1544-Pos Board B454

Chronic PKC Activation Inhibits the Repolarizing Cardiac Current by Decreasing the Functional ion Channel Expression at the Plasma Membrane

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Background: Cardiac arrhythmias are particularly common in heart failure patients. The slow delayed rectifier K+ current (IKs) is decreased in patients and in animal models of heart failure. However, the molecular mechanisms for IKs decrease are not known. PKC signaling is one of the most prominent pathways activated during heart failure, with both expression and activation of PKC being increased. Hypothesis: Chronic PKC activation inhibits IKs and delays cardiac repolarization, contributing to arrhythmogenic risk in heart failure. Methods: Human IKs channels, PKCalpha catalytic subunit (CA) and alpha1A-adrenoceptor (alpha1A-AR) were expressed in HEK293T cells. IKs current was measured by patch clamp. Subcellular localization of GFP-tagged IKs channels was observed by confocal microscopy. Results: IKs currents were strongly inhibited by either overexpression of PKCalphaCA or 2-hr alpha1A-AR stimulation by phenylephirne (Fig. 1A), although acute application of Ca2+-dependent PKC (cPKC) activator peptide or alpha1A-AR stimulation increased IKs. Plasma membrane localization of GFP-tagged IKs channels was significantly decreased by chronic alpha1A-AR-cPKC signaling activation (Fig.1B). Application of PKC inhibitors or mutation of a putative PKC phosphorylation site in the auxiliary KCNE1 subunit abolished this effect. Conclusion: Chronic PKC activation inhibits IKs currents by decreasing channel plasma membrane expression via direct phosphorylation of KCNE1 subunit. Our results suggest that cPKC-dependent phosphorylation of the IKs channel may contribute to QT prolongation and arrhythmogenesis in heart failure and implicate cPKC inhibitors as potential novel antiarrhythmic drugs.

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Hydrogen Peroxide Increases K_V Currents by Altering Intracellular Glutathione Redox Status in Rat Mesenteric Arterial Smooth Muscle Cell

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Hydrogen peroxide (H_2O_2) was reported as an endothelium-derived hyperpolarizing factor in mammalian arteries. However, the effect of H_2O_2 on voltage-gated K^+ (K_V) currents, which are reportedly the primary regulator of resting membrane potential in many arterial smooth muscles including rat mesenteric artery, is unclear.

In this study, using whole-cell patch-clamp technique, we examined the effect of H_2O_2 on the Kv currents of rat mesenteric arterial smooth muscle cells (MASMCs). H_2O_2 increased the Kv currents in major portion of MASMCs, whereas a slight decreasing effect of H_2O_2 on the Kv currents was evident in minor portion of MASMCs. As a reason for this heterogeneity, we hypothesized that heterogeneity exists in the basal intracellular redox status of MASMCs and that the effects of H_2O_2 on the Kv currents are intracellular redox-dependent. Pipette application of either H_2O_2 or oxidized glutathione (GSSG) resulted in the increased Kv currents compared with control. Under that condition, subsequent bath application of H_2O_2 decreased the Kv currents. Pipette application of glutathione reductase and NADPH for blocking conversion of GSH to GSSG prevented the increasing effect of H_2O_2 on the Kv currents. In organ chamber mechanics experiment, bath application of H_2O_2 relaxed arterial rings precontracted with norepinephrine. Pretreatment of dithiothreitol, a thiol-specific reducing agent prevented the relaxation by H_2O_2. Present results indicate that H_2O_2 activates K_V channels under reduced, physiologic conditions whereas it inhibits the channels under oxidized conditions in MASMCs. The glutathione redox system seems to primarily mediate the H_2O_2-regulation of the Kv channels.

Key words: Hydrogen peroxide; Mesenteric arterial smooth muscle cell; Kv channels; Redox; Glutathione.

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Phosphorylation Regulates Gating and Channel-Protein Interaction in the Slack Kna Channel

Matthew R. Fleming, Leonard K. Kaczmarek.

