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It is believed, but not without dispute, that activation of PKB is essential to obtain cardioprotection by ischemic preconditioning (IP). Here we have investigated the role of PKB activity in ischemic myocardial injury and IP using novel specific PKB inhibitors, examined whether any effect is species-dependent and determined its location in the transduction pathway. The specific PKB inhibitors VIII (0.05, 0.5 and 5μM) and XI (0.1, 1 and 10μM) were co-incubated with rat ventricular myocardium for 20min prior to 90min ischemia/120min reoxygenation at 37°C (n=6/group). CK release and cell necrosis and apoptosis (% of nuclei) were significantly decreased by more than 60% at all concentrations of both inhibitors. Similar protection was obtained with IP, results that were unaffected by PKB inhibitors. The PI-3K inhibitors LY294002 (10μM) and wortmanin (0.1μM) administered for 20min prior to ischemia induced identical results to those seen with PKB inhibitors. The protection afforded by PKB inhibitor XI was unaffected by the presumed mitoK_{ATP} channel blocker 5-HD (10μM) but was abrogated by the p38MAPK inhibitor SB203580 (10μM). Western Blot and Proteome Profiler studies confirmed a decrease in PKB phosphorylation in myocardium exposed to IP, wortmanin and PKB inhibitor XI. Studies using human myocardium also showed that both PKB inhibitor XI (1μM) and PI-3K inhibitor wortmanin (0.1μM) equally reduced CK release and cell necrosis and apoptosis. The diabetic myocardium, that could not be protected by IP or diazoxide (100μM), was however protected by PKB inhibitor XI and wortmanin, further suggesting that PKB is located beyond the mitochondria. In conclusion, inhibition of PKB activity is protective against ischemic injury of the rat and human myocardium and is as potent as IP. Importantly, PKB is downstream of the 5-HD target but upstream of p38MAPK.

3523-Pos Board B570
Pregnancy-induced Physiological Heart Hypertrophy Is Associated With Lower P38 Activity And Higher Phospho-akt Nuclear Labeling
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We have previously characterized physiological heart hypertrophy which occurs during pregnancy in mice. Hypertrophic stimuli, including volume overload, mechanical stretch, together with hormonal changes are potential triggers of pregnancy-induced heart hypertrophy[1]. The underlying molecular mechanisms of pregnancy-induced heart hypertrophy, which makes the heart work more efficiently, are not well understood. The mechanical stretch of cardiomyocytes can activate second messengers such as mitogen-activated protein kinase (MAPK). MAPK will facilitate the phosphorylation of extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK) and P38. The protein kinase Akt, which regulates the growth and survival of many cell types, has been proposed to be required for physiological heart hypertrophy. Here we performed Western Blot analysis together with high resolution confocal microscopy as to measure protein levels and subcellular distribution of cardiac MAPKs (P38, JNK1/2, ERK1/2) and Akt in non-pregnant (NP, at diestrus stage, as this stage has been exposed to low levels of estrogen for the longest time) and late pregnant (LP) mice. Western Blot analysis of heart lysates showed that only phospho-P38 protein levels were decreased ~ 2 fold at the end of pregnancy (n=7 NP and n=5 LP mice). High resolution confocal microscopy showed that P38, phospho-P38, JNK1/2, phospho-JNK, ERK1/2 and phospho-ERK were distributed in discrete clusters in the cytoplasm, T-tubules as well as in the nucleus, and their subcellular distribution did not change with pregnancy (n=3 NP and n=3 LP mice). As expected, for a protective Akt activity, nuclear phospho-Akt labeling was significantly higher in LP compared to NP, forming discrete aggregates in the nuclear region.
1. Eghbali M, Deva R, Alioua A, Minosyan TY, Ruan H, Wang Y, Toro L, Stefani E. Molecular and functional signature of heart hypertrophy during pregnancy. *Circ Res*. 2005;96:1208-16.

3524-Pos Board B571
Increased Activity of NADPH Oxidase Contributes to Enhanced LV Myocyte Contraction in nNOS$^{-/-}$ Mice
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Superoxide production from NADPH oxidases has increasingly been shown to play an important role in myocardial signalling. The activity of myocardial NADPH oxidases is known to be increased in the failing myocardium; however, whether this is a compensatory or maladaptive mechanism remains to be established. Gene deletion of the neuronal nitric oxide synthase (nNOS) is associated with an increase in myocardial superoxide production and with enhanced inotropy. Here we tested whether nNOS gene deletion leads to an increase in myocardial NADPH oxidase activity, which - in turn - causes a superoxide (O_2^-)-dependent increase in contraction.
As expected, O_2^- production (measured by lucigenin 5μmol/L -enhanced chemiluminescence) was greater in nNOS$^{-/-}$ LV myocytes than in their wild type littermates (nNOS$^{+/+}$). Pre-incubation of LV myocytes with the NADPH oxidase inhibitor apocynin (100 μmol/L, 30 min) reduced the level of O_2^- in nNOS$^{-/-}$ myocytes only, thereby abolishing the difference between genotypes. In agreement with these findings, apocynin significantly reduced cell shortening (%, field stimulation at 3Hz, 35°C) only in nNOS$^{-/-}$ myocytes. Inhibition of protein kinase A (amide 14-22, PKI, 2 μmol/L) reduced contraction to a larger extent in nNOS$^{-/-}$. The effects of PKA inhibition were abolished after pre-incubation with apocynin. NADPH oxidase stimulation by endothelin-1 (ET-1, 10 nM, 5-10 min) caused an increase in cell shortening in both nNOS$^{-/-}$ and nNOS$^{+/+}$ myocytes, which was abolished by apocynin. PKI significantly reduced the effect of ET-1 in both genotypes.
Taken together, these findings suggest that nNOS-derived NO may tonically inhibit the activity of NADPH oxidase in murine LV myocytes and indicate that production of O_2^- by this oxidase system may account for the PKA-dependent increase in cell shortening in nNOS$^{-/-}$ mice.

