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# Control of the Cardiac Consequences of Myocardial Ischemia and Reperfusion by L-Propionylcarnitine: Age-Response and Dose-Response Studies in the Rat Heart

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**ABSTRACT. We assessed the protective effects of L-propionylcarnitine, a liposoluble analogue of carnitine, in the isolated heart from rats of different ages subjected to global ischemia and reperfusion. Hearts from neonatal (3- to 7-d-old), immature (2- to 3-wk-old), and adult rats were retrogradely perfused with a modified Krebs bicarbonate buffer and subjected to ischemia and reperfusion. L-Propionylcarnitine was given either before ischemia and throughout reperfusion (protocol 1) or during reperfusion only (protocol 2). Coronary flow, heart rate, left ventricular developed pressure, and left ventricular end-diastolic pressure were measured throughout the perfusion period. Ventricular arrhythmias and creatine kinase leakage were measured at the time of reperfusion. Postischemic recovery of coronary flow and left ventricular developed pressure were age dependent and were not affected by L-propionylcarnitine, but recovery of heart rate was decreased in neonatal and immature hearts by $10^{-4}$ M and $10^{-5}$ M ($p < 0.05$), compared with controls (protocol 2). L-Propionylcarnitine always reduced creatine kinase leakage in the adult ($p < 0.05$) compared with controls (protocol 1). No effects on creatine kinase leakage were observed in neonatal and immature hearts. This study found that injury induced by ischemia and reperfusion was age dependent. Neonatal and immature hearts were more resistant to injury than adult hearts. The recovery of cardiac function was not affected by L-propionylcarnitine. However, in the adult rat hearts, L-propionylcarnitine given before ischemia and throughout reperfusion was protective by reducing creatine kinase leakage. (*Pediatr Res* 34: 465–470, 1993)**

Several studies carried out in isolated heart and intact animals have suggested that carnitine and its derivatives may protect against the cellular damage induced by ischemia and subsequent reperfusion of the ischemic myocardium (1–9). L-Propionylcarnitine has been shown to reduce the injury induced by lipid peroxidation on heart mitochondria membranes (10) and to improve the postischemic recovery of myocardial function by a reduction of oxidative stress due to oxygen-derived free radicals (4). Reactive oxygen intermediates are produced immediately upon reperfusion (11) when readmission of oxygen occurs.

The neonatal heart is believed to be more susceptible than the adult heart to the damaging effects of reperfusion as a consequence of an intrinsic immaturity of antioxidant enzymes (12, 13). Therefore, the aim of the present study was to assess the age response of L-propionylcarnitine on the consequences of ischemia and reperfusion in the isolated rat heart also in view of the fact that myocardial carnitine content decreases after myocardial ischemia (14) and the immature heart possesses a lower content of carnitine compared with the adult heart (15).

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## MATERIALS AND METHODS

*Animals.* Adult male and pregnant Sprague-Dawley rats were obtained from Charles River (Calco, Italy). Adult rats and litters were housed at room temperature (21–24°C with 55 ± 10%

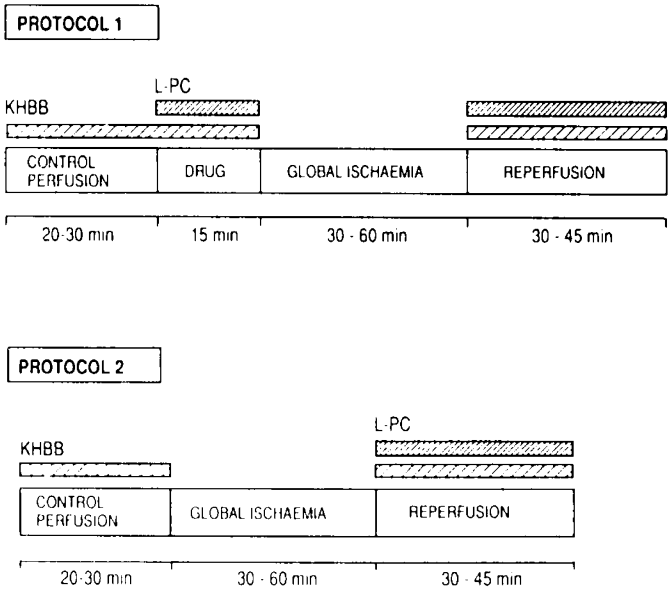


Fig. 1. Schematic representation of the protocol of studies. Isolated rat hearts were perfused with Krebs-Henseleit bicarbonate buffer (*KHBB*) with and without L-propionylcarnitine (*L-PC*) and subjected to global ischemia and reperfusion.

Table 1. *Exclusion criteria**

| Age group | CF (mL/min) | HR (beats/min) | LVDP (mm Hg) |
|---|---|---|---|
| Neonate | <0.5 | <180 | <40 |
| Immature | <1.0 | <200 | <50 |
| Adult | <6.0 | <220 | <80 |

* CF, coronary flow; HR, heart rate; LVDP, left ventricular developed pressure.

