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low unitary conductance, a sub-conductance level and a long open/closed dwell time[1]. From the effect of non-electrolyte polymers on this unitary conductance we estimate a narrow pore with a diameter of ca. 2.2 Å[2]. This value is similar to that reported for the central pore in the structure of the PLN pentamer[3]. Hence the PLN pentamer, which is in equilibrium with the monomer[4], is the most likely channel forming structure. Moreover, recent data on selectivity show that the channel follows the II Eisenmann sequence (Rb>Cs>K>Na>Li). This pattern reveals that the ion selectivity is regulated by the radius of the binding site and not by the ionic radius. Our results combined with computational data suggest the presence of a selectivity filter in the pentamer PLN.

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Ion Channels and Disease II

3851-Pos Board B579
TRPM4 Genetic Variants in Patients with Congenital Atrio-Ventricular Block

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Background: Transient Receptor Potential Subfamily Melastatin Member 4 (TRPM4) is a non-selective cardiac cation channel. Mutations in its gene cause cardiac conduction disease. The mechanisms underlying TRPM4-dependent conduction disorders are not fully understood.

Aim: To investigate genetic variants of TRPM4 from congenital atrio-ventricular block (cAVB) patients.

Methods and Results: Ninety-five cAVB patients were screened for candidate genes. We found 6 genetic variants in TRPM4. In whole-cell patch-clamp configuration, TRPM4 displays two current phases after patch rupture: transient (tr) and plateau (pl). Two cAVB mutants, p.A432T and p.A432T;G582S, showed lower protein expression ($29\pm4\%$ and $17\pm7\%$ n=4) and one, p.G582S, showed higher expression ($177\pm15\%$ n=4) compared to WT. Interestingly, only the plateau phase matches the protein expression profile (p.A432T 241 ± 23pA/pF n=6, p.G582S 994 ± 118pA/pF n=6 vs. WT 580 ± 66pA/pF n=20). Lower incubation temperature (28°C for 24h), which compromises protein quality control, rescued low expressing mutants, both at protein (p.A432T $72\pm8\%$ and p.A432T;G582S $65\pm7\%$ n=3) and functional (p.A432T 600 ± 114pA/pF n=6 vs. WT 639 ± 116pA/pF n=4) levels. We studied whether ubiquitylation plays a role in this loss-of-expression. Despite being ubiquitylated, no link between ubiquitylation and TRPM4 expression could be observed.

Conclusions: TRPM4 mutations in cAVB patients cause loss- or gain-of-function. Analogous to the supernormal conduction phenomenon, we speculate that increase or decrease of its activity in the conduction system may hyperpolarize or depolarize the resting membrane potential; thus, reducing the availability of sodium channels and subsequently leading to conduction slowing and clinical phenotype.

3852-Pos Board B580
The Contribution of Store Operated and Store Independent Calcium Entry to Migration in a Model of Neuroendocrine Cancer

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Orai1, the pore-forming sub-unit of a plasma membrane resident Ca^{2+}-channel and STIM-1, a Ca^{2+}-sensor localized to the endoplasmic reticulum are known to be involved in the canonical Store-operated Ca^{2+}-entry (SOCE) pathway. In recent years, this pathway has been implicated in cell migration of some types of cancers. However, it is not clear how near-maximal store depletion, a signal that is thought to be necessary for SOCE activation and generally considered pathophysiological, may effectively contribute to cell migration. Alternatively, a distinct, less well-studied store-independent channel type containing Orai1 and Orai3 has been shown to mediate Ca^{2+}-entry following activation by arachidonic acid. The relative contribution of store-operated and store-independent Ca^{2+}-entry pathways in cancer cell migration has not been adequately interrogated. The present study investigated this question in a model gastro-enteropancreatic neuroendocrine tumor (GEPNET) cell line by utilizing pharmacological and gene-silencing methods in combination with live cell fluorescence imaging and standard migration assays. It was revealed that these Ca^{2+}-entry pathways could be independently activated and regulated in these cells. While, classical SOCE required participation of Orai-1 and STIM1, the arachidonate mediated Ca^{2+}-entry pathway required in addition Orai3. Having identified both SOCE and arachidonate mediated Ca^{2+}-entry in GEPNET cells, their relative roles in cell migration were assessed using modified Boyden chamber assays. By selectively modulating channel conductances using pharmacological agents or sh-RNA to knockdown protein expressions, it was revealed that the arachidonate mediated Ca^{2+}-entry is the dominant pathway that induced migration in this type of cancer. Taken together, these data address the complex interaction between distinct Ca^{2+}-entry pathways that may induce cell migration yet utilize a common pool of molecular machinery.

