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Effect of L-Carnitine on Mitochondrial Acyl CoA Esters in the Ischemic Dog Heart

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A. KOBAYASHI AND S. FUJISAWA. Effect of L-Carnitine on Mitochondrial Acyl CoA Esters in the Ischemic Dog Heart. *Journal of Molecular and Cellular Cardiology* (1994) **26**, 499–508. Many studies have shown that L-carnitine has a positive effect on ischemic myocardium, probably by reducing accumulation of long-chain acyl coenzyme A (CoA) esters. Previous studies have involved whole-heart extracts and have not assessed changes of CoA ester levels in mitochondria, the site of translocase inhibition. To more precisely assess L-carnitine effects, we measured long-chain acyl CoA ester levels in cytosol and in mitochondria in the ischemic canine heart. Dogs were divided into four groups: a sham-operated control group; an untreated group; and high- and low-dose L-carnitine-treated groups (30 mg/kg and 100 mg/kg). After 60 min of ischemia, the heart was excised, and the cytosolic and mitochondrial fractions were isolated. CoA esters and the activity of carnitine palmitoylcarnitine transferase (CPT) I and II were measured in both compartments. Approximately 89% of cellular free CoA, 90% of cellular acetyl CoA, 97% of cellular shot-chain acyl CoA, and 92% of cellular long-chain acyl CoA were located in the mitochondrial space under the normal condition. Under the ischemic condition, mitochondrial free CoA was significantly decreased. Conversely, mitochondrial acetyl CoA and long-chain acyl CoA were significantly increased. Treatment with L-carnitine significantly decreased acetyl CoA and long-chain acyl CoA in the ischemic mitochondrial space in a dose-dependent manner. These results support the hypothesis that L-carnitine reduces accumulation of long-chain acyl CoA within the ischemic mitochondrial space and thereby improves mitochondrial function and adenine nucleotide translocation.

KEY WORDS: Carnitine; Mitochondria; Long-chain acyl CoA; Ischemic myocardium; Cellular distribution.

Introduction

It is well known that fatty acids are the preferred substrate in well-oxygenated hearts (Neely and Morgan, 1974). Under conditions of ischemia, impaired oxidation of fatty acids has been postulated to lead to accumulation of their long-chain acyl CoA esters (Folts *et al.*, 1978; Shug *et al.*, 1978; Liedtke and Ellis, 1979; Suzuki *et al.*, 1981). Long-chain acyl esters can, at low concentrations, inhibit translocation of adenine nucleotides across the inner mitochondrial membrane; this is reversible (Shug *et al.*, 1971).

Free fatty acids (FFAs) are converted into acyl-CoA at the cytoplasmic site of the outer mitochondrial membrane. The acyl groups are then transferred to carnitine to form acyl carnitine which can pass through the inner mitochondrial membrane to the matrix. Here, the acyl groups are transferred from carnitine to internal CoA, and then oxidized via the β-oxidation cycle (Opie, 1979). Thus, carnitine is essential for the transport of long-chain fatty acids across the inner mitochondrial membrane. A previous *in vitro* study showed that addition of L-carnitine to the mitochondrial reaction mixture at least partially reversed the inhibition of adenine nucleotide translocation (Shug and Shrago, 1973). In addition, many *in vivo* studies have demonstrated that L-carnitine has a protective effect on ischemic myocardium, probably by reducing the accumulation of long-chain acyl CoA (Folts *et al.*, 1978; Liedtke and Ellis, 1979; Suzuki *et al.*, 1981). In all previous studies, however, long-chain acyl CoA esters were measured in whole heart tissue and not analysed specifically in mitochondria, the site of effective translocase inhibition. Since CoA

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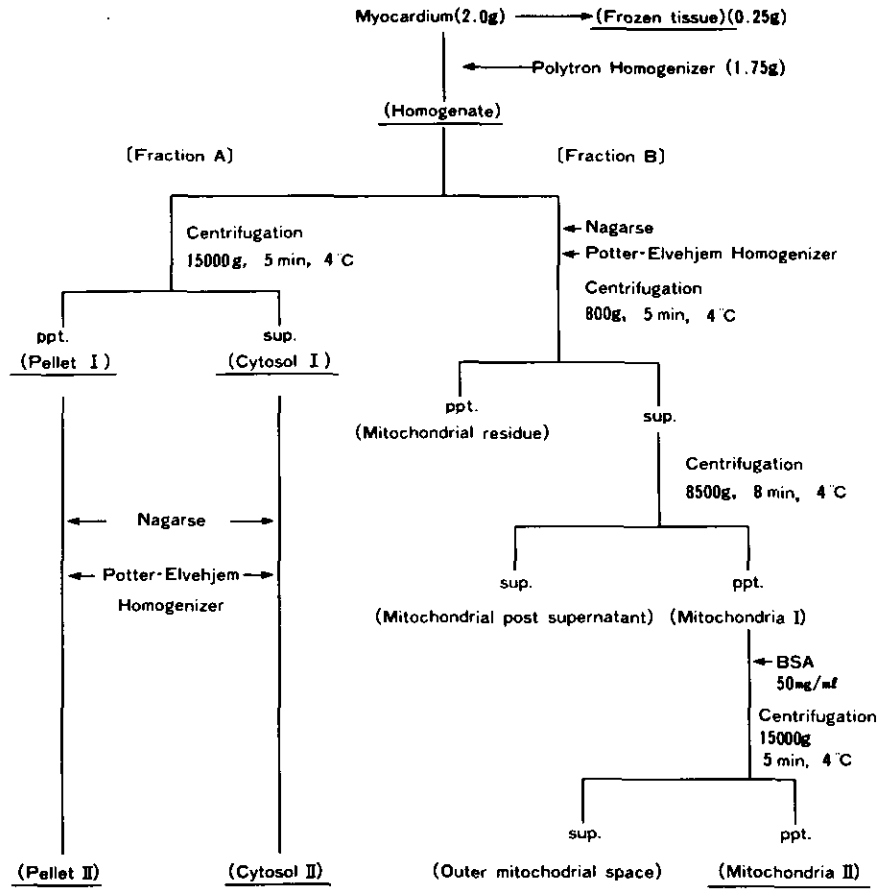

