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Hemodynamic and Metabolic Activities of Propionyl-L-Carnitine in Rats with Pressure-Overload Cardiac Hypertrophy

X. P. Yang, *M. Samaja, E. English, P. Benatti, †M. Tarantola, G. Cardace, †R. Motterlini, R. Micheletti, and ‡G. Bianchi

*Prassis Sigma-Tau Research Institute, *Department of Biomedical Science and Technology, University of Milan, †S. Raffaele Scientific Institute, and ‡Division of Nephrology, Dialysis and Hypertension, University of Milan, Milan, Italy*

Summary: Evidence has been put forth that a number of human and experimental cardiomyopathies are associated with a lower myocardial carnitine content. This study was performed to test the hypothesis that the correction of carnitine deficiency by a naturally occurring carnitine derivative, propionyl-L-carnitine (PLC), may improve cardiac function. Repeated administration of PLC was compared to saline with respect to cardiac function in rats with pressure-overload cardiac hypertrophy and low myocardial carnitine levels. Cardiac hypertrophy was induced by abdominal aorta constriction in rats. Separate groups of rats were used for (a) determination of myocardial carnitine content, (b) evaluation of in vivo hemodynamics, and (c) evaluation of performance and metabolic state of Langendorff perfused hearts. Results showed the following: (i) The myocardial carnitine content was inversely correlated to cardiac hypertrophy ($r = 0.68$, $p < 0.05$) and PLC treatment (50 mg/kg i.a. for 4 days) restored it to normal values (ii) The PLC effect on cardiac function was significantly and directly related to cardiac hypertrophy [correlations between heart weight and percent changes in cardiovascular parameters: cardiac output (CO), $p < 0.001$; cardiac work (CW), $p < 0.01$, stroke volume (SV) and stroke work (SW), $p < 0.02$]. In animals with heart weight >1,400 mg, the effect of PLC on CO, CW, SV, SW, and total peripheral resistance (TPR) was significantly different from that of saline (CO, CW, SV, and SW, $p < 0.005$ each; TPR, $p < 0.05$). The effect was observed 24 h after the first PLC administration and significantly diminished following a 4 day suspension of the treatment. (iii) Perfused hearts from PLC-treated rats displayed a significantly lower left ventricular end-diastolic pressure ($p < 0.01$) and greater relaxation rate ($p < 0.05$) than those from control rats. Moreover, in PLC-treated hearts, the content of creatine phosphate, ATP, and total adenine nucleotides (ATP + ADP + AMP; TAN) was significantly increased (CP, $p < 0.05$; ATP and TAN, $p < 0.01$ vs. control). These data show that PLC exerts a stimulatory activity on hearts with hypertrophy and low carnitine content, implying that carnitine deficiency may contribute to the depression of cardiac function in this model. **Key Words:** Carnitine—Propionyl-L-carnitine—Heart performance—High-energy phosphates.

Long-chain fatty acids are the preferred substrate for the production of metabolic energy required for cardiac performance (1). Carnitine plays an essential role in the transport of long-chain fatty acids from cytosol to the mitochondria, where oxidation of fatty acids takes place (2–4). Under normal conditions, myocardial carnitine content remains constant, at a level about 50–100 times greater than that found in plasma (5,6).

Markedly reduced levels of myocardial carnitine have been found in a number of human and experimentally induced cardiomyopathies (7–15). In some of these conditions, administration of carnitine or of its naturally occurring derivative, propionyl-L-carnitine (PLC), has resulted in a beneficial effect (8,15–22). With respect to carnitine, PLC has a greater affinity for carnitine acetyltransferase (23), is able to replenish mitochondria with interme-

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Address correspondence and reprint requests to Dr. G. Bianchi at Division of Nephrology, Dialysis and Hypertension, S. Raffaele Hospital, Via Olgettina 60, 20132 Milan, Italy.

diates of the citric acid cycle (24), with a greater stimulatory effect on palmitate oxidation by heart homogenates (19), and displays a greater positive inotropic activity both in mechanical recovery after ischemia and in intact isolated hearts (15,19,22,25).

However, there are no data on the activity of PLC on the hemodynamics of conscious rats with an experimentally induced cardiomyopathy, or on the relationship between such an activity and PLC effect on the metabolic status and mechanical performance of the isolated heart removed from the same experimental model. This information is indispensable in order to establish a possible role of this naturally occurring substance in the therapy of human cardiomyopathies. The present study was therefore performed in attempt to answer the following questions: (a) Does PLC produce persistent hemodynamic changes in conscious rats with pressure overload and cardiac hypertrophy consistent with the previously reported cardiac stimulatory activity, and do they disappear after suspending PLC administration? (b) Is the mechanical performance of isolated hearts obtained from rats pretreated with PLC compatible with the hemodynamic effect seen in vivo? (c) Is the pharmacological activity of PLC paralleled by changes in the content of myocardial carnitine and high-energy phosphates?

For practical reasons, the different aspects were studied in three separate sets of experiments.

Results showed that myocardial carnitine content is inversely related to the degree of cardiac hypertrophy. PLC treatment restored myocardial carnitine to normal levels and, in conscious rats, produced a hemodynamic effect compatible with a cardiac stimulatory activity and that was directly related to the degree of cardiac hypertrophy. Finally, isolated hearts from PLC-treated rats displayed an improved diastolic function and increased levels of high-energy phosphates.

METHODS

Aortic constriction

Male Wistar–Kyoto rats (Charles River, Calco, Italy) weighing 200–250 g were housed under standard conditions (12 h light–dark cycle, 22 ± 2°C) for 1 week before being used. Animals were fed a standard laboratory diet containing approximately 55 nmol/g of total carnitine (Altromin MT, Rieper, Vandois, Italy). Cardiac hypertrophy was produced by banding the abdominal aorta above the renal arteries with a silver clip. The degree of constriction, estimated from preliminary evaluation of the abdominal aorta size in a separate group of animals, was of about 50%. Surgery was performed under ether anesthesia. A lateral incision was made in the left lumbar region at the costophrenic level, the abdominal cavity was opened, and a silver band 0.7 mm internal diameter, 0.2 mm thick, 3 mm wide, was fitted around the aorta. Mortality for aortic-constricted (AC) animals was about 30%. Sham-operated animals were similarly treated, with the exception that the clip was not fixed into place.

Three different protocols were used for (a) measurement of carnitine levels, (b) in vivo hemodynamic studies, (c) in vitro functional and metabolic studies (Fig. 1).

