Increase of Cardiac β-Adrenergic Receptors in Young Spontaneously Hypertensive Rats

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SUMMARY

The authors studied the number of myocardial β-adrenergic receptors and the cyclic nucleotide concentration in both male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) at 4 to 5, 10 to 15, 20 to 25 and 35 to 55 weeks of age. A potent β-adrenergic antagonist, (125I) iodohydroxybenzylpindolol was used to estimate the number and affinity of β-adrenergic receptors. β-adrenergic receptors in cardiac membranes from SHR of 4 to 5 weeks and 10 to 15 weeks numbered 63.1±4.6 and 51.6±4.6 f mol/mg protein, respectively. These were significantly (p<0.02) greater than the number in WKY at 4 to 5 weeks and 10 to 15 weeks (42.2±5.1 and 31.5±5.4 f mol/mg protein, respectively). The dissociation constant in the membranes was the same in WKY and SHR, and no significant differences were found in the number of receptors and affinity of SHR and WKY at 20 to 25 weeks or 35 to 55 weeks of age. Also, there was no difference in the concentration of myocardial cyclic nucleotides at the various ages. Since cardiac hypertrophy in SHR had appeared before the onset of hypertension at about 7 weeks, the present results suggest that the SHR heart is hypersensitive to catecholamines and hemodynamically hyperkinetic due to the increased numbers of β-receptors in the pre- and early stages of hypertension.

Additional Indexing Words:
C-AMP (125I) Iodohydroxybenzylpindolol Hyper-beta-adrenergic states SHR WKY
ALTHOUGH the spontaneously hypertensive rats (SHR) developed by Okamoto and Aoki show several differences from the hypertensive patient, they have remained the most suitable animal model of hypertension. One of the most consistent vascular findings in the adult SHR and in established essential hypertension in man is an increased total systemic peripheral resistance associated with elevated arterial pressure and vascular structural changes. However, total systemic peripheral resistance has been shown to be normal in the young SHR and in borderline hypertension. Moreover, Pfeffer has suggested that the same hyperkinetic states (increased cardiac output, heart rate and myocardial contractility) are present in the young SHR 9 to 12 weeks of age. These hyperkinetic states were also reported in pre-hypertensive SHR by Yamori. Thus, in the pathogenesis of long-term hypertension, it has been postulated that these hyper-beta-adrenergic states produce an elevated arterial pressure in pre- and early hypertension of SHR and that increased total peripheral resistance rather than hyper-beta-adrenergic states maintain elevated arterial pressure in the established hypertension of SHR. We studied myocardial $\beta$-receptor density and myocardial c-AMP concentration of SHR and Wistar Kyoto (WKY) strains at various ages in order to account for this physiological phenomenon, because an increase of $\beta$-receptor numbers in some tissues implies hypersensitivity of those tissues to catecholamines.

**Materials and Methods**

Male spontaneously hypertensive rats were used for these experiments along with age-matched male Wistar Kyoto rats. Both types of rats were divided into 4 groups according to age (group 1: 4 to 5 weeks of age; group 2: 10 to 15; group 3: 20 to 25; group 4: 35 to 55). The SHR corresponded to the F9 generation from the Aoki-Okamoto strain of Kyoto University. They were inbred under identical conditions at the Laboratory Animal Center of Fujita Gakuen University, School of Medicine. The age-matched WKY corresponded to the F12 generation and were inbred under the same environment as the SHR. Systolic blood pressure was determined weekly in the unanesthetized state by the tail plethysmographic method.

Wistar rats were also used to study the effects of isoproterenol administration on $\beta$-receptor numbers in the cardiac membrane. They were divided into 4 groups and injected subcutaneously with isoproterenol at doses of 0.1, 1 and 10 mg/Kg, respectively, 5 times every 12 hours; control rats were injected with the same amount of distilled water. All rats were sacrificed at
1 hour after the final injection.

