THE CARDIAC ORNITHINE DECARBOXYLASE ACTIVITY AND POLYAMINE CONTENT IN CARDIOMYOPATHIC HAMSTERS

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ABSTRACT

The cardiomyopathic Syrian hamster is a well-known animal model for human cardiomyopathy. In this study, we measured the level of cardiac ornithine decarboxylase, polyamines (putrescine, spermidine and spermine) and nucleic acids in 80-day-old cardiomyopathic hamsters (Bio 14.6). The cardiac ornithine decarboxylase activity exhibited a circadian fluctuation, rising in the morning and falling in the evening, in both cardiomyopathic and control hamsters. The enzyme activity, putrescine and spermidine content, spermidine/spermine ratio and DNA content were higher in cardiomyopathic hamsters than in controls throughout the day. Subcutaneous injection of isoproterenol (5 mg/kg body weight) significantly increased the cardiac ornithine decarboxylase activity 4 h after the injection in both cardiomyopathic and control hamsters. The ornithine decarboxylase activity in cardiomyopathic hamsters was about two times higher than in controls. Treatment with an α -blocker (bunazosin, 10 mg/kg body weight) or a β -blocker (metoprolol, 10 mg/kg body weight) for 11 days decreased the cardiac ornithine decarboxylase level in cardiomyopathic hamsters, but not in control hamsters. The results suggest that the heart of the cardiomyopathic hamster has a high proliferation capacity, which may result from its hypersensitive sympathetic function.

The cardiomyopathic Syrian hamster is an animal model for myocardial hypertrophy and cardiac failure. Progressive lesions have been observed in myocardial cells of this animal. By 1 to 2 months after birth, focal necrosis in the myocardium is observed. By 3 to 5 months, necrotic areas undergo fibrosis and the residual myocardial cells become hypertrophic. It is during this period that myocardial cells exhibit the most intensive hypertrophy. After 6 months, the remaining myocardial cells become atrophic and fibrosis becomes prominent (2, 30).

Many studies have been attempted to clarify the genesis of the cardiomyopathy. An important pathogenetic role has been attributed to myocyte calcium overload. An abnormal cardiac response to sympathetic stimulation was also observed in these hamsters (1, 29).

The polyamine content is known to increase in rapidly proliferating cells and tissues (22, 31). Fig. 1

shows the pathway of polyamine synthesis in mammalian cells. Ornithine decarboxylase (ODC), the key enzyme in polyamine synthesis, turns over rapidly with a half-life ranging from 10 to 60 min (22, 31). In response to various growth stimuli, the activity of this enzyme is induced rapidly prior to DNA synthesis, making it a useful marker for cell growth. Furthermore, polyamines may affect several membrane functions and exert significant effects on various physiological parameters of the heart. We therefore examined the cardiac polyamine content and ODC activity in 80-day-old cardiomyopathic hamsters and compared them with age-matched control hamsters.

MATERIALS AND METHODS

Animals

Cardiomyopathic Syrian hamsters (Bio 14.6) and

Pathway of polyamine biosynthesis and interconversion

Fig. 1 The pathway of polyamine synthesis in mammalian cells

control hamsters (F1B) were obtained from Bio Breeders (Watertown, MA). We used male hamsters 71-90 days old at the time of sacrifice. Animals were fed a commercial laboratory chow *ad libitum* for 2 weeks in a temperature-controlled environment ($25 \pm 1^\circ\text{C}$) with a 12-h light and 12-h dark cycle (lighted from 07 00 to 19 00). Then hamsters were divided into 4 groups.

Group I: Hearts were removed at four different time points (04 00, 10 00, 16 00 and 22 00) to measure the ODC activity and the content of polyamines and nucleic acids (RNA and DNA). Five cardiomyopathic and five control hamsters were used for respective time points.