Figure 1 Method of tissue preparation.

esters are located in both the mitochondrial and the cytosolic compartments (Opie, 1979), it is difficult to assess changes in their mitochondrial distribution by studying whole heart extracts. If technically feasible, determining the level of long-chain acyl CoA esters levels in mitochondria and cytosol after administration of L-carnitine would be extremely important. Idell-Wenger *et al.* (1978) and Hütter *et al.* (1990) have reported a method of determining the intracellular distribution of CoA esters in the rat heart. The purpose of this study was to assess the effect of L-carnitine administration on the cellular distribution of acyl CoA esters in the ischemic canine heart.

Materials and Methods

Animals

Thirty-two male beagle dogs weighing 10–15 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.), followed by infusion of the same agent at 5 mg/kg/h during the experiment. The left anterior descending coronary artery (LAD) was isolated immediately distal to the first diagonal branch. After a stabilization period (about 30 min), the LAD was occluded for 60 min to produce acute myocardial ischemia.

The dogs were divided into four groups: a sham-operated control group ($n=8$), a nontreated control group ($n=8$), and two L-carnitine-treated groups ($n=16$). The L-carnitine groups included a low dose group (30 mg/kg, $n=8$) and a high dose group (100 mg/kg, $n=8$). In the L-carnitine groups, L-carnitine was administered as a bolus injection 5 min before LAD occlusion. The nontreated control group received an equivalent volume of 7.26% NaCl solution.

After occlusion of the LAD for 60 min, the heart was excised as rapidly as possible. Samples of myocardium were obtained from the left ventricular free wall. A part of each sample was rapidly freeze-clamped with a Wollenberger clamp cooled in liquid nitrogen (Fig. 1; Frozen tissue). The remainder of each sample was immediately chilled in ice-cold isolation buffer, containing the following: 225 mM D-mannitol, 75 mM sucrose, 10 mM

HEPES, and 1 mM EGTA (pH 7.4). These samples were used for isolation of the cytosolic and mitochondrial fractions (Fig. 1).

Isolation of cytosolic and mitochondrial fractions

We studied the cellular distribution of CoA and its derivatives in the ischemic dog hearts according to the modified method of Idell-Wenger *et al.* (1978). Myocardial tissue was added to four volumes of ice-cold isolation buffer (pH 7.4). The tissue suspension was homogenized twice for 10 s with a Polytron tissue homogenizer (PT-10, Kinematica), and the homogenate was divided into two fractions (Fig. 1; Fraction A and B).

Fraction A

A 2 ml portion of the homogenate was centrifuged at 15 000 × **g** for 5 min at 4°C, and part of the supernatant (Cytosol-I) and the pellet (Pellet-I) were immediately stored in liquid nitrogen. The remaining pellet and supernatant were treated with Nagarse. After standing at 0°C for 1 min, 13 ml of isolation medium was added, and the mixture was homogenized with a Potter-Elvehjem homogenizer at 500 rpm. After standing again at 0°C for 1 min, the supernatant and pellet were stored in liquid nitrogen (Cytosol-II and Pellet-II).

Fraction B

The homogenized tissue suspension Fraction B was treated with Nagarse (Fig. 1). After standing at 0°C for 1 min, 13 ml of isolation medium was added, and the mixture was homogenized with a Potter-Elvehjem homogenizer at 500 rpm. The homogenate was centrifuged at 800 × **g** for 5 min at 4°C, and then centrifuged at 8500 × **g** for 8 min at 4°C. The mitochondrial fraction was collected from the sediment by centrifugation (Mitochondria-I). This sediment was then rinsed in bovine serum albumin (BSA) to remove carnitine and its acyl derivatives which combined with the outside of the mitochondrial membrane, and centrifuged at 15 000 × **g** for 5 min at 4°C. The supernatant (outer mitochondrial space) and pellet (mitochondria II) were immediately frozen in liquid nitrogen for measurement of CoA and its derivatives. Oxygen consumption and the respiratory control ratio (RCR) of the isolated mitochondria (Mitochondria-I) were measured polarographically using the method described by Estabrook (1967).

