Voltage-gated Na Channels I

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The Plant-Derived Alkylamide, Hydroxy-Alpha-Sanshool, Induces Analgesia through Inhibition of Voltage-Gated Sodium Channels

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Many native cultures use extracts from *Xanthozylum* plants to topically treat toothache and joint pain. One active component of these extracts is the alkylamide, hydroxy-α-sanshool, which induces tingling and numbing paresthesia when applied to the skin or tongue. To understand the physiological mechanisms underlying paresthesias, we sought to identify the molecular targets of sanshool in the somatosensory system. We first measured the analgesic properties of sanshool using mouse models of somatosensory behavior. Topical application of sanshool on the hind paw of naïve mice did not alter their sensitivity to noxious thermal or mechanical stimuli. However, in a model of neurogenic inflammation, sanshool acutely suppressed inflammatory hypersensitivity to mechanical force whereas it did not suppress hypersensitivity to heat. These data suggest that sanshool inhibits activity of a subset of sensory neurons that transduce mechanical, but not thermal, stimuli. In cultured dorsal root ganglion (DRG) neurons from mice, sanshool inhibited action potential (AP) firing in a subset of medium-to-large diameter neurons, which are thought to mediate mechanotransduction. In contrast, sanshool did not inhibit AP firing in small-diameter sensory neurons, which predominantly transduce noxious heat. In addition to size, sensory neurons are distinct in their expression of sensory neuron-specific voltage-gated sodium channels. Thus the differential effect of sanshool on sensory neurons may be due to selective activity of sanshool on different sodium channels. To test this idea, we compared the effects of sanshool on two sodium channel subtypes that are expressed in sensory neurons, Nav1.7 and 1.8. Sanshool reduced the magnitude of Nav1.7 and Nav1.8 currents but caused a hyperpolarizing shift in the steady-state inactivation curve of Nav1.7 only. Thus intrinsic molecular differences between sensory neurons, such as expression of different sodium channel subtypes, may underlie specificity of sanshool action.

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Unique Type of Sodium Channel Inhibition by Riluzole

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Riluzole is a persistent-selective sodium channel inhibitor (SCI), which has a therapeutic potential to treat several neurological and psychiatric disorders. SCIs form a large and diverse group, which includes widely different modes of action. Individual SCIs may be very potent in one protocol, but ineffective in another. We have developed a method for testing the ''personality'' of SCIs, i.e., we assess their potency under different voltage protocols. The pattern of their relative potencies gives a characteristic fingerprint which shows correlation with specific chemical properties of molecules, and may also predict their therapeutic profile. We have identified distinct modes of action for specific groups of SCIs. Riluzole was found to have a unique ''personality'' type; therefore, in this study we performed a detailed analysis of its mode of action. The classic SCIs lidocaine and carbamazepine as well as the persistent-selective ranolazine were used as reference compounds. We observed a paradoxical inverse use-dependence and an apparent transient facilitation on sodium channels in the presence of riluzole (but not of other drugs) when currents were evoked by short depolarizations: in such protocols, riluzole appeared to be ineffective. On the other hand, in protocols with prolonged moderate depolarizations the drug was remarkably potent; suggesting that it strongly enhanced closed state inactivation. Recovery from fast inactivation was significantly impeded by riluzole, while recovery from slow inactivated state was - remarkably - even slightly accelerated. As a possible mechanism we propose that riluzole has an exceptionally fast binding kinetics, a high affinity for pre-open closed- and fast inactivated states, while a low affinity for slow inactivated state.

