Carnitine Palmitoyltransferase in Cardiac Ischemia
A Potential Site for Altered Fatty Acid Metabolism

Daniel F. Pauly, Katharine A. Kirk, and Jeanie B. McMillin

The sensitivity of carnitine palmitoyl coenzyme A (CoA) transferase I to inhibition of its activity by malonyl-CoA is progressively reduced in mitochondria isolated from ischemic cardiac cells as blood flow decreases to 30% or less of the preocclusion flow. The activity of carnitine palmitoyl-CoA transferase I in mitochondria isolated from nonischemic cardiac cells demonstrates incomplete inhibition, even at high concentrations of malonyl-CoA. Kinetic analyses of these data gave results most consistent with the expression of two overt enzyme activities: one activity that is sensitive to inhibition by malonyl-CoA and one activity that demonstrates little or no sensitivity to such inhibition. The decrease in malonyl-CoA-sensitive activity associated with ischemia results from a 13% decrease in the activity of the sensitive component and a corresponding 13% increase in the activity of the insensitive component. Decreased sensitivity of ischemic carnitine palmitoyl-CoA transferase I to inhibition by malonyl-CoA, together with potential fluctuations in the content of malonyl-CoA in tissue, would increase the synthesis of palmitoylcarnitine during ischemia and facilitate return to the use of fatty acid as a preferred metabolic fuel on reperfusion. This apparent conversion occurs concomitantly with a decrease in the free protein thiol content of the mitochondrial membranes isolated from ischemic cardiac cells. Treatment of the mitochondria from ischemic cardiac cells with dithiothreitol in vitro partially reverses the loss in sensitivity to malonyl-CoA, suggesting the possible role of thiol oxidation in the altered metabolism of ischemic mitochondria. Western blot analysis of these mitochondria using an antibody against carnitine palmitoyltransferase II purified from beef heart demonstrates a 68-kDa protein, which under ischemic conditions apparently is decreased by 2 kDa. These results are more indicative of a modification in protein folding of carnitine palmitoyltransferase than proteolytic changes during ischemia. (Circulation Research 1991;68:1085–1094)

Long-chain fatty acids extracted from the plasma of cardiac cells that are subsequently activated to form fatty acyl coenzyme A (CoA) either can proceed to glycerolipid synthesis or be transesterified to carnitine and translocated into the mitochondrial matrix for \(\beta\)-oxidation. Carnitine palmitoyl-CoA transferase represents this branch point and, thus, has been studied extensively as a site of metabolic regulation and rate limitation. The activity of the overt carnitine palmitoyltransferase (CPT-I) is modulated by the lipogenic substrate, malonyl-CoA, in the heart and in organs that contain pathways for de novo synthesis of fatty acid. Alterations in the sensitivity of CPT-I to malonyl-CoA are proposed to be a regulatory mechanism for controlling production of palmitoylcarnitine (see Figure 1 for reaction sequence). Changes in the sensitivity of CPT-I to malonyl-CoA have been reported to occur during fasting and in streptozotocin-induced diabetes. Differences in sensitivity also have been observed with gender, pregnancy, feeding regimen, diet transition, weaning, and chronic ethanol ingestion. Whereas most of these studies involved liver mitochondria, some of these effects also were reported in studies involving heart and skeletal muscle. A decrease in sensitivity of CPT-I to malonyl-CoA would blunt the regulatory influence of malonyl-CoA and result in increased quantities of palmitoylcarnitine synthesized for any given concentration of malonyl-CoA (Figure 1). In the heart, malonyl-CoA may be produced by a cardiac-
specific isofrom of acetyl-CoA carboxylase.13 When measured in heart tissue, malonyl-CoA is present at a concentration of 11–12 μM,11 which is well above the Kᵢ of CPT-I for malonyl-CoA.4 This factor suggests that malonyl-CoA may play a significant role in the regulation of CPT-I in the heart, where little or no fatty acid synthetase is present.

