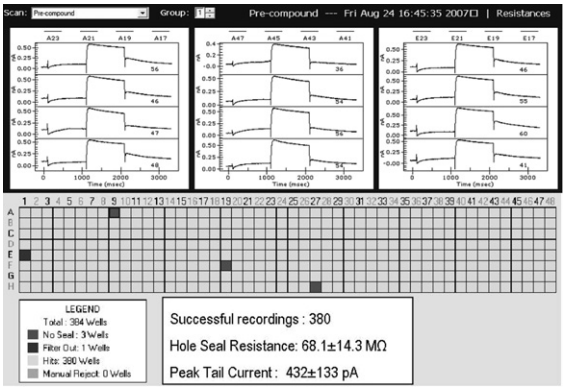
Voltage-gated K Channels - Gating I

970-Pos Board B849

Improved Early Detection of HERG Channel Liability in Lead Optimization Programs with Automated Patch-Clamp Technology

Arturo Picones, Kendra Kim, Jason Munderloh.
Exelixis, Inc., South San Francisco, CA, USA.

The HERG channel is the molecular entity directly involved in the majority of Long QT Syndrome cardiopathology cases, constituting a possible risk of life threatening arrhythmias (Torsades de Pointes). Avoidance of HERG liability is a central regulatory issue aiming to prevent costly withdrawals of potential or existing therapeutic agents. HERG safety pharmacology is an indispensable step and a high quality assay (patch-clamp electrophysiology considered as the gold standard) must accompany IND applications. Analysis of ion channel activity has been potentiated by automated planar patch-clamp within the drug discovery industry. At Exelixis we have significantly improved the early detection of HERG liability at the stage of lead optimization programs. For this report perforated-patch whole-cell recordings from cryopreserved CHO cells heterologously expressing HERG channels were obtained with IonWorks Quattro™. Channel expression was unaltered for >3 months time (>450 pA/well). Population patch-clamp wells with seal test >35 MOhms and peak currents >100 pA were included for analysis. Overall success rate consistently obtained was >95%. These improvements enabled a timely implementation of qSAR for lead compound selection and prioritization, thus avoiding potential QT prolongation liabilities at early stages.



971-Pos Board B850

Mechanism Of Action For The hERG Potassium Channel Activator, PD-118057

Matthew D. Perry, Michael C. Sanguinetti.
University of Utah, Salt Lake City, UT, USA.

Drug-induced long QT syndrome is a disorder characterized by impaired repolarization of the ventricular action potential which can lead to arrhythmia and sudden death. The most common form involves block of hERG1a channels which encode Ikr current in myocytes. Compounds which activate hERG1a channels could then provide an effective treatment for this disorder. We have studied a recently identified hERG1a activator, PD-118057, using the two microelectrode voltage clamp technique to analyze its affect on hERG1a current expressed in *Xenopus* oocytes. At 10 microM, PD118057 enhanced wild-type hERG1a current by slowing the rate of inactivation and shifting the voltage dependence to more positive potentials. Consistent with an inactivation perturbing mechanism, PD118057 failed to enhance the current magnitude of an inactivation removed hERG1a mutant channel, G628C/S631C. Unlike other hERG1a activators such as RPR260243, PD118057 did not alter the rate of hERG1a deactivation, suggesting a distinct binding site on the channel protein. Wild-type dEAG1 does not exhibit C-type inactivation and, as expected was unaffected by 10 microM PD118057. A single mutation in the S6 domain (Ala478Gly) of dEAG1 introduced a fast inactivation mechanism with similar time constants as for hERG1a channels. Ala478Gly dEAG current magnitude was enhanced by PD118057 in a concentration dependent manner through removal of the fast inactivation process. In contrast, other S6 mutations that introduce a marginally slower inactivation mechanism did not exhibit sensitivity to PD118057.

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Direct Binding Of Divalent Cations To The S4 Region In Eag Family K^+ Channels Reveals A Closed Conformation Shared With Kv1 Channels

Xiaofei Zhang[1], Badry Bursulaya[2], Christian C. Lee[2], Bihan Chen[1], Timothy Jegla[1].
[1]Dept. of Cell Biology, Institute of Childhood and Neglected Diseases, The Scripps Research Institute, La Jolla, CA, USA, [2]Genomics Institute of the NOvartis Research Foundation, La Jolla, CA, USA.

