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

Andrea Ciobotaru, Mansoureh Eghbali, Ligia Toro, Enrico Stefani.
UCLA, Los angeles, CA, USA.

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

Min-Ji Song[1], Su Bun Jeon[1], Inji Baek[1], Enyue Yang[1], In Kyeom Kim[1,2].
[1]Department of Pharmacology, Kyungpook National University School of Medicine, Daegu, 700-422, Republic of Korea, Department of Cardiovascular Research Institute, Kyungpook National University School of Medicine, Daegu, 700-422, Republic of Korea.

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

Enyue Yang[1], Su Bun Jeon[2], Inji Baek[2], Min-Ji Song[2], In Kyeom Kim[2].
[1]Department of Pharmacology, Kyungpook National University School of Medicine, Daegu, Republic of Korea, [2]Kyungpook National University School of Medicine, Daegu, Republic of Korea.

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

Ruichong Ma[1], Andrew Robertson[1], Pawel Swietach[2], Lijun Wang[1], David J. Paterson[1], **Richard D. Vaughan-Jones**[1].
[1]Oxford University, Oxford, United Kingdom, [2]Oxford, Oxford, United Kingdom.

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.
Work supported by the British Heart Foundation and Wellcome Trust.

3214-Pos Board B261

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

David R. Scriven[1], Parisa Asghari[1], Marco Lau[1], Ruth Westenbroek[2], William A. Catterall[2], Edwin D. Moore[1].
[1]University of British Columbia, Vancouver, BC, Canada, [2]University of Washington, Seattle, WA, USA.

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.
1. Chen-Izu, Y, et al. (2006) Biophys. J 91: 1-13.
2. Scriven, DR, et al. (2000) Biophys. J. 79: 2682-2691.

3215-Pos Board B262

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

Kirsten Hoyer[1], James Balschi[2], John Shryock[1], Luiz Belardinelli[1], Joanne S. Ingwall[2].
[1]CV Therapeutics, Inc., Palo Alto, CA, USA, [2]Brigham and Women's Hospital, Harvard Med School, Boston, MA, USA.

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-