

EFFECTS OF L-CARNITINE ON VENTRICULAR ARRHYTHMIAS AFTER CORONARY REPERFUSION

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Effects of L-carnitine on ventricular arrhythmias and myocardial metabolism in a reperfused ischemic myocardium were studied in 35 anesthetized mongrel dogs.

The left anterior descending coronary artery was ligated for 40 min and then reperfused for 15 min. L-carnitine (100 mg/kg) was administered intravenously 5 min before the coronary ligation and infused continuously at a rate of 20 mg/kg/min from 5 min before the reperfusion to the end of the experiment. Electrocardiograms were recorded continuously throughout the experiment. Transmural myocardial samples were obtained from both the ischemic and the nonischemic areas after 15 min of reperfusion and used for the determination of ATP, free carnitine, long chain acyl carnitine and long chain acyl CoA. L-carnitine significantly reduced the incidence rate of ventricular fibrillation after reperfusion (from 29% in the controls to 5%, $p < 0.05$). ATP in the ischemic myocardium in the L-carnitine group was significantly higher than that in the control group ($p < 0.05$). Free carnitine in the control group significantly decreased in the ischemic area as compared with the nonischemic area ($p < 0.01$). In L-carnitine group, on the other hand, no difference was observed between them. Long chain acyl CoA in the control group significantly increased in the ischemic area as compared with the nonischemic area ($p < 0.01$). In L-carnitine group, on the other hand, no difference was observed between them. Thus, the accumulation of long chain acyl CoA in the ischemic myocardium was reduced by the L-carnitine treatment.

These data suggest that L-carnitine has protective effects on ventricular arrhythmias and on metabolic changes after coronary artery reperfusion following coronary artery occlusion.

FREE fatty acids are substrates for oxidative metabolism in the normal heart, but an increase in plasma free fatty acids which occurs

frequently in patients with acute myocardial infarction has been postulated to precipitate life-threatening complications.¹ Although its mechanisms are not well known, it has been suggested that an accumulation of intermediate substances due to impaired free fatty acid oxidation may be involved because these substances have inhibitory effects on intracellular enzyme activities and on the membrane transport systems.²⁻⁴

Key Words:

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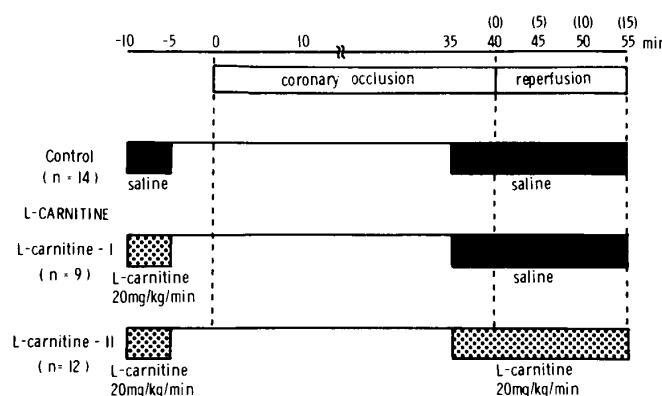


Fig.1. Experimental protocol.

TABLE I SCORING SYSTEM FOR VENTRICULAR ARRHYTHMIAS

0	No VPBs
1	Isolated unifocal VPBs < 5/min
2	Isolated unifocal VPBs > 5/min
3	Multifocal VPBs
4	Couplets or salvos VPBs
5	Early VPBs (R on T)
6	Ventricular tachycardia (VT)*
7	Ventricular fibrillation (Vf)

VPB = ventricular premature beat.

*More than 5 consecutive VPBs were considered VT.