Like starvation and diabetes, cardiac ischemia also reflects a state of altered availability of substrate. After coronary occlusion and reflow, carbohydrate oxidation is enhanced, whereas the utilization of fatty acid may be delayed in the canine heart.14 Because fatty acid is a poor substrate for the production of energy in the ischemic heart, we tested the hypothesis that changes in the regulation of CPT-I by malonyl-CoA may occur when energy demands are altered. There already is evidence that the level of acylcarnitine increases with myocardial ischemia15 and remains elevated for hours after reperfusion of the ischemic heart.16–18 Since acylcarnitine has been implicated in the inhibition of the deacylation of lysophospholipids,19 it is possible that lysophospholipids accumulate simultaneously with acylcarnitine and produce deleterious cellular and arrhythmogenic effects.20–24 Therefore, either an elevation in the activity of CPT-I or an alteration in its regulation may keep acylcarnitine levels high, and either of these could be a factor contributing to the delayed recovery of contractile function in the reperfused, ischemic heart.

Materials and Methods
Experimental Model
Regional myocardial ischemia was produced in dogs using the method we described previously.25 The dogs were anesthetized with 30 mg/kg pentobarbital sodium, intubated, and ventilated with a respiratory pump (Harvard Apparatus, South Natick, Mass.). A thoracotomy was performed at the level of the left fifth intercostal space, and the circumflex coronary artery was isolated for placement of a reversible ligature and downstream Doppler flow probe. The dogs were monitored postoperatively with a standard surface electrocardiogram. In experiments in which regional blood flow was quantitated using radioactive microspheres, the dogs were instrumented with lines inserted into the left atrial appendage and right femoral artery. Two sets of 15-μm microspheres labeled with different isotopes, chosen to minimize radioactive spill-over, were injected into the atrial appendage before and after 60 minutes of coronary occlusion. Reference blood was withdrawn from the femoral artery over a 3-minute interval after injection of each microsphere set, and the blood was weighed and counted. The dogs were then killed. After the hearts were excised, tissue was taken from transmural sections of the left ventricular free wall posterolateral to the posterior papillary muscle (ischemic tissue) and from the nonischemic left ventricular free wall anterolateral to the anterior papillary muscle (remote tissue). Control experiments were also carried out on mitochondria isolated from the tissue of hearts that were harvested immediately after the dogs were anesthetized. All tissue samples were immersed in a buffer (described below) to prepare them for isolation of mitochondria by polyanion homogenization and centrifugation. The pellet obtained initially by low-speed centrifugation contained 95% of the microsphere activity. Gamma counting was performed on this pellet using a dual label program with the windows set for the two isotopes used.

Mitochondrial Isolation and Assay
Mitochondria were isolated from control and ischemic cardiac cells according to the method of Palmer and coworkers26 as modified by Wolkowicz et al.25 The mitochondria were suspended in a medium containing (mM) mannitol 220, sucrose 70, MOPS 5, and EGTA 1 (pH 7.4) at a protein concentration of approximately 30 mg/ml. Mitochondrial protein was determined by the biuret procedure27 with bovine serum albumin used as the standard.

Mitochondrial respiration was measured at 30°C in an oxygen monitor (model 53, Yellow Springs Instrument Co., Yellow Springs, Ohio) as previously described.28 Phosphorylating respiration was initiated by adding 225 μM ADP. The sensitivity of palmitoyl-CoA–supported respiration to malonyl-CoA was monitored in mitochondria isolated from remote and ischemic tissues using an assay containing 3.75 mM malate; 0.75 or 1.5 mM ADP; 2 mM L-carnitine; 1, 3, or 10 μM malonyl-CoA; and 1 mg mitochondrial protein. After the malate-supported rate of respiration was established, oxygen consumption was stimulated by adding 15 μM palmitoyl-CoA.