Table 2. *Body weight, ventricular wet weight, and preischemic cardiac function in hearts from rats of various ages (mean ± SEM)**

| Age group | No. rats/ group | Protocol | Group | Trial | BW (g) | VW (mg) | VW/BW (mg/g) | CF (mL/min/g) | HR (beats/min) | LVDP (mm Hg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Neonate | 6 | 1 | 1 | Control | 15 ± 1 | 61 ± 5 | 4.1 ± 0.2 | 21 ± 4 | 294 ± 14 | 52 ± 2 |
| | 6 | | 2 | $10^{-5}$ M | 15 ± 1 | 58 ± 5 | 3.9 ± 0.1 | 21 ± 2 | 214 ± 10 | 55 ± 3 |
| | 6 | 2 | 3 | Control | 12 ± 1 | 62 ± 3 | 5.2 ± 0.2 | 24 ± 3 | 213 ± 12 | 48 ± 2 |
| | 6 | | 4 | $10^{-6}$ M | 14 ± 1 | 66 ± 4 | 4.9 ± 0.1 | 21 ± 4 | 227 ± 10 | 44 ± 1 |
| | 6 | | 5 | $10^{-5}$ M | 13 ± 1 | 61 ± 2 | 4.8 ± 0.3 | 23 ± 3 | 237 ± 14 | 43 ± 2 |
| | 6 | | 6 | $10^{-4}$ M | 13 ± 1 | 52 ± 4 | 4.1 ± 0.2 | 19 ± 3 | 206 ± 7 | 52 ± 2 |
| Immature | 6 | 1 | 7 | Control | 48 ± 2 | 150 ± 10 | 3.2 ± 0.2 | 22 ± 4 | 277 ± 13 | 60 ± 2 |
| | 6 | | 8 | $10^{-5}$ M | 46 ± 2 | 150 ± 10 | 3.2 ± 0.2 | 21 ± 3 | 279 ± 20 | 58 ± 5 |
| | 6 | 2 | 9 | Control | 32 ± 3 | 100 ± 10 | 3.0 ± 0.3 | 35 ± 4 | 284 ± 14 | 72 ± 11 |
| | 6 | | 10 | $10^{-6}$ M | 30 ± 2 | 90 ± 10 | 2.6 ± 0.5 | 28 ± 3 | 289 ± 12 | 60 ± 3 |
| | 6 | | 11 | $10^{-5}$ M | 39 ± 4 | 130 ± 30 | 3.1 ± 0.3 | 30 ± 5 | 326 ± 12 | 70 ± 7 |
| | 6 | | 12 | $10^{-4}$ M | 33 ± 1 | 110 ± 10 | 3.3 ± 0.2 | 31 ± 4 | 331 ± 20 | 68 ± 6 |
| Adult | 6 | 1 | 13 | Control | 283 ± 8 | 800 ± 30 | 2.8 ± 0.1 | 16 ± 1 | 291 ± 8 | 122 ± 5 |
| | 5 | | 14 | $10^{-6}$ M | 273 ± 6 | 820 ± 50 | 3.0 ± 0.1 | 13 ± 1 | 295 ± 5 | 126 ± 1 |
| | 5 | | 15 | $10^{-5}$ M | 299 ± 2 | 810 ± 10 | 2.7 ± 0.1 | 13 ± 1 | 274 ± 15 | 116 ± 2 |
| | 5 | | 16 | $10^{-4}$ M | 293 ± 14 | 780 ± 30 | 2.7 ± 0.1 | 17 ± 1 | 322 ± 10 | 113 ± 5 |
| | 5 | 2 | 17 | Control | 288 ± 6 | 890 ± 30 | 3.1 ± 0.1 | 15 ± 2 | 307 ± 18 | 112 ± 4 |
| | 6 | | 18 | $10^{-5}$ M | 285 ± 5 | 860 ± 30 | 3.0 ± 0.1 | 12 ± 2 | 286 ± 22 | 102 ± 22 |

* BW, body weight; VW, ventricular wet weight; CF, coronary flow; HR, heart rate; LVDP, left ventricular developed pressure.

