Excitation Contraction Coupling I

1568-Pos Board B478
BK Channels Regulate Contraction Secondary to M2 Muscarinic Acetylcholine Receptor-Mediated Depolarization
Airway smooth muscles (ASM) contraction is primarily mediated by M2 and M3 muscarinic acetylcholine receptors. We and others have shown that one of the functions of large conductance Ca\(^{2+}\)-- and voltage-activated (BK) potassium channels and the accessory β1 subunit is to moderate ASM excitation-contraction coupling by opposing cholinergic evoked depolarization and reducing activation of L-type voltage-dependent calcium channels. Here, we have employed airway contraction studies, and a mouse knockout of the BK channel β1 subunit to investigate functional consequences specifically downstream of M2 signaling. Our results indicate that effects of BK channels are downstream of M2 signaling since M2 receptor antagonist (100 nM AF-DX 116) eliminates the enhanced contraction of β1 KO trachea. We found that the mechanism of M2 antagonist evoked relaxation of β1 KO is a reduction of excitation-contraction coupling since pretreatment with L-type Ca\(^{2+}\) channels blocker Nifedipine or with low extracellular potassium solution that hyperpolarizes membrane potential occludes effect of M2 antagonist. M2 mediated membrane depolarization and the role of BK/β1 in controlling ASM membrane potentials were directly confirmed using sharp electrode recording of membrane voltage. In summary, these results indicate that the important role of BK/β1 during M2 activation is to oppose membrane depolarization and thereby attenuating Ca\(^{2+}\)-influx through voltage dependent Ca\(^{2+}\)-channels. Blocking M2 signaling largely made BK channels superfluous to control of ASM contraction.

1569-Pos Board B479
Diverse Effects of Alpha1 Adrenoceptor Activation on E-C coupling/CA\(^{2+}\) Cycling in Canine Atrial Myocytes
Hemantha K. Koduri, Angela Kwiatek, Paloma Pina, Rishi Arora, Gary Aistrup.
Activation of α1-adrenoceptors (ARs) by phenylephrine (PE), reportedly produces both transient negative inotropy and sustained positive inotropy in ventricles, but only sustained positive inotropy in atria. Differential contributions of α1A- and α1D-ARs and coupling to G\(_1\alpha\) and/or G\(_{βγ}\) proteins has been invoked to explain such responses. Using Ca\(^{2+}\) fluorescence confocal microscopy, we have investigated the PE action produces on Ca\(^{2+}\) transients (CaTs) in canine atrial myocytes. We found that PE (10μM) typically induced a transient decrease, followed by a sustained increase in CaT peak amplitude in these myocytes (Fig. 1A), the CaT decrease was often quite brief, and the sustained CaT increase was usually accompanied by the development of subcellular CaT alternans. Repeated PE exposure could sometimes induce acute ablation of CaTs, with occasional large “escapes” (Fig. 1B). Ongoing studies are focused on elucidating the mechanistic basis for these diverse α1-AR actions using a combination of selective α1A-AR antagonists and Galphai/ii, C-terminal peptides. Findings should augment understanding of cardiac α1-AR-G-protein coupled signaling, explain the ineffectiveness/side effects of present α1-AR drugs used in heart failure and/or ischemic cardiomyopathy, as well as demonstrate the need for tissue-specific targeted drug therapy.

1570-Pos Board B480
CAMKII Mediates Cardiac Glycoside Toxicity
Luis Gonano, Yanina Rico, Alicia Mattiazzi, Martin Vila Petroff.
The positive inotropic effect produced by inhibition of Na+/K+ ATPase (NKA) with digitalis has been used for the treatment of heart failure (HF) for over 200 years. However, digitalis toxicity-related death in HF patients undermines the beneficial effect of digitalis treatment. Digitalis-induced Na\(^{+}\) accumulation results in an increase in Ca\(^{2+}\)- via the reverse mode of the Na\(^{+}\)/Ca\(^{2+}\)- exchanger (NCX) leading to enhanced SR Ca\(^{2+}\)-load. The resulting increase in SR Ca\(^{2+}\)-load would then be responsible for the positive inotropic effect and the toxic arrhythmogenic effects of glycosides. Digitalis-induced increase in Ca\(^{2+}\)-, could also activate CaMKII which has been shown to have proarrhythmic effects. Here we investigate whether CaMKII underlies glycoside arrhythmogenic effects and if so, which are the subcellular mechanisms involved. In electrically paced rat ventricular myocytes (0.5 Hz), 50 μM ouabain increased contraction amplitude by 160 ± 5%. Continued exposure to ouabain resulted in spontaneous contractile activity and Ca\(^{2+}\)-waves which persisted even in the absence of electrical stimulation. Ouabain treatment was associated with activation of CaMKII (P-CaMKII) and phosphorylation of CaMKII downstream targets, site Tr17 of phospholamban and site Tr 2835 of the ryodine receptor (RyR). Ouabain-induced spontaneous activity was prevented by inhibition of CaMKII with 1 μM KN93 but not by 1 μM of the inactive analogue KN92. Similar results were obtained using the structurally different CaMKII inhibitor, AIP (1-2.5 μM). Ouabain treatment was associated with an increase in SR Ca\(^{2+}\)-content and Ca\(^{2+}\)- spark frequency, indicative of enhanced SR Ca\(^{2+}\)- leak. KN93 suppressed the ouabain-induced increase in Ca\(^{2+}\)- spark frequency without affecting SR content. These results suggest that CaMKII mediates ouabain-induced arrhythmogenic effects probably by phosphorylating its SR targets. We speculate that CaMKII mediated phosphorylation of the RyR resulting Ca\(^{2+}\)- leak from the SR could be the underlying mechanism involved.

