

1289-Pos Board B133**Mexiletine-responsive Erythromelalgia Due To A New Na_v1.7 Mutation Showing Use-dependent Block**

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Inherited erythromelalgia (IEM), characterized by episodic burning pain and erythema of the extremities, is produced by gain-of-function mutations in sodium channel Na_v1.7, which is preferentially expressed in nociceptive and sympathetic neurons. Most patients do not respond to pharmacotherapy, although a few patients have been reported as showing partial relief with lidocaine or mexiletine. We report here a new IEM Na_v1.7 mutation and its favorable response to mexiletine. SCN9A exons from the proband were amplified and sequenced. Whole-cell patch-clamp analysis was used to characterize wild-type and mutant Na_v1.7 channels in mammalian cells. A 7-year-old girl, with a two-year history of symmetric burning pain and erythema in her hands and feet, was diagnosed with erythromelalgia. Treatment with mexiletine reduced the number and severity of pain episodes. We identified a single nucleotide substitution (T2616G) in exon 15, not present in 200 ethnically-matched control alleles, which substitutes valine 872 by glycine (V872G) within DII/S5. V872G shifts activation by -10 mV, slows deactivation, and generates larger ramp currents. We observed a stronger use-dependent inhibition by mexiletine of V872G compared to wild-type channels. The Na_v1.7/V872G mutation provides a molecular basis for DRG hyperexcitability which can produce pain. While most IEM patients do not respond to pharmacotherapy, this patient displayed a favorable response to mexiletine, which appears to be due to use-dependent block of mutant channels. Continued relief from pain, even after mexiletine was discontinued in this patient, might suggest that early treatment may slow the progression of the disease.

1290-Pos Board B134**Biophysical Characterization Of Duloxetine Activity On Voltage-gated Sodium Channels Involved In Pain Transmission**

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Duloxetine, an inhibitor of the serotonin-norepinephrine reuptake system, is widely used for the treatment of major depression and has also been found to be effective in reducing neuropathic pain. This latter effect is believed to be related to the role of the two neurotransmitters in inhibiting pain signals at the level of spinal and supraspinal neural circuits. However the possibility that duloxetine also acts through alternative mechanisms has not yet been investigated. In this study we focused on the potential effect of duloxetine on neuronal isoforms of voltage-gated sodium channels (NaV1.3 and NaV1.7) that have a pivotal role in the generation and propagation of pain signals. Conventional whole-cell patch-clamp recordings were performed on CHO-cells expressing hNaV1.3 and hNaV1.7. Duloxetine exerted a concentration-dependent resting block, measured by the reduction of the sodium current elicited by a single depolarizing step from -100mV to 0mV for 10 ms, with IC₅₀ values of 5 and 16 microM for NaV1.3 and NaV1.7, respectively. These values were halved at holding potentials that reduce channel availability of both channels by 50%, indicating a preference for the inactivated state of sodium channels. Furthermore we observed that 10 microM duloxetine induced a 10mV negative shift of the steady state inactivation curves, and preliminary results indicate that this effect is correlated with stabilization of slow inactivation. Finally, for both sodium channel isoforms, an additional 20% inhibition over the resting block was observed at concentrations close to the IC₅₀ when the stimulation frequency was increased up to 10Hz. Thus, the antidepressant duloxetine exhibits most of the state-dependent mechanisms of a classic sodium channel blocker. These results may add valuable information about the pharmacological spectrum of duloxetine, an issue of high clinical impact considering its increasingly widespread use.

