functions carried out by PKD that can best be explained by a new model that requires specific spatially-resolved subcellular targeting.

3210-Pos Board B257

Higher Aromatase Expression In Female Heart May Underline Its High Estrogen Content Resulting In Cardioprotection

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Estrogen (E2) is a well-known cardioprotective steroid hormone. Although heart has all the machinery to biosynthesize estrogen from testosterone by cytochrome P450 aromatase, little is known about the role of local heart E2 concentration [E2] in cardioprotection. We hypothesized that high heart [E2] in females could be one mechanism for the higher cardioprotection in females. We optimized the radioimmunoassay technique to measure heart [E2] in whole homogenate by diethyl ether extraction. Male mouse hearts have significantly higher E2 levels (35 ± 3 pg/ml, n=6) than plasma (12 ± 0.9 pg/ml, n=5). Heart [E2] in female mice at estrus and diestrus (diestrus 20.2 ± 1.5 pg/ml n=4; estrus 17.2 ± 0.9 pg/ml, n=4) were very similar to plasma [E2]. Interestingly, in the proestrus stage, heart [E2] was extremely high ~ 170 ± 4 pg/ml, almost 3 times higher than plasma [E2]. The final heart [E2] will depend on the testosterone level as well as the efficiency of the aromatase to convert testosterone to E2. As females have much lower levels of testosterone (~40pg/ml at estrus and diestrus and ~240 pg/ml in proestrus) compared to males (2 ng/ml), much higher heart [E2] in females at proestrus compared to male lead us to hypothesize that the aromatase expression/activity is much higher in females than males. We performed real time PCR and western blot analysis to quantify transcript and protein levels of aromatase in male and in female mice at estrus stage, as this stage is under the control of the preceding estrogen peak at proestrus. Aromatase transcript levels were similar in males and females at estrus, but aromatase protein levels were two fold higher in estrus compared to male. We speculate higher aromatase expression in females may underline its high estrogen content, thus resulting in cardioprotection.

3211-Pos Board B258

Glyceollin Attenuates Vascular Contraction By Inhibiting Rhoa/rho Kinase Pathway

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Isoflavones such as genistein and daidzein prevented agonist-induced vascular contraction in isolated rat aortic rings. Glyceollins are derived from the parent isoflavone daidzein through a series of pterocarpan intermediates. We hypothesized that glyceollin attenuates vascular contractions through inhibition of RhoA/Rho kinase pathway. Rat aortic rings were denuded of endothelium, mounted in organ baths and treated with either glyceollin (20 or 100 μM) or vehicle (DMSO) for 60 min after submaximal contraction by NaF (8.0 mM). The phosphorylation level of the myosin light chain (MLC_{20}), myosin phosphatase target subunit 1 (MYPT1) and protein kinase C (PKC)-potentiated inhibitory protein for heterotrimeric myosin light chain phosphatase of 17-kDa (CPI17) were determined by means of the Western blot. Glyceollin not only inhibited vascular contractions induced by NaF (8.0 mM), but also decreased the activation of RhoA and subsequent phosphorylation of $MYPT1^{Thr855}$ and $CPI17^{Thr38}$. These results indicate that glyceollin attenuates vascular contraction by inhibiting RhoA/Rho-kinase signaling.

3212-Pos Board B259

17β-Estradiol Attenuates Vascular Contraction Through Inhibition Of RhoA/Rho Kinase Signaling Pathway

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It is now well-known that 17β-estradiol has an endothelium-independent, non-genomic vasorelaxant action. In the present study, we hypothesized that 17β-estradiol attenuates vascular contraction by inhibiting RhoA/Rho kinase signaling pathway in rat aorta. Rat aortic rings were denuded of endothelium, mounted in organ baths, and contracted with 30 nM U46619 or 8.0 mM NaF 30 min after pretreatment with 17β-estradiol (30 and 100 μM) or vehicle. We measured the amount of GTP RhoA and the level of phosphorylation of the myosin light chain (MLC_{20}), myosin phosphatase targeting subunit 1 (MYPT1) and PKC-potentiated inhibitory protein for heterotrimeric MLCP of 17 kDa (CPI17). Pretreatment with 17β-estradiol not only inhibited U46619- or NaF-induced vasocontractions and the phosphorylation of MLC_{20} but also inhibited activation of RhoA. 17β-Estradiol also decreased the level of phosphorylation of $MYPT1_{Thr855}$ and $CPI17_{Thr38}$, downstream effectors of Rho-kinase. In conclusion, 17β-estradiol attenuates vascular contraction, at least in part, through inhibition of RhoA/Rho kinase signaling pathway.
Key Words: 17β-estradiol, RhoA, Rho kinase, CPI-17, MYPT1, vasorelaxation