**Cardiac membrane preparations:**
Rats were anesthetized with ether, and the thorax was opened with scissors. The hearts were removed immediately and placed in 4 to 5 volumes of cold homogenate buffer (0.25 M sucrose, 5 mM Tris/HCl, pH 7.4, 1 mM MgCl₂). Cardiac membranes were prepared for an iodohydroxybenzylpindolol binding study according to the procedure of Williams. The left ventricle (two ventricles of 4 to 5 week rats) was removed from one rat heart with scissors and minced. The mince in the cold buffer was then homogenized twice with a Polytron PT-10 at a setting of 6.5 for 15 sec. The homogenate was centrifuged at 480 g for 10 min at 4°C. The pellets were discarded and the supernatant was again centrifuged at 30,000 g for 10 min. The pellet was washed twice in 10 ml of cold incubation buffer (50 mM Tris, pH 7.5, 10 mM MgCl₂) by resuspension and centrifugation and was finally resuspended at a concentration of 0.3 to 0.5 mg of protein/ml in incubation buffer. The yields of final membrane preparation per Gm of initial heart weight from SHR and WKY were similar at about 3 mg of membrane protein/Gm of initial wet weight.

**β-receptor binding study:**
For the β-receptor binding study, the 200 μl membrane preparations and 100 μl of (125I)iodohydroxybenzylpindolol (IHYP) were incubated for 60 min at 37°C. The 100 μl of IHYP used ranged from 20,000 to 400,000 CPM (Boxy Brown Co, specific activity, 2,100 Ci/mmoll). Incubations were terminated by diluting the incubation mixture with 2 ml of ice cold incubation buffer and rapid vacuum filtration through Whatman GF/C filters. The filters were immediately washed with 20 ml of ice cold incubation buffer. Radioactivity on the filters was measured in an Aloka Autowell gamma-counter. Nonspecific binding was determined by filtered aliquots of membranes which had been incubated in the presence of 2×10⁻⁵ M (±)propranolol. Specific binding was determined by subtracting nonspecific binding from total binding. Specific binding constituted 80% of the total binding at the lowest levels of IHYP and 60% at the highest levels of IHYP. Binding data were assessed by the method of Scatchard. The relative purity of cardiac membranes was checked by determining Na⁺⁻K⁺ ATPase activity.

**Myocardial cyclic-AMP assay:**
The myocardial tissues (10 to 20 mg wet weight of the left ventricle) were obtained simultaneously when the heart was removed for β-receptor
assay, and frozen immediately by liquid nitrogen. The concentrations of myocardial c-AMP were measured thereafter by a sensitive radioimmunoassay according to the method of Cailla et al.,14) modified by Honma et al.15) The protein concentration in the membrane fractions was measured by the Lowry method.16)

The statistical significance of differences between groups was determined by unpaired Student's t-test. All numerical results were expressed as mean ± standard error. A value of p less than 0.05 was considered significant.

RESULTS

Blood pressure and cardiac hypertrophy of SHR:

Fig. 1 shows the systolic blood pressure of SHR, together with their appropriate controls (WKY). The blood pressure of SHR began to elevate from about 7 weeks of age and continued to elevate gradually with aging until 20 to 25 weeks of age. Each difference in value of the blood pressure between WKY and SHR was significant (p<0.001) in groups 2, 3 and 4 (BP in group 2: 112.2±2.8 mmHg in WKY and 160.3±3.6 in SHR; in group 3: 124.3±3.0 in WKY and 184.2±4.5 in SHR; in group 4: 117.3±3.2 in WKY and 169.8±3.2 in SHR). Thus, SHR at 4 to 5 weeks of age (group 1) were considered to be in the pre-stages of hypertension (BP in group 1: 119.6±1.4 in WKY and 119.7±1.4 in SHR, NS). However, cardiac hypertrophy in SHR had already been established at 4 to 5 weeks after birth as shown in Fig. 2 (heart weight/body weight×10³ in group 1: 3.55±0.07 in WKY and 4.27±0.13 in SHR, p<0.001).

Fig. 1. The systolic blood pressure in SHR began to increase from about 7 weeks of age as shown on the abscissa and then continued to increase until 20 to 25 weeks. In control WKY, the blood pressure remained constant throughout life.
Fig. 2. The heart weight per body weight in SHR has already increased as compared with WKY at 4 to 5 weeks. Except at 35 to 55 weeks of age in SHR, significant myocardial hypertrophy was recognized throughout life.