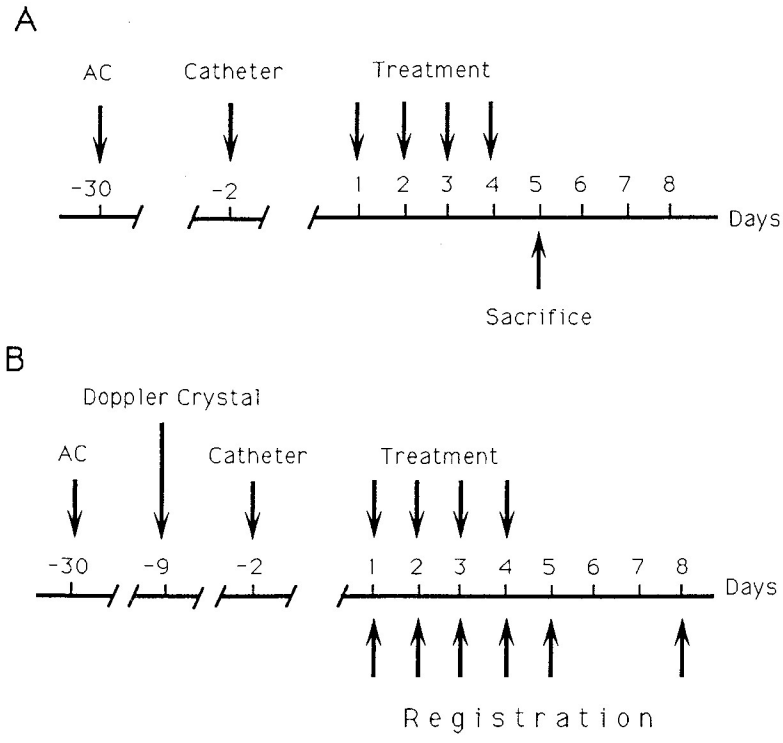

FIG. 1. Scheme of experimental protocols. **A:** Scheme for measurement of myocardial carnitine content and for Langendorff perfused hearts. Rats underwent sham operation or aorta constriction (AC) 4 weeks before implanting an aortic catheter for drug injection. Treatment with PLC (50 mg/kg i.a.) or saline was from day 1 to 4. Killing was on day 5. **B:** Scheme for hemodynamic studies. Three weeks after AC, rats were implanted with a Doppler crystal probe on the ascending aorta, and 1 week later with the aortic catheter. Treatment was as above. Data from Figs. 5 and 6 refer to registrations performed on day 2 to 5 and on day 8, respectively.

Measurement of carnitine levels

Four weeks after aortic constriction, under ether anesthesia, AC rats were implanted with a polyethylene catheter (PE 50) filled with heparinized 35% dextrose into the descending aorta via the left carotid artery (Fig. 1A). The catheter was tunneled subcutaneously, exteriorized at the back of the neck, and used for drug administration and blood pressure measurements, according to protocol. Carnitine levels were measured in sham-operated and in AC rats. The latter were untreated or treated with saline or PLC. PLC, 50 mg/kg dissolved in saline or saline alone were administered over 1 min via the catheter for 4 days. Rats were killed 24 h after the last treatment (Fig. 1A). Under urethane anesthesia, the heart was quickly excised, cleaned of adhering tissue, and rinsed in ice-cold saline. The total heart weight was measured after blotting dry; then, a portion of the left free ventricle wall was frozen in liquid nitrogen and stored at −80°C. Free and total acid-soluble L-carnitine content was determined in a neutralized perchloric acid extract of tissue homogenate (ref. 26, with minor modifications). Carnitine levels were expressed as nmol/mg of noncollagenous protein (NCP). NCP was extracted into 0.05 *N* NaOH and the protein was assayed by the method of Lowry (27).

Hemodynamic studies

The experimental protocol is summarized in Fig. 1B. Three weeks after abdominal aorta constriction, animals were intubated and ventilated by a positive pressure respirator (Harvard Rodent Respirator Model 683, South Natick, MA, U.S.A.) under pentobarbital anesthesia (50 mg/kg i.p.). A left thoracotomy was performed through the fourth intercostal space. A 20 MHz pulsed Doppler flow probe (Crystal Biotech, Hopkinton, MA, U.S.A.) was placed around the ascending aorta for aortic blood flow (cardiac output, CO) measurement. The lungs were inflated by increasing positive end-expiratory pressure and the wound was rapidly closed. The lead wires of the crystals were tunneled subcutaneously and exteriorized at the back of the neck. One week later, a catheter was implanted into the ascending aorta (Fig. 1B). The mean blood pressure (MBP, mm Hg) was measured by a pressure transducer (Statham P 23XL, Spectramed BV, Bilthoven, The Netherlands), deriving the heart rate (HR, beats/min) from the pulsed waveform. Values are expressed as the average of data sampled every 2.5 s, and elaborated by Dataflow program (Crystal Biotech). All parameters were recorded on a Gould polygraph (Gould RS 3800, Cleveland, OH, U.S.A.). CO was measured as Doppler frequency shifts (kHz). Because the absolute value of the Doppler shift depends on the sensitivity of the crystal, CO values are expressed as percent changes from basal. Total peripheral resistance (TPR), cardiac work (CW), stroke volume (SV), and stroke work (SW) were calculated as follows: TPR units = MBP (mm Hg)/CO (kHz), CW units = MBP (mm Hg) × CO (kHz), SV units = CO (kHz)/HR (beats/min), and SW units = MBP (mm Hg) × SV (units).

Experimental protocol for hemodynamic studies

Three days after catheter implant, animals were assigned randomly to treatment with PLC or physiological saline (control group). The experiment was performed in conscious, mildly restrained animals, after they were familiarized with the testing environment. Rats were treated for 4 days (Fig. 1B). The effect of treatment suspension was determined 4 days later. MBP, HR, and CO were recorded daily for 1 h before injection of compounds. Records of the 30 min following injection were obtained to evaluate the acute response to compound administration. On any day, values at 5-min intervals obtained from the 60 min record before compound administration were averaged and constituted the measurement of the response to the previous day's treatment. After killing, the heart was removed, rinsed, blotted dry, and weighed.

In vitro perfused heart

Hearts were removed on day 5, 24 h after the fourth administration of PLC or saline, according to the scheme described above (Fig. 1A). Rats were heparinized and anesthetized with i.p. sodium thiopental (100 mg/kg). The chest was opened, the venae cavae ligated, the heart excised, and in less than 45 s the aorta was mounted onto a stainless-steel cannula and retrograde perfusion was started. Buffer contained (in m*M*) 116 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 0.5 EDTA, 1.2 Na_2SO_4, 28.5 $NaHCO_3$, 3 $CaCl_2$, 1.2 $MgCl_2$, 16.6 glucose, 1.2 palmitate, and 3% (*w/v*) bovine albumin. PLC at 2.5 μ*M* and 25 μ*M* L-carnitine were also present in the perfusion fluid. The pH was 7.4, and the temperature was maintained at 37°C. The coronary flow rate was controlled by a peristaltic pump. The coronary sinus return was collected through an outflow cannula in the pulmonary artery. A latex balloon was introduced into the left ventricle through the left auricular appendage, and connected to a pressure transducer. Functional evaluation included coronary pressure (CP) and myocardial performance, both monitored by pressure transducers (Harvard Apparatus 52-9966, Natick, MA, U.S.A.) connected to the aortic cannula and to the intraventricular balloon, respectively. The signal from the latter transducer was supplied to a signal conditioner and to a six-channel strip chart recorder (Kipp & Zonen BD101, Delft, The Netherlands) for continuous monitoring of the left ventricular end-diastolic pressure (LVEDP), left-ventricular developed pressure (LVDP), HR, and maximal rates of heart contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$).

Experimental protocol for in vitro studies

Hearts were stabilized for 30 min at a coronary flow rate of about 15 ml/min, with the volume of the ventricular balloon adjusted to achieve a LVEDP of 0 ± 1 torr. This is the reference volume referred to as the zero filling volume. To compare hearts of widely different weights, the coronary flow rate was adjusted to achieve a coronary pressure of 80 ± 5 torr. At the end of the stabilization, baseline values were recorded and the intraventricular balloon was filled with accurately measured volumes of saline (steps of 55 μl each) using a glass gas-tight 1 ml syringe (Hamilton CO., Reno, NV, U.S.A.) and a micrometer. After each step, the hearts were allowed to stabilize (about 5 min) and a complete series of measurements was performed.

