflammatory cytokines. Atomic force and electron scanning microscopy were used for profilometry of the released particle and post-cleaning titanium surface. RT-qPCR indicated that titanium particles originating from sand-blasted/acid etched (SLA) implants stimulated gene expression of pro-inflammatory cytokines to a greater extent than LPS. We compared the profile, size and quantity of titanium particles obtained from different surface types. BMDM were then cultured with the particles released from each surface type. Particles originating from SB implants yielded the most severe inflammatory response. Our results showed that both amount and shape of particles have an effect on the inflammatory response. These data suggest that scaling of titanium implants, intended at preventing peri-implantitis, may in fact aggravate the osteolytic inflammatory response by causing an accumulation of Ti particles in the peri-implant environment. This adverse reaction is likely to be even more severe around roughened Ti implants.

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Spatio-temporal characteristics of Ca^{2+} fluxes in axons of layer 5 pyramidal neurons

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Abstract: In cortical pyramidal cells, action potentials (AP) elicit Ca^{2+} elevation in soma, dendrites and presynaptic terminals. AP evoked Ca^{2+} fluxes were also revealed in axon initial segments (AIS) of cortical cells where they were thought to take part in burst firing. The characteristics of Ca^{2+} influx in the AIS and in the more distal, myelinated portion of the axon have never been studied. We explored the spatial pattern of AP elicited Ca^{2+} and Na^{+} fluxes using a combination of patch-in-slice recording and high-speed fluorescence imaging. Axons were distinguished from the other fine processes by their distinctive Na^{+} transients. The boundaries of the AIS, of the first myelinated internode and of proximal nodes of Ranvier were determined by the characteristic amplitude and time course of their Na^{+} signals. Peak amplitude of Ca^{2+} transients declined gradually from soma to the end of the AIS. Small Ca^{2+} transients were observed in myelinated internodes up to a distance of ~120 μm from the cell body. Peak amplitude of the nodal Ca^{2+} transients was ~2 times larger than in the neighboring internodes. The calcium influx in the AIS and internode is partially blocked by T and L type VGCC blockers and Ryanodine (100□M), a ryanodine receptor blocker.

The AIS Ca^{2+} influx, was significantly reduced when the APs were elicited from -70 mV as compare to -80 mV. Assuming that the amplitude and shape of AP in presynaptic terminal and AIS are similar, the opposite effect of depolarization on the AIS Ca^{2+} influx, most probably, reflects the channel subtype dependent differences in steady-state inactivation and deactivation kinetics of Ca^{2+} channels.

The axonal Ca^{2+} transients, most probably, are mediated by molecularly distinct functionally segregated subtypes of Ca^{2+} channels. The Ca^{2+} flux might play a role in regulation of the activity-dependent axonal plasticity and of the neuro-metabolic feedback.

ESTABLISHING THE RELEVANCE OF HETEROMERIC GIRK CHANNELS IN NEURONAL PROCESSES.

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G protein coupled inwardly rectifying K^+ (GIRK) channels control the excitability of many cell types including neurons in the peripheral and central nervous system. The opening of the channel is controlled by the Gbg subunits of Gi/o protein family released upon activation of the GPCR. GIRK channel consists of 4 subunits (GIRK1-4) which form homo or hetrotetrameric functional channels. Few compositions of functional channel exist in the brain. GIRK2 can form homotetramers while GIRK1 and GIRK3 must associate with other family members to form functional heterotetrameric channels like GIRK1/GIRK2 and GIRK3/GIRK2.

YTI mice are a knock-in line in which Venus was fused to the first coding exon of GIRK1.*YTI* mice tend to have spontaneous seizures or seizures which are evoked by minor stress such as moving the mouse from one cage to another, light, noise etc. A set of behavioral experiments performed by our lab showed that *YTI* mice are significantly more active and have stronger anxiety related behavior compared to their *wt* litter mates. Behavioral experiments performed on Girk1-4 knock out strains showed that both Girk1 and Girk2 knock out strains have increased locomotor activity (Pravetoni & Wickman 2008). While total deletion of one of these subunits may explain this phenotype, it is harder to explain why a functioning subunit of Girk1 (Riven et al. 2003) fused to a fluorescent tag may cause a similar phenotype. We have showed that both GIRK1 and GIRK2 are expressed on the cell membrane in *YTI* brains. Whole cell recordings have demonstrated that GIRK currents are significantly smaller compared to the *wt* currents. We suggest that *YTI* strain is a GIRK1/2 hetrotetrameric channel functional knock down. This strain may help to establish the relevance and importance of GIRK1/2 heterotetramers function in neuronal processes.

MODULATION OF K2P CHANNELS BY MONOTERPENES

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Background. K2P potassium channels display constant conductance in the physiological membrane potential range. Their activity affects numerous physiological processes such as cardiac function, pain perception, and depression, neuroprotection, and cancer development. Channel activity is highly regulated by both chemical and physical modulators such as mechanical stretch, pH, and phosphorylation.

Terpenes form a family of compounds, mostly produced in plants. Several monoterpenes were found to affect the activity of a variety of ion channels. To date, the effect of terpenes on K2P channels has not been studied.

Aims. Our goal is to investigate the effect of terpenes on human K_{2P} channels, and to elucidate their mechanism of action.

Results. Human K2P channels were expressed in *Xenopus* oocytes and measured using the two-electrode voltage clamp technique. K2P2.1 currents were enhanced by five, four, four and twofold using menthol, carvacrol, thymol, and camphor, respectively. When tested on K2P5.1 channels, carvacrol and cinnamaldehyde enhanced currents by 15 and 13-fold respectively, while menthol decreased the currents by two-fold. When tested on the *Drosophila* K2P channel, K2P0, both carvacrol and menthol reduced the currents by two-fold. By measuring proton sensitivity with and without menthol, it was shown that the menthol affects the stability of K2P2.1 gate. Furthermore, using channels mutated at a cytoplasmatic phosphorylation site, the participation of the regulatory carboxy-terminal domain was demonstrated.

Conclusions. Our results demonstrate, for the first time, that terpenes might be powerful modulators of K2P potassium channels and that the mechanism of action by which they exert their effect may involve both the selectivity filter gate and the C-terminus regulatory domain.

FUNCTIONAL STUDY OF MITOCHONDRIAL NCLX IDENTIFIES RESIDUES IN THE α-CATALYTIC DOMAINS WHICH CONTROL MONOVALENT CATION SELECTIVITY

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Na^+/Ca^{2+} exchanger (NCX) is a bidirectional transporter which mediates exchange of Ca^{2+} at the expense of Na^+ across the cell membrane. Mitochondrial NCX, found in the inner mitochondrial membrane is unique in that it facilitates Li^+-dependent Ca^{2+} transport in addition to Na^+ and therefore designated as $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX). Thus, NCLX is distinct from plasma membrane NCX in terms of spatial distribution, molecular identity as well as ion selectivity.

The present study was undertaken in an endaevour to gain an insight into the molecular basis of cation transport of NCLX and hence to identify the catalytic residues engaged in ion transport as well as to evaluate their selectivity towards Na^+ and Li^+.

Recently, in a seminal breakthrough, high-resolution structural model of an archaebacterial NCX homolog (NCX_Mj) has revealed an intriguing architecture consisting of two inversely oriented transmembrane domains, each comprising five helices. The two transmembrane domains possess internal similarity and therefore termed α1 and α2 repeats, which share sequence homology among different NCX isoforms. The structure also suggested four putative ion binding sites in the α repeats: one for Ca^{2+} and three that likely bind Na^+.

Using the NCX_Mj as a templet for generating a 3D model of NCLX, several amino acids of potential importance were selected for site-specific mutation in the α repeats of NCLX. A mitochondria-selective calcium indicator, ratiometric pericam, was used for mitochondrial Ca^{2+} imaging. Mitochondrial Ca^{2+} efflux was monitored in digitonin permeabilized cells in presence of Na^+ or Li^+. This functional study with wild type and mutant NCLX has enabled us to put forward a cation (Na^+ and Li^+) selectivity profile for distinct amino acids, located in the putative transmembrane segments of NCLX, which could well be postulated to take part in the ion transport process of the exchanger.

CROSSTALK OF ASIC1A CHANNEL AND MITOCHONDRIA CONTROLLED BY Na^+ AND Ca^{2+} SIGNALS IS RELATED TO THE CHANGE IN MITOCHONDRIAL METABOLIC ACTIVITY

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A major hallmark of neuronal metabolic activity is the buildup of lactic acid resulting in mild drop in pH. The decrease in extracellular pH can be sensed by acid-sensing ion channels (ASIC). ASIC1a, which is main ASIC subunit in neurons, is predominantly Na^+ conducting channel and is expressed on both plasma membrane and inner mitochondrial membrane. Previous studies linked ASIC1a to pathophysiological insults in particular brain ischemia, which is followed by a very large pH drop. Here we ask how physiological pH decrease can affect mitochondrial Na^+ and Ca^{2+} signals as well as mitochondrial metabolic activity and if ASIC1a is related to these processes. We hypothesize that ASIC1a is a metabolic sensor that by communicating with the mitochondria can control metabolic activity of neurons.

We show that lowering of extracellular pH to 7.0 (physiological range) triggers increase in both cytosolic and mitochondrial Na^+ influx in cortical neurons and HEK293-T ASIC1a-overexpressing cells. We further demonstrate that the mitochondrial Na^+ accumulation is accompanied by cytosolic and mitochondrial Ca^{2+} influx and mitochondrial depolarization. Psalmotoxin 1 (PcTx 1), a selective inhibitor of ASIC1a, attenuates both the cytosolic and mitochondrial Na^+ loading, as well as Ca^{2+} accumulation in mitochondria, supporting the role of ASIC1a in this scenario. More importantly, PcTx 1 strongly reduces basal respiration, maximal respiration and ATP production in cortical neurons at ~ pH 7.0, suggesting ASIC1a channel via propagation of Na^+ and Ca^{2+} signals, plays significant role in the modulation of mitochondrial metabolic activity.

