

595-Pos**Divergent Pharmacological Properties of SCN1A Splice Variants**

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Voltage-gated sodium channels undergo alternative mRNA splicing. In the human neuronal Nav1.1 channel encoded by SCN1A, a common genetic variant affecting an intron splice donor site alters the proportion of transcripts that incorporate the canonical exon 5 (exon 5A) or an alternative (exon 5N) encoding portions of the S3 and S4 segments of domain I. Epileptic subjects with this genetic variant require lower doses of anticonvulsant drugs such as phenytoin compared with individuals lacking this variant. Because this genetic variant is associated with a larger proportion of exon 5N containing transcripts in brain, we hypothesized that differences in function and pharmacology of Nav1.1 channels containing either exon 5N or 5A account for the observed divergence in anticonvulsant dose requirements. To examine differences in drug efficacy of SCN1A splice variants, we performed whole-cell recording on tsA201 cells transiently co-transfected with either Nav1.1-5A or Nav1.1-5N and two accessory subunits ($\beta 1, \beta 2$). We examined voltage-dependence of activation, steady-state inactivation, and recovery from fast inactivation and observed no significant differences between splice variants. We also measured both steady-state block and use-dependent block (10Hz) by phenytoin, carbamazepine, and lamotrigine. Nav1.1-5N channels exhibited greater steady-state block by phenytoin (100 μ M) ($16 \pm 5\%$ vs. $2 \pm 6\%$) and lamotrigine (200 μ M) ($25 \pm 4\%$ vs. $14 \pm 2\%$) compared to Nav1.1-5A. Additionally, Nav1.1-5N exhibited greater use-dependent block by phenytoin ($39 \pm 5\%$ vs. $24 \pm 4\%$) and lamotrigine ($29 \pm 6\%$ vs. $18 \pm 2\%$). We tested cells stably transfected with either Nav1.1-5A or Nav1.1-5N and both β subunits using an automated planar patch clamp system (Patchliner, Nanion Inc.) to perform concentration-response curves to determine steady-state and inactivated state affinities for lamotrigine. Similar to conventional patch clamp experiments, lamotrigine exhibited greater steady-state and inactivated state affinity for Nav1.1-5N than Nav1.1-5A. These results suggest SCN1A transcripts containing the alternative exon 5N encode channels that are more sensitive to multiple anticonvulsant drugs.

596-Pos**From Plastic-Bottle-Toxin to Sodium Channel Blocker: A New Role for Bisphenol A**

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Bisphenol A has reached public attention due to its presence in food and beverages following leaching from plastic containers. It is a monomer that is polymerized to manufacture polycarbonate plastic food wrappings and it is detectable in the blood of human populations in developed countries (Palanza et al. 2008, Environmental Research). In animal studies and in vitro bisphenol A was shown to have estrogenic effects. Data link bisphenol A exposure to a variety of diseases including miscarriage, menstrual pain and cardiovascular syndromes.

The human heart voltage-gated sodium channel hNav1.5 was expressed in HEK293t cells to determine the effect of bisphenol A on sodium channel function.

With whole-cell patch clamp analysis, we show that bisphenol A reduces the peak sodium current through hNav1.5 from a holding potential of -120 mV with an EC₅₀ of 54 ± 8 μ M. This concentration is considerably higher than has been found in beverages from plastic bottles. However, compared to other known sodium channel blockers, such as lamotrigine or lidocaine, its efficacy is approximately ten-fold higher.

Bisphenol A shifts steady-state fast inactivation to more hyperpolarized potentials, whereas voltage-dependence of activation is unaffected. As with local anesthetics, bisphenol A binds preferentially to the inactivated state. The association time constant, as determined by a single exponential fit of peak current decline induced by 30 μ M bisphenol A is approximately 14 times faster than that induced by 300 μ M lidocaine.

In conclusion, we have determined that bisphenol A has blocking effects on hNav1.5 sodium currents that are more pronounced than those of known blockers, such as lidocaine or lamotrigine.

