Molecular Mechanism of Hypertrophied Failing Heart
— Abnormalities of the diastolic properties and contractility —

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The clinical syndrome of heart failure occurs as a consequence of the limitation of compensatory mechanisms, such as cardiac hypertrophy. To clarify transcriptional changes in specific genes in failing hearts, we examined the expression of cardiac Ca$^{2+}$ + Mg$^{2+}$-dependent ATPase in the sarcoplasmic reticulum and transforming growth factor β genes in the ventricles of rat hypertrophied heart, and the expression of guanine nucleotide-binding protein and "fetal" contractile protein genes in the ventricles of cardiomyopathic Syrian hamsters of Bio14.6. Northern blot analysis of total cellular RNA revealed that the mRNA levels of Ca$^{2+}$ + Mg$^{2+}$-dependent ATPase were decreased by pressure overload and became 32% of sham in 1 month, and were correlated with corresponding protein levels. Transforming growth factor β mRNA, a potent activator of collagen synthesis, was increased by pressure overload. The expression levels of the Gsα mRNA, which stimulated the adenylate cyclase, in Bio14.6 ventricles were lower than the levels in ventricles of the F1B hamster strain, and decreased as the stage of cardiomyopathy progressed. Moreover, re-expression of fetal mRNA was observed in the ventricle of cardiomyopathic Syrian hamsters of the Bio14.6 strain. These results indicate that reprogramming of cardiac gene expression both of myofibrillar and nonmyofibrillar components might occur in the failing heart. *(Jpn Circ J 1992; 56: 694–700)*

Heart failure is frequently caused by a defect in myocardial contraction. But in some patients, heart failure may be brought about by conditions in which the normal heart is suddenly presented with a load that exceeds its capacity, or in which ventricular filling is impaired! In the presence of a disturbance in myocardial contraction or an excessive hemodynamic burden placed on the ventricle, or both, the heart depends upon three principal compensatory mechanisms for maintenance of its pumping function: (a) the Frank-Starling mechanism, in which an increased preload acts to sustain cardiac performance; (b) increased release of catecholamines by adrenergic cardiac nerves and the adrenal medulla, which augment myocardial contractility; and (c) myocardial hypertrophy with or without cardiac chamber dilatation, in which the mass of con-

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- G-protein

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tractile tissue is augmented. The clinical syndrome of heart failure occurs as a consequence of the limitations and/or the ultimate failure of these compensatory mechanisms.

Many changes in mechanical performance and excitation-contraction coupling phenomena have been observed in the hypertrophied heart. In cardiac hypertrophy induced by pressure overload, there is a decrease in the maximal velocity of shortening and in peak isometric tension development, and a prolonged duration of isometric contraction and time-to-peak tension. Abnormalities of the diastolic properties of the left ventricle, especially diastolic relaxation and compliance, also exist under cardiac hypertrophy. A ventricle subjected to an abnormally elevated load for a prolonged period, however, may fail to maintain compensation despite the presence of ventricular hypertrophy, and as a result of reduced contractility, pump failure may ultimately occur. Although the most striking feature of the cardiac hypertrophic response is quantitative, resulting in the addition of new sarcomere units within the cell, there are also qualitative changes in the composition of contractile proteins in hypertrophic cells.

In the present study, we investigated the expression of Ca^{2+} + Mg^{2+}-ATPase (Ca^{2+}-ATPase) and transforming growth factor β (TGFβ) gene in hearts during pressure-overload-induced hypertrophy, and guanine nucleotide-binding proteins (G-proteins) and fetal protein genes in the ventricles of myopathic Syrian hamsters.

MATERIALS AND METHODS

Animals and surgical procedures
To make pressure overload-induced hypertrophy, male Wistar rats (40 d old, weighing 150–180 g) were anesthetized with diethyl ether and the upper part of the abdominal aorta was constricted with a hemoclip. Of 204 operated rats, 158 survived the procedures and were killed at predetermined times after the operation (0.5, 2, 4, 8, 12, 24, 48, and 72 h, 1 wk, and 1 month; n = 16, 18, 16, 18, 14, 18, 15, 13, 15, and 15, respectively). Sham-operated animals underwent identical procedures except for placement of the hemoclip and were killed after different time intervals postoperatively.

