Cardiac Adaptation and Its Limitation in an Experimental Model of Congestive Heart Failure

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To elucidate mechanisms of adaptation and maladaptation in heart failure, abnormalities of left ventricular function and their relationships to myocardial contractile protein were studied in the Syrian hamster Bio 14.6.

Left ventricular and heart weights were both increased in 20-week-old cardiomyopathic hamsters, indicating cardiac hypertrophy as a compensatory mechanism to the disease process of cardiomyopathy. However further increase in the left ventricular weight was not observed in older (40-week-old) cardiomyopathic hamsters. On the other hand left ventricular volume and volume/mass ratio were increased progressively. Correspondingly, V_3 type myosin was increased and myosin sliding velocity was decreased. Left ventricular function of cardiomyopathic hamsters evaluated using an isovolumically beating perfused heart preparation was depressed, and this functional impairment was also progressive. Chronic administration of metoprolol, a β -blocking agent, induced further increase in left ventricular volume and mass without changing left ventricular function and myosin isozyme pattern.

Thus in cardiomyopathic hamsters, left ventricular function progressively deteriorates in spite of a variety of adaptive mechanisms, and remodeling occurs. β -blocking agents may modify this process. (*Jpn Circ J* 1992; **56**: 475–481)

CARDIOMYOPATHIC hamsters (CM) are known to develop various pathological changes, including myocardial necrosis, subsequent fibrosis, and hypertrophy!^{1–3} Since typical congestive heart failure is developed in the late stage,⁴ this model is very suitable to study mechanisms of cardiac adaptation and maladaptation in congestive heart failure. However studies on functional

abnormalities of the whole heart of CM have been limited because of the small size of these animals. Recently we developed an isovolumically beating perfused heart preparation in CM.⁵ Using this model we sought to elucidate the process of adaptation and maladaptation of ventricular function in cardiomyopathy leading to congestive heart failure. We also studied the altered characteristics of myosin and their relation to the ventricular function in CM.

Key words:

Cardiomyopathy
Congestive heart failure
Myosin isozyme
Ventricular function
Metoprolol

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Densitometric scans of pyrophosphate gels of cardiac myosin from control and cardiomyopathic Syrian hamsters (Bio 14. 6)

Fig.1. Representative myosin isozyme distribution pattern in a control heart and a myopathic heart.

MATERIALS AND METHODS

Materials: Twenty-week and 40-week-old male CM Syrian hamsters (Bio 14.6, Bio Research, Cambridge, MA) and 20-week-old male control hamsters (Flb) were used.

Drug administration protocols: To study the effects of β -adrenergic antagonism, metoprolol 0.5 mg/kg/day was given orally for 30 weeks starting from 10 weeks after birth.

Isovolumically beating perfused heart preparation: Hamsters were sacrificed and their hearts were excised rapidly. The aorta was then cannulated with a plastic tube and retrograde perfusion was initiated with oxygenated Krebs-Henseleit buffer containing (mM) NaCl 118, NaHCO_3 25, KCl 4.7, KH_2PO_4 1.2, MgSO_4 1.2, CaCl_2 2.0, Na_2EDTA 0.4, D-glucose 5.5, and lactate 1.0 at 37 °C. The perfusion rate of the buffer was adjusted using a roller pump (Masterflex, Cole-Parmer, Inc.) to maintain coronary perfusion pressure at 90 mmHg. A latex balloon was inserted into the left ventricle from the left atrial appendage, and left ventricular pres-

sure was monitored with a Statham P23 ID transducer via a plastic tube connected to the balloon. The left ventricular pressure was recorded on both recording paper and magnetic tapes using a thermal recorder (Fukuda-denshi) and a data recorder (Sony Magnescale Inc.).

Assessment of left ventricular systolic and diastolic function: From digitized left ventricular pressure data (12 bit A-D converter), left ventricular developed pressure (left ventricular peak systolic pressure – left ventricular end-diastolic pressure), peak positive dP/dt ($+dP/dt$), peak negative dP/dt ($-dP/dt$), and the time constant (T) of left ventricular isovolumic relaxation were calculated with the use of a personal computer (Packet IIe, Anritsu Co.). The time constant (T) was calculated by a monoexponential method⁶. Left ventricular systolic and diastolic pressure-volume relations were assessed by plotting pressure-volume data obtained during stepwise changes of the left ventricular balloon volume.

