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L-PROPIONYLCARNITINE AND SYNCHRONIZATION OF SPONTANEOUS ACTIVITY IN RAT ISOLATED PORTAL VEIN

R. MANCINELLI

Istituto di Fisiologia Umana, Università Cattolica del S. Cuore, Largo F. Vito, 1, 00168 Roma, Italy

INTRODUCTION

L-carnitine (β-hydroxi-γ-trimethyl-aminobutirric acid) (LC) is an intrinsic carrier of activated long-chain fatty acids across the mitochondrial membrane towards the matrix where β-oxidation of fatty acid occurs (7).

An exhaustive review of carnitine metabolism and its implications in clinical chemistry has been recently reported by Siliprandi *et al.* (25). As pointed out by these authors, interorgan transport of carnitine intermediates and its quantitative assessment might help to define carnitine deficiency syndromes.

Besides its mostly recognized role of long chain acyl carrier, carnitine acts likely as an exporter of short-chain acyls from the inner mitochondrial space to the outside and also it exerts its role by trapping and eliminating unphysiological acyl groups.

Myocardial ischemia and the ensueing derangement of fatty acid oxidation has suggested the possible role of carnitine in modulating the cytric acid cycle, preventing accumulation of fatty acid intermediates, and as a membrane stabilizing agent (11, 12, 20).

In addition, L-carnitine has received attention because of its antiarrhythmic effects in dog (18-26) and human (10) heart.

One of the most potent analogues of carnitine, presumably because of rapid transport entry, is L-propionylcarnitine (LPC) which has been identified in kidney and in liver of various animal species (6). In isolated heart L-propionylcarnitine has been shown to enhance mechanical and metabolic performance (24).

It has also been reported that LPC is most effective in protecting against mitochondrial dysfunction induced by elevated blood levels of long-chain acyl CoA (9). According to Liedtke *et al.* (19) LPC has presumably positive inotropic properties. Barbieri *et al.* (4) have recently observed in guinea pig that LPC exerts antiarrhythmic effect versus reoxygenation-induced arrhythmias.

LPC has been extensively tested on cardiac physiopathology and metabolism. Our research effort has been directed toward assessing whether LPC has a broader action on the cardiovascular system.

It is well known that longitudinal smooth muscle of portal vein from various species shows spontaneous contractile activity; therefore, it has been extensively

studied as an example of spontaneous contracting vascular muscle. We have considered the portal vein as a convenient model to analyze the effect of pharmacological substances on venous smooth muscle; the electrophysiological and mechanical action of LPC on isolated portal vein of rat has been inquired.

METHODS

General procedures. — Isolated preparations of the portal vein from Wistar rats (350-400 g) were studied in 71 experiments. After ether anesthesia, the abdomen was opened with a parasagittal incision and the portal vein, 12-15 mm «in situ» length, was carefully dissected from surrounding tissue and cut at the level of the gastrosplenic vein and of the bifurcation of the hepatic hilus. The vessel was then rapidly removed and immediately placed in a 10 ml organ bath containing Krebs solution with a millimolar composition of NaCl 118, KCl 4.70, $CaCl_2$ 2.52, $MgSO_4$ 1.64, $NaHCO_3$ 24.88, glucose 5.55. This solution was bubbled with a mixture of 96% O_2 and 4% CO_2, pH 7.40, and maintained at a constant temperature of 37°C. Veins were either used as intact cylinders or slit along their longitudinal axis to form a sheet.

For measuring mechanical and electrical events, three different experimental arrangements were utilized.

Mechanical recordings. — One end of the portal vein was attached to a muscle holder in a 10 ml organ bath. The spontaneous activity of the longitudinal smooth muscle of the vessel was recorded under isometric conditions by attaching the other end to a force-displacement transducer (Grass FT03). The muscle was stretched to its approximate «in situ» length which gave a passive tension of about 600 mg.