Assessment of metabolic state: pO_2 in the arterial inflow and in the coronary sinus return was monitored for the calculation of the O_2 consumption (VO_2) (YSI 5300 oxygen Monitor, Yellow Springs Inc., Yellow Spring,

OH, U.S.A.). At the end of the experiments, hearts were freeze-clamped in liquid nitrogen, weighed, and transferred to a tube containing 2 ml of 0.5 *M* cold perchloric acid. The contents were homogenized using an OMNI 1000 (OMNI International, Waterbury, CT, U.S.A.) operating at 20,000 rpm. After 15 min, the suspension was centrifuged, 0.5 ml of the supernatant was neutralized with 0.4 ml of 0.5 *M* KOH and 0.1 ml of 1 *M* KH_2PO_4, centrifuged again 30 min later, and filtered through a 0.22 μm pore size membrane (Nihon Millipore, Yonezawa, Japan). ATP, ADP, AMP, creatine, and creatine phosphate in myocardial extract were analyzed by high-performance liquid chromatography (HPLC). The equipment (Kontron Instruments, Milan, Italy) was composed of two mod. 420 pumps and a mod. 432 UV/Vis detector set at 210 nm. The 3 μm Supelcosil LC18 column (Supelco, Bellefonte, PA, U.S.A.) was equilibrated with 0.1 *M* KH_2PO_4 and 5 m*M* tetrabutylammonium sulfate; the sample (20 μl) was injected and eluted using a composed gradient with a buffer containing 0.1 *M* KH_2PO_4, 4 m*M* tetrabutylammonium sulfate, and 90% (*v/v*) CH_3CN. The analysis was completed in 25 min and data were analyzed with Kontron's dedicated software.

PLC was synthesized at Sigma Tau Chemical Dept. (batch 2649). Drug solutions for animal injection were prepared immediately before use, dissolving PLC in saline and buffering the pH to that of saline.

Studies conformed to the guiding principles of the National Society for Medical Research and the "Guide for the Care and the Use of Laboratory Animals" (NIH Publication No. 80-23, revised 1978).

Data analysis

Values are expressed as mean ± SEM. Between-group comparison of heart weight (HW), body weight (BW), and metabolic variables was by either Dunnet's *t* test, two-tailed unpaired Student's *t* test, or analysis of variance (ANOVA) as appropriate. Functional data from perfused hearts were analyzed according to a mixed factorial ANOVA for repeated measurements. In order to take into account the nonindependence of the error term, the Greenhouse and Geisser procedure for degrees of freedom correction was used (28). Results of in vivo hemodynamic variables are expressed as percent change from basal level. Between-group analysis was by split-plot ANOVA (29) for unbalanced replication. The total variance was partitioned into that associated with treatment, time, and their possible interaction. Within-group comparisons were performed using paired Student's *t* test. Statistical significance was defined as an error probability equal or less than 0.05

RESULTS

Relationship between cardiac carnitine content and hypertrophy

Initial and final body weights for sham-operated and AC rats are reported in Table 1. AC did not significantly affect body growth. However, the maneuver significantly increased the total heart weight of AC rats when compared with hearts of sham animals (+43%, p = 0.0001); hence the HW/BW ratio of AC animals was significantly elevated (p = 0.0001, Table 1), indicating the presence of cardiac hypertrophy.

TABLE 1. *Heart and body weights of rats with aortic constriction (AC) and sham operation used for myocardial carnitine determination*

	n	Body weight (g) Initial	Body weight (g) Final	Heart weight (mg)	Heart weight/body weight (mg/g × 100)
Sham	14	244 (12)	307 (8)	1033 (38)	336 (7)
AC	11	233 (7)	293 (7)	1481 (51)[a]	509 (23)[a]
AC + saline	11	248 (2)	282 (3)	1419 (61)[a]	503 (23)[a]
AC + PLC	11	257 (4)	287 (11)	1482 (84)[a]	520 (34)[a]

Values are given as mean (SEM).
[a] p = 0.0001 vs. sham-operated rats.

Cardiac hypertrophy was associated with a reduction in left ventricle carnitine content. Total carnitine in the AC group was decreased by 37% (Fig. 2), due to a similar reduction in both free and short-chain carnitines: from 4.35 ± 0.14 to 2.65 ± 0.20 nmol/mg of NCP and from 2.56 ± 0.36 to 1.70 ± 0.22 nmol/mg of NCP, respectively. Myocardial NCP levels in sham-operated and AC rats did not differ (158 ± 5.3 vs. 160 ± 4.3 mg/g of wet tissue). Carnitine depletion was more marked in those animals with the greatest hypertrophy. In fact, a significant inverse correlation could be shown between total carnitine content and the degree of cardiac hypertrophy (Fig. 3).

The effect of saline or PLC administration (50 mg/kg i.a. for 4 days) on AC rats was also investigated. Heart hypertrophy in these animals was strictly comparable to that reported above (Table 1). PLC restored the myocardial total carnitine content to a level not significantly different from that of

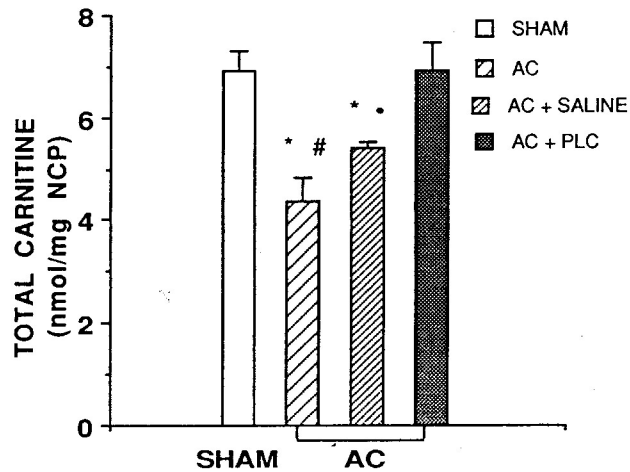

FIG. 2. Total carnitine content (nmol/mg of noncollagenous protein, NCP) in left ventricle free wall of sham-operated and aortic-constricted (AC) rats. PLC was administered at 50 mg/kg i.a. for 4 days. *p < 0.01 vs. sham; #p < 0.01 vs. AC + PLC; •p < 0.05 vs. AC + PLC (ANOVA).

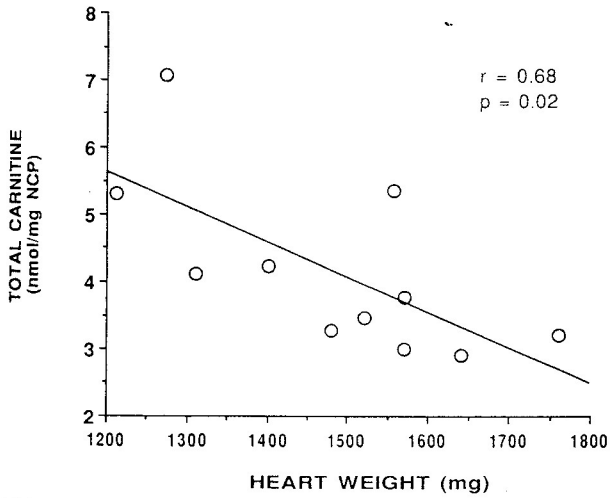

FIG. 3. Scatterplot of relationship between heart weight and total carnitine content (nmol/mg of noncollagenous protein, NCP) of left ventricle free wall in rats with constriction of abdominal aorta ($y = 11.867 - 0.005x$).

sham-operated rats, whereas saline treatment did not induce significant changes (Fig. 2).

Thus, cardiac hypertrophy due to pressure overload is correlated to left ventricle carnitine depletion, while PLC administration restores the carnitine content to normal values.

