Physiological and pathological cardiac hypertrophy induce different molecular phenotypes in the rat

MOTOYUKI IEMITSU,1 TAKASHI MIYAUCHI,1 SEIJI MAEDA,1 SATOSHI SAKAI,1 TSUTOMU KOBAYASHI,1 NOBUHARU FUJII,2 HITOSHI MIYAZAKI,2 MITSUO MATSUDA,3 AND IWAO YAMAGUCHI1

1Cardiovascular Division, Department of Internal Medicine, Institute of Clinical Medicine, 2Gene Experiment Center, Institute of Applied Biochemistry, and 3Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-0006, Japan

Received 23 April 2001; accepted in final form 27 July 2001

Physiological and pathological cardiac hypertrophy induce different molecular phenotypes in the rat. Am J Physiol Regulatory Integrative Comp Physiol 281: R2029–R2036, 2001.—Pressure overload, such as hypertension, to the heart causes pathological cardiac hypertrophy, whereas chronic exercise causes physiological cardiac hypertrophy, which is defined as athletic heart. There are differences in cardiac properties between these two types of hypertrophy. We investigated whether mRNA expression of various cardiovascular regulating factors differs in rat hearts that are physiologically and pathologically hypertrophied, because we hypothesized that these two types of cardiac hypertrophy induce different molecular phenotypes. We used the spontaneously hypertensive rat (SHR group; 19 wk old) as a model of pathological hypertrophy and swim-trained rats (trained group; 19 wk old, swim training for 15 wk) as a model of physiological hypertrophy. We also used sedentary Wistar-Kyoto rats as the control group (19 wk old). Left ventricular mass index for body weight was significantly higher in the SHR group than in control and trained groups. Expression of adrenomedullin mRNA in the heart was significantly lower in the control group than in control and trained groups.

We demonstrated for the first time that the manner of mRNA expression of various cardiovascular regulating factors participate in pathological cardiac hypertrophy. We also used the spontaneously hypertensive rat; swim training; hypertension; athletic heart; spontaneously hypertensive rat; swim training; hypertension

CHRONIC EXERCISE TRAINING causes cardiac hypertrophy, which is defined as athletic heart (7, 26). The athletic heart is a physiological cardiac hypertrophy, which is an induced beneficial adaptive response of the cardiovascular system, i.e., decreased resting and submaximal heart rates and increased filling time and venous return (1, 22, 28, 35). Together, these adaptations can help the myocardium satisfy the increased demands of exercise while maintaining or enhancing normal function (1, 22, 28, 35). Although it has been considered that exercise training-induced cardiac hypertrophy is partly caused by the increase in mechanical load by repeated bouts of exercise (28), the precise mechanisms are not known.

Hypertension and cardiac valvular disease induce pathological cardiac hypertrophy caused by pressure overload (24, 28). Pathological cardiac hypertrophy is a compensatory adaptation to an increase in workload of the heart (24). Pathological cardiac hypertrophy reduces cardiac function in the left ventricle (28). Furthermore, it has been reported that the progression of pathological cardiac hypertrophy results in heart failure (24, 27). Thus there are differences in cardiac properties between physiological and pathological cardiac hypertrophy (athletic heart).

Recently, it has been reported that some cardiovascular regulating factors participate in pathological cardiac hypertrophy (27). Recent in vivo studies have suggested that ANG II is a growth factor for pathological cardiac hypertrophy (16, 40). ANG II is converted from ANG I by angiotensin-converting enzyme (ACE). It has also been reported that ANG II plays an important role in the pathogenesis of heart failure (25). Furthermore, increased expression of ACE has been reported in the failing heart (25). Endothelin-1 (ET-1) also induces cardiac hypertrophy (11, 36). We previously reported that the tissue level of ET-1 is markedly increased in the failing heart of rats with congestive heart failure due to myocardial infarction (30, 31). Activation of the myocardial β1-adrenergic pathway also induces cardiac hypertrophy (27, 42). Furthermore, pressure overload hypertrophy and failing heart cause an increase in mRNA expression of brain natriuretic peptide; angiotensin; angiotensin-converting enzyme; adrenomedullin; endothelin-1; β1-adrenergic receptor; β1-adrenergic receptor kinase; mRNA; cardiac hypertrophy; athletic heart; spontaneously hypertensive rat; swim training; hypertension