Mechanical and electrical recordings. — To essay both mechanical and electrical activities, the vascular segment was put horizontally in a 100 ml bath with the mesenteric end connected to a hook and the hepatic end to a Grass FT03 force-displacement transducer. The extracellular electrical activity of the longitudinal muscle was recorded by a glass pressure electrode (tip diameter 150-200 μm) filled with 3 M KCl solution. The electrode was kept in position by means of a spring able to follow displacements of the segment wall.

The electrical signal was processed in parallel with two different AC preamplifiers Grass 7P5: one with a lower cut-off frequency of the filter set to 0.35 Hz (time constant 0.45 s) for detecting both slow and fast activity; the other with a lower cut-off frequency 4 Hz (time constant 0.04 s) to enhance only the fast activity.

Experiments on synchronization of mechanical activity. — In these experiments a small slide branch at the middle part of the portal vein (see scheme in Fig. 5), was anchored to a muscle holder in a 20 ml organ bath. The two ends, refferred to as the hepatic (h) and mesenteric (m) end, were connected to two transducers (Grass FT03) and a passive stretch of 0.2-0.3 mg was applied. The angle at the central point of attachment was such that each of the two ends could be stretched and could contract independently, without influencing mechanically the opposite end.

Portal vein response to changes in extracellular osmolarity. — Osmolarity of Krebs solution and of the various LPC doses was assessed by means of an osmometer (Ormostat OM 6020). Measured osmolarity of the basic solution was 290-292 mOsm and the highest increase, produced by 8×10^{-3} M of LPC, was 14 mOsm. In order to verify the effect of altered osmolarity «per se» on the spontaneous contractile activity, 5 experiments were performed by adding 14 mM sucrose to the Krebs medium.

For all the preparations an accomodation period of about one hour was allowed before the experiment.

Mechanical and electrical activities were simultaneously recorded by a Grass D7 polygraph.

Pharmacological trials. — LPC (Sigma-Tau S.p.A., Pomezia, Italy) was diluted in physiological solution, just before each experiment, and introduced directly in small volumes into the organ bath. In order to evaluate the effect of LPC on basal tone and on the spontaneous mechanical activity, an amount of drug ranging from 10^{-5} to 10^{-2} M was added to the physiological bath. The doses are expressed as the final concentration in the bath.

Each venous segment was utilized for a single dose of drug; each dose was tested on five preparations. The results here reported were obtained in experiments on a total of 71 portal vein preparations, of which 63 were used for mechanical experiments and 8 for simultaneous recordings of electrical and mechanical activity.

Data analysis. — Besides the direct recordings of mechanical activity on Grass polygraph, the signal was stored on a magnetic tape (Rachal, store 4 DS). This permitted the replay of the signal on an electronic integrating device giving deflection proportional to the area of the active tension.

The rhythmic mechanical activity was integrated over 2 min periods, 20 min before and after LPC administration. The effect of the drug was calculated as a percentage of the control activity. Changes of amplitude and frequency values were also evaluated using analysis of variance with a probability of less than 0.05 regarded as significant.

RESULTS

1. *Mechanical spontaneous activity recordings.* — Typical mechanical spontaneous activity of the rat portal vein recorded under isometric condition is illustrated in the control period of Fig. 1. Regular contractions (20% of preparations) were recorded from the longitudinal muscle of vessel (Fig. 1 A). However, in the majority of preparations (80%), waves of irregular phasic contraction were recorded. A single contraction was usually oscillatory in shape containing two or more peaks, more or less separated. The number of oscillations and the overall pattern of these waveforms changed considerably in the course of some experiments (Fig. 1 B). Fig. 2 illustrates the summarized data of variation of both amplitude and

