Impaired Gene Expression of β_1 -Adrenergic Receptor, but Not Stimulatory G-Protein $G_s\alpha$, in Rat Ventricular Myocardium Treated with Isoproterenol

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We investigated the gene expression of β_1 -adrenergic receptor (β_1 AR) and stimulatory G-protein $G_s\alpha$, important signal transduction elements for regulating heart rate and contractility, in ventricle after chronic treatment with isoproterenol (ISO) in rat. Rats were treated with ISO (4 mg/kg, intraperitoneal) twice a day for 4 d. Ventricle weight of the heart and ventricle weight/body weight ratio were increased by 23% and 25% compared with control, respectively. Positive inotropic responses to ISO in left atrial muscle preparations isolated from ISO-treated rats were markedly decreased. Northern blot hybridization showed that the mRNA transcript of β_1 AR was significantly decreased in ventricle of ISO-treated rats, whereas $G_s\alpha$ mRNA level was unchanged. Present results demonstrate that the gene expression of myocardial β_1 AR, but not $G_s\alpha$, was decreased in rat myocardium of ISO-induced cardiac hypertrophy, and suggesting that decrease in the gene expression of β_1 AR may be one of the mechanisms responsible for the diminished cardiac function.

Key words β_1 -adrenergic receptor; G-protein; cardiac hypertrophy

Cardiac hypertrophy is induced by a variety of diseases, such as hypertension, valvular diseases, myocardial infarction and endocrine disorders, and often associated with an increased sympathetic adrenergic activity.¹⁾ Although cardiac hypertrophy is initially compensatory for an increased workload, prolongation of this process leads to congestive heart failure, arrhythmia and sudden death.²⁾

Among the many mechanisms that control cardiac function, the stimulation of the frequency and force of contraction through β -adrenergic receptors (β AR) is the most prominent and relevant pathway.³⁾ The β_1 -adrenergic receptor (β_1 AR) is a principal subtype of β AR regulating heart rate and contractility.⁴⁾ Stimulation of cardiac β_1 AR by catecholamines results in the activation of the stimulatory G-protein $G_s\alpha$, which in turn, activates the adenylyl cyclase and promotes the production of cAMP. Cardiac hypertrophy induced by chronic β AR stimulation shares many similarities with end-stage human heart failure. Chronic treatment of isoproterenol (ISO), a β AR agonist, to rat shows the alternation of β_1 AR signaling system and the induction of hypertrophy in myocardium.⁵⁾ It has been reported the reduced density of β_1 AR and basal or agonist-stimulated adenylyl cyclase activity in rat myocardium of ISO-induced cardiac hypertrophy.⁶⁾ However, precise mechanisms of β_1 AR and post-receptor abnormalities remain unclear.

In contrast to the several functional studies, such as β_1 AR density and adenylyl cyclase activity, examining the response to induction of hypertrophy, surprisingly few studies have examined the gene expression. Eschenhagen *et al.*⁷⁾ found that the expression of $G_s\alpha$ mRNA was not changed in rat myocardium of ISO-induced cardiac hypertrophy. However, change in the gene expression of myocardial β_1 AR is not still clear. Therefore, we investigated the gene expression of β_1 AR and $G_s\alpha$ in rat myocardium following chronic treatment of ISO.

MATERIALS AND METHODS

Animals Male Wistar rats (7 to 9 weeks-old, Nihon Clea, Tokyo) were housed in standard cages, and were maintained on a standard laboratory diet and tap water and exposed to a 12 h light–dark cycle. Ambient temperature during the study was maintained at about 23 °C. After all animals used in the present study had been cared for in accordance with the guidelines for animal treatment of Kitasato University, which meet the principals of laboratory animal care. ISO-induced cardiac hypertrophy was made as described by Kimura *et al.*⁶⁾ ISO (Sigma Chemical Co., St. Louis, MO, U.S.A.) was administered intraperitoneally twice-daily injections with 4 mg/kg for 4 d. The control rats were injected with physiological saline (1 ml/kg) in same regimen with ISO treatment. After 4 d of the experimental regimen, the hearts were excised under sodium pentobarbital (50 mg/kg, i.p.) anesthesia and separated into the atrial and ventricular tissues.