Na+-activated potassium (KNa) channels encoded by the Slack and Slick genes contribute to neuronal adaptation during sustained stimulation and regulate the accuracy of timing of action potentials. Activation of protein kinase C (PKC) increases the amplitude of Slack-B currents and slows their rate of activation. We have now identified a specific residue that is required for modulation by PKC and have found that phosphorylation alters the interaction of Slack channels with other components at the plasma membrane. Using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) on immunopurified Slack-B protein, in combination with site-directed mutagenesis, we have found that at least three specific residues in the Slack protein are phosphorylated in the basal state, and that only one site (S407) is absolutely required for PKC activation to alter the gating of Slack-B channels. The Slack protein is known to interact with a variety of cytoplasmic signaling molecules, including FMRP, the Fragile-X Mental Retardation Protein. We have now also found that phosphorylation may modify interactions of Slack with these cytoplasmic components, Using resonance wavelength grating optical biosensors (the Corning Epic system and the SRU Biosciences BIND system), we have determined that the pharmacological activation of Slack channels by bithionol produces a sustained decrease in mass distribution close to the plasma membrane. Changes in mass distribution are specific to Slack channel because activation of the very closely related Slick channel with bithionol does not alter mass distribution. Moreover, when Slack channels are treated with bithionol after pretreatment with an activator of PKC, the change in mass distribution is very significantly reduced. Our findings suggest that, in addition to regulating channel gating, phosphorylation of Slack modulates its protein-protein interactions with cytoplasmic components.

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A novel Voltage Gated Proton Channel in a Dinoflagellate

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A decade before the first voltage-clamp study of voltage gated proton channels, Fogel & Hastings (1972, *Proc. Natl. Acad. Sci. USA* 69:690-693) proposed the existence of voltage gated proton channels in dinoflagellates, where they are thought to mediate proton flux from vacuole into the scintillon, triggering a bioluminescent flash in response to mechanical stimulation. We identified a candidate proton channel gene from a *Karlodinium veneficum* cDNA library based on homology of key regions of known proton channel genes. *K. veneficum* is a predatory, non-bioluminescent dinoflagellate notorious for producing a variety of toxins (Sheng et al, 2010, *Proc. Natl. Acad. Sci. USA* 107:2082-2087); its blooms coincide with fish kills in the coastal waters of four continents. This species was recently proposed as a source of biodiesel production (Fuentes-Grünewald et al, 2009, *J. Ind. Microbiol. Biotechnol.* 36:1215-1224). We have expressed the *Karlodinium* gene heterologously in both HEK-293 and COS-7 mammalian cell lines. Patch clamp studies confirm that this gene codes for a genuine voltage gated proton channel that is activated by depolarization and is extremely selective for protons. Similar to other known voltage gated proton channels, the g_H-V relationship shifts positively when pH_o is decreased or pH_i is increased. The time constant of activation (τ_{act}) exhibits non-monotonic voltage dependence, increasing with depolarization just above $V_{threshold}$ and then decreasing with larger depolarization. In most respects, the properties of *Karlodinium* proton channels qualitatively resemble those of other proton channels, but a number of distinctive properties are currently under investigation.

1548-Pos Board B458

The Conformational Transition of Kv1.2 Voltage Sensor Domain from Molecular Dynamics Simulations

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Voltage-gated potassium channels undergo gating conformational transitions in response to variations of the potential across the membrane. They are homotetrameric proteins with each monomer formed by two structurally and functionally different domains, the Voltage Sensor Domain (VSD, helices S1 - S4), and the Pore Domain (PD, helices S5 and S6). The transition starts at the VSD, where charged residues, four arginines on S4 in particular, respond to changes in the transmembrane electric field, and is then propagated to the pore. The open and closed states of the Kv1.2 full-channel have recently been modeled and refined via extensive molecular dynamics (MD) simulations, starting from the available X-ray structure in the open state. However, the atomic details of the sequence of events along the transition connecting these states are still unknown. To investigate this transition, we employ the string method with swarms-of trajectories with all-atom MD simulations. In the string method, the transition path is represented via a chain of states, or images, in the space of a large set of collective variables, called a string. Given an initial guess for the string, this is evolved to the most probable path by using the average dynamical evolution of the collective variables at each image. Once the string has converged, we use it to compute the free energy and the rate for the transition by applying a recent variation of the milestoning method. We study the conformational transition for an isolated VSD as well as for one VSD in the full-length channel starting from the open

conformation, in the presence of explicit water-membrane environment. [Supported by NIH grant GM062342 and GM067887]