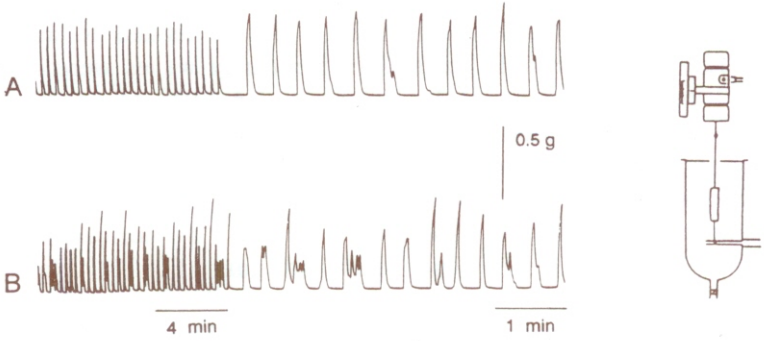

Fig. 1 – *Typical spontaneous mechanical activity of isolated rat portal vein recorded in Krebs solution at 37°C.*

The pattern of mechanical activity observed in our experiments was present in 20% (A), and in 80% (B) of preparations. The baseline of the traces, which is the same also for the Figs. 3, 4 and 6, represents a resting tension of about 600 mg applied to the muscle by passive stretch. The scheme depicts the experimental arrangement.

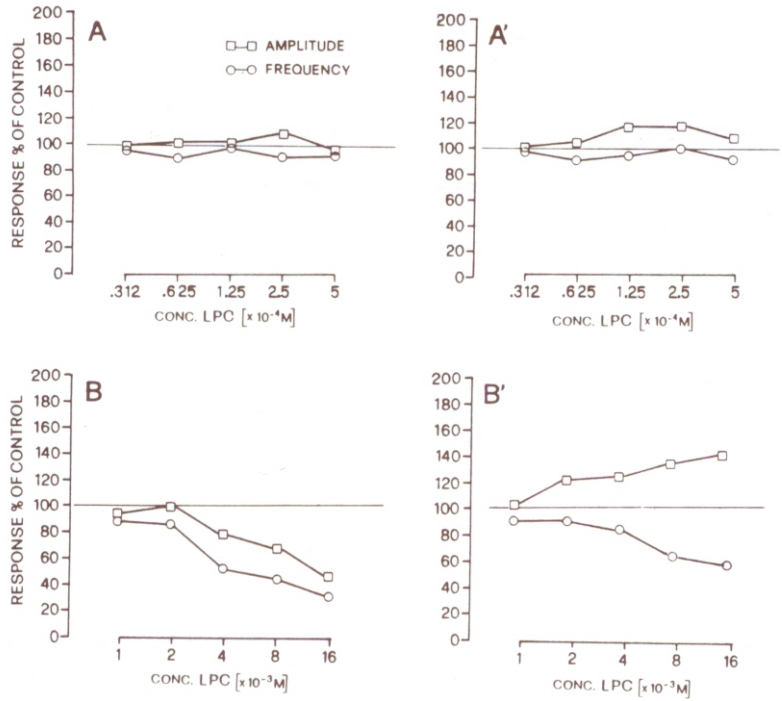

Fig. 2 – *Effect of increasing doses of LPC on amplitude and frequency of spontaneous contraction waves.*

AA' shows the effect of low concentrations (3.12×10^{-5}-5×10^{-4} M) and BB' that of higher doses (10^{-3}-1.6×10^{-2} M) observed in the first 10 min (A and B) and in the successive 10 min (A' and B') after LPC administration. Each point represents the mean value percentage of control value of 5 different preparations to a single dose.

frequency parameters of the spontaneous contractile activity of 50 preparations produced by all LPC tested concentrations (10^{-5} M—10^{-2} M).

Concentrations from 10^{-5} M to 10^{-3} M produced no significant changes in these parameters, although 12.5×10^{-5} M and 2.5×10^{-4} M tended to augmenting the force amplitude; no appreciable changes in frequency waves were observed (Fig. 2 AA'). LPC concentration from 2×10^{-3} M to 1.6×10^{-2} M produced, after an initial period of total inhibition, a significant increase ($P<0.01$) of the force waves and a reduction ($P<0.01$) of the contraction frequency (Fig. 2 BB'): the maximal effects were reached at 8×10^{-3} M. These parameters reached a stable pattern, depending on the concentration used after 7-10 min, in particular the frequency value of the contraction waves were nearly constant (Fig. 3). No modification of basal tone was observed at all concentrations of LPC used.