Address for reprint requests and other correspondence: T. Miyauchi, Cardiovascular Div., Dept. of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan (E-mail: t-miyauc@md.tsukuba.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpregu.org 0363-6119/01 $5.00 Copyright © 2001 the American Physiological Society
uretic peptide (BNP) and a shift of isozyme from α- to β-myosin heavy chain (MHC) (10, 27, 29). It has been reported that adrenomedullin, which is produced in cardiac myocytes, inhibits hypertrophy of cardiac myocytes in vitro (5, 37). Therefore, it is considered that various cardiovascular regulating factors, such as ANG II, ET-1, BNP, adrenomedullin, β1-adrenergic pathway, and MHC, participate in the development of pathological cardiac hypertrophy. Although the athletic heart exhibits physiological cardiac hypertrophy, it is unknown whether the various cardiovascular regulating factors participate in the development of athletic heart induced by chronic exercise training.

Because there are differences in cardiac properties between pathological and physiological cardiac hypertrophy, we hypothesized that the manner of mRNA expression of various cardiovascular regulating factors in the rat heart differs between these two types of hypertrophy. Therefore, we investigated whether the mRNA expression of various cardiovascular regulating factors differs between physiological cardiac hypertrophy (athletic heart) and pathological cardiac hypertrophy. In the present study, we used the spontaneously hypertensive rat (SHR; 19 wk old) as a model of pathological hypertrophy and swim-trained rats (trained group; 19 wk old, swim training for 15 wk, 5 days/wk) as a model of physiological hypertrophy. We also determined whether hemodynamic features differ in the trained and SHR groups.

METHODS

Animals and protocol. Experimental protocols were approved by the Committee on Animal Research at the University of Tsukuba. Male 4-wk-old Wistar-Kyoto (WKY) rats and SHR were obtained from Charles River Japan (Yokohama, Japan) and cared for according to the Guiding Principles for the Care and Use of Animals, based on the Helsinki Declaration of 1964. The rats were maintained on a 12:12-h light-dark cycle and received food and water ad libitum. Eight WKY rats were exercised by swimming for 5 days/wk (trained group) in a tank of water at 30–32°C with a surface area of 1,960 cm² and a depth of 50 cm. The rats swam for 5 min/day for the first 2 days, and then the swim time was increased gradually over the 2-wk period from 5 to 75 min/day. Thereafter, the trained group continued swim training for 13 wk. Therefore, the trained group received 15 wk of swim training. Eight SHR (SHR group) and seven WKY rats (control group) remained confined to their cages but were handled daily. After swim training for 15 wk, the body weight and hemodynamic parameters of the animals were measured, and the heart was removed, weighed, and frozen in diethyl pyrocarbonate-treated water, treated with DNase I with 75% (vol/vol) ethanol. The resulting RNA was isolated and purified using a Polytron tissue homogenizer (model PT10SK/35, Kinematica, Lucerne, Switzerland). The precipitated RNA was extracted with chloroform, precipitated with isopropanol, and washed with 75% (vol/vol) ethanol. The resulting RNA was resolved in diethyl pyrocarbonate-treated water, treated with DNase I (Takara, Shiga, Japan), and extracted again with Isogen to eliminate the genomic DNA. The RNA concentration was determined spectrophotometrically at 260 nm.

Total tissue RNA (10 μg) was primed with 0.05 μg of oligo(dT)18 and reverse transcribed by avian myeloblastosis virus RT using a first-strand cDNA synthesis kit (Life Sciences). The reaction was performed at 43°C for 60 min.

