Effect of D,L-carnitine on the response of the isolated heart of the rat to ischaemia and reperfusion: relation to mitochondrial function

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1 The effect of 100 μM (20 $\mu\text{g ml}^{-1}$) of D,L-carnitine was studied on the isolated heart of the rat subjected to 30 min of low flow ischaemia followed by reperfusion.

2 In untreated hearts ($n = 30$) ischaemia produced an almost total loss of contractility ($P < 0.05$ compared with non-ischaemic time control) which was accompanied by an increase in resting tension of approximately 235% ($P < 0.05$). Ventricular arrhythmias developed during ischaemia in 100% ($P < 0.05$) of untreated hearts studied. Following reperfusion, untreated hearts recovered 16.3% of contractile function and demonstrated a 60% elevation in resting tension. The incidence of reperfusion-associated ventricular fibrillation was 60%.

3 Carnitine treatment produced no effect on either the contractile depression or the elevation in resting tension during ischaemia but did significantly decrease the incidence of arrhythmias at the termination of ischaemia to 63.3% ($n = 30$, $P < 0.05$). In the presence of carnitine, contractile recovery at the end of reperfusion was significantly increased to 30.2% ($n = 10$, $P < 0.05$) and the elevation in resting tension was decreased to 30% ($n = 10$, $P > 0.05$). The incidence of ventricular arrhythmias during reperfusion was significantly reduced by carnitine.

4 Two populations of mitochondria, subsarcolemmal (SLM) and interfibrillar (IFM) isolated at the end of the ischaemic period exhibited an overall increase in oxidative phosphorylation rates as well as uncoupled oxygen consumption; both phenomena were more pronounced with IFM. Carnitine generally potentiated this response. A 29% and 38% inhibition in atractyloside-sensitive ADP uptake was observed in SLM and IFM, respectively, following ischaemia, which was partially prevented by carnitine.

5 After 10 min of reperfusion, adenosine diphosphate (ADP) uptake in SLM was further reduced to 55% of control whereas with IFM, uptake was not different from that seen at the end of ischaemia. Mitochondria isolated from hearts after 30 min of reperfusion revealed a significantly depressed oxidative phosphorylation as well as ADP/ATP translocase activity. These defects were partially reversed in hearts perfused with carnitine.

6 Our study demonstrates that D,L-carnitine protects the rat isolated heart against injury associated with ischaemia and reperfusion through a mechanism associated with improved mitochondrial function.

Introduction

Myocardial ischaemia is associated with the intracellular accumulation of various factors known to be associated with tissue injury, including long-chain acyl CoA (Opie, 1979; Piper & Das, 1987), inorganic phosphate (Pi) (van Belle *et al.*, 1986) and free radicals (Garlick *et al.*, 1987). These factors have been

shown to exert deleterious effects on isolated mitochondria including inhibition of oxidative phosphorylation and adenine nucleotide (ADP/ATP) translocase activity (Opie, 1979; Shug, 1979; Ferrari *et al.*, 1988). Various investigators have shown that the deleterious effects of a number of these compounds could be prevented by carnitine or its analogues (Opie, 1979; Shug & Subramanian, 1987). These observations suggest the possibility that carnitine may protect the ischaemic reperfused heart through a mechanism possibly involving the preservation of mitochondrial function (Shug & Sub-

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ramanian, 1987). This concept has not been substantially documented and is complicated by a number of factors. First, although the possible protective effect of carnitine is attractive, a number of studies have shown a distinct lack of protection (Dipalma 1986; Bahl & Bressler, 1987), and indeed a toxic influence of this compound on the ischaemic and reperfused heart (Hearse *et al.*, 1980) has been reported. A second important point to consider is the fact that the relative contribution of mitochondrial dysfunction to the ischaemic reperfused myocardium still remains controversial, particularly with respect to acute ischaemic-reperfusion conditions (Reimer & Jennings, 1986). Thus, a quantitative description linking mitochondrial and cardiac function is lacking. This issue is complicated by numerous factors such as the existence of multifactorial contributing mediators to ischaemic-reperfusion injury (Reimer & Jennings, 1986), the heterogeneous responses of cardiac subsarcolemmal (SLM) and interfibrillar mitochondria (IFM) to ischaemia and reperfusion (van Jaarsveld & Lochner, 1982; Duan & Karmazyn, 1989) as well as the fact that severity of injury may determine the response of the heart to cardioprotective intervention by various factors including carnitine (Paulson *et al.*, 1984). The purpose of the present study, therefore, was to examine the potential effect of a relatively low concentration of D,L-carnitine on the ischaemic reperfused isolated heart. A major focus of the study was to examine this pharmacological intervention on mitochondrial function after subjecting the heart to various treatments and, in particular, to assess the relationship between mitochondrial function and cardiac recovery after reperfusion. Because of the identification of two populations of cardiac mitochondria (Palmer *et al.*, 1977) as well as the prior suggestion of differential responses of these populations to ischaemic and reperfusion insults, both SLM as well as IFM populations were evaluated.