Table 3. *Postischemic recovery of coronary flow (CF) and heart rate (HR) in isolated heart from rats of various ages**

| Age group | No. rats/group | Protocol | Group | Trial | CF (%) | HR (%) |
|---|---|---|---|---|---|---|
| Neonate | 6 | 1 | 1 | Control | 119 ± 14 | 77 ± 10 |
| | 6 | | 2 | $10^{-5}$ M | 108 ± 11 | 95 ± 5 |
| | 6 | 2 | 3 | Control | 113 ± 12 | 94 ± 7 |
| | 6 | | 4 | $10^{-6}$ M | 130 ± 10 | 87 ± 4 |
| | 6 | | 5 | $10^{-5}$ M | 148 ± 13 | 81 ± 5 |
| | 6 | | 6 | $10^{-4}$ M | 111 ± 14 | 74 ± 7† |
| Immature | 6 | 1 | 7 | Control | 149 ± 22 | 107 ± 8 |
| | 6 | | 8 | $10^{-5}$ M | 172 ± 13 | 107 ± 10 |
| | 6 | 2 | 9 | Control | 166 ± 18 | 101 ± 4 |
| | 6 | | 10 | $10^{-6}$ M | 153 ± 25 | 103 ± 9 |
| | 6 | | 11 | $10^{-5}$ M | 137 ± 16 | 79 ± 6† |
| | 6 | | 12 | $10^{-4}$ M | 125 ± 8 | 91 ± 4 |
| Adult | 6 | 1 | 13 | Control | 75 ± 4 | 90 ± 5 |
| | 5 | | 14 | $10^{-6}$ M | 77 ± 5 | 92 ± 4 |
| | 5 | | 15 | $10^{-5}$ M | 72 ± 5 | 93 ± 6 |
| | 5 | | 16 | $10^{-4}$ M | 71 ± 6 | 61 ± 21 |
| | 5 | 2 | 17 | Control | 62 ± 4 | 90 ± 4 |
| | 6 | | 18 | $10^{-5}$ M | 67 ± 6 | 99 ± 12 |

* Data are percentages of control values, corrected for time-matched controls (mean ± SEM).
† $p < 0.05$ *vs* control group (analysis of variance and Dunnett's test).

humidity). Adult rats were fed standard animal food and maintained on a 12:12 h light:dark schedule with the light on from 0700 h. Neonatal rats were kept with the dam who fed them until weaning (approximately 3 wk).

Procedures involving animals and their care have been conducted in conformity with the institutional guidelines in compliance with National and International Laws and Policies (EEC Council Directive 86/609, OJ L 358.1, Dec. 12, 1987; *NIH Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 86-23, revised 1986).

*Surgical Preparation and Perfusion Technique. Neonatal hearts.* Male rats 3–7 d old were anesthetized with diethyl ether and 50 IU sodium heparin was administered intrahepatically. After the induction of whole-body hypothermia [by partial immersion of the animal in cold (4°C) perfusion fluid for 1 min], the chest wall was removed and the thoracic cavity filled with cold perfusion fluid (4°C). The pulmonary artery was incised near its origin, the left atrium was removed, and the aorta was cannulated *in situ* with a blunted 20-gauge needle. The heart was then excised, mounted on the perfusion apparatus via the aortic cannula, and installed in a temperature-controlled chamber at 37°C.

*Immature and adult hearts.* Immature (2- to 3-wk-old) and adult (2- to 3-mo-old) male rats were anesthetized with diethyl ether and 100 IU sodium heparin was administered intrahepatically in the first group and into a peripheral vein in the latter group. Thirty s later, hearts were excised and placed in cold (4°C) perfusion buffer until contraction ceased (approximately 15 s). Each heart was then cannulated through the aorta.

*Perfusion procedures.* Perfusion was carried out in the Langendorff mode at a constant pressure. Perfusion pressure was set at 60 cm $H_2O$ (neonatal hearts), 80 cm $H_2O$ (immature hearts), or 100 cm $H_2O$ (adult hearts). These values were selected to correspond with the *in vivo* mean arterial blood pressure in rats of the various age groups (16).

Perfusion was with a bicarbonate buffer solution containing (in mM): NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.8, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $CaCl_2$ 1.4, and glucose 11.0. The perfusion fluid was filtered (5-μm pore size) before use and was continuously gassed with 95% oxygen plus 5% carbon dioxide (pH 7.4 at