3213-Pos Board B260

Modulation Of Cardiac Na+/H+ Exchange Activity By Muscarinic Agonists, Nitric Oxide and Cyclic GMP

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Na^+-H^+ exchange (NHE) is the principal acid-extrusion mechanism in cardiac myocytes. Its activity has been linked to myocardial ischaemia-reperfusion injury, arrhythmia and the development of cardiac hypertrophy. NHE is modulated acutely by intracellular pH (pH_i), as well as through phosphorylation by kinases. Nitric oxide (NO) is an important regulator of cardiac function. It is synthesised by NO synthases (NOS), which are activated by muscarinic (M_2) receptors. NO targets proteins, partly via protein kinase G, which is activated by cyclic GMP (cGMP). We studied the effect of this regulatory pathway on NHE activity in rat ventricular myocytes. Myocytes were loaded with the acetoxymethyl-ester of carboxy-SNARF-1 (a pH-reporter dye) and superfused with Hepes-buffer at 37°C. Applying a 4min, 20mM NH_4Cl prepulse deposits an intracellular acid-load that stimulates NHE. The membrane-permeant cGMP analog 8Br-cGMP (20μM), the NO donor sodium nitroprusside (1mM) and the M_2 agonist carbachol (100μM) reduced NHE activity (*vs* paired controls). At a common pH_i of 6.6, NHE inhibition was 26%, 29% and 18%, respectively ($P>0.05$). We also transfected adult myocytes with the nNOS gene using an adenoviral system (5×10^{10} viral particles, incubated overnight) to increase NO production capacity. To confirm successful gene-transfer, eGFP was transfected in separate experiments and fluorescence was detected in >90% of cells. NHE activity at pH_i=6.6 was not significantly different in nNOS-transfected cells (*vs* sham-transfected cells). However, on addition of carbachol (100μM), NHE activity was reduced by 50%. These findings illustrate an important role for the NO/cGMP pathway in modulating pH_i homeostasis.
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3214-Pos Board B261

Colocalization Of RyR And $Ca_v1.2$ In Ventricular Myocytes Is Independent Of The Physical Orientation Of The Cell

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We have investigated the effect of cell orientation on the observed colocalization of the ryanodine receptor (RyR) with the L-type calcium channel ($Ca_v1.2$) in adult rat ventricular myocytes. Cells were embedded in 2% agarose[1] and visualised with a 60X 1.2 NA water immersion objective on an Olympus FV1000 confocal microscope; all images were deconvolved before analysis. We imaged cells oriented both parallel and perpendicular to the coverslip. We found that colocalization between RyR and $Ca_v1.2$ in the two orientations was not significantly different from each other, and similar to values previously reported[2]. Cells oriented perpendicular to the coverslip provided additional details of the colocalization: The colocalized region between RyR and $Ca_v1.2$ was often surrounded by an area of RyR fluorescence, implying that the calcium channels within a dyad cover a smaller area than do the RyR. RyR that had no corresponding $Ca_v1.2$, and are presumed to be extra-dyadic, were distributed in the Z disk with no discernable pattern.
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3215-Pos Board B262

Blocking the Late Sodium Current Reduces Intracellular Sodium Accumulation During Sodium Pump Inhibition

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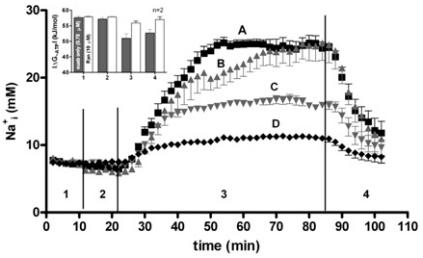
Impairment of Na^+/K^+-ATPase activity reduces sodium efflux and leads to an increase in intracellular Na^+ (Na^+_i). We tested the hypothesis that Na^+ accumulation caused by ouabain, a Na^+/K^+-ATPase inhibitor, would be reduced by concurrent inhibition of the late sodium current (I_{NaL}). We measured Na^+_i, high energy phosphates, and chemical driving force ($\Delta G_{\sim ATP}$) in isolated guinea pig hearts in real time with ^{23}Na- and ^{31}P- NMR. Hearts were pre-