2. *Mechanical and electrical activity recordings.* — The spontaneous mechanical and electrical activity of the isolated portal vein of the rat was recorded in 8 experiments. Fig. 4 A shows the typical traces of mechanical and electrical activity during the control period. Mechanical activity shows force waves with variable amplitude, duration, frequency and morphology. Electrical activity is characterized both by slow and fast response; each contraction is accompanied by a burst of

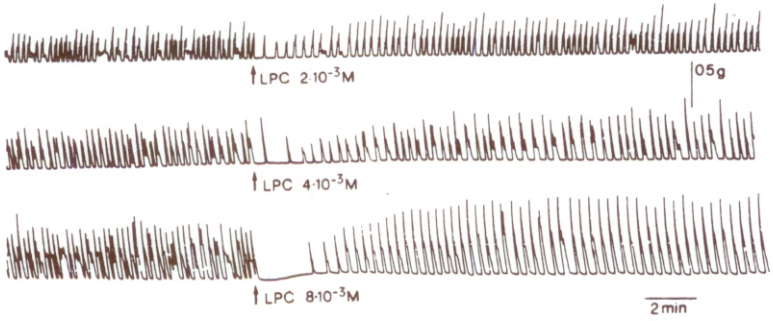

Fig. 3 – *Typical traces of mechanical response to increasing LPC doses.*

spike potentials whereas, during the quiescent period, only a slow component may be recorded.

Mechanical and electrical responses to LPC (8×10^{-3} M) is shown in Fig. 4 B. The drug provokes an increase in the amplitude of spontaneous contraction together with a parallel significant decrease in frequency. It has also been osberved a change in morphology of the force waves which become monophasic.

Electrical activity during LPC exposure shows a rhythmic regular slow wave with the same frequency of the mechanical one. Furthermore, the fast component exhibits an increment of number, frequency and amplitude of the spike potentials.

3. *Experiments on synchronization of mechanical activity.* — In 8 experiments the mechanical activity was simultaneously recorded from the hepatic (h) and the mesenteric (m) end of the vessel.

Fig. 5 shows a recording representative of the whole series of experiments. In the control period (Fig. 5 A) the hepatic and the mesenteric sections of the vein start to contract at different rhythms and show irregular and asynchronous contrac-

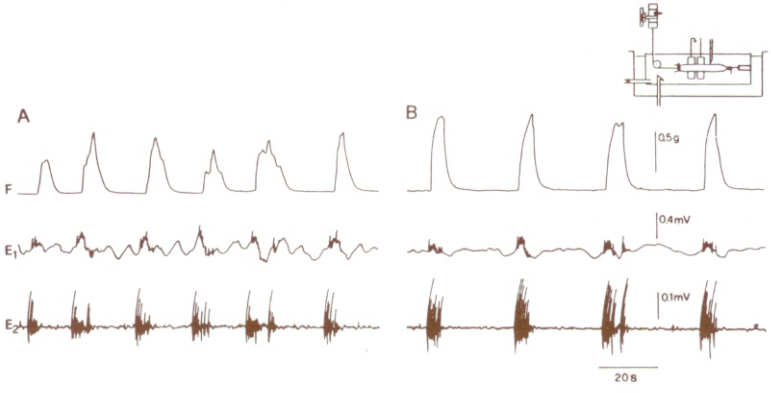

Fig. 4 – *Simultaneous recording of mechanical and electrical responses to 8×10^{-3} LPC administration.*

A: control period; B: after 10 min of drug exposure.