At the end of each perfusion experiment whole heart weight and left ventricular weight were measured and normalized by body weight.

Myosin isozyme pattern: Myocardial myosin was extracted as described by Hoh et al⁷ and separated as describe by Martin et al⁸. Myosin isozyme pattern of the gel was analyzed by densitometry. Relative amounts of the α and β heavy chains were calculated according to an assumption:

$$\alpha (\%) = \frac{(V_1 + 0.5 \times V_2)}{(V_1 + V_2 + V_3)} \times 100,$$

$$\beta (\%) = \frac{(V_3 + 0.5 \times V_2)}{(V_1 + V_2 + V_3)} \times 100$$

Myosin sliding velocity: To measure myosin sliding velocity an *in vitro* motility assay⁹ was used. Briefly, latex beads (2 μm in diameter) were coated with extracted purified myosin. In the presence of Mg-ATP, the latex beads were induced to an internodal cell model of an algae, *Nitellopsis obtusa*, which has actin cables. Movements of the myosin beads were recorded on a video tape and analyzed for the sliding velocity ($\mu\text{m/s}$). An averaged sliding velocity was obtained from repeated runs of the myosin coated beads.

Statistical analysis: Indices of left ven-

Fig.2. Relationships between myosin sliding velocity and relative amount of α -heavy chain (% α -MHC). Note decreases in the myosin sliding velocity and myosin α -heavy chain in the cardiomyopathic hamster.

TABLE I INDICES OF LEFT VENTRICULAR FUNCTION

	<i>F1b 20w</i>	<i>Bio 20w</i>	<i>Bio 40w</i>
DP (mmHg)	131.3 $\pm$ 13.2	82.0 $\pm$ 15.2**	72.9 $\pm$ 9.0**
+dP/dt (mmHg/sec)	2908 $\pm$ 291	1864 $\pm$ 321**	1806 $\pm$ 186**
-dP/dt (mmHg/sec)	2014 $\pm$ 262	1268 $\pm$ 324**	1155 $\pm$ 130**
T (msec)	27.8 $\pm$ 3.1	33.7 $\pm$ 3.8*	35.6 $\pm$ 3.6**

Data were obtained at the same level of left ventricular end-diastolic pressure (10 mmHg).

F1b 20w=20-week-old control F1 hamsters

Bio 20w=20-week-old cardiomyopathic hamsters

Bio 40w=40-week-old cardiomyopathic hamsters

DP=developed pressure

T=time constant of left ventricular pressure decline calculated by a monoexponential method.

* $p < 0.05$, ** $p < 0.01$ vs *F1b 20w*.

tricular function, heart weight, and relative amount of myosin isozyme among the different groups were first compared using an analysis of variance, and then individual data were compared using a modified t-test. Changes of each parameter in the same group of animals were also analyzed first with an analysis of variance and then by a paired t-test. Data are shown as the mean $\pm$ SD, and p values less than 0.05 were regarded as significant.

RESULTS

Heart weights of control and cardiomyopathic hamsters: In CM, both heart

weight and left ventricular weight were higher than those in control hamsters at 20 weeks of age (heart weight; 3.09 ± 0.15 g/kg body weight in control hamsters and 4.27 ± 0.47 g/kg body weight in CM, left ventricular weight; 2.30 ± 0.10 g/kg body weight in control hamsters and 3.19 ± 0.36 g/kg body weight in CM hamsters, both $p < .01$). However further increases in heart weight and left ventricular weight did not occur in older animals (40-week-old) CM (heart weight= $4.36 \pm .24$ g/kg body weight, left ventricular weight= 3.29 ± 0.23 g/kg body weight, both ns vs 20-week-old CM). Chronic administration of metoprolol increased left ventricular weight (heart

