Control of Fatty Acid Metabolism in Ischemic and Hypoxic Hearts*

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The effects of whole heart ischemia on fatty acid metabolism were studied in the isolated, perfused rat heart. A reduction in coronary flow and oxygen consumption resulted in lower rates of palmitate uptake and oxidation to CO₂. This decrease in metabolic rate was associated with increased tissue levels of long chain acyl coenzyme A and long chain acylcarnitine. Cellular levels of acetyl-CoA, acetylcarnitine, free CoA, and free carnitine decreased. These changes in CoA and its acyl derivatives indicate that β oxidation became the limiting step in fatty acid metabolism. The rate of β oxidation was probably limited by high levels of NADH and FADH₂ secondary to a reduced supply of oxygen. Tissue levels of neutral lipids showed a slight increase during ischemia, but incorporation of [U-¹⁴C]palmitate into lipid was not altered significantly.

Although both substrates for lipid synthesis were present in higher concentrations during ischemia, compartmentalization of long chain acyl-CoA in the mitochondrial matrix and α-glycerol phosphate in the cytosol may have accounted for the relatively low rate of lipid synthesis.

Fatty acids represent a major substrate for energy metabolism in cardiac muscle and under aerobic conditions, from 60 to 90% of total oxygen consumption may be used to oxidize fatty acids (1, 2). Rates of uptake and oxidation depend on the concentration of exogenous fatty acid and on the oxidative state of the tissue (3). At low extracellular concentrations (below approximately 0.3 mM bound to 3% albumin) the rate of uptake is limited by extracellular availability. At higher concentrations, the rates of uptake and oxidation are limited by the activity of the citric acid cycle. The rate of oxidation is linearly related to the rate of oxidative phosphorylation and it increases as cardiac work is elevated (3) but decreases with a reduction in oxygen supply (4). Since the rates of oxidative phosphorylation and flux through the citric acid cycle are tightly coupled (5), much of the control of fatty acid metabolism in aerobic hearts occurs secondary to regulation of these pathways (3). Control of the pathway in oxygen-deficient hearts is not understood. β Oxidation appeared to limit utilization by isolated liver mitochondria under anoxic conditions (6). This may also apply to oxygen-deficient cardiac muscle. Anoxic hearts have decreased levels of acetyl coenzyme A (7) and ischemic myocardium has increased levels of long chain acyl-CoA derivatives (8).

The purpose of the present study was to determine the regulation of fatty acid metabolism in isolated rat hearts made oxygen-deficient. The contribution of fatty acid oxidation to total oxidative metabolism was determined in ischemic hearts. In addition, rate-limiting steps of fatty acid oxidation in ischemic and hypoxic hearts were determined by measuring the tissue levels of acyl-CoA and acylcarnitine intermediates.

METHODS

Perfusion Technique—Hearts from 200- to 250-g male Sprague-Dawley rats were excised and cannulated as described for the isolated, working rat heart (9). In the working heart apparatus, cardiac output and ventricular pressure development can be controlled by regulating left atrial filling pressure or resistance of the aortic outflow tract (or both). Coronary flow in this preparation was a function of aortic pressure and, thus, changed in direct relation to aortic ventricular pressure development. Whole heart ischemia was induced by use of a one way valve in the aortic outflow tract which markedly restricted coronary flow during ventricular diastole but did not influence aortic output and ventricular pressure development initially (10). The hearts were perfused for 10 min as a Langendorff preparation and for 5 min as a working control prior to inducing ischemia. Two degrees of ischemia were established by changing the height of a minimal flow bubble trap which bypassed the one way valve in the cannula arrangement. By this system, coronary flow was prevented from declining further as pressure development of the heart deteriorated.

The perfusate was Krebs-Henseleit bicarbonate buffer gassed with 95% O₂, 5% CO₂. The buffer contained 11 mM glucose and 0.4, or 1.2 mM palmitate bound to 3% bovine serum albumin (Pentex, Fraction V). The concentration of palmitate was determined on the final perfusate by the Ducomble procedure (11). Palmitate was bound to albumin by converting the free acid to the sodium salt and injecting the salt into a warm albumin solution. This solution was dialyzed overnight against a large volume of buffer and filtered through a 0.8-μM Millipore filter before use.