Fig. 3. This figure indicates that (125I) IHYP binding was saturable and reversible. When cardiac membranes (0.3–0.5 mg of protein/ml, 200 μl) were incubated with IHYP (1.5 × 10^5 CPM), the binding reached a maximum within 40 min. Half-maximal binding time was about 10 min at 37°C. On addition of 10 μM (-)propranolol, IHYP binding was dissociated reversibly with a half-time of about 30 min.

β-receptor properties of cardiac membranes:

As shown in Fig. 3, (125I)iodohydroxybenzylpindolol binding was rapid with half-maximal binding at about 10 min at 37°C and maximal binding at 40 min in cardiac membrane preparations. The binding was reversible, when 10 μM propranolol was added at 60 min, and the dissociation of binding showed a half-time of about 30 min. Cardiac membranes were incubated with IHYP (1.5 × 10^5 CPM) in the presence of each agonist, and specific binding was determined (Fig. 4). Adrenergic agonists competed with IHYP for the binding sites in the following order: isoproterenol, epinephrine, norepine-
Fig. 4. Cardiac membranes (0.3-0.5 mg/ml, 200 μl) were incubated with IHYP (1.5 × 10^6 CPM, 100 μl) in the presence of each agonist and specific binding was determined. Adrenergic agonists competed with IHYP for the binding sites in the following order: isoproterenol, epinephrine, norepinephrine, as shown in the upper figure. In the lower figure, stereospecificity for antagonist binding (propranolol) with IHYP was determined; (−) isomer had more affinity than (+) isomer. All the measurements were done in duplicate.

Phrine. Agonist binding was characteristic of β-receptor properties described in the literature. The lower the concentration of agonist that inhibits IHYP binding, the more potent is its β-activity.

Again, stereospecificity of binding revealed that (−) propranolol was about 100 times more active than (+) propranolol. When each stereoisomer of propranolol was incubated with membranes and IHYP, a lower concentration of (−) propranolol (10^{-8} M) than of (+) propranolol (10^{-6} M) produced 50% inhibition of IHYP binding.
Na⁺–K⁺ ATPase activity of cardiac membranes from SHR and WKY:

In order to check the relative purity of cardiac membranes, Na⁺–K⁺ ATPase activity was determined as a marker enzyme. As shown in Fig. 5, no significantly different enzymatic activity was shown in SHR (40.0±4.0 μmol Pi/mg protein/hour) and WKY (34.5±4.0) of 4 to 5 weeks.

Effects of isoproterenol administration on β-receptor numbers from cardiac membranes:

Myocardial β-receptor numbers of rats administered isoproterenol were compared with controls. As shown in Fig. 6, the number of β-receptors in the group administered 10 mg isoproterenol was 38.4±4.9 fmol/mg protein which was significantly decreased (p<0.05) compared with controls (62.8±6.9). Those in the groups administered 1 mg and 0.1 mg showed 55.1±4.8 and 60.6±7.6, respectively. No significant differences were observed among the 1 mg, 0.1 mg and control groups. The dissociation constant (Kd) was not significantly different among groups. Kd was 0.121±0.009 in 10 mg, 0.148±0.02 in 1 mg, 0.109±0.08 in 0.1 mg and 0.132±0.02 in the control group.
Fig. 6. Each dose of isoproterenol shown on the abscissa was injected subcutaneously into Wistar rats 5 times every 12 hours. The Wistar rats were sacrificed at 1 hour after final injection and the myocardial membrane preparations were used for β-receptor assay. β-receptors were somewhat greater in number in controls, but there were significantly fewer β-receptors only in the group given 10 mg/Kg isoproterenol.

Fig. 7. The decreases in total β-receptor numbers seen in SHR with age are shown on the abscissa (from 63.1 f mol/mg protein to 35.0) in comparison with those in WKY. There were significant (p<0.02) increases of Rt in SHR at pre- and early stages before hypertension was observed but after myocardial hypertrophy had already been recognized.