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Molecular Determinants of Human Voltage-Gated Sodium Channels Blockade by Lubeluzole

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Lubeluzole displays neuroprotective activity in vitro and in vivo. Blockade of sodium channels (NaCh) was proposed as a main mechanism for preclinical and clinical efficacy. We studied the molecular determinants for lubeluzole action on whole-cell sodium currents in HEK293 cells expressing hNav1.4 NaChs, using patch clamp technique. Lubeluzole and derivatives were synthesized in our laboratories. Effect of racemic lubeluzole and enantiomers on NaChs was dose- and use-dependent, with IC_{50} values of 30 μM at 0.1 Hz stimulation frequency and 2 μM at 10 Hz using an holding potential (hp) of −120 mV. These are ~8 and ~18 times lower than the respective IC_{50} values for the well-known NaCh blocker mexiletine. The affinity of lubeluzole for the closed (K_R) and inactivated channel (K_I) were 840 and 0.03 μM, compared to 800 and 2 μM for mexiletine. Use-dependent block by lubeluzole was inhibited only partially by F1586C mutation at the local anesthetic molecular receptor, suggesting that lubeluzole may bind at a different but overlapping receptor. Indeed K_R and K_I values for lubeluzole binding to F1586C channel were 700 and 0.7 μM. To go further in details, we synthesized two lubeluzole derivatives, each containing about one half of the parent compound. The aryloxypropanolamine moiety recalls the structure of clenbuterol, while the benzothiazole moiety is similar to riluzole, both known NaCh blockers. However, both derivatives displayed very poor use-dependent block, with IC_{50} values greater than 800 μM at the hp of −120 mV. In conclusion, lubeluzole is a very potent blocker of inactivated sodium channels, which explains its huge use-dependent action. Lubeluzole probably utilizes binding interactions distinct from those of local anesthetic-like drugs, which may open the way for the development of new compounds with peculiar activity on sodium channels (Supported by Telethon-Italy).

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Ranolazine Reduces Central Neuron Excitability by Slowly Interacting with Na_V Channels

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Ranolazine inhibits the increased persistent Na^+ current (persistent I_{Na}) conducted by $Na_V1.1$ channels encoding epilepsy and migraine associated mutations. We therefore determined the effects of ranolazine on the electrical activity of cultured rat hippocampal neurons using empirical and computational modeling approaches. Ranolazine (3μM) produced a 24% reduction in the number of action potentials (APs) evoked in response to repetitive (1sec, 0.67Hz) depolarizing current injections (21 ± 4 for control and 16 ± 3 for ranolazine, pulse 9, $p<0.05$). With a single current injection of 4sec, spike cessation occurred at 2403 ± 220 msec in the presence of 10μM ranolazine (4000 ± 0 msec for control). Similar results were observed for the anticonvulsants phenytoin (3μM, 1387 ± 184 msec) and lacosamide (30μM, 2441 ± 53 msec), which bind to fast and slow-inactivated states of Na^+ channels, respectively. Ranolazine enhanced the development of Na^+ channel fast and slow inactivation evaluated with conditioning pre-pulses of either 100, 1000 or 10000 msec, consistent with progressive binding to inactivated states. Recovery of Na^+ channel activity assessed using fast and slow inactivating voltage protocols was also delayed in the presence of ranolazine. Interestingly, the use-dependent inhibition (25Hz) of Na^+ channel activity by ranolazine (10μM) was dependent on the duration of the voltage step (3.0 ± 2.0% for 2ms and 33.8 ± 13.5% for 20ms, $p<0.05$) suggesting the drug bound to inactivated state(s). Similar to phenytoin, ranolazine exhibited slow binding kinetics to HEK293 cells stably expressing $hNa_v1.2$ (K_{ON}= $1M^{-1}msec^{-1}$ and K_{OFF}= $5e^{-5}msec^{-1}$). Computational simulations predicted equal inhibition of neuronal APs regardless of whether ranolazine binding was constrained to fast-inactivated or slow-inactivated states of the Na^+ channel. Ranolazine had no or minimal effects on neuronal K_V channels, GABA or NMDA neurotransmission. In summary, ranolazine inhibits the excitability of hippocampal neurons by slowly stabilizing the inactivated states of Na^+ channels.

