Changes in Cardiac Beta-Adrenoceptor Concentrations in Spontaneously Hypertensive and Experimental Renal Hypertensive Rats

MASATO KUCHI, M.D., KAZUYA FUKUDA, M.D., TAKUZO HANO, M.D.
HIDEYO OHTANI, M.D., OSAMU MOHARA, M.D.
ICHIRO NISHIO, M.D., AND YOSHIKI MASUYAMA, M.D.

Cardiac β-adrenoceptors were studied in membrane fractions from spontaneously hypertensive rats (SHR) and rats with two-kidney, one clip hypertension (2K, 1C HT), using radioligand binding method. β-Adrenoceptor concentration measured by [3H]-dihydralprolol (DHA) binding was significantly lower in cardiac membranes from two months old SHR than those from Wistar-Kyoto rats (WKY) (38.2 ± 2.6 vs 45.1 ± 1.8 fmol/mg protein, means ± SEM, p < 0.05). Cardiac membranes from 2K, 1C HT rats had also a lower concentration of β-adrenoceptors than those from the sham-operated control rats at a week after operation (30.9 ± 2.2 vs 47.8 ± 1.6 fmol/mg protein, p < 0.01). But receptor affinity remained unchanged.

These reduced concentrations of β-adrenoceptors were restored to control levels at 12 months old in SHR and at 6 weeks after operation in 2K, 1C HT rats, although age-dependent decrease in β-adrenoceptor was observed. The decrease in β-adrenoceptor was associated with increase in plasma noradrenaline levels during the earlier stages of hypertension. But there is no correlation between β-adrenoceptor concentrations and plasma noradrenaline levels in the chronic stages of hypertension.

No significant difference was found in activities of 5'-nucleotidase, which is a marker enzyme of cell membrane, in membrane fractions between the hypertensive hearts and the controls, suggesting that the cardiac hypertrophy is not a determinant factor for change in β-adrenoceptor.

The observed decrease in β-adrenoceptor concentration may reflect an increase in sympathetic nerve activity during development of hypertension. In the chronic stages of hypertension, additional factors may be involved in the restoration of β-adrenoceptors.

Key Words:
Cardiac β-adrenoceptor
Spontaneously hypertensive rat
Two-kidney, one clip hypertension
Plasma noradrenaline level
Aging

Division of Cardiology, Department of Medicine, Wakayama Medical College, Wakayama, Japan
Address for reprints: Masato Kuchi, M.D., Division of Cardiology, Department of Medicine, Wakayama Medical College, 1, 7-Bancho, Wakayama City, Wakayama 640, Japan

