Changes in Tissue Alpha- and Beta-Adrenergic Receptors in Renal Hypertension in the Rat

ELIZABETH A. WOODCOCK, PH.D., AND COLIN I. JOHNSTON, M.B.B.S., F.R.A.C.P.

SUMMARY Myocardial membranes prepared from renal hypertensive rats contained reduced concentrations of both α- and β-adrenergic receptors. The decrease in α-receptor concentration measured by [³H]-dihydroergocryptine binding was from 80 ± 6 (SEM) to 52 ± 2 fmol/mg. Beta-receptor concentration measured by [¹²⁵I]-iodohydroxybenzylpindolol binding also decreased by about half from 80 ± 16 to 41 ± 9 fmol/mg. The affinities of the receptors were unchanged. There was no change in either concentration or affinity of β receptors in membranes prepared from the lungs or kidneys of these hypertensive rats. These results demonstrate that the observed receptor changes are tissue-specific. Cardiac adrenergic receptor alterations are therefore not part of a generalized adrenergic receptor decrease associated with elevated circulating plasma catecholamine concentrations, but probably reflect a specific increase in cardiac sympathetic drive. (Hypertension 2: 156-161, 1980)

KEY WORDS • adrenergic receptor • experimental hypertension • cardiac hypertrophy • sympathetic nervous system • heart • alpha receptor • beta receptor

The myocardium of hypertensive animals has a reduced inotropic and chronotropic response to adrenergic stimulation.¹⁻³ This reduced contractility is most likely the net result of a number of changes in the hearts of hypertensive animals. Depressed cAMP-dependent Ca⁺⁺ transport has been described in the cardiac sarcoplasmic reticulum of spontaneously hypertensive rats.⁴ Hearts of spontaneously hypertensive animals also contain a reduced concentration of cAMP,⁵ and cardiac membranes from DOCA-salt and renal as well as spontaneously hypertensive animals show a reduced isoproterenol stimulation of adenylate cyclase activity.⁶⁷ Recent studies have demonstrated decreased concentrations of β-adrenergic receptors in the myocardium of hypertensive animals.⁷⁻¹⁰ To determine whether these changes are specific to β-adrenergic receptors, cardiac α-adrenergic receptors have also been measured in hearts from renal hypertensive rats.

Depression of myocardial adrenergic receptors may be part of a generally reduced adrenergic receptor concentration caused by the raised plasma catecholamine levels found in renal hypertensive rats.¹¹ Alternatively, it may result from a specific increase in cardiac sympathetic drive demonstrated in renal hypertensive rats.¹² To distinguish between these two mechanisms, β receptors have been measured in kidneys and lungs as well as in hearts of renal hypertensive rats.

Materials and Methods

We obtained [¹²⁵I]-iodohydroxybenzylpindolol, [³H]-dihydroergocryptine, and [³H]-dihydroalprcnolol from Searle Nucleonics; D, L-isoproterenol HCl, L-epinephrine HCl, and D, L-norepinephrine HCl from the Sigma Chemical Company, St. Louis, Missouri; D-epinephrine HCl from KEK Fine Chemicals; propranolol HCl from ICI Australia; and phentolamine mesylate from CIBA Geigy. All other chemicals were A.R. grade.

One-Kidney, One Clip Hypertension (Goldblatt Model)

Male Sprague-Dawley rats weighing 70-90 g were anesthetized with ether. The right kidney was removed. The left renal artery was dissected free, and a silver clip 2 mm wide with a 0.2 mm gap was used to
constrict the artery. Control rats were unilaterally nephrectomized and the left renal artery dissected free but not clipped. Systolic blood pressure of conscious animals was measured by a tail plethysmographic method.

**Membrane Preparation**

Rats were killed by cervical dislocation. Tissues were removed immediately, trimmed, and washed in 0.9% saline at 0°C. Tissues were minced with scissors and homogenized with a Potter/Elvejhem homogenizer in 3–5 vol of the appropriate buffer containing 0.25 M sucrose. The buffers used were: 1) beta-receptors binding in cardiac membranes using [125I]-iodohydroxybenzylpindolol ([125I]-HYP) in 50 mM sodium phosphate, pH 7.4, 4 mM MgCl₂; 2) beta-receptor binding in renal and pulmonary membranes using [3H]-dihydroalprenolol ([3H]-DHA) in 50 mM tris-HCl, pH 8.1, 10 mM MgCl₂; 3) alpha-receptor binding in cardiac membranes using [3H]-dihydroergocryptine ([3H]-DHE), in 50 mM tris-HCl, pH 7.7, 10 mM MgCl₂.

