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occurs during ischemic events, may alter the interaction between ranolazine and the channel. In this study, we performed whole-cell patch clamp experiments with extracellular solution titrated to pH 7.4 or pH 6.0 using HEK cells expressing NaV1.5 and CHO cells expressing NaV1.2. We found that ranolazine modulates these sodium channels with onset/recovery kinetics and voltage-dependence resembling slow inactivation. In this way, ranolazine increases use-dependent inactivation of the channel and decreases window currents. At pH 6.0, ranolazine interaction with the sodium channel is slowed approximately 4-5 fold in both NaV1.2 and NaV1.5. Despite the slowed kinetics, ranolazine remains effective at steady-state during acidic conditions. At low extracellular pH ranolazine rescues the voltage-dependence of slow inactivation at a therapeutically relevant concentration (10μM). Our results suggest that, at pH 6.0, ranolazine compensates for proton-induced impaired slow inactivation and remains effective at reducing persistent currents.

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Correlation of Sodium Channels Transcripts of Single DRG Neuron to their Electrophysiological and Pharmacological Profiles

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Introduction

Voltage gated sodium channels (VGSCs) play an important role in nociceptive transmission. They are implicated in the genesis and the conduction of the neuronal action potential firing. Four different isoforms (Nav1.3, Nav1.7, Nav1.8, and Nav1.9) have been linked to the nociceptive responses. However, their specifics implication on the genesis and transmission of the nociceptive response remain to be refined. The aim of this study is to achieve a better understanding of the synergy between the different isoforms in a single neuron that confer a unique electrophysiological profile to the neuron.

Hypothesis

Intrinsic properties of the VGSCs isoform contribute to the electrophysiological profile of the small (<25μM) DRG neurons.

Method

We analyzed and correlated the composition of VGSCs in a single neuron and their electrophysiological properties. We used whole-cell configuration of the patch-clamp to record sodium currents or action potentials from acutely dissociated small DRG neurons from adult rats, before and after tetrodotoxin application, followed by a single-cell qPCR from the same neuron.

Results and conclusion

There is a strong correlation between sodium currents and the mRNA quantification in single neuron. Voltage-clamp experiments show that TTX-S currents correlate with Nav1.7 mRNA transcripts and the TTX-R current correlate with Nav1.8 and Nav1.9 mRNA transcripts with correlations over 0.85. Current-clamp experiments show that Nav1.7 contributes to increase the overshoot, maximum rate of rise and the maximum decay (dv/dt max). Interestingly, the proportion of Nav1.7 and Nav1.8 were not implicated in the firing frequency of the neuron. Those results improve our current understanding on the specific implication of the different isoform on the electrophysiological profile of the small DRG neurons and have strong implication in the comprehension of the remodeling of VGSCs that occurs in different pathological pain.

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A Voltage Gated Dependent Na^+ Channel is Activated during Apopoptosis in Xenopus Oocytes

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Ion channels in the plasma membrane are important for the apoptotic process. In previous studies we and other have found that different voltage-gated ion channels are upregulated early in the apoptotic process and that block of these channels prevents or delays apoptosis. In the present investigation we examined whether ion channels are upregulated in oocytes from the frog Xenopus laevis during apoptosis. The two-electrode voltage-clamp technique was used to record endogenous ion currents in the oocytes. During staurosporine-induced apoptosis a voltage-dependent Na^+ current was upregulated. This current activated at voltages more positive than 0 mV with a mid-point of the open-probability curve around +50 mV. The current was resistant to the Na^+-channel blockers tetrodotoxin (1 uM) and amiloride (10 uM), while the Ca^{2+}-channel blocker verapamil (200 uM) in the bath solution completely blocked the current. Oocytes treated with staurosporine in the presence of verapamil, or in the absence of extracellular Na^+ (replaced with Choline+) did not show an upregulated Na^+ current (when measured in the absence of verapamil, or in the presence of Na^+ respectively). Low extracellular Na^+ concentration also prevented caspase-3 activation. Taken together, this suggests that the influx of Na^+ is a crucial step in the apoptotic process in Xenopus oocytes.

