current blockade signature that gives a direct evidence of a binding event. Nanopore observation of molecular species may prove to be much more sensitive than mobility shift analysis, but the sensitivity and sequential nature of the channel current signal limit the range of applicability of the method. In practice, nanopore detection may offer an augmentation to mobility shift analysis, by taking a 'band' of concentrated analyte of interest in isoelectric focusing experiment, and placing it in a nanopore detector for more detailed analysis. In this way subtle isoforms may be discerned that only appear as a single band in electophoretic separation. In addition to the possibility of monitoring the free probe concentration, it may also prove possible with sophisticated nanopore cheminformatics methods to determine the concentration of complexes not directly interacting with nanopore.

Voltage-gated Na Channels

3081-Pos State-dependent Interaction of Paired Substituted Cysteine Residues in the Voltage Sensor of NaChBac Enhances Activation and Inactivation

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Board B384

Activation of voltage-gated ion channels is initiated by depolarization-dependent outward movement of the S4 voltage sensor driven by its conserved gating charges. The 'sliding helix' model of voltage sensing, as implemented at the structural level with the Rosetta algorithm (Yarov-Yarovoy, 2006), proposes that S4 arginine gating charges make sequential electrostatic interactions with negatively-charged residues of S2 and/or S3 segments, which catalyze their transmembrane movement during activation. To provide evidence for such voltage-dependent interactions, we replaced the 3rd arginine (R3) of S4 and the first negatively charged residue (D60) of S2 in the homotetrameric bacterial Na^+ channel NaChBac with cysteines. Whole-cell Na^+ currents were recorded from tsA-201 cells transfected with NaChBac WT, single mutants (D60C or R3C) and double-mutant channels (R3C:D60). Repeated depolarizations caused pulse-wise reduction of Na^+ current that did not recover at the holding potential (−120 mV) for R3C:D60C but not for WT or single residue mutants, suggesting disulfide bond formation between the S2 and S4 cysteines. We hypothesize that Na^+ current reduction results from enhanced inactivation subsequent to locking of one or more voltage sensors in the activated conformation. Consistent with such activation-locking, deactivation of the R3C:D60C was slowed upon repolarization and recovery from inactivation was shifted to much more negative membrane potentials. The voltage-dependence of the activation-locking interaction ($V_{1/2}$ = −10 ± 8mV) is more positive than channel activation ($V_{1/2}$ = −30 ± 6mV), suggesting that substantial voltage sensor movement is required for activation-locking of these residues. These data indicate that S4 moves relative to S2 upon channel activation, placing R3C sufficiently close to D60C (~2Å) to allow rapid disulfide bond formation, and thus are consistent with predictions of the Rosetta sliding helix model of voltage sensing.

3082-Pos Selectivity of the Bacterial Sodium Channel, NaChBac, Studied by Experiment and Simulation

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Board B385

The prokaryotic sodium channel, NaChBac, from *Bacillus halodurans*, is a homotetrameric Na_V channel, with each monomer having 6 putative transmembrane helices. The selectivity filter in the ion-conducting pore of NaChBac is thought to be lined by a ring of 4 glutamate residues (EEEE). We expressed NaChBac channels in HEK293 cells and studied their selectivity to monovalent organic and alkali cations. Reversal potential shifts were determined from whole-cell currents, following substitution of a test cation for extracellular sodium, and relative permeabilities (P_X/P_{Na}) were calculated using the Goldman-Hodgkin-Katz equation. Among the alkali cations, only Na and Li (P_{Li}/P_{Na} = 0.73 ± 0.07, n=3) were measurably permeant. Of 13 organic cations, at pH 7.3, only hydrazinium (HZ; P_{HZ}/P_{Na} = 0.37 ± 0.02, n=3) was permeant, and notably, in contrast to eukaryotic Na_V channels, ammonium was not. In preliminary experiments, two manipulations made NaChBac less selective. At pH=6 for WT NaChBac, both K and hydroxylammonium (HA) were permeant; For NaChBac E191D mutant at pH=7.3, both K and ammonium were permeant. Lacking a high-resolution structure or verified model, we have built a homology model of the NachBac pore domain, based on a reported model of an L-type Ca channel, and several K-channel crystal structures. After molecular dynamics simulations to check stability in a lipid bilayer bathed in explicit solvent, we performed free energy calculations to test the selectivity of the initial model. The resulting selectivity sequence (Na>Ca >K) encourages us to develop the model further to explore the complexities of NaChBac selectivity shown by our data. Overall, our results suggest that the simpler, tetrameric NaChBac channel is even more selective for Na than are eukaryotic Na_V channels, and that NaChBac achieves this high selectivity by a unique molecular strategy.

3083-Pos Structural Model of the Voltage-Dependent Gating of NaChBac

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Board B386

Voltage-gated sodium channels control the generation and propagation of action potentials in excitable cells. Despite recent progress in determining the high-resolution structures of the voltage-gated potassium channels, the high-resolution structure of the voltage-gated sodium channels remains unknown. We used the Rosetta-Membrane method (Yarov-Yarovoy et al. (2006) Proteins *62*, 1010–1025) and recently developed models of the Kv1.2 channel in the open and closed states (Yarov-Yarovoy et al. (2006) PNAS *103*, 7292–7297, Pathak et al. (2007) Neuron *56*, in press) to construct

homology/de novo models of the bacterial voltage-gated sodium channel, NaChBac. The resulting structural models suggest a molecular mechanism of the voltage-dependent activation of NaChBac in which the S4 segments are in transmembrane orientation in both resting and activated states. During activation, the S4 segment rotates clockwise (as viewed from the extracellular side of the membrane) and translates outward, as proposed in the 'sliding helix' model of gating, while the S1, S2, and S3 segments move around the S4 segment in a counterclockwise direction. This outward motion of the S4 segment leads to movement of the S4–S5 linker and corresponding movements of the S5 and S6 segments that open the intracellular gate of the ion conduction pathway through the pore. Structural similarities and differences between the mechanisms of activation of the voltage sensors of voltage-gated sodium and potassium channels will be discussed.