Estimation of Palmitate Uptake, Oxidation, and O₂ Consumption—Hearts were perfused with buffer containing 1.2 mM [U-¹⁴C] palmitate. Coronary venous samples were collected without exposure to air by cannulating the pulmonary artery and collecting the effluent perfusate under mineral oil. Arterial samples were collected from the left atrial perfusate just prior to entry into the heart (9). For estimating fatty acid uptake, the samples of perfusate were acidified and exposed to air for 24 h to remove ¹³C²O₂ and counted, and uptake was calculated from the arterial-venous difference in [U-¹⁴C] palmitate. For ¹³C₂O₂ production, 1-ml samples of perfusate were injected into a 25-ml stopped, Erlenmeyer flask containing 0.5 ml of 6 N H₂SO₄. The ¹³C₂O₂ released was collected in a center well containing 0.4 ml of 1 M Hyamine hydroxide. After 2 h the wells were removed, placed in toluene scintillator, and counted. Rates of palmitate oxidation were calculated from the arterial-venous difference in C₂O₂, specific activity of the perfusate palmitate, and rate of coronary flow. The rate of release of ¹³C₂O₂ reached a steady state after 8 min of perfusion with [U-¹⁴C]palmitate. During this time, the amount of label that accumulated in the tissue as acid-soluble metabolites, C₂O₂, and H₂CO₃ reached a maximum value. Therefore, rates of ¹³C₂O₂ release into the perfusate during the steady state should reflect the rate of palmitate oxidation. These rates were determined only after 10 min of perfusion with the labeled fatty acid. Oxygen consumption was estimated from rates of coronary flow and the arterial-venous differences in perfusate P0₂, which was measured on a radiometer model PHM 27 blood gas monitor.

Estimation of Tissue Levels of Metabolic Intermediates—At the
end of perfusion, hearts used to determine levels of metabolic intermediates were frozen, while being perfused, with a Wollenberger clamp cooled to the temperature of liquid nitrogen. The frozen tissue was powdered in a freezing mortar, extracted in cold, 6% (w/v) HClO, and centrifuged at 0°C. The supernatant was neutralized with KOH and used for estimation of tissue levels of free CoA, acetyl-CoA, free carnitine, and acetylcarnitine. The precipitate was washed with 0.6% HClO, and used for estimating tissue content of long chain acyl-CoA and acylcarnitine derivatives.

Acetyl-CoA, acetylcarnitine, and free CoA were assayed as free CoASH and carnitine after alkaline hydrolysis of the washed 6% perchloric acid precipitates (15). Long chain acyl-CoA was hydrolyzed at pH 11.8 to 12.0 for 12 min at 55°C in the presence of 10 mM dithiothreitol. Long chain acylcarnitine was hydrolyzed at pH 12.8 to 13.0 for 2 h at 70°C. The pH of the hydrolysates was then decreased to approximately 1 with 75% HClO, to precipitate any residual protein. After centrifugation at 0°C, the supernatants were neutralized with KOH and assayed as above.

Measurements of Tissue Lipids—At the end of perfusion, hearts used for lipid analysis were cut from the cannula and the ventricles were trimmed of atria and connective tissue before freezing. The frozen ventricles were powdered at liquid nitrogen temperatures and extracted with chloroform:methanol (2:1). After drying under nitrogen, the extract was dissolved in chloroform. Neutral and phospholipids were separated by running the chloroform extract over a silica acid column (Bio-Gel HA, 325 mesh). The column was washed with 2 volumes of chloroform to remove neutral lipids and the eluates were combined and dried. The neutral glycerides were saponified by heating at 60°C for 30 min after adding 4% KOH in 95% ethanolic and their content was estimated by measuring glyceral release (16).

Incorporation of [U-14C]palmitate into lipid was determined by counting a fraction of the chloroform eluate from the silica acid column. Specific activity of perfusate fatty acid was determined by extracting the fatty acid with Dole’s solution which was counted for labeled palmitate and by determining the fatty acid concentration by estimating free carnitine levels (13).