β-receptor numbers in cardiac membranes prepared from SHR and WKY:
Myocardial β-receptor numbers from SHR were significantly increased (p<0.02) compared with those from WKY in groups 1 and 2 as shown in Fig. 7. They were 42.2±5.1 f mol/mg protein from WKY and 63.1±7.2 from
Table I. $(^{125}$I)IYP Binding for $\beta$-Adrenergic Receptor Assay

<table>
<thead>
<tr>
<th>Age</th>
<th>WKY (n=9)</th>
<th>SHR (n=13)</th>
<th>WKY (n=6)</th>
<th>SHR (n=6)</th>
<th>WKY (n=5)</th>
<th>SHR (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5 weeks</td>
<td>119.6±1.4</td>
<td>119.7±1.4</td>
<td>112.2±2.8</td>
<td>160.3±3.6**</td>
<td>117.3±3.2</td>
<td>169.8±3.2**</td>
</tr>
<tr>
<td></td>
<td>3.55±0.07</td>
<td>4.27±0.13**</td>
<td>3.04±0.08</td>
<td>3.85±0.20**</td>
<td>2.99±0.18</td>
<td>3.04±0.20**</td>
</tr>
<tr>
<td></td>
<td>42.2±5.1</td>
<td>63.1±7.2*</td>
<td>31.5±5.4</td>
<td>51.6±4.6*</td>
<td>34.9±4.0</td>
<td>35.0±3.8</td>
</tr>
<tr>
<td></td>
<td>0.080±0.010</td>
<td>0.080±0.010</td>
<td>0.072±0.017</td>
<td>0.105±0.015</td>
<td>0.064±0.004</td>
<td>0.073±0.009</td>
</tr>
</tbody>
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$* p<0.02, ** p<0.001.$

SHR in group 1, and 31.5±5.4 from WKY and 51.6±4.6 from SHR in group 2, respectively. The binding densities decreased gradually with aging and reached almost the same density as WKY. Thus, in group 3, 40.5±2.9 f mol/mg protein from WKY and 46.2±5.0 from SHR were not significantly different. No significant difference between $\beta$-receptor numbers in group 4 (34.9±4.0 from WKY and 35.0±3.8 from SHR) was observed either. Although $\beta$-receptor numbers in SHR were already elevated in pre- and early stages of hypertension as compared to those in WKY (group 1 and group 2), the dissociation constant (inverse value of the affinity between IYP and $\beta$-receptor) was not significantly changed between SHR and WKY. These were 0.080±0.010 n mol from WKY and 0.080±0.010 from SHR in group 1, 0.072±0.017 from WKY and 0.105±0.015 from SHR in group 2, 0.110±0.005 from WKY and 0.121±0.010 from SHR in group 3, and 0.064±0.004 from WKY and 0.073±0.009 from SHR in group 4, respectively, as shown in Table I.

Myocardial cyclic AMP concentration:

As shown in Fig. 8, myocardial c-AMP concentrations were 0.88±0.05 n mol/Gm wet weight from WKY and 0.96±0.04 from SHR in group 1, 1.38±0.12 (WKY) and 1.63±0.18 (SHR) in group 2, 1.31±0.17 (WKY) and 1.08±0.09 (SHR) in group 3, and 1.46±0.26 (WKY) and 1.58±0.20 (SHR) in group 4. A tendency toward an age-dependent increase was observed, but no significant differences in myocardial c-AMP concentrations
Fig. 8. Myocardial c-AMP measurements from SHR and WKY were done in the same specimens used for determining the number of $\beta$-receptors. Although the c-AMP concentrations in rats of 4 to 5 weeks of age were lower than those seen at a more advanced age, no significant difference between SHR and WKY was found.

were observed between SHR and WKY.

**DISCUSSION**

The findings of the present study, in which biochemically determined myocardial $\beta$-receptor densities of SHR of various ages (4-55 weeks) were compared with those of normotensive WKY, accounted for the hyperkinetic phenomenon; young SHR (pre- and early stages of hypertension) initially had increased numbers of $\beta$-receptors, as measured with IHYP, but $\beta$-receptor numbers of older SHR (established stages of hypertension) gradually decreased with age to equal those of WKY. Since this increase in $\beta$-receptor numbers in SHR antedates the rapid rise in blood pressure, it cannot be ascribed merely to the secondary effects of increased intraluminal pressure. In addition, SHR of 4 to 5 weeks age had already shown cardiac hypertrophy\(^{18}\) and this may have augmented the number of genetically determined $\beta$-receptors in cardiac membranes. However, there were some inconsistencies in our findings, especially relating to the $\beta$-receptor regulatory mechanism. Recently, evidence has accumulated as to changes in $\beta$-receptors under different physiological and pathological conditions. The adrenergic agonist induced a decrease in the number of $\beta$-receptors ("down regulation"),\(^{19,20}\) whereas the antagonist caused their increase ("up regulation").\(^{21}\)