FUNCTIONAL EVIDENCE FOR DOMAIN SWAPPING IN K_{2P} CHANNELS

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Background. K_{2P} potassium channels have a key role in setting the resting membrane potential and their activity is regulated by numerous extra- and intracellular stimuli. K_{2P} channels function as dimmers, each having 2 pore domains and 4 trans-membrane helices (TM1-4). It has been recently demonstrated that in crystallized channels, TM1 from each of the subunits is located closer to TMs2-4 of the opposite subunit.

Aims. Our objective in this study domain swapping in functional, membrane-embedded channels. Domain swapping could modulate channel gating and function.

Results. Our strategy was to detect the formation of a disulfide bond between residues on TM1 (E28C) and TM2 (T115C). A formation of this bond was previously shown to reduce the current in our model channel, KCNK0. To test whether the bond is formed between subunits (and thus confirming the crystallographic observation) or within the same subunit (and thus rejecting the observation) several chimeric channel dimmers were formed. Channel activity was tested after expression in *Xenopus laevis* oocytes using the two-electrode voltage clamp technique. A formation of a disulfide bond was detected when a cysteine residues were present on the TM1 of one subunit and TM2 of the other subunit. Accordingly, no indication for a bond was detected when both systems were on the same subunit.

Conclusions. Our results support the existence of domain swapping in functional K_{2P} channels, as was predicted by the crystallographic data. We cannot rule out the possibility that some of the channels form dimers without swapped domains.

REVEALING THE NEUROTOXIC ARSENAL OF THE SCORPION BUTHACUS LEPTOCHELYS LEPTOCHELYS

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Background. Scorpion venoms are a rich source of bioactive peptides. Most of these peptides are small (23-78 amino acids-long), well packed by several disulfide bridges and affect ion channel function in excitable and non-excitable cells. The venom of each scorpion is a complex mixture of dozens or even hundreds of mainly neurotoxic peptides, interacting specifically with various ionic channels. As such, these venoms represent a potential resource for drug discovery to treat a wide range of pathological conditions, such as pain, cancer, diabetes and cardiovascular diseases.

Aims. Our goal was to establishing a comprehensive dataset of all putative neurotoxins in the venom of the Israeli scorpion *Buthacus ieptochelys leptochelys* using next generation sequencing (RNA-seq). The dataset will enable discovery of novel native and synthetic toxins-based peptides with desired activities, with an emphasis on potassium channel modulators.

Results. Two cDNA libraries were constructed for analysis, one comprising RNA extracted from the venom glands and one comprising RNA extracted from the abdomen segments of the same scorpions. Libraries were sequenced using a HiSeq 2000 instrument (Illumina), yielding ~200 million 100 bp paired-end reads and 24,300 contiges. 182 contigs were annotated as putative toxin-coding genes, out of which 50 encode putative potassium channel blockers.

Conclusions. The information gained here will allow us, using bioinformatics, to refine our strategy of predicting the active sites of toxins based on evolutionary considerations. Furthermore, using a yeast expression system, we will be able to detect novel selective potassium channel blockers. These peptides could serve as valuable research tools, as well as to provide the basis for developing therapeutic drugs

REGULATION OF THE NEURONAL KCNQ2 CHANNEL BY SRC – A DUAL REARRANGEMENT OF THE CYTOSOLIC TERMINI UNDERLIES BIDIRECTIONAL REGULATION OF GATING

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Neuronal M-type K+ channels are heteromers of KCNQ2 and KCNQ3 subunits, and are found in cell bodies, dendrites and the axon initial segment, regulating the firing properties of neurons. By contrast, presynaptic KCNQ2 homomeric channels directly regulate neurotransmitter release. Previously, we have described a mechanism for gating downregulation of KCNQ2 homomeric channels by calmodulin and syntaxin1A. Here, we describe a new mechanism for regulation of KCNQ2 channel gating that is modulated by Src, a non-receptor tyrosine kinase. In this mechanism, two concurrent distinct structural rearrangements of the cytosolic termini induce two opposing effects: upregulation of the single-channel open probability, mediated by an N-terminal tyrosine, and reduction in functional channels, mediated by a C-terminal tyrosine. In contrast, Src-mediated regulation of KCNQ3 homomeric channels, shown previously to be achieved through the corresponding tyrosine residues, involves the N-terminal-tyrosine-mediated downregulation of the open probability, rather than an upregulation. We argue that the dual bidirectional regulation of KCNQ2 functionality by Src, mediated through two separate sites, means that KCNQ2 can be modified by cellular factors that might specifically interact with either one of the sites, with potential significance in the fine-tuning of neurotransmitters release at nerve terminals.

TRACKING Ca^{2+}-DEPENDENT AND Ca^{2+} -INDEPENDENT CONFORMATIONAL TRANSITIONS IN SYNTAXIN 1A DURING EXOCYTOSIS IN NEUROENDOCRINE CELLS

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A key issue for understanding exocytosis is elucidating the various protein interactions and the associated conformational transitions underlying soluble N-ethylmeleimide-sensitive factor attachment protein receptor (SNARE) protein assembly. To monitor dynamic changes in syntaxin 1A (Syx) conformation along exocytosis, we constructed a novel fluorescent Syx-based probe that can be efficiently incorporated within endogenous SNARE complexes, support exocytosis, and report shifts in Syx between 'closed' and 'open'conformations by fluorescence resonance energy transfer analysis. Using this probe we resolve two distinct Syx conformational transitions during membrane depolarization-induced exocytosis in PC12 cells: a partial 'opening' in the absence of Ca^{2+} entry and an additional 'opening' upon Ca2+ entry. The Ca^{2+}-dependent transition is abolished upon neutralization of the basic charges in the

juxtamembrane regions of Syx, which also impairs exocytosis. These novel findings provide evidence of two conformational transitions in Syx during exocytosis, which have not been reported before: one transition directly induced by depolarization and an additional

transition that involves the juxtamembrane region of Syx. The superior sensitivity of our probe also enabled detection of subtle Syx conformational changes upon interaction with VAMP2, which were absolutely dependent on the basic charges of the juxtamembrane

region. Hence, our results further suggest that the Ca^{2+}-dependent transition in Syx involves zippering between the membrane-proximal juxtamembrane regions of Syx and VAMP2 and support the recently implied existence of this zippering in the final phase of SNARE

assembly to catalyze exocytosis.

AN INTERVAL OF SLEEP ENHANCES THE CONSOLIDATION OF LINGUISTIC SKILLS

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Memory consolidation is a generative process that can result in delayed, "offline" performance gains. In some tasks, notably in movement sequence production, sleep may be necessary to complete the process of consolidation, as expressed in "off-line" gains. Here, we tested whether the acquisition of linguistic knowledge benefits from post-training sleep. Young adults learned a miniature artificial language, Brocanto3, in laboratory settings, in the context of a novel board-game. Participants completed an instruction and game-playing session and an immediate post-training test, which were afforded either in the morning or in the evening. All participants were retested either 10-12 or 24 hours later, and again 10-18 days later for long-term retention. Irrespective of the time-of day in which the training was afforded, participants showed similar learning in the 1st session and exhibited good long-term retention. However, participants who had a night's sleep during the post-training interval (irrespective of its proximity to the training session) showed significant "offline" improvement in speed of performance in grammaticality judgement, comprehension and in production (describing game moves) of Brocanto3 phrases with no costs in accuracy. Participants who had no sleep in the 10-12 hours post-training interval showed significantly smaller 'offline' gains in grammaticality judgement, no improvement in comprehension or production, and even tended to regress. These preliminary data suggest that sleep plays a role in enhancing procedural memory consolidation processes triggered by new language learning in young adults. Moreover, consolidation processes can be completed in a subsequent nights' sleep even after a day-long interval in the wake state, compensating for temporary day-time attrition effects. Sleep may be more critical in supporting consolidation processes for language production skills.

NA,K-ATPASE AS A MODEL FOR SPECIFIC PROTEIN-LIPID INTERACTION AND THEIR FUNCTIONAL CONSEQUENCES

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Since the proposal of the Fluid Mosaic Model of Singer and Nicolson (1972) bilayer lipids has been thought primarily to serve as a passive environment of membrane proteins that can affect their function by virtue of changes in the physical properties of the lipid. The Na,K-ATPase is the primary active transporter for Na^+ and K^+ in all animal cells and its activity is influenced by the lipid environment via both general and specific lipid interactions. We have studied the specific effects of various lipids on activity, stability and kinetic properties of recombinant detergent soluble Na,K-ATPase using activity assays, mutant analysis, stopped flow measurements and native mass spectrometry.

We were able to distinguish three different lipid-mediated effects namely (1) stabilization against thermal inactivation by phosphatidylserine and cholesterol (2) stimulation of pump turnover by polyunsaturated phosphatidylethanolamine and (3) inhibition of ATPase activity by sphingomyelin.

Using a mutant of the thermolabile α2-isoform we show that cholesterol and PS bind to a crevice formed by three C-terminal transmembrane helices and stabilize the pump. A cholesterol molecule has recently been identified in a crystal structure but no PS-molecule was present. However, using native mass spectrometry we could show that one molecule of PS binds specifically to the Na,K-ATPase α-subunit.

Stimulation is specific for neutral phospholipids and depends on a polyunsaturated fatty acyl chain in sn-2 position, e.g. 18:0/22:6 PE. The effect is independent of enzyme stabilization and results from the acceleration of the E1->E2 conformational transition. As an opposite effect, inhibition of enzyme turnover by 70% is caused by reducing the E2->E1 transition rate by saturated PC or sphingomyelin.

