590-Pos

Using Patchxpress to Screen Blockers of the Cardiac Sodium Channel (Nav1.5) for Effects on Late INA, Peak INA, and Channel Kinetics

Kim W. Chan[1], Hilary Zou[1], Josephine Salcedo[1], Malcolm McGregor[2], Dmitry Koltun[1], Elfatih Elzein[1], Eric Parkhill[1], Xiaofen Li[1], Tetsuya Kobayashi[1], Rao Kalla[1], Robert H. Jiang[1], Jeff A. Zablocki[1], John Shryock[1], Luiz Belardinelli[1], Cathy Smith-Maxwell[1].
[1]Gilead Sciences, Foster City, CA, USA, [2]Accelrys, Inc, San Diego, CA, USA.

The Nav1.5 channel is responsible for the upstroke of the action potential in cardiac myocytes. Congenital mutations in hNav1.5 have been shown to impair fast inactivation, leading to an increase in persistent sodium current (late INa) and LQT3. Growing evidence suggests that an increase in late INa can contribute to the pathogenesis of several cardiac diseases. With the goal of finding small molecules that selectively target cardiac late INa over peak INa and have rapid unbinding kinetics, we developed three novel assays on PatchXpress using HEK-293 cells stably expressing hNav1.5. The first assay measures block of late INa generated by tefluthrin, a pyrethroid that impairs fast inactivation. We chose tefluthrin over another late INa activator, the sea anemone toxin ATX-II, because with tefluthrin late INa reaches steady state faster and more reliably. Importantly, block obtained with tefluthrin and ATX-II is usually similar. The second assay measures tonic and use-dependent block of peak INa stimulated at 0.1 Hz and 3 Hz, respectively. To reduce voltage clamp errors on PatchXpress due to large inward peak currents and access resistance, we used 50% series resistance compensation and reduced Na+ in the extracellular solution to 20 mM. The third assay is designed to determine the rate at which compounds dissociate from the channel and is calculated from the time-course of the decrease in peak INa when the stimulation frequency is reduced from 10 to 1 Hz. All three assays on PatchXpress were validated by comparing IC50s or off-rates for several standard Nav1.5 blockers (eg. flecainide, mexiletine, quinidine and lidocaine) to those obtained from manual patch clamp. IC50s agree within two-fold and off-rate rankings are identical.

591-Pos

A Comparative Automated Electrophysiology Study of 43 Sodium Channel Inhibitors. Distinct Types of Inhibition Correlate with Chemical Properties of Drugs

Nora Lenkey[1], Robert Karoly[1], Peter Lukacs[1], E. Sylvester Vizi[1], Morten Sunesen[2], Laszlo Fodor[3], Arpad Mike[1].
[1]Inst. of Experimental Medicine, Budapest, Hungary, [2]Sophion Bioscience A/S, Ballerup, Denmark, [3]Gedeon Richter Plc., Budapest, Hungary.

A comparative electrophysiological study of 43 drugs was carried out using rNav1.2 expressing HEK 293 cells and the QPatch automatic patch-clamp instrument. IC50 values were calculated for all drugs, and for the 35 drugs which caused substantial inhibition of sodium channels, onset/offset kinetics, reversibility and use-dependence were also determined. Based on these properties, sodium channel inhibitor drugs could be classified into distinct types. We have observed that properties of inhibition correlated with chemical properties, and that categories based on properties of inhibition often concurred with therapeutic categories. For example, a subset of antidepressants (tricyclic compounds and selective serotonin reuptake inhibitors) inhibited sodium channels in a special way (with high affinity, slow kinetics, poor reversibility and use dependence), which was clearly different from the inhibition caused by classic sodium channel inhibitors (low affinity, fast kinetics, and good reversibility). Recording multiple properties of inhibition did not make our measurements more costly or time consuming, but provided additional information. By the help of this extra information, we have established that sodium channel inhibitors are heterogeneous, delineated specific types of inhibition, and observed correlations both with chemical properties and therapeutic profile that would be hidden if the IC50 alone was determined.

592-Pos

Light Control of Cell Excitability Using a Photochromic Blocker for Voltage-Gated Ion Channels

Timm Fehrentz[1,2], Alexandre Mourot[2], Michael Kienzler[1], Makoto Tsunozaki[2], Matthew Banghart[3], Diana Bautista[2], Dirk Trauner[1], Richard H. Kramer[2].
[1]Ludwig-Maximilians-Universität, München, Germany, [2]University of California, Berkeley, CA, USA, [3]Harvard Medical School, Boston, MA, USA.

Rendering proteins light sensitive gives the opportunity to control various cellular functions with great spatial and temporal precision. Native proteins can be photosensitized using photochromic ligands (PCLs), small light sensitive molecules that can be reversibly converted between active and inactive forms with light. We engineered a new PCL for voltage-gated ion channels, QAQ, which consists of an Azobenzene photoswitch flanked by two Quaternary ammoniums. Azobenzenes rapidly photoisomerize between cis and trans configurations upon 380 or 500 nm light irradiation, respectively. Quaternary ammoniums are internal blockers for voltage-gated sodium, potassium and calcium channels. Our studies show that intracellular QAQ blocks indiscriminitely those three types of ion channels in its trans state, while photoisomerization to the cis configuration relieves block.