THE sympathetic nervous system may contribute to the pathogenesis of various types of experimental hypertension via postganglionic nerve activity and adrenoceptor responsiveness. Increased sympathetic nerve activity in experimental hypertension has been shown by the elevation in plasma catecholamine levels and tissue metabolism of noradrenaline.1,2 Whereas, the responsiveness of various tissues to adrenergic
TABLE I SYSTOLIC BLOOD PRESSURE, HEART WEIGHT AND HEART TO BODY WEIGHT RATIOS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND TWO-KIDNEY, ONE CLIP HYPERTENSIVE RATS (2K, 1C HT)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood pressure (mmHg)</th>
<th>Heart wt (mg)</th>
<th>heart/body wt (\times 10^3)</th>
<th>No. of exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR 2M</td>
<td>155.5 ± 3.8**</td>
<td>792 ± 36</td>
<td>3.99 ± 0.09**</td>
<td>6</td>
</tr>
<tr>
<td>WKY 2M</td>
<td>110.3 ± 4.2</td>
<td>765 ± 25</td>
<td>3.31 ± 0.08</td>
<td>9</td>
</tr>
<tr>
<td>SHR 6M</td>
<td>202.9 ± 6.0**</td>
<td>1364 ± 28*</td>
<td>3.46 ± 0.04**</td>
<td>9</td>
</tr>
<tr>
<td>WKY 6M</td>
<td>128.8 ± 6.1</td>
<td>1218 ± 46</td>
<td>2.44 ± 0.13</td>
<td>8</td>
</tr>
<tr>
<td>SHR 12M</td>
<td>217.5 ± 6.4**</td>
<td>1342 ± 55</td>
<td>3.43 ± 0.21**</td>
<td>4</td>
</tr>
<tr>
<td>WKY 12M</td>
<td>126.0 ± 5.2</td>
<td>1321 ± 34</td>
<td>2.07 ± 0.05</td>
<td>4</td>
</tr>
<tr>
<td>2K, 1C HT 1W</td>
<td>179.9 ± 5.7**</td>
<td>746 ± 20</td>
<td>4.12 ± 0.14**</td>
<td>11</td>
</tr>
<tr>
<td>Controls 1W</td>
<td>111.0 ± 2.7</td>
<td>705 ± 37</td>
<td>3.39 ± 0.24</td>
<td>7</td>
</tr>
<tr>
<td>2K, 1C HT 3W</td>
<td>199.8 ± 10.3**</td>
<td>947 ± 40*</td>
<td>4.10 ± 0.14**</td>
<td>4</td>
</tr>
<tr>
<td>Controls 3W</td>
<td>107.8 ± 3.8</td>
<td>807 ± 23</td>
<td>3.07 ± 0.07</td>
<td>4</td>
</tr>
<tr>
<td>2K, 1C HT 6W</td>
<td>195.7 ± 5.9**</td>
<td>1311 ± 55**</td>
<td>3.97 ± 0.12**</td>
<td>7</td>
</tr>
<tr>
<td>Controls 6W</td>
<td>127.6 ± 3.6</td>
<td>1114 ± 39</td>
<td>2.85 ± 0.15</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SEM. * p < 0.05, ** p < compared with controls.

stimulation is also altered in hypertension\(^3\) and the decreased sensitivity of the heart to catecholamines was observed in hypertension\(^4\)\(^5\). Recently, several observations have suggested that these changes were partly explained by alterations in the concentration\(^6\)\(^7\)\(^8\)\(^9\) or affinity\(^10\) of cardiac \(\beta\)-adrenoceptors. However, many factors can lead to changes in the number of cardiac \(\beta\)-adrenoceptors, including sympathetic nerve activity, aging, drugs and other hormones\(^11\). In the heart, chronic stress, such as hypertension and/or hypertrophic growth, may also be important\(^12\)\(^13\). In this study, we have examined the cardiac \(\beta\)-adrenoceptors in spontaneously hypertensive rats and in rats with experimental renal hypertension, and compared with plasma noradrenaline levels and cardiac hypertrophy in the different stages of hypertension.

MATERIALS AND METHODS

Experiments were carried out for two types of experimental hypertension.

(1) Spontaneously hypertensive rats (SHR). 2, 6 and 12 months old male SHR of the Okamoto-Aoki strain were studied with age-matched Wistar-Kyoto rats (WKY) used as controls.

(2) Two-kidney, one clip hypertension (2K, 1C HT). Male Wistar rats weighing 150—180g were anesthetized with pentobarbital (30 mg/kg, intraperitoneally). The left renal artery was dissected free and a silver clip (2 mm wide, 0.17—0.20 mm gap) was placed around the left renal artery. The contralateral kidney was left intact. A loose renal artery clip (0.5 mm gap) was applied to the left renal artery for control rats. Cardiac \(\beta\)-adrenoceptors were studied at 1, 3 and 6 weeks after operation.

Systolic blood pressure was measured by a tail-cuff plethysmographic method.

Blood sampling: Rats were allowed to acclimate to surroundings in the laboratory for an hour before sacrifice and excessive handling was avoided. These rats were killed by the decapitation with guillotine and the first 2.5 ml of blood from the trunk was collected in the tubes containing 2.5 mg of EDTA-2Na through a funnel for plasma noradrenaline determination\(^14\). Under these conditions, blood collected after decapitation has a similar noradrenaline content to arterial blood sampled from conscious unrestricted rats\(^15\).