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3084-Pos Assay Development And State-dependent Screening For Modulators Of Human Sodium Channels Using Automated Electrophysiology

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Board B387

Electrophysiology based screening methodology has significantly increased the tractability of ion channels as a class of proteins that can be targeted for the discovery and development of new drug therapies. We have combined IonWorks® Quattro™ Population Patch Clamp recording, a focused library of 20,935 compounds and a multi-step assay protocol to identify blockers of a human voltage-gated sodium channel stably expressed in HEK293 cells. The library was compiled from the BioFocus DPI SoftFocus® ion channel libraries and a further trained selection of compounds from the BioFocus DPI general screening collection, chosen using property filtering and subsequent Kernel Discriminant Analysis. Each screening plate comprised 320 test compounds with 24 wells reserved for evaluation of a standard compound IC_{50} and assessment of control amplitude stability. Quality control measures were enforced and wells not meeting criteria for seal resistance (>30M), current run up (<20%) and current amplitude (<−0.2nA) were rejected from the analysis and compounds re-screened. For wells passing quality control analysis mean seal resistance was 52.6 ± 13.2M , mean current amplitude for a 25ms voltage step from −90mV to 0mV was −1.4 ± 0.3nA and the standard compound IC_{50} was 5.3 ± 3.0μM (all mean ± standard deviation). The screening assay protocol involved 9 separate voltage steps and was validated to reveal tonic, use-dependent and inactivated state block. The primary screen identified 640 compounds worthy of progression to further validation studies. We will present data obtained from the screen and highlight the advantages and challenges associated with identifying sodium channel modulators using complex recording protocols.

3085-Pos Modification of Gating Differentiates Rested State Block From Use-Dependent Block for Lidocaine Interactions with Na Channels

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Board B388

The most important amino acid contributing to high affinity block of voltage-gated sodium (Na) channels by local anesthetics has been identified as a Phe in the DIV-S6. Substitutions here by both natural and unnatural amino acids produce channels in which use-dependent block is markedly or completely attenuated while rested state block is unaffected. We have previously reported that high affinity local anaesthetic block was associated with dramatic alterations in gating charge movement (Q_{max} is reduced by nearly 40% with a shift in the Q-V half-point by ~−20mV), which occurred because DIII-S4 was stabilized in an outward position and DIV-S4 was partially inhibited and its movement altered. We tested charge in two $Na_V1.5$ channels with Phe mutations, F1759A (neutral) and F1759K (positive charge). ON-charge for F1759K and F1759A channels were like WT. When mutant channels were exposed to 1 mM lidocaine, they exhibited rested state block of I_{Na} of ~ 50%, a value similar to WT, but they lacked use-dependent block in contrast to complete block of WT within 10 depolarizations. We tested whether rested state block by lidocaine was obligatorily associated with alterations in gating charge. Unlike WT, 10 or 20mM lidocaine exposure for cells expressing F1759K and F1759A channels produced no change in Q_{max} and a <3mV shift in Q-V half point, although these concentrations produced rested-state channel block similar to that measured in WT. Thus, rested state block is not associated with modification of channel gating in contrast to use-dependent block, which is dependent upon interactions of drug with the Phe in DIV S6. This residue links drug binding to channel gating and underlies stabilization of the non-conducting state associated with the high affinity action of lidocaine and lidocaine-like drugs.

3086-Pos Unique Gating Modification by ProTxII in Voltage-Gated Na Channels

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Board B389

ProTxII, a peptide toxin isolated from the venom of the tarantula *Thrixopelma puriens,* modifies voltage-dependent activation in both voltage-gated sodium (Na) channels and T-type calcium (Ca) channels. There exists an extensive literature on the activation mechanisms of voltage-gated Na channels, however relatively little is known about the parallel process in T-channels. We therefore compared the effects of ProTxII on the voltage-gated Na channel ($Na_v1.5$) and the T-type Ca channel $Ca_V3.1$. For both exposure to 5μM toxin positively shifted activation (~15mV, n=4–9 cells). In $Na_v1.5$ this shift was accompanied by a 25.3%±4.95% (n=5–9 cells) reduction in voltage dependence of activation and a speeding of tail current decay at voltages >-90mV. Neither the change in voltage dependence nor the effect on tail currents was observed in $Ca_V3.1$. Because ProTxII carries a net positive charge, we tested the hypothesis that both channel types share a common interaction with the toxin by which the binding of toxin neutralizes surface charge. To test this we neutralized surface charge by adding excess divalent cations to the bath. Neutralization of surface charge precluded the shift in the voltage-dependent activation of $Ca_V3.1$ by ProTxII. In $Na_V1.5$ this treatment precluded the activation shift, but not the decrease in voltage dependence. Surface charge neutralization also precluded the ability of ProTxII to speed the slow component of tail current decay while the fast component was unaffected. These results are consistent with the hypothesis that ProTxII shares a common mechanism between channel types, i.e. binding shifts activation positively by affecting the voltage seen by the gating machinery. In addition, although the toxin is interacting with each type of channel similarly, there are unique binding sites or features in the activation gating mechanism of Na channels that are revealed by ProTxII.

3087-Pos SCN5A Polymorphism Decreases Arrhythmogenic Events in a Family Carrying a LQT3 Mutation

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Board B390

Defects in the cardiac sodium channel gene, *SCN5A*, can cause Long QT3 syndrome (LQT3). A family which exhibited a mild LQT3 phenotype was genotyped. The *SCN5A*-P2006A mutation was found. Additionally, the patients were found to be homozygous for the sodium channel polymorphism H558R. Therefore, based on the unusual LQT3 phenotype that the patients exhibited, we hypothesized that the H558R-*SCN5A* polymorphism could modify gating kinetics in mutated sodium channels. Wild-type (WT) and mutant channels expressed in HEK293 cells were characterized. P2006A exhibited significantly increased persistent sodium currents. P2006A also displayed significant depolarizing shifts in voltage dependence of inactivation and faster recovery from inactivation. Interestingly, when the mutation was expressed in the presence of the H558R polymorphism, the sodium currents behaved like WT. These findings can explain the prolongation of QTc interval found in patients who carry this LQT3 mutation. Interestingly, the H558R polymorphism can modify the gating kinetics in this mutation which may explain the mild LQT3 phenotype seen in the patients that carry both a mutation and a polymorphism. These observations may provide a plausible mechanism for decreased arrhythmogenic events in LQT3 patients that also carry a polymorphism.