3853-Pos Board B581
Inhibition of Mitochondrial Na^+/Ca^{2+} Exchanger Prevents Stress-Induced Arrhythmia in the Isolated Guinea Pig Failing Heart

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The rate of sudden cardiac death (SCD) in patient with heart failure (HF) is about 9 times higher than that in the general population; however, the mechanisms of HF-associated SCD are poorly understood. We previously demonstrated that stress (isoproterenol chanllenge) induced a burst of reactive oxygen species (ROS) in association with delayed afterdepolarizations (DAD) in isolated failing cardiomyocytes, and inhibition of mitochondrial Na^+/Ca^{2+} exchanger (mNCE) prevented ROS production and DAD. Here, we tested the hypothesis that isoproterenol challenge induces arrhythmias in the failing heart and that the mNCE inhibitor, CGP-37157 (CGP), has anti-arrhythmic effect. The guinea pig HF model, ACi, was produced with aorta constriction combined with daily isoproterenol injection for 4 weeks. Perfused shami (sham with daily isoproterenol injection), ACi, and ACi+CGP hearts were subjected to the following electrocardiographic recording protocol: 10-minute baseline, 15-minute with 10 μM isoproterenol, and 20-minute after washout of isoproterenol (post-iso). In ACi+CGP group, 1 μM CGP was added to the buffer at the beginning of baseline recording. Arrhythmias were evaluated with heart rate variability (HRV) analysis. Isolated ACi hearts did not show significant arrhythmia at baseline. Surprisingly, isoproterenol challenge did not induce overt arrhythmias during the period of maximum LVPD in either group. However, HRV was dramatically increased during post-iso state in ACi heart. SDNN (standard deviation of the normal beat RR interval), RMSSD (root mean square of successive differences in RR), and non-linear parameters, SD1 and SD2 during post-iso was 42.08 ± 8.51, 65.60 ± 15.60, 46.418 ± 11.048, and 36.542 ± 6.167 in ACi, respectively, whereas they were 3.85 ± 1.39, 0.88 ± 0.29, 0.626 ± 0.207, 5.394 ± 1.968 in shami, respectively, $p\leq0.05$. CGP-37157 treatment prevented HRV increase in ACi heart during post-iso. Our results suggest that mNCE is an important therapeutic target for HF-associated ventricular arrhythmia and SCD.

3854-Pos Board B582
Identification and Functional Role of Calpain Cleavage Site in Na^+-Ca^{2+} Exchanger 1 (NCX1)

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Altered Ca^{2+} homeostasis is a key determinant of cardiac remodeling and contractility during chronic heart disease. Aberrant activation of calpain, a ubiquitous Ca^{2+}-dependent protease can contribute to loss of Ca^{2+} control in cardiomyocytes. Calpain cleaves NCX1, but the underlying significance of the direct cleavage of NCX1 by calpain remains to be determined.

By bioinformatics and mutational analysis we identified M369 as a putative calpain cleavage site residing within the α-catenin-like domain (CLD) in NCX1. Importantly, the cleavage of NCX1 at M369 corresponded to a proteolytic fragment of 75 kDa in left ventricular biopsies from aorta stenosis (AS) patients and in the failing left ventricle of rats following aortic banding (AB). Moreover, calpain binding in the CLD and Ca^{2+} -binding domain (CBD1) of

NCX1 protein were identified by overlay assays. In order to investigate the specific function of M369 cleavage and avoid auxiliary and indirect functions of calpain, we utilized the protease Tobacco Etch Virus (TEV) to investigate site-specific cleavage. The TEV protease recognition site was inserted at the location of M369 in NCX1. By employing the patch clamp technique on transfected HEK cells, we observed a significant reduction of NCX1 current following TEV cleavage. In conclusion, we have identified and investigated the functional role of the calpain cleavage site at M369 in the CLD of NCX1. Our findings show that cleavage of the NCX1 at M369 reduces the total current. This reduction of NCX1 activity could potentially function to compensate for altered Ca^{2+} homeostasis associated with NCX upregulation during heart failure.