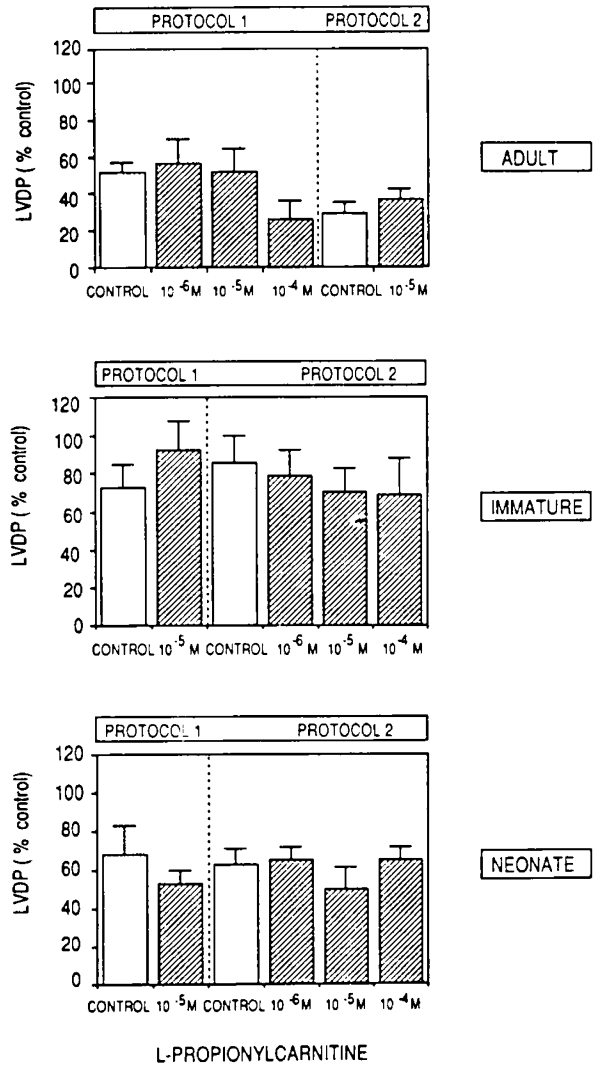


Fig. 2. Postischemic recovery of left ventricular developed pressure (*LVDP*) in the isolated hearts (five to six per group) from rats of different ages, after ischemia and reperfusion with and without L-propionylcarnitine. Data are expressed as percentages of the relative time-matched control values (mean ± SEM).

37°C). Partial pressures of $CO_2$ [34 ± 2 mm Hg (5 kPa)] $O_2$ [507 ± 64 mm Hg (68 ± 9 kPa)], and pH (7.4 ± 0.02) were periodically measured with a gas analyzer and found constant throughout the experiment.

After 5 min, an intraventricular balloon was introduced into the left ventricle through the mitral valve. The ultrathin balloon was constructed to match the internal dimensions of the left ventricle (17). The balloon was filled with saline and attached to a pressure transducer through a saline-filled rigid cannula. Using a microsyringe attached to a side arm of the transducer, the volume of the balloon was adjusted to give a left ventricular end-diastolic pressure of 2–8 mm Hg. After 20 min of control perfusion with bicarbonate buffer, values for cardiac function were recorded. Hearts that satisfied predefined exclusion criteria were then assigned to protocol 1 or protocol 2.

*Experimental Protocol.* There are several analogues of carnitine, and L-propionylcarnitine was chosen for these studies because it promptly enters the myocyte because of its high liposolubility (4, 6). We did not investigate the effects of the propionyl moiety in controls, because previous studies have shown that propionic acid given to isolated hearts was devoid of any effect on cardiac function and antioxidant enzyme (4). Therefore, in this study, control hearts were perfused with oxygenated bicarbonate buffer. Two treatment protocols were used, represented schematically in Figure 1.

*Protocol 1:* L-propionylcarnitine given before ischemia and throughout reperfusion. After 20 min of control perfusion, neonatal, immature, and adult hearts ($n$ = 5–6 per group) were perfused for 15 min with either normal bicarbonate buffer (control group) or buffer containing L-propionylcarnitine ($10^{-5}$ M). Hearts from adult rats were also perfused with $10^{-4}$ M and $10^{-6}$ M of L-propionylcarnitine. Values for cardiac function were recorded at the end of the 15-min perfusion period to detect any cardiac effect of the drug. Each heart was then subjected to 30 min (adult heart) or 60 min (neonatal and immature heart) of normothermic global ischemia. Reperfusion was carried out with normal bicarbonate buffer (control group) or with buffer containing the same concentrations of L-propionylcarnitine as before ischemia.

*Protocol 2:* L-propionylcarnitine given during reperfusion only. After 20 min of control perfusion, the hearts from neonatal, immature, and adult rats ($n$ = 5–6 per group) were subjected to 30 min (adult heart) or 60 min (neonatal and immature heart) of normothermic global ischemia. Reperfusion was performed with normal bicarbonate buffer (control group), with buffer containing $10^{-4}$ M and $10^{-6}$ M L-propionylcarnitine (neonatal and immature heart), or with $10^{-5}$ M L-propionylcarnitine (neonatal, immature, and adult heart).

The duration of ischemia was selected to achieve approximately 50% recovery of contractile function in each age group. This was expected on the basis of pilot studies. A recovery of 50% in controls allows scope for the detection of deterioration or protection of contractile function, whichever should occur.

*Exclusion Criteria.* Predefined exclusion criteria were applied before the start of the study and after 20 min of control perfusion. The minimal acceptable values for coronary flow, heart rate, and left ventricular developed pressure in the neonatal, immature, and adult heart are listed in Table 1. Any heart that was excluded was immediately replaced by another. In the course of the study, 24% of the hearts studied were excluded.