The homogenates were centrifuged at 3000 g for 5 minutes in the SS34 rotor of a Sorval RC-2B centrifuge at 5°C. The supernatant was centrifuged at 30,000 g for 15 minutes and the pellet membranes washed twice and resuspended in the appropriate buffer at a final protein concentration of 2–5 mg/ml for [125I]-HYP binding and 15–20 mg/ml for [3H]-DHE and [3H]-DHA binding. Protein concentration was determined by the method of Lowry et al. using bovine serum albumin as standard.

**Adrenergic Receptor Analysis**

**Cardiac Beta Receptors**

Cardiac membranes (0.05 ml) were incubated with [125I]-HYP for 40 minutes at 37°C in a total volume of 0.15 ml in 50 mM sodium phosphate, pH 7.4, 4 mM MgCl₂, 0.05% ascorbic acid, 10–250 nM isoproterenol, 100 nM phentolamine. Incubations were terminated by rapid filtration through Whatman glass fiber filters (GF/C). Filters were washed immediately with 20 ml of 20 mM phosphate, pH 7.4, 4 mM MgCl₂ at 37°C, dried, and counted in a Packard Autogamma Scintillation Spectrometer. Specific binding was defined as that percentage of [125I]-HYP bound which was displaceable by 10–500 nM isoproterenol. Specific binding ranged from 50–70% of the total counts bound per filter. Results were analyzed by the method of Scatchard.

**Cardiac Alpha Receptors**

The method used was similar to that described by Williams and Lefkowitz. Cardiac membranes (0.05 ml) were incubated with [3H]-DHE for 20 minutes at 25°C in a total volume of 0.15 ml containing 50 mM tris, pH 7.7, 10 mM MgCl₂, 0.05% ascorbic acid. Binding was terminated by filtration and the filters were washed with 10 ml of buffer. Filters were dried and counted using a triton X-100/toluene scintillation fluid in a Packard liquid scintillation spectrometer.

Specific binding was defined as [3H]-DHE bound that was displacable by 10–500 nM phentolamine. Specific binding ranged from 40–70% of the total counts bound per filter. Results were analyzed by the method of Scatchard.

**Renal and Pulmonary Beta Receptors**

Membranes were incubated with [3H]-DHA for 10 minutes at 37°C in a total volume of 0.2 ml containing 50 mM tris, pH 8.1, 10 mM MgCl₂, 0.05% ascorbic acid, 10–250 nM isoproterenol, 100 nM phentolamine. After termination of binding by filtration, filters were washed with 10 ml ice-cold buffer. Specific binding was defined as binding displaced by 10–500 nM isoproterenol and ranged from 40–60% in renal membranes and from 90–95% in pulmonary membranes. Results were analyzed by the method of Scatchard.

**Measurement of Plasma Membrane Marker Enzyme Activities**

The method of Kidwai et al. was used to measure 5'-nucleotidase and ouabain-inhibited p-nitrophenylphosphatase. Ouabain-inhibited activity was taken as the difference between incubations containing 10–250 nM ouabain and those containing no inhibitor. Adenylate cyclase was measured as described by Harden et al., using [3H]-labelled ATP as substrate. Separation of cAMP was achieved by the method of Krishna et al.

**Results**

**Blood Pressures and Cardiac Hypertrophy of Hypertensive Rats**

Figure 1 shows the systolic blood pressures and ventricular weight. Receptor measurements were made 3 weeks after surgery when the blood pressure elevation was stable.

**Characterization of Alpha- and Beta-Receptor Binding Sites**

**Cardiac Alpha Receptors**

Binding of [3H]-DHE to myocardial membranes was saturable, rapid, and rapidly reversible. Stereoselectivity was demonstrated by the 10–50 times-greater potency of L-epinephrine than D-epinephrine in competing for specific binding sites. The order of potency of catecholamines in inhibiting [3H]-DHE binding was in agreement with α-receptor activity; thus, norepinephrine = epinephrine >> isoproterenol = dopamine. The relative potencies of agonist and antagonist compounds are listed in table 1.

In these experiments, binding of [3H]-DHE to cardiac membranes has been measured only at high concentrations of [3H]-DHE (5–50 nM) where the Scatchard plot is linear. At low concentrations of [3H]-DHE, nonlinear Scatchard plots were obtained.
Figure 1. Increase in systolic blood pressures (BP) and heart weights of renal hypertensive (one-kidney Goldblatt, IKG) and control (C) rats.

Cardiac, Renal, and Pulmonary Beta Receptors

Similar receptor concentrations were found in heart, kidney, and lung, whether measurements were made using $[^{125}\text{I}]-\text{HYP}$ or $[^{3}\text{H}]-\text{DHA}$. In these experiments, cardiac $\beta$ receptors were measured with $[^{125}\text{I}]-\text{HYP}$ so that smaller amounts of tissue could be used, which allowed $\alpha$ receptors to be measured in the same membrane fraction. Binding of either $[^{125}\text{I}]-\text{HYP}$ or $[^{3}\text{H}]-\text{DHA}$ was saturable, rapid, and rapidly reversible. Stereospecificity was demonstrated by the potencies of the L-isomers of epinephrine and propranolol being 50-100 times greater than their respective D-isomers in competing for binding. The relative potencies of adrenergic agonists and antagonists for binding sites in the membrane preparations from the three different tissues are shown in Table 1. In all cases the relative activities were those expected of $\beta$-receptor binding activity.