1549-Pos Board B459

Gate Closure in Kv1.5 Channels is not Dependent on the Status of the Selectivity-Filter

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Voltage-gated potassium (Kv) channels are tetramers of 6-transmembrane domain (S1-S6) α-subunits. The activation gate that seals off the ion conducting pore is under control of the voltage-sensing domain and locates at the bundle crossing of the S6 segments. After channel opening most Kv channels display slow inactivation, a process that involves rearrangements of the selectivity filter (SF) resulting in a non-conducting channel although the S6-gate is open. Recent evidence argued for a strong coupling between inactivation and activation: after gate opening the S6 segment undergoes structural rearrangements that would be transmitted up to the level of the SF destabilizing its conformation. Substituting in Kv1.5 residue T480 that locates at the bottom of the SF by an alanine generated mutant channels which instead of inactivating displayed a second open state, characterised by slowly increasing current. Several molecular dynamics (MD) simulations showed a MD trajectory that displays a hydrophobic collapse of the central cavity with S6 dynamics decreasing the pore radius of the S6-gate. The simulations further showed that the SF did constrict in WT channels but not in the mutant. Ionic current measurements of the mutant T480A channels showed that after prolonged depolarizations - pushing the channels into the second conducting state - the activation gate could close and reopen with the channel remaining in the second conducting state. To convert the channels back to their original conducting state longer repolarizing times were needed. Thus, using the T480A pore mutant we could directly determine from ionic currents that gate closure in Kv1.5 channels does not depend on the status of the SF which, as suggested by Deutsch et al. implies the existence of a channel state with both a closed gate and inactivated selectivity filter. (Support: FWO-G025608)

1550-Pos Board B460

Genetic Algorithm Based Computer Simulation Reveals a New Property of Kv1.3 Inactivation

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Kv1.3, a voltage-gated potassium channel found in human T cells, enters a non-conducting state during prolonged depolarization by the slow P/C type inactivation, whose exact mechanism is yet to be determined. We have previously shown that acidic extracellular pH (pH_e) slows the inactivation process in the presence of low extracellular potassium concentration ($[K^+]_e$), whereas speeds it in the presence of high $[K^+]_e$. Our aim was to generate a gating scheme, based on a Markov model, which is able to explain the experimental results as a continuous function of pH_e and $[K^+]_e$.

We developed a new computer program based on the Gillespie algorithm that is able to generate simulated records from given gating schemes and compare the resulting curve to the original recording, i.e. it provides an indicator for the goodness of a given scheme. Optimizing of the fitted parameters was achieved using a genetic algorithm. The algorithm was implemented in parallel and run in multi-core and multi-processor environments.

As the program enabled us to optimize the gating scheme for more than one measurement record at a time, we could conclude that our simple model having two open microstates and one inactivation pathway from both of them was unable to explain the effects of pH_e and $[K^+]_e$. Introduction of a new binary parameter and therefore the duplication of inactivation pathways was needed to fully explain the experimental results. We therefore assume that not only the binding of a single potassium ion influences the speed of inactivation, but the conducting pore can have two conformations with different conduction and inactivation properties depending on the $[K^+]_e$.

1551-Pos Board B461

Molecular Rearrangements During Slow Inactivation of the Shaker-Ir Potassium Channel