Group II: Animals were subcutaneously injected with isoproterenol (5 mg/kg) at 15 00 to examine the influence of β -adrenoceptor stimulation. Cardiac ODC activity was measured 0.5, 2, 4 and 8 h after the treatment. Five cardiopathic and control hamsters each were used for respective time points.

In group III, animals were treated with a daily subcutaneous injection of an α -adrenoceptor blocker, bunazosin (10 mg/kg), and in group IV, with a β -adrenoceptor blocker, metoprolol (10 mg/kg). On the 11th day of the α - or β -blocker treatment, hamsters were sacrificed at 18 00. Five hamsters were used in each group.

Determination of the Cardiac Polyamine Contents

The heart was weighed and homogenized in 0.25 M sucrose containing 1 mM dithiothreitol (DTT) using a polytron. The homogenate was then used to measure the content of polyamines and nucleic acids, and the ODC activity.

The homogenate was treated with 2% perchloric acid, and after centrifugation at 8,000 g for 30 min, the supernatant was analyzed by high performance liquid chromatography (HPLC) (Shimadzu model LC-3A) using an LC column (ISC-05/so 504). The chromatography was performed at 70°C at a flow rate of 0.7 ml/min, using 0.16 M sodium citrate and 1.6 M NaCl. The eluate was mixed with 6 mM *o*-phthalaldehyde dissolved in a solution containing 0.38 M Na_2CO_3 , 0.108 M K_2SO_4 , 0.22 M H_3BO_3 , 0.1% Brij 35, and 2.9 mM 2-mercaptoethanol, and passed through the flow cell of a fluorometer at 0.5 ml/min (26).

The content of putrescine was expressed in nmol per mg of DNA, and that of spermidine and spermine as μmol per mg DNA.

Determination of Cardiac ODC Activity

The supernatant (65 μl) was added to a reaction

Table 1 *The Body and Heart Weight and the Heart/Body Weight Ratio in Control and Cardiomyopathic Hamsters*

Group	Body weight (g)	Heart weight (mg)	Heart weight (mg)/Body weight (g)
Control (F1B) n=19	109.3 $\pm$ 7.3	411.8 $\pm$ 33.3	3.8 $\pm$ 0.3
Cardiomyopathy (Bio 14.6) n=20	77.4 $\pm$ 6.5*	359.7 $\pm$ 25.6*	4.7 $\pm$ 0.4*

Values are means $\pm$ SD. *Significantly different from control ($P < 0.001$)

mixture consisted of 0.125 μ Ci of DL-[1- 14 C]ornithine, 0.4 mM L-ornithine, 20 μ M pyridoxal phosphate, 5 mM DTT, and 40 mM Tris/HCl, pH 7.4. The final reaction volume was 115 μ l and incubation was carried out at 37°C for 1 h. The ODC activity was determined by measuring the decarboxylation of L-[1- 14 C]ornithine (19). One unit of enzyme activity was defined as the amount that catalyzes the release of 1 nmol of CO₂ per hour.

Determination of the Cardiac Nucleic Acid and Protein Content

Cardiac RNA and DNA were extracted by Schneider's method (24). The homogenate was mixed with cold 10% trichloroacetic acid (TCA), and centrifuged at 3,000 rpm for 10 min. This was repeated twice. The residue was then resuspended in 1 ml of 5% TCA and centrifuged. The residue was suspended in 1 ml of 5% TCA, heated at 90°C for 15 min, cooled, and centrifuged. RNA concentration was determined spectrophotometrically by the orcinol reaction using yeast RNA as a standard. DNA concentration was determined by the diphenylamine reaction using calf DNA as a standard. The content was expressed in mg per g of heart tissue.

The protein content was determined by the biuret method (16), using bovine serum albumin as a standard.

Statistics

Statistical analysis of the circadian changes was made by the two way analysis of variance (ANOVA). To assess the statistical significance of the effect of adrenoceptor agonist or antagonist, we used the Student's *t*-test. Results are expressed as the mean $\pm$ SD.