Assay of CoA and its derivatives

CoA and its derivatives were assayed in the following fractions: Cytosol I, Cytosol II, Pellet II, and Mitochondria II. After the addition of 60% perchloric acid solution, the reaction mixture was centrifuged at 12 000 × **g** for 5 min at 4°C. Then 200 μl of distilled water, 10 μl 0.1 M dithiothreitol (DTT), and 50 μl 400 mM phosphate buffer (pH 7.0) were added to 200 μl of the supernatant. After neutralization with KOH, the reaction solution was centrifuged at 12 000 × **g** for 5 min at 4°C. Then, 15 μl 400 mM K-acetate buffer solution (pH 4.8) and 15 μl 1 mM $CuSO_4$ were added to 300 μl of the supernatant. The reaction mixture was then left to stand for 30 min at 25°C. Tissue oxaloacetate was removed by treating this mixture according to the method of Pande and Caramancion (1981), and the resultant solution was used to measure acetyl CoA and free CoA levels.

For the measurement of acid-soluble CoA, 60% perchloric acid was added to samples, and centrifugation was performed in the same manner as for the assay of free carnitine and free CoA. Then 200 μl distilled water and 10 μl 0.1 M DTT were added to 200 μl of the supernatant. After titration to pH 12.0 with KOH, the reaction mixture was hydrolysed at 55°C for 15 min. Then 40 μl 60% perchloric acid was added to the mixture. After centrifugation, 400 μl of the supernatant was neutralized with KOH, and tissue oxaloacetate was removed by the above-mentioned method. This solution was used for the measurement of acid-soluble CoA levels. The short-chain acyl CoA level was calculated by subtraction of the acetyl CoA and free CoA levels from the acid-soluble CoA level.

For the measurement of acid-insoluble CoA, the pellet was rinsed twice with 6% perchloric acid and then suspended in 500 μl of 6% perchloric acid solution. Then 100 μl of 0.1 M DTT and 200 μl of 3 N KOH were added to the reaction mixture and hydrolysation was allowed to procede for 15 min at 55°C. To the reaction mixture, 40 μl 60% perchloric acid was added, followed by centrifugation at 12 000 × **g**. Then 400 μl of supernatant was neutralized with KOH, and this solution was used for the measurement of acid-insoluble CoA levels.

CoA was assayed by the radioisotope procedure involving a two step method for determining reduced CoASH, in which [^{14}C]citrate is the final product (Rabier *et al.*, 1983; Pande *et al.*, 1981). In the first step of the reaction, phosphotransacetylase was used to catalyse the synthesis of acetyl-CoA from reduced coASH and acetylphosphate. Acetyl-CoA was then condensed with [^{14}C]oxaloacetate by

citrate synthase to give [^{14}C]citrate. At the end of the reaction, the excess of [^{14}C]oxaloacetate is transaminated to [^{14}C]asparatate in the presence of L-glutamateoxalo-acetate transaminase and asparatate removed with a cation-exchange resin.

Activity of carnitine palmitoylcarnitine transferase

Activities of carnitine palmitoylcarnitine transferase I (CPT I) and CPT II were determined by the method of Saggerson *et al.* (1983). CPT I activity was calculated by subtraction of the CPT II activity from the total CPT activity, which was determined in the absence of malonyl-CoA.

Assay of marker enzymes

The activity of citrate synthase (a mitochondrial marker enzyme) was determined by the method of Idell-Wenger *et al.* (1978). Lactate dehydrogenase activity (a cytoplasmic marker enzyme) was determined by the methods of Amador *et al.* (1963) and Babson *et al.* (1965). The activity of Na^+, K^+-ATPase (cell membrane marker enzyme) was determined by the method of Michael *et al.* (1984). The tissue protein concentration was measured by the method of Biuret (1949).

Protective effect of L-carnitine on the ischemic myocardium

We investigated the effect of L-carnitine on the ischemic myocardium using two indices. The mitochondrial respiratory control index (RCI) was measured polarographically by the method of Estabrook (1967). The ATP level was measured by an enzymatic method (1985).

Reagents

The L-carnitine used was purchased from Kongo Chemical Co. (Japan). Amersham Japan Co. supplied [^{14}C]-acetyl CoA and L-[methyl-^{14}C]-carnitine hydrochloride, and L-[1-^{14}C]-palmitoyl carnitine chloride was obtained from New England Nucleonic Research Products. All other reagents and buffers were obtained from commercial sources and were of the highest grade available.

Statistical analysis

All data are given as the mean ± S.E.M. Significant differences ($P < 0.05$) were determined by unpaired Student's *t*-test.