Hemodynamic studies

Twenty-five rats were included in the study: 14 animals were assigned to i.a. PLC treatment and 11 animals to saline treatment. Their basal values for MBP and HR did not differ: MBP was 179 ± 12 vs. 191 ± 9 mm Hg and HR was 412 ± 12 vs. 399 ± 17 beats/min. The mean heart weight was similar in the two groups: 1,570 ± 60 mg in the PLC group vs. 1,553 ± 75 mg in the saline group. Values were slightly, but not significantly, higher than those of hearts used for carnitine measurements, possibly because rats were killed 1 week later.

Neither PLC nor saline produced acute effects on MBP, HR, and CO during the 30 min measurement following each administration (data not shown). However, an increase in CO occurred from 24 to 48 h after the first PLC administration and persisted throughout treatment. Similar changes could be found in CW, SV, and SW; the TPR was significantly decreased, while the HR and MBP were unchanged (see below). These responses to PLC treatment appeared to be related to the degree of cardiac hypertrophy. As shown in Fig. 4, there was a significant correlation between the maximum PLC effect on CO, CW, SV, and SW and the HW. On the contrary, no such relationship was found for the saline-treated group (CO: $r = 0.13$; CW: $r = 0.04$; SV: $r = 0.03$; SW: $r = 0.14$). Because the PLC effect was directly related to the degree of hypertrophy, and to greater carnitine deficiency (see above), we analyzed separately the cardiac performance of rats with a higher hypertrophic response. Data were collected from rats with heart weight greater than 1,400 mg. Statistical evaluation was made of the effect of treatment, time, and their interaction. The serial changes (mean of percent variation from basal) in hemodynamic and cardiac function observed for the selected PLC-treated rats ($n = 10$) and for the saline group ($n = 9$) are illustrated in Figs. 5 and 6. Figures also show changes recorded on the fourth day after suspension of treatment. [Note that in some rats of either groups (two in the PLC, four in the saline group), the aortic catheter failed to give reliable measurements of the pulse blood pressure on days 4 and 5, preventing calculation of related parameters.] In comparison with saline treatment, PLC administration significantly increased the CO, CW, SV, and SW, and decreased TPR (Figs. 5 and 6). No significant differences between days of treatment were found. Similarly, there was no significant interaction between treatment and time. Moreover, 4 days after discontinuing PLC, the CO, CW, SV, and SW were significantly decreased compared to the last day of treatment (see the legend to Figs. 5 and 6), whereas the TPR was not significantly changed ($p > 0.1$).

In summary, PLC administration in conscious AC rats with large cardiac hypertrophy was able to increase the CO, CW, SV, and SW without changing the MAP and HR, but decreasing the TPR. The effects were already present after the first day of administration and were reduced 4 days after treatment suspension.

Isolated heart

The mean heart weight in the two experimental groups was 2.4 ± 0.1 g (PLC, $n = 9$) and 2.3 ± 0.1 g (saline, $n = 8$). Since the heart weight could only be determined at the end of the experiment, when it was greatly influenced by the perfusion procedure, values are not directly comparable with those from above-reported experiments.

Table 2 shows the functional parameters measured both at the balloon volume corresponding to zero intraventricular pressure and with increasing filling volumes. Differences were found in two parameters: in hearts from PLC-treated rats, the LVEDP increased less than in control hearts (Fig. 7). Statistical analysis showed a significant global treatment effect ($p = 0.005$), and a significant interaction between treatment and volume ($p < 0.01$), indicating that the rate of increase of LVEDP with increasing filling volumes was lower in PLC than in saline hearts. Moreover, the maximal rate of relaxation ($-dP/dt_{max}$) was significantly affected by PLC treatment: at the three largest balloon volumes, $-dP/dt_{max}$ was significantly larger in the PLC hearts than in control hearts ($p < 0.05$, Fig. 7). The possibility that LVEDP increases as a conse-

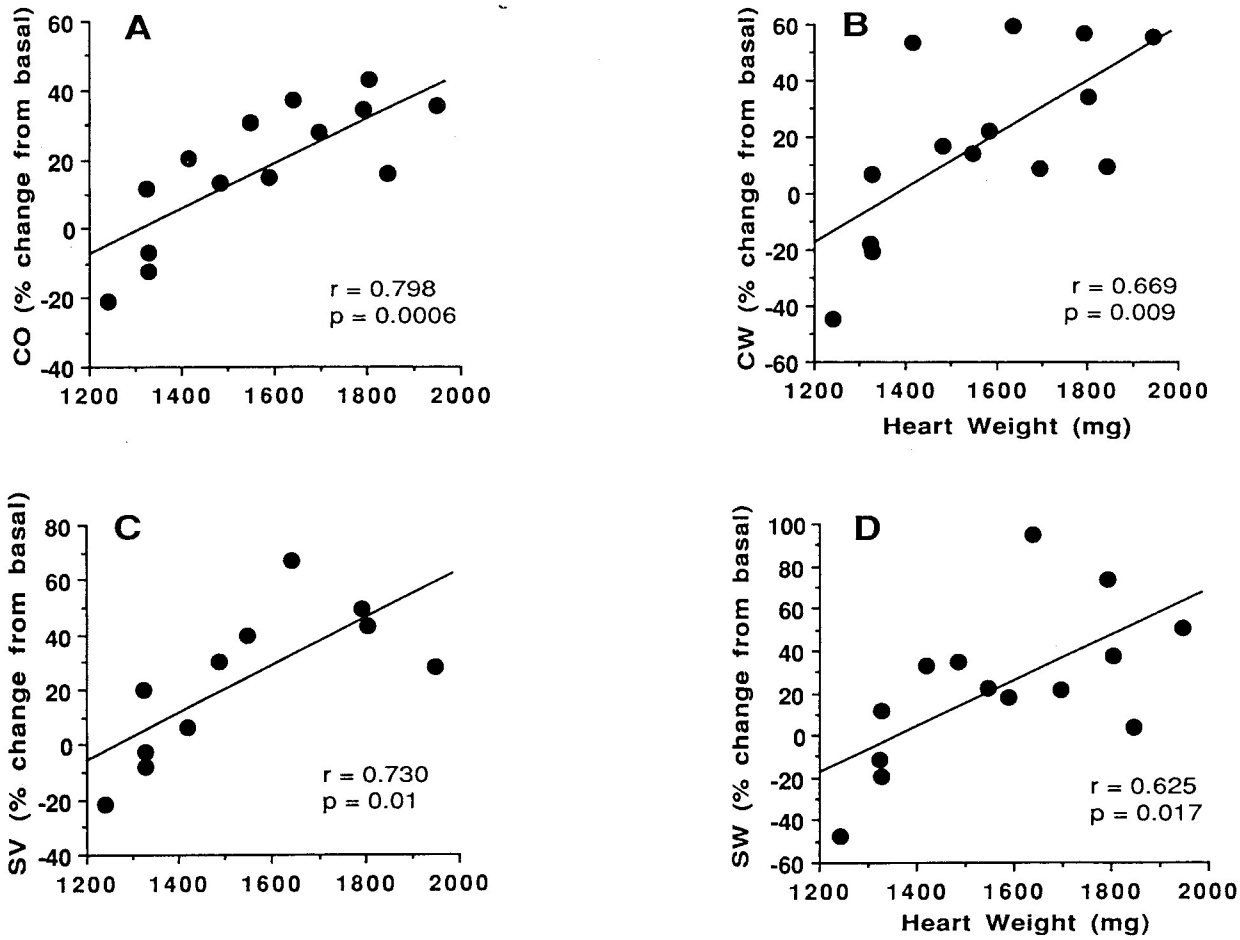

FIG. 4. Scatterplot of relationship between heart weight and hemodynamic variables in rats with abdominal aorta constriction treated with PLC, 50 mg/kg i.a. for 4 days. Values are the maximum percent change recorded in any of the 4 days of treatment. **A:** Cardiac output ($y = -90.6 + 0.069x$). **B:** Cardiac work ($y = -130.4 + 0.095x$). **C:** Stroke volume ($y = -84.9 + 0.068x$). **D:** Stroke work ($y = -135.9 + 0.10x$).

quence of an ischemic injury seems unlikely, as the coronary pressure and therefore probably vascular resistance did not significantly change during the perfusion and between treatments (Table 2).