	WT (n=12)	P2006A (n=12)	P2006A + H558R (n=20)
Persistent Current	0.15%	1.20% *	0.30%
Recovery from Inactivation (ms)	8.9 ± 0.4	4.4 ± 0.3 *	8.8 ± 0.4
Steady-State Inactivation (mV)	−91.2 ± 0.8	−80.6 ± 1.8 *	−88.1 ± 1.0

3088-Pos The partial Opioid-receptor Agonist Buprenorphine potently blocks Voltage Gated Na^+ Channels via the Local Anaesthetic Binding Site

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Board B391

The partial opioid-receptor (OR) agonist buprenorphine is used clinically for the treatment of severe acute and chronic pain. Buprenorphine exerts both a strong antinociceptive and a long lasting *antihyperalgesic* effect, which is uncommon for opioids but known for local anesthetics. The μ-OR is essential for buprenorphine-induced antinociception, however, the molecular mechanisms underlying the antihyperalgesic effect are unknown. We demonstrate here that buprenorphine displays common features of local anaesthetics. In the *ex-vivo* skin-nerve preparation, buprenorphine (100– 400 μM) reversibly blocked electrically evoked action potentials of unmylelinated C-fibers in the presence of naloxone (10 μM). Whole cell patch clamp recordings of voltage-gated Na^+ currents in cultured mouse dorsal root ganglion (DRG) neurons revealed that buprenorphine blocked Na^+ currents in DRG neurons in a concentration-dependent (IC_{50} = 22 ± 1 μM) and reversible manner and was more potent than the local anaesthetic lidocaine (IC_{50} = 231 ± 38 μM). In ND7/23 cells, IC_{50}-values for tonic Na^+ channel block block were 29 ± 3 μM at −140 mV and 5.3 ± 0.5 μM at −80 mV, suggesting state-dependent block. Accordingly, buprenorphine evoked a pronounced use-dependent block at 10 Hz (3 μM: 44 ± 5% and 30 μM: 81 ± 4%) and a strong block of the inactivation-deficient mutant rNav1.4-WCW (IC_{50} = 1.7 ± 0.1 μM). Use-dependent block by buprenorphine was strongly attenuated in the local anaesthetic-insensitive mutants $Na_v1.4$–434K (9.3 ± 3.7 %), $Na_v1.4$– 1280K (21 ± 3%) and $Na_v1.4$–1579K (15 ± 2%) compared to wild-type $Na_v1.4$ (75 ± 3%, n= 5). We conclude that buprenorphine acts as a potent local anaesthetic by blocking voltage gated

Na^+ channels via the local anaesthetic binding site. This action might be a relevant OR-independent mechanism contributing to the antihyperalgesic effect of buprenorphine.

3089-Pos A Novel Evolutionary Determinant of Reduced TTX-Sensitivity in Molluscan Sodium Channels

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Board B392

Bivalve molluscs exposed to red tides can cause paralytic shellfish poisoning (PSP) in humans and can show dramatic inter-and intra-species variation in their capacity for accumulating PSP toxins (PSTs). Previous work in softshell clams (*Mya arenaria*) identified a natural mutation in a single amino acid residue in domain II of the sodium channel that, when substituted in $Na_v1.2$, reduced TTX and STX sensitivity 3000 fold and 1500 fold, respectively. While this mutation accounted for inter-population variation in PSP resistance within a species, it did not explain why even the nerves of sensitive *M. arenaria* are much more resistant to STX and TTX than toxin-sensitive Na^+ channels from vertebrate central neurons and those from the squid (*Loligo opalescens*), another mollusc. In the Na^+ channel pore loops, where STX and TTX bind, only two amino acids differ between *M. arenaria* and these toxin-sensitive channels. We therefore tested the hypothesis that one of these amino acid differences is responsible for the μM affinity of STX and TTX of the Na^+ channels of *M. arenaria* relative to the nM affinity to TTX of toxin-sensitive Na^+ channels from squid and vertebrates. We engineered these two amino acid substitutions into $Na_v1.2$ using site-directed mutagenesis and conducted whole-cell voltage clamp recordings from transfected tsA-201 cells. We found that F1421Y had an IC_{50} for TTX of 2.8 μM, while WT channels had an IC_{50} of 12.8 nM. The second mutant channel, T1711C, had an IC_{50} for TTX similar to WT. These results further elucidate the molecular basis for the evolution of toxin resistance of *M. arenaria* and other molluscs, and provide insight into a novel site of TTX interaction in voltage-dependent Na^+ channels.

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3090-Pos Characterization of Na Currents From Intact Mouse Skeletal Muscle

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Board B393

Mutations in *SCN4A*, the gene encoding the skeletal muscle Na^+ channel (NaV1.4), cause several disorders of skeletal muscle excitability. The functional consequences of these NaV1.4 mutations have been extensively characterized in heterologous expression systems (HEK cells and Xenopus oocytes). Although these studies have significantly advanced our understanding of the pathophysiologic basis of these disorders, animal models are now available in genetically engineered mice and thereby provide an opportunity to examine channel function in mature, intact skeletal muscle. We optimized a two-electrode voltage clamp protocol to improve the fidelity of recording Na^+ currents from intact acutely dissociated mouse fibers by using: (1) short FDB muscle fibers to minimize space-clamp errors (2) osmotically detubulated muscle fibers to reduce capacitance and eliminate the series resistance error of the T-system (3) Cl^- - free bath to reduce leakage currents, (4) low external Na^+ to decrease peak inward Na^+ current. Computer simulation, employing measured capacitance and ionic current densities, was used to confirm sufficient voltage control and distortion-free Na^+ currents. The gating properties of endogenous Na^+ currents were measured and found to differ substantially from Na^+ currents in HEK cells expressing NaV1.4 plus the accessory β1 subunit. The voltage-dependence of activation had a hyperpolarized shift of ~25 mV in muscle compared to typical values from HEK cells, and steady-state fast inactivation exhibited a similar leftward shift of ~16 mV. A possible contribution from NaV1.5 channels in our mouse muscle preparation was excluded by RT-PCR and TTX-sensitivity. These data suggest that studies of NaV1.4 gating properties *in situ* will render new insights to the pathophysiology of disorders of skeletal muscle excitability.

3091-Pos Effect of Local Anesthetics on Sodium Channel Voltage Sensor Dynamics

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Board B394

Voltage gated sodium channels initiate action potentials and are, therefore, targeted by many drugs such as local anesthetics which modulate excitability. Local anesthetics (LA) block ionic currents in a state-dependent manner. Gating current measurements have shown that the binding of LA to the sodium channel also affects the movement of gating charge. In this study, we use site-specific fluorescence recordings to examine the effect of LA on the dynamics the sodium channel voltage-sensors. Specific residues in each of four voltage sensors were fluorescently labeled and the conformational changes in those voltage sensors were monitored in presence and absence of LA. These measurements were obtained under both use-dependent and resting state block condition. Our data shows that LA binding to the sodium channel pore primarily affected the movement of the voltage-sensor of domain III in both conditions. The binding of LA shifted the fluorescence-voltage relationship of labeled residues in S4-domain III by approximately 50 mV in the hyperpolarized direction. These findings suggest that the binding of

LA to the inner pore of sodium channel mainly stabilizes the voltage-sensor of domain III in an activated state.