Results

Mitochondrial and cytosolic fractions

The pellet and supernatant were treated with Nagarse to allow collection of mitochondria. Mitochondrial function was not affected by Nagarse. Table 1 shows the cellular content of mitochondrial fraction. The mitochondrial recovery rate was 81.6 ± 0.8%, and the respiratory control index in the nonischemic state was 4.47 ± 0.02. We confirmed by electron microscopy that pure mitochondria were obtained by this method. Cytosolic Na^+, K^+-ATPase activity was 0.98 ± 0.07 μmol Pi/g wet tissue/h. Since the activity in the homogenate was 19.7 ± 0.3 μmol Pi/g wet tissue/h, the cytosolic fraction had about 5% contamination by cell membrane components. Table 2 shows the activities of mitochondrial and cytosolic marker enzymes in each fraction. Lactate dehydrogenase activity was not detected in the mitochondrial fraction, indicating that the mitochondrial fractions were not contaminated with cytosolic components. The citrate synthase activities in Mitochondrial I and II fractions were very similar, indicating that citrate synthase was not extracted from the mitochondrial space by washing in an isolation buffer containing BSA.

Exogenous [^{14}C]-carnitine, [^{14}C]-palmitoyl carnitine, [^{14}C]-acetyl CoA and [^{14}C]-palmitoyl CoA were completely recovered in the mitochondrial post supernatant fraction and mitochondrial residual fraction, whereas neither form of labeled carnitine was detected in the Mitochondrial II fraction (Table 3). Mitochondrial II was obtained from Mitochondrial I. [^{14}C]-Palmitoyl carnitine in the Mitochondrial I was removed by rinsing with bovine serum albumin (BSA). This result shows that exogenous added [^{14}C]-palmitoyl carnitine combined loosely with the outside of the mitochondrial membrane. Therefore, we measured metabolites in the mitochondrial fraction-II as changes in mitochondrial fraction. These results indicate that carnitine, acetyl CoA and palmitoyl CoA were not transferred from the cytosolic space to the mitochondrial space during the tissue preparation. We calculated the content of CoA and its derivatives in mitochondrial and cytosolic fractions per g of myocardium.

Changes in metabolites of CoA during tissue preparation

The content of metabolite in mitochondria per g of myocardium (C) is calculated as follows:

Table 1 Cellular content of mitochondrial fraction

Parameter	Tissue fraction	Sham-operated control	Ischemia 60 min
Citrate synthase activity	Homogenate (units/g of wet tissue)	63.1 ± 1.2	64.1 ± 0.9
	Mitochondria I (units/mg mitochondria protein)	2.07 ± 0.03	2.12 ± 0.03
Protein content	Mitochondria I (yield) (mg/g of wet tissue)	24.9 ± 0.3	24.2 ± 0.3
	Mitochondria (calculated total) (mg/g of wet tissue)	30.5 ± 0.3	30.2 ± 0.5
	Recovery of mitochondria (%)	81.6 ± 0.8	80.1 ± 0.6
Respiratory activity	Respiratory control index (RCI)	4.47 ± 0.02	2.07 ± 0.05**
	ADP/O ratio	1.97 ± 0.01	N.D.
	State III QO_2 (natoms of oxygen/min/mg of protein)	281.9 ± 12.2	139.8 ± 13.8**

Protein content (P) of mitochondria (calculated total) was calculated by the following formula:

$$P = \frac{\text{Citrate synthase activity in homogenate}}{\text{Citrate synthase activity in mitochondria I}}$$

**$P < 0.01$: significance of change in Sham-operated control *v* Ischemia. N.D. = not detectable.

Table 2 Activities of mitochondrial and cytosolic marker enzymes

Fraction	Citrate synthase activity (units/g of wet tissue)		Lactate dehydrogenase activity (units/g of wet tissue)	
	Sham-operated control	Ischemia 60 min	Sham-operated control	Ischemia 60 min
Homogenate	63.1 ± 1.2	64.1 ± 0.9		
Fraction A				
Cytosol I	3.3 ± 0.1	3.4 ± 0.1		
Pellet I	59.6 ± 1.4	61.1 ± 0.7		
Pellet II	59.6 ± 1.3	60.8 ± 0.9		
Cytosol I + Pellet I	62.8 ± 1.4	64.5 ± 0.6		
Fraction B				
Mitochondrial residue	7.0 ± 0.1	7.5 ± 0.9	N.D.	N.D.
Mitochondrial post supernatant	4.2 ± 0.2	3.9 ± 0.3	176.0 ± 1.7	169.7 ± 1.6**
Mitochondria I	51.7 ± 1.0	51.2 ± 0.7	N.D.	N.D.
Mitochondria II	51.7 ± 1.0	50.9 ± 0.8	N.D.	N.D.
Outer mitochondrial space (wash with 50 mg/ml BSA)	N.D.	N.D.	N.D.	N.D.
Mitochondrial residue + Mitochondrial post supernatant + Mitochondria I	62.7 ± 1.0	62.7 ± 0.9	176.0 ± 1.7	169.7 ± 1.6**

There is low activity of mitochondrial marker enzyme in the control I fraction, whereas there is no activity of cytosolic marker enzyme in the mitochondrial fraction.
**$P < 0.01$: significance of change in Sham-operated control *v* Ischemia. N.D. = not detectable.

$$A = \text{CS in homogenate/CS in mitochondria I fraction} \quad (1)$$

$$C = A \times B \quad (2)$$

Where CS is citrate synthase activity, A is the content of mitochondria per g of myocardium, and B is the content of metabolite per mg protein of mitochondria.

