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Mechanism of the Preferential Block of the Atrial Sodium Current by Ranolazine

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Introduction: Atrial-selective inhibition of cardiac sodium channel has been shown to underlie the effectiveness of ranolazine in the suppression of atrial fibrillation. This study investigates the mechanism responsible for differences in the interaction of ranolazine with sodium channels in atrial vs ventricular myocytes. **Methods:** Whole cell sodium current was recorded at 15^0C in isolated canine atrial and ventricular myocytes, and in *HEK*293 cell line expressing SCN5A. Protocols were designed to determine interaction of ranolazine with resting, inactivated, and open states of sodium channels. Single pulses and trains of 40 pulses were elicited over a range of holding potentials in the absence and presence of ranolazine to yield tonic and use-dependent block. Development of block during pulse trains in myocytes was analyzed using guarded-receptor theory. **Results**: Tonic block was negligible at holding potentials up to -100 mV, suggesting minimal drug interaction with resting and inactivated states. However, use-dependent block was increased with more depolarized holding potentials, indicating ranolazine trapping by the inactivation gate. Train protocols demonstrated significant effect of shorter diastolic intervals to increase use-dependent block, but a lack of effect of longer pulse durations. Effects in atrial and ventricular myocytes, and in *HEK293* cells followed a similar pattern.

Conclusions: Ranolazine is a potent open-state blocker of sodium channels that unbinds from the resting channels unusually fast and is trapped in the inactivated state. Kinetic rates of ranolazine interaction with different states of atrial and ventricular sodium channels are similar. Ranolazine inhibition of sodium-channels is atrial-selective due to a more negative position of the steady-state inactivation curve, more positive resting membrane potential, as well as more positive take-off potential and shorter diastolic interval in atrial vs ventricular myocytes at fast rates.

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Determination Of The Specificity For FHF/Na Channel Interactions

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Specific FHFs (Fibroblast growth factor homologous factors) regulate voltage-gated sodium channel (VGSC) function by modulating activation, inactivation and/or subcellular distribution through binding to the C-terminus (CT) of specific VGSC alpha subunits. The effect upon VGSC function varies greatly depending upon the particular FHF isoform and its VGSC binding partner, but the specificity of these pair wise interactions are not understood. To identify the determinants for interaction between specific FHFs and VGSC pairs, we studied binding in a recombinant bacterial co-expression system. We show that FHF1b (aa 1- 144), which contains the conserved FHF β-trefoil core domain and is 60-70% homologous to the other members of the FHF family, bound to a $Na_V1.5$ CT containing aa 1773-1940, but not to a $Na_V1.5$ CT comprised of aa 1773-1878. Since the part of the CT necessary for binding contains the calmodulin (CaM) interaction site, we tested whether CaM and FHF1b competed for binding and found that all three components could form a complex; thus, CaM and FHF do not compete for interaction with the $Na_V1.5$ CT. A full length FHF1b and FHF2b also bound to the $Na_V1.5$ CT (aa 1773-1940) but not $Na_V1.5$ CT (aa 1773-1878), suggesting that the region between aa 1878-1940 is necessary for FHF interaction. FHF1b also bound to $Na_V1.6$ (aa 1767- 1926), but did not bind $Na_V1.1$ (aa 1787-1948) nor $Na_V1.2$ (aa 1772-1937). Since the distal regions among the tested $Na_V1.x$ CTs are less similar than the proximal regions, we predict that the specificity for interaction and the consequent particular effects upon VGSC function derives at least in part from this less-well conserved $Na_V1.x$ region.

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Role of L1462 in $Na_v1.5$ Channel DIII-S6 in Voltage-dependent Gating and Antiarrhythmic Block