Blood samples were immediately cooled in an ice bath. After centrifugation at 3,000 rpm for 15 min, 1 ml of plasma was separated and kept frozen until assay.

Membrane Preparation

The venticles were weighed, minced with scissors and homogenized in ice cold medium (0.25 M sucrose, 1 mM MgCl\(_2\), 5 mM Tris-HCl, pH 7.4) using a Polytron PT10 (Kinematica). The homogenates were filtered through double layered gauze and centrifuged at 400 x g for 10 min. The supernatant was centrifuged at 36,000 x g for 10 min and the pellet was washed.

\(\text{Japanese Circulation Journal Vol. 45, September 1981}\)
The 10th Conference on the Pathogenesis of Hypertension

![Graph](image)

**Fig.1.** Scatchard plot of $[^3]$H-dihydroalprenolol (DHA) binding to cardiac membranes from spontaneously hypertensive (SHR) and age-matched Wistar Kyoto (WKY) rats at the age of 2 and 12 months.

Twice with incubation buffer consisting of 50 mM Tris-HCl, 10 mM MgCl₂ at pH 7.4. The final pellet was resuspended in the same buffer to a final protein concentration of 2−4 mg/ml. Protein concentration was determined by the method of Lowry et al.

**β-Receptor Assay**

β-Adrenergic receptors in cardiac plasma membranes were assayed using the ligand $[^3]$H-dihydroalprenolol (DHA, New England Nuclear Company, specific activity 34−48 Ci/mmol) according to the method of Williams et al. One hundred μl of membrane suspension (0.2−0.4 mg protein) and $[^3]$H-DHA (1−10 nM) were incubated with shaking for 12 min at 37°C in a total volume of 150 μl of the incubation buffer. At the end of incubation, binding was terminated by adding 2 ml of ice cold buffer and immediately filtered through Whatman GF/C glass fiber filter within 10 sec. The filters were rapidly washed with 3 aliquots (5 ml each) of the same cold buffer. After drying in counting vials, 10 ml of Triton-toluene based scintillation fluid was added and counted. Specific binding to the β-adrenoceptor was defined by subtracting the radioactivity bound in the presence of 10 μM (±)-propranolol from the total radioactivity bound. Binding data were analyzed according to the method of Scatchard.

Plasma noradrenaline concentration was measured by the radioenzymatic method of Henry et al.

The 5'-nucleotidase activity was determined according to the method of Emmelot et al. and checked for the relative purity of cardiac membrane preparations.

All values are expressed as means ± SEM. Statistical significance was evaluated using the Student's t-test or Welch's test.

**RESULTS**

**Blood Pressure and Heart Weights**

The systolic blood pressure and heart weights in SHR and 2K, 1C HT rats are shown in Table I. In SHR, blood pressure elevated gradually and heart weight increased with age until 6 months of age. Thereafter, heart weights of both groups

**TABLE II**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Cardiac β-adrenoceptor conc.</th>
<th>affinity</th>
<th>Plasma NA conc.</th>
<th>5'-nucleotidase activity</th>
<th>No. of exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR 2M</td>
<td>38.2 ± 2.6*</td>
<td>1.1 ± 0.1</td>
<td>1.33 ± 0.36*</td>
<td>3.10 ± 0.72</td>
<td>6</td>
</tr>
<tr>
<td>WKY 2M</td>
<td>45.1 ± 1.8</td>
<td>1.4 ± 0.1</td>
<td>0.68 ± 0.09</td>
<td>3.42 ± 0.82</td>
<td>9</td>
</tr>
<tr>
<td>SHR 6M</td>
<td>30.0 ± 1.4*</td>
<td>1.3 ± 0.1</td>
<td>0.97 ± 0.13</td>
<td>— e</td>
<td>9</td>
</tr>
<tr>
<td>WKY 6M</td>
<td>36.8 ± 1.8</td>
<td>1.0 ± 0.1</td>
<td>0.80 ± 0.08</td>
<td>— e</td>
<td>8</td>
</tr>
<tr>
<td>SHR 12M</td>
<td>22.8 ± 0.8</td>
<td>1.3 ± 0.2</td>
<td>0.92 ± 0.07</td>
<td>2.80 ± 0.25</td>
<td>4</td>
</tr>
<tr>
<td>WKY 12M</td>
<td>22.1 ± 0.8</td>
<td>1.2 ± 0.2</td>
<td>1.10 ± 0.10</td>
<td>2.88 ± 0.61</td>
<td>4</td>
</tr>
</tbody>
</table>