Treatment: A: ouabain only (1.3μM), n=7; B: Ran (3 μM) + ouabain, n=5;
C: Ran (10 μM) + ouabain, n=8; D: TTX (1 μM) + ouabain, n=5
Timeline: 1: baseline, 2: pretreat ± drug, 3: ouabain ± drug, 4: wash out

treated for 10 min with no drug (control) or with the I_{NaL} inhibitors ranolazine (Ran; 3, 10 μM) or tetrodotoxin (TTX, 1 μM), then additionally exposed for 60 min to ouabain (1.3 μM in ^{23}Na- and 0.75 μM in ^{31}P- NMR experiments), after which all drugs were washed out for 20 min. Na^+_i was not changed by TTX or Ran alone. However, Ran (10 μM) and TTX significantly attenuated effects of ouabain to increase Na^+_i and decrease $\Delta G_{\sim ATP}$ (see Figure). The findings suggest that I_{NaL} contributes significantly to Na^+_i accumulation during exposure of myocytes to cardiac glycosides.

3216-Pos Board B263

Clinically Relevant Concentrations Of Di (2-ethylhexyl) Phthalate (dehp) Uncouple Cardiac Syncytium

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Di(2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer found in a variety of polyvinyl chloride (PVC) medical products. The results of studies in experimental animals suggest that DEHP leached from flexible PVC tubing may cause health problems in some patient populations. While the cancerogenic and reproductive effects of DEHP are well recognized, little is known about the potential adverse impact of phthalates on the heart. This study used preparations of confluent, synchronously beating cultures of neonatal rat cardiomyocytes to examine possible adverse effects of clinically relevant concentration of DEHP on cardiac tissue. Seventy two hour-long exposure to 50 μg/ml DEHP led to a marked decrease in conduction velocity and asynchronous cell beating in DEHP-treated samples but not in time-matched controls. The mechanism behind DEHP-induced changes was a loss of junctional connexin-43, documented using western blot analysis, dye-transfer assay and immunofluorescence. Use of organelle-specific connexin-43 antibodies, IF1 and CT1, allowed for further analysis of changes in intracellular distribution of connexin-43. In DEHP-treated samples the amount of gap-junctional connexin-43 (IF1-sensitive) was significantly decreased as compared to the controls. In contrast, Golgi and perinuclear (CT1-sensitive) staining was more pronounced. The data suggests that DEHP modifies connexin-43 trafficking and protein assembly into functional gap junctions, which impairs the electrical behavior of a cardiac cell network. Applicability of these findings to human patients remains to be established.

3217-Pos Board B264

Carbon Monoxide Pollution Affects Cardiac Function In Normal And Failing Hearts

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Objective : Epidemiological studies usually linked atmospheric pollution and cardiovascular events of sensitive population, mainly patients with heart failure. Accordingly, the present study tested the effect of chronic carbon monoxide (CO) pollution exposure on cardiac contractile function in normal and myocardial infarcted (MI) rats.

Methodology : 7 weeks after the left coronary artery ligation, MI and sham wistar rats were exposed for 4 weeks to ambient air or CO environment (constant 30 ppm with 5 peaks of 1 hour at 100 ppm, levels reached in regular urban area and during peak pollution, respectively). Cardiac morphology and function were evaluated by echocardiography. ECG recording were performed for investigating arrhythmias events. Excitation contraction coupling (ECC) was investigated in intact cardiomyocyte (shortening, electrophysiology, and Ca^{2+} transient) and skinned preparation (myofilament Ca^{2+} sensitivity).

