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Short communication

Positive action of propionyl-L-carnitine on mechanical performance of papillary muscle from Syrian hamsters with hereditary dilated cardiomyopathy

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Abstract

Propionyl-L-carnitine has been shown to exert a beneficial effect on cardiac function in different experimental models of cardiomyopathy in the rat, most likely by improving cardiac metabolism and energy production. We have previously shown that, in a strain of hamsters with hereditary dilated cardiomyopathy (BIO TO.2), the mechanical activity of papillary muscle (length-tension, velocity of shortening, shortening, work and power relationship) is significantly depressed when compared to the same parameter in normal hamsters (BIO F1.B). The repeated oral treatment with propionyl-L-carnitine (60 mg/kg per os for 7 weeks) to BIO TO.2 hamsters had a significant positive inotropic effect, as indicated by an increase in developed tension up to the levels observed in papillary muscles from normal hamsters. This action is most likely associated with metabolic effects similar to those observed in rats.

Keywords: Propionyl-L-carnitine; Papillary muscle; Cardiomyopathy; (Hamster)

1. Introduction

The results so far available clearly show that propionyl-L-carnitine improves cardiac function in different experimental cardiomyopathies induced in the rat, while it seldom modifies the function of normal hearts. Propionyl-L-carnitine has been shown to prevent most of the haemodynamic alterations induced in myocardial performance by pressure overload with a direct effect on cardiac muscle contractility (Micheletti et al., 1994; Yang et al., 1992). Left ventricular function and energy turnover were severely depressed in chronically volume-overloaded rat hearts; propionyl-L-carnitine administration restored to almost normal levels mechanical performance and oxygen consumption rate (El Alaoui-Talibi and Moravec, 1993). Mechanical recovery during reflow was positively affected by propionyl-L-carnitine in pig heart with coronary ligature (Liedtke and Nellis, 1988). A number of cellular, metabolic or biochemical effects of propionyl-L-carnitine have been described that support the hypothesis of an effect of this compound on cardiac metabolism and energy production (Yang et al., 1992; Ferrari et al., 1991; El Alaoui-Talibi and Moravec, 1989; Lopashuck et al., 1983; Di Lisa et al., 1989; Siliprandi et al., 1990; Paulson et al., 1986). A strain of Syrian hamster (BIO TO.2) develops a dilated cardiomyopathy exhibiting hypodynamic performance, metabolic cardiac alterations (Lochner et al., 1970; Hoppel et al., 1982; Whitmer, 1986; Fedelesova and Dhalla, 1971) and reduced levels of cardiac carnitine (Hoppel et al., 1982). Cardiac damage in this strain of hamster, characterized by thin ventricular walls and dilated chambers, progresses

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with time. The purpose of the present investigation in the dilated cardiomyopathy hamster (BIO TO.2 strain, 8 months old) was to assess the capacity of repeated oral treatment with propionyl-L-carnitine to counteract the above reported changes in the mechanical properties of the isolated papillary muscle. Normal hamsters (BIO F1.B strain) of the same age were used as controls.

2. Material and methods

2.1. Animals and treatment

Cardiomyopathic male hamsters (BIO TO.2 strain) with dilated cardiomyopathy, and a group of normal hamsters of the BIO F1.B strain (8 months old) were used. All the animals were obtained from the BIO Research Institute, Cambridge, MA, USA. The hamsters were kept in our animal house, under controlled environmental conditions (12-h light-dark cycle, 22–24°C, 40–50% humidity); they had food (LP Altromin) and water ad libitum. Cardiomyopathic hamsters were divided into two groups (5 animals in each group). The first group received a dose of 60 mg/kg of propionyl-L-carnitine by gavage once a day for 7 weeks. The compound, synthesized by Sigma-Tau, Pomezia, Italy, was dissolved in distilled water and the pH was adjusted at 4–4.5 with NaOH. The volume given to animals was 2 ml/kg. The second group was treated with an equal volume of acidified distilled water. A group (5 animals) of BIO F1.B normal hamsters treated with acidified distilled water was utilized as a control.

2.2. Papillary muscle preparation

At the end of treatment, the animals were killed by ether overdose and their hearts were removed. After the heart was washed free of blood, the atria and their connecting vessels were removed. The left ventricle was opened and the papillary muscle was carefully removed by means of a dissecting microscope and was transferred to a bath containing modified Tyrode solution with the following composition (mmol/l): NaCl 136.9, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 11.1. The bath was continuously and vigorously bubbled with a gaseous mixture of 95% O_2 and 5% CO_2, pH 7.3. The bath temperature was maintained at 25°C.