Left Atrial Muscle Preparation The preparations were electrically stimulated at 2 Hz with square-wave pulses of 5 ms duration at a voltage approximately 50% above the threshold with a pair of platinum field-stimulation electrodes.⁸⁾ The resting tension was adjusted to 1.0 g. Developed tension was continuously monitored with a force-displacement transducer (model TB651T, Nihon Koden Kogyo, Tokyo) and a Power Lab 4/20 computer system (ADInstruments, Colorado Springs, CO, U.S.A.). The concentration-response curves for ISO were generated on the preparations of control and ISO-treated rats.

Preparation of Hybridization Probes and Northern Blotting To prepare the digoxigenin (DIG)-labeled cRNA probes, the polymerase chain reaction (PCR) primers for rat $G_s\alpha$ (GenBank accession number; M12673, 5'-AAGTG-GATCCAGTGCTTCAATGAT-3' and 5'-TCCACGGCGCA-

GGTAAAGTG-3') and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GenBank accession number; M17701, 5'-GCTGATGCCCCCATGTTTG-3' and 5'-GCCATGAGG-TCCACCC-3') were designed. The β_1 AR primer sequences (5'-TCGTGTGCACCGTGTGGGCC-3' and 5'-AGGAAAC-GGCGCTCGCAGCTGTTCG-3') were obtained from Krief *et al.*⁹⁾ Preparation of DIG-labeled cRNA antisense or sense probes and Northern blotting were performed as described previously.¹⁰⁾

Statistical Analysis All data were presented as mean and S.E.M. Statistical significance was analyzed using Student *t*-test, and a value of $p < 0.05$ was considered significant.

RESULTS

Chronic β AR stimulation with ISO produced no change in body weight in the both groups (Table 1). On the other hand, ventricle weight of the heart and ventricle weight/body weight ratio in ISO-treated rats were significantly increased by 23% and 25%, respectively. Overt signs of congestive heart failure, such as pleural effusion and peripheral edema, were absent.

To evaluate the effect of chronic ISO treatment on cardiac function, we examined the positive inotropic effect of ISO in isolated left atrial muscle preparations (Fig. 1). Basal developed tension before addition of ISO was not statistically significant in control (405.4 ± 48.6 mg in tissue, $n = 10$) and ISO-treated rats (518.8 ± 64.8 mg in tissue, $n = 10$). In the control group, the positive inotropic responses to ISO were increased in a concentration-dependent manner. However, developed tension was reduced in the preparations of ISO-treated rats at 30–300 nM ISO.

To examine the myocardial β_1 AR and $Gs\alpha$ gene expression following chronic treatment with ISO, we performed Northern blot hybridization (Fig. 2). DIG-labelled antisense β_1 AR, $Gs\alpha$ and GAPDH cRNA probes detected each mRNA transcript in ventricles of the control and the ISO-treated rats (Fig. 2A). No signal was obtained when a sense probe was used during hybridization (data not shown). The intensity was determined by the densitometric analysis of the autoradiogram. After normalization of each mRNA level to GAPDH mRNA, the bands corresponding to β_1 AR mRNA was significantly decreased in the ISO-treated rats compared with the control, whereas $Gs\alpha$ mRNA levels were not different in the both groups (Fig. 2B).

DISCUSSION

The present study demonstrated first that the gene expression of β_1 AR was altered in rat myocardium after chronic treatment with ISO. However, it was not accompanied by altered gene expression of $Gs\alpha$. Prolonged treatment with ISO in rats has been widely used as a model of pharmacologically induced cardiac hypertrophy and heart failure.^{5–7)} In ISO-treated rats, the significant increase in the ventricle weight/body weight ratio observed in the present study revealed hypertrophic effects of chronic ISO treatment consistent with other reports.^{5–7)} Furthermore, dose-dependent inotropic responses with ISO were also decreased in the left atrial muscle preparations isolated from the ISO-treated rats. These results indicate that chronic treatment with ISO caused cardiac

Table 1. ISO-Induced Changes in Body Weight and Ventricle Weight

Parameters	Control	ISO
Body weight (g)	267.1 ± 3.6	263.5 ± 5.7
Ventricle weight (mg)	909 ± 42	$1117 \pm 42^{**}$
Ventricle weight/body weight (mg/g)	3.40 ± 0.11	$4.24 \pm 0.16^{**}$

Values are mean $\pm$ S.E.M. of 6 rats in each group. ISO, isoproterenol. $^{**}p < 0.01$ as compared to the control group.