The cDNA was diluted in a 1:10 ratio, and 1 μl was used for PCR. Each PCR contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, dNTP at 200 μM each, gene-specific primer at 0.5 μM each, and 0.025 U/μl Taq polymerase (Takara). The gene-specific primers were synthesized according to the published cDNA sequences for each of the following: BNP (15), ACE (14), ET-1 (33), adrenomedullin (32), β1-adrenergic receptor (19), β1-adrenergic receptor kinase (2), muscarinic M₂ receptor (8), MHC (17), and β-actin (23). The sequences of the oligonucleotides were as follows: 5'-TA-
were scanned by CanoScan 600 (Canon, Tokyo, Japan), and quantification was performed by a computer with MacBAS software (Fuji Film, Tokyo, Japan) according to the method described previously (9, 13, 20, 29, 38, 39). The cDNAs for the verification of the semiquantitative PCR analysis were prepared from each gene PCR product of rat cDNA. Each PCR product was purified, quantified, and used as a positive-control cDNA. We performed semiquantitative PCR analysis to evaluate the expression levels of BNP mRNA, ACE mRNA, ET-1 mRNA, adrenomedullin mRNA, β1-adrenergic receptor mRNA, β1-adrenergic receptor kinase mRNA, muscarinic M2 receptor mRNA, MHC mRNA, and β-actin mRNA. To demonstrate that our semiquantitative PCR strategy was valid, serial dilutions of each positive-control cDNA were amplified by PCR and quantified by a scanner.

Statistical analysis. Values are means ± SE. Statistical analysis was carried out by analysis of variance followed by Scheffé’s F test for multiple comparisons. P < 0.05 was accepted as significant.

RESULTS

Hemodynamic parameters in control, trained, and SHR groups. Body weight was significantly lower in the trained group than in the control and SHR groups (Table 1). Left ventricular mass index for body weight was higher in the SHR and trained groups than in the control group (Table 1). Resting HR was lower in the trained group than in the control and SHR groups and significantly higher in the SHR group than in the control group (Table 1). Systolic and diastolic blood pressure were significantly higher in the SHR group than in the control and trained groups (Table 1). SV was lower in the SHR group than in the control and trained groups (Table 1). Pressure-rate product, which is an index of cardiac workload, was higher in the SHR group than in the control and trained groups (Table 1). These results suggest that the heart of trained rats exhibited physiological cardiac hypertrophy (athletic heart), and the heart of SHR exhibited pathological cardiac hypertrophy.

mRNA expression of cardiovascular regulating factors in heart. Expression of BNP mRNA, ACE mRNA, and ET-1 mRNA in the heart was significantly higher in the SHR group than in the control and trained groups (Fig. 1). There was no significant difference between the control and trained groups in expression of BNP mRNA, ACE mRNA, and ET-1 mRNA (Fig. 1). Expression of adrenomedullin mRNA in the heart was significantly higher in the SHR and trained groups than in the control group (Fig. 1).

Table 1. Body weight, tissue weight, and hemodynamic parameters in SHR, trained, and control rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 7)</th>
<th>SHR (n = 8)</th>
<th>Trained (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>391.7 ± 5.8</td>
<td>379.0 ± 6.9</td>
<td>338.8 ± 4.4†</td>
</tr>
<tr>
<td>Left ventricle wt/body wt, mg/g</td>
<td>2.34 ± 0.19</td>
<td>2.76 ± 0.11*</td>
<td>2.63 ± 0.14†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>354.5 ± 16.1</td>
<td>408.6 ± 7.6*</td>
<td>317.4 ± 4.2†</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>130.8 ± 5.8</td>
<td>241.8 ± 2.0†</td>
<td>129.8 ± 3.2†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>102.8 ± 5.7</td>
<td>183.1 ± 1.8*</td>
<td>103.1 ± 2.5†</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>362.0 ± 15.5</td>
<td>281.6 ± 14.9*</td>
<td>414.5 ± 34.6†</td>
</tr>
<tr>
<td>Pressure-rate product, mmHg·beats·min⁻¹</td>
<td>43,391.2 ± 3,156.4</td>
<td>87,990.5 ± 2,060.7*</td>
<td>38,170.5 ± 1,201.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE. SHR, spontaneously hypertensive rat; trained, swim-trained (15 wk) rat. *P < 0.05 vs. corresponding value in control rats. †P < 0.05 vs. corresponding value in SHR rats.

