Characterization of cardiac malonyl-CoA decarboxylase and its putative role in regulating fatty acid oxidation

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Characterization of cardiac malonyl-CoA decarboxylase and its putative role in regulating fatty acid oxidation. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H2122–H2129, 1998.—Malonyl-CoA is a potent inhibitor of fatty acid uptake into the mitochondria. Although the synthesis of malonyl-CoA in the heart by acetyl-CoA carboxylase (ACC) has been well characterized, no information is available as to how malonyl-CoA is degraded. We demonstrate that malonyl-CoA decarboxylase (MCD) activity is present in the heart. Partial purification revealed a protein of ~50 kDa. The role of MCD in regulating fatty acid oxidation was also studied using isolated, perfused hearts from newborn rabbits and adult rats. Fatty acid oxidation in rabbit hearts increased dramatically between 1 day and 7 days after birth, which was accompanied by a decrease in both ACC activity and malonyl-CoA levels and a parallel increase in MCD activity. When adult rat hearts were aerobically reperfused after a 30-min period of no-flow ischemia, levels of malonyl-CoA decreased dramatically, which was accompanied by a decrease in ACC activity, a maintained MCD activity, and an increase in fatty acid oxidation rates. Taken together, our data suggest that the heart has an active MCD that has an important role in regulating fatty acid oxidation rates.

An important protein involved in the regulation of fatty acid oxidation in the heart is carnitine O-palmitoyltransferase (CPT) I. This protein is located within the outer mitochondrial membrane and is a key regulatory enzyme involved in the first committed step of fatty acid oxidation (16, 19). Malonyl-CoA, which is produced by acetyl-CoA carboxylase (ACC), is a potent inhibitor of CPT I (16, 17). Unlike the liver, in which a 88-kDa isoform of CPT I predominates, the heart predominantly expresses a 82-kDa isoform of CPT I (4) that is much more sensitive (10–50 times) to inhibition by malonyl-CoA (16–18). We have observed that under conditions in which fatty acid oxidation can vary widely the IC50 of mitochondrial CPT I for malonyl-CoA does not change (9). Rather, the actual levels of malonyl-CoA drop, resulting in an increase in CPT I activity. Therefore, existing evidence suggests that actual changes in malonyl-CoA levels appear to be the key factor regulating changes in fatty acid oxidation in the heart, as opposed to changes in sensitivity of CPT I to malonyl-CoA inhibition.

Studies from our laboratory (9–11, 14) and others (1) showed that ACC in the heart functions as a key regulator of fatty acid oxidation, secondary to the production of malonyl-CoA (13). For example, in rabbit hearts the activity of ACC decreases between 1 day and 7 days after birth (14). This is accompanied by a dramatic decrease in malonyl-CoA levels and an increase in fatty acid oxidation rates (14). In the postischemic heart, a decrease in ACC activity is also accompanied by a decrease in malonyl-CoA levels and an increase in fatty acid oxidation rates (9). Although the importance of malonyl-CoA in regulating myocardial fatty acid oxidation has been firmly established, to our knowledge no previous studies have addressed how fatty acid oxidation is effected by malonyl-CoA degradation in the heart. One possibility is that malonyl-CoA is decarboxylated by malonyl-CoA decarboxylase (MCD), thereby removing CPT I inhibition.

MCD was previously described as a mitochondrial enzyme that is involved in protecting certain mitochondrial enzymes such as malyl-CoA mutase and propionyl-CoA carboxylase from inhibition by mitochondrial-derived malonyl-CoA (5, 12, 21). Although very little is known about mammalian MCD, two isoforms of MCD have been identified in the goose uropygial gland (2). These proteins originate from the same gene, although they have separate start sites of transcription and translation. These alternate start sites create two proteins, one targeted to the mitochondrial matrix and the other to the cytoplasm. In addition to the uropygial gland, nonmitochondrial levels of MCD have also been detected in low levels in the liver. To date, MCD activity and subcellular localization have not been characterized in the heart.