Lipidomic analysis revealed that PS and PE-lipids identified here are present in Na,K-ATPase enriched kidney membranes. Transport properties of PS/PE/cholesterol-purified Na,K-ATPase resemble those of native renal pump, which makes the study highly physiological relevant. In summary this study highlights the importance of specific protein-lipid interactions over physical membrane interactions for Na,K-ATPase transport activity and stability.

EPIGENETIC MODIFICATIONS AFFECT THE EXPRESSION OF CORTICOTROPHIN-RELEASING-HORMONE (CRH) WHICH IS INVOLVED IN THE MECHANISM UNDERLYING THE BALANCE BETWEEN HEAT STRESS RESILIENCE AND VULNERABILITY

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Determining whether heat exposure will lead to future heat-resilience or vulnerability depends on a delicate balance of a probably adjustable heat response set-point. The adjustment of this set-point is most likely effective during sensory postnatal development and involves the hypothalamus-pituitary-adrenal (HPA) axis. Here we demonstrate that heat stress during the critical period of thermal control establishment in 3-day-old chicks, renders habituated or sensitized response, a week later, depending on the ambient temperature i.e. moderate heat lead to future heat resistance while harsh temperatures lead to heat vulnerability. Furthermore, these changes might be governed by epigenetic modifications, specifically DNA methylation.

The mRNA expression of CRH in the hypothalamic paraventricular nucleus and plasma corticosterone were elevated a week after heat conditioning in chicks which were trained to be vulnerable to heat, while it declined in chicks that were trained to be resilient, demonstrating correlative changes in the HPA axis. Interestingly, the DNA methylation pattern along the CRH gene changed significantly between the groups, a week after their heat conditioning. In order to study the role of plasticity in the HPA axis, CRH or antisense to CRH were intracranialy injected into the third ventricle. CRH caused an elevation both in body temperature and plasma cort level, while CRH-antisense caused an opposite response. These opposite responses were memorized a week later. This effect was used to reverse resilience into vulnerability and vise versa. Chicks that have been injected with CRH followed by exposure to mild heat stress, normally inducing resilience, demonstrated a very high elevation in body temperature and CRH expression while chicks that were injected with CRH-antisense and exposed to harsh ambient temperature were reversed and responded instead of the expected vulnerability in long-term heat resilience.

These results demonstrate a role for CRH in determining either heat resilience or vulnerability response later in life.

STRUCTURE-BASED ANALYSIS AND RE-DESIGN OF RGS ACTIVITY AND SPECIFICITY TOWARDS G-PROTEINS.

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Background: RGS proteins play a critical role in many G protein-dependent signaling pathways. The biological activity of RGS proteins is to turn off heterotrimeric G proteins, thereby determining the lifetime of the activated G-protein molecular switch. RGS proteins recognize specific and individual G proteins, but previous studies have not identified the full complement of residues that are responsible for this specificity.

Purpose and methods: We extrapolated from a quantitative analysis of crystallographic complexes of G-alpha subunits with RGS proteins to identify the residues responsible for specificity in all 20 members of the RGS family. We further analyzed available 3D structures to better understand the role of specificity-determining residues. We validate our predictions using loss-of-function mutagenesis and single turnover GTPase assays.

Results: Our computational map predicts the activity of previously uninvestigated RGS proteins, and maps the residues that encode for this specificity. In addition, we classified and figured out the mechanistic roles of RGS residues in the interaction with the unique helical domain of the G-alpha subunits.

Conclusion: We identified selective RGS residues that interact with the G-alpha helical domain and contribute to specificity toward G-alpha subunits. These results will be used to redesign RGS proteins and G-proteins with different specificities, which in turn can be used to investigate the interactions of these proteins in cells and tissues.

SPECIFICITY AND MECHANISM OF RGS17 INTERACTIONS WITH G PROTEINS

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Background: Regulator of G protein signaling (RGS) proteins turn off heterotrimeric G proteins by binding directly to activated Gα subunits and accelerate their intrinsic GTPase activity allosterically. RGS proteins thereby function as GTPase-activating proteins (GAPs), and thereby determine the timing of G protein signaling. While RGS17 was suggested to show specificity towards different Gα subunits, its quantitative activity varies between reports. A recent study identified 7 amino acids in four distinct sites in RGS17 that contributed to low GAP activity towards $G\alpha_{i/o}$. Notably, only simultaneous substitution of all four sites led to a gain-of-function, but the mechanism for this all-or-none effect is not understood.

Purpose and methods: We aim to understand the amino-acid level determinants of RGS17 specificity by using an integrated computational and experimental approach.

Results: We analyzed the specificity-determinants of RGS17 by 3D structure-analysis and energy calculations and propose mechanistic roles for each of the seven RGS17 residues that were suggested to contribute to its specificity.

Conclusion: Revealing how the specificity of RGS17 towards G proteins is determined at the amino acid level can lead to mechanistic understanding of this interaction specificity. This will enable to guide RGS17 mutant design to study how RGS17 function in a physiological context and in pathologies.

CHARACTERIZATION OF THE FUNCTIONAL ROLE AND SPECIFICITY OF Gα HELICAL DOMAINS RESIDUES TOWARD RGS PROTEINS

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Background: Heterotrimeric G-proteins regulate intracellular signaling cascades in response to GPCR. The G-alpha switch is turned "on" by exchange of GDP for GTP, and turned "off" by the intrinsic hydrolysis of GTP – controlled by RGS proteins that accelerate GTP hydrolysis. Many RGS proteins can regulate the activity of more than one G-alpha subunit, while some RGS proteins display specificity towards their interacting partners. Previous studies from our lab identified amino acids that determine RGS specificity towards G-alpha proteins, but little is known about the determinants of G-alpha specificity towards their interaction partners.

Purpose and methods: We aim to understand the determinants of interaction specificity between G-alpha subunits and their partners using a comparative structural and energetic analysis of multiple 3D complexes of these interaction partners.

Results: We produced a residue-level computational map that identified particular residues in the G-alpha subunits, and in particular in their helical domains, which participate in interactions with multiple RGS proteins and are predicted to modulate specific interactions with these proteins partners.

Conclusion: our work provides a framework for understanding the determinants of selective G protein interactions with RGS proteins and enables structure-based redesign of protein-protein interactions. It can be extended to design a variety of G protein and RGS protein mutants with distinct activities and selectivities as tools to decipher G protein signaling networks in living cells.

REPROGRAMMING CANCER CELL METABOLISM, REVERSING ONCOGENIC PROPERTIES AND INDUCING DIFFERENTIATION IN GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is an aggressive brain cancer with high rates of relapse and mortality that lacks effective treatment. Like many cancers, GBM cells acquire oncogenic properties, including metabolic reprograming, vital for growth and motility. As such, tumour metabolism is an emerging avenue for cancer therapy. Here, using siRNA, we targeted VDAC1, a mitochondrial protein found at the crossroads of metabolic and survival pathways and overexpressed in glioblastoma. In GBM xenografts, VDAC1 depletion led to reprogramed metabolism, inhibition of cell proliferation, tumour growth, angiogenesis, stemness, invasiveness, and induced differentiation into non-replicating end-stage astrocyte- and neuron-like cells. These VDAC1 depletion-mediated effects involved alterations in transcription factors regulating signalling pathways associated with cancer hallmarks. VDAC1 thus offers a target for GBM treatment via attacks on the interplay between metabolism and oncogenic signalling networks, leading to tumour cell differentiation into non-replicating end-stage neurons- and astrocytes-like cells, thereby preventing tumour invasion and relapse. Simultaneously attacking all of these processes, VDAC1 depletion can replace several anti-cancer drugs separately targeting angiogenesis, proliferation or metabolism. Such treatment overcomes GBM heterogeneity and recurrence, offering an innovative and potent therapeutic strategy.

ON THE ROLE OF THE PRO-APOPTOTIC PROTEIN SMAC/ DIABLO IN CANCER: DEFINING A NEW FUNCTION AND USE AS A POTENTIAL BIOMARKER OF CANCER PROGRESSION

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SMAC/Diablo, a mitochondrial pro-apoptotic protein, plays an important role in apoptosis by negatively regulating members of the inhibitor of apoptosis (IAP) family. Upon activation of apoptosis, mature SMAC/Diablo is released from the mitochondria to the cytosol where it neutralizes the anti-apoptotic effects of IAP (cIAP1, cIAP2, XIAP and surviving) and prevents caspase activation. SMAC/Diablo is controlled by several cellular proteins, such as members of the Bcl-2 family of proteins, Erk1/2 and c-Jun NH_2-terminal kinase. Yet, despite the pro-apoptotic function of SMAC/Diablo, it is over-expressed in various cancers, such as colorectal-, gastric-, renal- and adeno-carcinomas. This suggests that in addition to its roles for in cell death, SMAC/Diablo may also perform additional non-apoptotic functions, such as regulators of cell survival in cancer cells. In this study, we observed high expression levels of SMAC/Diablo in lung cancer tissue. Moreover, although SMAC/Diablo is a mitochondrial protein, we detected very high levels in the nucleus. This further suggests that SMAC/Diablo fulfills additional functions not related to apoptosis. To define such functions, we silenced SMAC/Diablo expression using h SMAC/Diablo-specific siRNA. This resulted in a marked decrease in cell growth in both HeLa and A549 cancer cells. This cell growth arrest may result from a decrease in cellular ATP levels. As expected, SMAC/Diablo over-expression resulted in cell death in both HeLa and A549 cells. The function of over-expressed SMAC/Diablo in cancers, its nuclear translocation and importance for cell growth point to an as yet unidentified activity of SMAC/Diablo other than countering the activities of IAP inhibitors. This new putative function of SMAC/Diablo is the focus of our present research.