1291-Pos Board B135**Biophysics Of Inhibition Of hNav1.3 And hNav1.7 Channels By A-803467**

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It has been recently published that a small molecule, A-803467, selectively inhibits hNav1.8 sodium channel subtype and is also efficacious in a variety of pre-clinical pain models (ref. 1). While A-803467 blocks hNav1.8 with an IC₅₀ value of 8 nM (at half-maximal inactivation), it also inhibits other sodium channel subtypes with a selectivity ratio of 30-1000-fold. In particular, it inhibits hNav1.3 and hNav1.7 channels, which have connection to pain sensation

in animal models (ref. 2), with IC₅₀ values around 1 μM. We suggest that inhibition of these channels may also contribute to analgesic efficacy of A-803467. We therefore were interested in studying biophysical parameters of interaction of A-803467 with hNav1.7 and hNav1.3 channels. We present the data on the affinity of binding of the drug to the resting and inactivated states of the channels, as well as the on-rate of binding to inactivated channels, retardation of repriming and use-dependent block of trains of depolarizing pulses. The biophysical profile of A-803467 suggests interaction with a site distinct from the "classical" local anaesthetic receptor site in Nav channels. This profile is compared with the parameters measured for other clinically relevant analgesics.

(1) Jarvis MF, et al. Proc Natl Acad Sci USA 2007; 104:8520-85.

(2) Dib-Hajj SD, et al. Trends in Neurosciences 2007; 30 (11):555-63.

1292-Pos Board B136**Counting EGFP-Marked Ion-Channels of Voltage-Clamped Oocytes to Deduce the Single Channel Gating Charge**

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Recently we indirectly determined the gating charge of single Na-channels (qNa) to be about 6 e (Gamal El-Din et al., 2008). We compared the ratios of total fluorescence intensity to total gating charge (Ft/Qt) of EGFP marked K- and Na-channels expressed in *Xenopus laevis* oocytes. This allowed to deduce qNa from the better known K-channels (qK = 13-14 e) without the need to know the fluorescence intensity of a single EGFP (Fs). Since we used EGFP to mark and count both channel types, Fs cancelled out in the comparison.

We now attempt to determine and apply Fs to obtain the total number of channels (Nt = Ft/Fs) and from that q = Qt/Nt directly. As outlined in the above cited study, Ft was extrapolated to the whole oocyte's surface from a defined region along the circumference in order to minimize contributions from sub-membraneous channels and cytoplasmic autofluorescence. Micrographs of EGFP solutions of known concentration in a hemocytometer chamber provided a figure for Fs. However, this figure cannot simply be transferred to the situation of the oocyte images because it holds true only for EGFP molecules in frontal areas, accessible for the entire opening of the microscope objective. At lateral areas of the oocyte the fluorescence intensity is attenuated. The attenuation was determined by measuring the fluorescence intensity of control oocytes whose surface was strongly and homogeneously stained with a fluorescent dye such that autofluorescence from the cytoplasm could be neglected. Comparison of the extrapolation from either the lateral or the frontal area to the total fluorescence of the oocyte (Ft) gave an attenuation factor of 3 to 6 depending on the optics. This factor was used to obtain the number of EGFP marked channels.

1293-Pos Board B137**Measuring The Contribution Of S4 Charges On Gating Currents Of A Sodium Channel**

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Upon membrane depolarization, conformational changes in the S4 voltage sensors results in the opening of the voltage-gated sodium channel. The movement of the positively charged residues on the four distinct voltage-sensors in the membrane electric field generates a measurable transient current referred to as the gating current. The ON gating currents of the Na⁺ channel has two kinetic components and the fast component correlates well with the kinetics of fluorescence changes of probes attached to the voltage sensors of domains I, II, and III. The slow component of the gating current, however, matches with the fluorescence kinetics of probes on domain IV voltage-sensor (Chanda and Bezanilla JGP 2002 120; 629-45). In an attempt to specify the molecular origin of the slow and fast components of the gating current, we neutralized the first three charged residues (Q3 mutants) in each of the four voltage-sensing segments of the sodium channel. Our data supports the hypothesis that the voltage-sensor of domain IV primarily contributes to the slow component of the gating current and provides support to the notion that the movement of this voltage sensor is slower than the opening of the pore.

1294-Pos Board B138**Gateless Gating Model vs. Gated Pore Model: Phase Pinning of Guanidinium Toxins in Sodium Channels**

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Unlike the gated pore model, which assumes the existence of a movable gate within the molecule, the gateless gating model (H. R. Leuchttag, *Voltage-Sensitive Ion Channels*, Springer 2008) (VSIC) explains ion-channel gating as a phase transition. Under this model, the closed channel conformation imposed