3855-Pos Board B583

Na/K ATPase Function Declines Before Changes to Calcium Handling in a Guinea-Pig Model of Progressive Heart Failure

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Previous work shows that intracellular [Na^+] is increased in cardiac myocytes isolated from failing hearts but there is less unanimity on the causes. Some studies suggest the increase in intracellular [Na^+] is a result of greater diastolic Na^+ influx with unaltered Na^+/K^+-ATPase function, while others report a decrease in the amount or function of Na^+/K^+-ATPase. These different findings may be model, species or timing-dependent. This study evaluated Na^+/K^+-ATPase function by measuring dihydro-ouabain and strophanthidin-sensitive current during voltage clamp at three different time points (30, 60 and 150 days) during the progression of cardiac dysfunction following aortic constriction (AC) on guinea-pigs. In this model, compensated hypertrophy develops 60 days after AC. After 150 days heart failure is well defined. Na^+/K^+-ATPase current decreases after the initial development of hypertrophy (1.10 ± 0.16 $A.F^{-1}$, N=15 in sham vs 0.54 ± 0.10 $A.F^{-1}$, N=13 in 60 day AC, $P<0.05$; 0.86 ± 0.13 $A.F^{-1}$, N=25 in sham vs 0.51 ± 0.06 $A.F^{-1}$, N=15 in 150 day AC, $P<0.05$). There is no change in ratio of dihydro-ouabain and strophanthidin-sensitive currents suggesting similar decreases in function of the $\alpha1$ and $\alpha2$ isoforms of the Na^+/K^+-ATPase.

Preliminary confocal immunocytochemistry of the $\alpha1$ and $\alpha2$ subunits correspond with the observed current changes. For $\alpha1$, staining density was 26.9 ± 3.0 a.u., N=6 in sham vs 2.9 ± 0.5 a.u., N=7, in 60 day AC, $P<0.001$; and 20.0 ± 3.0 a.u., N=5 in sham vs 9.3 ± 0.8 a.u., N=12, in 150 day AC, $P<0.001$. For $\alpha2$, staining density was 34.9 ± 5.6 a.u., N=11 in sham vs 11.7 ± 1.0 a.u., N=11 in 60 day AC; and 17.8 ± 1.6 a.u., N=10 in sham vs 8.3 ± 0.8 a.u., N=12 in 150 day AC.

The Na^+/K^+-ATPase function declines early in the progression of disease and may trigger changes in Ca handling and contractile function.

3856-Pos Board B584

Impaired Bone Formation in *TRIC-B*-Knockout Mice

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Osteogenesis imperfect (OI) is a heritable bone dysplasia characterized by bone fragility, but has divergent phenotypic manifestations, suggesting heterogeneous OI-responsible genes in the human genome. Most of OI cases result from defected type I collagen; structural mutations and altered post-translational modifications lead to its insufficient content, folding and trafficking errors, and also compromised matrix incorporation. Recently, a homozygous deletion mutation in the *TRIC-B* (also referred to as *TMEM38B*) locus has been identified in Saudi Arabic and Bedouin Israeli OI pedigrees. The OI mutant gene encodes a truncated TRIC-B protein lacking its C-terminal half, and is likely associated with severe impairment of TRIC-B channel activity in various cell types. However, pathological mechanism is still unknown in the OI pedigrees.

