and is detrimental to myocardial function. We previously demonstrated that high levels of peroxynitrite decrease myocardial contraction by reducing phospholamban (PLB) phosphorylation through a protein phosphatase-dependent mechanism. However, we did not examine the direct effect of peroxynitrite on protein phosphatase activity in the myocardium or the specific protein phosphatase which is activated. Here we test: 1.) the effect of SIN-1 (peroxynitrite donor) on protein phosphatase activity in whole heart homogenates using a colorimetric assay, and 2.) the effect of SIN-1 on the interaction of PLB with protein phosphatase 1 (PP1) and protein phosphatase 2a (PP2a) using co-immunoprecipitation. SIN-1 induced a 63% increase in total protein phosphatase activity (1.6 ± 0.2 vs. 2.6 ± 0.3 nmol/min/mg, $p<0.05$ vs. Control), which was abolished with specific PP1/PP2a inhibition using okadaic acid (1.4 ± 0.2 nmol/min/mg, $p<0.05$ vs. SIN-1). Since okadaic acid prevented the effects of SIN-1, we next examined the effect of SIN-1 on the interaction of PLB with PP1 and PP2a. SIN-1 increased the interaction of PLB with PP2a by 350% ($0.6 \pm$ 0.3 vs. 2.7 ± 0.7 A.U., $p<0.05$ vs. Control), but had no effect on the interaction with PP1. The peroxynitrite scavenger, urate, prevented both the SIN-1-induced increase in protein phosphatase activity and the interaction of PLB with PP2a, thus implicating peroxynitrite as the causal species. The results of this study provide further insight into the mechanism through which high levels of peroxynitrite serve to decrease PLB phosphorylation and myocardial contraction. Therefore, increased peroxynitrite production may play a key role in heart failure where protein phosphatase activity is increased and PLB phosphorylation is decreased, ultimately leading to contractile dysfunction.

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Epac Effect on the Cardiac RyR: Involvement of PLC, PKC and IP3R

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Epac is a protein directly activated by cAMP whose actions are independent of PKA. We recently show that Epac induces activation of CaMKII and phosphorylation of the Ca^{2+} release channel, the RyR, in rat cardiac myocytes. The effects included an increase in the Ca^{2+} sparks frequency and a slight decrease in the $[Ca^{2+}]_i$ transient amplitude. Here we investigated the signaling cascade from Epac activation to its effects on Ca^{2+} release. Ventricular myocytes were enzymatically isolated from rat heart ventricles. Cells were loaded with the fluorescence Ca^{2+} indicator Fluo-3 AM and viewed by confocal miscroscopy. $[Ca^{2+}]_i$ transients were evoked by field stimulation at 1 Hz. Ca^{2+} sparks were recorded in quiescent cells and SR Ca^{2+} load was estimated by rapid caffeine exposure. Epac activation was analyzed in presence of 8-CPT and of various antagonists. The possible involvement of Rap was checked on cells infected with adenoviruses coding for Rap-GAP and GFP. The results show that Rap is not involved in Epac effect on cardiomyocyte Ca^{2+} release. Inhibition of PLC by U73122 completely prevented Epac actions on Ca^{2+} sparks and $[Ca^{2+}]_i$ transients, indicating that PLC is involved in Epac actions. Blocking PKC by chelerytrine completely prevented Epac effect on $[Ca^{2+}]_i$ transient but not on Ca^{2+} sparks, suggesting that there are two separates pathways. Because PLC activation produces IP3, we checked whether activation of IP3 receptors (IP3R) is involved in Epac actions. Blockade of IP3R by 2-APB attenuated the effects of Epac on Ca^{2+} release events. Thus we conclude that activation of Epac by cAMP leads to Ca^{2+} release events modulation via a cascade involving PLC, PKC and IP3R. The resulted increase in the local Ca^{2+} release might be involved in the prohypertrophic actions of Epac on cardiac myocytes.