The isotope-forward assay to measure the activity of CPT-I was carried out according to the method of

<table>
<thead>
<tr>
<th>Orientation to mitochondrial inner membrane</th>
<th>Reaction sequence</th>
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<tbody>
<tr>
<td>Malonyl-CoA(↓)</td>
<td>CPT-I(↑)</td>
</tr>
<tr>
<td>Outside:</td>
<td>Palmitoyl-CoA + carnitine ⇄ Palmitoylcarnitine(↑) + CoA</td>
</tr>
<tr>
<td>Inside:</td>
<td>Palmitoylcarnitine + CoA ⇄ Palmitoyl-CoA(↑) + Carnitine(↓)</td>
</tr>
</tbody>
</table>

(©) Fluctuations of malonyl-CoA in tissue regulate production of palmitoylcarnitine by inhibiting CPT-I.
(©) Sensitivity of CPT-I to malonyl-CoA determines the extent to which production of palmitoylcarnitine is inhibited by malonyl-CoA.
(©) One mole of palmitoylcarnitine is transported into the mitochondria for exchange with one mole of carnitine from the matrix.
(©) Although CPT-II has been purified and sequenced, its identity with CPT-I is controversial.
(©) Production of palmitoyl-CoA initiates β-oxidation.

**Figure 1.** Schematic diagram showing how the production of palmitoylcarnitine is regulated by heart mitochondrial carnitine palmitoyltransferase (CPT). CoA, coenzyme A; CPT-I, overt CPT; CPT-II, latent CPT.
Saggerson and Carpenter and modified as described previously. Palmityl-CoA and malonyl-CoA in the presence of bovine serum albumin were added at the concentrations stated in the legends to Figures 2–4 and 6. L-Carnitine was present at 0.525 mM with 0.30 μCi d-[14C]carnitine (specific activity, 10 Ci/mmol) used as a tracer. For experiments in which malonyl-CoA or 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) was present, reactions were initiated by adding palmityl-CoA and radiolabeled carnitine to protein first exposed to the effector.

When experiments were carried out in the presence of a constant amount of albumin and a fixed amount of mitochondrial protein, the level of palmityl-CoA bound to the mitochondria was used to reflect more accurately the effective concentration of substrate. The $K_m$ values were determined by either fitting palmityl-CoA concentrations as micromolar values and then converting the $K_m$ term to nanomoles per milligram bound or by converting each data point to nanomoles per milligram bound and then fitting to the model. These methods are known to produce identical kinetic results.

Kinetic parameters of ischemic carnitine palmitoyltransferase (CPT) activity were calculated based on a kinetic model fit to data from experiments on mitochondria of control canine heart, using 42 different combinations of malonyl-CoA and palmityl-CoA (see “Statistics and Data Analysis”). The mitochondrial preparations used for kinetic modeling were active and well coupled with phosphorylating and resting rates of respiration of 366±23 and 35.2±1.9 $\mu$atoms/min/mg, respectively, and ADP/O of 2.81±0.06 with glutamate used as a respiratory substrate. A model of partial competitive inhibition, in which two forms of activity are expressed (one form is unaffected by malonyl-CoA and one is inhibited by it), gave a significantly improved fit over both the simple-competitive and partial-inhibition models (one enzyme form). The finding that this model does not conform to classical competitive kinetics is consistent with prior kinetic interpretations of changes in the malonyl-CoA-sensitive activity of CPT-I.

Mitochondrial Protein Thiols

Aliquots of mitochondria (500 μl) were rapidly frozen in liquid nitrogen and osmotically shocked by thawing and homogenizing in 8 vol ice-cold, nitrogen-saturated buffer containing 10 mM 2-(N-morpholino)ethanesulfonic acid (MES) and 2 mM EDTA, pH 6. Each aliquot was then centrifuged at 8,000g for 10 minutes, and the resulting pellets were suspended in the same buffer and assayed for protein according to the method of Layne. To verify depletion of the matrix contents, the levels of glutathione in the supernatants were determined by anion-exchange high-performance liquid chromatography and detected with an on-line postcolumn reaction according to the method of Alpert and Gilbert. Membrane protein thiols were quantitated spectrophotometrically by reacting the mitochondrial membranes with DTNB: 1 mg protein was added to 2.9 ml buffer containing 10 mM MES, 2 mM EDTA, and 1% sodium dodecyl sulfate at pH 6, followed by 100 μl of 50 mM DTNB. Changes in absorbance due to the 5-thio-2-nitrobenzoic acid anion were monitored at 412 nm.