2.3. Papillary muscle mechanical parameter recording

The experimental procedures used were similar to those described in detail in a previous paper concerning the mechanical properties of smooth muscle (Pescatori et al., 1979). The papillary muscles were stimulated at a frequency of 30/min with rectangular pulses, 3 ms in duration and 10% above threshold. The stimulus was delivered by means of two platinum electrodes arranged longitudinally on each side of the muscle. The transverse electrical current flowing along the whole length of the preparation was continuously monitored by means of an oscilloscope.

2.3.1. Length-tension determination

The lower non-tendinous end of the papillary muscle was firmly fixed to the bottom of the bath, its upper free end was tied to a Grass FT 03 tension transducer (compliance $20 \cdot \text{kg m}^{-1}$). The transducer could be raised or lowered by a micrometric screw, allowing a minimal length increase of 5 μm, thus permitting passive changes in muscle length. After a 1-h equilibration period, the muscle was carefully stretched until increased length resulted in a tension increase of 0.025 g. The length at this point, measured by a microscope, was defined as 'initial length' and referred to as L_o. The papillary muscle was continuously stimulated and its length was increased stepwise with increments of 0.02 L_o from its resting length to 1.14 L_o. At this point a significant stress-relaxation phenomenon occurred as we have previously observed in hamsters (Mancinelli et al., submitted). In all the experiments on length-tension determinations, it was thus convenient that the papillary length be increased up to 1.14 L_0. Passive and active isometric tension was recorded on a Grass polygraph.

2.3.2. Force-velocity determination

The force-velocity determination was carried out by the classic afterload isotonic technique. Muscle shortening was measured by a Basile 7006 linear displacement transducer (moment of inertia 35 g/cm^2, breakaway torque < 0.1 g/cm). Transducer lever arm (fulcrum–organ ring length: 10 cm, operating range: $\pm 15°$) was made of a thin wall of carbon fibre conical tubing. Lever arm loading was provided by a tungsten alloy cylinder counterweight moving along a scale providing a load variation of 0.01 g/step. Repetitive stimulations were delivered to the papillary muscle. The shortening was recorded at each shock; the load was increased stepwise by a constant amount corresponding to 0.05 g. The procedure went on until the displacement could not be reliably distinguished from the noise. The isotonic contractile response of the papillary muscle was analyzed with a Hewlett-Packard 5480 signal analyzer. For each load the average of 8 high-velocity sweeps (50 ms/cm^{-1}) was recorded and photographed with a Polaroid camera. The maximum rate in each contraction was obtained by extrapolating the steepest linear part of the isotonic shortening wave. The values for the mean velocity were obtained by dividing the

total change in length by the time elapsed from the beginning of shortening.

2.3.3. Work and power determination

Work and power of the papillary muscle were calculated from the data obtained for the force-velocity determination. Work was expressed as $P \times L$, where P is the load and L the extent of shortening. Power was expressed as $P \times V$, where V is the velocity of shortening.

2.4. Statistical methods and analysis

The resting and the developed tensions and their total were summarized and graphically represented in terms of mean values as observed at the different increasing lengths. In order to verify the differences among experimental groups, an analysis of covariance (ANCOVA) was performed on the resting, developed and total tension. The terms included in the model were: the relative length as a continuous one, the treatment and the within-muscle effect. The last term was included in the model in order to remove the variability of the response due to the different papillary muscles. The relative length was considered a covariate term, in order to verify the relation of the tension as a function of the different lengths. The mechanical parameters were represented graphically in terms of mean values as observed at the different increasing afterloads (the preload was 25 mg). As for the comparison between propionyl-L-carnitine and H_2O in BIO TO.2, the following analysis was performed on the velocity of shortening, the power and the work: for each muscle the maximum among the values observed at the different increasing afterloads was considered and the maximum observed value for the three above-mentioned variables was submitted to Student's t-test for two independent samples. All the statistical tests were two-sided and $P < 0.05$ was considered significant. We analyzed the maximum for each variable, and not the single values over the different increasing afterloads due to small sample size.

3. Results

3.1. Length-tension determinations

Length-tension relationships observed on papillary muscle obtained from the BIO F1.B normal group and from two dilated cardiomyopathic groups (BIO TO.2) treated with vehicle or propionyl-L-carnitine (5 samples/group) are reported in Fig. 1. With an increase of the initial length by 0.02 L_0 in each group, the resting tension rose in a non-linear manner with respect to the increasing length. Developed tension also increased with each increment of length. Both active tension and total tension were markedly depressed in papillary muscles of the vehicle-treated cardiomyopathic group as compared with those of normal hamster hearts ($P < 0.001$). Active and total tension values increased significantly ($P < 0.001$) in papillary muscles of hamsters treated with propionyl-L-carnitine. At final length, the values of developed tension (mean ± standard deviation) were 0.69 ± 0.12 g, 0.24 ± 0.080 g, 0.90 ± 0.16 g for normal, H_2O- and propionyl-L-carnitine-treated cardiomyopathic animals, respectively. The resting tension values were significantly ($P < 0.05$) higher (0.15 ±

