Antiarrhythmic and Arrhythmogenic Effects of L-Carnitine in Ischemia and Reperfusion

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Isolated rat hearts were subjected to 30-min coronary artery occlusion followed by 120-min reperfusion. The hearts (n=8-12) were perfused with Krebs—Henseleit solution enriched with L-carnitine (0.5, 2.5 and 5 mM) for 10 min before and after ischemia or reperfusion and for the whole period of ischemia and reperfusion. Two-hour perfusion with L-carnitine during ischemia/reperfusion markedly (p<0.05) and dose-dependently decreased the incidence of ventricular tachycardia (VT, maximum 65%). The incidence of reperfusion ventricular fibrillation (VF) also decreased from 63% (control) to 17% in hearts perfused with 5 mM L-carnitine, as reflected by a significant (p<0.05) decline in VF duration from 218±99 sec in control to 19±19 sec. Perfusion of etomoxir (palmitoyl-carnitinetransferase-1 inhibitor) along with L-carnitine reversed the antiarrhythmogenic action of L-carnitine. Interestingly, short time preischemic administration of L-carnitine produced a concentration-dependent arrhythmogenic effects on both ischemia and reperfusion-induced arrhythmias. These results show that L-carnitine produced a protective effect against reperfusion arrhythmias only when it was perfused for the whole period of the experiment. This protective action was reversed by concomitant use of etomoxir, suggesting that the efficacy of L-carnitine is due to its mitochondrial action but cannot be solely attributed to increased fatty acid oxidation.

Key Words: *L-carnitine; ischemia; reperfusion; arrhythmias; isolated rat heart*

Carnitine is an essential cofactor for the transport of fatty acyl groups from the cytoplasm to the mitochondrial matrix where they undergo β-oxidation to produce ATP. This pathway within the mitochondria is the major source of energy for the heart. This compound is vital in the process of long chain fatty acid oxidation and reduces intracellular accumulation of toxic metabolites during ischemia. Several experimental studies showed that L-carnitine reduces myocardial injury after ischemia and reperfusion by counteracting the toxic effect of high levels of free fatty acids, which occurs in ischemia, and by improving carbohydrate metabolism [3]. It was also shown that propionyl-L-carnitine which penetrates into myocytes faster than L-carnitine is effective in inhibiting reperfusion ventricular fibrillation (VF) and production of free radicals [2,6]. In this study, the effect of L-carnitine on ischemic and reperfusion-induced arrhythmias was investigated on isolated rat hearts.

MATERIALS AND METHODS

Male Sprague-Dawley rats (280-320 g) were pretreated with intraperitoneal (i.p.) injection of 300 U heparin and then anaesthetized with sodium pentobarbital (50-60 mg/kg, i.p.) [1,5,11]. As soon as deep anesthesia was achieved, the thoraxes were opened [12] and the hearts were rapidly and care-

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fully excised and mounted via the aorta on a standard Langendorff perfusion apparatus with a perfusion pressure of 100 cm H_2O. Modified Krebs—Henseleit buffer solution containing (in mM): 118.5 NaCl, 25.0 $NaHCO_3$, 4.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 12.0 D-glucose, and 1.7 $CaCl_2$ saturated with 95% O_2 and 5% CO_2 at 37°C (pH 7.4) was used as the perfusion medium throughout the experiment. A compliant left ventricular balloon was inserted to measure left ventricular systolic and diastolic pressure and heart rate [5,11]. The diastolic pressure was initially set at 4 mm Hg, which resulted in systolic pressure of 90-120 mm Hg. Epicardial ECG during the experiment was recorded on a polygraph using two silver electrodes attached directly to the heart. A 6/0 braided silk suture was placed around the left anterior descending coronary artery. After 20-min stabilization, coronary occlusion (30 min) was achieved by threading loose ends of the ligature through a polyethylene occluder and clamping in place. Release of the clamp allowed reperfusion of the previously ischemic tissue (120 min). Based on the Lambeth conventions, ECG was analyzed to determine the total number of ventricular ectopic beats (VEBs), the number of beats occurring as ventricular tachycardia (VT), and the incidence and duration of VT and VF during ischemia and reperfusion [13].

Rats (n=8-12) were allocated randomly to one of the following groups: drug-free control (group 1); hearts perfused with 0.5, 2.5 and 5 mM of L-carnitine-enriched Krebs—Henseleit solution for the whole period of ischemia and reperfusion (protocol A, group 2); hearts perfused with 0.5, 2.5 and 5 mM of L-carnitine-enriched Krebs—Henseleit solution for 10 min before and 10 min after ischemia (protocol B, group 3); hearts perfused with 0.5, 2.5 and 5 mM of L-carnitine-enriched Krebs—Henseleit solution for 10 min before and 10 min after reperfusion (protocol C, group 4); infusion of 1 μM etomoxir-enriched Krebs—Henseleit solution in the absence or presence of L-carnitine (2.5 mM) for the whole period of ischemia and reperfusion (groups 5 and 6, respectively).