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Epilepsy Alters the Activity and Adrenergic Response of the Cardiac Sodium Current

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Abnormalities in cardiac rhythm or repolarization are observed in epilepsy. They appear during or shortly after seizure and include atrioventricular (AV) block, atrial fibrillation, supraventricular tachycardia, ventricular premature depolarization and bundle-branch block. The ECG recordings often show altered heart rate dynamics, indicative of changes in sympathovagal balance in epileptic patients. It has been suggested that autonomic imbalance induced remodelling of the heart and altered cardiac sympathetic response. To test this hypothesis we measured the response of the cardiac sodium current I_{Na} to isoproterenol in the rat epilepsy model (kainic acid). Using patch clamp technique on isolated ventricular myocytes we found that epilepsy by itself increased maximum I_{Na} density by 76% from 59 ± 7 pA/pF to 104 ± 20 pA/pF. Isoproterenol increased I_{Na} more importantly in epileptic rats than in control animals with respective changes of 93% and 127%. Mid-potential for steady-state activation was shifted from $-42.9 \pm 0{,}3$ mV in control to -55 ± 1 mV in epileptic rats. Steady state mid-inactivation went from -79 ± 1mV to -84 ± 1 mV. Isoproterenol attenuated the differences in steady state parameters by bringing the activation and inactivation mid potential to -63 ± 1 mV and -89 ± 1mV respectively in sham and epileptic rats. We conclude that epilepsy modifies I_{Na} activity in a manner consistent with an increase in cardiac excitability. Moreover, the changes in gating parameters increase sodium entry into ventricular myocytes and may lead to calcium overload and associated delayed after-depolarizations. These mechanisms may explain in part the arrhythmias observed in epileptic patients.

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Rufinamide Modifies the Gating Process of Human Voltage-Gated Sodium Channels

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Lennox-Gastaut Syndrome (LGS) is a severe form of epilepsy manifesting during early childhood. Treatment of the seizures and the ensuing behavioral and mental health problems commonly associated with LGS requires multiple anticonvulsant therapeutics, often with deleterious effects on the patient. Although the relatively new orphan drug rufinamide is gaining importance as an adjunct therapy for LGS, its mode of action remains speculative. To establish the molecular mechanism underlying rufinamide efficacy, we compared its effect on a subset of neuronal human voltage-gated sodium (Nav) channel isoforms (hNav1.1, hNav1.2, hNav1.3, and hNav1.6), several of which are thought to be involved in epilepsy. As a result, we found that a clinically relevant concentration inhibits hNav1.1 activation whereas the recovery from fast inactivation of hNav1.1, hNav1.2, hNav1.3, and hNav1.6 is slowed down. Moreover, experiments with related triazole compounds revealed drug variants with effects comparable to those of rufinamide and suggest that modifications of particular side chains may be exploited to obtain an isoform-specific drug-induced effect on hNav1.1 activation.

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Mutagenesis Studies Investigating Slow Inactivation of Sodium Channel Nav1.6

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Slow inactivation (SI) determines the availability of voltage-gated sodium channels and effects action potential firing patterns. Neuronally-expressed Nav1.6 was suggested to play an important role in maintaining high firing frequencies, possibly due to a reduced SI compared to other sodium channel subtypes. With this study we characterized SI of WT Nav1.6R and introduced mutations that severely altered the channel's propensity to slow inactivate. Conserved residues in the S6 segment of DII and DIII were identified as SI-sensitive hot spots: V966C in DII and N1455A in DIII.

WT and mutant channels were transiently expressed in ND7/23 cells and patch-clamped. The N1455A mutation greatly enhanced SI whereas the V966C mutation shifted its voltage-dependence in the depolarizing direction by ~25 mV. Fast inactivation was shifted slightly to more hyperpolarized potentials (~3 mV for V966C and ~8.5 mV for N1455A).

We applied trains of depolarizing stimuli to imitate high-frequency firing. At a stimulation frequency of 50 and 100 Hz, only the N1455A mutation displayed enhanced run down, whereas V966C behaved similar to WT, probably due to the slight enhancement of fast inactivation of V966C.