Fig. 1. The Positive Inotropic Effect of ISO in Left Atrial Muscle Preparations Isolated from ISO-Treated Rats

Left atrial muscle preparations of control (○) and ISO (●) groups were suspended vertically in a temperature-controlled bath. Cumulative concentration–response curves for the positive inotropic effect of ISO was generated by increasing the ISO concentration at 2 min intervals. Data are given as the mean $\pm$ S.E.M. ($n = 10$). $^{*}p < 0.05$ as compared to the control group.

Fig. 2. Expression of β_1 AR and $Gs\alpha$ mRNA in Ventricle of ISO-Treated Rats

Representative Northern blot of β_1 AR, $Gs\alpha$ and GAPDH in the ventricles of control and ISO-treated rats (A). Densitometric analysis of β_1 AR and $Gs\alpha$ mRNA levels (B). The levels were normalized to GAPDH. Data are given as the mean $\pm$ S.E.M. ($n = 5$). $^{*}p < 0.05$ as compared to the control group.

hypertrophy in rats.

It has been reported that chronic ISO treatment caused reduction of β_1 AR density on myocardial membrane, which is measured by using radioligand binding assay, and the reduced cAMP production was also observed.⁶⁾ We also observed a decrease in the positive inotropic responses to ISO in the heart isolated from the ISO-treated rat. The concentration–response curves of the positive inotropic responses to ISO may suggest that decrease in maximum response and unchange in the ligand-receptor affinity in the left atrial preparations from ISO-treated rats. This suggestion is supported by the report that the chronic treatment with ISO caused re-

duction of β_1 AR density.⁶⁾

Although chronic treatment with ISO caused the alternations in the β_1 AR system in myocardium has been known for several years, few studies have examined the changes in the β_1 AR system-related gene expression. Eschenhagen *et al.*⁷⁾ reported that the levels of $Gs\alpha$ mRNA were not changed in rat myocardium of ISO-induced cardiac hypertrophy. Furthermore, it was observed that the type V and VI of adenylyl cyclase mRNA levels were slightly decreased in mice after chronic ISO treatment.¹¹⁾ The present observations confirmed the no change in $Gs\alpha$ mRNA expression. On the other hand, Kimura *et al.*⁶⁾ showed that the level of $Gs\alpha$ protein measured by Western blotting was decreased in myocardium of ISO-induced cardiac hypertrophy. The possible reason for the apparent discrepancy between gene and protein expression is not clear, but it may be due to translational or post-translational regulation of $Gs\alpha$, as shown in previous report.¹²⁾ However, the decreased myocardial β_1 AR mRNA followed by the reduction of β_1 AR density after chronic treatment with ISO may causes the reduction of cAMP production, and then elicit the dysfunction of cardiac contraction.

The precise molecular mechanism underlying the inhibition of β_1 AR gene expression is not clear in the present study. It has been reported that regulation of β_1 AR gene transcription is mediated by activation of Sp1 transcription factor.¹³⁾ The β_1 AR gene promoter of human, rat, mouse and porcine has a consensus Sp1-binding site, and Sp1 stimulates the expression of the β_1 AR promoter.¹³⁾ In addition to binding Sp1, the β_1 AR gene promoter has early growth response-1 (Egr-1) transcription factor binding site, and Sp1 and Egr-1 binding sites are partially overlapping. Furthermore, Egr-1 suppresses the transcription of the β_1 AR gene by competing with Sp1 for binding to their overlapping sites.¹³⁾ On the other hand, it has been reported that Egr-1 was overexpressed in hypertrophied hearts of mice treated with chronic ISO treatment,¹⁴⁾ and Egr-1 deficient mice revealed a blunted ISO-induced hypertrophy response.¹⁵⁾ Therefore, it is possible that the reduction of β_1 AR mRNA expression may be mediated by the increased expression of Egr-1 in ISO-in-

duced cardiac hypertrophy. Further studies are necessary to understand the gene regulation of β_1 AR in relation to ISO-induced cardiac hypertrophy.

In summary, we have demonstrated that the gene expression of β_1 AR was decreased in rat ventricular myocardium after chronic treatment with ISO, whereas the gene expression of $Gs\alpha$ was not altered. Present results suggest that abnormalities of β_1 AR system in myocardium of ISO-induced cardiac hypertrophy may stem, at least in part, from decrease in the gene expression of β_1 AR.

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