Under a variety of physiological and pathophysiological conditions, malonyl-CoA levels in the heart can rapidly decrease (9, 13, 14, 20). A decreased synthesis of malonyl-CoA can partly explain this, although a degradation of the malonyl-CoA would also be required to see an overall decrease in tissue levels. We hypothesize that MCD is the mechanism by which this occurs. To support this hypothesis we report the development of a new and reliable MCD assay that detects acetyl-CoA.
produced by MCD. With this assay system, we have identified and characterized MCD activity in the heart. Similarly, we have identified MCD as one enzyme that may be responsible for reducing malonyl-CoA levels after birth in the rabbit heart or after myocardial ischemia in the rat heart.

**METHODS**

Tissue preparation. Male Sprague-Dawley rats (300–350 g) were anesthetized with pentobarbital sodium, and the heart was quickly excised. Hearts were then immediately homogenized for measurement of MCD, immediately used for preparation of mitochondria (see Partial purification of MCD), frozen in liquid N₂, or used for isolated working heart perfusions. Hearts were also obtained from anesthetized 1- and 7-day-old New Zealand White rabbits for isolated heart perfusions (as described in Heart perfusions).

Frozen hearts (10–15 mg of tissue) used for MCD measurements were homogenized for 2 s, chilled in a buffer consisting of KCl (75 mM), sucrose (20 mM), HEPES (10 mM), and EGTA (1 mM), with or without NaF (50 mM) and sodium pyrophosphate (NaPPi; 5 mM). A fraction of this homogenate corresponding to 2 mg of tissue was used in the MCD assay.

Partial purification of MCD. Rat hearts were excised and separated from their atrias. The ventricles were then minced and 7-day-old New Zealand White rabbits for isolated heart perfusions (as described in Heart perfusions).

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To initiate the MCD assay, heart homogenates or mitochondrial preparations were incubated in a 210 µl reaction mixture (0.1 M Tris, pH 8, 0.5 mM dithiothreitol, 1 mM malonyl-CoA) for 10 min at 37°C, in the presence or absence of NaF (50 mM) and NaPPi (5 mM). The reaction was stopped by the addition of 40 µl of perchloric acid (0.5 mM), neutralized with 10 µl of 2.2 M KHCO₃ (pH 10) and centrifuged at 10,000 g for 5 min to remove precipitated proteins. The incubation of the heart sample with malonyl-CoA allowed for the conversion of malonyl-CoA to acetyl-CoA, which was then combined with [¹⁴C]oxaloacetate (0.17 µCi/ml) to produce [¹⁴C]citrate. All reactions were carried out in the presence of N-ethylmaleimide, which removes excess CoA remaining in the latter stages of the reaction so that the citrate present cannot generate non-MCD-derived acetyl-CoA. Unreacted [¹⁴C]oxaloacetate was removed from the reaction mixture by the addition of sodium glutamate (6.8 mM) and aspartate aminotransferase (0.533 µU/µl), followed by a 20-min incubation at room temperature. This allowed for transamination of unreacted [¹⁴C]oxaloacetate back to [¹⁴C]aspartate. The resulting solution was then sterilized in a 1:2 suspension of Dowex AG 50W-8X resin (100–200 mesh) and centrifuged at 400 g for 10 min. The pelleted Dowex fraction removed [¹⁴C]aspartate, whereas the supernatant contained [¹⁴C]citrate. The supernatant was then counted for ¹⁴C present in the form of [¹⁴C]citrate. The amount of acetyl-CoA produced by MCD was then quantified by comparison to acetyl-CoA standard curves that had been subjected to the identical assay conditions as described above. A standard acetyl-CoA concentration curve was run with each experiment. These curves were always found to be linear (r = 0.99; data not shown).

