

## **Effects of L-Carnitine on Phospholipids in the Ischemic Myocardium**

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### **SUMMARY**

To evaluate the protective effects of L-carnitine on the ischemic myocardium, the effects of its administration on tissue levels of high energy phosphate and phospholipids were studied in ischemic dog hearts. Myocardial ischemia was induced by the ligation of the left anterior descending coronary artery for 40 min. In the experiment, L-carnitine (300 mg/kg) was administered intravenously prior to coronary artery ligation. Mitochondrial phospholipids were extracted from nonischemic and ischemic regions of the myocardium and subsequently analyzed. In ischemic myocardial tissues, levels of adenosine 5'-triphosphate (ATP) were reduced. The decrease was significantly elevated by L-carnitine pretreatment. The mitochondrial fractions obtained from ischemic myocardia had significantly lower levels of phospholipids than those obtained from nonischemic tissues. Moreover, the amounts of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine were significantly decreased in ischemic myocardial tissues. L-carnitine-pretreatment prevented the reduction of these phospholipids. Lysophosphatidylethanolamine and sphingomyelin did not show statistically significant decreases. This may explain why the administration of carnitine has beneficial effects on ischemic myocardium.

### **Additional Indexing Words:**

Phospholipid metabolism    Ischemic myocardium    L-carnitine  
Phospholipid composition    Mitochondria

**F**ATTY acids are an important energy source of normal heart muscle. The oxidation of fatty acids in mitochondria requires the formation of

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acyl carnitine which is translocated across the inner mitochondrial membrane. It is known, however, that the concentration of carnitine decreases whereas those of long chain acyl carnitine and long chain acyl coenzyme A (CoA) increase in the ischemic myocardium.<sup>1)</sup> The latter intermediates in fatty acid oxidation are thought to be toxic to the myocardium while free carnitine is protective against the toxic effects of ischemia.<sup>2)</sup> Rapid, abnormal increases in free fatty acids (FFA) in the serum are reported to be mainly responsible for severe arrhythmias.<sup>3)-5)</sup>

Phospholipids are major components of biomembranes, including mitochondrial inner membranes, and are essential for the integrity of the functioning membranes. Consequently, any alteration in their amounts and composition may have major effects on membrane function and cell metabolism.

In this work we examined changes in the total amount and composition of mitochondrial phospholipids and the effect of administered free L-carnitine on ischemic myocardia.

#### MATERIALS AND METHODS

Fifteen mongrel dogs of either sex, each weighing 8–15 kg, were anesthetized with sodium pentobarbital (30 mg/kg) given intravenously. Under artificial respiration, the chest of each dog was opened by incision between the fourth and the fifth ribs. The left anterior descending artery (LAD) was ligated immediately distal to the first diagonal branch. The left cervical vein was cannulated for infusion of L-carnitine (provided by Earth Chemical Co., Ltd.). Some dogs received 300 mg/kg of L-carnitine, while control animals were given 5.9% NaCl intravenously for 5 min, starting 10 min prior to coronary artery ligation. The artificial occlusion was maintained for 40 min based on preliminary experiments. Soon after the ligation, the ischemic area appeared as a sharply delineated cyanotic region. Dogs which showed no epicardial cyanosis were excluded from the present study. At 40 min, the beating hearts were quickly removed from all animals and transmural tissues involving ischemic areas (occluded LAD area) and nonischemic areas (circumflex coronary artery area) were rapidly excised. They were processed to yield mitochondrial preparations. ATP content was determined as described below.

##### *ATP determination:*

To determine ATP, excised tissues were immediately frozen with a Wallenberger clamp cooled in liquid nitrogen. The procedure, from removal of the heart until freezing the excised tissue, took less than 30 sec and the frozen samples were stored at  $-80^{\circ}\text{C}$ .



Fig. 1. A partitioned mitochondria from normal dog heart.  $\times 1,000$ .  
Bar = 1  $\mu$ m.