Carnitine, a water-soluble naturally occurring amino acid, is essential for fatty acyl derivatives to penetrate across the inner mitochondrial membrane and to be transported to the sites of oxidation in the mitochondria^{5,6}. A reduction of tissue carnitine levels has been demonstrated in the ischemic⁷⁻¹⁰ and the free fatty acid supplemented heart² and is considered to aggravate the ischemic damage of the myocardium, presumably by accelerating the accumulation of fatty acyl derivatives. From this point of view, protective effects of exogenous carnitine on the metabolic derangements in the ischemic heart have been reported by several investigators.¹⁰⁻¹³ Furthermore, carnitine has recently been found to have antiarrhythmic properties.¹⁴⁻¹⁶ Antiarrhythmic effects of exogenous carnitine *in vivo* have been reported only in the case of ventricular fibrillation using dogs with coronary artery occlusion.^{11,14} However, ventricular fibrillation occurs not only during coronary artery occlusion but also after reperfusion.¹⁷ It has been reported that, as compared with coronary artery occlusion, reperfusion is more likely to provoke ventricular fibrillation.¹⁸

The purpose of this study was to evaluate the

effects of L-carnitine on ventricular arrhythmias and on metabolic changes after reperfusion following coronary artery occlusion.

MATERIALS AND METHODS

Thirty-eight mongrel dogs (weighing 10–15 kg) were anesthetized with intravenous sodium pentobarbital, and their ventilation was maintained using a Harvard animal respirator with room air. A left thoracotomy was performed at the 4th intercostal space. The pericardium was opened and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free below the first diagonal branch and ligated for 40 min and then reperfused for 15 min. Electrocardiograms were recorded continuously at a paper speed of 5 mm/sec throughout the experiment in order to observe the frequency and severity of ventricular arrhythmias. Two dogs in the control group and one dog in the L-carnitine group died during coronary artery occlusion and these dogs were excluded from the present study. The dogs which developed ventricular fibrillation were left to die without any resuscitation because such procedures may affect cardiac metabolism. Therefore, the present study was performed on 35 dogs which survived for 40 min of artery occlusion. These 35 dogs were divided into 2 major groups, i.e., the control group and the L-carnitine group. Furthermore, the L-carnitine group was divided into 2 subgroups in order to evaluate whether or not L-carnitine has a dose-dependent effect on ventricular arrhythmias. 1) The control group (n = 14) received saline only. 2) In the L-carnitine group I (n = 9), 100 mg/kg of L-carnitine (provided by Otsuka Pharmaceutical Factory Inc., Japan) was administered intravenously over 5 min. After an additional 5-min period, the coronary artery was