A schematic of the MCD assay is shown below

Tissue homogenate (MCD)

\[ \text{malonyl-CoA} \quad \longrightarrow \quad \text{acetyl-CoA} \]

(with or without the phosphatase inhibitors: NaF, NaPPi)

\[ \text{aspartate} \]

\[ \text{aminotransferase} \]

\[ \text{L-}[^{14}C]\text{aspartate} + 2-\text{oxoglutarate} \quad \longrightarrow \quad[^{14}C]\text{oxaloacetate} + \text{L-glutamate} \]

\[ \text{citrate synthase} \]

\[[^{14}C]\text{oxaloacetate} + \text{acetyl-CoA} \quad \longrightarrow \quad[^{14}C]\text{citrate} + \text{CoASH} \]

Determination of CoA esters. Detection and quantification were performed by extracting CoA esters from powdered tissue into 6% perchloric acid and measuring with a modified HPLC procedure (14). Essentially, each sample (100 µl) was run through a precolumn cartridge (C₁₈, size 3 cm, 7 µm) and a Microsorb Short-one column (type C₁₈, particle size 3 µm, size 4.6 × 100 mm) on a Beckman System Gold HPLC. Absorbance was set at 254 nm and flow rate at 1 ml/min. A gradient was initiated using buffer A (0.2 M NaH₂PO₄, pH 5.0) and buffer B (0.25 M NaH₂PO₄ and acetonitrile, pH 5.0) in a ratio of 80:20 (vol/vol). Conditions were maintained at initial levels for 2.5 min (97% buffer A and 3% buffer B) and were changed thereafter to 18% buffer B over 5 min using Beckman’s curve no. 3. The gradient was changed linearly at 15 min to 37% buffer B over 3 min and subsequently to 90% buffer B over 17 min. At 42 min the composition was returned linearly back to 3% buffer B over 0.5 min, and at 50 min column equilibration was complete. Peaks were integrated by the Beckman System Gold software package.

ACC assay. Approximately 200 mg of frozen heart tissue were homogenized, centrifuged, and dialyzed as previously described (14). Twenty-five microliters of dialysate were added to a reaction mixture (final vol 160 µl) containing 60.6 mM Tris acetate, pH 7.5, 1 mg/ml bovine serum albumin, 1.32
µM β-mercaptoethanol, 2.12 mM ATP, 1.06 mM acetyl-CoA, 5.0 mM magnesium acetate, 18.2 mM NaHCO₃, and 10 mM magnesium citrate. Samples were incubated at 37°C for 0, 1, 2, 3, or 4 min, and the reaction was stopped by the addition of 25 µl of 10% perchloric acid. Samples were then spun for 2 min at 13,000 rpm, and the malonyl-CoA concentration in the supernatant was measured using the HPLC procedure described in Determination of CoA esters.

Western blot analysis. Samples were subjected to either nondenaturating PAGE or SDS-PAGE and transferred to nitrocellulose as described (14). Membranes were immunoblotted with either rat anti-MCD antibody or to bovine anti-catalase antibody (Chemicon) in 1% milk powder. The antibodies were visualized using the Amersham Enhanced Chemiluminescence Western blotting detection system.

Heart perfusions. Isolated perfused hearts were obtained either from newborn New Zealand White rabbits (1 and 7 days after birth) or from adult Sprague-Dawley rats. One-day-old rabbit hearts were isolated and perfused, as described previously, at a coronary perfusion pressure of 30 mmHg (14). Seven-day-old hearts were perfused, as described previously, at 7.5-mmHg left atrial preload and 30-mmHg aortic afterload (14). This preload and afterload were chosen because we previously showed them to represent a moderate workload in the isolated 7-day-old rabbit heart (14). Rat hearts were perfused at a 11.5-mmHg left atrial preload and 80-mmHg aortic afterload (9).

The newborn rabbit hearts were perfused with Krebs-Henseleit solution containing 3% bovine serum albumin, 0.4 mM [1-14C]palmitate, 11 mM glucose, and 100 µU insulin/ml. Hearts were perfused for a 40-min period, and fatty acid oxidation was measured as described below. The rat hearts were perfused with Krebs-Henseleit solution containing 11 mM glucose, 1.2 mM palmitate, and 100 µU/ml insulin. Hearts were perfused for a 60-min aerobic period; a 30-min aerobic and a 30-min global ischemic period; or a 30-min aerobic, a 30-min global ischemic, and a 60-min reperfusion period.