Table 3 shows that the myocardial levels of ATP, total adenine nucleotides (TANs), and creatine phosphate were higher for the PLC-treated hearts compared to the control hearts. It appears that the higher ATP content was accompanied by a higher content of myocardial TAN rather than by higher energetic charge. Indeed, the energetic charge and phosphocreatine to creatine ratio (not shown) were unaltered in the two groups.

Finally, irrespective of the treatment, the content of high-energy phosphates was directly correlated to the relaxation rate and inversely correlated to the end-diastolic pressure ($p < 0.001$, both; not shown).

In conclusion, compared with controls, hearts from PLC-treated rats had a lower ventricular end-diastolic pressure and a higher relaxation rate, accompanied by a greater content of high-energy phosphates.

DISCUSSION

The objectives of the present studies were to investigate the effect of PLC, a naturally occurring derivative of L-carnitine, in a model of cardiac hypertrophy characterized by reduced carnitine content and to gain information about the underlying mechanism of action. Collectively, the data presented here show that repeated administration of PLC had a clear beneficial effect on the cardiac function of rats with myocardial hypertrophy induced by pressure overload. Hemodynamic studies evidenced that the cardiac stimulating action of PLC was related to the degree of hypertrophy. This finding is consistent with the results from in vitro experiments, showing that the diastolic function was improved and the myocardial content of carnitine and high-energy phosphates was increased.

In conscious rats, PLC produced an increase in CO, CW, SV, and SW, associated with a decrease in TPR and no significant changes in MBP. The decrease in afterload represented by the TPR does

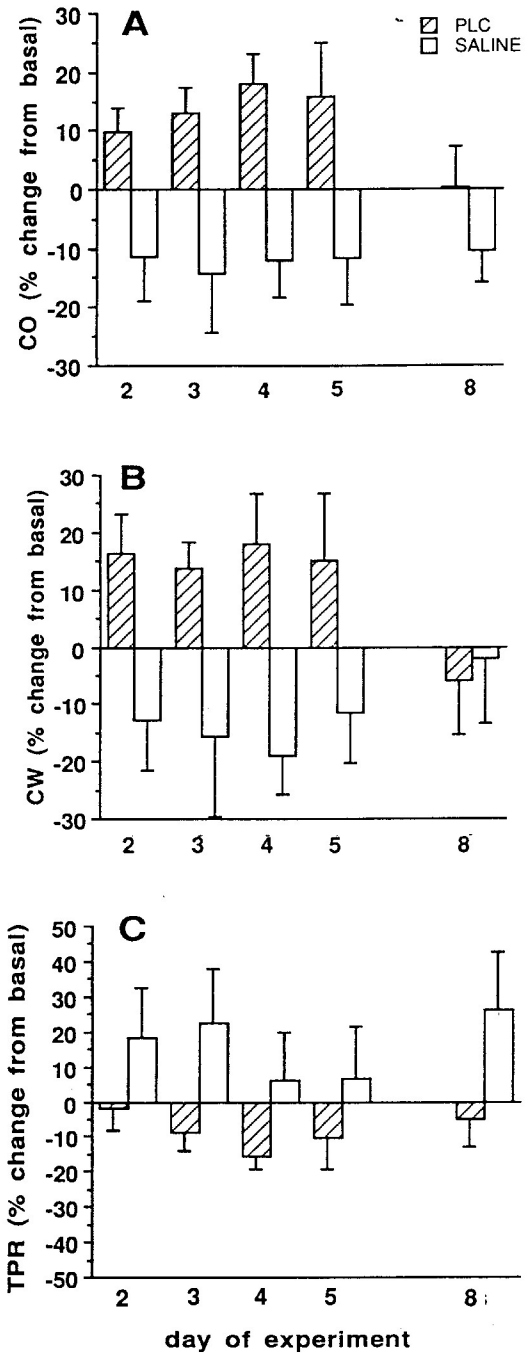

FIG. 5. Histograms of time course of hemodynamic changes observed during PLC or saline treatment, and on the fourth day after treatment withdrawal, in rats with abdominal aorta constriction. Data refer to rats with a heart weight >1,400 mg. Values are expressed as a percentage of pretreatment baseline values and are plotted as means ± SEM. **A:** Cardiac output (CO); **B:** cardiac work (CW); **C:** total peripheral resistance (TPR). Comparison between treatments was by split-plot ANOVA. $p < 0.005$ for CO and CW; $p < 0.05$ for TPR. Differences between fifth and eighth day were analyzed by two-tailed paired Student's test. CO, $p = 0.02$; CW, $p = 0.007$; TPR, $p = 0.11$.

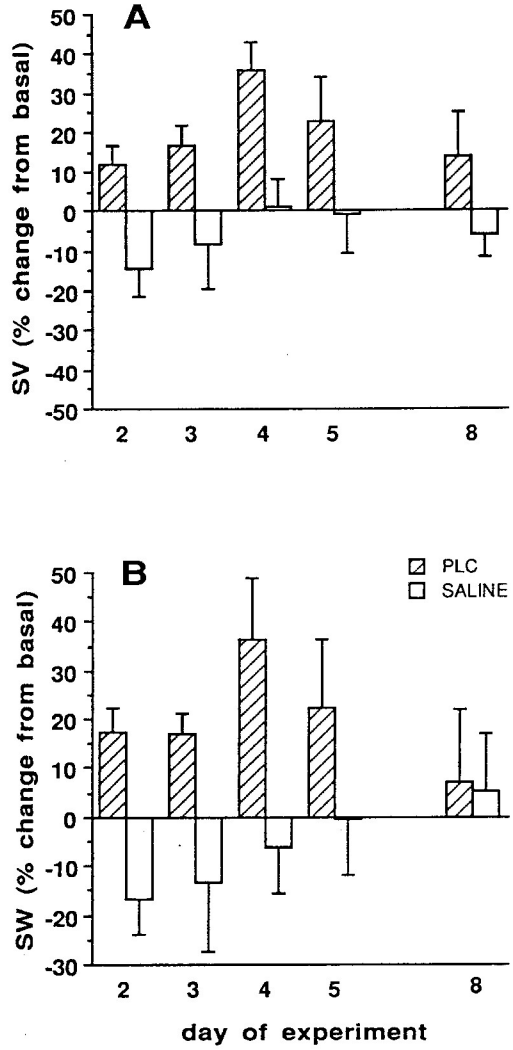

FIG. 6. Same as Fig. 5. **A:** Stroke volume (SV); **B:** stroke work (SW). For both parameters, $p < 0.005$ between treatments; $p = 0.04$ between fifth and eighth day of experiment.

not seem to account for the increase in CO, because the CW was actually enhanced. Although these data do not exclude the fact that PLC affected CO by increasing venous tone and thus venous return, a more likely explanation, supported by results obtained in isolated hearts, is a direct cardiac effect. In fact, perfused hearts obtained from animals pretreated with PLC displayed a lower diastolic pressure for any given filling pressure and a faster relaxation rate compared to controls. It is conceivable that this improvement of diastolic relaxation may favor the diastolic filling of the heart in vivo, thus explaining the increased SV and SW observed. On the other hand, the use of fixed volume loads in the left ventricle balloon under in vitro conditions prevented any increase in diastolic filling, possibly accounting for the lack of increment in $+dP/dt$.