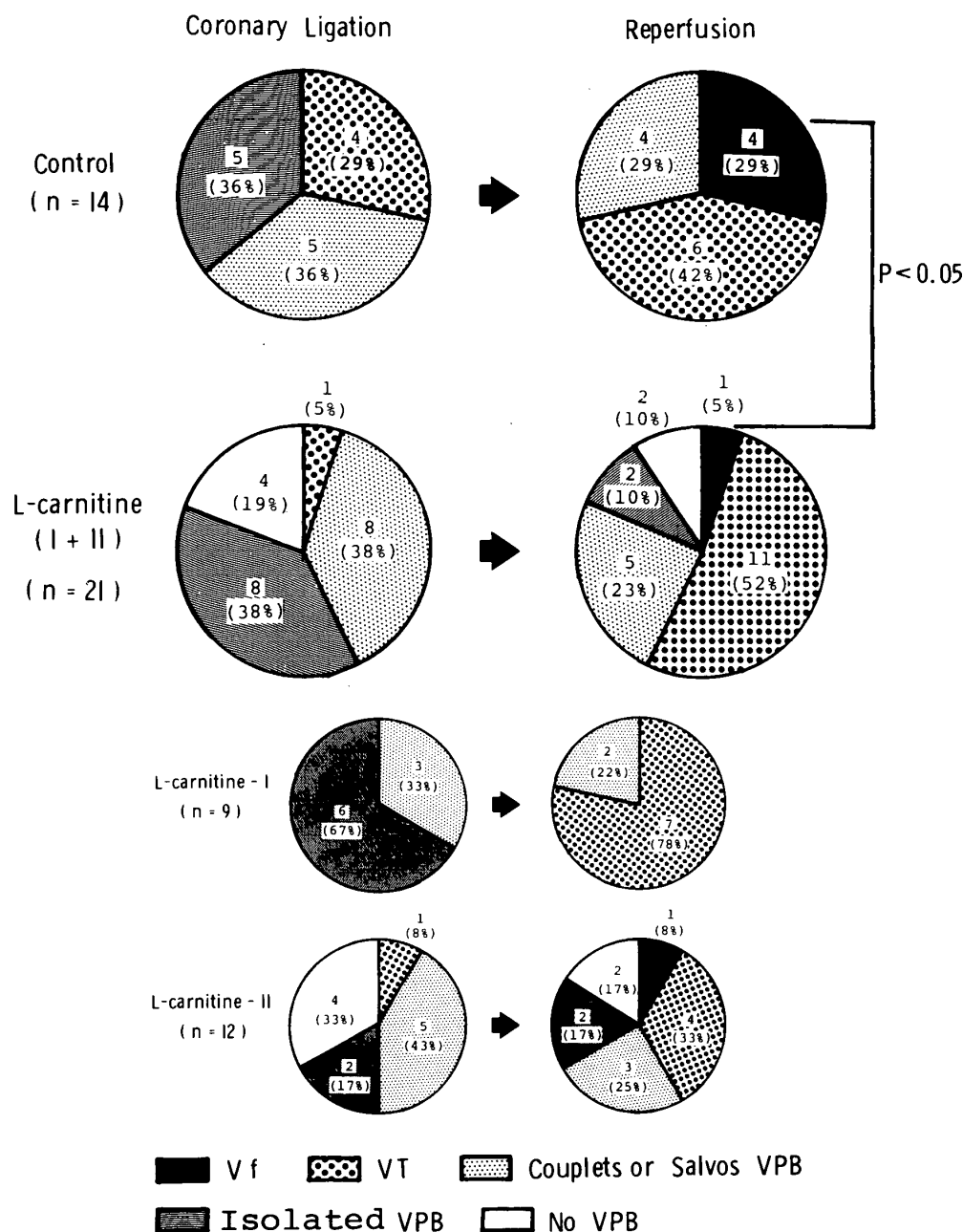


Fig.2. Incidence of reperfusion ventricular arrhythmias.

Vf = ventricular fibrillation; VT = ventricular tachycardia;
VPB = ventricular premature beat

ligated for 40 min. 3) In L-carnitine group II (n = 12), 100 mg/kg of L-carnitine was administered intravenously prior to the coronary artery ligation similarly as in L-carnitine group I and infused continuously at a rate of 20 mg/kg/min from 5 min before the reperfusion to the end of the experiment (Fig. 1). Fifteen minutes after the reperfusion the beating hearts were removed from the animals and transmural myocardial samples representing the ischemic area (supplied by the ligated left descending coronary artery) and the nonischemic area (supplied by the

circumflex coronary artery) were rapidly excised. Tissue samples were immediately frozen with Wollenberger clamp cooled to the temperature of liquid nitrogen for the determination of the metabolites. Free carnitine was determined enzymatically using carnitine acetyl transferase by the method of Marquis and Fritz.¹⁹ Long chain acyl carnitine were assayed as free carnitine after alkaline hydrolysis by the method of Pearson et al.²⁰ Long chain acyl coenzyme A (CoA) was assayed as free CoA after alkaline hydrolysis by the method of Veloso and Veech.²¹