Myopathic Animals
Myopathic hamsters of the Bio 14.6 strain were divided by age into 3 separate groups of 5 animals each: a 10-week-old group, a 22-week-old group, and a 35-week-old group. These age groups corresponded to the stages of acute myolysis, healing, and dilatation or hypertrophy. Hamsters of the F1B strain, matched for age, were used as controls.

RNA Preparation
Hearts were excised and separated into atria and ventricles. They were rinsed with cold saline and quickly frozen in liquid nitrogen. Total RNA was extracted from the myocardium by the lithium urea method.

Hybridization Analysis
Total RNA (10 μg) was denatured at 60 °C for 7 min, fractionated by electrophoresis on a 1.2% agarose gel, and transferred to nylon membranes. The membranes were exposed to ultraviolet rays for 2.5 min, prehybridized, and hybridized at 42 °C with 32P-labeled Ca^{2+}-ATPase, TGFβ, G-proteins,
TABLE I  YIELD OF SR FRACTIONS, Ca\textsuperscript{2+}-ATPase CONTENT, AND Ca\textsuperscript{2+} UPTAKE

<table>
<thead>
<tr>
<th></th>
<th>SR yield (mg/g)</th>
<th>Ca\textsuperscript{2+}-ATPase content (%)</th>
<th>Ca\textsuperscript{2+} uptake (nmol/mg per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.84±0.10</td>
<td>100</td>
<td>102±7</td>
</tr>
<tr>
<td>Sham</td>
<td>0.90±0.12</td>
<td>112±10</td>
<td>105±6</td>
</tr>
<tr>
<td>2 wk after banding</td>
<td>0.89±0.10</td>
<td>92±7</td>
<td>96±8</td>
</tr>
<tr>
<td>1 mo after banding</td>
<td>0.94±0.16</td>
<td>73±8</td>
<td>76±5</td>
</tr>
<tr>
<td>15-d fetus</td>
<td>0.12±0.08</td>
<td>90±6</td>
<td>96±5</td>
</tr>
</tbody>
</table>

\*p<0.01, \*p<0.05.

Fig.2. Stimulation of TGF\(\beta\) gene expression by pressure overload.

Fig.3. Stimulation of proline incorporation into collagen by TGF\(\beta\) in cardiac fibroblasts.

and fetal protein complementary DNA probes; complementary DNA probe coding for pHA6, TGF\(\beta\), Gs\(\alpha\) (pG5N),\(\textsuperscript{11}\) Go\(\alpha\) (pGI23), Gi\(\alpha\) 1 (pBG1), Gi\(\alpha\) 2 (pGI13), and Gi\(\alpha\) 3 (pGI14), from rat C6 glioma cells, ALC1, \(\alpha\)-skA, and ANP. Prehybridization was performed in a solution containing x5 saline-sodium-phosphate-EDTA buffer, x5 Denhardt's solution, 50% formamide, 1% sodium dodecyl sulfate, 10% dextran sulfate, and 100 \(\mu\)g/ml heat-denatured salmon sperm DNA for 12–24 h at 42°C. Hybridization was performed in the same solution with the addition of 5\(\times\)10\(^6\) cpm/ml \(^{32}\)P-labeled probe for 24–36 h at 42°C. Probes were prepared by random priming procedures. Membranes were washed twice at 42°C with \(\times\)1 saline sodium citrate buffer and 0.1% sodium dodecyl sulfate and twice at 48–52°C with \(\times\)0.1 saline sodium citrate and 0.1% sodium dodecyl sulfate, air dried, and exposed to X-ray film (Kodak XAR-5) for 24 h or 5 days with an intensifying screen.
at $-70^\circ$C. Relative amounts of Goα, Gsα, and Giα gene expression were determined by a densitometric scanner. Control hybridizations were carried out using a 1.1 kilobase (kb) mouse α-actin complementary DNA.