RESULTS

The effects of ischemia on oxygen consumption and oxidation of palmitate are discussed below. Myocardial oxygen consumption occurs in direct proportion to the rate of oxygen delivery over a wide range of ischemic coronary flow rates (17). In the present study, oxygen consumption and palmitate oxidation were determined at normal flows and at two rates of coronary flow which result in ventricular failure. Control hearts received a 10-min washout perfusion with 15 ml/min (solid circles), as ischemic hearts where coronary flow was allowed to decrease to 1.0 ml/min (open triangles). The perfusate was allowed to pass through the hearts only once. Rates of fatty acid (FA) oxidation were measured as 14CO2 production from [U-14C]palmitate and are expressed as micromoles of palmitate oxidized per g of dry heart tissue per min. This was calculated as follows:

\[
\text{micromoles of palmitate oxidized/g/min} = \text{cpm of } 14\text{CO}_2/\text{ml of perfusate} \times \text{coronary flow (ml/min)/specific activity perfusate palmitate (cpm/\mu mole)} \times 10^{-3} \text{g of dry tissue}
\]

reduced (Fig. 2). In control hearts, disappearance of [U-14C]palmitate from the perfusate averaged about twice as much as oxidation. Presumably, the remainder was incorporated into tissue lipids and other metabolic intermediates. In ischemic hearts receiving 5 ml/min of coronary flow, disappearance of palmitate decreased. After 20 min of perfusion, the rate of uptake was about equal to the rate of oxidation, indicating that conversion of fatty acid to other metabolites was also reduced.

In order to identify the rate-controlling steps of fatty acid metabolism, tissue levels of acyl-CoA and acylcarnitine derivatives were measured at various times after inducing ischemia. Levels of acetyl-CoA decreased rapidly while those of long chain acyl-CoA derivatives increased (Fig. 3). Very similar changes occurred in the levels of carnitine derivatives; acetylcarnitine decreased and long chain acylcarnitine increased (Fig. 4). The changes in acyl-CoA derivatives were essentially maximum after 5 min, but long chain acylcarnitine was still increasing after 20 min. Levels of long chain acyl-CoA increased to about the same extent during ischemia regardless of the concentration of exogenous fatty acid (Table I). In hearts perfused with glucose as the only exogenous substrate, long chain acyl-CoA levels increased to almost the same extent as when either 0.4 or 1.2 mM palmitate was included in the perfusate. Long chain acylcarnitine also increased with all
Fig. 2. Effects of coronary flow on palmitate uptake. The rate of palmitate uptake by control (solid line) and ischemic hearts with coronary flows of 5 ml/min (dashed line) was estimated by measuring the disappearance of labeled palmitate from the perfusate. Hearts were perfused with buffer containing glucose (11 mM) and [U-¹⁴C] palmitate (1.0 mM) bound to albumin (5%). Coronary effluent was collected from the pulmonary artery after a single pass through the heart to obtain venous [U-¹⁴C]palmitate content and coronary flow rate at the points indicated. Arterial perfusate samples were simultaneously collected and used to determine arterial [U-¹⁴C]palmitate content. The data are expressed as micromoles of palmitate uptake per min per g of dry heart tissue. Each value represents the mean ± S.E. for five to six hearts.

Fig. 3. Effects of perfusion time on acetyl-CoA and long chain fatty acyl-CoA levels. Levels of acyl-CoA were measured in control (solid lines) and ischemic hearts with coronary flows of 5 ml/min (dashed lines) at the times indicated. Perusions were conducted as described in Fig. 1. The data are expressed as nanomoles per g of dry heart tissue. Each value represents the mean ± S.E. for six to seven hearts.

Fig. 4. Effects of perfusion time on acetylcarnitine and long chain fatty acylcarnitine levels. Perusions were conducted as described for Fig. 1. The solid lines represent control hearts (coronary flow of 15 ml/min) and dashed lines represent ischemic hearts (coronary flow of 5 ml/min). The data are expressed as micromoles per g of dry heart tissue. Each value represents the mean ± S.E. for six to seven hearts.

substrates, but the levels were much higher in those hearts receiving palmitate. Tissue lipids are known to be utilized in hearts perfused with glucose as the only substrate. Decreased oxidation of the fatty acids derived from breakdown of tissue lipids probably resulted in higher levels of long chain acyl derivatives in ischemic hearts receiving only glucose. Levels of acetyl-CoA and acetylcarnitine were low in control hearts receiving either 0 or 0.4 mM palmitate but were greatly elevated when palmitate was increased to 1.2 mM. A decrease in
Fatty Acid Metabolism in Ischemic Hearts

**Effects of fatty acid concentration on tissue levels of CoA and carnitine intermediates in control and ischemic hearts**

Hearts were perfused with buffer containing 11 mM glucose and the concentration of palmitate indicated. Coronary flows were maintained at approximately 15 ml/min in control and 5 ml/min in the ischemic hearts. Perfusions were continued for 20 min under the conditions indicated. The data are expressed as nanomoles per g of dry heart tissue. Each value represents the mean ± S.E. for five to six hearts.