*Values are means ± SEM.
$a = [^3]$H-Dihydroalprenolol (DHA) bound (fmol/mg prot.),
$b = nM$
$c = ng/ml of plasma$
$d = μ moles of Pi released/mg prot./hr$
$e = Not determined$
$* = p < 0.05; compared with WKY$
$** = p < 0.01 difference between respective age groups of either SHR or WKY$

*Japanese Circulation Journal Vol. 45, September 1981*
were not increased. There are no significant differences in net weight of heart between SHR and control rats at 2 and 12 months of age, since the hypertensive rat grew slowly and reached lower mature weight than the control. Consequently, the heart/body weight ratios were consistently higher in the hypertensive rats than in the control groups.

In the renal hypertension, the blood pressure was elevated significantly a week after operation and slightly increased thereafter. However, the difference in heart weight between the renal hypertensive and control rats was not evident a week after operation. Significant cardiac hypertrophy was observed in hypertensive rats 3 and 6 weeks after operation (Table I).

**Cardiac β-Adrenoceptor Concentration**

Binding of [3H]-DHA to rat cardiac membranes was rapid and saturable and rapidly dissociated by the addition of 10 μM (±)-propranolol.

The (−)-isomer of propranolol was about a hundred times more potent than the (+)-isomer to inhibit [3H]-DHA binding, indicating the stereospecificity.

The specific [3H]-DHA binding to cardiac membranes from SHR and WKY rats were examined for β-receptor concentration and their affinity. Fig. 1 shows a Scatchard analysis from a typical experiment. As summarized in Table II, β-receptor concentration in cardiac membranes was significantly decreased with age in both the hypertensive and control rats (p < 0.01). Cardiac membranes from the hypertensive rats contained lower concentrations of β-receptor than that of control rats at the age of 2 and 6 months (p < 0.05), while this difference was not found in the myocardium of the 12 months old rats. Their affinities for [3H]-DHA were the same for both the hypertensive and normotensive rats regardless of age.

In 2K, 1C HT rats, the cardiac β-adrenoceptor concentration was significantly reduced compared to that of the control rats until 3 weeks after operation (p < 0.01), although the magnitude of the difference was reduced at 3 weeks. At 6 weeks, these differences could not found between hypertensive and control rats. The affinity for [3H]-DHA binding was unchanged.

<table>
<thead>
<tr>
<th>TABLE III</th>
<th>TIME COURSE OF CONCENTRATION AND AFFINITY IN CARDIAC β-ADRENOCEPTORS, PLASMA NORADRENALINE LEVEL AND 5'-NUCLEOTIDASE ACTIVITY IN TWO-KIDNEY, ONE CLIP HYPTERTENSIVE AND CONTROL RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks after operation</td>
<td>Heart adrenoceptor conc.</td>
</tr>
<tr>
<td>2K, 1C HT 1W</td>
<td>30.9 ± 2.2**</td>
</tr>
<tr>
<td>Controls 1W</td>
<td>47.8 ± 1.6</td>
</tr>
<tr>
<td>2K, 1C HT 3W</td>
<td>41.6 ± 2.0**</td>
</tr>
<tr>
<td>Controls 3W</td>
<td>49.8 ± 2.6</td>
</tr>
<tr>
<td>2K, 1C HT 6W</td>
<td>42.3 ± 3.0</td>
</tr>
<tr>
<td>Controls 6W</td>
<td>42.9 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SEM.  
\( a = [3H]\)-Dihydroalprenolol (DHA) bound (f mol/mg prot.),  
\( b = \text{nmol}, \  c = \text{ng/ml of plasma}, \  d = \mu \text{moles of Pi released/mg prot./hr} \),  
**p < 0.01: compared with controls