*Variables Measured.* Left ventricular systolic and end-diastolic pressures were obtained from high-speed recordings (25 mm/s) of the pressure trace and left ventricular developed pressure was calculated as the difference between these two values (COUPSYS R-04, Experimetria, Hungary). All signals were stored for further analysis on magnetic tape (model 141 Racal recorder, UK). Heart rate was also derived from the pressure trace and coronary flow was measured by direct timed collection of coronary effluent. Incidence, magnitude, time to onset, and time to peak ischemia-induced contracture were also recorded. Coronary flow, heart rate, and left ventricular developed pressure were measured throughout the perfusion period. Left ventricular end-diastolic pressure and creatine kinase leakage were measured throughout reperfusion. Reperfusion-induced arrhythmias were classified according to the Lambeth Convention (18). Conventional electrodes could not be used for electrographic recordings because the neonatal and immature rat hearts were too small. It was not possible to distinguish between sinus bradycardia and atrioventricular block. However, it was possible to detect ventricular arrhythmias (tachycardia, fibrillation, and premature beats) from the pressure tracing. The incidence of ventricular tachycardia and ventricular fibrillation were recorded. Creatine kinase levels in the coronary effluent were measured by a CK-NAC activated Monotest kit (Boehringer Mannheim, Mannheim, Germany) and with a spectrophotometer (DU-65 Beckman).

*Expression of Variables.* Results are expressed as mean ± SEM. Control preischemic variables are expressed as absolute values. Coronary flow and creatine kinase leakage are normalized per g of wet heart weight. Postischemic recovery of function is expressed as a percentage of the values recorded during the 20-min preischemic control period and corrected on the basis of the respective time-matched control value. The magnitudes of is-

Table 4. *Indices of ischemia- and reperfusion-induced injury in isolated heart from rats of various ages (mean ± SEM)**

| Age group | No. rats | Protocol | Group | Trial | Ischemia-induced contracture TTO (min) | TTP (min) | PEAK (mm Hg) | PEAK (% LVDP) | EDP (mm Hg) |
|---|---|---|---|---|---|---|---|---|---|
| Neonate | 6 | 1 | 1 | Control | 25 ± 3 | 34 ± 3 | 9 ± 1 | 19 ± 4 | 17 ± 3 |
| | 6 | | 2 | $10^{-5}$ M | 26 ± 3 | 38 ± 1 | 12 ± 2 | 22 ± 3 | 26 ± 6 |
| | 6 | 2 | 3 | Control | 27 ± 4 | 34 ± 4 | 9 ± 1 | 17 ± 3 | 15 ± 6 |
| | 6 | | 4 | $10^{-6}$ M | 23 ± 2 | 32 ± 3 | 13 ± 1 | 29 ± 3† | 17 ± 5 |
| | 6 | | 5 | $10^{-5}$ M | 28 ± 4 | 35 ± 3 | 8 ± 2 | 19 ± 3 | 12 ± 6 |
| | 6 | | 6 | $10^{-4}$ M | 31 ± 2 | 39 ± 2 | 10 ± 1 | 18 ± 2 | 15 ± 2 |
| Immature | 6 | 1 | 7 | Control | NM | 22 ± 2 | 17 ± 4 | 29 ± 7 | 24 ± 7 |
| | 6 | | 8 | $10^{-5}$ M | NM | 21 ± 3 | 18 ± 4 | 31 ± 7 | 18 ± 7 |
| | 6 | 2 | 9 | Control | 18 ± 4 | 26 ± 5 | 33 ± 12 | 41 ± 8 | 20 ± 5 |
| | 6 | | 10 | $10^{-6}$ M | 20 ± 2 | 29 ± 2 | 18 ± 4 | 31 ± 7 | 24 ± 5 |
| | 6 | | 11 | $10^{-5}$ M | 16 ± 4 | 23 ± 4 | 33 ± 10 | 44 ± 7 | 25 ± 7 |
| | 6 | | 12 | $10^{-4}$ M | 14 ± 3 | 21 ± 3 | 34 ± 13 | 43 ± 14 | 29 ± 9 |
| Adult | 6 | 1 | 13 | Control | 10 ± 0 | 17 ± 0 | 65 ± 3 | 54 ± 3 | 48 ± 3 |
| | 5 | | 14 | $10^{-6}$ M | 11 ± 0 | 17 ± 1 | 57 ± 3 | 45 ± 3 | 40 ± 8 |
| | 5 | | 15 | $10^{-5}$ M | 11 ± 1 | 17 ± 1 | 63 ± 3 | 55 ± 3 | 47 ± 7 |
| | 5 | | 16 | $10^{-4}$ M | 11 ± 1 | 17 ± 1 | 61 ± 5 | 55 ± 7 | 68 ± 7† |
| | 5 | 2 | 17 | Control | 11 ± 1 | 17 ± 1 | 57 ± 5 | 51 ± 4 | 60 ± 3 |
| | 6 | | 18 | $10^{-5}$ M | 12 ± 1 | 16 ± 1 | 56 ± 5 | 56 ± 6 | 58 ± 5 |

* TTO, time to onset; TTP, time to peak; LVDP, left ventricular developed pressure; EDP, end-diastolic pressure; NM, not measurable.
† $p < 0.05$ *vs* control group (analysis of variance and Dunnett's test).

chemic contracture and left ventricular end-diastolic pressure are expressed as absolute values (mm Hg).