As the coronary resistance of isolated hearts was similar in both groups, under our experimental con-

TABLE 2. *Functional parameters measured in* in vitro *perfused hearts from saline and PLC-treated rats at 0 end-diastolic pressure (filling volume = 0) and at the filling volumes indicated*

	Treatment	Filling volume (μl)				
		0	55	110	165	220
HR (beats/min)	Saline	207 (22)	219 (22)	206 (19)	211 (22)	191 (23)
	PLC	223 (11)	218 (14)	217 (13)	220 (12)	213 (15)
CP (torr)	Saline	85 (6)	87 (6)	91 (7)	99 (8)	104 (10)
	PLC	83 (3)	82 (2)	83 (3)	87 (3)	91 (4)
LVEDP (torr)	Saline	0.2 (0.3)	15.9 (1.1)	29.9 (1.7)	53 (3.1)	85 (6)
	PLC	0.2 (0.3)	10.5 (1.1)	24.3 (2.0)	42 (3.2)	63 (5)
LVDP (torr)	Saline	142 (18)	170 (18)	164 (14)	162 (15)	166 (10)
	PLC	124 (8)	164 (5)	169 (6)	165 (7)	163 (9)
$+dP/dt_{max}$ (torr/s)	Saline	3,438 (207)	4,414 (215)	4,653 (307)	4,529 (347)	4,605 (280)
	PLC	3,413 (188)	4,437 (127)	4,731 (174)	4,632 (191)	4,645 (186)
$-dP/dt_{max}$ (torr/s)	Saline	2,187 (136)	2,700 (154)	2,672 (143)	2,492 (146)	2,378 (90)
	PLC	2,279 (110)	2,744 (146)	3,096 (131)	2,952 (152)	2,844 (155)
VO_2 (μ*M*/min/g)	Saline	4.7 (0.2)	4.8 (0.2)	4.8 (0.3)	4.8 (0.3)	5.1 (0.2)
	PLC	4.6 (0.1)	4.8 (0.2)	5.0 (0.2)	5.0 (0.2)	5.3 (0.2)

HR, heart rate; CP, coronary pressure; LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; $+dP/dt_{max}$, maximal rate of contraction; $-dP/dt_{max}$, maximal rate of relaxation; VO_2, oxygen consumption.
Values are given as mean (SEM).

ditions two mechanisms may account for the lower end-diastolic pressure and the faster rate of relaxation: a change in the active relaxation process or a change in the passive stiffness of the left ventricular wall. A change in the former event is consistent both with the significantly lower slope of the pressure–volume curve in the PLC-treated hearts and with the greater availability of high-energy compounds. These may improve active relaxation by favoring calcium reuptake into the sarcoplasmic reticulum (30). In fact, abnormalities of diastolic relaxation rather than systolic performance may be an earlier and more sensitive marker of a deficient energy state in heart failure (31). This might be the reason why, in isolated hearts, PLC pretreatment affected the diastolic function, but not systolic performance. An effect of PLC on left ventricular stiffness can not be excluded, particularly in view of the recent observation that inhibition of fatty acid oxidation by 2-tedradecylglycidic acid resulted in left ventricular hypertrophy and an increase in the left chamber stiffness constant (32,33). PLC stimulation of fatty acid oxidation might thus decrease stiffness.

An amelioration of diastolic dysfunction is consistent with the improvement of exercise capacity caused by PLC in patients with heart failure (I. S. Anand, personal communication), considering that diastolic dysfunction has been suggested as one of the major determinants of the fall in exercise capacity in patients with heart failure (34).

A relevant finding was the positive relationship between the cardiac stimulatory activity of PLC in vivo and the degree of heart hypertrophy. The most hypertrophied hearts also exhibited lower levels of carnitine and we showed that PLC treatment restored the carnitine content. Thus, PLC seems to act by correcting carnitine deficiency. It could then be presumed that cardiac function is negatively affected by carnitine deficiency, as in the case of congenital myocardial deficiency responsive to carnitine treatment (8,16,18).

Finally, it can not be excluded that at the higher levels of hypertrophy, some degree of failure is also present (see below). The positive relationship found here between the increase in heart external work and heart hypertrophy is similar to that described for digitalis, which exerts a greater cardiac stimulatory activity in patients with a higher degree of heart failure (35,36).

The data presented allow one to gain some information on the mechanism of action of PLC. The clear-cut increase in ATP and TAN points to a metabolic effect, further strengthened by the features of the time course of PLC activity. In fact, in vivo experiments provided evidence that PLC administration did not modify the hemodynamic parameters for at least 30 min (i.e., the recording time). On the other hand, the PLC effect lasted over 24 h, as shown by the performance of isolated hearts and the levels of carnitine and high-energy phosphates, studied 24 h after the last PLC administration. Finally, it should be noted that the positive correlation between heart hypertrophy and hemodynamic response indicates that PLC tends to correct a dysfunction of hypertrophied cardiomyocytes rather than to modulate the activity of a "normal" cellular function. The following previously published observations are also relevant in this context: (a) In vitro perfused hypertrophied hearts have altered substrate metabolism in comparison to normal hearts, as shown by the accelerated deoxyglucose uptake and decreased extraction of a fatty acid analogue (37). (b) Fatty acid oxidation is lower in homogenates of failing hearts than in those of normal hearts

(19). Both carnitine and PLC stimulate this oxidation, with the latter compound showing greater activity (19).

Collectively, these observations suggest that the hemodynamic activity of PLC is somehow linked to its metabolic effect, i.e., stimulation of fatty acid oxidation with consequent preservation of TAN and increase in ATP and creatine phosphate production. This is in keeping with the suggestion that PLC induces a catalytic stimulation of the tricarboxylic acid cycle leading to an increase in the overall efficiency of myocardial energy production (38). In fact, since VO_2 and VO_2/LVDP × HR (not shown) were comparable in PLC- and saline-treated hearts, it is unlikely that the higher myocardial content of ATP, creatine phosphate, and TAN in PLC hearts is due to a higher O_2 supply.

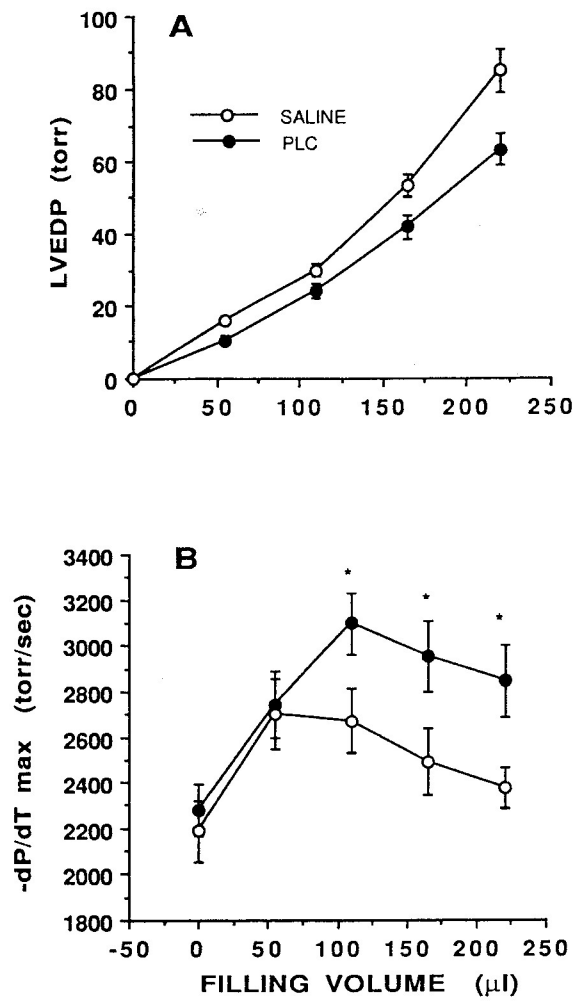

FIG. 7. In vitro perfused hearts from aortic constricted rats, obtained after 4-day treatment with saline or PLC (50 mg/kg i.a.) **A:** Relationship between intraventricular filling volume and left ventricular end-diastolic pressure (LVEDP). Significance between treatments: $p = 0.005$; treatment × volume interaction: $p < 0.01$. **B:** Relationship between intraventricular filling volume and maximal rate of relaxation ($-dP/dt_{max}$). Significance between treatments: $p < 0.05$. *$p < 0.05$ vs. control hearts.