At the end of all perfusions, hearts were frozen with tongs cooled to the temperature of liquid N₂. Frozen ventricular tissue from perfused hearts was weighed and powdered in a mortar and pestle cooled to the temperature of liquid N₂. A portion of the powdered tissue was used to determine the dry weight-to-wet weight ratio of the ventricles. The atrial tissue remaining on the cannula was removed, dried in an oven for 12 h at 100°C, and weighed. With the dried atrial tissue, total frozen ventricular weight, and ventricular dry weight-to-wet weight ratio, the total dry weight of the heart was determined.

Measurement of palmitate oxidation. Steady-state rates of palmitate oxidation were measured in both newborn rabbit hearts and in aerobic and reperfused ischemic hearts by quantitatively collecting 14CO₂ produced from hearts perfused with 11 mM [1-14C]palmitate (~50,000 dpm/ml buffer). Collection of 14CO₂ released as gas in the oxygenation chamber and the 14CO₂ trapped in the NaHCO₃ in the perfusate was performed as described previously (9).

Statistical analysis. The unpaired t-test was used for the determination of statistical difference of two group means. For groups of three, analysis of variance followed by the Neumann-Keuls test was used. A value of P < 0.05 was considered significant. All data are presented as means ± SE.

RESULTS

Characterization of MCD in heart. To study the activity of heart MCD we developed a new assay that quantified the amount of acetyl-CoA formed by MCD (see METHODS). The assay was optimized for use in heart homogenates as well as for isolated mitochondrial preparations. A tissue protein standard curve using heart homogenates demonstrated that 2 mg of tissue produced MCD activity that was linear for up to 20 min of incubation (data not shown). Similarly, a time course standard curve using a 10-min incubation time produced rates that were linear when 0–10 mg of tissue were used (data not shown). We therefore used 2 mg of tissue homogenate and a 10-min incubation period in all subsequent experiments. This also usually gave acetyl-CoA values that were in the middle of the acetyl-CoA standard curve. Control experiments were also run to determine whether contaminant acetyl-CoA or acetyl-CoA derived from other sources was competing with the assay. Background levels of the assay were equal to time 0 on our standard curve, indicating that exogenous acetyl-CoA from tissue homogenates did not skew our results. Furthermore, when tissue samples were run in the absence of added malonyl-CoA, no acetyl-CoA was formed over the course of the 10-min incubation period.

With this MCD assay we identified the existence of MCD activity in heart homogenates obtained from rats (Table 1). Rates of MCD activity were found to be significantly higher than previously observed rates of ACC (9). Because malonyl-CoA synthesis from ACC is under phosphorylation control, we also performed experiments to determine whether MCD was under phosphorylation control. Isolation of tissue under conditions that preserved the phosphorylated state of the enzyme did not alter rates of MCD activity compared with enzyme isolated under conditions that did not attempt to preserve the phosphorylated state seen in vivo (Table 1). However, incubation of heart homogenates in vitro with alkaline phosphatase resulted in an increase in MCD activity. This suggests that MCD is under phosphorylation control, with phosphorylation of the enzyme resulting in a decrease in enzyme activity. The actual kinases and phosphatases involved in controlling either form of MCD, however, have yet to be identified.

To characterize some of the physical properties of MCD we performed Western blot analysis using an