**Isolation of SR, gel electrophoresis, and assay of Ca\(^{2+}\) uptake**

SR was isolated using a method of Nakashishi et al.\(^{12}\) Eighty 15-d-old fetal hearts, four 40-d-old adult hearts that received pressure overload for 2 wk or 1 month, and four sham-operated hearts were used for SR isolation. The left ventricles were minced with scissors and homogenized 3 times with the isolation solution. The SR fractions were separated by electrophoresis with 7.5% acrylamide according to the procedure of Lammli\(^{13}\) and stained with Coomassie blue. The density of the protein band corresponding to the 100,000-D Ca\(^{2+}\)-ATPase protein was measured by a densitometric scanner. Ca\(^{2+}\) uptake by SR was measured by the Millipore technique at Ito et al.\(^{14}\)

**Proline incorporation into collagen by TGFβ in cardiac fibroblasts**

Primary cultures of cardiac myocytes and cardiac fibroblasts were grown from the ventricles of day-old Wistar rats essentially according to the method of Simpson et al.\(^{15}\) A cardiac fibroblast rich fraction was obtained by preplating the cells into 60-mm dishes for the first hour. The culture medium was changed 24 h after seeding to a serum-free, chemically defined solution consisting of DMEM, 1 mg/ml of insulin and 5 mg/ml of transferrin. After 2 days in the serum-free medium, 10$^{-11}$ to 10$^{-9}$ M TGF β and 1 μCi/ml $[^3]$H]proline were added to the culture dishes. Then after 2 days of stimulation, $[^3]$H]proline processing into the cells was determined with intracellular trichloroacetic acid (TCA)-insoluble radioactivity. The total TCA-insoluble radioactivity in each dish was determined by liquid scintillation counting.

**RESULTS AND DISCUSSION**

**Ventricular Relaxation and Diastolic Properties of the Ventricle**

Northern blot hybridization analysis of Ca\(^{2+}\)-ATPase in pressure overload-induced hypertrophy and Gel electrophoretic analysis of SR and assay of Ca\(^{2+}\) uptake

In pressure overload-induced hypertrophy, there is now much evidence that not only contraction force but also myocardial relaxation is impaired\(^{3-7}\) Morgan et al reported that the hypertrophied myocardium demonstrated a prolonged duration of isometric contraction that correlated with a similar prolongation of the calcium transient. They interpreted that the rate of sequestration and perhaps release of calcium by intracellular
Fig. 6. RNA blot hybridization analysis of fetal isogenes in normal hamster hearts.

stores was decreased in the hypertrophied hearts. There have been many studies on the SR function in states of cardiac hypertrophy. Although SR function was dependent on the species and ages of the experimental animals, or the duration of pressure overload, most studies showed the depression in calcium uptake and calcium ATPase of SR in the hypertrophied hearts. In this study we also showed that Ca\(^{2+}\) uptake by SR was decreased by pressure overload for 1 mo in parallel to a decrease in the content of Ca\(^{2+}\)-ATPase protein. These results suggest that the abnormality of Ca\(^{2+}\) metabolism might be due to a reduced number of functional Ca\(^{2+}\)-ATPase molecules per unit of membrane. The mRNA levels of Ca\(^{2+}\)-ATPase decreased from 4 h after aortic banding and gradually progressed to become 32% at 1 month compared with those of sham-operated animals (Fig. 1). The protein content of Ca\(^{2+}\)-ATPase decreased to 65% of sham operation by pressure overload for 1 month (Table I). The discrepancy between mRNA and protein-decreased levels of Ca\(^{2+}\)-ATPase might imply translational or post-translational regulation of Ca\(^{2+}\)-ATPase in cardiac hypertrophy. If the Ca\(^{2+}\)-ATPase protein had a long half-life, the discrepancy might be due to the differences between the half-lives of the mRNA and protein. However, since the mRNA levels decreased markedly, the reduction of the Ca\(^{2+}\)-ATPase molecule at the pretranslational level might be one of the major reasons for the decrease of the Ca\(^{2+}\)-ATPase protein and its decrease might be the molecular mechanism for the impaired sequestration of intracellular Ca\(^{2+}\) in hypertrophied hearts.