Heat acclimation memory: do the kinetics of the deacclimated transcriptome predispose to rapid reacclimation and cytoprotection?

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Adaptation to environmental heat is evolutionary conserved feature, allowing enhanced thermal tolerance, cross tolerance and adaptive memory to adverse environments, while employing powerful physiologically-controlled and molecular coping tools. To achieve these features, the organism undergoes long-term adaptive process involving temporally changing short and long-term molecular and cellular programs. Using the heat acclimated cardio-phenotype (HA-CP) as a model system, in this presentation these programs are discussed. The HA-CP, shows qualitative changes in excitation-contraction coupling, is more metabolically efficient and increases compliance to complying with larger volume of venous return when heat acclimated. Enhanced thermal tolerance and cytoprotection involves augmented HSPs and HIF-1α reserves and mitochondrial remodeling of the electron transport chain. The onset of acclimation is of transient nature. Up/down-regulated genes linked to maintenance of DNA integrity, decreased histone H1 transcript level and increased miRNAs emphasize the strain at the onset of this acclimation phase. We consider that these orchestrated initial events act as the molecular 'on-switch' of acclimation. Histone H3 phosphorylation at this acclimation phase, and in-turn constitutive histone H4 acetylation and HSF1 binding at the HSPs 72kD and 90kD genes is only one among the mechanisms of enhanced cytoprotection. HA-CP has a cytoprotective memory. During acclimation-decline, dynamic transcriptional changes establish a "dormant memory" state, predisposing to rapid re-acclimation. Specific molecular network, leading to constitutive up-regulation of P38 MAPK, AKT and other kinases signaling targets is activated. Up/down regulation of epigenetic markers such as linker histones (histones H1 cluster), associated with nucleosome spacing, transcriptional chromatin modifiers, molecules linked to chromatin compaction and miRNAs imply maintenance of euchromatin and proteostasis and thus enable fast physiological re-gain of the HA-CP.

MITOCHONDRIAL VDAC1- BASED PEPTIDES AS A POTENTIAL THERAPEUTICS FOR GLIOBLASTOMA

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Glioblastoma multiforme (GBM), a common primary brain malignancy characterized by high morbidity, relapse rate and mortality, is highly invasive, proliferative, and resistant to chemo- and radiotherapies and for which effective therapeutic options are lacking. GBMs undergo metabolic reprograming and develop cell survival strategies, involving anti-apoptotic defense mechanisms, hallmarks of numerous cancers. As such, tumor metabolism and apoptosis resistance are emerging avenues for cancer therapy. The outer mitochondrial membrane protein voltage-dependent anion channel 1 (VDAC1) is a central player in cell energy metabolism and assumes a key role in mitochondria-mediated apoptosis.

Here, we demonstrate that VDAC1-based cell-penetrating peptides induce cell death in several brain cancer-derived cell lines, including GBM and GBM-derived stem cells. The mode of action of the VDAC1-based peptides involved induction of mitochondria-mediated apoptosis and impairment of energy and metabolic homeostasis. In a xenograft glioblastoma mouse model, VDAC1-based peptides, markedly inhibited tumor growth and induce apoptosis. Strikingly, peptide-treated tumors showed down-regulation of the glucose transporter, glycolytic enzymes and VDAC1 expression. In contrast, the levels of apoptotic proteins, such as p53 cytochrome *c* and caspases, increased. Moreover, peptide tumor treatment induced dramatic decreases in cell proliferation and eliminated stem cells. These findings show that VDAC1-based peptides dramatically inhibit cancer cell growth and tumor development, eliminate cancer stem cells and trigger apoptosis, raising the possibility of a more effective pipeline of anti-glioblastoma drugs designed to overcome GBM stemness, invasiveness and relapse.

THE MITOCHONDRIAL ANTI-VIRAL SIGNALING PROTEIN, MAVS IS OVEREXPRESSED IN MANY CANCERS: POSSIBLE FUNCTION

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The mitochondrial anti-viral signaling protein (MAVS) plays an important role in host defense against viral infection via coordinating the activation of NF-κB and interferon regulatory factors (IRF3 and IRF7). Recent studies suggest the involvement of MAVS in viral-induced apoptosis via its predicted interaction with the outer mitochondrial membrane protein VDAC1. VDAC1 is a dynamic regulator of global mitochondrial function in both health and disease. VDAC1 controls cellular energy production and metabolism by mediating the exchange of metabolites and ions between mitochondria and the rest of cell, thereby regulating cell survival. VDAC1 has also been proposed to function as a key player in apoptosis. We have found that MAVS is over-expressed in many cancer types, including lung and cervical cancers, chronic lymphocytic leukemia and others. This study aimed to explore the unclear function of MAVS in cancer. Using several approaches, we demonstrate the direct interaction of MAVS with VDAC1 and with the pro-apoptotic proteins caspase-8 and tBid. MAVS prevented the cleavage of Bid by caspase 8 to produce apoptosis inducer, tBid. We further demonstrate that MAVS offered protection against apoptosis as induced by various means, although this effect was most pronounced with TNF-α, acting via the extrinsic apoptotic pathway leading to caspase 8 activation and tBid formation that subsequently activates mitochondria-mediated apoptosis. These results suggest that MAVS acts as anti-apoptotic protein by interacting with caspase-8, VDAC1 or tBid to interfere with their pro-apoptotic activities. Furthermore, the findings presented here point to MAVS-VDAC1 and MAVS-tBid interactions as new targets for cancer therapy.

PKA PHOSPHORYLATION OF NCLX REVERSES MITOCHONDRIAL CALCIUM OVERLOAD AND DEPOLARIZATION PROMOTING SURVIVAL OF PINK1 DEFICIENT DOPAMINERGIC NEURONS

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Mitochondrial Ca^{2+} overload is a critical, preceding event in neuronal damage encountered during neurodegenerative and ischemic insults. We found that loss of PTEN-induced putative kinase 1 (PINK1) function, implicated in inherited form of Parkinson's disease, inhibits the mitochondrial Na^{+}/Ca^{2+} exchanger (NCLX) leading to impaired mitochondrial Ca^{2+} extrusion. NCLX activity was, however, fully rescued by activating the protein kinase A (PKA) pathway. We further show that PKA rescues NCLX activity by phosphorylating serine 258, a putative regulatory NCLX site. Remarkably, a constitutively active phosphomimetic mutant of NCLX ($NCLX^{S258D}$) prevents mitochondrial Ca^{2+} overload and mitochondrial depolarization in PINK1 KO neurons, thereby enhancing neuronal survival. Our results identify mitochondrial Ca^{2+} transport regulatory pathway that protects against mitochondrial Ca^{2+} overload. Since mitochondrial Ca^{2+} dyshomeostasis is a prominent feature of multiple disorders, this link between NCLX and PKA may offer a therapeutic target.

Neurogenesis-promoting effect of weak static magnetic fields in vitro and in vivo

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Low intensity static magnetic fields (SMFs) interact with various biological tissues, thereby affecting key biological processes. In the CNS, magnetic fields affect gene expression, cell proliferation and differentiation, thereby regulating cell fate, by initiating a primary physical interaction with a particular molecular target resulting in its modulated biological activity. We have previously demonstrated the neuroprotective capacity of SMFs in primary cortical neuronal cultures, where exposure to an intensity of 50 G decreased the extent of toxin-induced apoptotic cell death. We now show that SMF exposure may also affect plasticity by promoting neurogenesis in vitro and in vivo. SMF exposure of primary cortical neuronal cultures stimulated cells' proliferation, directing newly born cells into a neuronal lineage. The proportion of newly -generated early post-mitotic neurons, co-expressing the immature neuronal marker Doublecortin (DCX) and the proliferation marker 5-ethynyl-2'-deoxyuridine (EdU), was higher amongst SMF-exposed cultures, compared with control (4.14±0.40 % vs. 1.81±0.17 %, respectively. $P<0.001$). SMF exposure further supports the differentiation of newly born neurons into mature neurons, culminating in an increased proportion of neurons co-expressing the mature neuronal markers Neuronal Nuclei (NeuN) and Neuron Specific Enolase (NSE) along with proliferation markers, compared with control (2.10±0.43-fold - and 3.14±0.47-fold of appropriate controls. $p<0.05$ and 0.001, respectively). SMFs' pro-neurogenic activity in cortical neurons was accompanied by activation of the basic helix loop helix (bHLH) pro-neuronal transcription factors, and mediated by voltage-gated Ca^{2+} channels. Interestingly, SMF exposure stimulated constitutive neurogenesis known to occur at the Sub-ventricular zone (SVZ) of the lateral ventricles in the adult brain, inducing a 63.2±5.2% increase in the number of proliferating SVZ migrating neuroblasts (co-expressing DCX and EdU), in rats transplanted with magnetic disc generating 45-50 G SMF intensity at the SVZ.

CARBENOXOLONE CAN SIGNIFICANTLY DECREASE THE FORMATION OF LUNG METASTASES.

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Purpose: Lung cancer is one of the deadliest cancers in the US and throughout the world, with statistics pointing out metastases as the main reason for the poor survival rates. Carbenoxolone is a steroid-like compound which is prescribed for treating esophageal ulcerations. Based on the previously published data regarding the anti-cancerous attributes of Licorice and its extracts, such as glycyrrhizin and Glycyrrhizinic acid, we aimed to investigate the effect of Carbenoxolone on lung cancer for the first time.

Results: In this study we used both *in-vitro* and *in-vivo* models to look at the effect of therapeutic concentration of the drug on the proliferation, migration, invasion, cell anchorage and adhesion of LLC (Lewis Lung Carcinoma), murine lung cancer cell line. Different *in-vivo* models were employed, i.e. orthotopic model and S.C. tumor implantations, to assess the reaction of the tumor to the drugs in a microenvironment that would most resemble its natural one the most. Data presented here show that Carbenoxolone has significantly decreased the formation of lung metastases using both *in-vitro* and *in-vivo* models.