Table 1. Malonyl-CoA decarboxylase activity in aerobically perfused rat hearts

<table>
<thead>
<tr>
<th>Malonyl-CoA Decarboxylase Activity, nmol·g dry wt⁻¹·min⁻¹</th>
<th>Control (±NaF, ±NaPP,)</th>
<th>Uncontrolled (–NaF, –NaPP,)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (±NaF, ±NaPP,)</td>
<td>7,176 ± 1,269</td>
<td>7,672 ± 1,311</td>
</tr>
<tr>
<td>Uncontrolled (–NaF, –NaPP,)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Uncontrolled (–NaF, –NaPP, ± alkaline phosphatase)</td>
<td>12,433 ± 914*</td>
<td></td>
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<tr>
<td>(n = 4)</td>
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Values are means ± SE of at least 4 hearts. Heart homogenates were assayed for malonyl-CoA decarboxylase activity in presence or absence of NaF and NaPP, and with or without alkaline phosphatase treatment. *Significant differences between groups of hearts.
existing MCD antibody (5). In rat heart and liver tissue this antibody cross-reacted with high affinity with at least one other protein. Because this other protein is known to be catalase (unpublished observations), we clarified the results obtained with the MCD antibody using an antibody directed against catalase. Rat liver MCD was partially purified using the procedure described in METHODS. The partially purified protein was loaded onto two lanes of a nondenaturing gel and subjected to electrophoresis. After electrophoresis, one lane was transferred to nitrocellulose and blotted with our MCD antibody, whereas the other lane was stored in water for future use. In a Western blot from a nondenaturing gel (Fig. 1A), the MCD antibody reacted with one large band that we suspect is \( \sim 160-190 \) kDa, as predicted by Kim and Kollattukudy (5). The position of the band was aligned with the nontransferred lane of the nondenatured gel, and the region containing MCD was extracted from the gel. The protein from the gel slice was eluted and either assayed for MCD activity or subjected to SDS-PAGE, transferred to nitrocellulose, and blotted with the anti-MCD antibody. The eluted protein exhibited a large MCD activity and when subjected to Western analysis, reacted with the MCD antibody (data not shown). The same semipurified MCD sample was also subjected to Western blot analysis using the MCD antibody (Fig. 1A, lane 2). The blot was then stripped and reprobed with anti-catalase antibody to ascertain which band corresponded to the catalase protein(s) (Fig. 1A, lane 3). This experiment allowed us to determine that the molecular mass of rat liver MCD is \( \sim 50 \) kDa. This molecular mass has been confirmed by the recent purification of rat liver MCD (J. R. B. Dyck, L. Berthiaume, A. J. Barr, and G. D. Lopaschuk, manuscript in preparation). Our results are also consistent with the previous conclusion that the native enzyme is a tetramer (5).

Western blot analysis was also performed on denatured, semipurified heart samples (Fig. 1B). Under these conditions the 50-kDa band was identified in both liver and heart tissue samples (Fig. 1B, lanes 1 and 2). When samples were treated with alkaline phosphatase and subjected to SDS-PAGE and Western blot analysis, we did not detect any shift in molecular mass of the protein (data not shown). Interestingly, we did observe a slightly larger protein that reacted to our MCD antibody in the whole heart extracts and not in the semipurified samples (see Fig. 4). This suggests that another isoform of MCD may be present in fractions containing cytosolic proteins.

Role of MCD in regulating fatty acid oxidation. We previously demonstrated that myocardial fatty acid oxidation increases dramatically between 1-day and 7-day rabbit hearts (14). As shown in Table 2, a significant increase in fatty acid oxidation rates is seen in hearts from 7-day-old rabbits compared with 1-day-old rabbits. This is accompanied by a decrease in malonyl-CoA levels and a decrease in ACC activity. However, to substantially decrease malonyl-CoA levels in the heart, a decreased rate of synthesis would have to be accompanied by a simultaneous degradation of malonyl-CoA. We therefore measured MCD activity in 1- and 7-day-old rabbit hearts. As shown in Fig. 2, the MCD activity in 7-day-old rabbit hearts was significantly elevated compared with 1-day-old hearts. In light of the amount of malonyl-CoA present, these high rates of MCD activity suggest a rapid turnover of malonyl-CoA. When hearts were dephosphorylated in
Table 2. Malonyl-CoA content, acetyl-CoA carboxylase activity, and palmitate oxidation rates in 1- and 7-day-old isolated, perfused rabbit hearts

<table>
<thead>
<tr>
<th></th>
<th>Malonyl-CoA Content, nmol·g dry wt⁻¹</th>
<th>ACC Activity, nmol·mg protein⁻¹·min⁻¹</th>
<th>Palmitate Oxidation Rates, nmol·g dry wt⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day (n = 5)</td>
<td>108.5 ± 2.5</td>
<td>0.208 ± 0.057</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>7 day (n = 5)</td>
<td>3.6 ± 0.25</td>
<td>0.036 ± 0.003</td>
<td>46.7 ± 15.7</td>
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</table>