Methods

Animals

Male Sprague-Dawley rats weighing 250–300 g were purchased from Canadian Hybrid Farms (Centreville, Nova Scotia, Canada). Animals were housed in Dalhousie University's Animal Care Facilities in accordance with guidelines of the Canadian Council on Animal Care (Ottawa, Ontario, Canada).

Heart perfusion

Rats were killed by decapitation and the hearts were immediately excised and immersed in a small crucible containing ice-cold Krebs-Henseleit buffer which

produced an immediate cessation of contraction. The composition of the buffer (in mM) was as follows: NaCl 120, KCl 4.63, NaHCO₃ 20, KH₂PO₄ 1.17, MgCl₂ 1.2, CaCl₂ 1.25, and glucose 8 as substrate; pH was 7.4. The heart was squeezed a few times with fingers to dislodge any clotted blood in the coronary vasculature. The heart was mounted via the aorta on a stainless steel cannula and perfusion with Krebs-Henseleit buffer was started immediately at a constant flow rate of 10 ml min⁻¹ regulated by a Watson-Marlow peristaltic pump. The buffer was continuously gassed with a 95% O₂: 5% CO₂ mixture and the entire system was regulated at 37°C. The atria were removed and the ventricles were electrically stimulated at 2 times threshold voltage at a frequency of 5 Hz. Contractile force and coronary perfusion pressure were recorded on a Grass Model 7 polygraph using standard techniques (Karmazyn, 1986a). Surface electrical activity was obtained by inserting one fine gauge platinum electrode into the apex while a second electrode was connected to the steel cannula. Hearts were considered arrhythmic when the incidence of extrasystoles was greater than 20 per min and was of at least one min duration.

Following an initial 30 min equilibration period at normal flow rates, low-flow global ischaemia was induced for 30 min by reducing the flow rate to 1 ml min⁻¹ with or without a further 30 min of reperfusion at 10 ml min⁻¹. Initial experiments were done in which carnitine was present throughout ischaemia and reperfusion, reperfusion only or ischaemia only. These preliminary studies revealed a complete absence of any effect of carnitine when it was present only during the latter 2 treatments; we have therefore described here results obtained with carnitine present during the complete perfusion sequence. Time control hearts were perfused for a similar duration as experimental hearts at normal flow rates.

Mitochondrial isolation

Two populations of cardiac mitochondria were isolated after the appropriate perfusion protocol according to the procedure of Palmer *et al.* (1977) except that identical isolation buffer containing (in mM) KCl 100, MOPS 50, EGTA 2 and 0.2% bovine serum albumin, pH 7.4 was used for both populations. The final suspension medium for both populations consisted of (in mM) KCl 125, MgCl₂ 5 and Tris 2, pH 7.4.

Oxidative phosphorylation

Mitochondrial oxidative phosphorylation was determined in a Gilson oxygraph equipped with a Clark electrode as described in detail previously (Karmazyn, 1986b). Oxidative phosphorylation was

assessed in terms of state 3 respiration rate (QO_2) following the addition of 600 nmol adenosine diphosphate (ADP), respiratory control index (RCI), ADP: oxygen ratio (ADP:O) and oxidative phosphorylation rate (OPR). Either pyruvate-malate (10 mM–2.5 mM) or glutamate-malate (10 mM–2.5 mM) was used as substrate. Uncoupled oxygen consumption was determined in the presence of 0.1 mM 2,4-dinitrophenol with glutamate-malate as substrate.

