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1283-Pos Board B127

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

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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.

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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.