3525-Pos Board B572
Activation of the Cardiac Sarcolemmal ATP-sensitive Potassium Channel by A_1 and A_3 Receptor Agonists: Confirmation from knockout mice studies
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Activation of the A_1 adenosine receptor (AR) provides cardioprotection against ischemia/reperfusion injury most likely by facilitating opening of the cardiac sarcolemmal K_{ATP} (sarcK_{ATP}) channel. Recently, A_3AR agonists have also been reported to protect the myocardium against ischemia/reperfusion injury. Though the functional coupling between the A_1AR and sarcK_{ATP} is well documented, the coupling between the A_3AR and the sarcK_{ATP} channel is unknown due to a lack of direct evidence. In the present study, we characterized the ability of the respective AR agonists to elicit opening of the sarcK_{ATP} channel. To activate A_1 or A_3AR, CPA (1μM) or CP-532,903 (1μM), respectively, were used. Whole-cell sarcK_{ATP} channel current, I_{KATP}, was recorded from ventricular myocytes enzymatically isolated from hearts obtained from wild-type (WT) and A_1 and A_3AR gene knock-out (A_1KO and A_3KO, respectively) mice. In all studies, potential input from A_{2A} and A_{2B}ARs was blocked by the extracellular application of ZM 241385 (100nM) and PBS 663 (100nM). In WT myocytes, CPA and CP-532,903 elicited I_{KATP} with current densities of 2.6 ± 0.7pA/pF (mean ± SEM, n=6) and 2.4 ± 0.7pA/pF (n=7), respectively. To confirm the effects of the respective agonists, experiments were repeated in A_1KO and A_3KO myocytes. In the A_1KO myocytes, CP-532,903, but not CPA, elicited I_{KATP} with a current density of 2.2 ± 0.4pA/pF (n=6). This confirmed that the activation of I_{KATP} by CP-532,903 was via the A_3AR. On the other hand, in the A_3KO myocytes, CPA, but not CP-532,903, elicited I_{KATP} with a current density of 2.3 ± 0.7pA/pF (n=4). These results provide strong evidence of the functional coupling between A_3AR and the sarcK_{ATP} channel. They further confirm the specificities of the A_1AR and A_3AR agonists to activate the sarcK_{ATP} channel via the A_1 and A_3AR, respectively.

3526-Pos Board B573
The C-terminus of 5-HT_{2A}R Directly Interacts with the N-terminal Half of c-Src by a Tyrosine Phosphorylation Independent Mechanism
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We recently reported that the functional coupling of c-Src with 5-HT_{2A}R is an early and critical step in 5-HT-induced vascular contraction and that both proteins strongly associate with each other. Because the C-terminus of 5-HT_{2A}R can serve for signal transduction, and the N-terminal half of c-Src (residues 1-251) contains SH2 and SH3 domains known to bind associating partners, we hypothesized that the association between 5-HT_{2A}R and c-Src may occur via these domains. To address whether SH2 and SH3 domains are sufficient for c-Src interaction with 5-HT_{2A}R, a truncated c-Src construct containing SH2 and SH3 but lacking the kinase-regulatory domain (c-Src_{1-251}) was made. Coimmunoprecipitation (co-IP) showed that both wild type c-Src (c-Src_{WT}) and c-Src_{1-251} can be pulled down by 5-HT_{2A}R underscoring a role for the c-Src domain containing SH2 and SH3 in 5-HT_{2A}R-c-Src association. Additionally, it indicates that c-Src phosphorylation activity is not essential for c-Src and 5-HT_{2A}R association. However, when c-Src_{WT} and 5-HT_{2A}R are co-IPed, tyrosine phosphorylation (pY) Ab recognizes a phosphorylated protein with molecular mass identical to 5-HT_{2A}R, which is absent when c-Src_{1-251} lacking its phosphorylation catalytic domain is used. Together the data indicate that 5-HT_{2A}R interaction with c-Src and the c-Src-mediated