Chemicals

DL-Isoproterenol, phenylephrine, orcinol, diphenyl-

amine, yeast RNA, calf DNA and bovine serum albumin were obtained from Sigma Chemical. Bunazosin hydrochloride was obtained from Eisai Chemical, and metoprolol tartrate was from Fujisawa Astra Chemical.

RESULTS

Body Weight and Heart Weight

Table 1 shows the body weight, heart weight, and heart/body weight ratio in cardiomyopathic and control hamsters. The body weight of the cardiomyopathic hamster was significantly less than that of the control hamster. The heart weight was also significantly lower in the cardiomyopathic than in the control hamster. However, the heart/body weight ratio was significantly higher in the cardiomyopathic than in the control hamster.

Circadian Fluctuation in the Cardiac ODC Activity

The cardiac ODC activity was highest at 10 00 and lowest at 22 00 in the cardiomyopathic hamster, and highest at 10 00 and lowest at 04 00 in the control hamster (Fig. 2). The ODC activity was higher in the cardiomyopathic hamster than in the control hamster ($P < 0.01$).

Absence of Circadian Changes in the Cardiac Polyamine Content

Cardiac polyamines did not exhibit any circadian changes in their content (Fig. 3). The putrescine content was higher throughout the day in the cardiomyopathic than in the control hamster ($P < 0.01$). The spermidine content was always higher in the cardiomyopathic hamster ($P < 0.01$). No difference was observed in the spermine content between cardiomyopathic and control hamsters. The spermidine/spermine ratio was always higher in the cardiomyopathic hamster ($P < 0.01$).

Fig. 2 Circadian change in the cardiac ODC activity in control (F1B) and cardiomyopathic (Bio 14.6) hamsters. □, light; ▨, dark

The Content of Cardiac Nucleic Acids

The cardiac DNA and RNA content did not exhibit any significant changes throughout the day (Fig. 4). The cardiac DNA content was significantly higher in the cardiomyopathic hamster than in the control hamster ($P < 0.01$). The RNA content and the RNA/DNA ratio did not significantly differ between the two hamster groups.

Effect of an Adrenoceptor Agonist

Fig. 5 shows changes in the cardiac ODC activity after the injection of isoproterenol (5 mg/kg body weight) at 15 00. Four hours after the injection, the cardiac ODC activity in the cardiomyopathic hamster was approximately two times higher than in the control ($P < 0.001$). However, 8 h after the injection, the cardiac ODC activity returned to the pretreatment level in both groups.

Effects of Adrenoceptor Antagonists

The cardiac ODC activity was determined after the administration for 11 consecutive days of an α -adrenoceptor antagonist (bunazosin; 10 mg/kg body weight) or of a β -adrenoceptor antagonist (metoprolol; 10 mg/kg body weight) (Fig. 6). The cardiac ODC activity in the cardiomyopathic ham-

Fig. 3 The level of various cardiac polyamines throughout the day in control (F1B) and cardiomyopathic (Bio 14.6) hamsters; (A) putrescine, (B) spermidine, (C) spermine and (D) spermidine/spermine ratio. □, light; ▨, dark

ster treated with bunazosin or metoprolol was decreased compared with that in the cardiomyopathic hamster that received saline injection. No significant activity changes were observed in the control hamster.

DISCUSSION

Many enzymes and metabolites in animals are

Fig. 4 The cardiac nucleic acid content throughout the day in control (F1B) and cardiomyopathic (Bio 14.6) hamsters; (A) DNA, (B) RNA, (C) RNA/DNA ratio. □, light; ▨, dark

known to display a circadian rhythm. We measured the cardiac ODC activity and the polyamine content in cardiomyopathic and control hamsters to determine whether they exhibit circadian changes. The cardiac ODC activity was shown to display a significant circadian fluctuation in both cardiomyopathic and control hamsters with a peak value at 10 00. The ODC activity in the liver, kidney, and small intestine of the rat has been demonstrated previously to exhibit circadian changes which peak at night (9, 11, 20-22). These were related to food intake at night rather than to the light-dark cycle of the environment. On the other hand, the cardiac ODC activity showed circadian changes

which peaked during the day, in confirmation of the previous reports by Waldrop *et al.* (32) and ourselves (27). The cardiac ODC activity is known to be affected by exercise, sympathetic stimulation, insulin, thyroid hormone, growth hormone and other cardiostrophic hormonal agents (8, 22, 31).