Values are means ± SE; n, no. of hearts. Malonyl-CoA content and acetyl-CoA carboxylase (ACC) activity were measured as described previously (14), and palmitate oxidation rates were determined as outlined in methods. *Significant differences between appropriate groups.

vitro with alkaline phosphatase we observed no change in MCD activity (data not shown). This suggests that unlike adult rat hearts, MCD activity is not under the same degree of phosphorylation control as seen in the newborn rabbit heart. To determine whether alteration in MCD protein expression may be responsible for alterations in MCD activity we performed Western blot analysis on 1- and 7-day-old rabbit hearts. However, the rabbit MCD did not have any antigenicity to our antibody. The reason for this is unknown; however, because our antibody does not have any cross-reactivity to the goose MCD it is possible that species-specific forms of MCD are present and are immunologically dissimilar. As a result, further studies are required to determine whether the difference in MCD activity between these two age groups is caused by differences in the amount of MCD expression.

As shown in Table 3, reperfusion of adult rat hearts after a 30-min period of global no-flow ischemia results in a dramatic drop in malonyl-CoA levels and a parallel decrease in ACC activity. During this time we also observed a slight increase in fatty acid oxidation rates. Because cardiac work is significantly inhibited during reperfusion of ischemic hearts, however, a large increase in fatty acid oxidation per unit work was observed. Moreover, because the drop in malonyl-CoA occurs in such a short period of time, we believe that the reduction in malonyl-CoA synthesis by ACC would have to be aided by a simultaneous degradation by MCD. We therefore measured MCD activity in aerobic, ischemic, and ischemic-reperfused hearts (Fig. 3). Levels of MCD activity were maintained at the end of ischemia and/or reperfusion. Treatment of samples with alkaline phosphatase also resulted in an increase in MCD in all groups. Combined, these data demonstrate that the drop in malonyl-CoA postischemia can be explained by a decrease in ACC activity and maintained MCD activity.

Figure 4 shows the MCD protein levels in samples extracted from aerobic, ischemic, and ischemic-reperfused rat hearts. Levels of MCD protein were not altered during the perfusion protocols, nor did the molecular masses of the MCD shift between the different groups, suggesting that MCD is not modified posttranslationally by phosphorylation (see Discussion). Also, when whole heart extracts were used for Western analysis, we detected another protein of a slightly larger molecular mass than seen with semipurified MCD from mitochondria (Fig. 4, lanes 3–8). It is entirely possible that samples that contain cytoplasmic extracts (instead of purely mitochondrial extracts) also possess the cytoplasmic form of MCD. In the goose uropygial gland the cytoplasmic form of MCD is ~55 kDa whereas the mitochondrial form is processed posttranslationally into a 50-kDa molecular mass protein (2). Whether this processing occurs in the rat heart is yet to be established, but our Western blots do support this possibility.

DISCUSSION

The importance of malonyl-CoA in regulating fatty acid uptake into the mitochondria has been well documented (16, 17, 19). Studies from both our laboratory (8–11, 13) and others (1) also established the role of a cytoplasmic ACC in synthesizing malonyl-CoA in the heart. Although regulation of ACC activity is very important in controlling malonyl-CoA levels and fatty acid oxidation rates, we propose that MCD also plays a key role. We were able to demonstrate significant MCD activity in hearts. At least one isoform of MCD is expressed in the heart and can be phosphorylated and inhibited by a yet unidentified kinase. We also demonstrate that under experimental conditions in which ACC activity decreases, MCD activity is either stimulated or maintained. We propose that this active degradation process occurs in parallel with decreased ACC activity and can result in a rapid decrease in malonyl-CoA levels and an acceleration of fatty acid oxidation rates.