Conclusions: Our results are of major clinical implications; not only did we use therapeutic concentrations, but this is a drug that was already approved and the anti-metastatic effect it displays has great ramifications for cancer patients and even healthy human beings throughout the world.

THE ROLE OF PGD2/CRTH2 AXIS IN THE MC-EOS ALLERGIC EFFECTOR UNIT

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Prostaglandin D_2 (PGD_2), the main mast cell (MC) COX metabolite, is involved in the ethiopathology of allergy. PGD_2 acts on immune cells via the chemoattractant receptor-homologous molecule expressed on Th2 cells ($CRTH_2$). We have defined a functional Allergic Effector Unit (AEU) between MCs and eosinophils (Eos), involving both cell-cell contact (surface receptors/ligands) and secreted mediators. Here we hypothesized that the PGD_2-$CRTH_2$ axis could have a role in the AEU. And investigated its role in cell survival, chemotaxis and activation by using the potent and selective $CRTH_2$ antagonist QAV680. We used co-cultures of human cord blood derived mast cells (CBMC) and human peripheral blood eosinophils (pbEos) (1:1) (up to 72h). IgE-sensitized CBMC were activated with anti-IgE antibodies (1h). Cell survival (PI), pbEos chemotaxis (Boyden chambers), $CRTH_2$ expression (FACS), tryptase and PGD_2 release (Enzymatic and ELISA assays) were evaluated in the absence/presence of QAV680 (0.1-1µM).

We confirmed that pbEos express the $CRTH_2$, and that CBMC do not express it. We showed that the expression of $CRTH_2$ on pbEos is stable in co-culture and that CBMC released PGD_2 following activation both in monoculture and co-cultured with pbEos. pbEos were chemoattracted by PGD_2 in a dose-dependent manner. Similarly resting and even more IgE-activated CBMC induced significant Eos migration and this effect was partially blocked by QAV680. Moreover pbEos enhancement in tryptase release from IgE-activated CBMC, was found to be partly mediated by PGD_2 since addition of the antagonist significantly reduced it. Additionally QAV680 inhibited pbEos survival both in monocultures and co-cultures indicating that pbEos survival under our cultures is partly dependent on PGD2.

In conclusion we identified PGD2 as an additional important player in the AEU. Since the AEU is an enhancing cross-talk our data further demonstrate the validity to inhibit $CRTH_2$ for downregulating the allergic response.

Evolutionary Optimization of Allosteric Regulation

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Allosteric regulation of protein function is a fundamental phenomenon of major importance in many cellular processes. Such regulation is commonly a result of synergy between protein subunits traditionally evaluated using the Hill formalism. The derived Hill coefficient value denotes the strength of such allosteric interactions. Here, we offer the hypothesis that the magnitude of such protein interactions is a singular optimum value that reflects evolutionary optimization of the protein mechanistic parameters to meet the system's physiological requirements. To test this hypothesis we performed a mechanistic meta-analysis of the hemoglobin model allosteric protein, a protein for which expanded physiological datasets are available. In this study, we found that hemoglobin has evolved to function within an allosteric parameter range that enables optimal protein function of oxygen binding and release. We also show that diverse mammalian hemoglobins are fixed on different optimal cooperativity points that are in correlation with particular animal physiological characteristics. We suggest that evolutionary tuning of hemoglobin optimal cooperativity serve to set the dynamic range for physiology adaptation to meet the particular requirement of different animal tissue for oxygen supply.

ROLE OF 'SUMO'YLATION ON MITOCHONDRIAL NCX TRAFFICKING

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Small ubiquitin-like modifier (SUMO) proteins belong to the family of ubiquitin-like peptides. SUMOylation, the attachment of SUMO, modifies target localization or function and is implicated in several physiological processes such as nuclear import, and the control of transcription factors etc. SUMOylation is also shown to be involved in various neurological disorders such as in Alzheimer's disease and Parkinson's disease. SUMO subtypes, SUMO-conjugating enzyme, and SUMO proteases are conserved from yeast to humans. The predicted site for SUMOylation has the sequence Ψ**K**XE where Ψ is any hydrophobic amino acid. Very similar to ubiquitination, attachment of SUMO to the Lysine residue on the target protein is mediated by SUMO conjugating enzyme Ubc9 (1,2).

Mitochondrial Ca^{2+} overload is a critical factor for neurodegenerative diseases. Our group, for the first time, identified Na^{+}/Ca^{2+} exchanger (NCLX) as the major protein for mitochondrial Ca^{2+} efflux (3). Recent data from Feligioni et al (4) shows that SUMOylation of presynaptic target proteins regulates calcium homeostasis and neurotransmitter release.

"SUMOplot" analysis of human NCLX shows several SUMOylation site with differential probability score. Among these, we have found that alteration of a putative SUMOylation site by mutating specific lysine residue on NCLX impairs its localization to mitochondria. This is corroborated with a decrease in mitochondrial Ca^{2+} efflux through NCLX. These preliminary results demonstrate that SUMOylation may play an important role for the turnover of NCLX which in turn can be an important aspect for mitochondrial Ca^{2+} overload.

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Evaluating the Efficacy of VDAC1-based Peptides in DEN-induced Liver Cancer in Mice

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Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related deaths. The incidence rate of HCC is 2–3 times higher in developing countries than in the developed world. The incidence of HCC is increasing, despite progress in identifying risk factors, better understanding of disease etiology and the development of anti-viral strategies. Still, therapeutic options are limited and survival after diagnosis is poor. Therefore, better preventive, diagnostic and therapeutic tools are urgently needed. Accordingly, we have tested the effect of Retro-TF-D-LP4, a VDAC1-based peptide developed in our lab, on liver cancer. For our model, we chemically induced liver cancer in C57BL/6 mice using diethylnitrosamine (DEN) and tested the effect of the Retro-TF-D-LP4 peptide on tumor growth. Our results showed that the peptide dramatically reduced both the number and the size of the developed tumors. The peptide also induced alterations in the tumor micro-environment, as reflected in reduced numbers of macrophages in the tumor, and decreased fibrosis. These findings show that VDAC1-based peptides represent a promising therapeutic approach for liver cancer.

THE MOLECULAR CROSS-TALK IN A UNIQUE PARASITIC MANIPULATION STRATEGY

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Cockroaches (*Periplaneta americana*) can fall victim to the parasitoid Jewel Wasp (*Ampulex compressa*) which uses them as food supply for its larvae. The wasp injects its venom cocktail directly into the host's central nervous system (CNS). The sting of the parasitoid *wasp* is unusual in that it induces a long-term hypokinesia of the cockroach prey. During this hypokinetic state, specific behaviors of the prey are inhibited while others are unaffected. The intoxicated cockroach follows the wasp submissively to its nest, demonstrating a completely normal walking pattern. The wasp then lays an egg on the lethargic cockroach. The larva that hatches from the egg finds accessible fresh food needed for its development into an adult wasp.

The goal of the present work is to uncover the molecular mechanisms which underlie this unique parasite manipulation strategy. We approach this goal by investigating different biochemical aspects: the proteinic components of the venom, their molecular targets, and changes in the cockroach's CNS proteome after stung by the wasp.

By combining transcriptomics and proteomics we reveal that *Ampulex* venom contains at least 201 proteins. We then coupled the venom proteins to a NHS-activated column and by affinity chromatography we purified proteins from the cockroach CNS that are putative molecular targets for the venom. In addition, using quantitative mass spectrometry we identify proteome changes in the CNS of stung cockroaches.

Combining all this information brings us closer to understanding, at the molecular level, how a wasp can manipulate its host behavior.

Design of lithium transporting sodium-calcium exchanger based on structural templates of NCX_Mj and NCLX proteins

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The NCX gene family is one of five families belonging to the CaCA (Ca^{2+}/Cation Antiporter) superfamily. Although these proteins share structural similarities, different proteins show high selectivity for transporting Ca^{2+}, Na^{+}, H^{+}, K^{+} and/or Li^{+} ions. NCX and similar proteins contain ten transmembrane helices, which form two hubs (TM1-TM5 and TM6-TM10). The ion-binding pocket encompasses highly conserved α_1 and α_2 repeats with twelve ion-coordinating residues (four in TM2 and TM7, and two in TM3 and TM8). Notably, the ion-coordinating residues are highly conserved in proteins of the CaCA superfamily despite diverse ion selectivity. The mitochondrial Na^{+}/Ca^{2+} exchanger (NCLX) differs from the other NCX proteins in that NCLX can transport either Li^{+} or Na^{+} in exchange with Ca^{2+}, whereas nine ion-coordinating residues (among twelve conserved residues) are naturally substituted by other residues in NCLX. To elucidate the molecular determinants of ion selectivity, we generated the NCX_Mj/NCLX construct by introducing nine point mutations to WT NCX_Mj at positions corresponding to the naturally substituted critical residues of NCLX to mimic ion-coordination by NCLX. The NCX_Mj/NCLX construct was examined for its capacity to catalyze the Li^{+}/Ca^{2+} and Na^{+}/Ca^{2+} exchange reactions as compared with WT NCX_Mj. For this purpose, $^{45}Ca^{2+}$-uptake was measured in Na^{+} or Li^{+}-loaded vesicles, which were obtained from *E. coli* cell membranes containing the overexpressed WT NCX_Mj or NCX_Mj/NCLX. We found that while WT NCX_Mj has no significant activity for Li^{+} transport, the NCX_Mj/NCLX construct exhibits similar V_{max} and K_m values for Na^{+} or Li^{+} transport in exchange with $^{45}Ca^{2+}$. The V_{max} for Na^{+}/Ca^{2+} of the NCX_Mj/NCLX construct is 2-3 fold lower compared to that of WT NCX_Mj. The NCX_Mj/NCLX construct can serve as an excellent model for studying the structure-dynamic determinants of ion selectivity and ion-coupled alternative access in NCX, NCLX and similar proteins.