1571-Pos Board B481
Erk1-Mediated Development of Left Ventricular Cardiac Hypertrophy in a P21 Activated Kinase-1 (Pak1) Knockout Mouse Model
Domenico M. Taglieri, Ivana I. Knezevic, Jonathan Chernoff, R. John Solaro, Yunbo Ke.
Pak1, a serine/threonine protein kinase, plays a critical role in cardiac excitation-contraction. We hypothesize that Pak1 prevents or attenuates the development of left ventricular (LV) cardiac hypertrophy. Six wild-type (WT) and six Pak1-knockout (KO) mice were randomized into four groups of three mice each to receive subcutaneously 25μg/g/day of isoproterenol (ISO) or saline (CTRL) for seven days. Thalluspheric echocardiography showed in the Pak1-KO/ISO group vs. WT/ISO group, respectively: i) increased LV fractional shortening (%)(61.65 ± 2.72 vs. 48.02 ± 4.01; p = 0.048); ii) reduced LV chamber volume (μL) in diastole (36.77 ± 4.19 vs. 73.92 ± 3.95; p = 0.0004); iii) increased isoproterenol-induced LV cardiac hypertrophy in Pak1-KO mice (40% increase in LV mass, p = 0.049) vs. WT mice (15.2 % increase in LV mass, p = 0.07); iv) enhanced early filling deceleration time (mm/s) (−80460 ± 13620 vs. −21550 ± 3560; p = 0.014). We also generated novel evidence that Erk1 and Pak1 establish protein-protein interaction in whole cardiac tissue, as assessed by co-immunoprecipitation. Western immunoblotting of Erk1 phosphorylation in whole cardiac tissue showed maximal Erk1 activation in Pak1-KO/ISO mice vs. all other groups (phosphoErk1/totalErk1 ratio in Pak1-KO/ISO 0.80 ± 0.005 vs.: i) WT/CTRL 0.042 ± 0.02171, p<0.0001; ii) WT/ISO 0.3086 ± 0.0924, p=0.006; iii) Pak1-KO/CTRL 0.6200 ± 0.046, (p=0.013), whereas Erk1 phosphorylation was consistently reduced in lysates obtained from adrenovially-infected adult rat cardiomyocytes that express constitutively active Pak1 vs. dominant negative Pak1 (phosphoErk1/actin ratio: 7.06% vs. 73.73%, respectively). In conclusion, Pak1-null mice develop LV cardiac hypertrophy due to increased Erk1 activation, indicating a role for Pak1 as a natural inhibitor of Erk1 and a novel anti-hypertrophic signaling molecule.

1572-Pos Board B482
AAV Mediated Knockdown(KD) of Histidine Rich Calcium Binding Protein (HRC) Showed Deterioration of Cardiac Function after Transverse Aortic Constriction-Induced Heart Failure(TAC-HF)
Chang Sik Park, Hye Seon Cha, Mi Young Seo, Woo Jin Park, Do Han Kim.
HRC is a SR luminal protein that binds to both triadin and SERCA, and affects Ca\(^{2+}\) cycling in the SR (Arvanitis et al., Am. J. Physiol. Heart. Circ. Physiol. 293: H1581, 2007). In the present study, we attempted to characterize the function of HRC by AAV-mediated KD using TAC-HF model. We expected that HRC KD could recover cardiac function in failing heart (HF), because HRC by itself could enhance Ca\(^{2+}\)-uptake and Ca\(^{2+}\)-release by increased activities of SERCA2 and RyR2 in HL-1 cells. Unexpectedly, AAV-mediated HRC KD in TAC-HF showed decreased fractional shortening and increased cardiac fibrosis compared with control TAC-FH. We found that phospho-RyR2, phospho-CaMKII, phospho-p38MAPK and phospho-PLB Th17 were significantly up-regulated in HRC-KD TAC-FH. The cardiac cell death markers such as LC3A/B and active caspase 9 were also increased, consistent with our results of TUNEL assay. Collectively, the increased cytosolic Ca\(^{2+}\)- level could activate CaMKII and hence phosphorylation of p38MAPK causing the enhanced mitochondrial death pathway in TAC-FH. Our results show evidence that down-regulation of HRC is linked to deterioration of cardiomyocytes in the pathological stage. (Supported by Korea NRF Grant (2010-0002159), GIST Systems Biology Infrastructure Establishment Grant (2010) and KISTI-KREONET (2010)).

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Calumenin Inhibits Ca\(^{2+}\)-Release from Sarcoplasmic Reticulum in Murine Cardiomyocytes through a Direct Interaction with RyR2
Sanjaya K. Sahoo, In-Ra Seo, Taeyong Kim, Do Han Kim.
Sarcoplasmic reticulum (SR) luminal proteins play an important role in calcium buffering and in regulation of Ca\(^{2+}\)- release. Recently, we showed that