3092-Pos Species-dependent Expression Patterns of Voltage-gated Na^+ Channels in the Mammalian Heart

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Board B395

The cardiac Na^+ current is mainly generated by TTX-resistant $Na_v1.5$ channels. Neuronal and skeletal muscle isoforms are also expressed in the myocardium, but their functional significance for the cardiac action potential is still a matter of debate. In the present study we addressed the question whether myocardial expression of TTX-sensitive Na^+ channels occurs in a species-dependent manner. The composition of the Na^+ channel transcript pool including $Na_v1.1$ to $Na_v1.5$ was analyzed by RT-PCR in mouse, rat, pig and human hearts. Our PCR data indicate that the expression patterns in the heart are strictly species dependent. The relative transcript level of TTX-sensitive channels decreased with increasing heart size (30% for mouse, 8% for rat, and 4% for both pig and human). Considering transcript levels of individual isoforms, human and pig hearts were nearly indistinguishable whereas significant differences existed between human and mouse as well as between mouse and rat. E.g., $Na_v1.1$ levels were highest in rat (4.6%), but much lower in pig (0.6%) and human (0.5%). High $Na_v1.4$ levels were only found in the mouse heart (6.8%), whereas this isoform was detected only at negligible levels in the rat heart (0.1%). We also noticed that both the alternative splicing of $Na_v1.1$ and $Na_v1.5$ and the age-dependent Na^+ channel expression occurred in a species-dependent manner in the mammalian heart. Our data suggest (1) that some of the TTX-sensitive Na^+ channels may play an important role in the heart of small rodents and (2) that species-dependent expression differences of cardiac Na^+ channels should be considered when discussing results from such animal models with respect to the situation in the human heart.

3093-Pos A Critical Role of the Conserved Domain III Lysine Residue in the Energetics of Voltage-Gated Na Channel Selectivity

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Board B396

The putative selectivity filter of the voltage-gated Na channel uses a different mechanism to achieve selectivity than the K channel. Molecular modeling of the protein sequence and point mutations have shown that Na channel selectivity results from a narrow ring at the bottom of an outer vestibule composed of the side chains of aspartate, glutamate, lysine, and alanine of domains I–IV (DEKA motif). Using molecular dynamics simulation with our previously developed molecular model of this selectivity region, we found that the critical lysine (III) shares a hydrogen bond with the glutamate (II) carboxylate, locking the monovalent binding site. In the modeled AEKA configuration only strong alkali cations can compete with lysine for interaction with this binding site. When Na+ (r = 0.95 Å) is added to the simulation, it binds electrostatically to the glutamate stronger than lysine, displacing the lysine amino group. K+ (r = 1.33 Å) interaction energy with glutamate is about equal to the electrostatic repulsion of lysine, reducing its binding and conductance. Lysine easily expels Rb+(r = 1.48 Å), which is unable to bind to glutamate. We suggest that lysine's electrostatic repulsion of cations critically distinguishes between Na+ and K+, resulting in the Na channel's selectivity. The repulsive energy of lysine is very precise; reduction of repulsion by substitution of arginine for lysine abolished selectivity.

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3094-Pos $hNa_V1.4$ Gating Model with Temperature Dependence Obtained from a Single Set of Experimental Data

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Board B397

Mutations in the voltage-gated sodium channel $hNa_v1.4$ have been correlated to various muscle diseases, such as Paramyotonia Congenita (PC), a disease clinically characterized by attacks of muscle stiffness mainly triggered by exposure to cold. Mutations causing PC share a common gating defect: slowed inactivation from the open state that mainly accounts for the disease symptoms. Nevertheless, the origin of the temperature dependence and the implication of other possible gating alterations for PC are still contradictorily discussed. Here we perform a very detailed investigation of WT and R1448H, a typical PC mutation, gating in a broad temperature range (5 – 30°C) by performing whole-cell patch-clamp experiments on HEK-293 cells, stably expressing $hNa_v1.4$. To better understand disease patho-physiology and to allow incorporation in more general systemic muscle models, we tested different gating schemes considering all gating transitions altered by PC mutations and fitted these models to our measurements. Rate constants were obtained by a simultaneous fit to several different data sets measured on the same cell: 6 current traces of an activation protocol, a steady-state inactivation curve, recovery curves at 3 different potentials and entry into fast inactivation curves at 4 different potentials. To gain inside in the temperature dependency of WT and R1448H sodium channel gating, we calculated the rate constants for measurements at several temperatures. In summary, we present a model that describes the gating of WT and R1448H sodium channels and confirms previous findings, that the relationship between inactivation and

deactivation may be decisive for muscle excitability or weakness. In addition, we focus on the role of the altered closed-state inactivation kinetics that has not been considered until now.

3095-Pos Exploring Nav1.7 Use-Dependent Phenomena Through the Application of IonWorks and Computational Chemistry Methods

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Board B398

Voltage-gated sodium (NaV) channels comprise a family of nine structurally related proteins that have a ubiquitous distribution and are found in neuronal, neuroendocrine, skeletal muscle and cardiac tissues. They open in response to membrane depolarisation and are responsible for the near-diffusion influx of sodium ions that is essential for the initiation and propagation of action potentials.

Altered NaV1.7 activity and expression levels in injured peripheral neurons is associated with the development of neuropathic and inflammatory pain, or with complete loss of pain perception, thus making NaV1.7 an attractive therapeutic target for the treatment of pain.

There are a number of drugs that are used in the treatment of pain, yet their clinical use is restricted by limited efficacy, as they generally lead to the block of all NaV family subtypes, which in turn can lead to undesirable side effects. The most successful drugs to date exhibit state and/or use dependencies which are responsible for the preferential targeting of aberrant subtypes.

In this study we identify chemical features that contribute to state and use dependent properties in NaV1.7 blockers through the application of computational chemistry methods and optimised electrophysiological assays using the IonWorks Quattro screening platform.

3096-Pos Vgsc-mediated Refractory Periods Control Neuronal Encoding And Synaptic Transmission

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Board B399

The neural codes controlling well-organized cognition and behaviors rely on the meaningful encoding of action potentials at neurons, the efficient output of spikes at synapses and the coordinate activities of neurons in the network. In order to read out the neural codes, it is critically important to figure out the determinants for generating these codes. Refractory periods after action potentials are phases when the neurons do not fire the spikes well in response to excitatory inputs. We have investigated how refractory periods influence the encoding of spikes and assign them into the effective neural codes (i.e., those spikes sufficient to initiate synaptic transmission) by using dual-recording in mice brain slices. Absolute refractory period (ARP) process the best linear correlations with inter-spike intervals and standard deviation of spike timing. The smallest spikes at ARP are sufficient to evoke synaptic transmission, in which uEPSC amplitude, probability and patterns are not different to those evoked by normal spikes. The data imply that absolute refractory period is a primary determinant for spike encoding and synaptic transmission initiation. In terms of the mechanisms of refractory periods, we examined a role of Hodgkin-Huxley's theory that voltage-gated sodium channels (VGSC) are inactive after spikes and the fading of refractory period is the conversion of silent-into-functional VGSCs. With simultaneous whole-cell and cell-attached recording on a pyramidal neuron, we found that refractory periods are controlled by the conductance and number of functional VGSCs. Our data provide experimental evidence for Hodgkin-Huxley's theory, and link the dynamics of single VGSCs to writing effective neural codes, neuronal spike encoding and its signal flow over synapses.