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Ranolazine Effects on NaV1.2 and Modulation by pH

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Ranolazine is an anti-anginal drug previously shown to block persistent currents of the cardiac voltage gated sodium channel, NaV1.5. The effects of ranolazine, however, have not yet been described in all sodium channel isoforms. We studied the effects of ranolazine on the neuronal sodium channel isoform, NaV1.2, and its modulation by extracellular protons. Ionic currents were measured from Chinese Hamster Ovary (CHO) cells expressing the

α-subunit of NaV1.2, using whole cell patch clamp techniques. Voltage protocols were run with extracellular solutions of pH 7.4 and pH 6.0 before and after the addition of ranolazine. The addition of 100 μM ranolazine at pH 7.4 led to a significant decrease in late sodium current, faster rate of open state fast inactivation, and slower recovery from inactivation. Similar trends were seen in preliminary experiments at 10 μM and 30 μM ranolazine. In addition, we observed a tonic block of peak current and an increase in total use-dependent block. Many of these effects were different at low pH. Low pH led to a significant depolarizing shift of the conductance curve, and slowed the onset of fast inactivation. Adding ranolazine at pH 6.0 significantly decreased the rate of fast inactivation recovery, and increased the total use-dependent block and rate of open state inactivation. Although directions of the effects were unchanged, the magnitude of these effects was significantly different between the two pH values. Our results suggest that ranolazine stabilizes fast inactivation at both pH 7.4, and pH 6.0. It is possible that ranolazine and protons may compete for a common binding site or indirectly interact leading to decreased ranolazine efficacy at low pH. (Supported by a research grant from Gilead Sciences, Inc. and an NSERC Discovery Grant to PCR, and an NSERC URSA to CHP.)

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Contribution of Local Anesthetic Binding Site Residues F1760 and Y1767 to Block of the Cardiac Na^+ Channel, $hNa_V1.5$, by Ranolazine

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Amino acid residues F1760 and Y1767 in S6/Domain IV of voltage gated Na^+ channels are part of the local anesthetic binding site in the inner mouth of the pore. Mutagenesis experiments have shown that use-dependent block (UDB) of the cardiac $hNa_V1.5$ Na^+ channel by the Class Ia anti-arrhythmic drug lidocaine is significantly reduced by alanine substitutions at these two sites. The antianginal drug ranolazine, which has structural similarity to lidocaine, was shown previously to block the cardiac late sodium current (I_{Na}) and to block peak and late I_{Na} in a use-dependent manner (Rajamani et al., 2009). As with lidocaine, UDB of peak I_{Na} by ranolazine was reduced in the F1760A mutant channel (Fredj et al., 2006; Wang et al., 2008). We used the F1760A and Y1767A mutants to investigate further the block by ranolazine of the closed, inactivated, and open states of the channel. To measure affinity to the open state, we used an inactivation-deficient $hNa_V1.5$ channel (L409C/A410W, Edrich et al., 2005) in the presence and absence of the F1760A or Y1767A mutations. We found that both mutations (F1760A and Y1767A) significantly decreased UDB of peak I_{Na}, block of the open state, and block of late I_{Na} by ranolazine compared to WT channels (14-24 fold shift of the value of IC_{50} for ranolazine). The effect of the mutations on block by ranolazine of the resting and inactivated states was modest (1.3-1.9 fold shift of the value of IC_{50} for ranolazine). The results indicate that both the F1760A and Y1767A mutations reduce the effect of ranolazine to cause UDB and open state block of $hNa_V1.5$.

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Using Full Factorial Design of Experiments on an Automated Patch Clamp Instrument: A Case Study on the Ionworks Quattro and Nav1.5 Channel

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In the past decade, technological advancements have enabled automation of patch clamp recordings. Instruments like the Ionworks Quattro produces electrophysiological data from a 384 well plate in about an hour. However, they also require proprietary and expensive one-use consumables like the PatchPlate. Conversely, the large amount of data produced from these instruments also allows more than the ''one factor at a time'' experiments. Here we present an example of a full factorial experiment on the Nav1.5 channel using the Ionworks Quattro. A full factorial experimental design provides more information about the instrument by having a more precise estimation of each factor alone and by estimating the interactions between factors.