*Japanese Circulation Journal Vol. 45, September 1981*
through the time after operation (Fig. 2, Table III).

**Plasma Noradrenaline Levels**

The mean value of plasma noradrenaline in SHR was significantly higher than that of WKY only at the age of 2 months (p < 0.01). There were no significant differences in plasma noradrenaline levels at the age of 6 and 12 months (Table II).

In renal hypertensive rats, plasma noradrenaline levels were significantly higher than those in control rats at all times after clipping (p < 0.01) (Table III).

**5'-Nucleotidase Activity**

The activities in the membrane preparations from SHR were similar to those in WKY without regard to age differences (Table II). Also no significant difference was found in the enzyme activities between the renal hypertensive rats and the controls (Table III).

**DISCUSSION**

The alteration of cardiac responsiveness to adrenergic stimulation has been observed in several types of experimental hypertension\(^5\)–\(^9\) and was partly explained by the changes in adrenoceptor characteristics\(^6\)–\(^10\).

Recently, Woodcock et al. have reported that the cardiac membranes prepared from rats with hypertension or genetically hypertensive (New Zealand strain) have a reduced concentration of \(\beta\)-adrenoceptors without changes in their affinity\(^6\)–\(^9\). Limas and Limas\(^7\) also observed a similar reduction of \(\beta\)-adrenoceptor concentration in cardiac membranes from SHR of the Okamoto-Aoki strain with no age difference. While, Giachetti et al.\(^10\) showed a reduced affinity in cardiac membrane from one-kidney Grollman hypertensive rats. They suggested that these changes in \(\beta\)-receptor are attributed to a decrease in the responsiveness of cardiac \(\beta\)-adrenoceptor. We have found that, although concentrations of \(\beta\)-adrenoceptors decreased with age, they were significantly lower in SHR than those in age-matched WKY; this is in accordance with the previous reports.\(^7\) The reduced concentrations of \(\beta\)-adrenoceptors are also observed in cardiac membranes in rats with 2K, 1C HT. However, the time course of changes in receptor concentration is somewhat different from the results of other reports. In this experiment, the most pronounced decrease in cardiac \(\beta\)-adrenoceptor concentration was shown in the earlier stages of both hypertension models and thereafter, the concentration gradually restored to the control levels.

Many factors have been shown to influence the concentration of cardiac \(\beta\)-adreceptor, such as the sympathetic agonists or antagonists, aging, cardiac hypertrophy and abnormal levels of other hormones.\(^11\)–\(^13\) First, it is important to rule out the possibility that the observed changes in adrenoceptor number are secondary to the different amounts of the plasma membrane, because of the different heart weights. In this experiment, the activity of \(5'\)-nucleotidase, which is plasma membrane marker enzyme, was similar in membrane fractions from the hypertensive hypertrophied hearts and the controls. In addition, the difference in the number of \(\beta\)-adrenoceptors between the normotensive and the hypertensive rats was already evident before the development of significant cardiac hypertrophy. The decreased number of \(\beta\)-adrenoceptor returned to the control level, in spite of a significant increase in heart weight in renal hypertensive rats. Therefore, it is unlikely that the observed changes in adrenoceptor number are due to the difference in recovery of plasma membranes from hypertrophied heart and the decreased adrenoceptor appears to be a reflection of the reduced receptor density.