It is well known that myocardial norepinephrine turnover, dopamine $\beta$-hydroxylase and plasma norepinephrine levels are increased in young SHR,\(^{22}\) so a decrease in myocardial $\beta$-receptors in SHR seems to be an attractive explanation. However, since these experiments showing "down reg-
ulation" were done with high concentrations of injected catecholamine\(^{(19),(20)}\) and the possibility of \(\beta\)-receptor destruction in the cellular membranes could not be excluded, "down regulation" might not occur at physiological levels of catecholamines present in vivo. In fact, our findings showed "down regulation" only in the group receiving 10 mg/Kg isoproterenol injected subcutaneously. This dose was sufficient to induce apical infarction and necrosis in the Wistar rat heart.\(^{(23)}\) However, the administration of 0.1 mg/Kg of isoproterenol which results in physiologic concentrations and causes injuries in the Wistar rat myocardium did not induce a significant decrease in the number of \(\beta\)-receptors compared with control rats. Recently, Mukherjee et al reported that the SHR-SP (stroke prone SHR) 9 weeks of age failed to show fewer myocardial \(\beta\)-receptors, although there was an increased cardiac sympathetic drive in these animals (i.e., elevation of serum norepinephrine).\(^{(24)}\) The same authors demonstrated a failure of the normal "down regulation" phenomenon in the SHR-SP.

The present findings, too, suggest a failure of the down regulation phenomenon and show an increase in \(\beta\)-receptor numbers to explain the hyperhemodynamic states seen in young SHR. Limas et al demonstrated that all the SHR of various ages have decreased myocardial \(\beta\)-receptors by \((^3\text{H})\text{di}-\) hydroalprenolol (DHA) binding and ascribed it to an overflow of sympathetic drive in SHR.\(^{(17)}\) There is the consistent view that, at 4 weeks of age, sympathetic neuronal activity in SHR is increased. But this does not differ significantly from that of normotensive WKY.\(^{(22)}\) Moreover, our studies were done in male SHR and WKY. There was no information as to the gender of rats used in the Limas study. Also, we used IHYP as adrenergic ligands. Since IHYP has more affinity to \(\beta\)-receptors, a longer half-maximum saturable time and greater radioactivity than DHA, the discrepancy might be due in part to the differences in the radioligand used. There is no report other than ours which measured \(\beta\)-receptor numbers in SHR by IHYP binding. Again, Limas described the increased \(\beta\)-receptor numbers in hypertensive rats following abdominal aortic constriction.\(^{(25)}\) Thus, the discrepancy might be due in part to the different kinds of animals with experimental hypertension.

Other heterogenous factors, including phospholipid methylation,\(^{(26)}\) should be considered as mechanisms influencing the mechanical regulation of \(\beta\)-adrenergic receptors, since changes in fragility of cellular membranes of SHR-SP was proposed as a mechanism by Yamori et al.\(^{(27)}\) The changes in \(\beta\)-adrenergic receptors with aging may be related to the normalizing of cardiac output in elder SHR, and the decreased leukocyte \(\beta\)-receptor numbers in the elderly offers a possible explanation for the age-associated loss of adrenergic responsiveness.\(^{(28),(29)}\) Although several investigators have measured cardiac
and vascular concentrations of c-AMP in SHR, no consistent data have yet been obtained.\textsuperscript{30-33} An increase in myocardial c-AMP was to be expected since \(\beta\)-receptors increased in the SHR myocardium, but no significant difference was observed. Myocardial c-AMP concentration may not necessarily reflect directly the myocardial \(\beta\)-receptor numbers and their activity. Further studies are needed of \(\beta\)-receptors coupled with the adenylcyclase system. In conclusion, the present findings show that the cardiac membranes in SHR have about 50\% more \(\beta\)-receptors than those of WKY, and this might play an important role in the initiation of hypertension.

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**References**