<table>
<thead>
<tr>
<th>Condition</th>
<th>CoA</th>
<th>Acetyl-CoA</th>
<th>Long chain acyl-CoA</th>
<th>Carnitine</th>
<th>Acetylcarnitine</th>
<th>Long chain acylcarnitine</th>
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<tr>
<td>Control</td>
<td>478 ± 11</td>
<td>5</td>
<td>95 ± 6</td>
<td>6223 ± 119</td>
<td>123 ± 5</td>
<td>303 ± 14</td>
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<tr>
<td>Ischemic</td>
<td>320 ± 20</td>
<td>5</td>
<td>251 ± 15</td>
<td>5485 ± 224</td>
<td>298 ± 22</td>
<td>987 ± 26</td>
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<th>Condition</th>
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<th>Long chain acyl-CoA</th>
<th>Carnitine</th>
<th>Acetylcarnitine</th>
<th>Long chain acylcarnitine</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>408 ± 12</td>
<td>12 ± 1</td>
<td>93 ± 7</td>
<td>5954 ± 192</td>
<td>208 ± 15</td>
<td>638 ± 47</td>
</tr>
<tr>
<td>Ischemic</td>
<td>6 ± 11</td>
<td>15 ± 1</td>
<td>284 ± 10</td>
<td>389 ± 124</td>
<td>323 ± 32</td>
<td>2424 ± 177</td>
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<th>Condition</th>
<th>CoA</th>
<th>Acetyl-CoA</th>
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<th>Carnitine</th>
<th>Acetylcarnitine</th>
<th>Long chain acylcarnitine</th>
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<tr>
<td>Control</td>
<td>301 ± 18</td>
<td>188 ± 9</td>
<td>179 ± 4</td>
<td>2001 ± 262</td>
<td>1707 ± 166</td>
<td>872 ± 56</td>
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<tr>
<td>Ischemic</td>
<td>115 ± 8</td>
<td>22 ± 1</td>
<td>293 ± 11</td>
<td>1796 ± 131</td>
<td>925 ± 57</td>
<td>3078 ± 143</td>
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**Effects of coronary flow and hypoxia on tissue levels of acyl-CoA and acylcarnitine derivatives**

Tissue levels of CoA, acetyl-CoA, long chain fatty acyl-CoA, carnitine, acetylcarnitine, and long chain fatty acylcarnitine were measured after 20 min of perfusion. The perfuse contained 11 mM glucose and 1.0 mM palmitate. In control and ischemic hearts, the perfuse was gassed with 95% O2, 5% CO2. Coronary flow was maintained at approximately 5 ml/min in one group of ischemic hearts and was allowed to progressively deteriorate to 0.6 ml/min in a second group. Flow was maintained at 15 ml/min in hypoxic hearts and oxygen availability was restricted by gassing the perfuse with 20% O2, 5% CO2, 75% N2. The values represent the mean ± S.E. for 6 to 10 hearts.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Coronary flow</th>
<th>CoA</th>
<th>Acetyl-CoA</th>
<th>Long chain acyl-CoA</th>
<th>Carnitine</th>
<th>Acetylcarnitine</th>
<th>Long chain acylcarnitine</th>
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</thead>
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<tr>
<td>Control</td>
<td>12</td>
<td>302 ± 37</td>
<td>162 ± 17</td>
<td>178 ± 4</td>
<td>3459 ± 262</td>
<td>2002 ± 118</td>
<td>1307 ± 159</td>
</tr>
<tr>
<td>Ischemic</td>
<td>5</td>
<td>199 ± 20</td>
<td>38 ± 6</td>
<td>346 ± 20</td>
<td>1995 ± 146</td>
<td>470 ± 58</td>
<td>4353 ± 265</td>
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<tr>
<td>Hypoxic</td>
<td>0.6</td>
<td>112 ± 10</td>
<td>23 ± 2</td>
<td>333 ± 10</td>
<td>1829 ± 100</td>
<td>516 ± 43</td>
<td>4361 ± 241</td>
</tr>
</tbody>
</table>

