to wildtype animals. Upon induction of hyperglycemia in knockouts, expression of PKC δ was only slightly increased to the level found in non-diabetic wild-types. Expression, phosphorylation and translocation of PKC isoforms, however, remained unaffected by the induction of diabetes in Gα11-deficient mice. We conclude that Gα11 is central to the induction of myocardial hypertrophy in type 1 diabetes. Activation of PKC α and ζ appear to be important pathways in hypertrophic signaling via Gα11. The inhibition of this pathway may in part explain the strong therapeutic benefit of AT1 receptor blockade in diabetic patients.

3518-Pos Board B565

Methylglyoxal Impairs Brain Microvascular Endothelial Cell Function In Vivo and In Vitro

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The responsiveness of cerebral microvasculature to stimulation by intrinsic ligands is altered during diabetes. Studies attribute this to a defect in endothelial cell function. However, the etiology underlying endothelial cell dysfunction during diabetes remains poorly characterized. Here we show that methylglyoxal (MGO), a reactive carbonyl species whose production increases shortly after the onset of hyperglycemia, impairs rat brain endothelial cell function, *in vivo* and *in vitro*. When pial arterioles in anesthetized rats were exposed to adenosine diphosphate (10μM) and nitroglycerin (1μM), vessel diameters increased by 12.5 ± 0.7% and 22.0 ± 2.6%, respectively. Pre-treating arterioles with 25μM of freshly synthesized MGO for 30 min at 37^0C, significantly attenuated the response of vessels to stimulation by adenosine diphosphate (4.6 ± 1.1%), but the response to nitroglycerin was preserved (19.7 ± 1.7%). In live-cell confocal imaging employing relevant probes, acute exposure (15min) of rat brain microvascular endothelial cells to MGO (25μM-100μM) triggered dose-dependent increases in cytoplasmic and mitochondrial Ca^{2+} levels, and mitochondrial superoxide production. Acute exposure to MGO also reduced cytoplasmic nitric oxide level reminiscent of an increased cytoplasmic superoxide production as well. Incubating rat brain microvascular endothelial cells with MGO for 24hr at 37^0C MGO reduce cell viability in a dose-dependent manner with an EC_{50} of 75μM. MGO treatment also reduced the viability of brain microvascular smooth muscle cells, but the EC_{50} was shifted rightwards to 300μM. Taken collectively, these data suggest that increased production of MGO following early upregulation of semicarbazide-sensitive amine oxidase (also referred to as vascular adhesion protein1) may be an initiating cause for endothelial dysfunction during diabetes. (Work supported in part by grants from NIH to WGM and KRB)

3519-Pos Board B566

Adenosine A1 Receptor Stimulation in Mouse Heart Elicits Phosphorylation of Hsp27

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Adenosine in the heart manifests an anti-adrenergic action that limits adrenergic stimulation of the myocardium. One mechanism involves the stimulation of specific A_1 receptors (A1R) that reduces β-adrenergic-induced Gs protein cycling. This results in a reduction in adrenergic-enhanced adenylyl cyclase activity, cAMP levels, protein kinase A activation and contractile protein phosphorylation. Adenosine via A1R has been reported by this laboratory to induce the activation and translocation of PKCϵ to RACK2, and by others to induce phosphorylation and activation of p38-MAPKs. The latter exerts a negative inotropic effect possibly by phosphorylating heat shock protein 27 (Hsp27), which subsequently phosphorylates αB-crystallin resulting in a reduction in myofilament Ca^{2+} sensitivity. The hypothesis of this study is that another mechanism by which A1R limits contractile function involves the activation of a MAPK cascade and the phosphorylation of Hsp27. Mouse hearts were isolated and perfused with physiological saline. After stabilization, agents were administered for the designated times, whereupon hearts were freeze-clamped. Frozen heart proteins were resolved by SDS-PAGE, transferred and blotted with antibodies against Hsp27 and phospho-Hsp-27(Ser82). Stimulation of the heart with chlorocyclopentyladenosine (CCPA, 1μM), an A1R agonist, for 5 min increased the presence of phospho-Hsp27 by 2.6-fold, whereas treatment for 45 minutes increased it by 27-fold. Furthermore, 45 min of CCPA increased the ratio of phospho-Hsp27:total Hsp27 by 40-fold. Increases elicited by CCPA stimulation were inhibited by the A1R antagonist DPCPX (0.1 μM) and by the p38-MAPK inhibitor SB-203580 (2.64 μM). Together these data suggest that the A1R modulates heart function by eliciting the phosphorylation of Hsp27 by a signaling cascade involving p38-MAPK.

