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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

tyrosine phosphorylation of the receptor are two independent mechanisms. Examining the role of the 5-HT_{2A}R C-terminus in c-Src interaction, we found that c-Src was able to co-IP only with wild type 5-HT_{2A}R but not with a truncated mutant lacking the C-terminus indicating that 5-HT_{2A}R C-terminus carries the interaction site(s) to associate with c-Src. Furthermore, the purified recombinant 5-HT_{2A}R C-terminus pulled down purified c-Src demonstrating their direct interaction. In conclusion, 5-HT_{2A}R directly interacts with c-Src via the C-terminal end of the receptor explaining their tight functional coupling. Supported by NIH.

3527-Pos Board B574

Intracellular detection of Reactive Oxygen Species using single lanthanide nanoparticle imaging: application to vascular signaling

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Reactive oxygen species (ROS) in low concentrations mediate a variety of physiological processes. In the vascular system, Endothelin-1 (ET-1) and Platelet Derived Growth Factor (PDGF) regulate contraction and migration, respectively, by producing intracellular H_2O_2. How these signaling cascades sharing the same second messenger can lead to different physiological effects is still an open question. It possibly relies on a fine regulation of amplitude, timing and location of H_2O_2 production and thus requires an adequate sensor.

We here propose a novel method based on lanthanide nanoparticles, $Y_{1-x}Eu_xVO_4$, for the quantitative, dynamic and local detection of H_2O_2 generation in living cells. $Y_{1-x}Eu_xVO_4$ nanoparticles are photostable probes presenting a continuous emission due to fluorescence of Eu^{3+} ions. We demonstrated in vitro that photoreduction and chemical oxidation by H_2O_2 causes a fluorescence modulation. We identified the temporal response of these nanoprobes submitted to an oxidative signal and proposed a method to determine the H_2O_2 concentration based on the particle fluorescence for concentrations down to 1 μM, relevant to cell physiology, with temporal resolution down to 10-30 s. In addition, the capability of single-particle detection allows spatial resolution.

Imaging of these nanoparticles loaded in vascular smooth muscle cells by pinocytic influx revealed the production of H_2O_2 under stimulation (C_{PDGF}=7 μM, C_{ET1}=13 μM) and a notable timing difference between the two pathways. This points to a method for the cell to integrate distinct signals sharing secondary messengers. Pharmacological treatments, moreover, revealed that H_2O_2 production is partly due to rapid transactivation of EGF receptors. Such cross-talk between pathways is essential for the signal transduction.

These results constitute the first quantitative, time-resolved monitoring of H_2O_2 production and open new perspectives for the deciphering of complex signaling pathways in a variety of biological systems.

3528-Pos Board B575

Study of Electrophysiology of Thermal Shock in Higher Plants using High Speed Data Acquisition

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Vascular plants such as the soybean plant, the *Aloe vera* plant, and the *Mimosa pudica* plant have developed mechanisms in order to respond quickly to external stimuli. Throughout the history of plant electrophysiology research, several scientists have attempted to measure the speeds of electrical signal propagation in higher plants in response to thermal stress and other stressors. Several earlier researchers produced signals which were erroneously reported as 0.1 mm/s to 20 cm/s, much slower than actual propagation speeds. These incorrect signaling data resulted from aliasing effects of antiquated data acquisition systems. In this research study, new high-speed data acquisition systems were used to obtain accurate speeds for electrical signals in higher plants. Our results show solitary waves in response to localized thermal stress, with speeds of propagation measured from a few meters per second to approximately 105 m/s [1,2]. In this study, possible mechanisms for electrical signal propagation in response to heat shock are also introduced.

[1] Lang, R.D., A.G. Volkov (2008). Solitary waves in soybean induced by localized thermal stress. *Plant Signal Behav 3*, 224-228.
[2] Volkov, A.G., R.D. Lang, M.I. Volkova-Gugeshashvili. (2007). Electrical signaling in *Aloe vera* induced by localized thermal stress. *Bioelectrochem 71,* 192-197.