*Statistical Analysis and Expression of Results.* One-way analysis of variance was used for multiple comparisons when a significant $F$ value was obtained; comparison of means was followed by Dunnett's test to compare treated groups with the control group (neonatal and immature hearts in protocol 2, and adult hearts in protocol 1). The $t$ test was used to compare treated groups (adult hearts in protocol 2, neonatal and immature hearts in protocol 1) with the control group. The $t$ test for paired data was used to compare values for cardiac function before and after L-propionylcarnitine and to compare left ventricular end-diastolic pressure before ischemia and at the end of reperfusion. Binomially distributed variables such as incidence of ventricular premature beats, tachycardia, and fibrillation were compared by the $\chi^2$ test. The relation between postischemic recovery of contractile function and other indices of injury was compared by linear regression analysis. Differences are considered significant at $p < 0.05$.

## RESULTS

*Basal characteristics and function.* Body weight, ventricular weight, and preischemic cardiac function for each group are shown in Table 2. As expected, body weight, ventricular weight, coronary flow, and left ventricular developed pressure increased with age. Heart rate was lower in the neonatal heart than in the immature and the adult heart. Ventricular weight to body weight ratio decreased with increasing age. However, coronary flow normalized per g of tissue was different in the three age groups. Coronary flow, heart rate, and left ventricular developed pressure measured before and after administration of L-propionylcarnitine were not affected by the drug.

*Stability during perfusion and time-matched controls.* Contractile function in the isolated neonatal rat heart declines in a time-dependent manner (19). This contrasts with the adult heart preparation, in which contractility is stable for long periods of perfusion. To assess age-dependent changes in stability during aerobic perfusion, hearts ($n = 6$) from neonatal, immature, and adult rats were subjected to 50 min of continuous aerobic perfusion at 37°C (equivalent to the 20-min control perfusion plus the 30-min reperfusion). In the neonatal hearts coronary flow, heart rate, and left ventricular developed pressure, respectively, decreased by 7, 36, and 19%, and in the immature hearts, by 17, 4, and 16%. Therefore, data from these hearts were used to normalize ischemia/reperfusion data, whereas recovery of the adult heart was not corrected, for the reason mentioned above.

*Postischemic recovery of function.* Postischemic recovery of coronary flow, heart rate, and left ventricular developed pressure are shown in Table 3 and Figure 2. The immature heart was the most resistant to ischemia- and reperfusion-induced injury, as shown by the highest cardiac function recovery values; the adult heart was the least resistant. Recovery of coronary flow was not affected by L-propionylcarnitine (Table 3). Heart rate was significantly decreased in the neonatal and the immature heart by L-propionylcarnitine at concentrations of $10^{-4}$ M and $10^{-5}$ M, respectively (protocol 2). L-Propionylcarnitine did not significantly affect the recovery of contractile function under any of the conditions.

*Ischemia-induced contracture, postischemic diastolic state, and creatine kinase leakage.* Table 4 summarizes other indices of ischemia- and reperfusion-induced injuries. The neonatal rat heart was significantly more resistant than the adult heart to ischemia-induced contracture, as shown by the late onset and the lower peak of contracture. The immature heart showed values between those of the neonatal and adult heart. Ischemia-induced contracture was not affected by L-propionylcarnitine except for the neonatal heart, where L-propionylcarnitine at a concentration of $10^{-6}$ M significantly increased this variable compared with control (protocol 2) (Table 4).

Creatine kinase leakage in each group is shown in Figure 3. Age-dependent creatine kinase leakage (IU) in the coronary effluent persisted even after normalization (IU/g). In the adult heart, L-propionylcarnitine significantly reduced creatine kinase leakage in the coronary effluent (protocol 1) compared with control. Neonatal and immature heart were unaffected.

Left ventricular end-diastolic pressure (mm Hg) during reperfusion increased with age, but the age-dependent increase disappeared after normalization (as percent of preischemic left ventricular developed pressure) (Table 4). Independently of L-propionylcarnitine, left ventricular end-diastolic pressure significantly increased after reperfusion compared with preischemic values (set between 2 and 8 mm Hg in each heart). In the adult heart, left ventricular end-diastolic pressure was significantly increased by L-propionylcarnitine at the concentration of $10^{-4}$ M (protocol 1).