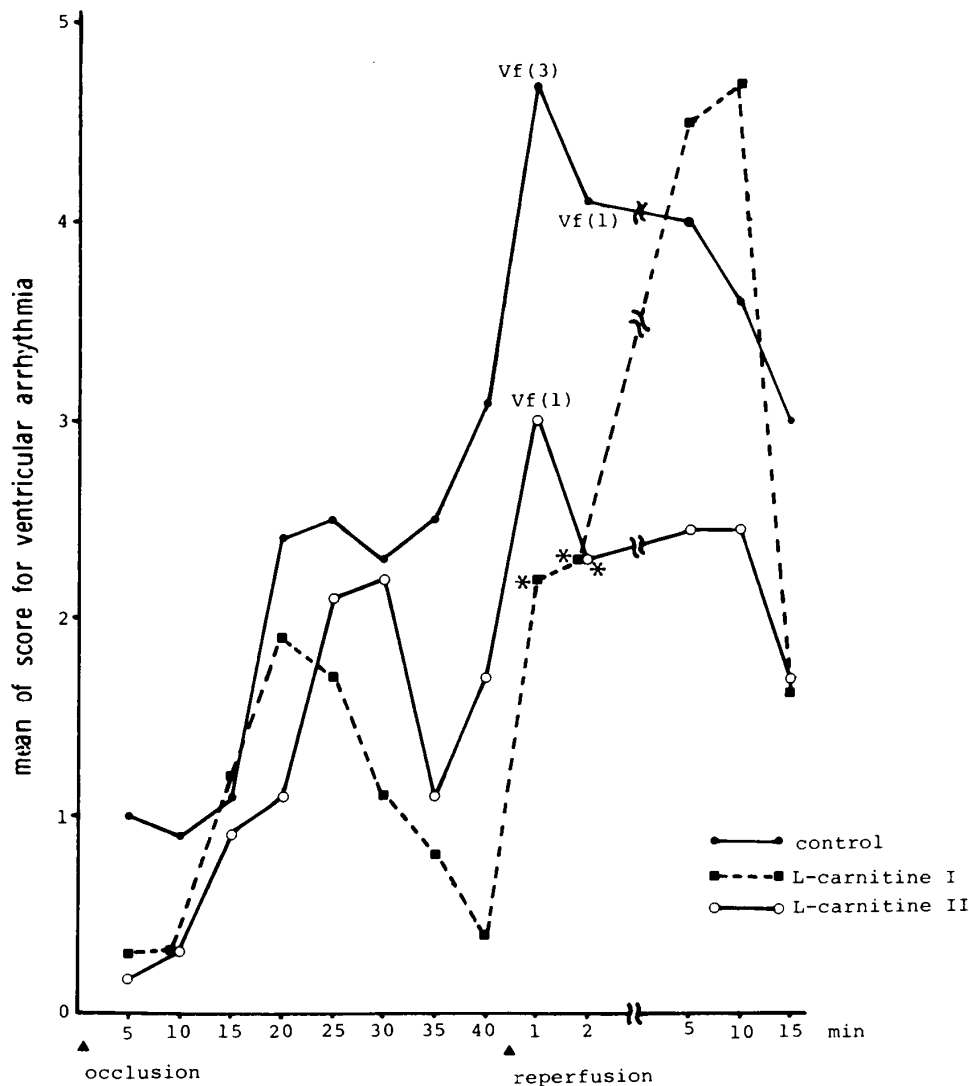


Fig.3. Changes in the mean score for ventricular arrhythmias. Vf () shows cases with ventricular fibrillation. Asterisks represent the significance of changes between the control group and the L-carnitine group ($p < 0.05$).

Adenosine triphosphate (ATP) was determined by enzyme assay using hexokinase and glucose-6-phosphate dehydrogenase²². Values of the metabolites were expressed as mean \pm SD.

Statistical analysis was made by the paired or non-paired Student's t -test, as appropriate. Ventricular arrhythmias were quantified by an arbitrary scoring system reported previously¹⁴ (Table I). The maximum score during every 5 min was analysed in each case. Statistical analysis of ventricular arrhythmias was made by the chi-square method and the Wilcoxon signed rank test, and $p < 0.05$ was considered significant.

RESULTS

Ventricular Arrhythmias after Reperfusion

The incidence of ventricular arrhythmias due to coronary artery ligation and reperfusion was shown in Fig. 2. In the control group, all 14 dogs had ventricular arrhythmias during coronary artery occlusion and 4 (29%) had ventricular tachycardia. After reperfusion 4 (29%) developed ventricular fibrillation and 6 (42%) developed ventricular tachycardia. Reperfusion arrhythmias occurred in early phase of reperfusion and were apt to develop into ventricular fibrillation. Four of 21 dogs (19%) treated with L-carnitine had no ventricular arrhythmias during coronary artery occlusion and one (5%) developed ventricular tachycardia. After reperfusion 2 (10%) had no ventricular arrhythmias and only one (5%) developed ventricular fibrillation. The incidence of ventricular fibrillation in the L-