Kinetic and equilibrium properties of NCX2 and NCX3 Ca^{2+}-binding regulatory domains

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In mammals, three sodium-calcium exchanger (NCX) protein isoforms (NCX1, NCX2, and NCX3) mediate Ca^{2+} fluxes across the membrane to maintain cellular Ca^{2+} homeostasis. NCX isoforms and their splice variants are expressed in a tissue-specific manner to meet physiological demands. NCX1 is ubiquitously expressed, NCX2 is expressed in the brain and spinal cord, and NCX3 is expressed in the brain and skeletal muscle. Eukaryotic NCXs contain two cytosolic regulatory Ca^{2+}-binding domains, CBD1 and CBD2, which form a two-domain tandem (CBD12) through a short linker. Ca^{2+} binding to the CBDs underlies allosteric regulation of NCX. Previous structural and functional studies in NCX1 have shown that the CBDs synergistically interact, where their interactions are modulated in a splice variant-specific manner by alternative-splicing at CBD2. Here, we analyze the equilibrium and kinetic properties of Ca^{2+} binding to purified preparations of CBD1, CBD2, and CBD12 from NCX2 and from NCX3 splice variants. CBD1 binds four Ca^{2+} ions in both NCX2 and NCX3, with similar high affinity in both isoforms. NCX2-CBD2 binds one Ca^{2+} ion with low affinity; NCX3-AC (expressed in skeletal muscle) does not bind Ca^{2+}; and B-exon containing NCX3-CBD2 splice-variants (CBD2-B and CBD2-BC, expressed in the brain) bind three Ca^{2+} ions with moderate affinity. We show that CBD1 interacts with CBD2 in the context of the CBD12 tandem in all NCX isoforms, where these interactions specifically modulate Ca^{2+} sensing at the high-affinity primary sensor of CBD1 to meet the physiological requirements. For example, the rate-limiting slow dissociation of "occluded" Ca^{2+} (underlying slow NCX inactivation upon $[Ca^{2+}]_i$ depletion) from the primary allosteric sensor of variants expressed in skeletal muscle is ~10-fold slower than that of variants expressed in the brain. Notably, these kinetic differences between NCX variants occur while maintaining a similar Ca^{2+} affinity of the primary sensor, since the resting $[Ca^{2+}]_i$ levels are similar among different cell types.

HUMAN PLACENTAL EXPANDED (PLX) MESENCHYMAL CELLS CONFER NEUROPROTECTION TO NERVE GROWTH FACTOR (NGF) DIFFERENTIATED PC12 CELLS EXPOSED TO ISCHEMIA BY SECRETION OF IL-6 and VEGF.

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Stroke afflicts a large segment of population and is a leading cause of death in the western world. Stroke develops when a part of the brain is deprived of oxygen and glucose. Mesenchymal stem cells are potent candidates in stroke therapy due to their ability to secrete protective anti-inflammatory cytokines and growth factors. In the present study we investigated the neuroprotective effect of PLX using an established ischemic model of NGF-differentiated pheochromocytoma PC12 cells exposed to oxygen and glucose deprivation (OGD) followed by reperfusion. Under optimal conditions, $2x10^5$ PLX cells, added in a trans-well system, conferred 30-60% neuroprotection to PC12 cells subjected to ischemic insult. PC12 cell death, measured by lactate dehydrogenase (LDH) release, was reduced by PLX cells or by conditioned medium derived from PLX cells exposed to ischemia, suggesting the active release of factorial components. Since neuroprotection is a prominent function of the cytokine IL-6 and the angiogenic factor VEGF165, we measured their secretion using selective ELISA of the cells under ischemic or normoxic conditions. IL-6 and VEGF165 secretion by co-culture of PC12 and PLX cells was significantly higher under ischemic compared to normoxic conditions. Exogenous supplementation of 10 ng/ml each of IL-6 and VEGF165 to insulted PC12 cells conferred neuroprotection, reminiscent of the neuroprotective effect of PLX cells or their conditioned medium. Growth factors as well as co-cultures conditioned medium effects were reduced by 70% and 20% upon pretreatment with 240 ng/ml Semaxanib (anti VEGF165) and/or 400 ng/ml neutralizing anti IL-6 antibody, respectively. PLX induced neuroprotection in ischemic PC12 cells partially explained by IL-6 and VEGF165 secretion. These findings may also account for the therapeutic effects seen after treatment with these cells in clinical trials.

Email**: adi.lahiani@mail.huji.ac.il; PL holds The Jacob Gitlin Chair in Physiology and is affiliated and partially supported by the David R. Bloom Center for Pharmacy and the Dr. Adolf and Klara Brettler Center for Research in Molecular Pharmacology and Therapeutics at The Hebrew University of Jerusalem, Israel.* *The authors greatly appreciate the financial support of Israel Ministry of Industry and Commerce, Magneton Programs (P L) and the Gulton Foundation NY.*

RECONSTITUTION AND INVOLVEMENT OF NGF RECEPTOR COMPLEX OF $p75^{NTR}$ and α9β1 INTEGRIN IN ADHESION, PROLIFERATION AND MIGRATION OF GLIOMA CELLS

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Nerve growth factor (NGF) receptor $p75^{NTR}$ is a major contributor to the invasiveness of brain glioma tumors. Since these tumors express high levels of α9β1 integrin, which was also identified as NGF receptor, we hypothesized that NGF modulation of glioma invasiveness involves contributions of both $p75^{NTR}$ and α9β1 integrin. To verify this hypothesis we selectively transfected glioma cell lines with these receptors, and found a strong, cation-independent association of α9, with $p75^{NTR}$ in a immunoprecipitation complex. Other integrin subunits such as α1, α2, α4, and even β1 were not found to be associated with $p75^{NTR}$. Förster resonance energy transfer experiments indicated that $p75^{NTR}$ and α9β1 are not in close association through their intracellular domains, most probably because of the molecular interference of paxilin. Interaction of α9β1 integrin with its ligand VCAM-1 was not modulated by the $p75^{NTR}$, whereas binding of NGF significantly increased when both receptors were present. NGF treatment of α9/$p75^{NTR}$ expressing cells stimulated to a higher degree Erk1/2 and Akt phosphorylation compared to cells expressing separated individual receptors. Present results suggest that α9/$p75^{NTR}$ complex is an important contributor to NGF- dependent glioma cell signaling, adhesion, proliferation and migration.

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HUMAN UMBILICAL CORD BLOOD (HUCB) DERIVED CD45$^+$ HEMATOPOIETIC CELLS PROTECT MICE BRAIN AFTER CLOSED HEAD INJURY *

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Car and motor accidents, sports' injuries are causes of mechanical traumatic brain injury (TBI), a major cause of morbidity and mortality in the Western World, with no therapeutic neuroprotective agents clinically available to date. We have already reported neuroprotective effect of HUCB mononuclear fraction in experimental TBI. This fraction contains different populations of stem cells, while an abundant population is the hematopoietic nucleated cells, which express the CD45 antigen. Here we demonstrate that HUCB derived CD45 positive hematopoietic cells (CD45$^+$) reduced neuronal deficits in TBI mice model. CD45$^+$ were isolated from HUCB mononuclear fraction by magnetic sorting. These cells were characterized by the expression of CD45^{high}, CD11b^{high}, CD34^{low}, CD133^{low} and CD184^{low} markers and negative expression of late differentiation markers such as CD4 and CD20 for lymphocytes as well as lack of mesenchymal markers CD73, CD90 and CD105. The CD45$^+$ cells were transplanted intravenously and were detected at the site of brain injury 2 hours after transplantation. The therapeutic effect was measured up to 35 days with a significant improvement from day 7 and was found correlative to the decrease in the brain lesion volume. A 3 fold decrease in the therapeutic effect was observed by using anti-human-CD45 antibody for CD45$^+$ cells antigen neutralization. Acute (3 days) and chronic (35 days after trauma) secretion of the neurotrophins BDNF, NGF and VEGF at the ipsilateral and contralateral hemispheres didn't differ between the positive and the negative CD45 populations. Preliminary results showed that presence of CD45$^+$ cells was associated with attenuation in the injured brains of both neural inflammation and gliosis. These findings demonstrate the neuroprotective potential of HUCB CD45$^+$ fraction in mice model of brain trauma and may serve the basis for clinical therapeutic applications.

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INTERMEDIATE CALCIUM ACTIVATED POTASSIUM CHANNELS SK4 PLAY A ROLE IN THE PACEMAKER ACTIVITY OF HUMAN EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES AND OF THE MURINE SINOATRIAL NODE

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We recently identified by real-time PCR, Western blotting and immunocytochemistry a previously unrecognized Ca^{2+}-activated intermediate K^+ conductance (IKCa, SK4) in young and old stage- cardiomyocytes derived from human embryonic stem cells (hESC-CMs). Under the voltage-clamp configuration of the patch clamp technique, the SK4 blocker Tram-34 and the opener NS-309 led respectively to a decrease or an increase of the SK4 isolated current recorded when a voltage ramp protocol was applied. Exposure to TRAM-34 produced a reversible depolarizing drift of the MDP accompanied by a cessation of the pacemaker activity of the cells. On the contrary, application of NS309, led to an increase in the beating rate and in the slope of diastolic depolarization as well as a decrease in the action potential duration. A specific immunoreactive band and an amplified fragment were observed when we performed Western blotting and reverse transcription PCR on lysates and cDNA from murine sinoatrial node (SAN). Like in hESC-CMs, we also identified TRAM34-sensitive SK4 currents in mouse isolated SAN cells. Clotrimazole but also TRAM-34 strongly decreased and eventually suppressed the spontaneous electric activity of isolated SAN cells. In vivo heart telemetric measurements showed that intraperitoneal injection of the SK4 blockers TRAM 34 or clotrimazole (20 mg/kg) produced a bradycardic effect in mice, thereby increasing the PP and PR intervals. These results suggest that SK4 Ca^{2+} activated K^+ channels play a critical role in normal pacemaker function.

