Voltage-gated Na Channels II

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Frequency-Dependent Inhibition of Sodium Channels by the General Anesthetic Isoflurane

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Voltage-gated sodium channels (Na_v) are important for initiation and propagation of the action potential in excitable cells, thus contributing to cell-to-cell communication via neurotransmitter release at the synapse. Na_v 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 Na_v, 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, Na_v 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_{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 recombinant and native Na_v during high-frequency firing. We expressed $Na_v1.2a$, 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_{Na} by enhancing apparent inactivation. To confirm these results in a more physiological preparation we studied the effects of isoflurane on native Na_v in rat neurohypophysial nerve terminals and show 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 $Na_v1.2$. Our data indicate that high-frequency neuronal firing potentiates isoflurane effects, which might contribute to selective modulation of fast firing neuronal networks.

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Pharmacology of Heterologously Expressed Human $Na_V1.9$ Channels

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The prevalence and impact of chronic pain is extensive and underscores the need to investigate the mechanisms that initiate and propagate neuronal pain signaling, and to identify new analgesic targets. Neuronal voltage-gated sodium (Na_V) channels are implicated in different chronic pain disorders, including inflammatory pain. The Na_V channels $Na_V1.8$ and $Na_V1.9$ are almost exclusively expressed in nociceptors, consistent with their involvement in pain signaling pathways. $Na_V1.9$ is responsible for TTX-resistant persistent currents in sensory neurons and is associated with inflammatory pain hypersensitivity making it a desirable drug target. However, difficulties with expressing $Na_V1.9$ in heterologous systems have limited the study of functional and pharmacological properties. We developed a stable cell line expressing full length human $Na_V1.9$ allowing the functional characterization of this channel. Here we present the sensitivity of $Na_V1.9$ to small molecules known to block TTX-sensitive and TTX-resistant Na_V 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_{50} values (in μM) for $Na_V1.9$ peak current block are: lidocaine = 295.7 ± 52.3; carbamazepine = 97.8 ± 12.4; vinpocetine = 17.1 ± 4.6; riluzole = 8.9 ± 1.0; and A-803467 = 1.9 ± 0.1. These results are the first pharmacological analysis of heterologously expressed $Na_V1.9$ and provide insight into what compounds are more effective. In addition, the results validate this cellular model for small molecule screening and drug discovery.

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Block of Nachbac by Cadmium and Lanthanum Ions

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The prokaryotic, voltage-gated sodium channel, NaChBac, has features common to both eukaryotic Nav and Cav channels, but also differs in important details. NaChBac assembles as a homo-tetramer, and it achieves Na^+-selective conduction with a pseudo-symmetric selectivity filter possessing a 4-glutamate motif (EEEE), which is common also to eukaryotic Cav channels, but is distinctly different from the asymmetric selectivity filter (DEKA) of eukaryotic Nav channels. Block by impermeant, or weakly permeant, ions offers functional clues as to the nature and location of ion-binding sites within the channel's conducting pathway. Here, we examine block by a transition metal (Cd^{2+}), or a lanthanide (La^{3+}), in the extracellular solution. Whole-cell patch-clamp experiments were performed using tsA201 cells transiently expressing NaChBac. Onset of block and washout were observed using repeated depolarizations to −10 mV, from a holding potential of −120mV (IC50s (mM, mean ± SEM): Cd, 2.0 ± 0.06; La, 0.81 ± 0.03. Two analyses examined voltage dependence of block and provided estimates of the ''apparent electrical distance'', δ, from external solution to the blocking site based on the analysis of Woodhull (J. Gen. Physiol., 1973). First, peak current-voltage relations were determined in the presence and absence of the blocker. Second, instantaneous I-V relations allowed calculation of fractional block of the conductance activated by a fixed, activating prepulse. In each analysis, a fit of fractional block, f_b, vs V yielded an estimate of δ, and the IC50(V=0). IC50 values were similar to those obtained directly from dose-response data (see above). Values for δ appeared to vary slightly with the method, but indicated only weak V dependence of block: Cd, 0.02 - 0.08; La, 0.02 - 0.13. These data are consistent with a superficial binding site, and only weak coupling of block to movements of permeant ions within the channel. Supported by: MOP-10053 (CIHR).

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Mechanism of Slow Repriming of Nav Channels by Lidocaine

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Local anaesthetics (LA) exert their action by prolongation of the time course of repriming of voltage-gated Na channels after repolarization of the action potential. This may result from slow drug-dissociation from fast inactivated states or, alternatively, from stabilization of a native slow inactivated state (I_M, Chen ZH et al. J Physiol. 524:37). The C-terminal part of transmembrane S6 segment of domain IV (DIV-S6) is an important determinant of inactivation gating and LA block. We performed serial cysteine scanning mutagenesis of positions 1575-1586 in DIV-S6 of $rNa_v1.4$ channels and investigated the relationship between changes in mutation-induced alterations of I_M and respective changes in LA-induced prolongation of recovery. The constructs were expressed in TsA201 cells and studied by means of whole-cell patch-clamp technique. We examined the time course of recovery from fast and slow inactivation produced by 50 ms and by 10 s conditioning pulses to −20 mV, respectively. The LA Lidocaine (500 μM; LIDO) significantly slowed recovery from fast inactivation in all tested constructs except F1579C. The time constant of LIDO - induced slow recovery was ~ 150 ms. Under LIDO-free conditions a similar time constant of slow recovery was found most constructs following 10 s depolarization, presumably reflecting recovery from native I_M. However, the fraction of channels recovering from I_M (F- I_M) varied greatly among the tested constructs. LIDO significantly increased F- I_M in most constructs. Interestingly, there was a significant *negative* correlation between F-I_M without LIDO and the LIDO-induced increase in F- I_M**(**R^2 = 0.91; P < 0.0001**).** Furthermore, in M1585C F- I_M was 0 under LIDO-free conditions but increased to 0.73 ± 0.02 with LIDO. The data suggest that in the tested constructs slow recovery with LIDO reflects slow dissociation from fast inactivation rather than recovery from I_M. Funding support: Austrian Science Fund W1232-B11.

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Fluoxetine Blocks $Na_V1.5$ Channels via a Mechanism Similar to that of Class 1 Antiarrhythmics

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The cardiac voltage-gated $Na_v1.5$ 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 antiarrhythmic drugs and a number of antidepressants. The purpose of the present study was to investigate the $Na_v1.5$ channel-blocking properties of fluoxetine, a selective serotonin re-uptake inhibitor and widely prescribed antidepressant.$Na_v1.5$ channels were stably expressed in HEK-293 cells, and Na^+ currents were recorded using the patch-clamp technique in the whole-cell configuration. Dose-resonse curves of racemic fluoxetine and its optical isomers had similar IC_{50} (40 μM for the (+) isomer and 47 μM for the (-) isomer). Norfluoxetine, a fluoxetine