AJP-Regulatory Integrative Comp Physiol • VOL 281 • DECEMBER 2001 • www.ajpregu.org
significantly lower in the trained group than in the control and SHR groups (Fig. 2). Expression of $\beta_1$-adrenergic receptor mRNA in the heart was significantly higher in the SHR and trained groups than in the control group (Fig. 3A). There was no significant difference between the SHR and trained groups in expression of $\beta_1$-adrenergic receptor mRNA (Fig. 3A). Expression of $\beta_1$-adrenergic receptor kinase mRNA, which inhibits $\beta_1$-adrenergic receptor activity, in the heart was markedly higher in the SHR group than in the control and trained groups (Fig. 3B). There was no significant difference between the control and trained groups in expression of $\beta_1$-adrenergic receptor kinase (Fig. 3B). There were no significant differences among the three groups in expression of muscarinic M2 receptor mRNA (Fig. 3C). Expression of $\alpha$-MHC mRNA in the heart was significantly higher in the trained group than in the SHR group (Fig. 4A). There were no significant differences among the three groups in expression of $\beta$-MHC mRNA (Fig. 4B).

**DISCUSSION**

We have demonstrated for the first time that the manner of mRNA expression of various cardiovascular regulating factors in the heart differed between physiological cardiac hypertrophy, which is induced by chronic exercise, and pathological cardiac hypertrophy, which is induced by hypertension. In the present study, the hearts of SHR and trained rats developed cardiac hypertrophy, as evidenced by an increase in left ventricular mass index for body weight. Trained rats received 15 wk of swim training, which caused enhancement of cardiac function, i.e., a decrease in resting HR and pressure-rate product and an increase in SV. SHR developed cardiac hypertrophy by hypertension and showed a decline in cardiac function, i.e., increase in resting HR and pressure-rate product and decrease in SV. Therefore, these results suggest that trained rats exhibited physiological cardiac hypertrophy (athletic heart) and SHR exhibited pathological cardiac hypertrophy.

The present study revealed that expression of BNP mRNA, ACE mRNA, and ET-1 mRNA in the heart was significantly higher in the SHR group than in control and trained groups. It has been reported that BNP is involved in pathological cardiac hypertrophy (10). Furthermore, pathological cardiac hypertrophy is partly induced by humoral cardiovascular regulating factors such as ANG II (16, 40), which is converted from ANG I by ACE, and ET-1 (11, 36). These humoral cardiovascular regulating factors activate mitogen-activated protein kinase by the activation of GTP-binding (Gq) protein (4, 41, 43), thereby resulting in cardiac myocyte hypertrophy (6, 27). Furthermore, cardiac hypertrophy and contractile dysfunction have been reported in $G_q$-overexpressing mice (6). Therefore, it is

---

**Fig. 1.** Expression of brain natriuretic peptide (BNP) mRNA (A), angiotensin-converting enzyme (ACE) mRNA (B), and endothelin-1 (ET-1) mRNA (C) in the heart (left ventricle) of control rats (n = 7), spontaneously hypertensive rats (SHR, n = 8), and trained rats (n = 8). **Top:** typical examples of RT-PCR analysis for levels of BNP, ACE, and ET-1 mRNA. **Bottom:** results of statistical analysis of levels of expression of BNP, ACE, and ET-1 mRNA by a densitometer. Expression of $\beta$-actin mRNA was determined as an internal control. Photos of PCR products were scanned by a densitometer, and ratios of BNP, ACE, and ET-1 mRNA to $\beta$-actin mRNA were calculated. Thus expression of BNP, ACE, and ET-1 mRNA was normalized by expression of $\beta$-actin mRNA. Values are means ± SE.