Many workers have reported unusual sympathetic responses in cardiomyopathic Syrian hamsters (1, 12-14, 28-30), including an increase in the cardiac sympathetic tone and an enhancement of cardiac sensitivity to adrenergic stimulation. In the present study, the cardiac ODC activity in the cardiomyopathic hamster was found to be much higher than in control hamsters throughout the day. It is known that catecholamines increase the cardiac ODC activity (3, 4, 6, 7, 10, 17, 18, 33). The higher activity of cardiac ODC in cardiomyopathic hamsters may reflect the high sympathetic tone in the heart and/or a hypersensitivity to cardiac sympathetic stimulation.

The administration of isoproterenol enhanced the cardiac ODC activity in cardiomyopathic hamsters to a greater extent than in control hamsters, suggesting that the myocardium of Bio 14.6 hamsters is hypersensitive to isoproterenol stimulation. The resting ODC activity in cardiomyopathic hamsters was depressed by both an α - and β -adrenergic blocker, given for 11 consecutive days.

It has been reported that the number of α - and β -receptors is increased in cardiomyopathic hamsters, at the age employed in our experiments (12, 13). Another report has suggested that the turnover of norepinephrine is increased in cardiomyopathic hamsters at this age (28). Our results are in line with these observations. Recently, an unusual coupling in the receptor GTP-binding protein-adenylate cyclase system has been reported in cardiomyopathic hamsters after both α - and β -adrenergic stimulation (14, 25).

The cardiac putrescine and spermidine content, and the cardiac DNA content are higher in cardiomyopathic hamsters than in control hamsters. These findings are consistent with the increased cardiac ODC activity, and suggest increased cardiac cell proliferation in cardiomyopathic hamsters.

The spermidine/spermine ratio usually rises when the extent of cell proliferation is increased (23). We previously reported that the cardiac spermidine/spermine ratio was very high in the heart of the spontaneous hypertensive rat (SHR) compared with the ordinary rats (27). The higher ratio of spermidine/spermine in cardiomyopathic hamsters is also in line with an increase in cardiac cell growth

Fig. 5 Effect of isoproterenol on the cardiac ODC activity in control (F1B) and cardiomyopathic (Bio 14.6) hamsters. Isoproterenol (5 mg/kg) was subcutaneously injected at 15 00. Values are mean $\pm$ SD, with the number of hearts in parentheses. ††† Significantly different from the pretreatment value ($P < 0.001$). *** Significantly different from controls ($P < 0.001$)

Fig. 6 Effect of bunazosin or metoprolol on the cardiac ODC activity. Bunazosin (10 mg/kg) or metoprolol (10 mg/kg) was subcutaneously given once a day for 11 consecutive days. Values are mean $\pm$ SD, with the number of hearts in parentheses. ††, ††† Significantly different from the saline-treated ($P < 0.01$ and $P < 0.001$, respectively). *, *** Significantly different from controls ($P < 0.05$ and $P < 0.001$, respectively)

in these hamsters.

In the cardiomyopathic Syrian hamster, myocyte calcium uptake and intracellular calcium concentration have been reported to be elevated (1, 29). Koenig *et al.* have reported that newly synthesized polyamines affect Ca^{2+} fluxes and membrane transport in myocytes (5, 15). The increase in cardiac polyamines biosynthesis in cardiomyopathic hamsters is possible to stimulate calcium transport and increase the concentration of myocyte calcium, leading to myocardial damage due to Ca^{2+} overload.

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