ADP/ATP translocase activity

ADP/ATP translocase activity was determined in a reaction medium containing (in mM) KCl 110, Tris 20 and EDTA 1 to which had been added 0.02 μ Ci of [14 C]-ADP, pH 7.4 using a modified procedure of Duee & Vignais (1969). The reaction was started by the addition of 0.5 mg mitochondrial protein and terminated at either 0 or 80 s later with 0.1 mM atractyloside. These durations were selected from initial studies showing saturation of the reaction after 40 s. The mixture was centrifuged at 25,000 g for 5 min and the supernatant discarded. The pellet was washed twice with medium, dissolved in 0.2 ml of 0.1 N NaOH and counted for radioactivity. ADP/ATP translocase activity was determined by the atractyloside-sensitive ADP uptake per mg mitochondrial protein.

Chemicals

Most drugs, chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.) and were of the highest purity available. Nagarse was purchased from Enzyme Development Corporation (New York, N.Y., U.S.A.). [14 C]-ADP was obtained from DuPont Canada Inc. (Dorval, Quebec, Canada).

Statistical analysis

Data were analyzed by one-way analysis of variance with Student-Newman-Keuls (SNK) test for inter-treatment comparisons, except results for mechanical function which were analyzed by Student's t test for unpaired data. Frequency of arrhythmias was analyzed using a 2×2 contingency table (Zar, 1984). Differences were considered significant when $P < 0.05$.

Results

Cardiac mechanical function and incidence of arrhythmias

In time control experiments, hearts subjected to 90 min of normal perfusion exhibited an average loss in contractile force of approximately 25% as com-

pared to initial values. Carnitine produced no effect on this loss of force. Resting tension remained at the initial 2 g setting over the entire 90 min perfusion period both in the presence and absence of carnitine (control data not shown).

The mechanical response to ischaemia and reperfusion is shown in Figure 1. The reduction in coro-

Figure 1 Response of the rat isolated heart to 30 min ischaemia and reperfusion in control hearts (○) or in hearts treated with carnitine (●). For mechanical data each point represents the mean with vertical bars indicating the s.e.mean. Data were pooled from all experiments including hearts not subjected to reperfusion; $n = 30$ for each treatment from 30 to 60 min; $n = 20$ at 70 min; $n = 10$ at 80 and 90 min. Where absent for contractile force and resting tension s.e.mean were smaller than the size of the symbol. * $P < 0.05$ compared with values in the absence of carnitine.

nary flow produced a rapid reduction in contractility and a concomitant progressive elevation in resting tension. The changes in both parameters were significant ($P < 0.05$) after 5 min of ischaemia and remained significant for the duration of the ischaemic, and indeed reperfusion periods. Carnitine produced no effect on either contractile force or resting tension changes associated with the initial ischaemic period. Reperfusion produced a gradual reduction in resting tension and recovery of contractile force; the response of both parameters was substantially influenced by the presence of carnitine. In untreated hearts recovery of force was 16.32% of pre-ischaemic values after 30 min of reperfusion. Carnitine significantly increased recovery to 30.2% of pre-ischaemic values. Furthermore, in the presence of carnitine resting tension was moderately reduced, particularly after 20 and 30 min of reperfusion; however differences were not statistically significant.

Figure 1 also shows the incidence of arrhythmias associated with ischaemia and reperfusion. In untreated hearts arrhythmias persisted throughout both ischaemic and reperfusion conditions. Most observable arrhythmias were in the form of extrasystoles, as described in Methods. With carnitine the incidence of all arrhythmias throughout ischaemia was significantly reduced and at the end of reperfusion arrhythmias were virtually totally abolished by carnitine (Figure 1). During ischaemia, ventricular fibrillation was observed in 29 of 30 untreated hearts whereas 6 of 10 hearts developed ventricular fibrillation during reperfusion; in 2 of these 6 hearts the fibrillation was persistent. In carnitine-treated hearts the incidence of all arrhythmias at the end of ischaemia was 63.3%, which was significantly lower than control. The total cumulative incidence of fibrillation during ischaemia in the presence of carnitine was 23 of 30 hearts (77%) whereas 4 of 10 hearts fibrillated during reperfusion although none of these were persistent. However, these reductions in carnitine-treated hearts were not statistically significant. No arrhythmias were ever observed in time control perfused hearts either with or without carnitine throughout the 90 min perfusion period (not shown).