Except for the incidences of VT and VF, all results are expressed as $M\pm m$. To compare the number of VEBs and duration of VT and VF between the groups, the Mann—Whitney nonparametric U test was employed. For analyzing the incidence of VT and VF, χ^2 test with Yates correction was used. The differences between the groups were considered significant at $p<0.05$.

RESULTS

Effects of L-carnitine on ischemic arrhythmias are shown on Fig. 1. In the hearts perfused with 0.5-5 mM of L-carnitine for the whole period of ischemia and reperfusion (protocol A), there were no significant changes in ischemic arrhythmias. However, administration of L-carnitine in concentrations of 2.5 and 5 mM before ischemia (protocol B) produced significant increases in the total number of ischemic VEBs from 667±116 in the control to 1227±161 and 1289±161 in the treated groups, respectively (Fig. 1). This increase was due to an increase in the number of VT. In this protocol, the incidence of VF was also increased from 18% in the control group to 67% in hearts treated with 5 mM L-carnitine, as reflected by a significant ($p<0.01$) raise in the total duration of VF (85±32 sec *vs*. 6±5 in the control). Perfusion of the isolated rat hearts with

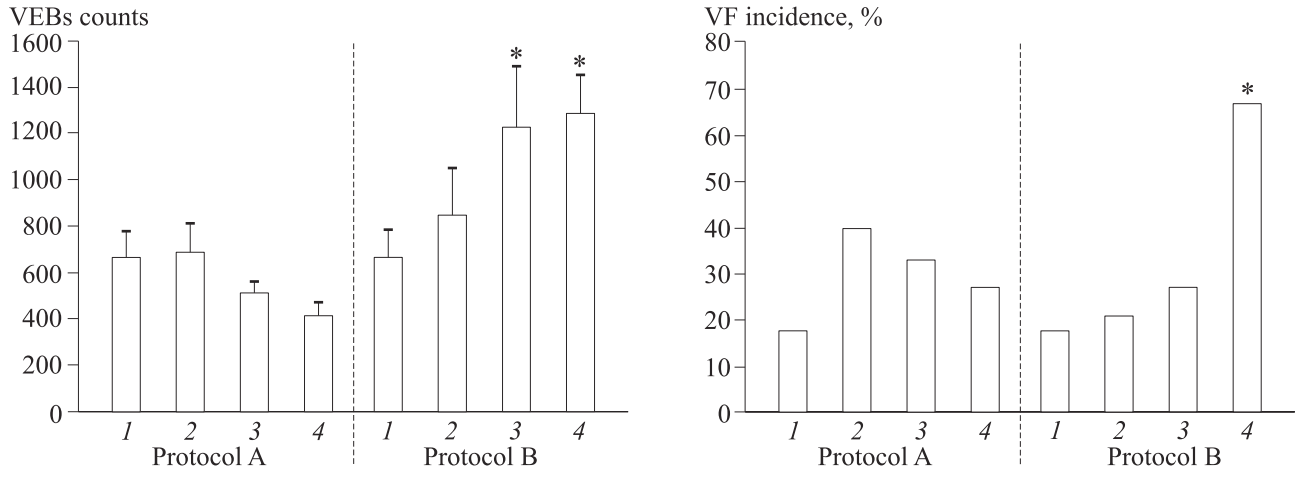

Fig. 1. Effects of L-carnitine on ischemic arrhythmias in protocol A and B. Total VEBs is the sum of arrhythmias occurring as singles, salvos and VT. Total incidence of VF (reversible and irreversible) was recorded. *1*) control; *2*) L-carnitine, 0.5 mM, *3*) L-carnitine, 2.5 mM, *4*) L-carnitine, 5 mM.*$p<0.05$ compared to the control.

TABLE 1. Effects of L-Carnitine on Reperfusion Arrhythmias

Group	VT Counts	VEBs Counts	VF Duration, sec	VF Incidence, %
Drug free control	154±29	349±73	218±99	63
L-Carnitine, 0.5 mM				
protocol A	68±31	212±99	269±90	33
protocol B	115±20	208±24	328±80	67
protocol C	327±76*	452±120	240±98	33
L-Carnitine, 2.5 mM				
protocol A	63±22*	208±75	37±27*	40
protocol B	311±60*	410±72	198±83	67
protocol C	197±60	352±93	260±97	57
L-Carnitine, 5 mM				
protocol A	55±19*	169±23	19±19*	17
protocol B	331±90*	443±100	457±70*	100
protocol C	100±40	278±73	373±106	84
Etomoxir (1 μM; protocol A)	7±7*	158±37	0*	25
Etomoxir(1 μM)+L-carnitine (2.5 mM; protocol A)	121±41+	235±40	421±105+	60