A NOVEL SITE OF COMPETITIVE PIP2 AND CALMODULIN INTERACTION TO KCNQ1 C-TERMINUS HELIX B IS CRUCIAL FOR I_{KS} CHANNEL ACTIVITY

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KCNQ1 and KCNE1 co-assembly generates the I_{KS} potassium current, which is crucial to the cardiac action potential repolarization. Mutations in their corresponding genes cause cardiac arrhythmias. In the KCNQ1 C-terminus (CT), proximal helices A and B form sites for calmodulin (CaM) binding. Studies identified basic residues in KCNQ1 at S2-S3 and S4-S5 intracellular linkers and proximal CT as PIP2 binding sites. Our recent crystallographic data showed that CaM embraces the two proximal anti-parallel helices B and A with its calcified N-lobe and apo C-lobe, respectively. Here we identified a novel site of competitive PIP2 and calmodulin interaction to helix B that is crucial for I_{KS} channel activity. PIP2 competes with CaM binding to purified His-tagged KCNQ1 CT in the presence of Ca^{2+} only. Conversely, recombinant WT Ca^{2+}-CaM or CaM3,4 but neither WT apoCaM nor CaM1,2 compete with PIP2 binding to purified His-tagged KCNQ1 CT. Recombinant WT Ca^{2+}-CaM or CaM3,4 but not WT apoCaM or CaM1,2 in the patch pipet significantly attenuate the decrease in I_{KS} current resulting from PIP2 breakdown by Dr-VSP. Molecular docking, dynamic simulation and subsequent biochemical and electrophysiological validation experiments indicate that K526 and K527 in helix B form a novel and crucial site for competitive PIP2 and calmodulin interaction. Our data suggest that upon PIP2 breakdown (e.g. following GPCR-Gq signaling), PIP2 unbinds from helix B and allows the calcified CaM N-lobe to replace it, which guaranties the maintenance of the channel open state in front of stressful PIP2 depletion events.

ZINC SIGNALING IN TAMOXIFEN RESISTANT BREAST CANCER CELLS

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Breast cancer is the most common and lethal cancer among women. Although estrogen-receptor positive breast cancers are treated with antihormones such as Tamoxifen, the development of resistance to such therapies presents a major obstacle in the treatment of breast cancer. Tamoxifen-resistant breast cancer cells (TAM-R) display morphology and repertoire of proteins that are different from those displayed by non-resistant cells (MCF-7). Interestingly, the expression of several zinc transporters is upregulated in the TAMR cells. Previous work in our lab identified a Gq-coupled receptor, the ZnR, which is activated by extracellular zinc. We show, here, that ZnR activity is present in the TAM-R cells but is significantly decreased in the MCF-7 cells. Fluorescent calcium imaging indicated that extracellular Zn^{2+}-dependent Ca^{2+} release is active in the TAM-R cells but not in the MCF-7 cells. Indeed, using real time PCR assays we show that TAM-R cells express at least 2-fold higher level of ZnR/GPR39 than MCF-7 cells. Dose response analysis showed that TAM-R cells have a Km of 19±8 µM to Zn^{2+}, within the physiological concentration. We also show that prolonged exposure to zinc induces desensitization, of ZnR in TAM-R cells. In accordance, Zn^{2+}-dependent activation of MAP and PI3 kinases is shown in the TAM-R but not in MCF-7 cells. Silencing GPR39 using siRNA or the IP3 pathway inhibitors in the TAM-R cells was followed by significantly decreased calcium release. TAM-R cells also exhibit enhanced proliferation following treatments with Zn^{2+}, while MCF-7 cells do not, suggesting that ZnR activation may enhance cell growth. These results suggest that ZnR/GPR39 signaling is present in TAM-R cells activating pathways that are closely linked to cell proliferation and survival. The ZnR-dependent activity, however, is absent in the MCF-7 cells. We therefore suggest that Zn^{2+} signaling, mediated by the ZnR, in TAM-R cells can enhance their malignant properties.

SK4 K+ channels regulate cardiac pacemaker in sinoatrial node and their blockade ameliorate arrhythmias in CPVT2 patient-derived iPSC and *in vivo* in CASQ2 knock-in and knock-out mice

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Catecholaminergic Polymorphic Ventricular Tachychardia (CPVT) is a stressed-provoked ventricular arrhythmia triggered by diastolic intracellular calcium leak which can lead to sudden death. While a lot of studies have been focused on ventricular tachyarrhythmia, very little is known about the pathological pacemaker activity in this disease. Recently, we identified the intermediate calcium-activated potassium channel SK4 (IK_{Ca}, $K_{Ca}3.1$) as a new player involved in the pacemaker activity of cardiomyocytes derived from human embryonic stem cells. Here we used human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) from healthy and CPVT2 (CASQ2 D307H) patients, mouse sinoatrial node (SAN) cells as well as knock-in (CASQ2 D307H) and knock-out mice to investigate the pacemaker arrhythmogenic properties of this disease. Combining current and voltage-clamp recordings and using TRAM-34, a selective blocker of SK4 channels, we could isolate SK4 currents in both wild-type and CPVT2-derived hiPS-CMs. We could observe delayed afterdepolarizations (DADs) in old CPVT2-derived hiPS-CMs cells following application of the beta-adrenergic agonist isoproterenol (Iso). Interestingly, the iso-provoked arrhythmias were markedly reduced by adding TRAM-34 (2-5 μM). Like in hiPS-CMs, we also identified TRAM34-sensitive SK4 currents in mouse SAN. A heart telemetry device was implanted in WT, knock-in (CASQ2 D307H) and knock-out mice for continuous ECG recording at rest and during exercise. Intraperitoneal injection of the SK4 blockers TRAM 34 or clotrimazole (20 mg/kg) produced a bradycardic effect in WT, knock-in and knock-out mice, thereby increasing the PP and PR intervals. Remarkably, TRAM 34 or clotrimazole dramatically reduced the ECG arrhythmia features of knock-in and knock-out mice like sustained and non-sustained ventricular tachycardia, ventricular premature contractions. These results suggest that SK4 Ca^{2+} activated K^+ channels play a critical role in normal and diseased pacemaker dysfunction and notably that found in CPVT patients. Our data also indicate that SK4 channel blockers could open new horizons in the management of CPVT rhythm disorders.

SENSORY DEPRIVATION IN EARLY LIFE TRIGGERS SYNAPTIC AND INTRINSIC PLASTICITY IN THE HIPPOCAMPUS

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Hippocampus, a temporal lobe structure involved in learning and memory, receives information from all sensory modalities. Despite extensive research on the role of sensory experience in cortical map plasticity, we still do not know if and how sensory experience regulates synaptic processing in the hippocampus. Here, we show that whisker deprivation early in mouse life depresses synaptic transmission in the CA3-CA1 hippocampal connections by decrease of release probability and drop in excitatory quantal amplitude. These changes were accompanied by an increase in the fraction of NR2B-containing NMDA receptors and reduction in the intrinsic excitability of CA3 neurons. As a result of sensory disuse during early development, synaptic facilitation was augmented at high frequencies, enhancing input discrimination by CA1. Notably, whisker deprivation in adult mice did not alter synaptic facilitation, indicating an existence of a critical period in the hippocampus. We suggest that sensory experience in early life regulates computations performed by the hippocampus by tuning the gain and filtering characteristics of hippocampal synapses.

THE ROLE OF ZIP4 N-TERMINAL DOMAIN IN TRANSPORT ACTIVETY AND ENDOCYTOSIS

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Zinc plays a contradictory role in cell physiology. On one hand it is incorporated in hundreds of enzymes and serves as a structural ion in transcription factors. On the other hand, high concentration of intracellular free Zinc may lead to cell death. Monitoring cellular Zinc levels are therefore essential for survival. Indeed, Zinc homeostasis is strictly maintained by two major Zn^{2+} transporter families: (i) The ZnT family, which mediates Zn^{2+} efflux. (ii) The Zip family, which mediates Zn^{2+} influx. A member of the Zip family, the human Zip4, is a membrane $Zn^{2+/}H^{+}$ simporter involved in dietary Zinc uptake from the intestinal lumen and the embryonic visceral yolk sac. Zip4 is likewise associated with the Acrodermatitis Enteropathica disorder, which causes skin lesions, growth retardation, immune system dysfunction and neurological disorders. Previous studies have shown that Zinc stimulates endocytosis and degradation of Zip4. Our study results suggest a novel ERK1- mediated endocytosis pathway, in which phosphorylation of Zip's P201 residue leads to Zip4 endocytosis and degradation.