Results: CO pollution increased posterior left ventricular wall thickness and decreased shortening fraction of the whole heart in Sham rats, and worsened MI rats cardiac remodeling (increase of posterior wall thickness, chamber dilation and alteration of contracting and relaxing ventricular index). The *in vivo* deleterious effects were associated with alterations of ECC: CO pollution decreased Ca^{2+} transient, involving both decrease of SR Ca^{2+} load and increase of I_{Ca}, reduced myofilament Ca^{2+} sensitivity in Sham cardiomyocytes, and aggravated the cellular alterations observed in MI cardiomyocytes. CO pollution also triggered arrhythmic events.

Conclusions: Chronic exposure to CO pollution altered cardiac morphology and function of sham rats, and worsened cardiac MI rats phenotype, by altering cellular ECC.

3218-Pos Board B265

Phosphodiesterase Activity Is Necessary But Not Sufficient For cAMP Compartmentation In Cardiac Myocytes

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The second messenger cAMP regulates a variety of activities in cardiac myocytes. However, different effectors respond to different cAMP concentrations. This supports the idea that cAMP signaling is compartmentalized. Our laboratory has previously used FRET-based biosensors to estimate cAMP levels in a membrane associated (caveolar) and bulk cytoplasmic compartments of intact adult ventricular myocytes. The results indicate that the cAMP concentration in the bulk cytoplasmic compartment is as much as 10 fold higher than that found in the caveolar compartment, even under basal conditions. The common assumption is that the concentration of phosphodiesterase (PDE) activity in specific subcellular locations is sufficient to explain such compartmentation. In the present study, we used a simple, two compartment, mathematical model to systematically evaluate the potential contribution of the following parameters in maintaining a significant cAMP gradient between membrane and bulk compartments: 1) membrane compartment volume, 2) membrane compartment surface area, 3) total adenylyl cyclase (AC) activity, 4) total PDE activity, 5) distribution of AC activity between compartments, 6) distribution of PDE activity between compartments, and 7) flux of cAMP between compartments. Although the results demonstrate that extreme heterogeneous distribution of PDE activity alone can theoretically explain cAMP gradients consistent with those observed experimentally, it requires the absolute number of PDE molecules present in the membrane compartment to exceed physical limits. Restricting the flux of cAMP between compartments can also explain observed cAMP gradients, but it requires the membrane compartment to be significantly larger than current estimates. Our results support the conclusion the PDE activity is necessary but not sufficient to explain cAMP compartmentation in cardiac myocytes. It is concluded that a flux rate significantly slower than free diffusion is also an essential factor involved in cAMP compartmentation.

3219-Pos Board B266

Anisotropic Diffusion Of Fluorescently Labeled Atp In Cardiomyocytes Determined By Raster Image Correlation Spectroscopy

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A series of experimental data point to the existence of profound diffusion restrictions of ADP/ATP in rat cardiomyocytes. To be able to analyze and estimate the role of intracellular diffusion restrictions on bioenergetics, the intracellular diffusion coefficients of metabolites have to be determined. The aim of this work was to develop a practical method for determining diffusion coefficients in anisotropic medium and to estimate the overall diffusion coefficients of fluorescently labeled ATP in rat cardiomyocytes. For that, we have extended raster image correlation spectroscopy (RICS) protocols. The extension of RICS that allowed us to study diffusion in anisotropic media is based on the fact that RICS relates spatial and temporal information in the analysis. By modifying the direction of the laser scan, we altered the relationship between spatial and temporal fluctuations. This allowed us to relate autocorrelation functions with direction of the scan thus discriminating between diffusion coefficients in different directions. Using this extended protocol, we estimated diffusion coefficients of ATP labeled with the fluorescent conjugate Alexa Fluor 647 (Alexa-ATP). In the analysis, we assumed that the diffusion tensor can be described by two values: diffusion coefficient along the myofibril and across it. The average diffusion coefficients found for Alexa-ATP were as follows: 83+/−14microm2/s in longitudinal and 52+/−16microm2/s in transversal directions (n=8, mean+/−SD). Those values are ~2 (longitudinal) and ~3.5 (transversal) times smaller than the diffusion coefficient value estimated for surrounding solution. Such uneven reduction of average diffusion coefficient leads to anisotropic diffusion in the rat cardiomyocytes. While the source for such anisotropy is uncertain, we speculate that it may be induced by ordered pattern of intracellular structures in rat cardiomyocytes.