Note. Total VEBs is the sum of arrhythmias occurring as singles, salvos, and VT. Total incidence of VF (reversible and irreversible) and duration of reversible VF were recorded. $p<0.05$ *compared to the control value, +compared to etomoxir and L-carnitine (2.5 mM; protocol A).

etomoxir (1 μM) either in the absence or presence of L-carnitine (2.5 mM) had no effect on ischemic arrhythmias during perfusion according to protocol A.

Table 1 summarizes the effects of different concentrations of L-carnitine on arrhythmia count, durations and incidences during reperfusion for all protocols. Application of 2.5 and 5 mM L-carnitine for the whole period of ischemia/reperfusion (protocol A) significantly reduced the number of reperfusion-induced VT. These concentrations also markedly decreased the duration of reversible VF (by 90%). In contrast, perfusion of the hearts with L-carnitine (2.5-5 mM) only for 10 min before and after induction of ischemia (protocol B) induced a significant increase in VT count and the duration of reversible VF during reperfusion. Compared to the control group, administration of L-carnitine 10 min before and 10 min after reperfusion (protocol C) with the exception of a significant increase in the number of reperfusion-induced VT with low concentration (0.5 mM) did not alter reperfusion arrhythmias. Isolated rat hearts perfused with etomoxir (1 μM) throughout the whole period of ischemia and reperfusion exhibited a significant reduction in reperfusion arrhythmias because of the decrease in the number of VT and incidence and duration of VF. However, concomitant application of L-carnitine (2.5 mM) and etomoxir reversed the antiarrhythmic effects of both drugs against reperfusion arrhythmias (Table 1).

The present study shows that L-carnitine produces concentration dependent antiarrhythmic effects only against reperfusion arrhythmias when it is used for the whole period of ischemia and reperfusion (protocol A). In contrast, short-time preischemic administration of carnitine has arrhythmogenic effects during both ischemia and reperfusion (protocol B). Cui *et al*. (2003) in an *in vitro* study investigated the effects of L-carnitine and propionyl-L-carnitine on the incidence of reperfusion-induced VF during 30-min global ischemia followed by 120-min reperfusion [2]. Their results showed that different concentrations of L-carnitine failed to reduce the incidence of VF. However, the incidence of reperfusion VF was reduced from its control value of 90 to 10% ($p<0.05$) in hearts perfused with 5 mM propionyl-L-carnitine. Suzuki *et al*., (1981) reported that intravenous pretreatment of ischemic dog heart with L-carnitine (100 mg/kg) reduced the severity of ventricular arrhythmias. They hypothesized that administration of L-carnitine might be beneficial for prevention of serious arrhythmias in ischemic heart disease, presumably by restoring impaired free fatty acid oxidation. During myocardial ischemia, depressed oxygen supply results in uncoupling of oxidative phosphorylation, leading to accumulation of β-hydroxy fatty acid intermediates and acyl CoA molecular species. The accumulation of fatty acids and their intermediates during myocardial ischemia can be deleterious to the recovery of myocardial function of the reperfused heart [4]. On the other hand, recovery after ischemia was improved by reducing the

availability of fatty acid during reperfusion [9], so that the increase in fatty acid oxidation can be detrimental to cardiac recovery during reperfusion in ischemic tissues [8]. Furthermore, recent findings suggest that carnitine is also crucial in the regulation of carbohydrate metabolism in addition to its role in the oxidation of fatty acids [7]. We hypothesized that preischemic application of L-carnitine for an inadequate time can be harmful for the heart because of incomplete metabolism of fatty acids and accumulation of their intermediates. However, in isolated perfused hearts treated with L-carnitine for the whole period of ischemia and reperfusion, myocardial recovery from the ischemic and reperfusion episodes is improved through a mechanism believed to involve shunting of fatty acids into mitochondria for β-oxidation. The antiarrhythmogenic action of L-carnitine against reperfusion arrhythmias was reversed by concomitant using of etomoxir (palmitoylcarnitinetransferase-1 inhibitor), which suggests that the efficacy of L-carnitine is determined its mitochondrial action but cannot be completely attributed to increased fatty acid oxidation. As well as removing the deleterious fatty acid metabolites from mitochondria [6] and limiting the necrotic area [10], depending on concentration and duration of administration, it appears that L-carnitine may be beneficial in myocardial ischemia and reperfusion by increasing pyruvate dehydrogenase activity and shifting cellular energy production from oxidation of fatty acids to glucose.

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