The tracings from top to bottom represent: isometric active force (F), electrical activity recorded with a time constant of 0.45 s (E_1) and of 0.04 s (E_2). In E_1 both slow and fast components are observed, while in E_2 only the fast component is detected. The scheme depicts the experimental arrangement.

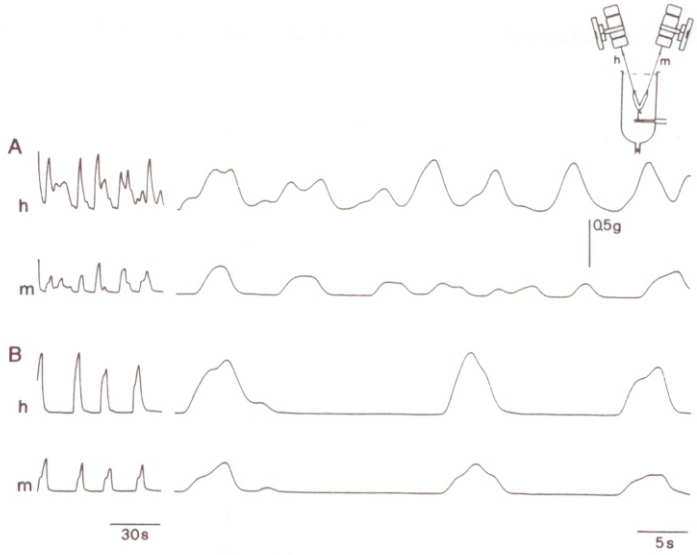

Fig. 5 – *Experiments on synchronization of spontaneous activity.*

In the scheme; experimental design used for studying synchronization of mechanical activity in isolated rat portal vein. Hepatic (h) and mesenteric (m) ends were anchored to a muscle holder and connected to a force transducer. The two recordings are mechanically independent.

A: asynchronized and irregular contraction in the two muscle portions were recorded in the control period.

B: synchronized and rhythmic activity was recorded at the hepatic and mesenteric ends 10 min after 8×10^{-3} M of LPC administration.

tion. The mesenteric end of this thinner preparation developed less tension than the hepatic counterpart.

Fig. 5 B illustrates the pattern of activity obtained 10 min after 8×10^{-3} M of LPC administration. The substance produced an increase of the amplitude and a decrease of the frequency of spontaneous contraction waves.

It has also been observed that the hepatic and the mesenteric sections of the portal vein seemed to contract in close synchrony. Furthermore, the contraction of the two ends of the muscle started simultaneously and the waves became monophasic.

4. *Portal vein response to changes in extracellular osmolarity.* — The portal vein response to changes in extracellular osmolarity was studied in 5 experiments. Fig. 6 shows the typical traces recorded after 14 mM sucrose and 8 mM LPC from the same preparation. Sucrose causes a decrease (45%-60%) of frequency of contraction waves. LPC provokes in the first 10 min a period of total inhibition of activity and spontaneous contractile waves start after 4-6 min with an increase of the wave force amplitude of 25-34%, as regards the control. Successively, in the second 10 min the frequency decreases of 30-35% and the amplitude values increases of 40-55%.

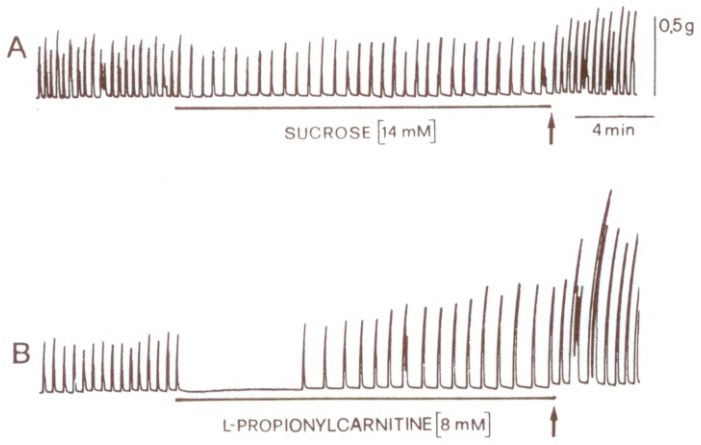

Fig. 6 – *Portal vein responses to changes in extracellular osmolarity.*

A: response to increased osmolarity of 14 mOsm produced by adding sucrose to the normal solution (292 mOsm).
B: response of the same preparation to 8×10^{-3} M (14 mOsm) of LPC.
The arrows indicate the return to normal solution.