Mitochondrial function

Effect of ischaemia Mitochondria isolated after 30 min of whole heart ischaemia exhibited few changes when pyruvate-malate was used as the respiratory substrate (Figure 2). Small increases in respiratory rates (state 3 Q_{O_2} and OPR) were found, particularly with IFM and in mitochondria isolated from carnitine-treated hearts. With glutamate-malate, the stimulation in respiration rates was more pronounced and under these conditions, significant

Figure 2 State 3 respiration (Q_{O_2}), respiratory control index (RCI), ADP: oxygen ratio (ADP:O) and oxidative phosphorylation rate (OPR) with pyruvate-malate as substrates for subarcolemmal (a) and interfibrillar (b) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia without reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

for both populations (Figure 3). Furthermore, a stimulation in RCI was also seen. Uncoupled oxygen consumption in mitochondria treated with 2,4-dinitrophenol exhibited increased oxygen uptake following ischaemia although this was significant in the SLM population only after carnitine perfusion (Figure 4a,b). Lastly, both mitochondrial populations isolated from ischaemic hearts showed a trend ($P > 0.05$) towards a reduction in atractyloside-sensitive ADP uptake capability, a phenomenon partially prevented by carnitine perfusion (Figure 4c,d).

Effect of reperfusion To assess the possible time-dependent effects of reperfusion on mitochondrial function, mitochondria were harvested following both early (10 min) as well as prolonged (30 min) reperfusion periods. Figure 5 shows oxidative phosphorylation values with pyruvate-malate of mitochondria isolated after 10 min of reperfusion. Virtually no effect was seen with the exception of a significant reduction in RCI of SLM and its prevention by carnitine perfusion (Figure 5). Although a slight, similar reduction and prevention by carnitine was seen with glutamate-malate, the effects were not

Figure 3 State 3 respiration (QO_2), respiratory control index (RCI), ADP: oxygen ratio (ADP:O) and oxidative phosphorylation rate (OPR) with glutamate-malate as substrates for subarcolemmal (a) and interfibrillar (b) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia without reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

significant (data not shown). IFM, however, exhibited significantly elevated respiration rates when isolated from these hearts. Figure 6 shows uncoupled oxygen uptake (a,b) and atractyloside-sensitive-ADP uptake values (c,d) in mitochondria isolated from 10-min reperfusion hearts. No effect on the former was evident. However, both populations demonstrated significantly depressed ADP uptake; with SLM, but not IFM, this depression was partially prevented by perfusing hearts with carnitine (Figure 6).

Oxidative phosphorylation data after 30 min of reperfusion are summarized in Figures 7 and 8. With both substrates, respiration was significantly depressed in both mitochondrial populations. The depression in respiration rates observed with pyruvate-malate was prevented by carnitine (Figure 7). With glutamate-malate (Figure 8), this effect of carnitine was evident only with IFM. Furthermore, this population demonstrated a significant, carnitine-reversible, inhibition in RCI when glutamate-malate was used as substrate (Figure 8).

After 30 min reperfusion, both mitochondrial populations demonstrated significantly depressed

Figure 4 Uncoupled oxygen consumption (a and b) and adenine nucleotide translocase activity (c and d) for subarcolemmal (a and c) and interfibrillar (b and d) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia without reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Studies with uncoupled mitochondria were carried out in the presence of 2,4-dinitrophenol and glutamate-malate as substrates. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

uncoupled oxygen uptake (Figure 9, top) which was partially prevented by carnitine. However, only with IFM did carnitine reverse this effect to values not significantly different from control. Atractyloside-sensitive ADP uptake (Figure 9, c,d) was significantly depressed in both populations of mitochondria isolated from hearts after 30 min reperfusion. Carnitine partially prevented this depression although values remained significantly reduced from control.