Immunoblotting

After the mitochondrial proteins were separated on 7.5% acrylamide–sodium dodecyl sulfate gels, they were electrophoretically transferred to nitrocellulose membranes in a buffer containing 25 mM Tris, 190 mM glycine, and 20% (vol/vol) methanol at 100 V for 1 hour or at 20 V overnight. The samples were then immunoblotted according to the procedure described in the immunoblot assay kit (BioRad, Richmond, Calif.). The primary antibody solution was prepared using a polyclonal immunoglobulin G fraction prepared in rabbits against CPT purified from beef heart that was made available to us by Dr. Loran Bieber of Michigan State University. After immunoblotting, the nitrocellulose membranes were washed and then incubated with goat anti-rabbit antibody conjugated to alkaline phosphatase. The color was developed with a procedure that uses 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

Statistics and Data Analysis

Results are expressed as mean±SEM. Student’s $t$ test for paired or unpaired variates was used to make comparisons between two groups. Analysis of variance was used when more than two groups were compared, followed by Fisher’s least significant difference test to locate particular differences. For kinetic analyses, data from 10 dogs at different combinations of malonyl-CoA and palmityl-CoA concentrations were fit to a surface predicted by two different forms of CPT (one form demonstrating little or no sensitivity to malonyl-CoA and the other form being competitively inhibited by it) using a nonlinear least-squares fitting program (Gauss-Newton algorithm, SAS/STAT statistical software package, SAS Institute, Cary, N.C.). The parameter solutions (enzyme activity [$V_{max}$], $K_m$, and $K_i$) describe the surface for which the sum of squared distances to the data points is minimized. The residual error sum of squares was used to indicate the remaining lack of fit of the data points to the model.

Results

Inhibition of mitochondrial respiration by malonyl-CoA in the presence of palmityl-CoA, carnitine, and ADP was measured in mitochondria isolated from ischemic and remote tissues of circumflex-ligated canine heart. The consumption of oxygen by mitochondria in the absence of malonyl-CoA was 169±12 nanomols of oxygen for those from remote tissues and 104±14 nanomols of oxygen for those from ischemic tissues ($p<0.01$). However, malonyl-CoA was less effective in inhibiting respiration supported by palmityl-CoA in mitochondria from the ischemic...
tissues when compared to those from nonischemic tissues (Figure 2).

Changes in the sensitivity of CPT-I to malonyl-CoA were confirmed in ischemic mitochondria by directly assaying the production of [14C]palmitoylcarnitine in the presence of varying concentrations of malonyl-CoA. A typical result is shown in the inset of Figure 3. The concentration of malonyl-CoA required to produce 50% inhibition of activity in mitochondria from the remote tissues produced only 26% inhibition in mitochondria from the ischemic tissues. Therefore, experiments were carried out to determine the relation between the inhibitory effect of malonyl-CoA and the percentage of reduction in ischemic blood flow from preocclusion values. The percentage of inhibition of ischemic CPT-I by malonyl-CoA at the concentration of malonyl-CoA that produced 50% inhibition in the remote tissues varied according to the degree to which the ischemic blood flow was reduced (Figure 2). Inhibition of CPT-I by malonyl-CoA decreased in ischemic tissues when blood flow was reduced to 30% or less of preocclusion flows.

Ischemia-induced alterations in the sensitivity of CPT-I to malonyl-CoA might result from a change in the amount of enzyme that is sensitive to malonyl-CoA. Another possibility is that a change in the affinity of the enzyme for malonyl-CoA has occurred. Data from control studies (see "Materials and Methods") are consistent with the presence of two forms of CPT-I activity that differ in sensitivity to inhibition by malonyl-CoA. Therefore, data on CPT velocity obtained from mitochondria isolated from remote and ischemic tissues were fit to a three-dimensional plot with the best-fit surface presented with lines demonstrating the residual distances from the data points to the fit surface (Figure 4).