Expression of BNP, ACE, and ET-1 mRNA in the heart was significantly higher in the SHR group than in control and trained groups.
considered that $G_q$ overactivation induces heart failure. In the present study, the mRNA expression of ACE and ET-1, which activate $G_q$ protein, in the heart was increased in the SHR group. Taken together, the development of pathological cardiac hypertrophy in SHR in the present study may be partly caused by ANG II- and ET-1-induced activation of $G_q$ protein. In the present study, the expression of ACE and ET-1 mRNA in the heart was not altered by 15 wk of swim training. Therefore, it is likely that activation of the $G_q$-signaling pathway by ANG II or ET-1 may not mainly participate in the development of physiological cardiac hypertrophy. Furthermore, the expression of BNP mRNA in the heart was not altered by exercise training. Therefore, it is possible that physiological cardiac hypertrophy (athletic heart) is induced by other mechanisms.

Cardiac myocytes, as well as vascular endothelial cells, produce adrenomedullin (5). It has been reported that adrenomedullin inhibits hypertrophy of cardiac myocytes in vitro (37). In the present study, the expression of adrenomedullin mRNA in the heart was significantly lower in the trained group than in the control and SHR groups. Therefore, the decrease in expression of adrenomedullin mRNA in the trained heart in this study may have accelerated the development of physiological cardiac hypertrophy in the trained rats. It is possible that a decrease in expression of myocardial adrenomedullin partly participates in development of the athletic heart.

The present study revealed that mRNA expression of $\beta_1$-adrenergic receptor, which is a receptor in the signal transduction pathway in sympathetic nerve stimulation, in the heart was significantly higher in the SHR and trained groups than in the control group. However, mRNA expression of $\beta_1$-adrenergic receptor kinase, which inhibits activation of the signal transduction system downstream of the $\beta_1$-adrenergic receptor by desensitization of the $\beta_1$-adrenergic receptor, in the heart was markedly higher in the SHR group than in the control and trained groups. These findings suggest that in the heart of trained rats the signal transduction system downstream of $\beta_1$-adrenergic receptor was activated, whereas in SHR the signal transduction system downstream of the $\beta_1$-adrenergic receptor was inactivated. Therefore, it is possible that the signal transduction system of the $\beta_1$-adrenergic receptor in the heart differs between the athletic heart (physiological cardiac hypertrophy) and pathological cardiac hypertrophy. Sympathetic nervous system activity has been implicated in development of cardiac hypertrophy (27). An in vivo study also indicated that stimulation of $\beta$-adrenergic receptors leads to development of myocardial hypertrophy independent of hemodynamic effects (42). Furthermore, Ji et al. (12) reported that $\beta$-adrenergic blockade prevented exercise-training-induced cardiac hypertrophy. On the basis of results from past studies plus the present results, it is considered that $\beta_1$-adrenergic system activity participates in development of the athletic heart (physiological cardiac hypertrophy). In the present study, there were no significant differences among the three groups in mRNA expression of muscarinic M2 receptor, which binds acetylcholine released from parasympathetic nerve endings.

In the present study, expression of $\alpha$-MHC mRNA in the heart was significantly higher in the trained group than in the SHR group. We also demonstrated that expression of $\beta$-MHC mRNA in the heart was slightly increased in the SHR group. It has been reported that hypertension in rats results in a shift of myosin isozymes from the predominant V1 to the V3 pattern and that physical training by swimming in rats results in an increase in the percentage of V1 myosin isozyme in cardiac myosin (34). These observations are consistent with our findings. Therefore, these results suggest that the myosin isozyme differs between the athletic heart (physiological cardiac hypertrophy) and pathological cardiac hypertrophy. Furthermore, we suspect that Ca$^{2+}$-ATPase activity accelerates in the athletic heart and is attenuated in pathological cardiac hypertrophy, because the V1 myosin isozyme accelerates Ca$^{2+}$-ATPase activity, whereas the V3 myosin isozyme attenuates Ca$^{2+}$-ATPase activity.

