Myocardial Substrate Oxidation During Cardioplegic Arrest With Potassium Chloride or Tetrodotoxin

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Introduction

Experimental studies have demonstrated myocardial protection from ischemia by inducing either polarized arrest (with agents such as tetrodotoxin [TTX]) or depolarized arrest (with hyperkalemia). Polarized arrest may reduce cellular ion fluxes and diminish sodium entry during ischemia (1), but may also lead to more avid uptake of fatty acids (2). Depolarized arrest reduces myocardial oxygen consumption but does not seem to influence uptake of long-chain fatty acids (3). However, changes in uptake of fatty acids by the myocyte may not translate into changes in oxidation rates. Substrate oxidation rates have not been compared in hearts arrested by these two techniques.

Methods

To study substrate oxidation under conditions of polarized and depolarized arrest, hearts from adult male Sprague-Dawley rats were excised and perfused retrograde (Langendorff) with a modified Krebs-Heinseleit (K-H) buffer containing 5.5 mM glucose, 0.17 mM acetoacetate, 0.12 mM pyruvate, 1.2 mM lactate and 0.35 mM long-chain fatty acids for 20 minutes. Hearts were then perfused for an additional 30 minutes with either (1) unaltered K-H (CONTROL group, n=8), (2) K-H with the KCl concentration increased to 20 mM (KCl Group, n=7), or (3) K-H with 28 mM TTX added (TTX Group, n=6). Substrates were uniquely labeled with ^{13}C (3-^{13}C lactate, 3-^{13}C pyruvate, 1,3-^{13}C acetoacetate, U-^{13}C mixed fatty acids, and unlabeled glucose) during this interval. Myocardial oxygen consumption (MVO_2) was measured from coronary flow and AV-O_2 difference.

At the end of the perfusion interval, hearts were freeze-clamped, extracted in KOH and reconstituted in D_2O. Proton-decoupled ^{13}C NMR spectra were acquired in a 9.4T Bruker spectrometer and substrate entry into the citric acid (TCA) cycle was determined by ^{13}C NMR isotopomer analysis (4). Isotopomer data were combined with MVO_2 values to calculate oxidation rates for each substrate class (5). Data are expressed as mean±SD and groups are compared by ANOVA.

Results

Cardiac function remained stable in the CONTROL group, and mechanical function ceased in both the KCl and TTX groups. Oxygen consumption (in µmol/min/g dry weight) was significantly lower in both the KCl group (12.3±3.1) and TTX group (19.1±15.4) compared to the CONTROL group (54.5±11.8, $p<0.05$). Changes in total TCA cycle flux (in µmol/min/g dry weight) paralleled changes in oxygen consumption (KCl group: 5.4±1.5; TTX group: 7.9±6.2; CONTROL group: 21.0±4.6 [$p<0.05$ KCl vs CONTROL and TTX vs CONTROL]).

Under conditions of both KCl and TTX arrest, myocardial substrate selection was markedly altered with a shift away from fatty acid utilization and towards ketone use. Percent contributions of each substrate to the total acetyl-CoA pool oxidized within the TCA cycle are shown in **Table 1** below. Calculated flux of each substrate through the TCA cycle is presented in **Table 2** below.

Discussion

These data reveal major shifts in substrate utilization under conditions of both polarized and depolarized arrest. The direction of these changes was similar in both forms of induced arrest, with suppression of fatty acid oxidation and increases in ketone oxidation. Changes in oxidation rates did not correlate with the predicted effects of membrane potential on fatty acid uptake (2,3), suggesting that other mechanisms play a role in substrate selection under conditions of low MVO_2. These substrate effects may play a role in the cardioprotective properties of potassium chloride and tetrodotoxin.

References

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Table 1. Percent Contribution of Substrates to Acetyl-CoA Oxidized Within the TCA Cycle.
[* p<.05 vs control, ¥ p<.05 vs KCl]

	Aceto-acetate	Lactate or Pyruvate	Fatty Acids	Unlabeled Substrates
Control	28±7	5±1	58±7	10±11
KCl	64±15*	3±1	18±5*	16±13
TTX	50±8*¥	4±2	29±15*¥	17±17

Table 2. Substrate Oxidation Rates (µmol/min/g dry weight)
[* p<.05 vs control]

	Aceto-acetate	Lactate or Pyruvate	Fatty Acids	Unlabeled Substrates
Control	5.78±1.23	0.94±0.24	12.21±3.22	2.19±2.58
KCl	3.53±1.70	0.15±0.08*	0.95±0.35*	0.78±0.83
TTX	3.67±2.08	0.35±0.25*	2.85±3.84*	1.01±0.91