When data obtained on mitochondria from the remote and ischemic tissues of canine hearts were fit to this model, they suggested that the decreased sensitivity of the ischemic mitochondria to malonyl-CoA does not result from a significant change in the $K_i$ for malonyl-CoA (Table 1). Rather, there is an apparent redistribution of the activity of CPT-I between the malonyl-CoA-sensitive and -insensitive forms. The amount of enzyme velocity due to the insensitive component ($V_{max}$) is significantly increased (by 13%) when taken as a percentage of the total activity of CPT-I measured. Similarly, there is a corresponding decrease (by 13%) in the velocity of the sensitive component ($V_{max}$). The total activity of CPT-I ($V_{max} = V_{max} + V_{max}$) is unchanged in mitochondria isolated from ischemic tissues (Table 1).
Thiol Modification in Ischemia and CPT-I

The sensitivity of mitochondrial CPT-I to malonyl-CoA has been shown to decrease in mitochondria after thiol oxidation with DTNB.29,37 Thiol oxidation has also been implicated as a mechanism of ischemic damage,38 and an 85% decrease in the glutathione redox potential has been observed in the matrix of mitochondria isolated from ischemic heart.28 To determine if thiol oxidation occurred in mitochondrial protein from ischemic heart, mitochondria isolated from ischemic and remote tissues of circumflex-ligated hearts and from control hearts were osmotically shocked to deplete the matrix of glutathione (see “Materials and Methods”) and presumably other matrix components. The matrix glutathione released (5.9±0.4 nmol reduced glutathione/mg and 0.7±0.19 nmol oxidized glutathione/mg) from the shocked mitochondria fully accounts for the matrix glutathione and indicates that the free sulfhydryl levels in the mitochondrial pellet reflect membrane protein thiols. A greater free thiol content was measured in mitochondrial membranes from the control and remote tissues when compared with the ischemic tissues (Figure 5). This observation is consistent with oxidative events that affect the proteins of the mitochondrial inner membrane.

To determine whether the change in the sensitivity of the ischemic CPT-I to malonyl-CoA could be attributed to sulfhydryl changes on the enzyme, the
mitochondria were treated with the thiol reductant, dithiothreitol (DTT), before the CPT-I assay. In the absence of malonal-CoA, the kinetic parameters that define the catalytic activity were unchanged when mitochondria from either the remote or ischemic tissues were treated with DTT (Table 2). In contrast, the sensitivity of the activity of CPT-I to malonal-CoA is significantly influenced by sulfhydryl reduction (Figure 6). Mitochondria from the remote tissues show significant sensitivity to 10 μM malonal-CoA, which remains high after treatment with DTT. When these mitochondria are treated with DTNB, their sensitivity to malonal-CoA is diminished, and this effect is reversed by DTT (Figure 5). Like the mitochondria from remote tissues treated with DTNB, mitochondria from ischemic tissues not exposed to DTNB demonstrate low sensitivity to malonal-CoA that is increased by treatment with DTT. However, the desensitization to malonal-CoA appears to be incomplete both in ischemic mitochondria and in control mitochondria after treatment with DTNB (Figure 5). There is a possibility that higher concentrations of DTT or longer pretreatment intervals might further restore sensitivity; however, it is also conceivable that thiol modifications may proceed to irreversible states of higher oxidation or that other mechanisms may account for the incomplete recovery. Thiol oxidation produces both reversible and irreversible components of inactivation of muscle glycogen phosphorylase. These data are similar to the results shown in Figure 6 and suggest either parallel or consecutive pathways for inactivation of the enzyme. One pathway is readily reversible; the second is irreversible and involves only a portion of the protein sulfhydryl. Initial modification of protein sulfhydryl groups may alter enzyme conformation. Additional groups are then exposed, resulting in

### Table 2. Overt Carnitine Palmitoyltransferase Kinetics in Mitochondria From Remote and Ischemic Regions of Circumflex-Ligated Heart After Treatment With Dithiothreitol

<table>
<thead>
<tr>
<th></th>
<th>Remote region</th>
<th>Ischemic region</th>
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<tbody>
<tr>
<td>Blood flow (ml/min/g)</td>
<td>1.26±0.49</td>
<td>1.0±0.40</td>
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<tr>
<td>Preocclusion</td>
<td>0.77±0.17</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Km (nmol/mg)</td>
<td>12.0±1.0</td>
<td>7.7±0.70</td>
</tr>
<tr>
<td>Vm,a (nmol/min/g)</td>
<td>11.3±0.2</td>
<td>7.1±0.06</td>
</tr>
<tr>
<td>−DTT</td>
<td>1.2±0.30</td>
<td>1.8±0.40</td>
</tr>
<tr>
<td>+DTT</td>
<td>0.8±0.15</td>
<td>1.9±0.10</td>
</tr>
</tbody>
</table>