TABLE II EFFECTS OF L-CARNITINE ON TISSUE LEVELS OF VARIOUS METABOLITES

	Free carnitine (nmole/g)	Long chain acyl carnitine (nmole/g)	Long chain acyl CoA (nmole/g)	ATP (μ mole/g)
<i>Nonischemic area</i>				
Control (n = 10)	1212 \pm 356	140 \pm 31	16.0 \pm 5.3	3.84 \pm 0.69
<i>L-carnitine treated</i>				
L-carnitine (I) (n = 9)	1384 \pm 387	215 \pm 75	17.0 \pm 7.0	4.40 \pm 0.42
L-carnitine (II) (n = 11)	3353 \pm 604 ^{###}	201 \pm 83	14.4 \pm 5.1	3.97 \pm 0.28
<i>Ischemic area</i>				
Control (n = 10)	951 \pm 292 ^{**}	160 \pm 40	19.2 \pm 6.0 ^{**}	1.89 \pm 0.68 ^{***}
<i>L-carnitine treated</i>				
L-carnitine (I) (n = 9)	1217 \pm 257	245 \pm 103	17.1 \pm 7.1	2.80 \pm 0.60 ^{***}
L-carnitine (II) (n = 11)	3641 \pm 796 ^{###}	255 \pm 114	16.2 \pm 4.2	2.66 \pm 0.77 ^{***} _#

Values are expressed per gram wet tissue weight and represented as mean \pm SD. Dogs which developed ventricular fibrillation after reperfusion were excluded. The significances of the changes are represented as follows: nonischemic area vs ischemic area (paired *t*-test): *** = $p < 0.001$; ** = $p < 0.01$; the control group vs L-carnitine group (non-paired *t*-test): ### = $p < 0.001$; # = $p < 0.05$.

carnitine group was significantly lower than that in the control group ($p < 0.05$).

The incidence of ventricular arrhythmias and ventricular tachycardia tended to be lower in the L-carnitine group II than in the L-carnitine group I, but this change was not significant. The changes in the mean score of ventricular arrhythmias in each group are shown in Fig. 3. In all groups, ventricular arrhythmias appeared within a few minutes after coronary artery ligation and the mean score increased gradually. During coronary artery ligation, the mean scores of L-carnitine groups were lower than that of the control group and they reached their maximum 20–25 min after coronary artery ligation and subsided thereafter up to 40 min. Ventricular fibrillation occurred within 2 min after reperfusion. The mean scores of L-carnitine groups were significantly lower than that of the control group within 2 min after reperfusion ($p < 0.05$). In the L-carnitine group I, which was pretreated with L-carnitine only before coronary artery ligation, reperfusion provoked frequent, repetitive and multifocal ventricular arrhythmias after 2 min of reperfusion but did not cause ventricular arrhythmias after 2 min of reperfusion but did not cause ventricular fibrillation.

Tissue Levels of Carnitine Derivatives, Acyl CoA and ATP

Effects of L-carnitine on tissue levels of various metabolites are shown in Table II. Tissue samples were obtained 15 min after reperfusion. In the control group, free carnitine decreased

significantly in the ischemic area as compared with the nonischemic area ($p < 0.01$, by paired *t*-test). In the L-carnitine groups, on the other hand, free carnitine increased in both the ischemic and the nonischemic areas and no difference was observed between them. Long chain acyl carnitine showed no significant change between the ischemic and the nonischemic area both in the control and the L-carnitine groups. In the control group, long chain acyl CoA in the ischemic area increased as compared with the nonischemic area ($p < 0.01$, by paired *t*-test). On the other hand, L-carnitine prevented the increase in long chain acyl CoA in the ischemic myocardium. ATP decreased significantly in the ischemic area of both the control and the L-carnitine groups ($p < 0.001$, by paired *t*-test). However, ATP content in the ischemic area was kept at a higher level in the L-carnitine group as compared with the control group ($p < 0.05$). Between the L-carnitine group I and the L-carnitine group II, there were no difference in the myocardial metabolic changes.