Discussion

In this study we have shown that 100 μ M D,L-carnitine modestly protects the ischaemic and reperfusion isolated rat heart. With respect to function, this protective influence was manifested by improved post-ischaemic ventricular contractile recovery and reduced resting tension although the latter was not statistically significant. Furthermore, carnitine

Figure 5 State 3 respiration (Q_{O_2}), respiratory control index (RCI), ADP:O ratio (ADP:O) and oxidative phosphorylation rate (OPR) with pyruvate-malate as substrates for subarcolemmal (a) and interfibrillar (b) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia and 10 min reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

reduced the incidence of ventricular arrhythmias during both low-flow and reperfusion conditions. As discussed in detail below, mitochondrial function was also improved by carnitine treatment.

The effects of carnitine on the ischaemic heart are controversial. A number of studies have reported a protective influence of carnitine or its analogues on the hypoxic or ischaemic isolated heart (Fanelli, 1978; Liedtke & Nellis, 1979; Liedtke *et al.*, 1981; Paulson *et al.*, 1984; 1986). Others however, failed to demonstrate any ameliorating effect of carnitine with respect to contractile function, metabolism or arrhythmogenesis in the ischaemic heart (Neely *et al.*, 1979; Paulson & Shug, 1982; Fazekas *et al.*, 1986). A partial explanation for the discrepant results may be related to concentration of carnitine employed. For instance, *in vitro* studies showing a lack of protective effect of carnitine have employed concentrations in the millimolar range (Neely *et al.*, 1979; Paulson & Shug, 1982). Indeed, it has been demonstrated that millimolar concentrations of car-

Figure 6 Uncoupled oxygen consumption (a and b) and adenine nucleotide translocase activity (c and d) for subarcolemmal (a and c) and interfibrillar (b and d) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia and 10 min reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Studies with uncoupled mitochondria were carried out in the presence of 2,4-dinitrophenol and glutamate-malate as substrates. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

nitine actually have a toxic influence and reduce ventricular recovery after reperfusion of the ischaemic heart (Hearse *et al.*, 1980). In initial experiments not reported in this study we found that carnitine concentrations of 200 μM or greater produced a marked elevation in coronary perfusion pressure indicative of coronary constriction and failed to improve contractile recovery or reduce the incidence of arrhythmias, thus confirming a critical concentration-dependency of this agent. The concentration used in the present study represents the lowest level devoid of coronary effects. The maximum protection was seen with carnitine present throughout the perfusion protocol, as reported here. A possible explanation for this is that adequate intracellular concentrations of carnitine

Figure 7 State 3 respiration (Q_{O_2}), respiratory control index (RCI), ADP: oxygen ratio (ADP:O) and oxidative phosphorylation rate (OPR) with pyruvate-malate as substrates for subarcolemmal (a) and interfibrillar (b) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia and 30 min reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

must be attained before a protective influence is observed. This may be particularly relevant in the present study where relatively low concentrations of the agent were used; normal physiological plasma levels of $44 \mu M$ (Neely *et al.*, 1982) have been reported. Thus the present study shows a physiologically relevant protective influence of carnitine.

The detailed assessment of mitochondrial function was based firstly on the well known importance of carnitine transport to cardiac mitochondrial fatty acid oxidation (Opie, 1979). A complex, and as yet unresolved issue is whether mitochondrial dysfunction participates in cardiac injury associated with ischaemia or reperfusion. Although mitochondrial injury in the ischaemic and reperfused myocardium has been reported, others have demonstrated either normal (Rouslin, 1987) or even stimulated (Pelikan *et al.*, 1987) mitochondrial respiration after ischaemia. Discrepancies in terms of mitochondrial function following ischaemia may reflect isolation artifacts following whole-heart treat-

Figure 8 State 3 respiration (Q_{O_2}), respiratory control index (RCI), ADP: oxygen ratio (ADP:O) and oxidative phosphorylation rate (OPR) with glutamate-malate as substrates for subarcolemmal (a) and interfibrillar (b) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia and 30 min reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

ment or a failure to separate the relative effect of pathological insult on the two identifiable mitochondrial populations which have been shown to possess distinct biochemical properties (Palmer *et al.*, 1977). In an effort to delineate more precisely the role of mitochondrial function to the carnitine-induced protection we undertook two primary approaches. First, mitochondria were harvested at the end of three specific points of the perfusion sequence, that is at the termination of ischaemia, after early (10 min) reperfusion, and finally after 30 min reperfusion. Secondly, we assessed possible differences in the response of two different mitochondrial populations.

