Potassium and water channels have been the wining channels for 2003 Nobel prize. The finding of the intrinsic aqueduct orifices and their vital functions in channel gating shows water flowing merges with ion activity. The orifices even exist in the newly determined atomic structure of NaK channel (Nature 440, 570-574, 2006), which belongs to another large ion channel family (cyclic nucleotide-gated channels).

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ChannelTools: A VMD Plugin For Manipulating, Visualizing and Measuring Ion Channel Properties

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A new set of software tools has been developed for manipulating, visualizing, and quantifying properties of ion channels. ChannelTools is a plugin for the Visual Molecular Dynamics (VMD) program and brings together native VMD commands and several independent software routines in one simple graphical user interface. ChannelTools allows quick and easy visualization of ion channels and employs the HOLE program to visualize the channel pore and pore lining surface. An option to apply the atomic co-ordinate standardization previously developed by the authors is implemented yielding a consistent channel orientation, axis, and geometric centre. By employing the ESFERA program, pore volume and surface area can be computed from HOLE program output at the click of a button. Cross-sectional area and radius profile data and plots can also be generated with a single button click. Several different routines for estimating channel conductance are also being developed.

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Assay Development And Screening For Modulators Of The Human Two-pore Domain Potassium Channel, TASK-3, Using Automated Electrophysiology

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The advent of high-throughput electrophysiology utilizing Population Patch Clamp™ (PPC) technology has allowed the screening of large compound libraries against ion channels representing novel targets in a variety of disease states. Here we report the design and implementation of an assay enabling the screening of 56,000 compounds against human TASK-3, a member of the two-P potassium channel family.

cDNA corresponding to the channel of interest was transfected into HEK-293 cells and a stably-expressing clone selected. Ion channel pharmacology was subsequently validated using Ruthenium Red, Lidocaine, Bupivicaine, Quinidine & pH, with all 5 standard inhibitors giving values within two-fold of reported literature values.

The biophysical properties of TASK-3 prevents the use of conventional methods of leak calculation, however, given the relatively low seal resistance routinely seen when using IonWorks® platforms in a PPC™ mode, some form of leak correction must be applied. Currents were recorded at 0mV in order to remove any effect of leak and a final addition of a supramaximal concentration of Ruthenium Red was used in order to calculate a current window from which to base the efficacy of compounds on.

Assays were performed at pH 7 (EC_{75}) in order to screen for potentiators as well as inhibitors. Data derived from the voltage step to 0mV was analysed using Genedata. Inhibitory compounds showing $<25\%$ control current and potentiating compounds showing current $>150\%$ of control were selected, giving a hit rate of 5%.

Using the approaches detailed above, this assay has provided a robust screening platform for large compound collections and could easily be configured for screening other channels where leak subtraction cannot be applied.

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Highly Parallel Automated Patch Clamp Platform For High Quality Recordings From Up To 96 Cells At A Time

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Ion channels have for several years now, received more and more interest as drug targets, because of their known involvement in chronic and acute disease. The frequently cited fact that 15% of the 100 top selling drugs target ion channels, motivates the pharmaceutical industry to search for techniques that accurately can determine ion channel function, associated with an acceptable data throughput and reasonable cost per data point. Patch clamp is the gold standard for obtaining highly relevant information about ion channels and their effectors, but is an extremely laborious technique. This has spurred the development of automated patch clamps that are capable of high quality recordings, at a much higher throughput. Up to now, several automated patch clamp platforms are commercially available which allow for high quality recordings. None of these meet the throughput requirements given by high throughput screening in drug discovery.

To meet the ever increasing demand for higher throughput in ion channel screening and safety testing we have developed a highly parallel patch clamp platform. The platform supports giga-seal recordings, continuous recording during compound application and addition of multiple compounds at each of the 96 cells recorded from at a time. The platform allows recordings in the whole-cell configuration with high success rates. Due to the open structure of the patch clamp substrate, dose-responses can be obtained, thus cutting the costs per data point to be compatible to industrial requirements. Data from different cell types and ion channels will be presented.

3470-Pos Board B517

Action Potential Peak Shape Analysis As A New Tool For Antiarrhythmic Drug Development

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Cardiac arrhythmia is a critical heart condition characterized by abnormal electrical activity of the heart. There are a wide variety of drugs which are approved for the treatment of arrhythmia; many of them are acting on voltage dependent sodium, potassium or calcium channels. One of the problems concerning determination of the major mechanism of action of antiarrhythmics is that measurement of their effects on the different ion channels is time-consuming and usually done utilizing different experimental conditions. In order to counter this problem, in this study we applied a novel method, action potential shape analysis, to determine the effect of selected antiarrhythmics on voltage dependent sodium, potassium and calcium channels without performing time-consuming voltage-clamp experiments on each ion channel. Our method is based on fitting ion channel parameters to intracellularly or extracellularly recorded action potentials in a realistic model of NG108-15 cells and quantifying drug effects through their action on the shape of the action potentials and consequently on the fitted ion channel parameters. For this study we selected four drugs, quinidine, lidocaine, encanide, and amidarone, representing Class Ia, Ib, Ic and Class III antiarrhythmics, respectively. Quinidine, encanide and amidarone blocked both sodium and potassium channels, while lidocaine, at the measured membrane potential, shifted the activation of sodium channels in a depolarizing direction. Amidarone showed profound calcium channel blocking properties. Our work is a first step towards establishing a new assay system, based on the analysis of the shape of intracellularly and eventually extracellularly recorded action potentials, which can be used for fast quantitative analysis of drug effects on ion channel currents and classification of antiarrhythmics, and also for measurement of possible cardiac side effects of drug candidates

3471-Pos Board B518

MarkoLAB: A Graphical Interface To Study Stochastic Channel Behavior

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The most studied feature of an ionic channel is the current flowing through it. This happens when the channel moves into the open state, but most of the time, as revealed by the low open probability values, the channel transitions between closed or inactive states. By focusing only when the channel enters or leaves the open state the most of its activity is being missed. To have a better representation of the total behavior of the channel we constructed a computer program (MarkoLAB) that creates a 3-D plot with all channel's states. In this graphical interface each state is represented with a column. The height of the column is proportional to the occupancy level. During voltage clamp simulation the transition between states are visualized as changes in the columns' height. This dynamic plot provides more complete information about the channel behavior and illustrates the stochastic nature of the transitions. Furthermore the macroscopic current is simultaneously shown allowing the user to link the single channel activity with the overall result of the ensemble currents. The program was developed in LabVIEW language and the stochastic transitions were implemented with a Monte Carlo simulation. This first version of the software covers three typical channels under voltage clamp conditions: The rapid sodium channel (I_{Na}), the slow activated potassium channel (I_{Ks}) and the L-type calcium channel. (I_{CaL}). MarkoLAB is an original tool to gain insight of the channel kinetics and illustrates more clearly concepts as recovery from inactivation or distinguish between voltage-dependent versus calcium-dependent inactivation. This novel representation of channel activity constitutes a powerful aid to demonstrate effect of gene mutations or drugs on the channel function.