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 ($3.0 \pm 2.0\%$ for 2ms and $33.8 \pm 13.5\%$ 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