3529-Pos Board B576

Plant Electrical Memory

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Electrical signaling, memory and rapid closure of the carnivorous plant *Dionaea muscipula* Ellis (Venus flytrap) have been attracting the attention of researchers since the XIX century. We found that the electrical stimulus between a midrib and a lobe closes the Venus flytrap upper leaf in 0.3 s without mechanical stimulation of trigger hairs. As soon as the 8 μC charge for small trap or a 9 μC charge for large trap is transmitted between a lobe and midrib from the external capacitor, the trap starts to close at room temperature. At temperatures 28-36°C a smaller electrical charge of 4.1 μC is required to close the trap of the *Dionaea muscipula*. The Venus flytrap can accumulate small subthreshold charges, and when the threshold value is reached, the trap closes. The cumulative character of electrical stimuli points to the existence of short-term electrical memory in the Venus flytrap. We also found sensory memory in the Venus flytrap. When one sustained mechanical stimulus was applied to only one trigger hair, the trap closed in a few seconds. Prolonged pressing of the trigger hair generates two electrical signals, which stimulate the trap of *Dionaea muscipula* to close.

Membrane Transporters & Exchangers II

3530-Pos Board B577

Activation of the $Na^+/K^+/2\ Cl^-$-Cotransporter in Mammalian Skeletal Muscle

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Skeletal muscle expresses a functional active isoform of the $Na^+/K^+/2\ Cl^-$-Cotransporter (NKCC). This cotransporter is activated by an increase of extracellular osmolality. Previous studies show a linear relationship between the NKCC activity and osmolality [1]. This is contradictory to the fact, that the transport rate should saturate, when the NKCC is activated to its maximum. The aim of this study was to determine the activation curve of the NKCC activity of mammalian skeletal muscle. **Methods and results:** Activation of the NKCC has an impact on the resting membrane potential. Therefore, we measured membrane potentials of rat diaphragm and flexor digitorum brevis muscles at different extracellular osmolalities. Histogram plots of the data revealed a bimodal distribution of membrane potentials - one fraction with high (HP) and one with low (LP) membrane potentials. The means of the LP fractions at different osmolalities were depolarized to values between −50 and −60 mV, those of the HP fractions represented a sigmoidal shaped curve. A computer model of an excitable cell, in which a volume-dependent NKCC activity was incorporated, was fitted to the HP data. In rat diaphragm the NKCC is maximal activated at an extracellular osmolality of 340 mOsmol, in rat FDB this maximum occurs at an osmolality of 320 mOsmol. **Conclusion:** The apparent linear relationship between osmolality and membrane depolarization caused by NKCC activation is based on the invalid calculation of means under the assumption of a monomodal distribution. Accounting for the real bimodal distribution, we successfully revealed the assumed sigmoidal activation curve.

[1] van Mil HG et al., Br J Pharmacol. 1997, 120(1):39-44.

3531-Pos Board B578

Water Transport By The Sodium Glucose Cotransporter

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In addition to transepithelial water flow along an osmotic gradient, isosmolal fluid absorption and water transport against the osmotic gradient were also observed. Two putative mechanisms of solute-solvent flux coupling may explain these findings: (i) local osmosis due to increased solute concentration in the poorly mixed water layers adjacent to the basolateral membrane; and (ii) ''molecular water pumping'' by secondary active cotransporters. The present work aims to identify the water transport mechanism of human sodium glucose cotransporter. Thus, we stably transfected MDCK cells with the hSGLT1-EGFP fusion protein and measured their osmotic water permeability by laser scanning reflection microscopy. We also assessed water flux through confluent cell monolayers, both in the presence and absence of an osmotic gradient by detecting tiny concentration changes with scanning fluorescence correlation spectroscopy (FCS). Fitting the solution of the differential equations for the osmotic drift and for back diffusion to the experimentally determined dye distribution adjacent to the epithelial monolayer allowed calculation of the osmotic water permeability. Assessment of the single transporter permeability coefficient p_f required the simultaneous determination of hSGLT1 abundance in the plasma membrane by FCS. With 4.6×10^{-14} cm^3/sec p_f is close to the single channel permeability of aquaporin-1. Consequently, even small osmolyte concentration differences between the cytoplasm and the basolateral buffer solution are sufficient to drive a substantial water flux. Thus, the physiological importance of secondary active water transport is doubtful.