ATP was determined using hexokinase and glucose-6-phosphate dehydrogenase according to Lamprecht and Trautshold.<sup>6)</sup>

*Mitochondrial preparation:*

Samples were trimmed of adipose tissue and minced with scissors in a cold room. The samples were suspended in about 5 vol of cold 0.25 M sucrose 50 mM Tris(hydroxymethyl) aminomethane (Tris), pH 7.4. The suspension was immediately homogenized for 20 sec with a Polytron tissue homogenizer (Kinematica PT10/35) with an external rheostat setting at 5.5. The homogenate was centrifuged at 1,000 g for 10 min. After centrifugation, the supernatant layer was recovered and passed through a cheesecloth. The supernatant was centrifuged at 12,000 g for 10 min. The sediment was suspended in 0.25 M sucrose 10 mM Tris, pH 7.7 and centrifuged at 24,000 g for 10 min. The resulting sediment was resuspended in 0.25 M sucrose 10 mM Tris, pH 7.7. Fig. 1 shows the morphology of the prepared mitochondria.

*Measurement of mitochondrial phospholipid content:*

Mitochondrial lipids were extracted using a modified method of Folch et al.<sup>7)</sup> A mitochondrial suspension (1 ml) was extracted with a 25 ml solution of  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  = 2:1 and filtered through filter paper. Four ml of

0.73% NaCl were added to the filtrate and the mixture centrifuged at 3,000 rpm for 10 min. The lower phase was vigorously mixed with 8 ml of the solution (0.73% NaCl:  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$ =47: 3: 48) and centrifuged at 3,000 rpm for 10 min. The resulting lower phase containing essentially all of the mitochondrial lipids was evaporated to dryness with a rotory evaporator. The amount of phospholipids in the mitochondria was determined by measuring the phosphorus content of the extract by Allen's method.<sup>8)</sup>

The lipid extract was analyzed with silica gel thin-layer plates (HPTLC-Fertigplatten Kieselgel 60, Merck, Darmstadt), and the developing solvent system ( $\text{CHCl}_3$ :  $\text{C}_2\text{H}_5\text{OH}$ :  $\text{H}_2\text{O}$ : Triethylamine=30: 34: 8: 35).<sup>9)</sup> The chromatogram was scanned at 600 nm with a chromatoscanner (Shimadzu, CS 920). Protein was determined by biuret reaction.<sup>10)</sup>

The amounts of the metabolites were expressed as mean  $\pm$  SD and statistical analysis was performed using Student's t-test. P values of less than 0.05 were considered significant.

## RESULTS

Fig. 2 shows the myocardial levels of ATP in the normal and the affected areas for both the control and the L-carnitine-pretreated groups. In the control group, the ATP levels in the ischemic myocardium ( $2.52 \pm 0.62 \mu\text{mol/g}$ ) were significantly ( $p < 0.001$ ) lower than in the nonischemic area ( $5.33 \pm 0.69$

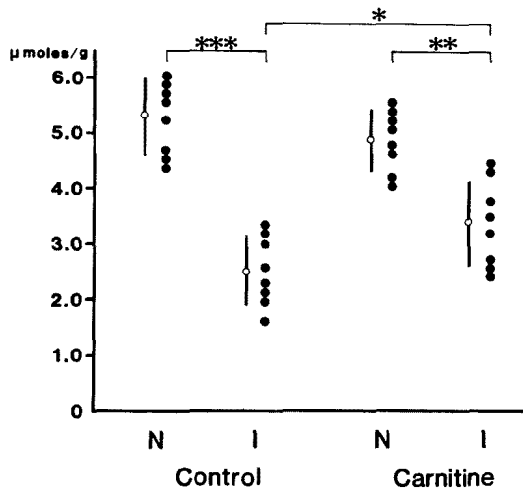


Fig. 2. Specific amounts of ATP in the normal (N) and ischemic (I) myocardial tissues of the control and the L-carnitine-pretreated animals. Values ( $\mu\text{moles}$  of ATP/g of tissue) are expressed as means (o)  $\pm$  SD (bars). N=nonischemic tissue; I=ischemic tissue.