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3097-Pos Block of the Closed Cardiac Sodium Channel by Cocaine, Tetracaine, and QX-314: Insight from Monte Carlo-Energy Minimization

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McMaster University, Hamilton, ON, Canada. Local anaesthetics block differently the closed and open/inactivated sodium channels.

Board B400

Conserved Phe_IVS6 is important for both the open and resting-channel block, while Tyr_IVS6 is important for the open, but not the resting-channel block by cocaine (Wright et al., 1998) and tetracaine (Li et al., 1999). To rationalize these observations, we docked cocaine, tetracaine, and the permanently charged QX-314 in the closed $Na_v1.5$ model. The S5 and S6 helices were modeled from the X-ray structure of KcsA, while the P-loops were modeled as in Tikhonov and Zhorov (2005). Energetically favourable complexes were searched by Monte Carlo-energy minimizations from thousands starting points. We found two categories of binding modes: vertical and horizontal. In both, the ligands' ammonium group is at the focus of the P-helices' macrodipoles. In the horizontal mode, all three drugs expand into the IIIS6/IVS6 interface and, in agreement with mutational data, their benzene rings interact with Phe_IVS6 but no interactions with Tyr_IVS6. The horizontal mode is readily occupied upon the ligand entering into the resting channel via the III/IV interface and is also the most stable mode in the presence of Na^+ in the selectivity filter. The vertical binding mode is preferable in the open channel (Tikhonov and Zhorov, 2007) and could remain upon the channel closure. Indeed, cocaine and QX-314 can adopt the vertical mode in the closed channel by pi-stacking with Tyr_IVS6, but tetracaine is too long to adopt this mode. According to calculations, QX-314 trapped in the closed channel in the vertical mode can escape through the III/IV interface by first transiting into the

horizontal mode. The energy barrier between the vertical and horizontal modes in the closed channel is as small as ~8 kcal/mol.

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3098-Pos Simulated Demand Ischemia Stimulates Myocyte Sodium and Calcium Loading via an Oxidative Stress-induced Increase in the Late Na^+ Current

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Board B401

The late Na^+ current is activated by ischemia and may elevate $[Na^+]_i$ and thus cause increased Ca^{2+} loading via Na^+/Ca^{2+} exchange (NCX). Rabbit ventricular myocytes were subjected to metabolic inhibition (MI) for 45 min with 2 mM cyanide and 0 mM glucose at 37° C. $[Ca^{2+}]$i (nM, Fluo 3) and $[Na^+]$i (mM, Na Green) were measured by flow cytometry during MI plus pacing at 0.5 Hz (P-MI), and in P-MI plus the relatively specific inhibitor of the late Na^+ current, ranolazine 1–50 μM..

P-MI increased $[Ca^{2+}]_i$ and $[Na^+]_i$ to 1825±202.6 nM and to 18.1± mM (means ±SEM, $p<0.01$, n=7, normal values of 117 nM and 5.0 mM respectively). Ranolazine 10 μM reduced $[Ca^{2+}]_i$ and $[Na^+]_i$ during P-MI to 1172.4±51.6 nM and 10.1±1.3 mM ($p<0.01$). The IC50 was1.8 μM for a ranolazine-induced reduction in $[Na^+]_i$ and 2.4 μM for a reduction in $[Ca^{2+}]_i$. The NCX inhibitor KB-R7943 (10 μM) reduced $[Ca^{2+}]_i$ during P-MI from 1544.8±81.5 to 877.7+/−45.1 nM (n=5, $p<0.01$), and inhibited the effect of 10μM ranolazine on $[Ca^{2+}]_i$ ($[Ca^{2+}]$i in P-MI+KB-R+Ran = 904+/−79.8 nM), but did not prevent a ranolazine-induced reduction in $[Na^+]_i$ during P-MI. Exposure to the superoxide scavenger Tiron 20 mM reduced $[Ca^{2+}]_i$ during P-MI from 1190.4±145.5 to 804.1±111.5 nM (n=5, $p<0.005$) and in the presence of Tiron, ranolazine 10 μM had no effect (P-MI+Tiron+Ran $[Ca^{2+}]_i$ =841.6±113.8 nM).

Thus increased Na^+ influx via a ranolazine-sensitive pathway, presumably the late Na^+ current, is activated by oxidative stress during simulated ischemia, and contributes to Ca^{2+} loading by enhancing Ca^{2+} influx by NCX. Reduction of Ca^{2+} loading by this process probably accounts for the anti-ischemic effects of ranolazine.

3099-Pos Functional Consequences of $Na_V1.2$ Mutations in Benign Familial Neonatal-Infantile Seizures

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Board B402

Mutations in *SCN2A*, the gene encoding the human brain voltage-gated sodium channel α-subunit $Na_V1.2$, are associated with inherited forms of epilepsy including benign familial neonatal-infantile seizures (BFNIS), benign familial infantile seizures, generalized epilepsy with febrile seizures plus, and a disorder similar to severe myoclonic epilepsy of infancy. To determine the functional consequences of BFNIS mutations on *SCN2A*, we examined three BFNIS alleles (R1319Q, L1330F, and L1563V) using whole-cell patch-clamp recording of heterologously expressed human $Na_V1.2$ with the human β_1 and β_2 accessory subunits. These mutations affect different regions of the protein structure. The R1319Q mutation causes replacement of a highly conserved arginine within the S4 voltage sensor of domain 3 (D3), L1330F occurs within the intracellular linker connecting the S4–S5 helices of D3, and L1563V affects the D4/S2 transmembrane helix. R1319Q exhibited a depolarizing shift in the voltage dependence of activation and a slowed recovery from fast inactivation. L1563V had minimal effects on activation, but caused impaired fast inactivation characterized by a positively shifted steady state channel availability curve, accelerated recovery from fast inactivation, and slower whole-cell current inactivation decay. The L1330F mutation did not affect the individually assessed parameters of activation or fast inactivation, but displayed an enhanced use-dependent reduction of peak current amplitude. Similar to other sodium channel mutations associated with inherited epilepsy syndromes, BFNIS appears to have a heterogeneous mix of gain and loss of function. Further, the mild clinical phenotype of BFNIS correlates well with the subtle biophysical defects associated with these $Na_V1.2$ mutations.

