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.

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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)

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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.

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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.

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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.

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

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