Fatty acid oxidation in rats fed with etomoxir enriched diet.

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Introduction

Etomoxir, an inhibitor of carnitine palmitoyl transferase I (CPT 1), has attracted intense interest because acute administration improves cardiac function after ischemia[1]. Studies during chronic administration of etomoxir, however, suggest that a cardiomyopathy may be induced[2]. Since studies with chronic etomoxir administration in humans are underway, we thought to determine if left ventricular function and metabolism are altered by chronic etomoxir administration. Because the fat content of human diets varies significantly, and fatty acid metabolites may have adverse effects, the interaction of diet with etomoxir was examined.

Methods

Rats were fed four different diets over four weeks; low fat, no etomoxir; low fat with etomoxir; high fat, no etomoxir; high fat with etomoxir. After an overnight fast, hearts were removed from animals under general anesthesia and perfused in working heart apparatus. The perfusate contained ^{13}C enriched long chain fatty acids, aceto-acetate, β hydroxybutyrate, pyruvate, lactate, and nonenriched glucose. Oxygen consumption, heart rate, developed pressure and cardiac output were continuously monitored. After 45 minutes, the heart was frozen and later extracted in perchloric acid. The extract was analyzed by proton decoupled ^{13}C NMR spectroscopy in a Varian 14.1 T system. The contribution of ^{13}C labeled substrates to production of acetyl CoA was analyzed using isotopomer steady state approach.[3]

In parallel set of experiments, mitochondria were isolated from the heart and CPT 1 activity in mitochondria was assayed as described previously[4].

Results

Oxygen consumption, cardiac output and rate pressure product were not significantly different in all four groups. The oxidation of fatty acids in hearts from rats on low versus high fat diets was similar when measured using both biochemical and NMR techniques. As expected, etomoxir suppressed CPT1 activity by 95% in mitochondria isolated from the myocardium. In contrast, the oxidation of fatty acids was reduced by almost 50% as measured by ^{13}C spectroscopy. (Table 1) The utilization of lactate was significantly lower in hearts of rats fed with low fat diet and etomoxir. Higher utilization of unlabeled sources was noted in both etomoxir groups. This could indicate enhanced carbohydrate utilization or oxidation of triglycerides that accumulated in myocardium during treatment with etomoxir.

Diets	Low Fat - E	High Fat - E	Low Fat +E	High Fat +E
CPT1 Activity nmol/min/mg protein	15.2±0.1	16.3±0.2	1.0 ±0.4	1.0 ±0.3
LCFA	60±2%	58±4%	47±1%	36±4%
La+ pyruvate	17±2%	20±4%	5±1%	19±4%
ketones	10±1%	8±1%	13±1%	9±1%
other	13±7%	14±6%	36±1%	36±6%

Table 1. Utilization of substrates in heart extracts.

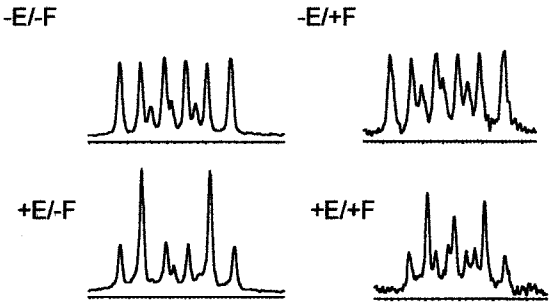

Fig1.
Proton decoupled ^{13}C spectra of the glutamate C4 resonance. The symbols + or – indicate presence of absence of etomoxir (E) or high fat diet (F)

Discussion and Conclusions

This study confirms that chronic administration of etomoxir causes complete inhibition of CPT1 activity in mitochondria isolated from the heart. Myocardial function was not substantially influenced by etomoxir, regardless of diet. Unexpectedly, ^{13}C NMR shows that oxidation of long chain fatty acid continued to provide a significant fraction of cardiac energy production in a working heart treated with etomoxir. We conclude that chronic therapy with etomoxir may provoke compensatory changes that circumvent classic mitochondrial β-oxidation. This study illustrates also the importance of testing conclusions from organelles in intact tissues.

References

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