3100-Pos Phenotypical Changes of Voltage-Dependent Sodium Channels During Myogenesis

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Board B403

Skeletal muscle is a contractile tissue which expresses a wide variety of ion channels. Skeletal muscle differentiation takes place as a result of several complex changes at both cellular and molecular level. During this process membrane potential hyperpolarizes. This phenomenon is generated by the coupled action of different types of ion channels. Voltage-dependent sodium channels (Nav) are one of the most important channels at the initial steps of muscle action potential propagation. Therefore, we decided to analyze the physiological regulation of Nav channels during proliferation and myogenesis processes in neonatal myocyte L6E9 cell line. We found that myoblasts solely express the isoforms Nav1.4 and Nav1.5. Electrophysiological and pharmacological properties, as well as mRNA expression indicated that both isoforms were induced during myoblast proliferation. By using Tetradotoxin we demonstrated that either Nav1.4 or Nav1.5 had no apparent role. On the other hand, myotube differentiation selectively induced Nav1.5, accounting for almost 90% of Na+ current in myotubes. Unlike proliferation, this protein would play a pivotal role in myogenesis. Differentiation also increased colocalization of Nav1.5 in caveolar rafts. Finally, we

demonstrated that the treatment of myoblasts with Neuregulins, which induce myotube formation and increase myocyte survival, further increased Nav1.5 expression. Our results indicate a differential physiological role of the two isoforms of Nav expressed in L6E9 cell line during myoblast proliferation and myotube formation.

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3101-Pos Fast And Slow Gating Modes Of Deactivation In Sodium Channels

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Board B404

Sodium channels close, or deactivate, in response to membrane hyperpolarization. Open-state deactivation is observed as a "tail current" during hyperpolarizing commands, after depolarization opens the permeation pathway. The model for open-state deactivation is that one S4 segment functionally closes the channel, resulting in monoexponential decay of tail current. In the present study, we elicited bi-exponential tail currents in NaV1.2 and NaV1.4 channels using protocols in which the conditioning depolarization was longer, more positive, or both. Several pieces of evidence suggest that the 2 components of the tail current reflect 2 deactivating transitions and not contamination of tail current decay with fast inactivation. The slow component was most pronounced at hyperpolarized commands. The slow component was not prohibited in the IFM to QQQ mutation. The slow component was attenuated, however, in the presence of Anthopleurin A, suggesting that DIVS4 is the origin of the second component of deactivation. Charge reversing mutations in DI - DIV voltage sensors indicate that DI and DII voltage sensors promote the fast movement of charge during deactivation, whereas translocation of DIIIS4, DIVS4, or both underly the second, slower mode of deactivation.

3102-Pos A kinetic model of Na channels in Raphe neurons

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Board B405

The voltage-dependent kinetics of Na channels are a critical factor in the generation of action potentials in neurons and other excitable cells. Here, we investigate the kinetic properties of Na channels in spontaneously spiking Raphé neurons, using a combination of voltage-clamp and dynamic clamp experiments. Our goal is to find a minimal kinetic model that explains both the voltage-clamp data and the shape of the action potential, and agrees with current biophysical knowledge. First, a kinetic model is built by globally fitting whole-cell data obtained with different voltage-clamp stimulation protocols. Then, the model is verified in a functional context, using a recently developed technique that allows fitting the action potential shape, using dynamic clamp. We find that the simple Hodgkin-Huxley model is inadequate for simultaneously describing the voltage-clamp data and the action potential shape, mostly by overestimating the rate of inactivation at sub-threshold potentials. In contrast, allowing for different inactivation rates along the activation pathway results in good fits and reproduces well the action potential waveform. The dynamic clamp experiments and the modeling work were done with the QuB software.

3103-Pos Field-Dependent Changes in Dielectric Permittivity Control Electrostatic Forces between Charged Residues, and Segment Dimensions, in Model Voltage-Sensitive Ion

Channels H. R. Leuchtag Retired

Bandera, TX, USA.

Board B406

Reported observations of dielectric permittivities ε as high as 8000 in tilted chiral smectic mesophases with molecules containing sidechains of the branched nonpolar amino acids (leu, ile, val)[1] suggest a physical explanation for the stochastic onset and termination of ionic avalanches in voltage-sensitive ion channels in response to transmembrane voltage changes. These liquid crystals possess ferroelectric properties, including spontaneous polarization. The high ε associated with a ferroelectric phase keeps the electrostatic forces between charged amino acid residues low in the resting phase, assumed under the gateless gating model to be ferroelectric.[2,3] Upon membrane depolarization leading to a phase transition, a sharp decrease of ε in a nonpolar phase would increase the mutually repulsive forces between positively charged residues (arg, lys) in the voltage-sensing S4 segments. These repulsions must cause cooperative increases in the lengths of the S4s and associated segments of the pore region. This length increase implies a widening of the H bonds of their α helices. Since Li^+, Na^+, K^+ and Ca^{2+} are capable of replacing H^+ in widened H bonds,[4] critical ion occupancy of interloop H-bond sites can initiate ion percolation by hopping down the electrochemical potential gradient, yielding single-channel ion currents. Substitutions of branched residues with unbranched in Na-channel S4s affect activation and inactivation.[5]

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3104-Pos Purification and Characterisation of Prokaryotic Voltage Gated Sodium Channels

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Board B407

Voltage gated sodium channels (VGSC) are integral membrane proteins that selectively transport sodium ions across cellular membranes in response to changes in membrane potential. In higher eukaryotes VGSCs are responsible for the propagation of action potentials in excitable cells such as neurons and muscle. In eukaryotes the pore-forming alpha subunit is a single polypeptide containing four homologous domains. Each domain contains six transmembrane helices (S1–S6) which form a voltage sensing domain (S1–S4) and a pore-forming domain (S5–S6). Prokaryotic analogues of eukaryotic VGSCs have been identified which have sequence identities of approximately 30% to various mammalian and lower eukaryotic VGSCs. The prokaryotic channels apparently are tetramers, each monomer corresponding to a single domain of the eukaryotic channels, and by analogy, each contains six transmembrane helices. Three members of the prokaryotic VGSC family (NaChBac, Na_vSP and Na_vPZ) have been overexpressed and purified in an *E.Coli* system. The purified proteins have thus far been characterised in detergent micelles by a range of biophysical techniques, including dynamic light scattering, circular dichroism spectroscopy and fluorescence spectroscopy and isothermal calorimetry, and are suitable for both three-dimensional and two-dimensional crystallisation.