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597-Pos**Characterization of the Reverse Use Dependent Block of Voltage Gated Sodium Channels**

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Some clinically used drugs interact *via* state-dependent inhibition of voltage-gated sodium channels. For example, cocaine, procaine or lidocaine preferentially interact with, and stabilize the inactivated conformation of the channel. Upon repetitive high frequency activation they cause a progressive inhibition during the pulse train which is termed use-dependent inhibition.

Here we describe compounds that show the opposite behaviour, i.e. the inhibition is diminished during the pulse train.

To adequately determine the state-dependent interactions of drugs with sodium channels, we developed a high-throughput electrophysiological assay using the IonWorks^(r) Quattro(tm) PPC platform. Compounds were tested against the brain Nav1.3 sodium channel expressed in CHO cells. A train of 10 depolarizing voltage steps from -90 mV to 0 mV for 20ms (10Hz frequency) was applied before and after compound addition. To evaluate the tonic block, inhibition of the peak current at the first pulse was measured while the use-dependent block was determined as the inhibition at the 10th pulse. Lidocaine shows the expected use-dependent inhibition. Surprisingly, we found compounds with the opposite profile: the compound with the most pronounced effect blocked the 1st and 10th pulses by $72.3 \pm 6.1\%$ and $42.9 \pm 6.9\%$ (mean \pm SD, n=5) at 10 μ M. In a second instance these compounds were tested against the cardiac Nav1.5 and the peripheral nervous and neuroendocrine systems Nav1.7 observing similar effects. An in-depth comparison between use-dependent and reverse use-dependent blockers was performed for parameters such as voltage-dependent activation and inactivation, recovery from inactivation, and frequency dependency. These data provide biophysical insights in the mechanism of reverse use-dependent inhibition for Nav channels.

598-Pos**Insights on the Mechanisms of the Fast Blockade of TTX-R Na⁺ Channels by Eugenol**

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OBJECTIVES. It was previously shown that eugenol, a phenylpropene, blocks fast and reversibly voltage-gated Na⁺ channels (NaV), but little concern was given to the blocker binding to different conformational states of channel molecule. Here we reported a detailed analysis of state-dependent effects of eugenol on tetrodotoxin-resistant (TTX-R) NaV isoforms, comparing them to those of lidocaine, a reference blocker.

METHODS. TTX-R Na⁺ currents were recorded in dorsal root ganglia neurons from newborn Wistar rats with whole-cell configuration of patch clamp technique. Tetrodotoxin-sensitive Na⁺ currents were blocked by TTX 100 nM in the extracellular solution.

RESULTS and CONCLUSIONS. A dose-dependent fast blockade due to eugenol was observed in 0.2 Hz time series depolarizations from a holding potential of -110 mV to a 0 mV pulse. This tonic blockage is due to eugenol binding to the closed state. The IC₅₀ was 2.28 ± 0.10 mM for eugenol compared to 0.44 ± 0.08 mM for lidocaine. The tonic NaV blockade was more effective when the membrane was held at more depolarized, still sublimar, holding potentials. This observation indicates a higher affinity of eugenol for closed substates dwelled at less hyperpolarized potentials. No consistent evidences for additional binding to open state were observed. A displacement of steady-state inactivation curve to more negative potentials, associated with a slower recovery from fast inactivation under eugenol indicates that this molecule also binds to fast inactivated state. For currents undergoing slow inactivation, a consistent reduction by eugenol indicates that the phenylpropene additionally binds to the slow inactivated state. A frequency-dependent blocking effect of eugenol on NaV was observed, but the effect is smaller than that induced by lidocaine. In conclusion, eugenol binds to several isoforms of TTX-R NaV and to the different states of the proteins, leading to a channel blockage.

599-Pos**A Residue (W756) in the P-Loop Segment of Sodium Channel is Critical for Primaquine Binding**

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The scope of our study on wild-type and mutant-types of the voltage-dependent sodium channel in rat skeletal muscle (Nav 1.4) was to examine the possible binding site of primaquine (PQ) using a combined design and experimental approach. We applied a standard voltage-clamp in oocytes and in-silico methods, mainly protein modeling and ligand docking. Previously, we demonstrate that PQ blocks the voltage-dependent sodium current in rat myocytes, and these block is concentration-dependent and voltage-independent fashion. Direct site mutagenesis in the P-loop segment (W756C, W1239C and W1531A at the