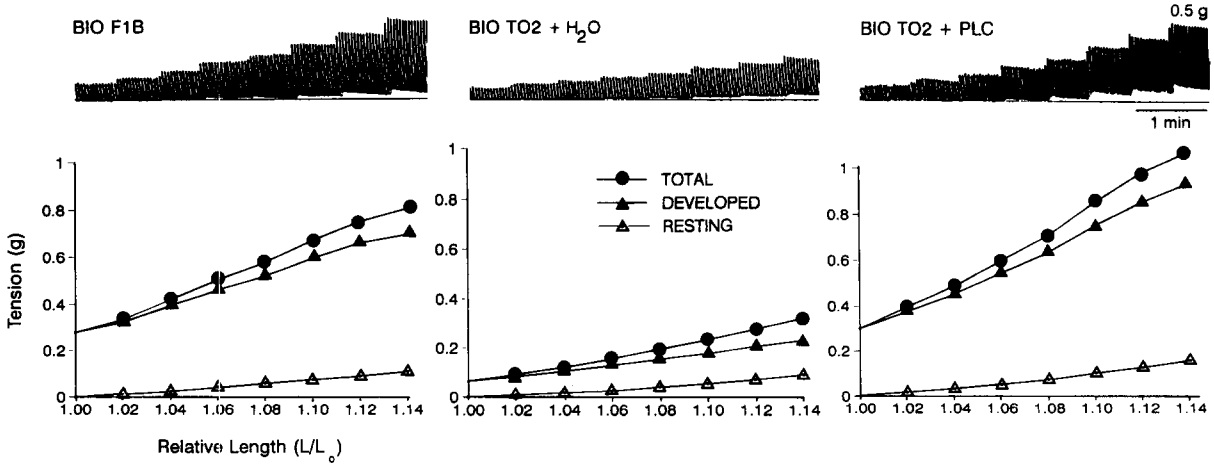

Fig. 1. Effect of increasing length on resting, developed and total tension of papillary muscle from normal (BIO F1.B) and H_2O- and propionyl-L-carnitine (PLC)-treated myopathic (BIO TO.2) Syrian hamsters (5 samples/group). The upper part of the figure shows the experimental traces for resting and developed tension with increasing length (0.02 L_0 steps) of the muscles. The lower part presents the isometric length-tension relationship.

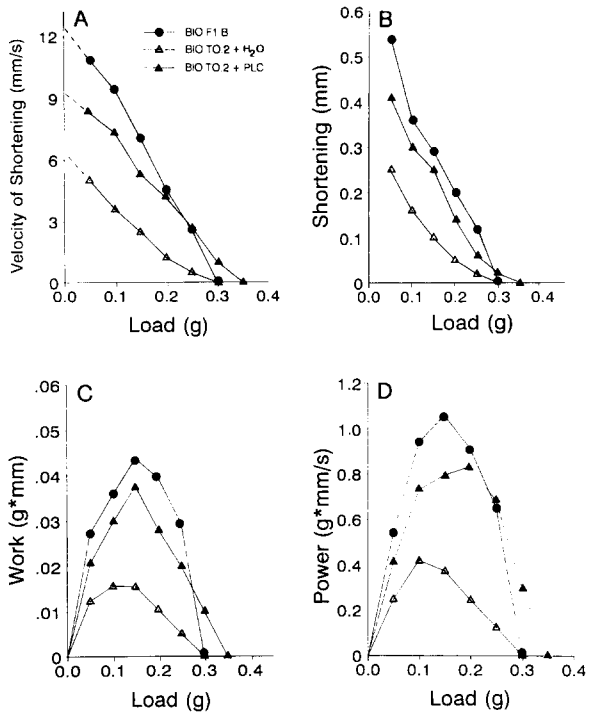

Fig. 2. Velocity of shortening (A), Shortening (B), Work (C) and Power (D) of normal and myopathic papillary muscles treated with H_2O and propionyl-L-carnitine (PLC) (3 samples/group). The curves were plotted as function of afterload (preload + increasing load). Initial muscle length with preload 0.025 g: 5 mm. Temperature 25°C, frequency: 30/min.

0.03 g) for the propionyl-L-carnitine-treated BIO TO.2 strain than for the H_2O-treated (0.09 ± 0.01 g) and normal hamsters (10 ± 0.01 g).