Table 3 Distribution of added ^{14}C-labeled carnitine and CoA during fractionation procedure

Fraction	Carnitine				CoA			
	Free		Palmitoyl		Acetyl		Palmitoyl	
	Normal	Ischemia 60 min	Normal	Ischemia 60 min	Normal	Ischemia 60 min	Normal	Ischemia 60 min
Homogenate	13627	13606	13437	13681	13560	13606	13710	13687
Mitochondrial post supernatant + Mitochondrial residue	13451	13440	12751	12880	13579	13440	13713	13690
Mitochondria I	N.D.	N.D.	686	512	N.D.	N.D.	N.D.	N.D.
Mitochondria II	—	—	N.D.	N.D.	—	—	—	—
Outer mitochondrial space (wash with 50 mg BSA)	—	—	690	517	—	—	—	—

^{14}C-labeled carnitine and CoA were added to myocardial tissue and then tissue samples were isolated mitochondrial fraction. Mitochondria I was rinsed in bovine serum albumin, and centrifuged at 15 000 × g. The supernatant (outer mitochondrial) and pellet (mitochondria II) were obtained. Units are disintegration per minute (d/min). N.D. = not detectable.

Table 4 Observed distribution of CoA

Group	Fraction	CoA (nmol/wwg)				
		Free	Acetyl	Acid-soluble	Acid-insoluble	Total
	Frozen tissue	21.7 ± 1.1	4.0 ± 0.2	30.8 ± 2.1	9.2 ± 0.2	40.0 ± 2.2
	Homogenate	21.9 ± 1.1	3.9 ± 0.2	30.9 ± 2.1	9.1 ± 0.2	40.0 ± 2.2
Sham-operated	Cytosol I	1.4 ± 0.2	0.2 ± 0.1	2.0 ± 0.2	0.5 ± 0.1	2.6 ± 0.2
control (n = 8)	Cytosol II	1.4 ± 0.2	0.2 ± 0.1	2.0 ± 0.3	0.5 ± 0.1	2.5 ± 0.3
	Pellet I	20.2 ± 1.2**	3.8 ± 0.2	29.2 ± 1.7**	8.7 ± 0.2**	37.9 ± 1.8
	Pellet II	23.6 ± 1.4**	3.7 ± 0.2	33.9 ± 2.0**	5.1 ± 0.1**	39.0 ± 2.0
	Mitochondria II	22.3 ± 1.0	3.6 ± 0.2	33.4 ± 1.2	5.0 ± 0.1	38.3 ± 1.3
	Frozen tissue	6.2 ± 0.3	10.5 ± 0.3	21.6 ± 0.5	24.3 ± 0.3	46.0 ± 0.8
	Homogenate	6.0 ± 0.2	10.5 ± 0.3	21.0 ± 0.8	24.4 ± 0.3	45.4 ± 1.1
Ischemia	Cytosol I	0.3 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	2.0 ± 0.1	3.3 ± 0.2
60 min (n = 8)	Cytosol II	0.3 ± 0.2	0.8 ± 0.1	1.3 ± 0.1	1.9 ± 0.2	3.2 ± 0.3
	Pellet I	5.3 ± 0.4**	9.9 ± 0.3	19.7 ± 0.7**	21.9 ± 0.5**	41.6 ± 1.2
	Pellet II	20.8 ± 0.6**	9.9 ± 0.3	35.2 ± 1.0**	6.7 ± 0.2**	41.9 ± 1.1
	Mitochondria II	20.8 ± 0.5	9.4 ± 0.2	34.6 ± 0.9	6.4 ± 0.2	41.0 ± 1.1

There are significant differences between Pellet I and II fractions in the Sham-operated and Ischemic groups. Therefore, it is necessary to make a slight correction to obtain the true content of CoA.

The content of metabolite in cytosol per g of myocardium (E) is calculated as follows:

$$D = C \times CS \text{ in cytosol I/CS in homogenate} \quad (3)$$

$$E = \text{cytosol I-}D \quad (4)$$

Where D is the content of metabolite in mitochondria which is mixed in cytosol I.

