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Comparison of Computationally and Experimentally Determined Single I_{Kr} Channel Activity During Pacemaking in Sinoatrial Node Cells

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Background: The contribution of the rapidly activating delayed rectifier current (I_{Kr}) to sinoatrial (SA) node pacemaking, is mainly derived from computational models. The mathematical representation of I_{Kr} therein, is based on voltage-clamp data in SA-node cells, but to what extent computational I_{Kr} activity accurately describes true dynamic behavior of I_{Kr} during the SA-node action potential (AP), remains to be established.
Methods: With the dual electrode patch clamp technique, we simultaneously recorded spontaneous APs (whole-cell) and single I_{Kr} channel activity (cell-attached-patch) from isolated rabbit SA-node cells. To allow comparison between measured I_{Kr} channel activity and computational I_{Kr} activity, a model rabbit SA-node cell (Zhang et al. Am J Physiol Heart Circ Physiol. 2000; 279: H397-H421) was action potential clamped by our experimentally recorded APs.
Results: In experiments, I_{Kr} channel openings were detected during AP repolarization and diastolic depolarization. The open probability (P_o) was very low (<0.15) early during repolarization, but rapidly increased towards final repolarization, reaching a maximum of 0.27 ± 0.03 (mean $\pm$ SEM, $n = 4$) shortly before the maximum diastolic potential (MDP). During the subsequent diastolic depolarization, P_o gradually declined to a value of 0.08 ± 0.02 (mean $\pm$ SEM, $n = 4$) just before take-off of the next AP. P_o obtained from the computational model followed a qualitatively similar course during SA-node automaticity, but values were doubled. That is: P_o rapidly increased from zero to a maximal value of 0.63 shortly before MDP, and then declined to 0.18 towards the end of diastolic depolarization.
Conclusions: The computational model well-described the time- and voltage dependent changes in P_o of I_{Kr} channels during SA-nodal pacemaking, however, P_o was over-estimated by a factor of 2 in all phases of the pacemaker cycle.

TRP Channels

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Modelling the Membrane Potential Dependence on Non-Specific Cation Channels in Canine Articular Chondrocytes

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In a previous report, we showed that the predominant ion channel in potassium-free solutions was a gadolinium III (Gd^{3+}) sensitive non-specific cation channel, with functional characteristics similar to that expected for transient receptor potential (TRP) type channels.
In this study reverse transcription-PCR (RT-PCR) was used to investigate the expression of TRP channels in canine articular chondrocytes. Both a mathematical model based on the Goldman-Hodgkin-Katz voltage equation and current clamp whole-cell electrophysiology were then used to investigate the effect of these channels on membrane potential (*Vm*).
Chondrocytes isolated from canine articular cartilage were cultured for 5 days in Dulbeccos Modified Eagles Medium with 10% Foetal Calf Serum. For RT-PCR analysis, total RNA was extracted from first passage cells. Electrophysiological recording was carried out on first to third passage cells.
RT-PCR analysis of chondrocyte mRNA, and subsequent sequencing of products, showed a member of the TRP vanilloid group of channels (TRPV4) to be present; sequence homology to the human TRPV4 was 94%. We have so far failed to find mRNA for the functionally similar TRPC3 and TRPC6 channels.
Using whole-cell and single-channel data from our own experiments and the literature, our model predicts the membrane to be heavily dependent on the activity of TRPV4. Simulated block of all non-specific cation channels in the chondrocyte membrane leads to a predicted −27mV change in *Vm*. This prediction closely matches our current clamp experiments with 100μM Gd^{3+} inducing a -32 ± 1mV ($n = 6$) change of *Vm*.
Previously, large-conductance chloride and voltage-sensitive potassium channels have been reported to be important in the maintenance of chondrocyte *Vm.* The data presented here showsthat the TRPV4 channel also has a significant contribution to maintenance of the chondrocyte *Vm*.