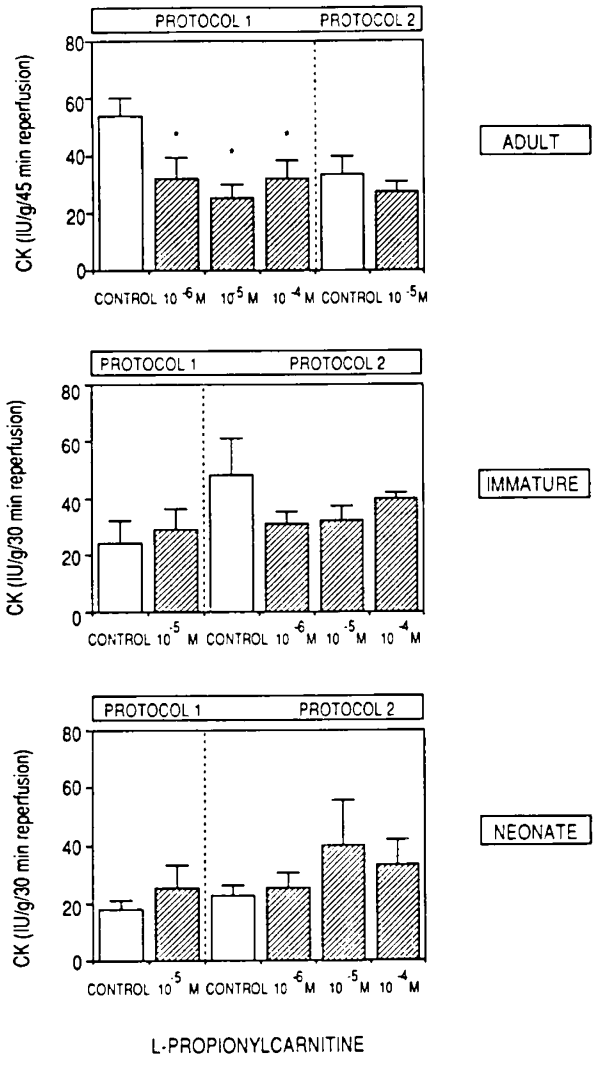


Fig. 3. Creatine kinase leakage (*CK*) during reperfusion in the coronary effluent in the isolated hearts (five to six per group) from rats of different ages perfused with and without L-propionylcarnitine (mean ± SEM).

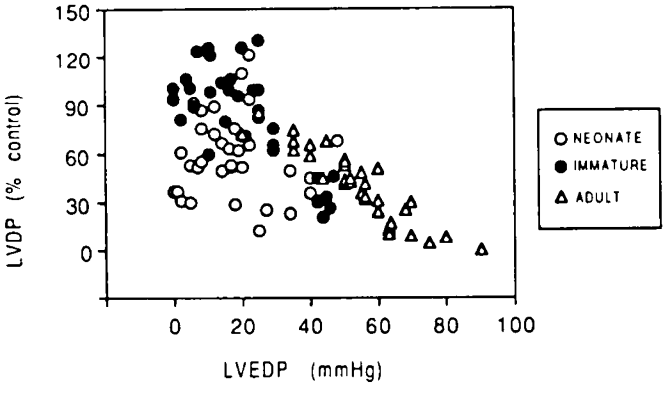


Fig. 4. Correlation between left ventricular end-diastolic pressure (*LVEDP*) at the end of reperfusion and postischemic recovery of left ventricular developed pressure (*LVDP*) in the isolated hearts (five to six per group) from rats of different ages (mean ± SEM).

*Reperfusion-induced arrhythmias.* Ventricular tachycardia and ventricular fibrillation were observed at the time of reperfusion only in the adult heart. In immature and in neonatal hearts, only premature ventricular contractions were observed. The incidence of ventricular arrhythmias in the adult rat hearts was not modified by L-propionylcarnitine, whereas the incidence of ventricular fibrillation was reduced from 43 to 20% at the concentration of $10^{-4}$ M (protocol 1) and from 67 to 33% at $10^{-5}$ M (protocol 2).

*Correlation between indices.* We examined the relationship between recovery of contractile function and other indices of injury such as ischemia-induced contracture, left ventricular end-diastolic pressure, and creatine kinase leakage. Recovery of left ventricular developed pressure correlated poorly with creatine kinase leakage, which was also independent from the postischemic recovery of coronary flow ($p$ = NS). Postischemic recovery of left ventricular developed pressure correlated with left ventricular end-diastolic pressure (Fig. 4), and a significant correlation was seen with values for the adult heart ($r^2$ = 0.848) and the immature heart ($r^2$ = 0.643) but not with the neonatal heart ($r^2$ = 0.014). Postischemic recovery of left ventricular developed pressure did not correlate with ischemia-induced contracture, nor did ischemia-induced contracture and left ventricular end-diastolic pressure.