**Effects of ischemia on metabolism of tissue neutral lipids**

Hypoxic hearts were perfused for 10 min as control working hearts and then for an additional 20 min under either control or ischemic conditions with perfuse containing 11 mM glucose and 1.0 mM [1-14C]palmitate. Rates of coronary flow were 15 and 5 ml/min for control and ischemic hearts, respectively. Absolute levels of neutral lipids were measured as micromoles of glycerol released after extraction and hydrolysis (see "Methods"). Incorporation of perfusate fatty acid into lipid was calculated as micromole equivalents of [1-14C]palmitate incorporated per μmol of lipid. The number of hearts in each group is indicated by the value in parentheses.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Tissue content</th>
<th>Incorporation of perfusate [1-14C]palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.7 ± 0.88</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>Ischemic (9)</td>
<td>11.3 ± 0.64*</td>
<td>0.37 ± 0.04</td>
</tr>
</tbody>
</table>

* p < 0.05.

**DISCUSSION**

The rate of β oxidation does not appear to control the overall rate of fatty acid utilization in aerobic hearts (3, 18). In oxygen-deficient tissue, however, β oxidation may limit the rate of utilization (6, 7, 10). In the present study, β oxidation appeared to be inhibited in both hypoxic and ischemic hearts. The rise in levels of long chain acyl-CoA and the decrease in acetyl-CoA in ischemic hearts in conjunction with a slower rate of palmitate oxidation indicated that β oxidation was inhibited in ischemic tissue. Similar changes in tissue levels of CoA derivatives occurred in hypoxic hearts, indicating that this type of oxygen deficiency also inhibited β oxidation.

These changes in tissue levels of intermediates can be interpreted to indicate inhibition of β oxidation only if the changes in CoA derivatives occurred within the mitochondrial matrix where the β oxidation enzymes are located. From the data available on cellular distribution of CoA and carnitine...
produced from glycolysis, the increase would be expected to times in ischemic tissue (20) and since this metabolite is the level of α-glycerol phosphate increases some 10 to 13 very high levels of lipids would accumulate in the ischemic resulted from the cellular compartmentalization of substrates. if this rate of lipid synthesis were continued for long periods, tissue. The rather slow rate of lipid synthesis may have increase incorporation of fatty acid into tissue lipids. These effects, however, have been minimal and a net increase in tissue lipids has been difficult to demonstrate. In the present study, ischemia resulted in only a small increase in tissue levels of neutral lipids during 20 min of perfusion. However, if this rate of lipid synthesis were continued for long periods, very high levels of lipids would accumulate in the ischemic tissue. The rather slow rate of lipid synthesis may have resulted from the cellular compartmentalization of substrates. The level of α-glycerol phosphate increases some 10 to 13 times in ischemic tissue (20) and since this metabolite is produced from glycolysis, the increase would be expected to occur in the cytosol. Although the level of long chain acyl-CoA increased some 3-fold, it is difficult to estimate the extent of acyl-CoA increase in the cytosolic compartment. Since most of the CoA is confined to the mitochondrial matrix (19), acyl-CoA may be largely unavailable for cytosolic synthesis of complex lipids.

The accumulation of long chain acyl derivatives may have severe consequences on the metabolism of ischemic hearts. Long chain acyl-CoA is known to inhibit a number of enzymes. This acyl derivative inhibits adenine nucleotide translocase (8) and it is thought that mitochondria isolated from ischemic tissue have a reduced rate of State 3 oxygen consumption due to the presence of high levels of acyl-CoA. Long chain acylcarnitine inhibits (Na⁺,K⁺)-ATPase activity at concentrations that are likely to be present in ischemic tissue (24). In addition, high levels of both acyl-CoA and acylcarnitine may have generalized detergent effects which could disrupt membranes and perhaps increase lysosomal enzyme activity. With reduced rates of fatty acid activation, it would be expected that tissue levels of free fatty acids would increase and fatty acids have also been shown to inhibit (Na⁺,K⁺)-ATPase (25).

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