Voltage-gated Na Channels II

1649-Pos Board B379 Frequency-Dependent Inhibition of Sodium Channels by the General Anesthetic Isoflurane

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Voltage-gated sodium channels (NaV) are important for initiation and propagation of action potential in excitable cells, thus contributing to cell-to-cell communication via neurotransmitter release at the synapse. NaV are potential presynaptic targets for inhaled volatile anaesthetics (VAs), which despite their widespread use have poorly understood mechanisms of action. Many drugs that act on NaV, including local anesthetics, show state-dependent effects, that is affinity of the drug depends on whether the channel is open, closed or inactivated. Upon action potential firing, NaV open and rapidly transition into a non-conducting, inactivated state. During high-frequency firing, inactivated channels accumulate as there is insufficient time to return to a closed state before subsequent action potential firing, resulting in frequency-dependent reduction of I\textsubscript{Na}. Therefore, drugs that affect the rates of channel state transitions can have a profound effect on neurotransmitter release and neuronal communication. We investigated the state-dependent effects of the prototypical VA isoflurane on human NaV1.9, a presynaptic NaV during high-frequency firing. We expressed NaV1.2a, a major isoform found in the central nervous system, in the neuronal mammalian cell line ND7/23 and found that isoflurane enhanced frequency-dependent reduction of I\textsubscript{Na} by enhancing apparent inactivation. To confirm these results in a more physiological preparation we studied the effects of isoflurane on native NaV in rat neuropsychopetal nerve terminals and showed similar frequency-dependent findings. These data led us to propose a simple pharmacological model of open channel block to account for the effects of isoflurane on NaV1.2a. Our data indicate that high-frequency neuronal firing potentiates isoflurane effects, which might contribute to selective modulation of fast firing neuronal networks. Supported by NIH grant GM 58055.

1650-Pos Board B380 Pharmacology of Heterologously Expressed Human NaV1.9 Channels

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Voltage-gated sodium channel (NaV) channels are implicated in different chronic pain disorders, including inflammatory pain. The NaV channels NaV1.8 and NaV1.9 are almost exclusively expressed in nociceptors, consistent with their involvement in pain signaling. Native NaV1.9 is responsible for TTX-resistant persistent NaV currents in sensory neurons and is associated with inflammatory pain hypersensitivity making it a desirable drug target. However, difficulties with expressing NaV1.9 in heterologous systems have limited the study of functional and pharmacological properties. We developed a stable cell line expressing full length human NaV1.9 allowing the functional characterization of this channel. Here we present the sensitivity of NaV1.9 to small molecules known to block TTX-sensitive and TTX-resistant NaV channels. Whole-cell currents were recorded from a holding potential of −120 mV and elicited with 50 ms pulses to −40 mV every 15 s in the continuous presence of 150 nM TTX to block endogenous TTX-sensitive sodium currents present in the host cells. Under these conditions, calculated IC\textsubscript{50} values (in mM) for NaV1.9 peak current block are: lidocaine = 295.7 ± 52.3; carbamazepine = 97.8 ± 12.4; vinpocetine = 17.1 ± 4.6; lidocaine = 9.9 ± 0.7; and A-803467 = 1.9 ± 0.1. These results are the first pharmacological analysis of heterologously expressed NaV1.9 and provide insight into what compounds are more effective. In addition, the results validate this cellular model for small molecule screening and drug discovery.

1651-Pos Board B381 Block of Nav1.5 by Racemic Fluoxetine

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The cardiac voltage-gated Na\textsubscript{v}1.5 channel is a membrane protein that is essential for the propagation of action potentials in the heart. Malfunctions of this channel are known to cause hereditary diseases such as long QT syndrome, Brugada syndrome, and conduction disorders. These channels are the prime target for class 1 antiarrhythmics and a number of antidepressants. The purpose of the present study was to investigate the Na\textsubscript{v}1.5 channel-blocking properties of fluoxetine, a selective serotonin re-uptake inhibitor and widely prescribed antidepressant. Na\textsubscript{v}1.5 channels were stably expressed in HEK-293 cells, and Na\textsuperscript{+} currents were recorded using the patch-clamp technique in the whole-cell configuration. Dose-response curves of racemic fluoxetine and its optical isomers had similar IC\textsubscript{50} (40 mM for the (+) isomer) and Na\textsuperscript{+} currents were blocked by fluoxetine. The results validate this cellular model for small molecule screening and drug discovery.