## DISCUSSION

Several studies have shown that hearts reperfused after myocardial ischemia exhibit irreversible cell injury or transient injury (stunning) by mechanisms in part attributable to the action of toxic oxygen-derived free radicals (20, 21), and it has been demonstrated that a variety of interventions with an ability to prevent free radical formation or scavenge hydroxyl radicals once formed may enhance recovery of contractile function and reduce loss of intracellular enzymes (22). This study was designed to characterize the protective effects of L-propionylcarnitine on ischemia- and reperfusion-induced injury mediated by oxygen free radicals in the isolated immature heart.

Carnitine is essential for normal fatty acid oxidation and myocardial energy production (23, 24). The neonatal capacity to oxidize long-chain fatty acid, carnitine myocardial content (15), and mitochondrial enzyme activities is lower compared with the adult heart (25, 26). Although under normal conditions FFA metabolism is not essential in the immature heart, because of a high glycolytic capacity, it should be remembered that global ischemia (zero flow ischemia) depresses the anaerobic glycolytic pathway (27). Thus, instead of simply using neonates, we compared three age groups—neonate, immature, and adult—and instead of simply supplying carnitine in its more soluble form (as L-propionylcarnitine) at the optimal concentration for adults, we used different doses. In addition, we used multiple indices to assess tissue injury and sought to correct functional results for the natural time-dependent deterioration of any isolated organ preparation by expressing recoveries in relation to time-matched aerobic controls.

*Effects of L-propionylcarnitine on indices of cardiac function of ischemic/reperfused heart.* Although our results do not support the view that L-propionylcarnitine improves the postischemic recovery of cardiac function, a number of important points need to be addressed.

The effects of carnitine and its derivatives on the ischemic heart are controversial. A number of studies have reported a protective effect of carnitine or its analogues (1–8, 28, 29). Others, however, have found no such effect (9, 30, 31). Liedtke *et al.* (2), using intact regionally ischemic swine hearts, found that infusion of L-carnitine did not improve the global and regional parameters of mechanical cardiac function. However, when the mechanical data for performance were expressed as a function of metabolic energy expenditure, L-carnitine significantly enhanced the mechanical efficiency of the heart compared with D-carnitine.

Ferrari *et al.* (4), using isolated rabbit hearts subjected to low-flow global ischemia (60 min), found that L-propionylcarnitine $10^{-7}$ M, infused throughout ischemia and reperfusion, significantly enhanced the postischemic recovery of left ventricular developed pressure from 17% (controls) to 38%. Because release

of reduced and oxidized glutathione in the coronary effluent was significantly reduced, these authors speculated that the improvement of cardiac function might be mediated by a reduction of oxidative stress caused by oxygen-derived free radicals.

Paulson *et al.* (6), using isolated rat hearts subjected to low-flow global ischemia (90 min), found that L-propionylcarnitine improved the postischemic recovery of cardiac output from 38% in controls to 67% and 87%, at doses of $5.5 \times 10^{-4}$ M and $1.1 \times 10^{-4}$ M, respectively. Duan and Karmazyn (1), using isolated rat hearts subjected to low-flow ischemia (30 min), observed that D,L-carnitine ($10^{-8}$ M) given throughout ischemia and reperfusion mildly improved contractility. If D,L-carnitine was given only during ischemia or only during reperfusion, no such protection was observed.

*Effects of L-propionylcarnitine on biochemical indices of ischemic/reperfused heart.* In our study, L-propionylcarnitine at all doses significantly protected the myocyte, as shown by the decrease of creatine kinase leakage in the coronary effluent. This effect was observed in the adult heart with L-propionylcarnitine given before ischemia and throughout reperfusion, in agreement with Duan and Karmazyn (1). The protection afforded by L-propionylcarnitine on creatine kinase was significant when ischemia- and reperfusion-induced injury was severe, as shown by the earlier appearance of ischemic contracture and the lower postischemic recovery of contractile function in the adult heart than in the immature heart. In contrast with the adult, creatine kinase leakage after ischemia in the neonatal and immature heart does not appear to correlate linearly with postischemic recovery of contractility. We do not know why there is a step function relationship between recovery of left ventricular developed pressure and enzyme leakage in the neonatal heart and a linear relationship in the adult heart, but overall the results for creatine kinase, recovery of function, and contracture also support the concept of a greater resistance in the neonatal heart (19).

In conclusion, this study found that the adult heart is more responsive to the protected effect of L-propionylcarnitine against ischemia- and reperfusion-induced biochemical injury than the neonatal heart. However, ischemia- and reperfusion-induced injury was less severe in the neonate than in the adult heart, in terms of postischemic recovery of contractile function, contracture, and creatine kinase leakage.

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