2647-Pos Board B617

A Quantitative Assessment Of Selective Pharmacological Inhibition Of Serca In Isolated Rabbit Working Hearts

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Decreased SERCA2a activity has been associated with contractile dysfunction in animal models of heart failure. An isolated working rabbit heart preparation and direct SERCA activity measurements were used to assess the level of SERCA inhibition necessary to terminate cardiac output under a standardised set of haemodynamic conditions. Hearts were perfused with a physiological extracellular solution whilst preload and afterload were set at $10cmH_2O$ and $85cmH_2O$ repectively. Ventricular function was assessed through the use of a pressure-volume catheter. Following initial stabilisation of cardiac function, 2.8μM thapsigargin (TG) was added to the circulating solution. Functional parameters were assessed continuously before and during application of TG. Cardiac function steadily declined in the presence of TG until the working heart configuration could not be sustained. The time to termination of aortic flow ranged from 15 to 60min. In the last minute prior to failure haemodynamic characteristics were markedly impaired (steady state *vs*. TG, n=4, $p<0.05$). Peak systolic pressure (107.78 ± 3.66 *vs*. 82.40 ± 2.15mmHg) and the maximum rate of rise of pressure (dp/dt_{max}) (1951 ± 177 *vs*. 995 ± 22mmHg.s^{-1}) were significantly reduced whilst dp/dt_{min} (-2422 ± 178 *vs*. -1470 ± 122mmHg.s^{-1}), relaxation time constant (0.028 ± 0.004 *vs*. 0.066 ± 0.009ms) and end diastolic pressure (8.33 ± 1.85 *vs*. 11.65 ± 0.62mmHg) were significantly increased. In all hearts coronary flow was maintained (80.5 ± 1.26 *vs*. 79.50 ± 1.7ml.min^{-1}). On cessation of aortic flow the left ventricle was snap frozen and homogenised in a protease-phosphatase buffer solution before biochemical analysis. Oxalate-dependent SERCA-mediated Ca^{2+}-uptake was used to assess SR Ca^{2+} uptake at a range of homogenate protein concentrations. Initial measurements indicate that termination of aortic flow occurs when SERCA activity (V_{max}) is reduced to <15% of control (DMSO vehicle). These data indicate the minimum level of SERCA activity required to sustain cardiac output in the rabbit working heart preparation.

2648-Pos Board B618

Contribution of Cycle Length History to Myocardial Contractility in Isolated Rabbit Myocardium under Physiological Conditions

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Modulation of contractile force via changes in heart rate can occur through processes that are either immediate (intrinsic) and/or through processes that involve prolonged exposure to a given situation and act via post-translational modification. Because the contractile strength of the steady state force-frequency relationship (FFR) and post-rest potentiation (PRP) involve both instant intrinsic responses to cycle length as well as slower acting components such as post-translational modification based mechanisms, it remains unclear how cycle length intrinsically affects cardiac contraction and relaxation. To dissect the intrinsic impact of cycle length changes from slower acting signaling components of the FFR, twitch contractions of right ventricular rabbit trabeculae at 5 different cycle lengths were randomized around a physiological stimulation baseline of 2.85 Hz. Patterns of previous cycle lengths that resulted in changes in force and/or relaxation times were identified. We found that the duration of the cycle length prior to the analyzed twitch contraction (primary) positively correlated with force. In sharp contrast, the cycle length one more removed from the analyzed twitch (''secondary'') was found to have a negative correlation with force. The ''tertiary'' cycle length impacted force similar to the secondary cycle length, albeit with a lesser magnitude. Using this novel stimulation protocol we can quantify the intrinsic effect of cycle length on contractile strength, as well as avoiding run-down and lengthiness that are often complications of FFR and PRP assessments. The data show that the history of at least 3 cycle lengths prior to a contraction influences myocardial contractility under near physiological conditions, and the secondary/tertiary cycle lengths affect cardiac twitch dynamics in the opposite direction than primary cycle length with decreasing importance as the cycle length is further removed from the current beat.

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Nitroxyl (HNO) Modifies Cysteine Residues in Phospholamban to Increase Myocyte Ca^{2+}-Cycling and Contractility

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HNO donors enhance cardiac inotropy by increasing SR Ca^{2+} re-uptake/release. Given its thiophylic nature, HNO likely modifyies critical cysteine residues in E-C coupling proteins. Phospholamban (PLN) is a potential target for HNO, and its genetic removal or mutation of PLN cysteines should abolish/blunt HNO cardiac effects. Cardiomyocytes were isolated from PLN knockout (PLN-/-) and wildtype (WT) mice, field-stimulated and assessed for Ca^{2+} transients and sarcomere shortening (SS). HNO effects on the SR-Ca^{2+}ATPase (SERCA2a) were evaluated by isolating SR vesicles from PLN-/- and WT mice and measuring Ca^{2+} uptake by stopped-flow mixing. Dephosphorylation of SERCA2a (a measure of E_2P hydrolysis) was investigated in ER microsomes from Sf21 insect cells expressing SERCA2a ± PLN (WT or Cys 36-41-46->Ala mutant). PLN-/- myocytes showed enhanced myocyte contraction and a blunted response to isoproterenol. When challenged with the HNO donor