Relationship among malonyl-CoA, ACC, and MCD. Although the steps involved in the reduction of malonyl-CoA by MCD are unknown, we previously demonstrated that a dramatic decrease in malonyl-CoA level occurs after birth in the rabbit heart. Part of this decrease is caused by decreased synthesis of malonyl-
MCD activity in aerobic, ischemic, and ischemic-reperfused adult rat hearts.

![Image](http://ajpheart.physiology.org/)

Malonyl-CoA content, ACC activity, cardiac work, palmitate oxidation rates, and palmitate oxidation rates/cardiomyocyte work in aerobic and reperfused adult rat hearts.

<table>
<thead>
<tr>
<th></th>
<th>Malonyl-CoA Content, nmol·g dry wt⁻¹</th>
<th>ACC Activity, nmol·mg protein⁻¹·min⁻¹</th>
<th>Cardiac Work, CO±PSP 10⁻²</th>
<th>Palmitate Oxidation Rates, nmol·g dry wt⁻¹·min⁻¹</th>
<th>Palmitate Oxidation/ Cardiac Work, nmol·min⁻¹·CW⁻¹</th>
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<tbody>
<tr>
<td>Aerobic</td>
<td>29.3 ± 4.2*</td>
<td>9.6 ± 3.2*</td>
<td>51.9 ± 6.4*</td>
<td>724 ± 64*</td>
<td>4.60 ± 0.63*</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Reperfused</td>
<td>0.31 ± 0.31</td>
<td>2.3 ± 1.6</td>
<td>14.2 ± 4.3</td>
<td>1,051 ± 227</td>
<td>21.32 ± 0.788</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
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Values are means ± SE of n hearts. CO, cardiac output; PSP, peak systolic pressure; CW, cardiac work. Malonyl-CoA content, ACC activity, and cardiac work were measured as described previously (14), and palmitate oxidation rates were determined as outlined in METHODS. *Significant differences between appropriate groups.

CoA by ACC (15) secondary to phosphorylation and inhibition of ACC by AMP-activated protein kinase. This decrease in ACC cannot account for the actual reduction in malonyl-CoA levels unless a concomitant degradation of malonyl-CoA is occurring. Therefore, because the activity of MCD is increased after birth, we suggest that MCD is also involved in the decrease in malonyl-CoA levels. How MCD activity increases in the newborn rabbit heart is still not known. The increase in MCD activity in 7-day-old rabbit hearts is, however, a significant finding in light of the changes observed in fatty acid oxidation during development. Our data show that the 7-day-old rabbit heart has a significant increase in MCD activity compared with 1-day-old rabbit hearts. Although a decrease in ACC activity is an important determinant of malonyl-CoA levels, the degradation of MCD also appears to be important. We propose that the observed decrease in malonyl-CoA levels is caused by the increased MCD in the 7-day-old rabbit hearts, thereby increasing fatty acid oxidation rates to meet the energy demands necessary to maintain contractile function.

A decrease in myocardial malonyl-CoA levels is also an important contributing factor to the high rates of fatty acid oxidation seen during reperfusion of previously ischemic hearts (see Ref. 13 for review). A marked decrease in malonyl-CoA levels during reperfusion of ischemic hearts removes an important inhibitory regulation of CPT I, thereby allowing accelerated fatty acid uptake into the mitochondria. As we have previously reported (9), the high fatty acid oxidation rates during reperfusion of previously ischemic hearts exceed rates seen in the nonischemic heart, resulting in a decrease in cardiac efficiency and an increase in the severity of ischemic damage. During reperfusion a decrease in ACC activity is an important contributing factor to the low levels of malonyl-CoA seen in the reperfused heart (9). Maintained degradation of malonyl-CoA in the presence of decreased synthesis is probably responsible for the marked decrease in malonyl-CoA levels. As a result, pharmacological inhibition of MCD may prove to be a beneficial approach to treating ischemic heart disease.

Existing studies have now convincingly demonstrated that malonyl-CoA is a very important regulator of fatty acid oxidation in the heart (1, 9, 10, 13–16). However, although considerable information is now available as to how malonyl-CoA is synthesized in the heart, no information has previously been published on how malonyl-CoA is degraded in the heart. We believe that MCD is an attractive candidate for this role, and we demonstrate in this study that the heart has a very...
active MCD. However, further studies still must be performed to unequivocally determine what role MCD has in regulating fatty acid oxidation.