Values are mean±SEM. −DTT, absence of dithiothreitol; +DTT, presence of dithiothreitol.
partial denaturation of the enzyme and suggesting cooperative behavior between the two pools of oxidizable thiols. Finally, sensitivity to malonyl-CoA is lost after treatment of mitochondria with detergent, an effect that is not antagonized by treatment with DTT (Figure 6); both findings are in agreement with other reports.

**Antibody Studies**

Because the loss in sensitivity to malonyl-CoA associated with ischemia represents a response to a pathological state as opposed to a physiological condition, we considered the possibility that proteolytic changes in the mitochondrial membrane proteins might reflect the altered response. Antibody against CPT purified from beef heart was provided by Dr. Loran Bieber. The antibody labels one major polypeptide with a molecular mass of 68.6±0.6 kDa in Western blots of mitochondrial protein from canine heart (data not shown). Western transfers using the antibody against this purified CPT were carried out on mitochondria isolated from uninvolved and ischemic heart after sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing and nonreducing conditions (Figure 7). In the reducing lanes, a small decrease in molecular mass of 2.2±0.4 kDa was observed in the ischemic CPT; however, no comparable change was evident when the samples were run under nonreducing conditions.

**Discussion**

Augmented perfusate fatty acids have been associated with impaired contractile performance and altered cardiac electrophysiology, particularly in the oxygen-deprived heart. In cardiac ischemia, accumulation of fatty acyl esters is associated with depressed β-oxidation and may contribute to the observed mechanical and electrical dysfunctions. However, the preferred metabolic fuel for restoration of contractile energetics in the previously ischemic heart is controversial. Reperfusion after transient ischemia in the canine heart was accompanied by delayed clearance of the positron tracer [13C]palmitate and increased accumulation of [18F]deoxyglucose in nonnecrotic myocytes. Conversely, others have observed a return to fatty acids as the preferred metabolic fuel in the reperfused, working swine heart. This apparent shift to a greater expression of the malonyl-CoA–insensitive form of CPT described in the present results would predict increased production of the fatty acylcarnitine available for β-oxidation when oxygenation is restored to tissues. The enhanced potential for oxidative metabolism of fatty acids may reflect diminished concentrations of malonyl-CoA in tissues, decreased sensitivity of CPT-I to malonyl-CoA, or a combination of these effects. Similar decreases in inhibition of the outer transferase by malonyl-CoA have been observed in liver mitochondria from ketotic, diabetic rats and from rats that were fasted for 24 hours. These effects in the liver were proposed to result from expression of a transferase enzyme that is less sensitive to malonyl-CoA.

Another explanation for the observation of a malonyl-CoA–insensitive component of CPT-I may be...
the expression of latent mitochondrial activities, for example, CPT-II, which is believed to be loosely associated with the inner surface of the mitochondrial inner membrane.46 This explanation would be consistent with the suggested fragility of mitochondria isolated from ischemic heart.47 However, we25,28 and others48 have demonstrated that ischemic mitochondria maintain high levels of coupled respiration and a full membrane potential, suggesting the absence of a membrane leak. Several other pieces of experimental evidence argue against the possibility of the presence of leaky mitochondrial membranes in preparations of isolated mitochondria. Similar changes in the malonyl-CoA sensitivity of CPT-I in isolated mitochondria have been measured in cells isolated from livers of diabetic and starved rats,49 indicating that the mitochondrial results2,6,45 are not an artifact of isolation. In isolated liver mitochondria, no significant activities of the latent enzymes, palmitoyl-CoA hydrolase or malonyl-CoA decarboxylase, could be measured, which could explain the decreased sensitivity of CPT-I to malonyl-CoA.2 Neither was any matrix palmitoyl-CoA hydrolase detected in the present studies on intact mitochondria from ischemic and remote regions of canine heart. It is also unlikely that the loss of malonyl-CoA inhibition is caused by expression of latent ischemic mitochondrial palmitoyl-CoA decarboxylase, since there was no change in the apparent $K_i$ for malonyl-CoA over an increase in the concentration of malonyl-CoA by three orders of magnitude. Nor can the presence of CPT-II from exposed matrix, known to be unaffected by malonyl-CoA, explain the partial reversal of the total sensitivity of ischemic CPT-I to malonyl-CoA by the sulphydryl reductant, DTT. It should also be noted that 30% of the CPT-I measured from remote regions is also insensitive to malonyl-CoA, an amount that would predict a significant contribution of broken mitochondria to the organellar preparations. This has been clearly shown not to be the case in both ischemic and control preparations.25,26

