depolarization of mitochondria may be altered by extracellular glucose. Oxidative phosphorylation requires the large $\alpha\Psi$mito and when the mitochondria are depolarized, oxidative phosphorylation becomes uncoupled and ATP production falls. Here we investigate the effect of hyperglycemia (30 mM versus the physiological glucose of 5 mM) on mitochondrial $\alpha\Psi$mito under photon-induced oxidative stress in rat ventricular myocytes. Using a buffered physiologic salt solution on quiescent ventricular myocytes, changing the [glucose] has dramatic effect on the rate of $\alpha\Psi$mito depolarization. The rate of depolarization was significantly decreased in the presence of high glucose (the time-to-50% depolarization was increased from 250 s to 500s). These results were carried out in the absence of insulin. We conclude that hyperglycemia appears to protect mitochondrial function in quiescent heart cells from photon-induced oxidative stress. It is not yet clear how this apparent protection may change as metabolic load and muscle work increases, nor is it clear whether the absence of fatty acid substrates will increase or decrease this seemingly protective effect.

2502-Pos Board B488

Mitochondrial Transhydrogenase: Yin and Yang of Antioxidative Capacity in Cardiac Myocytes

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Mitochondrial production of reactive oxygen species (ROS) contributes to the pathogenesis of various diseases and aging. Superoxide ($^{\bullet}O_2^-$) is generated as by-product of the electron transport chain (ETC), rapidly dismutated to H_2O_2 and eliminated by enzymes that require NADPH. The nicotinamide nucleotide transhydrogenase (Nnt) catalyzes the reaction $NADH+NADP^+ \rightarrow NADPH+NAD^+$, which is coupled to the proton motive force across the inner mitochondrial membrane ($\Delta\mu_H$). Thus, the Nnt is considered to play a key role in regenerating NADPH and maintaining mitochondrial antioxidant capacity. Recently, a loss-of-function mutation in the Nnt gene was discovered in C57BL/6J (J-) but not C57BL/6N (N-) mice, rendering this strain glucose-intolerant due to increased ROS production in pancreatic islet cells.

Here, we analyze the role of Nnt in cardiac mitochondria of N- and J-mice by applying various techniques including fluorescence imaging, patch-clamping and EPR spin-trap measurements on isolated mitochondria or cardiac myocytes. In the absence of ADP and Ca^{2+}, $^{\bullet}O_2^-$ and H_2O_2 formation were comparable in energized mitochondria from J- and N-mice. Accelerating NADH-coupled respiration with ADP or uncoupler oxidized both NADH and NADPH in N-mice, but only NADH in J-mice, indicating that Nnt mediates NADPH oxidation through its *reverse* reaction when NADH is consumed by the ETC. This was associated with *lower* H_2O_2 and $^{\bullet}O_2^-$ formation in uncoupled mitochondria from Nnt-deficient J-mice. In intact myocytes, however, an increase in work (β-adrenergic stimulation, 5 Hz stimulation frequency) was associated with similarly increased cytosolic and mitochondrial [Ca^{2+}] and accelerated NADH regeneration by the Krebs cycle. Under these conditions, lack of NADPH regeneration via Nnt in the *forward* mode provoked *increased* H_2O_2 formation in J- vs. N-mouse mitochondria.

We conclude that in cardiac mitochondria, the Nnt either *prevents* or *promotes* ROS production, depending on the energetic state of the cell.

2503-Pos Board B489

Stat3 Regulates Mitochondrial Superoxide Generation in Response to β-Adrenergic Stimulation

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Besides its role as a transcription factor, Signal transducer and activator of transcription 3 (Stat3) is present in mitochondria and regulates respiration (*Science* 2009;323:793-7). Furthermore, Stat3-deficient mice (Stat3-CKO) develop peripartum cardiomyopathy caused by oxidative stress.