Localization of malonyl-CoA in the heart. It is not yet clear whether mammalian tissues have cytoplasmic and mitochondrial forms of MCD, as previously shown by Kollattukudy and co-workers (2) in the uropygial gland of the goose. An important question, therefore, is, where is MCD localized in the heart, and how is cytoplasmic malonyl-CoA (which is presumably important in regulating CPT I activity) degraded? Although we propose that a cytoplasmic isoform of MCD exists, definitive data to prove this have yet to be provided. Although we have found MCD activity in cytoplasmic extracts of heart tissue, we have not accurately determined how much of this is cytoplasmic and how much originates from mitochondria broken during homogenization of the tissue. However, the fact that MCD activity increases in mitochondria by 14-fold after detergent solubilization yet only 6-fold in crude heart homogenates does suggest that some MCD is extramitochondrial in nature (data not shown). MCD activity can also be measured in intact mitochondria, suggesting that malonyl-CoA may have access to MCD on the outer mitochondrial membrane. However, further studies are needed to definitively determine the subcellular location of MCD in the heart. Our isolated perfusion studies do support our hypothesis that MCD is altering the levels of cytoplasmic malonyl-CoA, because decreases in malonyl-CoA levels were accompanied by a decrease in ACC activity and an increased or maintained MCD activity. This supports the existence of either MCD in the cytoplasm or mitochondrial MCD acting to reduce malonyl-CoA levels through some as of yet unidentified process. It has been suggested that cytoplasmic malonyl-CoA can enter the mitochondria as malonate and, once inside, be converted back into malonyl-CoA (3). The evidence for this comes from the experiments involving intracerebral injection of [1-14C]malonate and [2-14C]malonate. It therefore cannot be ruled out that malonyl-CoA in the cytoplasm of the heart is degraded to malonate and that the malonate then penetrates the mitochondria, is reesterified to malonyl-CoA, and is converted to acetyl-CoA by the mitochondrial MCD. However, this would be an extremely inefficient means of degrading malonyl-CoA, requiring two high-energy phosphates to resynthesize mitochondrial malonyl-CoA. It should also be pointed out that malonyl-CoA degradation is normally stimulated in the heart during times of increased energy demand. We therefore hypothesize that a cytoplasmic MCD exists and degrades the malonyl-CoA that has direct access to CPT I.

Phosphorylation control of MCD. We have shown that the activity of at least one form of MCD is under phosphorylation control and that a higher degree of phosphorylation results in a decrease in activity. When we attempted to completely dephosphorylate the enzyme with alkaline phosphatase, MCD reached a maximal activity in all three groups of hearts. This suggests that our measured activities are a result of a partially phosphorylated enzyme. Because active MCD is proposed to exist as a tetramer, it is possible that the phosphorylation site(s) may only be exposed when the protein is in a tetrameric form. Because our measured activities are not maximal, it is likely that some proportion of MCD is in its monomeric form and therefore not phosphorylated in vivo. Our Western blot analyses indicate that most of the individual subunits are not phosphorylated, because we do not detect any shift in protein mass on SDS-PAGE. Therefore, although MCD is under phosphorylation control, any alterations in protein mass caused by the increased phosphate group(s) cannot be observed using standard Western blot analysis. This may be caused by the small proportion of phosphorylated to dephosphorylated monomeric forms of MCD present. Further studies are necessary to determine the significance of this phosphorylation control of MCD.

In summary, we demonstrate that heart expresses a 50-kDa isoform of MCD that is important in regulating myocardial malonyl-CoA levels. An increased or maintained MCD activity in conjunction with a decrease in ACC activity is probably responsible for the decrease in malonyl-CoA levels and increased fatty acid oxidation seen in both the postnatal heart and the reperfused ischemic heart. These studies therefore implicate MCD as a putative regulator of myocardial fatty acid oxidation.

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