The expression of TGF\(\beta\) gene in the ventricles of hypertrophied hearts

The mRNA levels of TGF\(\beta\) significantly increased from 8 h after aortic banding and gradually progressed to become 6.8-fold at 72 h compared with those of sham-operated animals (Fig. 2). On the other hand, TGF\(\beta\) enhanced the synthetic rate of collagen in cardiac fibroblasts. Fig. 3 showed the stimulation of proline incorporation into collagen by TGF\(\beta\) in cardiac fibroblasts. These findings suggest that the expression of TGF\(\beta\) might induce collagen remodeling and accumulation of the pressure-overloaded, hypertrophied myocardium and relate to the impairment of diastolic relaxation.

Mechanisms Responsible for Depressed Contractility
G-protein mRNAs in cardiomyopathic hamsters

Guanine nucleotide-binding proteins couple membrane receptors to effectors. The best characterized G-proteins regulate adenylate cyclase activity. In the heart, the activity of adenylate cyclase is controlled by 2 G-proteins: Gs, which stimulates the enzyme, and Gi, which inhibits the enzyme. Recently, Gaα, Gsα, and 3 related forms of Giα (Giα1, Giα2, and Giα3) have been isolated by molecular cloning. Although there have been some reports on the expression of these G-protein genes in physiological states, their expression in pathological states is not well known. Syrian hamsters with hereditary cardiomyopathy have provided a valuable model of the pathological course and development of heart muscle diseases with ventricular hypertrophy and necrosis leading to heart failure. The expression of G-protein genes (Gsα, Gaα, Giα1, Giα2, and Giα3) was examined in the ventricle of cardiomyopathic Syrian hamsters of the Bio14.6 strain (10–35 weeks old). Northern blot analysis of total cellular RNA revealed that all G-protein genes except Giα1 were expressed in the ventricle of Syrian hamsters (Fig. 4). Gsα and Giα2 genes were abundantly expressed. The expression levels of the Gsα mRNAs in Bio14.6 ventricles were lower than the levels in ventricles of the F1B hamster strain; the abundance of Gaα and Giα3 messenger RNAs did not change markedly (data not shown. see Ref. 25). Moreover, the messenger RNA levels of Gsα decreased as the stage of cardiomyopathy progressed (Fig. 5). Since G-proteins are linked to adenylate cyclase, these alterations of G-protein messenger RNA levels may be related to reduced contractility of a cardiomyopathic and failing heart.

Reexpression of fetal type mRNAs in failing heart

Recently, reexpression of fetal isoforms has been described in the hypertrophic heart as an adaptation. We examined the changes in the cardiac gene expression in the heart of cardiomyopathic Syrian hamster, Bio14.6. Using gene-specific DNA probes, we examined gene expression of atrial myosin light chain 1 (ALC-1) and α-skeletal actin (α-skA) and atrial natriuretic factor (ANP) by Northern blot analysis. The mRNAs encoding myosin ALC-1 and α-skA, which were expressed in the fetal hearts (Fig. 6), were also expressed in the adult ventricle of 10 to 43 week-old cardiomyopathic hamsters but not in the ventricle of control hamsters (Fig. 7A, B). In addition, ANP mRNA, which was expressed in fetal and neonatal ventricle and not in the normal adult ventricle, was not only expressed in the atrium but also expressed abundantly in the ventricle of cardiomyopathic hamsters (Fig. 7C). These results indicate that reprogramming of cardiac gene expression both of myofibrillar and nonmyofibrillar components might occur in the failing heart of cardiomyopathic Syrian hamsters.

CONCLUSION

Reprogramming of cardiac gene expression, both of myofibrillar and nonmyofibrillar components, might influence and cause abnormalities of the diastolic relaxation and contractility in hypertrophied hearts.

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