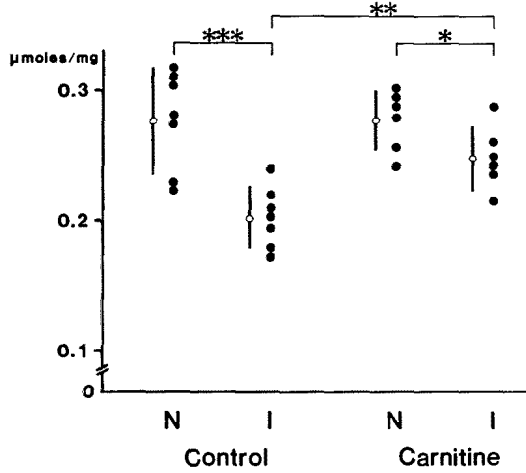


Fig. 3. Specific amounts of mitochondrial total phospholipids in the normal and the ischemic myocardial tissues of the control and the L-carnitine-pretreated animals. Values ( $\mu$ moles of P/mg of protein) are expressed as means  $\pm$  SD. N=nonischemic tissue; I=ischemic tissue.

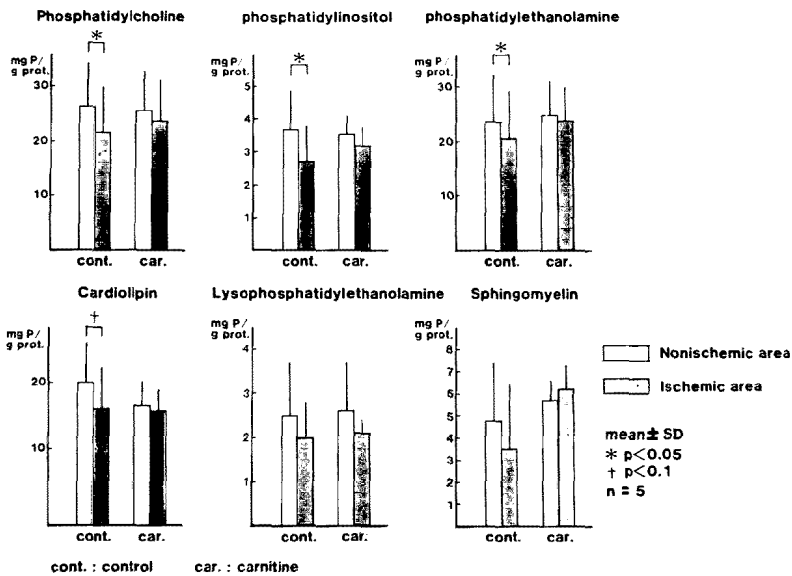


Fig. 4. Specific amounts of individual phospholipids in the mitochondria prepared from the normal and the ischemic myocardial tissues of the control and the L-carnitine-pretreated animals. Values (mg P/g of protein) are expressed as means  $\pm$  SD. cont.=control group; car.=carnitine.

$\mu\text{mol/g}$ ). In the L-carnitine-administered group, the ATP levels in the ischemic myocardium ( $3.40 \pm 0.77$ ) were significantly ( $p < 0.01$ ) elevated compared with those of the control group.

Fig. 3 shows the specific amounts of mitochondrial phospholipids in the normal and the ischemic areas of the 2 groups. In the ischemic myocardial mitochondria of the control group, total mitochondrial phospholipids decreased from  $0.28 \pm 0.04$  to  $0.20 \pm 0.02 \mu\text{mol/mg}$  ( $p < 0.001$ ). Pretreatment with L-carnitine (300 mg/kg) prevented the decrease ( $0.25 \pm 0.02 \mu\text{mol/mg}$ ,  $p < 0.05$ ).

The specific amounts of phospholipids were determined for the normal and the ischemic myocardial mitochondria of the control and the pretreated groups (Fig. 4). The amounts of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine were decreased significantly in the mitochondria of the affected tissues in the control group whereas that of cardiolipin showed a trend toward decreasing. The levels of sphingomyelin and lysophosphatidylethanolamine did not show statistically significant changes.