Fig.3. Left ventricular systolic and diastolic pressure-volume relations in control hamsters and cardiomyopathic hamsters. Each left ventricular pressure-volume datum was obtained at each level of the left ventricular end-diastolic pressure, (0, 10, 20, 30 and 40 mmHg) and averaged. Upper panel: Left ventricular systolic pressure (LVSP)-volume (LVV) relationships. Lower panel: Left ventricular end-diastolic pressure (LVEDP)-volume (LVV) relations in control and cardiomyopathic hamsters. Flb 20w (open circles)=20-week-old control hamsters Bio 20w (closed circles)=20-week-old cardiomyopathic hamsters Bio 40w (closed squares)=40-week-old cardiomyopathic hamsters Bio 40w +M (open squares)=40-week-old cardiomyopathic hamsters with metoprolol treatment ** $p < 0.01$ vs Bio 20w

weight = $4.59 \pm .06$ g/kg body weight, left ventricular weight = 3.82 ± 0.16 g/kg body weight, both $p < 0.01$ vs 40-week-old CM without medication)

Myocardial myosin isozyme: Fig. 1 shows typical patterns of myosin isozyme

distribution of left ventricular myocardium from a control hamster and a CM hamster. In 20-week-old CM, V_3 myosin isozyme was increased compared to control hamsters ($14.9 \pm 4.5\%$ in CM vs $7.0 \pm 1.4\%$ in control hamsters, $p < 0.01$). Further increases in V_3 myosin isozyme ($25.4 \pm 7.9\%$, $p < 0.01$ vs 20-week-old CM) was observed in 40-week-old CM. Long-term administration of metoprolol did not alter the distribution of myosin isozyme ($V_3 = 21.3 \pm 0.9\%$, ns vs 40-week-old CM without medication).

Myosin sliding velocity: Relationships between myosin sliding velocity and relative amount of α -myosin heavy chain in control hamsters and CM were shown in Fig. 2. In CM, lower sliding velocity accompanied less amount of α -heavy chain. Data obtained from control and hyperthyroid rabbits are also shown in the same figure. In hyperthyroid rabbits whose myosin heavy chain was α -dominant, myosin sliding velocity was increased.

Left ventricular function (Table I): In 20-week-old CM, left ventricular developed pressure, $+dP/dt$, and $-dP/dt$ were lower and the time constant of left ventricular relaxation was longer than in control hamsters, indicating impairment of ventricular relaxation as well as systolic function in CM. These changes in indices of left ventricular performance became more prominent in older (40-week-old) CM. Metoprolol did not affect these indices of left ventricular function. Left ventricular systolic pressure-volume relationships in control and CM are shown in the upper panel of Fig. 3. The relationship shifted downward and to the right in CM. The lower panel of Fig. 4 shows diastolic pressure-volume relations. Diastolic pressure-volume relation of 20-week-old CM was almost identical to that of control hamsters. However, it shifted to the right in 40-week-old CM. Metoprolol seemed to shift the systolic and diastolic pressure-volume relationship slightly to the right.

Left ventricular volume/mass ratio at the same level of left ventricular end-diastolic pressure (0 mmHg) was also compared. In 20-week-old CM, left ventricular volume/mass ratio was lower than that of control hamsters (0.24 ± 0.04 ml/g in CM hamsters vs. 0.32 ± 0.03 ml/g in control

hamsters, $p < 0.01$). However in 40-week-old CM, left ventricular volume/mass ratio was increased (0.42 ± 0.10 ml/g, $p < 0.01$ vs 20-week-old CM hamsters). In the metoprolol treated group, left ventricular volume/mass ratio (0.34 ± 0.08 ml/g) was lower than that in non-treated hamsters of the same age ($p < 0.05$).

DISCUSSION

In the present study the process of left ventricular remodeling in cardiomyopathic hamsters was demonstrated. Our data on left ventricular volume and weight indicated that left ventricular hypertrophy was at first concentric in 20-week-old cardiomyopathic hamsters. However, in 40-week-old hamsters, left ventricular dilatation became overt and data on volume/mass ratio indicated that wall thickness was probably decreased. Thus cardiac remodeling had occurred. Correspondingly, left ventricular function progressively deteriorated. Depressed myocardial function in cardiomyopathic hamsters has been demonstrated in the papillary muscle¹⁰ and isolated working heart.¹¹ However, detailed data on the left ventricular systolic and diastolic function of cardiomyopathic hamsters have not been reported, probably due to technical difficulties in evaluating left ventricular function in these small animals. In the present study, we demonstrated that left ventricular relaxation as well as systolic function was impaired in the cardiomyopathic hamsters. Regarding systolic ventricular function, both time related index ($+dP/dt$) and indices of the extent of contraction (developed pressure at the same level of preload, and end-systolic pressure-volume ratio) were depressed. The decrease in $+dP/dt$ may correspond to decreased myosin ATPase activity due to altered composition of the myosin as discussed later. The decrease in left ventricular developed pressure and the shift in systolic pressure-volume relationship to the right and downward may be due to loss of normal myocardium or impaired energy utilization.¹¹⁻¹³ Decreased rate of relaxation may be due to impaired Ca^{2+} handling by the sarcoplasmic reticulum.¹⁴ The shift of diastolic left ventricular pressure-volume relation to the right may be reflective of the