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Crosstalk between the activation and slow inactivation gates in Shaker potassium channels is now well-established. The activation gate perceives the conformation of the inactivation gate (Panyi and Deutsch, 2006, 2007). Closure of the inactivation gate speeds opening and slows closing of the activation gate, i.e., stabilizing the gate in the open configuration. If this coupling involves movement of the S6 transmembrane segment, then we predict state-dependent changes in accessibility of residues lining the channel cavity. We engineered cysteines, one at a time, at positions 470, 471, 472, 473, and 474 in a T449A Shaker-IR background and determined modification rates for the cysteine modifying reagents, MTSET and MTSEA, in the open, closed, and inactivated state of the channel. Neither reagent, applied from the intracellular side, modifies cysteines at 470-474 in the closed state. Both 470C and 474C are rapidly modified in the open state and at approximately one-tenth this rate in the inactivated state. In contrast, 471C is not modified in the open state but can be modified by MTSEA but not MTSET in the inactivated state. Residue 472C cannot be modified in any of the three states. Mutant 473C did not express current. Our findings are consistent with a rotation of S6 in the inactivated state, which increases the accessibility of residue 471 while simultaneously decreasing accessibility of residues 470 and 474. Any model of C-type inactivation in the Shaker Kv channel must conform to these experimental observations.

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Shakerir and Kv1.5 Mutant Channels with Enhanced Slow Inactivation also Exhibit [k+]O-Dependent Resting Inactivation

Yen May Cheng, Steven J. Kehl.

Previous studies have shown that in N-type-inactivation removed *Shaker* (*Shaker*IR) channels, the T449K and T449A mutations enhance slow inactivation. Additionally, these mutant channels show a loss of conductance in 0 K^+_o that was attributed to an inactivation process occurring from the closed, resting state and which we refer to as resting inactivation. Similar behaviour is also observed in Kv1.5 channels with a mutation in the turret (H463G). Although the time courses for the onset of and recovery from resting inactivation are unknown, a comparison of the kinetics for resting inactivation at -80mV and slow inactivation at 50mV may provide information on whether resting and depolarization-induced inactivation are mechanistically related. Thus, we performed an analysis of the kinetics for the onset of and recovery from resting and depolarization-induced inactivation of these mutant channels. Although the time constant for slow inactivation at 50mV (τ_{inact}) was reduced by the *Shaker*IR T449K, T449A and Kv1.5 H463G mutations, τ_{inact} was insensitive to changes in $[K^+]_o$, suggesting that the loss of conductance in 0 K^+_o was not due to accelerated slow inactivation. The time constant for resting inactivation (τ_{RI}), estimated by monitoring the decrease in the peak current evoked by a brief test pulse to 50mV after a variable exposure time to 0 K^+_o solution at -80mV, was at least an order of magnitude larger than τ_{inact}. Although the time courses for the onset of inactivation varied between mutants, for each mutant the time course of recovery from 0 K^+_o-induced resting inactivation was the same as that for recovery from slow inactivation. These results suggest that the 0 K^+_o-induced resting inactivation of these mutant *Shaker*IR and Kv1.5 channels is mechanistically related to slow inactivation.

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Voltage Sensor Immobilization in Kv1.2 Channels

Samuel Goodchild, Hongjian Xu, Christopher Ahern, David Fedida.

The voltage gated potassium channel family contains several functionally distinct isoforms which serve to shape the duration, frequency and timing of action potential firing in electrically excitable cells. Several mechanistic elements that contribute to the function of the voltage sensing apparatus are not yet fully described, especially in channels other than the prototypical *Shaker* potassium channel. We have studied voltage sensor rearrangements of Kv1.2 channels using two-electrode voltage clamp fluorometry and gating current recordings in mammalian cells. Fluorescence measurements reporting on voltage sensor movement revealed a transition into a relaxed state upon prolonged depolarization, causing a left shift in the fluorescence-voltage relationship. Gating current measurements of wild type Kv1.2 channels recorded in permeant ion free solutions ($NMDG^+_{int}$//TEA^+_{ext}) also displayed a left shifted Q-V after depolarizing pre-pulses, reflecting a stabilization of the activated state of the voltage sensor. To enable examination of the effects of different cations and processes of inactivation in Kv1.2 a non-conducting double mutant channel mimicking the permanently slow inactivated and non-conducting *Shaker* W434F, Kv1.2 (W366F, V381T) was created. Off-gating currents recorded from this channel in the presence of permeant ions displayed less voltage sensor stabilization in the activated state than the wild type channel. These data suggest that cations play a specific role in regulating voltage sensor dynamics in the Kv1.2 channel.