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3105-Pos Ankyrin-G Participates In Remodeling Of I_{Na} In Myocytes From The Epicardial Border Zone Of Infarcted Canine Heart

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Board B408

Altered Na^+ current (I_{Na}) function in myocytes from epicardial border zone (EBZ) of 5-day infarcted heart plays an important role in reentry that occurs in this arrhythmic substrate (Baba et al, 2006). Mohler et al reported that $Na_v1.5$ not only associates with ankyrin-G (AnkG) but also requires AnkG to target to excitable membrane. Therefore, we hypothesized that infarction-induced remodeling of $Na_v1.5$ may be associated with changes in AnkG in EBZ. Here western blot assay and immunostaining labeling were used to quantify $Na_v1.5$ and AnkG proteins and to determine their subcellular distribution in EBZ and REMOTE cells from infarcted hearts. I_{Na} was recorded in cultured newborn rat myocytes into which Rat or Human siRNA AnkG was delivered. Rat siRNA AnkG was employed to knockdown AnkG expression. Human siRNA AnkG was used as a Control. AnkG protein showed a time-dependent (24hr, 48hr, 5 day) significant increase in EBZ versus Remote while $Na_v1.5$ protein expression decreased. AnkG cell immunostaining was increased just below the sarcolemma in 5-day EBZ cells. In fact surface/core AnkG staining in EBZ (16.9±6.3) was significantly greater than that in Remote cells (4.3±1.1). Knockdown of AnkG altered I_{Na} kinetic properties in cultured newborn rat myocytes although I_{Na} density was similar to Control. A significant shift of I_{Na} activation curve occurred in Rat ($V_{0.5}$: −22.5±2.3 mV) versus Human siRNA AnkG cells (−13.8±1.3 mV). Decay of I_{Na} was significantly slowed (Rat (2.8±0.2 ms) vs Human siRNA AnkG cells (1.9±0.08ms)) and a significant shift of the steady-state inactivation was observed in Rat ($V_{0.5}$: −68.2±2.3 mV) versus Human siRNA AnkG cells ($V_{0.5}$: −62.6±1.2 mV). Thus in the acquired remodeling of I_{Na} in EBZ cells, AnkG expression plays an active role in myocyte electrical remodeling.

3106-Pos Mechanosensitivity of the Sporadic Long-QT Syndrome Related Mutant Nav1.5 Channel, R1623Q

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Board B409

The cardiac Nav1.5 mutation, R1623Q (most external domainIV-S4 arginine), causes a sporadic Long-QT syndrome. R1623Q has slowed, less steeply voltage-dependent inactivation kinetics (Kambouris et al 1998 Circulation 97:640; Chen et al 1996 J Gen Physiol 108:549). Since WT Nav1.5 channel activity is sensitive to bilayer mechanics, responding to membrane stretch with a reversible acceleration of both activation and inactivation (Morris & Juranka 2007 Biophys J 93:822), we investigated the mechanosensitivity of R1623Q. Cell-attached patch recordings of macroscopic currents were made on *Xenopus* oocytes expressing either WT or R1623Q channels. When depolarizing steps (voltage range −40 to −20 mV) from the holding potential (−120 mV) were applied before/during and after membrane stretch (−30 mmHg pipette suction was applied), R1623Q peak current increased but the effect was often only partially reversible. Maximal peak current increases were similar in R1623Q (4.5-fold) and WT (3.5-fold), as were the averages: 2.2±0.2 fold increase in R1623Q (n=24) and 2.2±0.2 fold in WT (n=12). Stretch accelerated both activation and inactivation in R1623Q, and in 62% of recordings (n=16) the two processes accelerated to the same extent (acceleration factor = 1.5±0.1), the same pattern as seen in WT. In the other R1623Q cases, the two processes seemed to be kinetically uncoupled: activation was accelerated by a factor 1.7±0.2 and inactivation by a factor 1.3±0.1. Effects of stretch on recovery from inactivation were also tested in WT and R1623Q channels. This process was not detectably affected when the membrane was held at −120 mV, but when −80 mV was used, stretch slowed recovery in both. If stretch-modulated Nav1.5 current contributes to cardiac mechano-electric feedback,

then for R1623Q patients, whose inactivation is already compromised, faster inactivation plus extra-slow recovery during stretch might explain some sporadic long-QT episodes.

3107-Pos Bubbles, Gating, and Anesthetics in Ion Channels

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Board B410

We suggest that bubbles are the bistable hydrophobic gates responsible for the on-off transitions of single channel currents. In this view, many types of channels gate by the same physical mechanism – dewetting by capillary evaporation – but different types of channels use different sensors to modulate hydrophobic properties of the channel wall and thereby trigger and control bubbles and gating. Bubbles nearly exist in bulk water. Experiments show thin gas layers on hydrophobic surfaces in water. Spontaneous emptying of channels has been seen in many simulations. Because of the physics involved, such (pseudo) phase transitions are inherently sensitive, unstable threshold phenomena difficult to simulate reproducibly and thus convincingly. We present a thermodynamic analysis of a bubble gate using morphometric density functional theory of classical (not quantum) mechanics. Thermodynamic analysis of phase transitions is generally more reproducible and less sensitive to details than simulations. Anesthetic actions of inert gases – and their interactions with hydrostatic pressure (e.g., nitrogen narcosis) – can be easily understood by actions on bubbles. A general theory of gas anesthesia may involve bubbles in channels. Only experiments can show whether, or when, or which channels actually use bubbles as hydrophobic gates: direct observation of bubbles in channels are needed.

3108-Pos Tetrodotoxin-sensitive Sodium Channels Contribute Significantly To The Cardiac Late Sodium Current In Dog Ventricles

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Board B411

Introduction: The role of the late sodium current (I_{NaL}) in hereditary sodium channelopathies such as Long QT syndrome (LQTS), Epilepsy and muscoloskeletal diseases has been well characterized. In most of these cases the sodium channel defect cause an increase in the sustained component of the sodium current, I_{NaL}, that significantly delays the repolarization of the target cells and tissues action potential. Interestingly, some of these neuronal and skeletal muscle diseases also display a clinical phenotype of prolonged QT interval on the electrocardiogram and cardiac rythm disturbance. These observations combined with others made since the 1970's indirectly suggest that a tetrodotoxin-sensitive (TTX) component contributes to cardiac I_{NaL}.

Methods: We investigated the contribution of TTX-sensitive sodium channels ($tNa_V s$) to I_{NaL} using patch clamp techniques and selective blockade of the cardiac sodium channel isoform $Na_V1.5$ in dog ventricular myocytes. The thiosulfonate reagent (2-aminoethyl) methanethiosulfonate (MTSEA) binds to a specific cysteine in the pore region of $Na_V1.5$ and selectively block this isoform. In expression studies, we looked at the distribution of $tNa_V s$ within the epicardial, midmyocardial and endocardial layers of the left venricle myocytes. Our results show that $tNa_V s$ contribute up to 40.18 ± 8.30 % of the late sodium current in dog cardiac myocytes. Immunoblot and mRNA data show that the molecular correlates of $tNA_V s$: $Na_V1.1$, $Na_V1.2$ and $Na_V1.4$ account for a significant portion of this contribution.