3520-Pos Board B567

Alteration of Gq-signaling in Human Heart Disease

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In this study we investigated putative correlations between key proteins of the Gq-coupled pathway and atrial arrhythmia (AA) in humans. For this purpose we used quantitative real time PCR to investigate transcription levels of various genes including: protein kinase C (PKC), phospholipase C and inositol-1,4,5-trisphosphate receptor. The mRNA was isolated from human auricles obtained from patients undergoing heart surgery that we divided into four groups:
1. patients with AA without cardio-specific medication
2. patients with AA receiving ACE- and/or beta-blocker
3. patients with sinus rhythm receiving ACE- and/or beta-blocker
4. patients with sinus rhythm without cardio-specific medication

Initial analysis of the mRNA appeared to suggest significant alterations of gene transcription with respect to the groups (1-4). Gene transcripts that were up-regulated during disease were found to be down-regulated in patients receiving cardio-specific medication. During atrial fibrillation structural remodeling occurs. Thus we investigated whether such remodeling was also reflected in the transcriptional activity of tissue specific marker genes. We found that e.g. the transcriptional activity of the PKCα gene strongly correlated with markers for endothelial cells and fibroblasts but did not show any correlation with myocyte specific markers. This finding suggested that in human atria PKCα is not expressed in the myocyte. Instead, transcriptional signals of this gene product most likely solely originate from non-muscle cells. Results such as the one described strongly suggest that qPCR analysis of entire cardiac tissue ought to be interpreted in light of possible changes of the tissue composition that usually accompanies cardiac diseases.

This work was supported by: German Science Foundation (DFG), SFB530 and KliFor196

3521-Pos Board B568

Distinct Signaling Pathways Regulate Membrane Architecture And Drive Bone Stem Cell Differentiation

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Recent research demonstrates that membrane domains on the plasma membrane of cells and the underlying cytoskeleton regulate subsequent receptor mediated signaling. However less is known about how the three dimensional plasma membrane architecture created by proteins, lipids and cytoskeleton is regulated by the cell itself. Influencing the architecture of the plasma membrane by distinct signaling pathways may be one crucial mechanism for the cell to selectively respond to external signaling stimuli. Therefore, signaling pathways must be identified that affect its assembly.

Recently, we showed that membrane receptor and domain aggregation and their dynamics drive bone stem cell differentiation toward osteoblastic lineage commitment, however we failed to identify pathways necessary for lipid, protein and cytoskeletal assembly. In order to identify these novel roles of classical pathways, we employed the Family of Image Correlation Spectroscopy, Atomic Force Microscopy followed by Fractal Analysis, roughness calculations, and molecular biology techniques to primary bone marrow stromal cells isolated from mice; C57BL/6 (B6, control) and B6C3H-1-12 (1-12, high peak bone density).

Our results revealed two signaling pathways that affect membrane morphology. These signaling pathways are activated at distinct steps during osteoblast differentiation and communication with surrounding cells. While triggering the first signaling cascade altered the topography of the cell membrane by modifying the maximum valley depth, the second pathway caused a change in the perimeter and area of distinct membrane regions. Our data suggest that osteoblast precursor cells and osteoblasts undergo physical changes in the membrane dependent on their stage of differentiation. These variations in the topography were triggered by distinct signaling pathways causing a rearrangement of the plasma membrane. These changes may be crucial for the response of the cells to external stimuli received at various timeopoints during the differentiation event.

3522-Pos Board B569

Specific Inhibition of Protein Kinase B Protects Against Ischemic Myocardial Injury in Rat and Man

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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