DISCUSSION

Under our experimental conditions, the rat isolated portal vein shows in 80% of cases asynchronous spontaneous contractions.

Hermsmeyer (14) reports that in more than half of experiments on isolated mesenteric-hepatic portal vein contractions are complex and postulates the existence of more than one pacemaker site. Also our rhythmic compound contractions recorded in the control period could be due to such an interaction between several pacemakers; multiple pacemaker sites have also been reported in intestinal muscle (8).

The typical and substantial response of rat isolated portal vein to LPC was obtained at doses ranging from 2×10^{-3} M to 8×10^{-3} M. This drug produces, after an initial period of total inhibition, a coordinated and rhythmic activity with an increase of the amplitude of force waves and a parallel decrease of the contraction frequency. The coordinated rhythmic activity implies that excitation initiates in a cyclic manner in some part of the tissue and then spreads to an extensive number of muscle fibers, so that a synchronized activation results.

Funaki and Bohr (13) and Axelsson *et al.* (3) report that single muscle cells in the rat portal vein preparation can exhibit a gradual depolarization which apparently leads to firing a burst of action potentials. The areas where the cell shows this behavior might serve as pacemakers, provided that their activity is conducted to other parts of the muscle (17, 21).

Biamino and Kruckenberg (5) suggest that the contraction frequency would then be dependent on the number of active pacemaker areas and on the rate of burst formation from each of them. Amplitude and duration of mechanical response,

in turn, are determined by the degree of synchronization of the smooth muscle activity. Both initiation and propagation of the spontaneous activity are due to myogenic events (16).

At the light of these events, we considered the trials in which hepatic and mesenteric end can contract independently. In these experiments in presence of LPC the contraction of the two vein sections starts simultaneously, the waves become monophasic and their amplitude increases. These results may suggest that LPC synchronizes the spontaneous activity and that the amplitude increase of the contraction wave is the consequence of synchronization of contractile elements in vasal muscle, the mechanism of which is presently unknown. Moreover, LPC could interfere with the pacemaker activity or with the mechanism responsible for a myogenic conduction in the longitudinal smooth muscle. In fact, regular rhythmic activity of constant frequency similar to the electrical and mechanical signals recorded in presence of 8×10^{-3} M LPC could be driven by a single pace-maker in the whole preparation, as described by Biamino and Kruckenberg (5).

The issue regarding the effect of osmolarity changes on spontaneous activity of isolated portal vein is controversial. Johansson and Jonsson (15) and Arvill *et al.* (2) report that an increase in osmolarity of 50-100 mOsm, produced by sucrose, depresses both frequency and amplitude of spontaneous contractions.

McKinley *et al.* (22) report that osmolarity increment of 80 mOsm obtained by NaCl or sucrose, causes a sustained decrease in frequency of spontaneous contraction, but only a transient depression of amplitude that, after 5 min, begins to increase.

In our experiments, the only effect of a 14 mOsm increase produced by sucrose was a frequency decrease. The effects reported by McKinley were obtained when the increase in osmolarity was six times higher than the corresponding increase induced by the highest dose of LPC.

Therefore, we may conclude that, even taking into consideration the influence of osmolarity on the frequency, the joint effect in frequency synchronization and amplitude on the spontaneous contractile activity should be ascribed to LPC.

