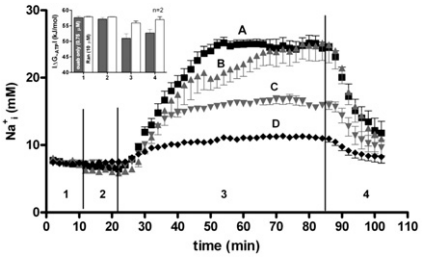

Treatment: A: ouabain only (1.3μM), n=7; B: Ran (3 μM) + ouabain, n=5;
C: Ran (10 μM) + ouabain, n=8; D: TTX (1 μM) + ouabain, n=5
Timeline: 1: baseline, 2: pretreat ± drug, 3: ouabain ± drug, 4: wash out

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.

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

Nikki Gillum, Zara Karabekian, Narine Sarvazyan.
The George Washington University, Washington, DC, USA.

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

Andre L.[1], J. Boissiere[1], C. Reboul[2], R. Perrier[1], S. Zalvidea[1], P. Obert[2], S. Richard[1], O. Cazorla[1].
[1]INSERM U-637, Physiopathologie Cardiovasculaire, Montpellier, France, [2]EA-2426, Physiologie et Physiopathologie des Adaptations Cardiovasculaires à l'Exercice, France.

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

Radu V. Iancu[1], Stephen W. Jones[1], **Robert D. Harvey**[2,1].
[1]Case Western Reserve University, Cleveland, OH, USA, [2]University of Nevada, Reno, Reno, NV, USA.

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

Marko Vendelin, Ardo Illaste, Rikke Birkedal.
Institute of Cybernetics, Tallinn University of Technology, Tallinn, Estonia.

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.