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Mutations of domain III S6 (DIII-S6) Leu-1465 of rat brain IIA Na^+ channel ($Na_v1.2$) or the equivalent (L1280) in the adult rat skeletal muscle isoform ($Na_v1.4$) affect local anesthetic and anticonvulsant block (Yarov-Yarovoy et al., 2001; Nau et al., 2003). We examined the role of the equivalent position (L1462) of the human heart isoform ($Na_v1.5$) in voltage-dependent gating and antiarrhythmic block. Whole-cell Na^+ currents were measured in HEK293 cells transiently expressing the recombinant wild type (WT) or mutant channels and the beta-1 subunit. Compared to WT, all the mutants of L1462 (L1462A, L1462C, L1462F) accelerated the current decay and shifted the voltage dependence of activation towards more positive direction. L1462 mutants had no effects on the voltage dependence of fast inactivation and the recovery from fast inactivation, but increased the fraction of intermediate inactivation component. Internal charged methanethiosulfonate, MTSES blocked L1462C/C373F with a high frequency of stimulation (20 Hz), where C373 is known to face the outer pore and be the critical residue for isoform differences in tetrodotoxin block. L1462F had no obvious effects on tonic block, but increased use-dependent block by the class Ic antiarrhythmic drug, flecainide, which binds preferentially to activated channels. L1462F increased affinity of activated channels for flecainide by 4-fold, while L1462F had little effects on affinity of inactivated channels. For internal membrane-impermeant QX314, L1462F increased use-dependent block and affinity for QX314 binding to activated channels by 5-fold. These results suggest that L1462 faces the pore in the open state and is involved in activated channel block by antiarrhythmic drugs as well as inactivated channel block by local anesthetics.

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Regulation Of Cardiac Na^+ Channel By NAD^+/NADH

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Introduction: Glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) mutations have been shown to reduce cardiac Na^+ current and cause Brugada Syndrome. Our previous work suggests that mutations in GPD1-L act through NAD(H) to alter Na^+ current. NADH results in downregulation of Na^+ current and NAD^+ can reverse the downregulation of Na^+ current by mutant (MT) GPD1-L or NADH. Here, we studied potential signaling pathways between GPD1-L, NAD(H), and Na^+ channel.

Methods: Currents were measured using whole-cell patch clamp of HEK cells stably expressing the human cardiac sodium channel with and without chelerythrine (a PKC inhibitor, $IC_{50} = 660$ nM), $PKAI_{6-22}$ (a PKA inhibitor, $IC_{50} = 1.6$ nM), apocynin (a NADPH oxidase inhibitor), or elevated intracellular Ca^{2+} in the pipette solution. MT GPD1-L (A280V) was co-transfected with red fluorescent protein with Fugene6 to HEK cells 40 hours prior to measuring current.

Results: The 2-fold reduction in Na^+ current mediated by NADH (100 μM) was inhibited by 200 μM apocynin ($50 \pm 6\%$ vs. $86 \pm 15\%$ of the control current, n=14, $p<0.001$), 6 mM $[Ca^{2+}]_i$ ($107 \pm 17\%$ of control, n=12, $p<0.001$), or 10 μM chelerythrine ($86 \pm 14\%$ of control, n=10, $p<0.05$). $PKAI_{6-22}$ blocked the NAD^+-mediated upregulation of Na^+ currents in the presence of MT GPD1-L (n=10, $p<0.001$). Chelerythrine (10-50 μM) or $PKAI_{6-22}$ (50 nM - 5 μM) alone did not affect the peak currents of Na^+ channels.

Conclusions: Our experiments suggest that NAD(H) can alter Na^+ currents. Downregulation by NADH seems to involve the NADPH oxidase and PKC. PKA appears to be involved in NAD^+-dependent current upregulation. This implies that redox/metabolic state can influence Na^+ current.

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Differential Regulation Of Navß Subunits During Myogenesis

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Voltage-gated sodium channels (Na_v) consist of a pore-forming alpha subunit ($Na_v\alpha$) associated with beta regulatory subunits ($Na_v\beta$). Adult skeletal myocytes primarily express $Na_v1.4$ channels. We found, however, using neonatal L6E9 myocytes, that myofibers acquire a $Na_v1.5$-cardiac-like phenotype efficiently. Differentiated myotubes elicited faster $Na_v1.5$ currents than those recorded from myoblasts. Unlike myoblasts, I_{Na} recorded in myotubes exhibited an accumulation of inactivation after the application of trains of pulses, due to a slower recovery from inactivation. Since $Na_v\beta$ subunits modulate channel gating and pharmacology, the goal of the present work was to study $Na_v\beta$ subunits during myogenesis. All four $Na_v\beta$ ($Na_v\beta1$-4) isoforms were present in L6E9 myocytes. While Navβ1-3 subunits were up-regulated by myogenesis, $Na_v\beta4$ subunits were not. These results show that $Na_v\beta$ genes are strongly regulated during muscle differentiation and further support a physiological role for voltage-gated Na^+ channels during development and myotube formation. Supported by BFI2002-00764 and BFU2005-00695 (to AF), SAF2004-06856, SAF2007-65868 and FIS RD06/0014/0006 (to CV). MD hold a FIS