Furthermore, effects of LPC at concentrations comparable to those used in our experiments were observed by other authors, in isolated organs. Pasini *et al.* (23) report that, in isolated and perfused rabbit and rat hearts, LPC concentrations ranging from 10^{-5} to 10^{-2} M induce a dose-dependent negative inotropic effect accompanied (at concentrations from 10^{-3} M to 10^{-2} M) by an increase of diastolic pressure, coronary perfusion pressure and of rate of CPK release.

Aomine *et al.* (1) studied the effect of LPC on action potentials of canine Purkinje fibers «in vitro» under acid condition. These authors report that the concentrations from 10^{-5} M to 3×10^{-3} M had no significant effect on action potential amplitude, maximal stroke velocity of phase 0 and resting potential, while higher concentrations of LPC (10^{-2} M and 3×10^{-2} M) decreased some of these action potential parameters and such high concentration consistently prolonged the action potential duration.

SUMMARY

The effects of L-propionylcarnitine (LPC) on spontaneous mechanical and electrical activity of rat portal vein have been studied «in vitro» by means of an isometric technique. Mechanical activity in normal Krebs solution consisted, in the majority of cases, in phasic contractions with variable amplitude, duration and frequency, while electrical activity showed both slow and fast spike potential components. By adding LPC to the medium at doses ranging from 10^{-5} M to 10^{-3} M, no effect has been observed while at concentrations from 2×10^{-3} M to 8×10^{-3} M, after an initial period of total inhibition, a dose-dependent increase in amplitude associated with a parallel decrease in frequency of contraction waves have been observed. The pattern of electrical activity was characterized by a regular slow wave component with the same frequency of the mechanical waves, and by an increase in number, amplitude and frequency of spike potentials. Experiments on synchronization of contractile activity showed that, in presence of LPC, hepatic and mesenteric regions of the vessel contract in close synchronism. These results suggest that LPC synchronizes spontaneous activity of rat portal vein by means of a mechanism which is at the present unknown.

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REFERENCES

1. Aomine, M., Nobe, S. and Arita, M. Electrophysiologic effects of a short-chain acyl carnitine, L-propionylcarnitine, on isolated canine Purkinje fibers. *Pharmacol.*, **13**: 494-501, 1989.
2. Arvill, A., Johansson, B. and Jonsson, O. Effects of hyperosmolarity on the volume of vascular smooth muscle cells and the relation between cell volume and muscle activity. *Acta Physiol. Scand.*, **75**: 484-495, 1969.
3. Axelsson, J., Wahlstrom, B., Johansson, B. and Jonsson, O. Influence of the ionic environment on spontaneous electrical and mechanical activity of the rat portal vein. *Circul. Res.*, **21**: 609-618, 1967.
4. Barbieri, M., Carbonin, P. U., Cerbai, E., Gambassi, G., Lo Giudice P., Masini, I., Mugelli, A. and Pahor, M. Lack of correlation between the antiarrhythmic effect of L-propionylcarnitine on reoxigenation-induced arrhythmias and its electrophysiological properties. *Br. J. Pharmacol.*, **102**: 73-78, 1991.
5. Biamino, G. and Kruckenberg, P. Synchronization and conduction of excitation in the rat aorta. *Amer. J. Physiol.*, **217**: 376-382, 1969.
6. Bohemer, T. and Bremer, J. Propionylcarnitine physiological variations in vivo. *Biochim. Biophys. Acta*, **152**: 559-567, 1968.