Table 4 shows the changes in the metabolites of CoA esters during tissue preparation. The differences in CoA metabolites detected in Pellet I and Pellet II were significant. The increase in free CoA and acid-soluble CoA almost equalled the reduction of acid-insoluble CoA. This indicates that the changes in CoA metabolites during preparation of mitochondria may have been due to conversion of acid-insoluble CoA to free CoA. Therefore, it was

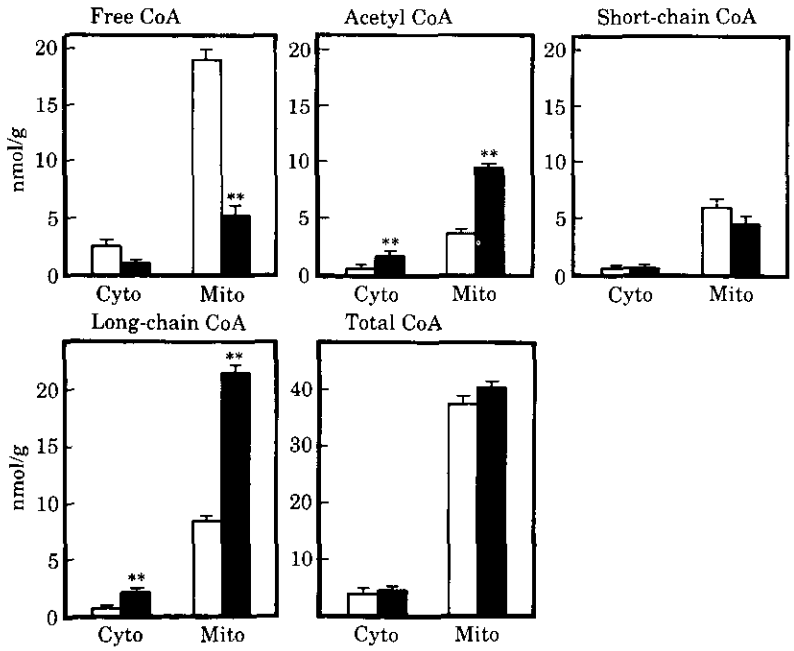

Figure 2 Cellular distribution of CoA in the heart. All values are expressed as nmol of wet tissue, and represented as mean ± S.E.M.; ($n=8$). $^{**}P<0.01$ *v* no ischemia. Cyto = cytosolic compartment, Mito = mitochondrial compartment.

possible to obtain the true contents of mitochondrial CoA and its derivatives using a corrected calculation. The true content of acid-soluble CoA in mitochondria (F) was calculated as follows:

$$F=K+(G-H) \quad (5)$$

Where K is the content of acid-soluble CoA in mitochondria per g of myocardium using the calculation of equation (2), and G and H are the contents of acid-soluble CoA in Pellet I and II.

The true content of acid-insoluble CoA in mitochondria (J) was calculated as follows:

$$J=L-(G-H) \quad (6)$$

Where L is the content of acid-insoluble CoA in mitochondria per g of myocardium using the calculation of equation (2).

Cellular distribution of CoA and its derivatives in ischemic myocardium

Figure 2 shows the contents of CoA and its derivatives in the mitochondrial and cytosolic fractions in nonischemic and ischemic canine myocardium. Under nonischemic conditions, approximately 89% of cellular free CoA, 90% of cellular acetyl CoA, 97% of cellular short-chain acyl CoA, and 92% of cellular long-chain acyl CoA were located within the nonischemic mitochondrial fraction.

Under ischemic conditions (for 60 min), the concentration of free CoA in the ischemic mitochondrial fraction significantly decreased. On the other hand, the concentrations of acetyl CoA and long-chain acyl CoA in the ischemic mitochondrial fractions significantly increased, compared to the values under non-ischemic conditions (both $P<0.01$).

Protective effect of L-carnitine on ischemic myocardium

Administration of L-carnitine increased the mitochondrial RCI of ischemic myocardium in a dose-dependent manner [Fig. 3 (a)]. Furthermore, L-carnitine treatment significantly increased the ATP content of ischemic myocardium in a dose-dependent manner [Fig. 3 (b)].

Effect of L-carnitine on cellular distribution of CoA and its derivatives in ischemic myocardium

The concentration of acetyl CoA in the ischemic mitochondrial fraction significantly decreased in a dose-dependent manner after administration of L-carnitine (Fig. 4). In particular, 100 mg/kg of L-carnitine reduced the acetyl CoA to almost the