To determine the role of Stat3 in regulating redox state and reactive oxygen species (ROS) production in cardiac mitochondria, myocytes from mice with cardiac-specific deletion of Stat3 (Stat3-CKO) and wild-type littermates (WT) were field-stimulated at 0.5 Hz and then exposed to a transition in workload (5 Hz, isoproterenol 30 nM). Redox-states of NAD(P)H/NAD(P)$^+$ and $FADH_2/FAD^+$ (autofluorescence) were similar in WT and Stat3-CKO at baseline (~65% reduced, respectively) and transiently oxidized upon increased workload (to ~53% after 3 min), suggesting similar increases in ADP-induced respiration. Mitochondrial membrane potential ($\Delta\Psi_m$; TMRM) was maintained during the transition in both groups. While superoxide production ($^{\cdot}O_2$-; MitoSox) remained stable in WT myocytes after elevated workload, it increased ~4-fold during the first minute after the transition in Stat3-CKO, but normalized thereafter. To test whether this affects the long-term response to β-adrenergic stimulation, Stat3-CKO and WT mice were treated with isoproterenol for 24h *in vivo* (osmotic mini-pumps). After this treatment, left ventricular function was maintained *in vivo*, but NAD(P)H/NAD(P)$^+$ and $FADH_2/FAD^+$ redox states were substantially oxidized in isolated myocytes at baseline (~35% reduced, respectively). After increasing workload, NAD(P)H/NAD(P)$^+$ and $FADH_2/FAD^+$ redox states were reduced to ~55% in WT, but further oxidized in Stat3-CKO myocytes (to 28%; $p<0.001$). This was associated with a sustained 2.3-fold increase in $^{\cdot}O_2$- formation in Stat3-CKO vs WT myocytes. Isoproterenol treatment *in vivo* for 14 days led to dilated cardiomyopathy in Stat3-CKO, but not WT mice.

We conclude that lack of Stat3 increases mitochondrial $^{\cdot}O_2$- production, predisposing to the development of heart failure in response to β-adrenergic stimulation.

2504-Pos Board B490

Influence of SERCA and Actomyosin ATPase on Respiration Kinetics in Permeabilized Rat Cardiomyocytes

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Compartmentalization of ATP and ADP by intracellular diffusion restrictions plays a role in heart energetics. While specific causes of diffusion restrictions are not known, intracellular structures are speculated to act as diffusion barriers. We did kinetic experiments on permeabilized rat cardiomyocytes under relaxing conditions and analyzed the data by mathematical modeling. We found significant diffusion restriction by the mitochondrial outer membrane and confirmed a functional coupling between mitochondria and a fraction of ATPases. Such coupling indicates restricted diffusion in the cytosol. In addition, we found evidence for a tight coupling between pyruvate kinase (PK) and some ATPases. However, it is not clear which ATPases are coupled with which cellular energetic sites. The aim of this work was to establish the role of SERCA and myosin ATPase as possible candidates for coupling with PK and mitochondria. First, the effect of SERCA was assessed by inhibition with thapsigargin (TG). We found no effect of TG on respiration and no change in ATPase activity was observed spectrophotometrically. This indicates that SERCA activity is minor in our preparation. Second, the role of myosin ATPase was assessed in cells where myosin had been extracted by incubation in high KCl solution. We recorded respiration rate dependence on exogenous and endogenous ADP, inhibition of ATP-stimulated respiration by endogenous and exogenous PK. In spectrophotometer, we measured activity of ATPases and endogenous PK. We found that while ATP respiration kinetics changed, ADP respiration kinetics were the same as control. To establish the role of actomyosin ATPase, the experimental results will have to be analyzed by mathematical models.

2505-Pos Board B491

Complex I of the Mitochondrial Electron Transport Chain is Dysfunctional in the Early Embryonic Heart

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Proper cardiac function is crucial to ensure embryonic survival. The heart is the first organ to become functional during embryonic development, with the onset of beating at 8 days post fertilization (E8). Within two days of this (E10.5), blood begins to circulate providing nutrients to the developing embryo. Early defects in the embryonic heart can result in embryonic death or severe deformities leading to death during or shortly following birth. Although structural cardiac anomalies rarely cause demise, functional cardiac defects are much more devastating *in utero*.

In the adult heart, mitochondria play an important role in proper function. Mitochondrial dysfunction can result in cardiac dysfunction and eventually death. While mitochondrial function is well studied in the adult heart, which relies on complex I as its primary source of electron entry, little is known about mitochondrial function in the developing embryonic heart.

Data generated in this lab show that at the early stages of embryonic development (E9.5) mitochondrial membrane potential ($\Delta\psi_m$) is low, and the potential for increased oxidative stress is high. A minimal change in $\Delta\psi_m$ is observed at E9.5 upon the addition of the complex I inhibitor rotenone. This observation is contrasted in E13.5 myocytes, which exhibit a higher sensitivity of $\Delta\psi_m$ to rotenone. In concert, these data suggest that at E9.5 complex I of the mitochondrial electron transport chain is non-functional. This study employs established bioenergetic and mitochondrial proteomic techniques, which have been adapted and applied in whole embryonic hearts and cardiomyocytes during cardiac organogenesis (E9.5, E11.5, E13.5) to support the hypothesis that at early stages of embryonic cardiac development, mitochondrial function is limited due to an immature complex I, thus resulting in decreased $\Delta\psi_m$ and increased potential for oxidative stress.