TABLE 3. *Metabolic status of Langendorff perfused hearts from saline and PLC-treated rats*

	n	ATP (µmol/g d.w.)	TAN (µmol/g d.w.)	Creatine phosphate (µmol/g d.w.)	Energetic charge
Saline	8	15.5 (1.0)	23.7 (1.36)	20.5 (2.45)	0.80 (0.01)
PLC	9	20.7 (1.27)[a]	30.5 (0.16)[a]	27.2 (1.54)[b]	0.80 (0.01)

d.w., dry weight; TAN, total adenine nucleotides.
Values are given as mean (SEM).
[a] $p = 0.007$ and [b]$p = 0.04$ vs. hearts from saline-treated rats.

If PLC hemodynamic activity depends on its metabolic properties, we could postulate that, at least in the experimental model used here, a deficiency of high-energy phosphates secondary to a depressed oxidation of fatty acids may contribute to diastolic dysfunction and impairment of the pumping activity of the heart. However, the role of a deficiency in high-energy phosphate compounds in the pathogenesis of heart failure is controversial (39,40). Recently, significantly lower myocardial high-energy phosphates (−20%) were detected at 2 and 4 weeks but not at 6 weeks after aortic banding in the rat (41). Belanger et al. reported that hypertrophied hearts show lower baseline levels of ATP and glycogen (and worse function) both under normal conditions and after ischemia (42). The tissue high-energy phosphate level may not be the most appropriate parameter to study the relationship between the overall energy production by the heart and its contractile status. In fact, during hypoxia, the myocardial high-energy phosphate content decreased to a much lesser extent than their turnover and the changes of this last parameter paralleled much more closely the contractile failure occurring during this condition (43).

The beneficial effects of PLC have also been demonstrated on the exercise capacity of patients with angina pectoris (44) and on the walking capacities of patients with peripheral arteriopathy (45). Skeletal muscle of the latter patients exhibited a deficiency of carnitine and energy production that was counteracted by PLC administration (46), lending further support to the notion of the PLC metabolic action. An additional property of PLC is its antiarrhythmic efficacy (47), a feature that is also rather unique for a compound possessing a cardiac stimulatory activity in vivo. Given the well-known association between hypertrophy and ventricular arrhythmias (48,49), there is a strong rationale to determine whether PLC is a promising compound for the treatment of heart failure, particularly in those conditions where ischemic or arrhythmic complications are present.

A few technical points need to be discussed. In order to assess properly the hemodynamic effect of PLC over a few days of treatment in an experimen-

tal model of cardiomyopathy where the hemodynamics may change with time, we need to record data both before and some days after discontinuing treatment. To our knowledge, there are no prolonged hemodynamic studies in rats with experimentally induced severe cardiomyopathy. We found that the Doppler probe was suitable for rats with large hypertrophied and possibly failing hearts. The Doppler system used did not allow measurement of absolute values of the hemodynamic parameters investigated, preventing both the comparison of basal values between saline- and PLC-treated rats and the evaluation of the effect of different degrees of hypertrophy on basal levels. However, the lack of calibration has been compensated for by expressing our data in arbitrary units as percentage changes over basal levels.

It has recently been reported (50) that the Doppler module with a pulse repetition frequency of 62.5 kHz (the same as we used) tends to blunt the peak changes (either increase or decrease) in flow. Since we actually detected a clear-cut increase in aortic flow, the effect of PLC we observed may have actually been underestimated.

Finally, one limitation is the lack of hemodynamic studies in normal animals. From the relationship linking the PLC effect and hypertrophy, we did not expect to see any activity of PLC in normal rats or nonhypertrophied hearts. In fact, PLC was not active in the few animals with little hypertrophy (HW $<$ 1,400 mg, see Fig. 4). Whether hypertrophied hearts of AC rats also have impaired function has not been directly proven. Ventricular impairment may be inferred by the stimulatory effect of PLC. Moreover, a further indication comes from experiments carried out on the same model of hypertrophy used here (R. Micheletti, unpublished results). We found that in about 80% of the hearts weighing over 1,400 mg, the right ventricle weight was 2 SD above the mean of sham-operated hearts. Furthermore, right ventricular hypertrophy was directly correlated ($p < 0.02$) with the LVEDP, strongly suggesting that hypertrophy of the right ventricle after aortic banding is indicative of left ventricular cardiac failure.

CONCLUSIONS

This study demonstrates that PLC administration to conscious rats with pressure-overload heart hypertrophy and decreased ventricular concentration of carnitine is able to improve cardiac function. The beneficial effect of PLC seems to have a well-defined metabolic basis that can be found at the level of the bioenergetic metabolic pathways. These findings are likely to be relevant to other experimental or human cardiomyopathies where the myocardial carnitine concentration is reduced.