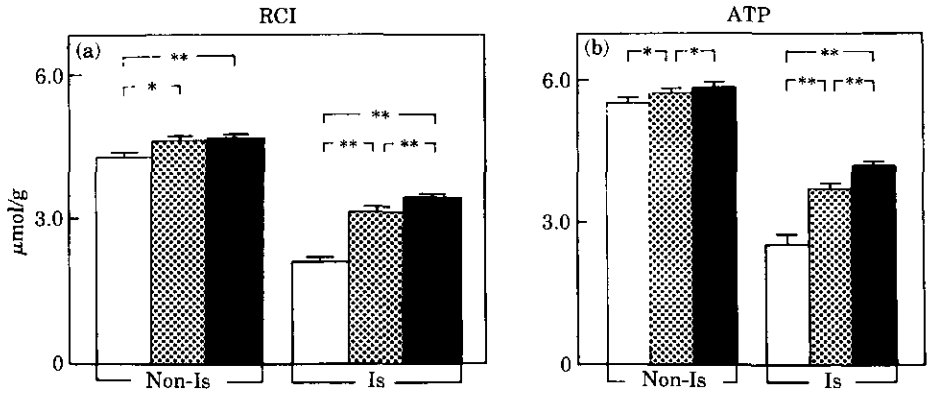

Figure 3 Effect of L-carnitine on the respiratory control index (RCI) and ATP levels of ischemic myocardium. (□) Control, (▨) 30 mg/kg carnitine, (■) 100 mg/kg carnitine. All values represent mean ± S.E.M.: ($n = 8$). $^{**}P < 0.01$, $^{*}P < 0.05$. Is = ischemic myocardial mitochondria, Non-Is = no ischemic myocardial mitochondria.

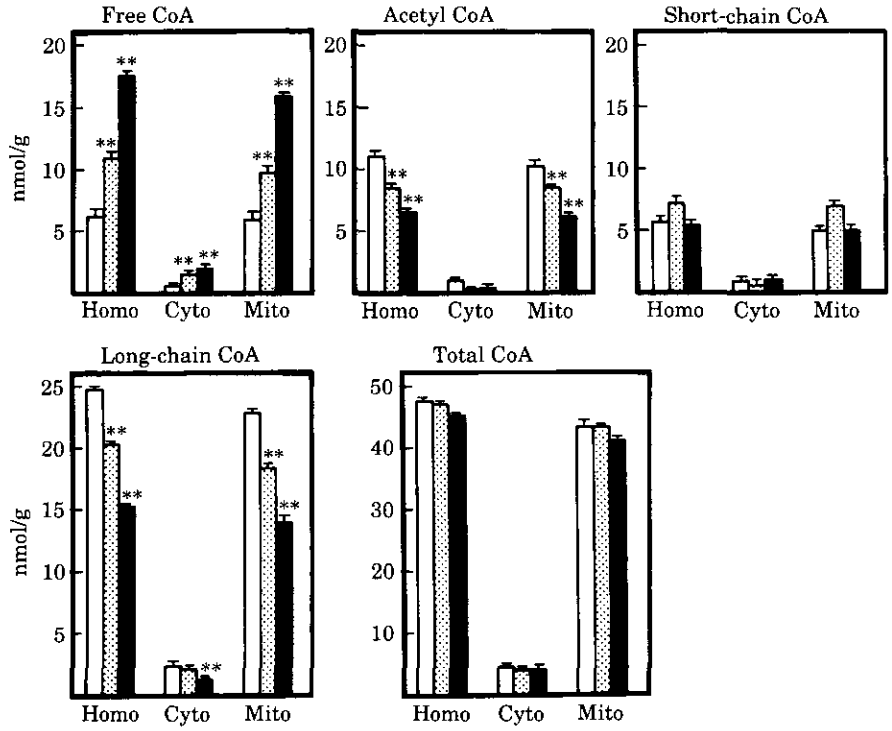

Figure 4 Effect of L-carnitine on the cellular distribution of CoA esters in the ischemic heart. (□) Control, (▨) 30 mg/kg carnitine, (■) 100 mg/kg carnitine. All values represent mean ± S.E.M.; ($n = 8$). $^{**}P < 0.01$ *v* nontreated group (control). Homo = homogenate (cytosol + mitochondria), Cyto = cytosolic compartment, Mito = mitochondrial compartment.

same level found in the nonischemic mitochondria (6.2 ± 0.1 *v* 5.1 ± 0.1 nmol/g wet tissue). The concentration of long-chain acyl CoA in the ischemic mitochondrial fraction significantly decreased in a dose-dependent manner (control *v* 30 mg/kg *v* 100 mg/kg; 22.4 ± 0.3 *v* 18.0 ± 0.2 *v* 13.9 ± 0.2 nmol/g wet tissue, both $P < 0.01$).

CPT activity

Table 5 shows the CPT I and II activities in nonischemic and ischemic myocardium. The activities in ischemic myocardium were significantly lower than those in nonischemic myocardium. In particular, the changes in CPT I were greater than those in

Table 5 Effect of L-carnitine on CPT I and CPT II

Group	CPT I (nmol/min/mg protein)	CPT II (nmol/min/mg protein)
Control		
Nonischemia	2.85 ± 0.04	0.63 ± 0.01
Ischemia	1.39 ± 0.03##	0.47 ± 0.01##
Carnitine-treated (30 mg/kg)		
Ischemia	1.39 ± 0.04##	0.47 ± 0.01##
Carnitine-treated (100 mg/kg)		
Ischemia	1.54 ± 0.03##	0.54 ± 0.02**

All values represent mean ± S.E.M.; ($n = 8$).
**$P < 0.01$ *v* nontreated ischemic group. ##$P < 0.01$ *v* nonischemic group. CPT = carnitine palmitoylcarnitine transferase.

