

*J. Physiol.* 586:2477-2486). Here we mutated two putative PKC phosphorylation sites on the human proton channel. When the mutant channels were expressed in LK35.2 cells, the response to PMA or GFX was ablated. These studies indicate that PKC phosphorylates the proton channel directly. Supported by the NIH-HLBI (HL61437), Philip Morris, and the Schmidtman Foundation.

#### 884-Pos Board B763 Differential Regulation Of The L-type Ca Channels And SERCA Pump By Type 3 Phosphodiesterase

Andriy Belevych, Dmitry Terentyev, Radmila Terentyeva, Sandor Gyorke. OSU, Columbus, OH, USA.

Inhibitors of phosphodiesterase type 3 (PDE3) are positive inotropic agents that are used for short-term management of acutely decompensated heart failure. However, the molecular mechanisms mediating the cardiac effects of PDE3 inhibitors are not clearly defined. The aim of the present study was to investigate the signaling pathways involved in the effects of PDE3 inhibition on cardiac excitation-contraction coupling in a large animal model. Confocal microscopy and patch-clamp techniques were used to monitor intracellular Ca cycling in isolated canine ventricular myocytes. PKA type 2 activity in live cells was recorded using FRET-based genetically encoded fluorescent probe. Application of cilostamide, a specific PDE3 inhibitor, resulted in a significant increase in the amplitude of depolarization-induced Ca transient. This effect had two phases: transient, developed within 10 min, and sustained, characterized by a smaller increase in the Ca transient persisting up to 3 hours. The same time-course was observed for cilostamide-mediated increase in Ca current. Cilostamide produced only a transient increase in SERCA and type 2 PKA activities. The rate of SR Ca leak measured in the presence of SERCA inhibitor, thapsigargin, was not altered by cilostamide, suggesting that ryanodine receptor function was not modified by PDE3 inhibition. Although inhibition of type 4 phosphodiesterase (PDE4) alone did not affect any of the recorded functional readouts, PDE4 inhibitors potentiated the effects of cilostamide and converted the cilostamide-mediated transient effects into sustained responses. These results suggest that PDE3 inhibition can produce the long-lasting effect on cardiac cytosolic Ca transients. This effect is predominantly mediated by an increase in the Ca current amplitude that occurs via type 1 PKA-dependent phosphorylation. The data also suggest that concomitant inhibition of PDE3 and PDE4 results in persistent stimulation of SERCA and type 2 PKA activities.

#### 885-Pos Board B764 IK<sub>s</sub> Is Activated By Both Ca<sup>2+</sup> Dependent And Independent Isoforms Of PKC

Jin O-Uchi, Alessandra Matavel, Coeli M.B. Lopes. University of Rochester School of Medicine and Dentistry, Rochester, NY, USA.

KCNQ1 is co-assembled with KCNE1 to form IK<sub>s</sub>, one of the main currents responsible for cardiomyocyte repolarization. Our data shows that IK<sub>s</sub> is regulated by stimulation of several Gq-coupled receptors both in native and heterologous systems, in a biphasic manner, showing an activation and an inhibition phase. For all receptors tested activation was blocked by the PKC inhibitor calphostin C. Mutation of a putative PKC phosphorylation motif (KCNE1(S102)) decreased 50% of the activation, suggesting phosphorylation of this residue is involved in the effect, but not precluding the contribution of other putative PKC phosphorylation sites present in the KCNQ1 subunit. Agonist-induced activation was observed in the presence and absence of intracellular Ca<sup>2+</sup> release, but the extent and kinetics of activation were dependent on intracellular Ca<sup>2+</sup> release. These results suggest possible roles for both Ca<sup>2+</sup>-independent and Ca<sup>2+</sup>-dependent PKC isoforms. To test for this hypothesis we used cell-permeable PKC activator peptides that specifically activate either the Ca<sup>2+</sup>-dependent classical PKC isoforms or the Ca<sup>2+</sup>-independent PKCδ isoform. The activator peptide for classical PKC isoforms significantly activated IK<sub>s</sub> current (@ 25% at +40mV) in HEK-293 cells within 1 min and shifted the voltage dependence of activation toward negative voltages (@ -60 mV). On the other hand, the PKCδ activator peptide strongly increased the maximal conductance of activation of the channel with slower kinetics (@ 160% at 4 min) without changing the channel voltage dependence. Our results suggest that both Ca<sup>2+</sup>-dependent and Ca<sup>2+</sup>-independent isoforms of PKC enhance IK<sub>s</sub> channel activity after GqPCR stimulation, but each isoform regulates the IK<sub>s</sub> channel in a distinct fashion, possibly through phosphorylation of different sites in the channel.

#### 886-Pos Board B765 Phosphorylation Of KAT1 C-terminus Modulates K<sup>+</sup> Uptake Activity

Aiko Sato<sup>1</sup>, Mitsutaka Taniguchi<sup>2</sup>, Hiroshi Miyake<sup>2</sup>, Taishi Umezawa<sup>3</sup>, Kazuo Shinozaki<sup>3</sup>, Derek B. Goto<sup>4</sup>, Nobuyuki Uozumi<sup>1</sup>.