PERTURBATION OF M-TYPE POTASSIUM CHANNEL ACTIVITY LEADS TO FAST HOMEOSTATIC PLASTICITY AT THE AXON INITIAL SEGMENT IN CULTURED HIPPOCAMPAL NEURONS

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The ability of the brain to undergo plastic changes in response to various input challenges is one of its remarkable trademark. In addition to Hebbian plasticity, neurons employ a variety of powerful homeostatic mechanisms to stabilize firing rates in response to bidirectional perturbations of network activity that deviate spiking frequency from a defined set point. The adaptative mechanisms include changes in intrinsic neuronal excitability, synaptic functions, synapse number and structure as well as alterations in structural organization of the axon initial segment (AIS). In this work, we induced a chronic imbalance of M-type potassium channels, which fine tune neuronal excitability and are prominently expressed in AIS, a site recently recognized to be crucial for homeostatic plasticity. Cultured hippocampal neurons were chronically treated with the M-channel blocker XE-991 (10 µM). Our results indicate that adaptive changes in the intrinsic properties of hippocampal neurons operate at relatively rapid time scale (1-4 hours). These homeostatic changes include the resting membrane potential, threshold current, input resistance, evoked spike discharge, action potential width and location of voltage-gated Na^+ channels (Nav) and Kv7.3 potassium channels in the AIS with respect to the soma. A 4 µm distal shift of Nav and Kv7.3 channels is observed following 1-4 hour incubation with the M-channel blocker, with no change in the AIS length. The data suggest that in addition to slow synaptic adaptive alterations of the neural network, fast homeostatic changes in intrinsic properties can help to stabilize neuronal excitability.

THE EFFECT OF $G\alpha^{GTP}$ ON THE GATING OF THE GIRK2 CHANNEL

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The G protein gated inward rectifying potassium (GIRK; Kir3.X) channel is an important channel in various physiological and pathophysiological conditions. According to the classical scheme, the channel is activated by the Gβγ subunit of the heterotrimeric G protein family released after GPCR activation by extracellular stimuli. Whether an activated $G\alpha^{GTP}$ participates in the gating of GIRK is unclear, with evidence raised for and against an active role of $G\alpha^{GTP}$. We have set to investigate the role of $G\alpha^{GTP}$ in the muscarinic 2 receptor (m2R) – G protein – homotetrameric GIRK2 channel signaling cascade using the Xenopus oocyte expression system. We have found out that coexpression of GIRK2 with $G\alpha_{i3}\beta\gamma$ in the presence of extracellular acetylcholine gives rise to significantly larger whole cell currents than by coexpressed Gβγ alone. By measuring single channels using cell-attached patch clamp we have found out that $G\alpha^{GTP}$ increases the maximal open probability of the channel while maintaining similar single channel amplitudes. Similar measurements with the $G\alpha_{i1}$ subtype have failed to find such an increase, hinting that this effect is specific to the $G\alpha_{i3}$ subtype. To conclude, we have shown direct evidence that support an active role of $G\alpha^{GTP}$ in the gating of GIRK. This role is specific to the $G\alpha_{i3}$ subtype, which hints to how specificity is maintained in the GIRK signaling cascade. Whether $G\alpha^{GTP}$ directly affects the gating or it exerts its effects via modulation of Gβγ gating of the channel is yet to be investigated.

DUAL-SPECIFICITY TYROSINE-PHOSPHORYLATION REGULATED KINASE 2 IS A NOVAL REGULATOR OF OSTEOCLAST FUSION

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During the differentiation of the osteoclast, it transform from a mono-nucleated cell to multinucleated cell by fusion of its precursors. Although, cell fusion is a complicated process which likely involves many regulating proteins, only a few osteoclast fusion regulating proteins were identified. We have identified Dual-Specificity Tyrosine-Phosphorylation Regulated Kinase 2 (Dyrk2) as a novel regulator of the osteoclast fusion process. Dyrk2 belongs to a family of protein kinases whose members are involved in cell cycle and cytoskeleton organization. We found that Dyrk2 mRNA expression increases, 48 hours after induction of osteoclast differentiation in vitro, a time point were fusion commence. Dyrk2 knock-down, increases osteoclast nuclei numbers, but not osteoclast numbers and area, in differentiating RAW 264.7 cells and primary bone marrow derived monocytes (BMMs), While, overexpression of Dyrk2 decreases the number of osteoclast and osteoclast nuclei numbers suggesting it is involved in regulation of the fusion process per se. In order to further determine if Dyrk2 is involved in the fusion mechanism and not in earlier differentiation steps that lead to a fusion competent state, we investigated its role in giant macrophage fusion which are induced by different signaling pathways but share a similar fusion mechanism. Knockdown of Dyrk2 in giant macrophages increased the number of nuclei per macrophage, supporting a more general role of Dyrk2 in limiting the fusion process. While attempting to uncover the mechanism at which Dyrk2 regulates fusion we found that the rate of fusion of multinuclear cells is faster in Dyrk2 deficient osteoclasts. Moreover an ectopic expression of a GFP-tagged Dyrk2 showed co localization of Dyrk2 and actin in osteoclasts actin rings known to regulate osteoclast fusion. Taken together, our data reveal a novel role for Dyrk2 as a negative regulator of osteoclasts and giant macrophage fusion likely through the modulation of the osteoclast cytoskeleton.

A novel interaction site of the GIRK channel with Gβγ

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The G protein-sensitive inwardly rectifying potassium channel (GIRK) mediate postsynaptic inhibitory effects of neurotransmitters via G protein-coupled receptors (GPCRs) in the atrium and in the brain. The main goal of this research is to determine the molecular interaction sites between GIRK and G protein subunits, and their role in the regulation of gating properties of GIRK. In order to determine the specific interaction sites between GIRK and G protein subunits (Gα, Gβγ) we used molecular modeling methods. The best model predicted that amino acids L95 and D186 in $G\beta_1$ are involved in binding to GIRK1. In order to explore the impact of these amino acids on GIRK gating in terms of basal activity, agonist-evoked responses and regulation by Gβγ, we generated point mutations in GIRK1, GIRK2 and Gβ. Every combination of GPCR-Gαβγ-GIRK is subjected to a standard set of functional measurements in *Xenopus* oocytes. Whole-cell parameters include I_{basal}, I_{evoked} (in our experiments acetylcholine-induced), $I_{\beta\gamma}$, R_a (extent of activation by agonist), and $R_{\beta\gamma}$ (extent of activation by Gβγ). We used a combined approach of whole-cell current monitoring and fluorescent imaging to explore how these parameters, as well as surface expression of the channel, were affected by mutations of GIRK and Gβγ. We examined the effect of each mutated residue by measuring the currents of GIRK1Δ121* or GIRK2 channel co-expressed with each of the mutated Gβ construct in comparison to Gβ WT. We found that GIRK1Δ121* and GIRK2 co-expressed with Gβ L95Q and D186R produced smaller I_{basal} and I_{evoked} compared to Gβ WT, at similar expression levels of the mutated Gβ. These findings identify a new important Gβ-binding site in GIRK1, different from that identified by others in the crystal structure of GIRK2-Gβγ complex and that proposed by a previous molecular model in GIRK1.

DOWN-REGULATION OF COX-2 BY BETA 2-ADRENERGIC RECEPTOR-BASED PEPDUCINS

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Cyclooxygenase 2 (COX-2) is one of two main isoforms that highly abundant in various pathologies, such as inflammation and cancer. Overexpression of COX-2 is characteristic of many chronic inflammatory diseases therefore reducing its levels may be a new strategy to fight these diseases. Previous results in our lab showed that certain G protein-coupled receptors (GPCRs) down-regulate the levels of COX-2 in a pathway that increases its levels of ubiquitination and accelerates its degradation via the proteasome. Here we show that co-expression of COX-2 and the β_2 adrenergic receptor (β_2AR) in HEK293 causes a significant reduction in COX-2 levels. This effect is specific to COX-2 because co expression of the ubiquitously expressed COX-1 with β_2AR is not affected. To further investigate which parts of the receptor are involved in the effect on COX-2 we screened 32 lipidated peptides (termed 'pepducins') derived from the three intracellular loops of the receptor for their ability to affect COX-2 expression. To this end, HEK293 were transfected with YFP-COX-2 in the presence of each pepducin, and the effect was evaluated 24 h later by flow cytometry.

Results: Of the 32 pepducins, most did not affect COX-2 (N=19). Three pepducins in ICL-2 elevated COX-2 by 1.5 fold or more. Three pepducins in ICL-1, two in ICL-2 and five in ICL-3 lowered COX-2 expression by 20% or more. Using western blot, we confirmed that the pepducins derived from ICL-3 lower COX-2 expression in the nanomolar range.

Conclusions: our findings indicated that there are a specific parts in the intracellular loops of the receptor that are involved in the regulation of COX-2, which may be good candidates for development of next generation therapeutics.

SPATIOTEMPORAL LOCALIZATION OF RNA-INDUCED SILENCING IN AXONS: A CLOSER LOOK AT HOW DICER AND miRNA MOVE AND LOCALIZE IN NEURONS.

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In order to survive and maintain normal function neurons depend on a dynamic system of spatial specificity and fidelity of signaling pathways that can respond to both internal and external changes over space and time. Local protein synthesis in axons far from the cell body plays an important role in this essential spatiotemporal signaling process. The molecular basis for this post-transcriptional regulation however, is still poorly understood. As microRNAs are key players in gene regulation and play a vital role in motor neuron survival and synapse maintenance, we tested the possibility that proteins involved in the biogenesis and silencing function of microRNA, namely Dicer and Argonaute, transport to axons, and locally regulate synthesis. First, we identified the expression of microRNAs, Dicer and Arogonaute in cultured embryonic motor neuron axons in vitro, as well as at the adult neuromuscular junction in vivo. Then, using live cell imaging techniques we track the transport and localization of Dicer in the distal axon. Within the distal axon, we observed that a subset of Dicer localizes to, and transports with mitochondria. This correlation between Dicer and mitochondria is altered in the absence of neurotrophic factors. Next, we used dorsal root ganglia neurons to characterize the transport of a fluorescently tagged mature neuronal miRNA in axons. Our work sheds new light on the spatiotemporal regulation of RNA-induced silencing in axons, and may have implications in axonal health and neurodegenerative disease

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