3.2. Velocity of shortening

A positive inotropic effect of propionyl-L-carnitine treatment was also observed on the velocity of shortening, shortening, work and power curves. Fig. 2A shows force-velocity curves plotted as a function of increasing afterload obtained from normal hamsters ($n = 3$) and two groups of dilated cardiomyopathic hamsters treated with H_2O or PLC (3 samples/group). At zero load, the velocity of shortening (V_{max}) in normal hamsters was 12 mm $\pm$ s^{-1}, while in papillary muscles of propionyl-L-carnitine- and water-treated cardiomyopathic hamsters, the velocity was 9.5 and 6.5 mm s^{-1}, respectively. Shortening of papillary muscles at various loads is shown in Fig. 2B. At the lowest afterload (0.05 g) the shortening of muscles from the normal BIO F1.B strain was 0.54 ± 0.14 mm; from the cardiomyopathic BIO TO.2 H_2O-treated and BIO TO.2 propionyl-L-carnitine-treated the shortening was 0.42 ± 0.11 mm and 0.35 ± 0.09 mm, respectively. The propionyl-L-carnitine-treated animals reached isometric condition at greater afterload (0.35 g) with respect to the animals treated with H_2O (0.30 g). The differences did not reach statistical significance, most likely due to the small size of samples.

3.3. Work and power

The work done by the papillary muscle at various loads is plotted in Fig. 2C. Maximum physical work was performed by normal and propionyl-L-carnitine-treated hamsters under a load which was about 1/2 of the greatest load which the muscle could lift, while in H_2O-treated animals the maximum work was obtained at 1/3 of the greatest load. In dilated cardiomyopathic animals the work amount differed significantly ($P < 0.001$) between the group treated with H_2O and the one treated with propionyl-L-carnitine. The maximum amount of work (0.038 ± 8.26 g · mm^{-1}) recorded in animals treated with propionyl-L-carnitine was 2.5 times greater than the one in animals treated with H_2O (0.015 ± 5.42 g · mm^{-1}). Concerning power (Fig. 2D), the maximum values (0.84 ± 16.3 g · mm · s^{-1}) were twice as high in the propionyl-L-carnitine-treated animals as in those treated with H_2O (0.42 ± 12.5 g · mm · s^{-1}).

4. Discussion

The present study was designed to evaluate the effect of repeated propionyl-L-carnitine oral treatment on the mechanical properties of isolated papillary muscle obtained from dilated hamster hearts. At approximately 90 days of age, the BIO TO.2 strain hamsters develop a dilated, i.e. congestive, form of cardiomyopathy characterized by thin ventricle walls and dilated chambers as compared to the normal animals. Recently, impairment of contractile behavior of papillary muscle, measured in terms of tension-length, velocity of shortening, work and power relationships has been observed in 8-month-old hamsters of the same strain (Mancinelli et al., submitted). The most significant finding of the present study was the significant positive inotropic effect of the administration of propionyl-L-carnitine on mechanical performance of papillary muscles from BIO TO.2 hamsters, as indicated by the increase in developed tension up to the levels observed in papillary muscles from normal hamster hearts (BIO F1.B). This finding clearly indicates that propionyl-L-carnitine administration restores the depressed cardiac functions in a genetic model of dilated cardiomyopathy in agreement with previous results obtained using pressure overload (Yang et al., 1992) and volume overload rat hearts (El Alaoui-Talibi and Moravec, 1993). The data reported do not provide a biochemical or metabolic explanation of the observed effect. It is known however that a deficit in oxidative phosphoryla-

tion occurs in the cardiac tissue of cardiomyopathic hamsters (Hoppel et al., 1982) along with other metabolic disturbances reminiscent of that observed in pressure or volume overload rat hearts. In the rat, these metabolic disturbances, as well as alterations of mechanical function, are corrected by propionyl-L-carnitine administration (Yang et al., 1992; Micheletti et al., 1994; El Alaoui-Talibi and Moravec, 1993). Furthermore, Whitmer (1986) showed that a major defect in dilated cardiomyopathy of hamsters may be due to a decrease in volume of sarcoplasmic reticulum calcium transport sites. Micheletti et al. (1994) suggested an action of propionyl-L-carnitine on sarcoplasmic reticulum function. A reduced level of myocardial carnitine (Hoppel et al., 1982) and an alteration of carnitine transport (York et al., 1983) have been described in cardiomyopathic hamsters. In rats with cardiac hypertrophy due to pressure overload, propionyl-L-carnitine prevents carnitine depletion and improves hemodynamic parameters (Yang et al., 1992). It seems reasonable to suggest that propionyl-L-carnitine administration to cardiomyopathic hamsters brings about metabolic effects similar to those observed in rats. Finally, the fact that the propionyl-L-carnitine treatment of dilated cardiomyophatic hamsters shifts the passive curve to the left as compared to that for the H_2O-treated group, could be ascribed to a decrease of connective tissue abnormalities that may contribute to the progressive loss of ventricular function in cardiomyophatic Syrian hamsters (Cohen-Gould et al., 1987).

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