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 −90mV to 0mV 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±6.1 % and 42.9±6.9 % (mean±sd, n=5) at 10µM. 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 phenylpropane, 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) Na+ 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 100µM in the extracellular solution.

RESULTS AND CONCLUSIONS. a dose-dependent fast blockade due to eugenol was observed in 0.2Hz time series depolarizations from a holding potential of −110 mV to a 0 mV pulse. this tonic blockade is due to eugenol binding to the closed state. the IC50 was 2.28±0.10mM for eugenol compared to 0.44±0.08mM for lidocaine. the tonic NaV blockade was more effective when the membrane was held at more depolarized, still sublimiar, holding potentials. this observation indicates a higher affinity of eugenol for closed substrates 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 blockade.