<sup>1</sup>Tohoku university, Sendai, Japan, <sup>2</sup>Nagoya University, Nagoya, Japan, <sup>3</sup>RIKEN Tsukuba Institute, Tsukuba, Japan, <sup>4</sup>Hokkaido university, Sapporo, Japan.

In plants, the stomata apertures respond to various environmental signals such as light, temperature, humidity and water potential. They control the loss of water through transpiration and CO<sub>2</sub> uptake for photosynthesis. Inward-rectifying potassium channels in stomatal guard cells have been suggested to provide a pathway to K<sup>+</sup> uptake into guard cells during stomatal opening. Phosphorylation is known to modulate many K<sup>+</sup> channels involved in signal transduction cascade. The Arabidopsis thaliana K<sup>+</sup> channel KAT1 is expressed primarily in guard cells and is expected to be regulated by phosphorylation. Several putative phosphorylation target residues exist in the cytosolic region of KAT1. In this study, in vitro and in gel kinase assays demonstrated that the C-terminal region of KAT1 acts as a phosphorylation target for an Arabidopsis protein kinase in guard cells. To identify the phosphorylation target sites, several KAT1 variants have been generated which contain point mutations at putative phosphorylation target sites. To elucidate the relationship between phosphorylation of KAT1 and channel function, K<sup>+</sup> transport activities of these variants were examined in *Xenopus* oocyte and yeast systems. Several variants showed loss of the K<sup>+</sup> uptake function in both systems. These results indicate that K<sup>+</sup> uptake activity of KAT1 may be regulated by the phosphorylation of its C-terminal region.

#### 887-Pos Board B766 Modulation of the Cardiac Transient Outward Potassium Current by Alpha1-Adrenoceptors Requires Caveolae Integrity

Aintzane Alday, Janire Urrutia, Monica Gallego, Oscar Casis. Universidad del Pais Vasco, Bilbao, Spain.

The alpha1-adrenoceptor is critically involved in controlling cardiac muscle contraction and excitability. In ventricular myocytes, alpha1-adrenoceptors stimulate Gs proteins and reduce the transient outward K<sup>+</sup> current (I<sub>to</sub>) via a cAMP/PKA-mediated pathway. This alpha1/cAMP response seems to be compartmentalized as adrenoceptor stimulation increases cAMP levels only in localized membrane regions. Moreover, alpha1-agonists have no effect on I<sub>to</sub> amplitude when myocytes are pre-treated with the microtubule-disrupting agent colchicine.

We tested the possibility that the I<sub>to</sub> channel forming proteins Kv4.2 and Kv4.3, as well as the components of the alpha1/cAMP pathway colocalize within the cholesterol enriched membrane microdomains named lipid rafts.

We used freshly isolated ventricular myocytes from Sprague Dawley rats. I<sub>to</sub> current recordings were made by the whole-cell patch-clamp technique. Membrane rafts were isolated by centrifugation in a discontinuous sucrose density gradient. The presence of the different proteins was visualized by western blot techniques, and protein-protein interactions were determined by coimmunoprecipitation experiments.

Patch-Clamp recordings show that cyclodextrine, colchicine and Ht31 block the alpha1-adrenoceptor effect on the I<sub>to</sub> current. These results indicate that the I<sub>to</sub> channel is locked to the PKA by an A Kinase Anchoring Protein (AKAP), and that the signalling complex is localized in a specific subtype of lipid rafts named caveolae. Separation in density gradients and coimmunoprecipitation experiments show that the components of the alpha1/I<sub>to</sub> pathway organize into two separated groups within the lipid rafts. AKAPm, PKA and the Kv4.2/Kv4.3 channel form a supramolecular complex that interacts with caveolin-3, whereas adrenoceptor, Gs protein and AC gather in a second group also connected to the caveolin. Caveolin-3, therefore, maintains both groups of assembled proteins in close proximity, allowing the functional response of the channel to the neurotransmitter.

#### 888-Pos Board B767 Human Ether á-go-go Gene Potassium Channels Are Regulated by EGFR Tyrosine Kinase

W. WU, C.P. Lau, H.F. Tse, G.R. Li.

University of Hong Kong, Hong Kong, Hong Kong.

Human ether á-go-go gene potassium channels (hEAG1) are expressed in brain and several types of human cancers and play a critical role in neuronal excitement and tumor progression. However, functional regulation of hEAG1 channels is not understood. The present study was designed to determine whether hEAG1 channels are regulated by EGFR kinase in HEK 293 cells expressing hEAG1 gene using a whole-cell patch clamp technique and a site-directed mutagenesis. It was found that EGF (100 ng/ml) slightly increased hEAG1 current in HEK 293 cells expressing WT-hEAG1. AG556 (an inhibitor of EGFR kinase) suppressed hEAG1 current in