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Involvement Of TTX-sensitive Na^+ Channels In Excitability Of Skeletal Muscle Arterioles

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The composition of ionic channels involved in electrogenesis of smooth muscle cells in arterioles of skeletal muscle is poorly understood. Here, we investigated inward currents responsible for the depolarizing phase of action potential. Although these currents are thought to be primarily through L-type Ca^{2+} channels, action potentials recorded in our experiments with perforated patch technique were not eliminated by removal of extracellular Ca^{2+} or by addition of L-type Ca^{2+} channel blocker nifedipine (10 μM). Na^+ channel blocker tetrodotoxin (TTX, 1 μM) abolished action potentials at low (~10 μM), but not normal (2 mM), extracellular Ca^{2+}. When recorded with 140 mM Cs^+ and 10 mM EGTA in the pipette and 145 mM Na^+ and 2 mM Ca^{2+} in the bath, the maximal whole-cell currents (at +10 mM) were 22 ± 2 A/F, n=6. The magnitude of slowly activating/inactivating currents that remained in the presence of 1 μM TTX was 4 ± 1 A/F. Na^+ currents recorded with 10 mM Ca^{2+} in the bath were at least two-fold smaller than those with ~10 μM Ca^{2+}. Na^+ currents recorded through perforated patch at 2 mM Ca^{2+} were reduced in the presence of 10 mM caffeine from 19 ± 3 to 9 ± 4 A/F, n=4.

Our results suggest that TTX-sensitive voltage-gated Na^+ channels contribute to depolarization of smooth muscle cells in skeletal muscle arterioles. Voltage-gated Na^+ channels appear to be under a tight control by intracellular Ca^{2+} signaling.

Voltage-gated Na Channels in Nerve

1284-Pos Board B128

Axon Amplifies Somatic Sequential Spikes

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Action potentials are an essential form of neuronal encoding. Sequential spikes in various amplitudes, to be effective neural codes, should be propagated securely via the axons to activate the synapses and drive postsynaptic neurons. We investigated how the axon propagates sequential spikes by simultaneously recording the axon vs. soma in same cortical neurons. The functionally intact axons enable somatic spikes in lower amplitudes be enlarged, which induce synaptic transmission patterns constantly. This facilitation of the axons to spike propagation is associated with shorter refractory periods of sequential spikes and of voltage-gated sodium channels on the axon vs. soma. Therefore, the axons facilitate the propagation of somatic sequential spikes to presynaptic terminals and set neuronal encoding in spike timing order.

1285-Pos Board B129

A Kinetic Model That Explains Slow Inactivation Properties Of Na Channels In Pacemaker Neurons

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Whole cell Na currents were recorded from neurons in raphé *n. obscurus*, using *in vitro* brainstem slices from P0-P4 rats. A Markov model was obtained by globally fitting macroscopic currents evoked by a variety of voltage clamp protocols. The model was based on the topology proposed by Kuo and Bean, with the addition of states to account for slow inactivation. The model explains well the available data, including the entry into and the exit from slow inactivated states, and the current flowing at subthreshold potentials and during the action potential waveform. The kinetic properties of the model and their effects on the firing properties of raphé pacemaker neurons were explored with the dynamic clamp technique, using the QuB software. We focused on subthreshold activation/inactivation, and on slow inactivation.

1286-Pos Board B130

Eugenol Blocks Tetrodotoxin-Resistant Nav Channels

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Eugenol, a phenylpropene synthesized by many angiosperms, acts as atimicrobial toxin and has analgesic properties for humans. Here we show that EUG has a blocking action on tetrodotoxin-resistant voltage-gated sodium channels. The effects of eugenol and lidocaine were thoroughly compared. Currents were recorded in dorsal root ganglia neurons from newborn rats, with patch-clamp technique, whole-cell configuration. The experiments were done in the presence of 100 μM tetrodotoxin.