We tested the potencies of several known blockers on the cardiac sodium channel (Nav1.5) and varied 3 factors: incubation time, mixing cycles, and cell density. From the experiment, we find that the IC50s of most compounds are affected by incubation time and cell density. We also observed interactions with the factors of incubation time/cell density and the number of mixing cycles/cell density. From this case study, we propose that a factorial design of experiments can be done effectively with an automated patch clamp instrument to optimize assay conditions with enriched information to the effect of reduction of consumables and shortened time of assay development.

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Functional Studies of Interaction Between Huwentoxin-IV and Voltage-Gated Sodium Channel Nav1.7

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Voltage-gated sodium channel 1.7 (Nav1.7) plays a role in the conduction of action potentials and is involved in the sensation of pain. Spider toxins such as Huwentoxin IV (HwTx-IV) are potent inhibitors of Nav1.7, functioning as a gating modifier trapping the voltage sensor in an inward closed conformation. HwTx-IV interacts with specific residues in the voltage sensor S3-S4 region of domain II. The purpose of the present study was to understand the residues important for interaction between HwTx-IV and Nav1.7. Computationally, molecular dynamics was used to study wild type HwTx-IV along with various alanine and cystine mutations to determine residues important for the stability of toxin and to obtain a model of the interacting surface. A homology model of Nav1.7 was built and the toxin docked to determine key interactions. Experimentally, alanine mutants of HwTx-IV were tested for functional activity using FLIPR, QPatch, and manual patch. Computational and experimental results suggest key residues critical for binding of the toxin to Nav1.7, particularly S25, W30 and K32. These studies provide a more clear idea of the nature of interaction between HwTx-IV and Nav1.7 and may therefore be a useful guide in designing novel peptides with improved selectivity for sodium channel subtypes.

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''Clip Detecting'' with Series Resistance Compensation using an Automated Patch Clamp System

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One of the significant challenges for automated patch clamp has been to incorporate amplifiers with the ability of adjusting to changes in membrane capacitance, series resistance (Rs) and voltage-clamp time constants without user intervention. If these adjustments are not made correctly there is a risk of generating un-precise data or even losing data due to oscillations in the recorded trace. Rs compensation capability of the patch clamp amplifier of QPatch is presented. A new ''clip detector feature'' allowing Rs to be temporarily turned off avoiding loss of cells due to fatal oscillations, and thereby increasing the data throughput while maintaining high quality recordings, is presented. Clamping whole-cells can be a challenge depending on the magnitude of Rs. The series resistance produces two undesired effect on whole cell voltage clamp recordings: i) The cells not being clamped to the desired potential due to the voltage drop over the Rs (V_{drop}= R_s x I), ii) The resolution of the voltage clamp in fast physiological processes being affected by the Rs. It is essential to reach adequate series compensation in a timely manner to obtain accurate recordings. As such, the quality of data depends on the quality of the patch clamp amplifier and its features. In order to make fast 100% series resistance compensation a fast patch clamp amplifier is needed. The embedded amplifier in the QPatch utilizes a patented technique invented by Adam Sherman (Alembic Instruments). QPatch data from human SK potassium and fast sodium channels are shown. The data demonstrates that i) QPatch measures the fastest sodium channels with accurate clamp, and ii) the patch clamp amplifier in QPatch has the required speed to record the correct current amplitude when the current through an open SK channel is reversed.

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Inhibition of Voltage-Gated Na^+ Currents in Sensory Neurons by the Sea Anemone Toxin APETx2

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ASICs are neuronal H+-gated channel that are transiently opened by extracellular acidification. Functional ASIC channels are made of homotrimeric or heterotrimeric complexes of different ASIC subunits (ASIC1a, −1b, −2a, −2b and 3). APETx2, a toxin from the sea anemone Anthropleura elegantissima inhibits homotrimeric ASIC3 with IC50<100 nM. In rat sensory neurons (DRG), where heterotrimeric channels are expressed, higher concentration are necessary to block ASIC3 containing channels (0.1-2μM). Several animal studies relied on APETx2 as a selective pharmacological tool to study ASIC3 physiological role.