left ventricular remodeling. A similar change was observed in patients with cardiomyopathy.¹⁵ However, in human dilated cardiomyopathy, left ventricular chamber stiffness was increased in spite of the dilated left ventricle.¹⁶ In the present study we avoid quantitative analysis of the left ventricular diastolic stiffness because calculation of indices including modulus of left ventricular stiffness from only several data points may be unreliable.

It is well known that ventricular myocardial myosin isozyme pattern and myosin ATPase activity are altered in experimental hypertrophy in small animals.¹⁷⁻²¹ Generally in hypertrophic hearts due to mechanical overload, V_3 isomyosin and β type myosin heavy chain are increased and myosin ATPase activity is decreased. Similar changes in myosin isozyme distribution and ATPase activity have also been reported in cardiomyopathic hamster hearts.^{22,23}

The results of myosin isozyme assay in our study are consistent with recent reports. The increase in V_3 myosin or β -form heavy chain became more prominent in older hamsters whose mechanical properties are markedly depressed. Decreased myosin sliding velocity in cardiomyopathic hamsters is reflective of decreased ATPase activity in V_3 dominant myosin in these animals, and may explain the decrease in time related index of left ventricular systolic performance ($+dP/dt$). Thus evaluation of myosin sliding velocity may be an important tool to investigate the relationships between characteristics of myocardial contractile protein and cardiac mechanical properties. The alteration of myosin isozyme from V_1 to V_3 and depressed myosin ATPase activity, however, may be beneficial to the failing heart. Cooper et al²⁴ demonstrated that in hypertrophic right ventricular muscle, O_2 consumption at the same level of generated force was lower than that in normal myocardium. More recently, Goto et al²⁵ demonstrated that efficiency of left ventricular mechanical work is better in the hypothyroid rabbit whose myosin isozyme pattern was V_3 dominant. These findings may suggest V_3 isomyosin is more efficient than V_1 in heart failure where oxygen delivery and energy utilization may be impaired. In addition, we reported that the left ventricle was tolerant to hypoxic in-

sult in terms of mechanical performance and energy metabolism in the cardiomyopathic hamsters.²⁶

Since we already reported that β -adrenergic receptors were upregulated with long term administration of metoprolol²⁷ we studied the effects of long-term metoprolol on ventricular function, myocardial hypertrophy and myosin isozyme pattern. Metoprolol (0.5 mg/kg/day) seems to increase left ventricular mass without increasing volume-mass ratio. This may indicate that metoprolol prevent left ventricular remodeling. However, myosin isozyme pattern was unchanged and left ventricular performance was also unchanged. Therefore the progression of left ventricular hypertrophy by metoprolol did not seem to accompany further depression of ventricular performance. Recently it has been reported that β -blocking agents, including metoprolol, improve clinical symptoms and left ventricular function in patients with dilated cardiomyopathy.^{28,29} In the present study, however, we could not obtain any evidence of improvement of left ventricular function by metoprolol. Further studies with different doses and periods of administration of β -blocking agents may be necessary to conclude that chronic β -blocker therapy improves left ventricular function in cardiomyopathic hamsters.

In summary, in spite of a variety of adaptive mechanisms including myocardial hypertrophy, changes in the pattern of myosin isoforms and increased tolerance to hypoxia, left ventricular function was progressively deteriorated and maladaptation became overt at the end stage of cardiomyopathy in the Syrian hamster Bio14.6. Chronic administration of β -adrenergic antagonists may modify this process.

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