DISCUSSION

A number of animal models have been used for the studies of the effectiveness of antiarrhythmic agents. Acute coronary artery ligation in open chest dogs is considered to be the simplest infarction arrhythmia model and the most malignant arrhythmia model is the coronary artery occlusion followed by reperfusion. During reperfusion after 15–45 min of occlusion, ventricular

fibrillation is common.²³ Balke et al²⁴ have suggested that the highest risk for reperfusion ventricular fibrillation is present after 20–30 min, when ischemic changes are most marked but infarction is minimal. Stephenson et al¹⁸ have occluded the left anterior descending coronary artery in 330 open chest dogs. Twenty-eight percent of these developed ventricular fibrillation during a 30-min period of coronary occlusion; among 239 dogs which survived 30-min occlusion, 71% developed ventricular fibrillation after reperfusion. In the control group in our study, on the other hand, 29% developed ventricular fibrillation after reperfusion following 40 min of occlusion. Dreifus et al²⁵ have pointed out that an incidence of ventricular fibrillation depends on how proximal the coronary artery is ligated. The difference in the incidence of reperfusion ventricular fibrillation between Stephenson's study and ours may be due to the differences in the site and the duration of coronary occlusion. We occluded the left anterior descending coronary artery more distally than that of Stephenson. Dreifus et al²⁵ have indicated that a model, in which reperfusion was preceded by a 30-min ligation of the proximal part of the left anterior descending coronary artery, may offer a too severe condition for testing potential antiarrhythmic agents. Therefore, we tried to evaluate the effects of L-carnitine on ventricular arrhythmias and myocardial metabolism in the reperfused ischemic myocardium after 40 min of coronary occlusion. In this study, L-carnitine was effective in preserving high energy store in the ischemic myocardium and in reducing the incidence of ventricular fibrillation after reperfusion. After 2 min of reperfusion, the mean score of ventricular arrhythmias in L-carnitine group II was lower than that in L-carnitine group I, although it was not statistically significant.

Carnitine has been reported to lower the incidence of ventricular fibrillation in dogs with coronary artery occlusion.^{11,14} In addition, it has been shown that carnitine increases the threshold for electrically induced atrial fibrillation in cats¹⁵ and for ventricular fibrillation in dogs.¹⁶ Carnitine was also shown to stimulate fatty acid oxidation in heart tissue homogenates²⁶ and to reduce the impairment of myocardial function in dogs.²⁷ It is not yet known how carnitine protects the ischemic myocardium. However, it seems likely that the protective effects of carnitine is related to its metabolic role as the requisite carrier of acyl esters across the mitochondrial membrane.⁴

In our previous study,¹³ after 15 min of coronary artery occlusion, tissue levels of free carnitine decreased in the ischemic myocardium, whereas long chain acyl carnitine and long chain acyl CoA increased. Shug et al² have observed that an accumulation of acyl CoA is accompanied by a decrease in adenine nucleotide translocase activity in acute myocardial infarction, and they suggested that the accumulation of long chain acyl CoA plays an important role in the mitochondrial dysfunction induced by ischemia. Pande²⁸ has reported that the inhibition of mitochondrial respiration by long chain acyl CoA is reversed by an administration of L-carnitine.

In this study, we demonstrated that L-carnitine significantly suppressed the decrease of free carnitine and ATP, and tended to suppress the increase of long chain acyl CoA. These observations indicate that an administration of L-carnitine may benefit the ischemic myocardium by increasing the depleted free carnitine levels towards normal, by reversing the inhibition of adenine nucleotide translocase activity by long chain acyl CoA and by restoring mitochondrial function.

It has been demonstrated that the recovery of cellular functions during reperfusion following ischemia correlates with the size of the residual adenine nucleotide pool.²⁹ Moreover, ischemia leads to a rapid decrease of high energy phosphate and to an increase of cellular and subcellular membrane permeability.³⁰ Changes in the membrane permeability are responsible for the effluxes of potassium and magnesium and the influxes of sodium and calcium in the reperfused ischemic myocardium. High acyl CoA levels may act as a detergent and cause conformational changes of the membrane and disruption of the lipid phase of the membrane, and eventually they make it weaker and more susceptible to physical rupture.²⁹ Thus, carnitine may have an antiarrhythmic effect through membrane-stabilizing action.

These results indicate that L-carnitine is effective to prevent the mitochondrial dysfunction and protect the myocardium against ventricular fibrillation in the ischemic reperfused myocardium.

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