Conclusions: We conclude that tNAVS are present in the cardiac ventricles of higher order mammals. In man, such contribution to I_{NaL}could explain the incidence of cardiac arrhythmias and QT prolongation observed in neuronal and musculoskeletal diseases and some of the cardiac secondary effects of neuroleptic drugs

3109-Pos Calcium And Sodium Handling In Mice Myocytes Carrying Sodium Channel Mutation

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Board B412

Background: Mutations of the sodium channel alter the electrophysiological characteristics of this channel. However, it is not known if mutations of the sodium channels alter sodium handling and secondarily calcium handling via the Na/Ca-exchanger.

Hypothesis: Alteration in sodium channel mutation influence sodium and calcium.

Methode: Intracellular sodium and calcium handling were measured in myocytes isolated from transgenic mice carrying the sodium channel mutation 1795insD and after mimicking characteristic of the sodium channels mutations in wild type myocytes. Veratridine and TTX were used to mimic sodium channels characteristics. Veratridine to induce a sustained sodium current throughout the action potential and TTX to reduce the sodium current. $[Na^+]_i$ and $[Ca^{2+}]_i$ were measured with SBFI and Indo-1.

Results: Calcium and sodium handling were not different in right and left ventricular myocytes. In addition, no differences were found in sodium and calcium handling between myocytes isolated from wild type mice compared to mice carrying the sodium channel mutation 1795insD. 50% reduction of the sodium channel current (1.7 uM TTX) and persistent sodium current $<1\%$ of the peak

current (veratridine< 0.5 umol/l) do not alter calcium and sodium handling in myocytes isolated from wild type mice.

Conclusion: The characteristics of sodium channel mutation can be mimicked with veratridine and TTX. Calcium and sodium handling is not different in ventricular myocytes isolated from wild type mice and mice carrying the 1795insD+ mutation.

3110-Pos Structure-function Relashionship Of Two Spider Toxins PnTx2-5 And PnTx2-6

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Board B413

The venom of the Brazilian solitary spider *Phoneutria nigriventer* contains potent polypeptide neurotoxins. Among them, a family of similar toxins (Tx2 type) causes excitatory symptoms such as salivation, lachrymation, priapism, convulsions, spastic paralysis and death. The toxins PnTx2-5 and PnTx2-6 differ only in five of their 48 amino acids and have 10 cysteine residues forming 5 disulphide bridges. We took advantage of this difference to investigate structure-activity relationship. Both toxins inhibit sodium current inactivation of GH3 cells, similar to the alpha-scorpion toxins, except that PnTx2-6 is not displaced by high depolarizing pulses. Furthermore, they partially compete with alpha-scorpion toxins in binding assays [personal communication]. PnTx2-6 has 3 times higher affinity than PnTx2-5 ($K_{0.5}$ = 32nM and 95nM, respectively). Conventional homology modelling was not possible, because of low sequence similarity with other known structures. However, as all short spider toxins present the same overall fold, templates were chosen according to their cysteine pattern in the spider neurotoxin classification proposed by Kozlov *et al.* in 2005. The cysteine connectivity pattern has been deduced by sequence alignment with all cysteine-rich short spider toxins whose 3D structures have previously been experimentally determined and present the Inhibitory-Cysteine-Knot (ICK) motif. Finally, a three-dimensional model of each toxin structure was performed using Modeller package version 8v2. The models were successfully tested by several evaluation software. Fluorescence spectroscopy confirms the exposition of Phe36 and Circular Dicroism spectra confirms the absence of alpha-helix and presence of beta-sheet and beta-turns, as predicted by the models. The amino acid residues that differ in the toxins are predicted to be exposed. Our models suggest a key role of Phe36 and Tyr41 in the binding of both toxins to the channel, while Tyr35, Trp37 and Trp40 can account for the higher affinity of PnTx2-6.

Voltage-gated Ca Channels

3111-Pos Different Structure-function Relationships Determine Biophysical Properties And Pharmacological Modulation Of T-type Calcium Channels Cav3.1 And Cav3.2

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Board B414

The family of T-type calcium channels consists of three isoforms, respectively $Ca_V3.1$(α1G), $Ca_V3.2$(α1H) and $Ca_V3.3$(α1I). Whole-cell recordings allow for an only limited discrimination between T-type calcium channel isoforms. For that, we followed these goals, respectively:

1. Comparison of $Ca_V3.1$ (GGGG) and $Ca_V3.2$ (HHHH) by their single-channel gating properties.
2. Single-channel recording of chimera constructs, composed of N-terminal domains DI and DII of $Ca_V3.1$ and C-terminal domains DIII and DIV of $Ca_V3.2$, respectively (construct 1, GGHH) and (construct 2, HHGG) for structure-function analysis.
3. A detailed analysis of interaction between single $Ca_V3.1$ and $Ca_V3.2$ with N_2O that has previously been described as a $Ca_V3.2$-selective blocker at the whole-cell level (Todorovic et al., *Mol Pharmacol 60*:603–610, 2001).

In HEK-cells we find baseline activity of single $Ca_V3.1$ pore-subunits (n=6) to be significantly lower compared to $Ca_V3.2$ (n=7) (e.g. I_{peak}: −8.2±2.1 fA vs. −19.8±3.6 fA; P_{open}: 2.2±0.2% vs. 4.5±0.5%). Interestingly, gating properties of construct 2 (HHGG, n=6), mirror that of $Ca_V3.1$ (I_{peak}: −6.8±1.5; P_{open}: 2.3±0.2%), while gating properties of construct 1 (GGHH, n=8) are similar to that of $Ca_V3.2$ (I_{peak}: −15.2±2.5 fA; P_{open}: 4.3±0.3%). These findings indicate that baseline activity is mediated by the C-terminal two domains DIII and DIV. We find N_2O to inhibit both T-type calcium channel isoforms $Ca_V3.1$ and $Ca_V3.2$ in a potential-dependent manner, with more pronounced block of $Ca_V3.2$. Preliminary experiments with chimeric α1 subunits suggest that the $Ca_V3.1$-like chimera (HHGG) is more susceptible to N_2O blockade, thus pointing at N_2O effects to be mediated by N-terminal structures of T-type calcium channels.

3112-Pos Two Distinct α_{1S} Residues In Bony Fishes Incompatible With $Ca_V1.1$ Ca^{2+}-conductance

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