Eugenol blocked tetrotoxin-resistant Nav channels fast and reversibly, in a concentration-dependent manner. The IC50 for eugenol was of 2.27 ± 0.22 mM and 0.44 ± 0.08 mM for lidocaine and inhibition is due mostly to binding to the channel resting state. Eugenol and lidocaine did not shift the steady-state activation curve along voltage axis. The steady-state inactivation curve was displaced to more negative voltages, reflecting some binding to the inactivated state, by both agents. Eugenol affects the kinetics of inactivation recovery, increasing the weight of the slow component from 21.3% to 27.8%. Eugenol effect is smaller than the lidocaine effect (from 18.0% to 30.7%). Both inhibitors prolonged the half-times of the slow component. In concentrations around IC50 the frequency-dependent blockade was less conspicuous for eugenol. The ratio of a remaining current peak for the 20th /1st pulse, frequency of 5 Hz, was 0,86 for eugenol and 0,58 for lidocaine. In conclusion, eugenol is a fast and reversible blocker of tetrodotoxin-resistant Na currents, with affinity 5 times lower than that of lidocaine for the same channel isoforms. Compared to lidocaine, eugenol has a higher relative affinity for the resting state and lower relative affinity for the open/inactive channel state, as unveiled by low dependence on voltage and frequency of the blocking action.

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Molecular Model of Anticonvulsant and Antidepressant Drug Binding to the Sodium Channel

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Voltage-gated Na channels are molecular targets for many classes of drugs, including local anesthetics, antiepileptics (anticonvulsants) and antidepressants. The anticonvulsants phenytoin and carbamazepine have a tricyclic rigid structure and are neutral, in contrast to the positively charged local anesthetics, but they all block the neuronal Na channels with similar affinities. Using a model of the inner pore of the Na channel, which we developed by homology with K channels, we have docked these anticonvulsants (including lamotrigine) with residues identified by mutagenesis as important for their binding. Anticonvulsants are too wide to fit into the modeled closed pore, but they do fit into the open/inactivated pore. The pharmacophore core of anticonvulsants contain amides or similar groups with a high polarity and large partial positive charge on their amines. When these molecules are docked in the pore, the amines participate in amino-aromatic interactions with the side chain of Phe-1764 of DIVS6 ($Na_V1.2$). One aromatic ring of the tricyclic ring interacts with Tyr-1771 of IVS6, and the second aromatic ring is located in the center of the pore, in proximity to domains I-III. It physically occludes the inner pore in contrast to local anesthetics, which do not but create an electrostatic barrier to ion permeation. Hydrophobic interactions with the second aromatic ring also contribute an important energetic component to binding for anticonvulsants, which compensate for the absence of positive charge in their structures. The antidepressants amitriptyline and nortriptyline are structurally similar to carbamazepine, but the side chain at the tricyclic ring is substituted by a tertiary amine. For these an additional cation interaction with Phe-1764 can contribute to their high binding affinity. Supported by NIH HL5-2016.

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pH-Dependent Regulation of rNaV1.2 Channel Inactivation

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Brain sodium channels play a crucial role in neuronal excitability. Ischemia leads to decreased sodium influx that, as suggested by previous studies, may be caused by channel protonation. Using whole-cell recordings we studied effects of low extracellular pH on the biophysical properties of rNaV1.2 stably expressed in CHO cells. We confirmed that acidification causes a decrease of peak Na currents with no measurable effects on the voltage dependence of activation. By contrast, low pH has a significant effect on rNaV1.2 inactivation properties. At pH 6.0 the effective charge of the steady state fast inactivation curve is significantly reduced, whereas the midpoint voltage is unchanged. The kinetics of fast inactivation are accelerated and shifted to less negative potentials, indicating a destabilization of the fast inactivated state. This was confirmed by first-order two-state Eyring model fit to $\tau(V)$ dependence. Slow inactivation is enhanced at low pH, as demonstrated by experiments using cumulative inactivation of rNaV1.2 with 45Hz stimulation. Thus, our data suggest Na+ channel fast and slow inactivation, but not activation, might be the target for protonation at low pH and might play role in rNaV1.2 down-regulation in low extracellular pH during ischemic events.