In the L-carnitine-pretreated group, decreases in the affected myocardial tissues were insignificant, i.e., prevented for all mitochondrial phospholipids examined.

## DISCUSSION

Long chain acyl CoA and long chain acyl carnitine accumulate in ischemic myocardia. On the other hand, free carnitine decreases. Long chain acyl carnitine inhibits several cellular functions such as  $\text{Na}^+\text{-K}^+\text{-ATPase}$  of sarcolemma<sup>11)</sup> and  $\text{Ca}^{2+}\text{-ATPase}$  of sarcoplasmic reticulum.<sup>12)</sup> Because high levels of long chain acyl CoA inhibit adenine nucleotide translocase activity,<sup>13),14)</sup> ATP availability for contraction in ischemic myocardium is impaired both by the reduced supply of oxygen and the accumulation of long chain acyl CoA. Suzuki et al<sup>2)</sup> reported that pretreatment with L-carnitine prevents both the depletion of free carnitine and ATP and the accumulation of long chain acyl CoA and long chain acyl carnitine in ischemic myocardium. Although the mechanism of protective effects of L-carnitine is a subject of controversy, it may involve maintenance of tissue ATP levels in the ischemic myocardium,<sup>15)</sup> the reduction of the accumulation of lipid metabolite intermediates<sup>2)</sup> and the release of inhibition of adenine nucleotidetranslocase by long chain acyl CoA.<sup>14),15)</sup> Recent studies have suggested that sarcolemmal alteration can contribute to the production of irreversible damage during myocardial ischemia.<sup>16),17)</sup> While the mechanism of the phospholipid degradation during myocardial ischemia is not clear at present, several studies in

an ischemic myocardial model have suggested that disturbances in phospholipid metabolism may be one of the critical alterations that produce irreversible injury during ischemia.<sup>18),19)</sup>

Sobel et al have shown that lysophosphoglycerides (hydrolysis products of membrane phospholipid) accumulate in ischemic myocardium, and that their accumulation plays an important role in the development of cellular dysfunction and ventricular arrhythmias.<sup>20)</sup> Katz et al emphasized the importance of the loss of membrane lipids induced by phospholipase activation as a cause of cellular damage.<sup>21)</sup> Using a cardiac sarcolemmal preparation, Chien et al recently reported a close correlation between phospholipid degradation and the irreversible changes of ischemic myocardium, and that chlorpromazine, a potent inhibitor of phospholipases, prevented the accelerated phospholipid degradation and the development of membrane dysfunction in ischemic myocardium.<sup>22),23)</sup>

Many authors have reported various changes in phospholipid composition in the ischemic myocardium. Bruce and Myers<sup>24)</sup> found that phosphatidylserine, phosphatidylcholine and cardiolipin decreased but lysophosphatidylcholine and phosphatidylethanolamine remained unchanged. Boime et al<sup>25)</sup> found that the phosphatidylcholine content was unchanged but the phosphatidylethanolamine content was decreased. Chien et al<sup>19)</sup> reported that the phosphatidylethanolamine and phosphatidylcholine levels were decreased, but without any corresponding increase in lysophospholipid content.

We found a significant decrease in the amount of total phospholipids, phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine in the mitochondrial fractions prepared from ischemic myocardia. Thus in the ischemic myocardium, the hydrolysis of membrane phospholipids may be increased in the environment of ATP depletion and the accumulation of long chain acyl CoA and long chain acyl carnitine.

These amphiphiles can produce changes in membrane function and structure by the insertion of monomers into the membrane by a detergent effect that depletes the membrane of some of its components<sup>26)</sup> and affects its integrity.

Decreases in ATP and total phospholipid levels were prevented by the administration of L-carnitine. Furthermore, the determination of individual phospholipids showed that the decreases in phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine were also prevented by the administration of L-carnitine.

The present results suggest that L-carnitine has preventive effects on the degradation of mitochondrial membrane phospholipids in ischemic myocardium and L-carnitine is a useful drug for the treatment of ischemic heart

diseases.

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