The mechanism underlying the differences in the mRNAs measured between the athletic heart and pathological cardiac hypertrophy remains to be elucidated. However, the following mechanism is possible. Trained rats (athletic heart) induce tachycardia only
Fig. 3. Expression of $\beta_1$-adrenergic receptor mRNA (A), $\beta_1$-adrenergic receptor kinase mRNA (B), and muscarinic M$_2$ receptor mRNA (C) in the heart (left ventricle) of control rats ($n = 7$), SHR ($n = 8$), and trained rats ($n = 8$). Top: typical examples of RT-PCR analysis for levels of $\beta_1$-adrenergic receptor, $\beta_1$-adrenergic receptor kinase, and muscarinic M$_2$ receptor mRNA. Bottom: results of statistical analysis of expression of $\beta_1$-adrenergic receptor, $\beta_1$-adrenergic receptor kinase, and muscarinic M$_2$ receptor mRNA by a densitometer. Expression of $\beta$-actin mRNA was determined as an internal control. Photos of PCR products were scanned by a densitometer, and ratios of $\beta_1$-adrenergic receptor, $\beta_1$-adrenergic receptor kinase, and muscarinic M$_2$ receptor mRNA to $\beta$-actin mRNA were calculated. Thus expression of $\beta_1$-adrenergic receptor, $\beta_1$-adrenergic receptor kinase, and muscarinic M$_2$ receptor mRNA were normalized by expression of $\beta$-actin mRNA. Values are means ± SE. Expression of $\beta_1$-adrenergic receptor mRNA in the heart was significantly higher in SHR and trained groups than in the control group. Expression of $\beta_1$-adrenergic receptor kinase mRNA in the heart was markedly higher in the SHR group than in control and trained groups.

Fig. 4. Expression of $\alpha$- and $\beta$-myosin heavy chain ($\alpha$-MHC and $\beta$-MHC) mRNA in the heart (left ventricle) of control rats ($n = 7$), SHR ($n = 8$), and trained rats ($n = 8$). Top: typical examples of RT-PCR analysis for levels of $\alpha$- and $\beta$-MHC mRNA by a densitometer. Bottom: results of statistical analysis of expression of $\alpha$- and $\beta$-MHC mRNA by a densitometer. Expression of $\beta$-actin mRNA was determined as an internal control. Photos of PCR products were scanned by a densitometer, and ratios of $\alpha$- and $\beta$-MHC mRNA to $\beta$-actin mRNA were calculated. Thus $\alpha$- and $\beta$-MHC mRNA expression was normalized by $\beta$-actin mRNA expression. Values are means ± SE. Expression of $\alpha$-MHC mRNA in the heart was significantly higher in the trained group than in the SHR group.
during exercise, whereas SHR (pathological cardiac hypertrophy) sustain tachycardia and hypertension at all times. The difference in periods of tachycardia and hypertension between the trained rats and SHR might cause a difference in workload on the heart. Therefore, it is possible that a difference in the persistence in the workload on the heart between the trained rats and SHR is one of the causal factors for the difference in mRNA expressions in these two types of hypertrophy.

In conclusion, we have demonstrated that the manner of mRNA expression of various cardiovascular regulating factors in the heart differs between pathological and physiological cardiac hypertrophy (athletic heart). The present study also demonstrated that hemodynamic features in the heart differed between pathological and physiological cardiac hypertrophy. We speculate that the different alterations in the molecular phenotypes between the athletic heart (physiological cardiac hypertrophy) and pathological cardiac hypertrophy are among the causal factors in the differences in hemodynamic features (e.g., SV and cardiac diastolic function) and the prognosis of cardiac hypertrophy (predisposition to heart disease or failure).

Perspectives

It is generally accepted that there are differences in cardiac function between physiological and pathological cardiac hypertrophy (24, 26, 28). The present study demonstrated a new molecular finding that the manner of mRNA expression of various cardiovascular regulating factors in the heart differed between physiological and pathological cardiac hypertrophy. These findings showed that a different molecular mechanism of formation of cardiac hypertrophy is possible between these two types of cardiac hypertrophy. Further studies to precisely reveal molecular features of physiological and pathological cardiac hypertrophy are needed, because these further studies will provide important clues to the mechanisms of the differences in hemodynamic features and the prognosis of cardiac hypertrophy.

We thank Dr. Wendy Gray for editing the English language of our manuscript.

This study was supported by grants-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan (00006781, 11557047, 12470147); and by a grant from the Miyaoi Project of the Center for Tsukuba Advanced Research Alliance at the University of Tsukuba.

REFERENCES