Ischaemia without subsequent reperfusion failed to depress oxidative phosphorylation and indeed a stimulation in respiration rates was routinely observed particularly in the presence of carnitine. This stimulation may be due to compensatory mechanisms of some mitochondrial enzyme systems as previously suggested (Pelikan *et al.*, 1987). For instance dephosphorylation or a small increase in intracellular Ca^{2+} may stimulate enzymes such as pyruvate dehydrogenase (Kerbey *et al.*, 1976). Fur-

Figure 9 Uncoupled oxygen consumption (a and b) and adenine nucleotide translocase activity (c and d) for subsarcolemmal (a and c) and interfibrillar (b and d) mitochondria isolated from non-ischaemic hearts (open columns), or from hearts subjected to 30 min ischaemia and 10 min reperfusion in the absence (solid columns) or presence (hatched columns) of carnitine. Studies with uncoupled mitochondria were carried out in the presence of 2,4-dinitrophenol and glutamate-malate as substrates. Each column represents the mean with vertical bars indicating the s.e.mean of $n = 10$. * $P < 0.05$ compared with non-ischaemic values.

thermore, an increase in the creatine:creatine phosphate ratio, as would be expected under ischaemic conditions has also been shown to stimulate mitochondrial respiration (Jacobus & Diffley, 1986). Although the effect was not statistically significant a reduction in atractyloside-sensitive ADP uptake was noticeable in both mitochondrial populations isolated from ischaemic hearts which was reversed by carnitine. This observation suggests that the adenine nucleotide translocase is more sensitive to ischaemic insult than oxidative phosphorylation and that a reduction in the former is possible without any resultant depression in oxidative phosphorylation. This concept was reinforced by our observation that mitochondria isolated from hearts reperfused for 10 min demonstrated a significantly depressed ADP uptake in the absence of substantial changes in oxidative phosphorylation.

Defective function of mitochondria was evident after their isolation from hearts subjected to reperfusion. The more severe nature of mitochondrial injury after 30 min of reperfusion suggests that mitochondrial injury is not immediate as a consequence of reflow but instead progresses as reperfusion duration increases. These results also show equal sensitivity in the response of either SLM or IFM to 30 min reperfusion.

The results presented here show that the consequences of carnitine treatment on mitochondrial function post-reperfusion are extremely complex. The significance of the early depression in RCI after 10 min reperfusion and its prevention by carnitine is suggestive of a protective effect, however the failure to observe differences in contractile recovery after 10 min reperfusion questions whether this prevention was relevant to contractile recovery. Furthermore, our study shows that the marked inhibition of adenine nucleotide translocase activity after only 10 min reperfusion was not significantly affected by carnitine treatment. In contrast, the more pronounced depression in mitochondrial function after 30 min reperfusion was substantially affected by carnitine, primarily with respect to oxidative phosphorylation. Thus depression in OPR was in most cases significantly prevented by carnitine, suggesting that improved ATP repletion may play a part in the enhanced recovery. Although carnitine has been shown to preserve ATP content during myocardial ischaemia *per se*, following coronary artery ligation in dogs (Folts *et al.*, 1978) improved repletion of high energy phosphates has not been demonstrated. Indeed, it has been shown that carnitine can improve postischaemic left ventricular recovery in dogs through a mechanism independent of tissue ATP content (Silverman *et al.*, 1985). Thus it is evident that the precise role of adenine nucleotides in mediating the effects of carnitine on the ischaemic and reperfused heart requires further investigation.

In summary, our study shows that carnitine can offer modest improvement in ventricular recovery after reperfusion of ischaemic isolated rat hearts through a mechanism possibly mediated by improved mitochondrial function. This study also shows that carnitine can protect the isolated heart, in contrast to previous suggestions that the protective effects could not be observed in this model due to poor washout of the drug during ischaemia (Silverman *et al.*, 1985).

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