7. Bremer, J. Carnitine metabolism and function. *Physiol. Rev.*, **63**: 1420-1480, 1983.
8. Christensen, J. and Hauser, R. L. Circumferential coupling of electric slow waves in circular muscle of cat colon. *Amer. J. Physiol.*, **221**: 1033-1037, 1971.
9. Di Lisa, F., Menabò, R. and Siliprandi, N. L-propionyl-carnitine protection of mitochondria in ischemic rat hearts. *Vasc. Cell. Biochem.*, **88**: 169-173, 1989.
10. Di Palema, J. R., Ritchie, D. M. and McMichael, R. F. Cardiovascular and arrhythmic effects of carnitine. *Arch. Int. Pharmacodyn. Ther.*, **217**: 246-250, 1975.
11. Ferrari, R., Ciampalini, G., Agnoletti, G., Carnioni, A., Ceconi, C. and Visioli, O. Effect of L-carnitine derivatives on heart mitochondrial damage induced by lipid peroxidation. *Pharmacol. Res. Comm.*, **20**: 125-132, 1988.
12. Folts, J. D., Shug, A. L., Kore, J. R. and Bittar, N. Protection of the ischemic dog myocardium with carnitine. *Amer. J. Cardiol.*, **41**: 1009-1014, 1978.
13. Funaki, S. and Bohr, D. F. Electrical and mechanical activity of isolated vascular smooth muscle of the rat. *Nature, Lond.*, **203**: 192-194, 1964.
14. Hermsmeyer, K. Multiple pacemaker sites in spontaneously active vascular muscle. *Circul. Res.*, **33**: 244-251, 1973.
15. Johansson, B. and Jonsson, O. Cell volume as a factor influencing electrical and mechanical activity of vascular smooth muscle. *Acta Physiol. Scand.*, **72**: 456-468, 1968.
16. Johansson, B. and Ljung, B. Spread of excitation in the smooth muscle of rat portal vein. *Acta Physiol. Scand.*, **70**: 312-322, 1967.
17. Johansson, B. and Ljung, B. Role of myogenic propagation in vascular smooth muscle. Response to vasomotor nerve stimulation. *Acta Physiol. Scand.*, **73**: 501-510, 1968.
18. Kotaka, K., Miyazaki, Y., Ogawa, K., Satake, T., Sugijama, S. and Ozawa, T. Protection by carnitine against free fatty acid-induced arrhythmia in canine heart. *J. Appl. Biochem.*, **3**: 292-300, 1981.
19. Liedtke, A. J., DeMaison, L. and Nellis, S. H. Effect of L-propionylcarnitine on mechanical recovery during reflow in intact hearts. *Amer. J. Physiol*, **255**: H169-H176, 1988.
20. Liedtke, A. J. and Nellis, S. H. Effect of carnitine in ischemic and fatty acid supplemented swine hearts. *J. Clin. Invest.*, **64**: 440-447, 1979.
21. Ljung, B. and Stage, L. Adrenergic excitatory influences on initiation and conduction of electrical activity in the rat portal vein. *Acta Physiol. Scand.*, **80**: 131-141, 1970.
22. McKinley, M. J., McKenzie, J. S. and Blair-West, J. R. Effects of maintained changes on rat portal vein spontaneous contractions. *Amer. J. Physiol.*, **226**: 718-723, 1974.
23. Pasini, E., Condorelli, E., Cargnoni, A., De Giuli, F., Ferrari, R. and Visioli, O. Effects of propionyl-L-carnitine on cardiac function of isolated rabbit and rat hearts. P. 16. In: *Focus on propionyl-L-carnitine*. Sigma Tau Ed., Roma, 1988.
24. Paulson, D. J., Traxler, J., Schmidt, M., Noonan, J. and Shug, A. L. Protection of the ischemic myocardium by L-propionylcarnitine: effect on the recovery of cardiac output after ischemia and reperfusion, carnitine transport, and fatty acid oxidation. *Cardiovasc. Res.*, **20**: 36-41, 1986.
25. Siliprandi, N., Sartorelli, L., Ciman, M. and Di Lisa, F. Carnitine: metabolism and clinical chemistry. *Clin. Chim. Acta*, **183**: 3-12, 1989.
26. Suzuki, Y., Kamilawa, T. and Yamazaki, N. Effects of L-carnitine on ventricular arrhythmias in dogs with acute myocardial ischemia and a supplement of excess free acids. *Jap. Heart J.*, **45**: 552-559, 1981.