Factors Affecting Fatty Acid Oxidation in the Potassium Arrested Heart

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Background: Myocytes preferentially oxidize fatty acids (FA) during normokalemic (N) perfusion. During cardiac surgery, when the heart is arrested with potassium cardioplegia (PC), FA oxidation is suppressed and ketones become the dominant fuel used. FA oxidation is associated with reactive oxygen species generation and cardiac dysfunction during reperfusion [1]. Therefore a metabolic shift away from oxidizing fats might be beneficial. Experimental evidence suggests that myocardial substrate selection under normokalemic conditions may be influenced by myocardial oxygen consumption (MVO_2) and the myocardial redox state [2,3]. The purpose of the current experiments was to determine the effects of altered MVO_2 and redox state on substrate selection by the heart under both normokalemic perfusion and potassium arrest.

Methods: Groups of male Sprague-Dawley rat hearts (n=8-10 per group) were equilibrated under Langendorff perfusion from a height of 100 cm H_20 for 20 minutes. The perfusate during equilibration consisted of Krebs-Heinseleit buffer containing physiologic concentrations of unlabeled fatty acids (.35mM), acetoacetate (.17mM), lactate (1.2mM), pyruvate (.12mM) and glucose (5.5mM). After stabilization, hearts were perfused for an additional 30 minutes with modified buffer to evaluate effects of redox state and MVO_2 on substrate oxidation during either N or PC (KCl=20mM) conditions. During this 30 minute interval, substrates included in the perfusate were labeled as follows: U-^{13}C labeled fatty acids, 1,3-^{13}C acetoacetate, 3-^{13}C lactate, 3-^{13}C pyruvate and unlabeled glucose to enable determination of relative substrate oxidation.

The effect of the cytosolic redox state on substrate oxidation was assessed by increasing pyruvate to 1.2mM and decreasing lactate to .12mM (ie reversing the lactate/pyruvate ratio) in the perfusate (N-PL and PC-PL groups). Mitochondrial redox state was altered by replacing acetoacetate with ^{13}C labeled 3-D-β-hydroxybutyrate (N-B and PC-B groups). In two other groups, 37.5μM of the mitochondrial uncoupler 2,4 dinitrophenol (DNP) was added to the buffer in an attempt to increase MVO_2 (N-DNP and PC-DNP groups).

Developed pressure, heart rate, coronary flow, inflow and outflow pO_2 were measured. MVO_2 was calculated from coronary flow and pO_2 data. After the final 30 minutes of perfusion, hearts were removed and freeze clamped in liquid nitrogen. Cardiac tissue was extracted with perchloric acid and lyophilized. Extracts were reconstituted in D_2O and high-resolution proton decoupled ^{13}C NMR spectra were obtained on a 14.1T Varian Inova spectrometer. Fractional substrate oxidation was determined by glutamate isotopomer analysis under non-steady-state conditions [4]. Groups were compared by one-way analysis of variance. Differences between groups, when present, were determined by the Fisher LSD.

Results: No difference in MVO_2 was noted between groups during the stabilization period. In the normokalemic (N) groups, MVO_2 increased from baseline values with reversal of the lactate/pyruvate ratio and with the addition of DNP. As expected, MVO_2 was decreased in all PC groups compared to N. MVO_2 was similar in all PC groups, but rose in the PC-DNP group compared to PC alone (p=0.09). See Table 1. **During N perfusion**, DNP did not affect fractional substrate utilization. Changes in cytoplasmic redox state profoundly suppressed FA oxidation and increased oxidation of lactate+pyruvate. Alterations in mitochondrial redox state did not affect FA oxidation but reduced ketone body metabolism. **Under PC conditions**, increased MVO_2 and changes in both cytoplasmic and mitochondrial redox state suppressed ketone oxidation compared to PC alone. FA oxidation was increased in both PC-DNP and PC-B groups. Oxidation of unlabeled substrate and lactate+pyruvate was stimulated in PC-B and PC-PL groups. See Figure 1.

Group	Final RPP (bpm x pressure)	Stabilization MVO_2 (μmol/min/g dry wt)	Final MVO_2 (μmol/min/g dry wt)
N	30874±1436*	57±5	58±4*
N-DNP	30825±1994*	53±3	60±2*,†
N-PL	31271±1112*	57±3	64±4*,†
N-B	31095±2330*	51±1	52±2*
PC	0	62±5	13±1†
PC-DNP	0	55±3	19±1†
PC-PL	0	59±3	17±2†
PC-B	0	58±3	12±3†

Table 1 Cardiac Function and Myocardial Oxygen Consumption
Data are mean ± SEM * - p < .05 vs all PC Groups, † - p < .05 vs Stabilization MVO_2

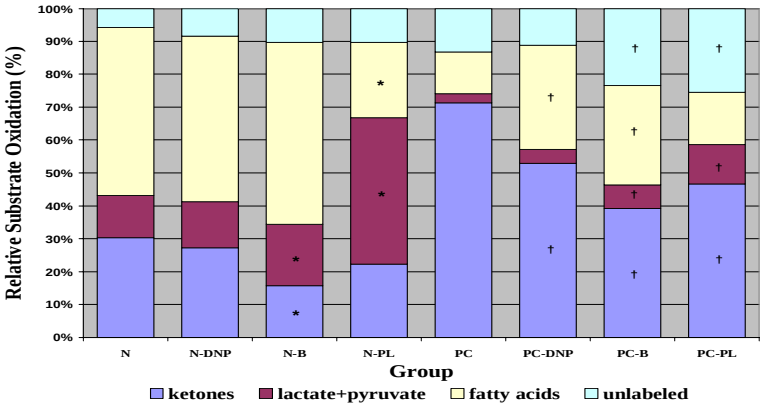

Figure 1 Myocardial Substrate Oxidation
* - p < .05 vs N Group, † - p < .05 vs P Group

Conclusion: These data suggest that alterations in MVO_2 and redox state affect myocardial substrate oxidation under working and arrested conditions. During normokalemic perfusion, FA oxidation can be suppressed by manipulating the cytoplasmic redox state in a manner predicted to increase the NAD/NADH ratio. During potassium cardioplegia, FA oxidation is low but may increase with changes in MVO_2 and mitochondrial redox state. These data suggest that fatty acid oxidation can be altered during both normal perfusion and during cardioplegic arrest. Further studies will be required to assess the affects of altered substrate oxidation profiles on functional recovery after ischemia and after controlled myocardial arrest during cardiac surgery.

References:

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