QAQ is a permanently charged, membrane-impermeable molecule that needs hydrophilic pathways to reach its site of action. Loading QAQ into neurons through a patch pipette can be used to control excitability of sub-cellular domains in a single cell. More importantly, QAQ can be loaded into multiple cells at a time, passing through open TRPV1 or P2X7 receptors, two non-selective cation channels that show pore-dilation upon prolonged activation. Experiments in HEK cells and hippocampal neurons show that QAQ can enter cells only upon expression AND activation of TRPV1 or P2X7R. QAQ can also be loaded into cells naturally expressing those channels, like the sensory neurons of the dorsal root ganglia that play a crucial role in pain sensation. QAQ acts like a local anesthetic, silencing sensory neurons by blocking voltage-gated cation channels. In this context, light can then be used to restore nociception. QAQ is therefore a very promising PCL molecule with both scientific and clinical applications.

593-Pos

Is the Skeletal Muscle Sodium Channel of the Batrachotoxin (BTX)-Producing *Phyllobates aurotaenia* Poison Dart Frog Resistant to BTX?

Ludivine Frezza[1], Santiago Castano[2,1], Helberg Asencio[2,1], Walter Sandtner[3,1], Leonardo Fierro[2], Francisco Bezanilla[1], Ana M. Correa[1].
[1]The University of Chicago, Chicago, IL, USA, [2]Universidad del Valle, Cali, Colombia, [3]The Medical University of Vienna, Vienna, Austria.

Batrachotoxin is a potent toxin found in skins of *Phyllobates* frogs. The skeletal muscle Na^+ channels of *Phyllobates aurotaenia* frogs have been proposed to be resistant to high concentrations of BTX (>1μM). In order to unravel the mechanism and structural elements that confer BTX-resistance to *P. aurotaenia*, we cloned its skeletal muscle Na^+ channel, *Pa*$Na_V1.4$. As reported last year, *Pa*$Na_V1.4$ has high homology (>70%) with other $Na_V1.4$ channels especially in the membrane spanning regions. Some residues that have been identified in mutagenesis studies as critical for BTX-channel interaction in mammalian Na_V are conserved in *Pa*$Na_V1.4$. To further address the issue, we have expressed *Pa*$Na_V1.4$ in *Xenopus laevis* oocytes. We report here the functional characterization of *Pa*$Na_V1.4$, studied under voltage-clamp, and its response to BTX.

Pa$Na_V1.4$ expresses robustly. While the general characteristics of the ionic currents were similar, at room temperature *Pa*$Na_V1.4$ tended to open at more depolarized voltages, inactivated faster and currents peaked earlier than *r*$Na_V1.4$. BTX, at concentrations as high as 10μM, had a significantly lower effect on *Pa*$Na_V1.4$ currents than on *r*$Na_V1.4$. The ratio of plateau to peak currents at 80 mV was ~0.2-0.5 in *Pa*$Na_V1.4$ while >0.95 in *r*$Na_V1.4$. BTX modification of *Pa*$Na_V1.4$ occurred at a slow rate. Both activation thresholds were negatively shifted. Because most of the residues proposed to participate in the BTX effect are located in the pore lining segment (S6) of Na_V, we have also studied *Pa*/*r*$Na_V1.4$ hybrid channels with domains, S6 segments or residues swapped or exchanged.

Supported by COLCIENCIAS1106-12-13836 (LF), AHA 0725763Z (WS), and NIH GM030376 (FB) and GM068044 (AMC).

594-Pos

Molecular Link Between Voltage-Sensor Modification and Local Anesthetic Block

Manoel Arcisio-Miranda, Yukiko Muroi, Baron Chanda.
University of Wisconsin, Madison, WI, USA.

Sodium channels are a major target for many toxins and drugs including local anesthetics (LA). Gating current (Sheets and Hanck, J. Gen. Physiol.; 121(2), 2003) and fluorescence measurements (Muroi and Chanda, J. Gen. Physiol.; 133(1), 2009) show that LA binding to the pore mainly stabilizes the voltage-sensor of domains III of sodium channel in an upward (activated) conformation. The half maximal (V1/2) of the fluorescence-voltage (F-V) curves of probes attached to the voltage-sensor of domain III are left shifted by as much as 50 mV upon LA binding. To address the molecular basis of stabilization of the activated S4 upon LA binding, we systematically introduced tryptophan (or alanine) residues in the S4-S5 linker, N-terminal of S5 and C-terminal of S6 of the domain III muscle sodium channel. We examined the effect of these substitutions by voltage-clamp fluorimetry and by gating current measurements. Mutations of specific residues on the S4-S5 linker and C-terminal of the S6 segment significantly reduced or eliminated the shifts in the F-V curve upon LA binding. Furthermore, the total gating charge in the presence of LA remained unchanged in these mutants. These findings may provide insight into the structure and interactions of intermediate states during the sodium channel gating process.

Support: National Institutes of Health, AHA and Shaw Scientific Award