The TRIC (trimeric intracellular cation) channel subtypes, namely TRIC-A and TRIC-B, form homotrimeric complexes to function as intracellular monovalent cation-specific channels. TRIC-A channels are predominantly expressed in muscle and brain, while TRIC-B channels are ubiquitously detected throughout excitable and non-excitable cell types. Based on our observations in knockout mice, TRIC channels seem to mediate, in part, counterion movements to support efficient Ca^{2+} release from the sarco/endoplasmic reticulum. *Tric-b*-knockout mice develop respiratory failure at birth, and the mutant alveolar epithelial cells exhibit compromised IP_3R-mediated Ca^{2+} release and insufficient handling of surfactant lipids. In skeleton preparations from *Tric-b*-knockout neonates, semi-translucent parts were frequently detected in major bones, indicating impaired bone formation. Indeed, insufficient bone mineralization was confirmed by computed tomography imaging and histochemical analysis. RT-PCR experiments detected decreased expression of osteocalcin and RanklmRNAs as osteoblast makers in *Tric-b*-knockout femoral bones, suggesting the possibility that *Tric-b* deficiency may affect osteoblast functions. Therefore, *Tric-b*-knockout neonates provide a useful model for OI bearing the mutant *TRIC-B* gene.

3857-Pos Board B585

H^+ Inhibits TRIC-B Channels Derived from Mouse TRIC-A Knockout Tissue

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Trimeric intracellular cation channels (TRIC-A/TRIC-B) are found in the endo/sarcoplasmic reticulum (ER/SR) and nuclear membranes. TRIC-B knockout mice die in respiratory failure following birth but there are additional diverse, severe pathological symptoms (Yamazaki D. *et al.* (2009)). The physiological roles of TRIC-B are not fully understood and few regulators of channel activity are known. We here investigate if TRIC-B function is sensitive to pH by incorporating light SR membrane vesicles from TRIC-A knockout skeletal muscle into artificial membranes under voltage-clamp conditions in symmetrical 210 mM K-PIPES, pH 7.2. Open probability was determined at ±30 mV when ≤2 channels were gating in the bilayer. With ≥3 channels present, noise analysis was performed because the complex sub-conductance state gating prevented accurate measurements. As previously observed (Venturi E. *et al.* (2009)), TRIC-B was more open at positive potentials but exhibited variable gating behaviour. For example, at pH 7.2, at +30 mV, noise analysis yielded a mean current of 1.25 ± 0.25 pA (SEM; n=23) but ranged from 0.025 to 3.36 pA. We observed that TRIC-B became more open as cytosolic pH was increased. At +30 mV, as cytosolic pH was raised from 7.2 to 9.2, we observed a 3.34 fold increase in mean current (SEM; n=7; $p<0.05$) whereas mean current decreased 3.33 fold when cytosolic pH was lowered from pH 7.2 to 6.2, A similar trend was observed when luminal pH was altered. Under acidic conditions where both cytosolic and luminal pH was lowered, TRIC-B channel opening was markedly inhibited and the effects of cytosolic and luminal [H^+] appeared to be additive.

Further experimentation is required to understand the mechanisms underlying pH regulation of TRIC-B function and the physiological/pathophysiological significance of TRIC-B sensitivity to pH.

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3858-Pos Board B586

Ion Channels Controlling Resting Membrane Potential of Nociceptive DRG Neuron Somata

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Purpose: Dorsal root ganglia (DRG) contain somata of peripheral nerve fibres. Somatic excitation is one of the main sources of ectopic firing, which underlies many types of chronic pain. Control of resting membrane potential (E_m) of a neuron is a key to its excitability, however, surprisingly little is known about the main ion channels maintaining E_m of nociceptive DRG neuron somata.

Methods: We used electrophysiological recordings from cultured nociceptive neurons and from acute DRG slices in rats. We characterized the effects of modulation of Kv, KNa, M-type and ATP-sensitive (KATP) K^+ channels; hyperpolarization-activated cyclic nucleotide-gated channels (HCN), T-type Ca^{2+} channels and TTX-sensitive and TTX-resistant voltage-gated Na^+ channels on the somatic resting Em. We then evaluated in vivo the effect of acute topical application of key modulators to DRG in a pain model.

Results: Although background activity of most of the ion channels has been detected, their efficacy and repartition differed. By the ability to induce