The decrease in the content of protein sulphydryl between control and ischemic mitochondria is approximately 8 mmol/mg. This 25% decrease in protein sulphydryl should distribute between all exposed sulphydryl residues in the mitochondrial membrane. However, not all proteins would lose catalytic activity, which is dependent on the location of the sulfur-containing amino acid in the peptide chain. If the most abundant inner membrane protein (10% of the total inner membrane protein), the adenine nucleotide translocase, is modified at one of its cysteine residues, the contribution of this protein to the decreased sulphydryl content can be calculated to be 8%. Because the translocase is present at 1.3 nmol/nmol cytochrome aa,50 and because concentrations of cytochrome aa in canine heart mitochondria are 0.5 nmol/mg,51 one cysteine on the translocase would be present at a concentration of 0.65 nmol/mg. If CPT-I constitutes 1% of the total mitochondrial membrane proteins, then less than 1% of the decrease in membrane sulphydryl would contribute to the modification of CPT-I.

The identity of the protein that recognizes the antibody prepared against CPT purified from heart has been controversial. Kerner and Bieber52 report that the antibody to CPT from beef heart inactivates malonyl-CoA-sensitive and –insensitive CPT of octyl glucoside–solubilized mitochondria from rat heart. Cross-reactivity of the antibody with CPT-I and CPT-II also was suggested by Brady and Brady,53 whose antibody (against CPT purified from liver) inactivates CPT in the supernatant and the pellet (enriched in CPT-I and CPT-II, respectively) of sonicated mitochondria from rat liver. In contrast, results from other investigations using tetradecylglycyl-CoA to inhibit CPT-I54 suggest that Tween 20 solubilizes the activity of CPT-I and separates it from the activity of CPT-I, which remains membrane bound. The CPT activity that reacts with tetradecylglycyl-CoA, apparently at the malonyl-CoA site, is labile to solubilization by detergent39,55 and does not react with the antibody to purified CPT.39,45,54

Although ischemia appears to increase the resistance of the outer CPT to inhibition by malonyl-CoA, it is still not clear whether the insensitive activity is the same form or a different isoform of CPT-I. Because our antibody to CPT-II was shown to retain malonyl-CoA-sensitive CPT-I on an antibody affinity column,52 we remain open to the possibility that cross-reactivity of the CPT-II antibody with the 68-kDa protein on the immunoblot may provide information about both CPT-II and the malonyl-CoA–sensitive enzyme. It may be that the transferase that is far less sensitive to malonyl-CoA becomes exposed on the outer surface of the mitochondrion, as suggested by others.2 On the other hand, if CPT-I manifests both malonyl-CoA–sensitive and –insensitive forms, the small change in molecular weight observed on the immunoblot may be more appropriately interpreted with respect to CPT-II. In addition to the reduced sensitivity of CPT-I to malonyl-CoA, the activity of CPT-II also may be altered with ischemia.56 The kinetic analysis of the overt activity of CPT suggests that two forms of the enzyme are expressed and that the malonyl-CoA–sensitive regulation of the overt enzyme is reduced in ischemia. The kinetic studies suggest that this reduction in regulation is due to switching between the malonyl-CoA–sensitive and –insensitive forms. Sulphydryl modification of CPT-I in ischemia may account in part for the decreased malonyl-CoA sensitivity.

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