As to aging, it was observed that the isolated cardiac muscle from old rats exhibits diminished inotropic response to catecholamines.\(^2\) The observed age-dependent decrease in \(\beta\)-adrenoceptor in SHR and WKY rats may provide a possible explanation for the age-related loss of adrenergic responsiveness. This finding is in accord with the observation that the \(\beta\)-adrenoceptors on lymphocytes gradually reduced with aging.\(^2\)

\(\beta\)-Adrenoceptor concentration is known to be regulated by the concentration of noradrenaline at neuroeffector junction. Prolonged exposure to \(\beta\)-adrenergic agonist resulted in a decrease of receptor and an inverse correlation has been shown between the receptor density and the extent of occupancy by agonist.\(^11\) Sympathetic nerve activity has been reported to be elevated in experimental hypertension.\(^12\) In SHR, an increase in plasma noradrenaline concentration was observed in young rats and these values returned to normal after development of hypertension.\(^2\) In our experiment, plasma noradrenaline concentration increased only at the age of 2 months,

but this is not evident thereafter. The reduction of β-adrenoceptor concentration observed in young SHR would, then, be a reflection of increased sympathetic nerve activity during the development of hypertension. However, a slight but significant decrease of β-adrenoceptor at 6 months old SHR was not associated with an raised plasma noradrenaline and can not be ascribed only to an increase of sympathetic nerve activity. This may be related to the other factors, such as a genetic factor.

We have previously reported that 2K, 1C HT rats with severe hypertension showed a marked increase in plasma noradrenaline levels in acute and chronic phases14 although it was reported that plasma noradrenaline was not altered in the two-kidney model with mild hypertension24 This study confirmed the consistent increase in plasma noradrenaline of the rats with this type of hypertension throughout the experiment. Therefore, a reduced concentration of cardiac β-adrenoceptor found in renal hypertensive rats at a week after operation can be explained by a reflection of the increase in cardiac sympathetic nerve activity. On the other hand, at 6 weeks after operation we failed to find any significant change in β-adrenoceptor concentration in the hypertensive hearts, when the plasma noradrenaline levels were still elevated. The lack of an inverse correlation between the concentrations of β-adrenoceptor and the plasma noradrenaline levels may represent free from ‘down-regulation’ mediated by the sympathetic nerve activity, suggesting that an additional factor may be involved in the regulation.

Recently, Limas25 reported that development of cardiac hypertrophy produced by the aortic constriction was associated with an increased adrenoceptor concentration and suggested that this increase of adrenoceptor was an adaptive change in response to tissue catecholamine depletion. Since the development of cardiac hypertrophy was shown to result in a decrease of tissue catecholamine content24 this possible mechanism could be responsible for the restoration of β-adrenoceptor concentration after development of cardiac hypertrophy.

Consequently, the observed decrease in cardiac β-adrenoceptor concentration could be the result of β-adrenoceptor overstimulation during the development of hypertension and may contribute to the reduced responsiveness of the heart. In the chronic stages of hypertension, an additional factor may be involved in the restoration of β-adrenoceptor concentration, which seems to be the result of a compensatory mechanism to prolonged pressure overload.

REFERENCES

7. LIMAS C, LIMAS CI: Reduced number of β-adrenergic receptors in myocardium of spontaneously hypertensive rats. Biochim Biophys Res Commun 83: 710, 1978
11. LEFKOWITZ RJ: Direct studies of adrenergic receptors; Biochemical, physiologic and clinical implication. Ann Int Med 91: 450, 1979

18. SCATCHARD G: The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51: 660, 1949


20. EMMELOT P, BOS CJ, BENEDETTZ EL, RUMKE PH: Studies on plasma membranes. I. Chemical composition and enzyme content of plasma membranes isolated from rat liver. BBA 90: 126, 1964


25. LIMAS CJ: Increased number of β-adrenergic receptors in the hypertrophied myocardium. BBA 558: 174, 1979