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Regulation by Calcium of Polycystin-2 (TRPP2) and Hetero-Complexes with TRPC1

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Polycystin-2 (PC2, TRPP2) and the canonical TRPC1 are TRP superfamily members that act as non-selective cation channels, with multiple subconductance states and permeability to calcium. Recent studies from our laboratory (Zhang et al., *Hum Mol Genet*, 2009) indicated that both channels, form homo and hetero-tetramers with distinct functional properties. Both TRP channels are implicated in calcium transport and signaling events, therefore, their regulation by calcium is relevant to their role in cell function. Here we studied the effect of cytoplasmic and external calcium on the regulation of PC2, TRPC1 and PC2/TRPC1, channel function. Lowering cytoplasmic calcium (0.3 nM) with either BAPTA or EGTA inhibited PC2 channel function. This inhibition was extrinsic to the channel complex, and was dependent of external calcium concentrations. Titration of cytoplasmic calcium recovered PC2 channel activity, with a Hill coefficient of 5 and an apparent affinity constant of 1-5 nM. The addition of either BAPTA (2 mM) or EGTA (1 mM) to TRPC1 to lower cytoplasmic calcium concentrations did not inhibit the current (single channel conductance) mediated by the TRPC1 homo-tetrameric channel. In contrast, low calcium decreased TRPC1's mean open probability by 50%. In addition, low cytoplasmic calcium did not inhibit PC2 channel function when hetero-complexed with TRPC1. These data suggest that the formation of structural hetero-complexes between PC2 and TRPC1 confers important regulatory changes to either channel's function, such as the sensory response to cytoplasmic calcium concentrations, thus providing functional diversity to their channel properties in endogenous environments such as the primary cilium or the plasma membrane, where they locate.

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Evaluation of the QPatch HT and HTX Systems As Methods for Ion Channel Screening

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The introduction of high throughput planar automated electrophysiology systems has brought the potential of ion channels as targets for pharmacological modulation to the forefront. Sophion Biosciences QPatch systems are fully automated electrophysiology platforms generating high resistance seals with resultant ion channel biophysical and pharmacological properties similar to conventional patch clamp recordings. In this study, using a CHO cell line stably expressing the TRPM2 channel, the performance of the QPatch HTX, which records from a population of 10 cells per recording site, was evaluated against the QPatch HT which records from a single cell per recording site.
TRPM2 can be challenging to measure, particularly when determining inhibitor potencies, due to poor recording stability characterised by current rundown. A compound set was screened against TRPM2 on the QPatch HT and HTX to assess their relative performances. There was a good correlation between the IC_{50} values generated on the two systems ($r^2 = 0.91$). The current rundown on the QPatch HTX was considerably reduced compared to the QPatch HT (19.5 $\pm$ 1.5% and 37.5 $\pm$ 3.0% respectively, mean $\pm$ SEM, $n = 4$ experiments, 767 cells profiled). This resulted in an increase in the number of successful recordings obtained from 51.6 $\pm$ 7.1% for HT to 83.1 $\pm$ 4.5% for HTX (Mean $\pm$ SEM, $n = 4$ experiments, 912 cells profiled) for a 5 point IC_{50} assay and from 67.2 $\pm$ 8.9% for HT to 98.6 $\pm$ 1.4% for HTX (Mean $\pm$ SEM, $n = 3$-4 experiments, 480 cells profiled) for a single concentration assay.
The QPatch HT has made a significant impact on the volume of high quality electrophysiology data that can be generated. This preliminary study suggests that the QPatch HTX system can further enhance throughput, particularly with channels where current stability is problematic.

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Activating Mutations Reveal a Role of TRPML1 in Lysosomal Exocytosis

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The contents of lysosomes undergo exocytosis (lysosomal exocytosis) in response to an increase of intracellular Ca^{2+}. Emerging evidence suggests that lysosomal exocytosis plays important roles in a variety of cell biological functions including neurotransmitter release, neurite outgrowth, and plasma membrane repair. The putative Ca^{2+} channel in the lysosome that mediates intralysosomal Ca^{2+} release, however, has not been identified. The mucolipin TRP (TRPML) proteins are a family of endolysosomal cation channels with genetically established importance in man and rodent. Mutations of human *TRPML1* cause type IV mucolipidosis, a devastating pediatric neurodegenerative disease. In this study, we found that although TRPML1-mediated currents can only be recorded in late endosome and lysosome (LEL) using the lysosome patch clamp technique, several proline substitutions in TRPML1 (such as $TRPML1^{V432P}$) display gain-of-function (GOF) constitutive activities at both