Voltage-gated K^+ channels share a common voltage sensor domain (VSD) consisting of four transmembrane helices, including a highly mobile S4 domain. Nevertheless, it is an open question as to whether functionally diverse voltage-gated K^+ channels, such as *Shaker* channels and the sub-threshold *ether-a-go-go* (EAG) family channels, will also share common conformational changes during voltage gating. EAG channels have a unique divalent binding site formed by two family-specific acidic charges in S2 and S3. However, the conformational similarity between this divalent-bound state and models of *Shaker* closed states has been difficult to assess in the absence of constraints for S4 position. We show here that divalent cations slow the activation rate of two EAG family channels (Kv12.1 and Kv10.2) by directly binding to the S4 domain and locking it to those acidic charges on neighboring helices. Histidine 328 in the S4 of Kv12.1 favors binding of Zn^{2+} and Cd^{2+}, while the homologous residue Serine 321 in Kv10.2 contributes to high affinity binding of Mg^{2+} and Ni^{2+}. This novel finding provides definitive constraints for the positions of S2, S3 and S4 in a deep closed state. Homology models of Kv12.1 and Kv10.2 VSD structures based on a closed state model of the *Shaker* family delayed rectifier K+ channel Kv1.2 match divalent binding constraints precisely. Thus our results suggest remarkable conformational conservation between EAG and *Shaker* family channels early in the activation pathway, despite large differences in voltage sensitivity, activation rates and activation thresholds.

973-Pos Board B852

Voltage-dependent Fluorescence Associated with Native-Cysteine Residues in hERG channels

Robert R. Fougere, HongJian Xu, Saman Rezazadeh, Moni Vaid, David Fedida.
University of British Columbia, Vancouver BC, BC, Canada.

Voltage-clamp fluorimetry (VCF) has been used to examine voltage sensor movement and the mechanism of coupling of activation to fast inactivation in the hERG potassium channel. Here, we used both tetramethylrhodamine maleimide (TMRM) and PyMPO maleimide as experimental fluorophores, as it is hypothesized that PyMPO experiences less steric hindrance than TMRM and is able to access cysteine residues with a greater sensitivity. Prior to examining constructs with strategically engineered cysteine residues, we looked at the fluorescence report from wild-type channels. We observed a robust fluorescence signal with a voltage-dependence that overlaid the conductance relationship. The cysteine residues readily accessible to labeling by thiol-reactive fluorophores in the wild-type channel, based on the accepted homology model, are C445 and C449; located on the S1-S2 linker. hERG channels with both C445V and C449V mutations show an attenuation of fluorescence signal. Consistent with prior reports, wild-type hERG channels treated with TMRM did not show detectable voltage-dependent changes in fluorescence. However, pretreatment with dithiothreitol (DTT), a compound that reduces disulfide bridges, prior to labeling with TMRM resulted in a resolvable voltage-dependent signal in oocytes with high levels of protein expression. The time- and voltage- dependent kinetics of the PyMPO fluorescence report from the native cysteines was investigated in terms of channel gating. The correlation between the F-V and the G-V relationship suggest that the environmental changes sensed by PyMPO attached to C445 and C449 reflect gating movements and channel opening. The contribution of these cysteine residues as a background signal must be considered when evaluating fluorescent reports from cysteine residues engineered into other domains of the channel designed to track voltage sensor movement and coupling of activation to fast inactivation.

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On the Nature of hERG Inactivation using KcsA, Shaker and Kv1.2 as Structural and Functional Models

Julio F. Cordero-Morales[1], Vishwanath Jogini[1], Anthony Lewis[1], Valeria Vasquez[1], Martin Tristani-Firouzi[2], Eduardo Perozo[1].
[1]University of Chicago, Chicago, IL, USA, [2]University of Utah, Salt Lake City, UT, USA.

In K^+ channels, C-type inactivation locks the selectivity filter in a non-conductive state. The prokaryotic proton-gated potassium channel KcsA undergoes a time dependent inactivation process. The interaction between Glu71 and Asp80 is one of the key driving forces that promote filter instability and inactivation. This interaction promotes a compression of the selectivity filter parallel to the permeation pathway, which biases it towards the inactivated