REFERENCES

1. Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu Rev Physiol* 1974;36:413–59.
2. Bremer J. Carnitine: metabolism and function. *Physiol Rev* 1983;63:1420–80.
3. Bieber LL. Carnitine. *Annu Rev Biochem* 1988;57:261–83.
4. Siliprandi N, Di Lisa F, Pivetta A, Miotto G, Siliprandi D. Transport and function of L-carnitine and L-propionylcarnitine: relevance to some cardiomyopathies and cardiac ischemia. *Z Kardiol* 1987;76(suppl 1):34–40.
5. Böhmer T. Conversion of butyrobetaina to carnitine in the rat in vivo. *Biochim Biophys Acta* 1974;343:551–7.
6. Rebouche CJ. Carnitine and tissue accretion in rats. *Biochim Biophys Acta* 1989;1033:111–3.
7. Regitz V, Shug AL, Fleck E. Defective myocardial carnitine metabolism in congestive heart failure secondary to dilated cardiomyopathy and to coronary, hypertensive and valvular heart disease. *Am J Cardiol* 1990;65:755–60.
8. Carnitine deficiency. *Lancet* 1990;335:631–3.
9. Gilbert EF. Carnitine deficiency. *Pathology* 1985;17:161–9.
10. Spagnoli LG, Corsi M, Villaschi S, Palmieri G, Maccari F. Myocardial carnitine deficiency in acute myocardial infarction. *Lancet* 1982;1:1419–20.
11. Suzuki Y, Masumura Y, Kobayashi Y, Yamazaki N, Harada Y, Osawa M. Myocardial carnitine deficiency in chronic heart failure. *Lancet* 1982;1:116.
12. Reibel DK, Uboh CE, Kent RL. Altered coenzyme A and carnitine metabolism in pressure-overload hypertrophied hearts. *Am J Physiol* 1983;244:H839–43.
13. El Alaoui-Talibi ZJ, Moravec J. Carnitine transport and exogenous palmitate oxidation in chronically volume-overloaded rat hearts. *Biochim Biophys Acta* 1989;1003:109–14.
14. Shug AL, Thomsen JH, Folts JD, et al. Changes in tissue levels of carnitine and other metabolites during myocardial ischemia and anoxia. *Arch Biochem Biophys* 1978;187:25–33.
15. Liedtke AJ, DeMaison L, Nellis SH. Effects of L-propionylcarnitine on mechanical recovery during reflow in intact hearts. *Am J Physiol* 1988;255:H169–76.
16. Tripp ME, Katcher ML, Peters HA, et al. Systemic carnitine deficiency presenting as familial endocardiofibroelastosis. A treatable cardiomyopathy. *N Engl J Med* 1980;305:385–90.
17. Rodrigues Pereira R, Scholte HR, Luyt-Houwen IEM, Vaandrager-Verduin MHM. Cardiomyopathy associated with carnitine loss in kidneys and small intestine. *Eur J Paediatr* 1988;148:193–7.
18. Tein I, De Vivo DC, Bierman F, et al. Impaired skin fibroblast carnitine uptake in primary systemic carnitine deficiency manifested by childhood carnitine-responsive cardiomyopathy. *Paediatr Res* 1990;28:247–55.
19. Paulson DJ, Traxler J, Schmidt M, Noonan J, Shug AL. Protection of the ischaemic myocardium by L-propionylcarnitine: effects on the recovery of cardiac output after ischaemia and reperfusion, carnitine transport, and fatty acid oxidation. *Cardiovasc Res* 1986;20:536–41.
20. Molaparast-Saless F, Nellis SH, Liedkte AJ. The effects of propionylcarnitine taurine on cardiac performance in aerobic and ischemic myocardium. *J Mol Cell Cardiol* 1988;20:63–74.
21. Leasure JE, Kordenat K. Effect of propionyl-L-carnitine on experimental myocardial infarction in dogs. *Cardiovasc Drugs Ther* 1991;5:85–95.
22. Ferrari R, Ceconi C, Curello S, Pasini E. Protective effect of propionyl-L-carnitine against ischaemia and reperfusion damage. *Mol Cell Biochem* 1989;88:161–8.
23. Clarke PRH, Bieber LL. Effect of micelles on the kinetics of purified beef heart mitochondrial carnitine palmitoyltransferase. *J Biol Chem* 1981;256:9869–73.
24. Davis EJ, Spyderold O, Bremer J. Pyruvate carboxylase and

propionyl-CoA carboxylase as anaplerotic enzymes in skeletal muscle mitochondria. *Eur J Biochem* 1980;110:255–62.
25. Pasini E, Ceconi C, Curello S, Cargnoni A, Condorelli E, Ferrari R. Effect of acute and chronic treatment with propionyl-L-carnitine on mechanical performance of isolated heart. *J Mol Cell Cardiol* 1990;22(suppl 3):S113.
26. Mc Garry JD, Foster DW. Free and esterified carnitine. Radiometric method. In: Bergmeyer J, Grable M, eds. *Methods of enzyme analysis*, Vol. 3, 3rd edition. Weinheim: Verlag Chemie GmbH, 1983:474–81.
27. Lowry OH, Rosebrough NJ, Far AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–75.
28. Greenhouse SW, Geisser SC. On methods of analysis of profile data. *Psychometrica* 1959;24:95–112.
29. Armitage P. In: *Statistical methods in medical research*. Oxford, U.K.: Blackwell Science Publ., 1971:256.
30. Chapman RA. Control of cardiac contractility at the cellular level. *Am J Physiol* 1983;245:H535–52.
31. Grossman W. Diastolic dysfunction and congestive heart failure. *Circulation* 1990;81(suppl III):III-1–7.
32. Bressler R, Gay R, Copeland, JG Bahl JJ, Bedotto J, Goldman S. Chronic inhibition of fatty acid oxidation: new model of diastolic dysfunction. *Life Sci* 1989;44:1897–906.
33. Litwin SE, Raya TE, Gay RE, et al. Chronic inhibition of fatty acid oxidation: new model of diastolic dysfunction. *Am J Physiol* 1990;258:H51–6.
34. Packer M. Abnormalities of diastolic function as a potential cause of exercise intolerance in chronic heart failure. *Circulation* 1990;81(suppl III):III-78–86.
35. Kelly RA. Cardiac glycosides and congestive heart failure. *Am J Cardiol* 1990;65:10E–6.
36. Lee DCS, Johnson RA, Bingham JB. Heart failure in outpatients: a randomized trial versus placebo. *N Engl J Med* 1982;306:699–705.
37. Kagaya Y, Kanno Y, Takeyama D, et al. Effects of long-term pressure overload on regional myocardial glucose and free fatty acid uptake in rats. A quantitative autoradiographic study. *Circulation* 1990;81:1353–61.
38. Siliprandi N, Di Lisa F, Menabò R. Propionyl-L-carnitine: biochemical significance and possible role in cardiac metabolism. *Cardiovasc Drugs Ther* 1991;5:11–6.
39. Katz AM. Cardiomyopathy of overload: a major determinant of prognosis in congestive heart failure. *N Eng J Med* 1990;322:100–10.
40. Braunwald E. Pathophysiology of heart failure. In: Braunwald E, ed. *Heart disease*. Philadelphia: Saunders, 1988:426–48.
41. Tanonaka K, Sanbe A, Takeo S. Myocardial energy metabolism of pressure-overloaded rats in vivo state. *J Mol Cell Cardiol* 1991;23(suppl II):S32.
42. Belanger R, Hiram C, Panos T, Wittnich C. Metabolic effect of pressure induced hypertrophy on left/right ventricles. *J Mol Cell Cardiol* 1990;22(suppl I):S22.
43. Bittl JA, Balshi A, Ingwall JS. Contractile failure and high-energy phosphate turnover during hypoxia: ^{31}P-NMR surface coil studies in living rat. *Circ Res* 1987;60:871—8.
44. Cherchi A, Lai C, Onnis E, et al. Propionyl carnitine in stable effort angina. *Cardiovasc Drugs Ther* 1990;4:481–6.
45. Brevetti G, Perna S, Sabbà C, et al. Superiority of L-propionylcarnitine in improving walking capacity in patients with peripheral vascular disease: an acute, intravenous, double-blind, crossover study. *Eur Heart J* 1992;13:251–5.
46. Brevetti G, Angelini C, Rosa M, et al. Muscle carnitine deficiency in patients with severe peripheral vascular disease. *Circulation* 1991;84:1490–5.
47. Barbieri M, Carbonin PU, Cerbai E, et al. Lack of correlation between the antiarrhythmic effect of L-propionylcarnitine on reoxygenation-induced arrhythmias and its electrophysiological properties. *Br J Pharmacol* 1991;102:73–8.
48. McLenachan JM, Henderson E, Morris KI, Dargie HJ. Ventricular arrhythmias in patients with hypertensive left ventricular hypertrophy. *N Engl J Med* 1987;317:787–92.
49. Levy D, Anderson KM, Savage DD, Balkus SA, Kannel WB, Castelli WP. Risk of ventricular arrhythmias in left ventricular hypertrophy: the Framingham study. *Am J Cardiol* 1987;60:560–5.
50. Gardiner SM, Compton AM, Bennett T, Hartley CJ. Can pulsed Doppler technique measure changes in aortic blood flow in conscious rats? *Am J Physiol* 1990;259:H448—56.