CPT II. The reduction of CPT II was significantly improved by treatment with 100 mg/kg L-carnitine.

Discussion

This study investigated the influence of L-carnitine on the cellular distribution of CoA and its derivatives in ischemic canine myocardium. We showed that L-carnitine significantly reduced the accumulation of long-chain acyl CoA in the mitochondrial space induced by ischemia.

Some researchers have suggested that L-carnitine's protective effect on ischemic myocardium may be secondary to its reducing the accumulation of long-chain acyl CoA (Folts *et al.*, 1978; Liedtke and Ellis, 1979; Suzuki *et al.*, 1981). Long-chain acyl CoA at high levels inhibits the action of adenine nucleotide translocase, which is involved in the transport of ATP. Therefore, the accumulation of long-chain acyl CoA may inhibit the formation and utilization of ATP in ischemic myocardium (Shug *et al.*, 1971). This study was undertaken to determine if L-carnitine reduces the accumulation of long-chain acyl CoA in the mitochondrial space.

Previous studies have shown the beneficial effects of L-carnitine in whole heart preparations. These whole heart studies cannot isolate the effects in the mitochondrial space, since CoA and its derivatives are also located in cytosolic compartments (Opie, 1979). Idell-Wenger *et al.* (1978) and Hütter *et al.* (1990) reported on the cellular distribution of carnitine, CoA, and their acyl derivatives in rat hearts. We adopted their methods in the present study. However, a major problem is clarification of the alterations in the degree of translocation between tissue compartments and/or catabolism of these esters during tissue processing.

In our study, the recovery of mitochondria was very high, and lactate dehydrogenase activity was not detected in the mitochondrial fraction. Furthermore, carnitine, acetyl CoA and palmitoyl CoA were not transferred from the cytosol to the intramitochondrial space during tissue preparation processing. However, processing did alter metabolites of CoA and its derivatives. The increase in free CoA and acid-soluble CoA equalled the reduction in acid-insoluble CoA. This indicates that the changes in CoA metabolites during preparation of mitochondria have been due to conversion of acid-insoluble CoA to free CoA. Therefore, it was necessary to correct the values obtained for CoA. The corrected values of metabolites in the mitochondrial and cytosolic fractions were almost equal to the values for frozen tissue. This finding suggests that it is possible to calculate the approximate amount of each compound in each cellular compartment.

Approximately 90% of the total cellular CoA was located in the mitochondrial space. This result corresponded closely to previous studies (Idell-Wenger *et al.*, 1978; Hütter *et al.*, 1990). Myocardial ischemia induced increases in acetyl CoA and long-chain acyl CoA levels in the mitochondrial space. On the other hand, L-carnitine administration significantly decreased the levels of acetyl CoA and long-chain acyl CoA in the mitochondrial space, the site of effective translocase inhibiton and β-oxidation of free fatty acids (FFAs) (Opie, 1979).

FFAs can freely cross the sarcolemmal membrane and convert to long chain acyl CoA in the cytosolic compartment. Long-chain acyl CoA then combines with carnitine to form long-chain acylcarnitine, which can transfer to the mitochondrial compartment. Since the uptake of FFAs by the myocytes increases as the blood FFA level rises, the long-chain acyl CoA content increases in the cytosolic compartment of ischemic myocardium (Opie, 1979). Exogenous L-carnitine raises the plasma carnitine content and then enhances intracellular carnitine content (Fujisawa *et al.*, 1992). In ischemic myocardium, carnitine palmitoylcarnitine transferases (CPT I and II) activities were decreased and acetyl CoA accumulated in the mitochondrial space. On the other hand, CPT II activity and free carnitine content in the mitochondrial space were increased after treatment with L-carnitine. Therefore, accumulated mitochondrial long-chain acyl CoA could be converted to long-chain acylcarnitine

by the action of CPT II and free carnitine in the ischemic myocardial mitochondria. These may be the mechanisms by which L-carnitine treatment prevented the accumulation of acetyl CoA and long-chain acyl CoA in the ischemic myocardial mitochondrial space.

In addition, L-carnitine reduces the acetyl CoA to CoA ratio of the intramitochondrial space, thereby contributing to maintaining pyruvate dehydrogenase in the active form (Hansford, 1976); at the same time, reduced levels of acetyl CoA in the cytosolic space enhance glycolysis (Randle, 1976). Previous studies have reported that carnitine enhances glucose metabolism in humans (Ferrannini *et al.*, 1988). These findings provide further evidence that carnitine has a beneficial effect on ischemic myocardium.

In conclusion, our results support the hypothesis that L-carnitine reduces the accumulation of mitochondrial long-chain acyl CoA and thereby improves mitochondrial function, and β-oxidation of FFA. In addition, L-carnitine increased the relative contribution of glycolysis and decreased the relative contribution of fatty acids in mycardial substrate metabolism, and has a protective effect on the ischemia myocardium.

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