Receptor Changes in Renal Hypertensive Rats

Cardiac Alpha and Beta Receptors

Myocardial membranes prepared from hypertensive animals contained lower concentrations of both $\alpha$ and $\beta$ receptors than membranes from control animals. The affinities of the receptors were unchanged. Figures 2 and 3 show the results from typical experiments. Results from all experiments are shown in table 2.

Renal and Pulmonary Beta Receptors

No change in either concentration or affinity of $\beta$ receptors was found in renal and pulmonary membranes from renal hypertensive rats. Results are summarized in table 2.

Table 1. Relative Affinities of Adrenergic Agonists and Antagonists in Competing for Binding Sites in Heart, Kidney, and Lung Membranes

<table>
<thead>
<tr>
<th>Compound</th>
<th>$[^{125}\text{I}]-\text{HYP}$ binding ($\beta$ heart)</th>
<th>$[^{3}\text{H}]-\text{DHE}$ binding ($\alpha$ heart)</th>
<th>$[^{3}\text{H}]-\text{DHA}$ binding ($\beta$ kidney)</th>
<th>$[^{3}\text{H}]-\text{DHA}$ binding ($\beta$ lung)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td>$2 \times 10^{-4}$</td>
<td>$&gt; 10^{-4}$</td>
<td>$1.8 \times 10^{-7}$</td>
<td>$1.6 \times 10^{-7}$</td>
</tr>
<tr>
<td>L-epinephrine</td>
<td>$1.3 \times 10^{-4}$</td>
<td>$2 \times 10^{-4}$</td>
<td>$1 \times 10^{-4}$</td>
<td>$4 \times 10^{-7}$</td>
</tr>
<tr>
<td>D-epinephrine</td>
<td>$&gt; 10^{-4}$</td>
<td>$3 \times 10^{-4}$</td>
<td>$&gt; 10^{-4}$</td>
<td>$&gt; 10^{-4}$</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>$2 \times 10^{-4}$</td>
<td>$1.3 \times 10^{-4}$</td>
<td>$3 \times 10^{-4}$</td>
<td>$3 \times 10^{-4}$</td>
</tr>
<tr>
<td>Dopamine</td>
<td>$&gt; 2.6 \times 10^{-4}$</td>
<td>$-$</td>
<td>$-$</td>
<td>$-$</td>
</tr>
<tr>
<td>L-propranolol</td>
<td>$8 \times 10^{-4}$</td>
<td>$2 \times 10^{-4}$</td>
<td>$10^{-4}$</td>
<td>$4 \times 10^{-4}$</td>
</tr>
<tr>
<td>D-propranolol</td>
<td>$2 \times 10^{-4}$</td>
<td>$&gt; 10^{-4}$</td>
<td>$5 \times 10^{-4}$</td>
<td>$8 \times 10^{-4}$</td>
</tr>
<tr>
<td>Atenolol</td>
<td>$1 \times 10^{-4}$</td>
<td>$-$</td>
<td>$1.3 \times 10^{-4}$</td>
<td>$4 \times 10^{-4}$</td>
</tr>
<tr>
<td>Butoxamine</td>
<td>$5 \times 10^{-4}$</td>
<td>$-$</td>
<td>$&gt; 10^{-4}$</td>
<td>$1.4 \times 10^{-4}$</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>$&gt; 10^{-4}$</td>
<td>$1.6 \times 10^{-4}$</td>
<td>$&gt; 10^{-4}$</td>
<td>$&gt; 10^{-4}$</td>
</tr>
</tbody>
</table>

*Values are expressed as $K_i$ in the formula $K_i = \frac{I_{50}}{I + L/K_L}$, where $I_{50}$ is the concentration of the drug causing 50% inhibition of binding, $L$ and $K_L$ are the concentration and the dissociation constant respectively of the radioactive ligand (Williams and Lefkowitz, 1978). Where no value is shown, the drug was not tested in that particular system.
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FIGURE 2. [3H]-dihydroergocryptine ([3H]-DHE) binding to myocardial membranes from control and renal hypertensive rats. Scatchard analysis of the binding data expressed as the ratio of [3H]-DHE bound (mol/mg protein) to [3H]-DHE free (mol/liter) plotted against increasing [3H]-DHE bound (fmol/mg protein).

FIGURE 3. [125I]-iodohydroxybenzylpindolol binding to myocardial membranes from control and renal hypertensive rats. Scatchard analysis of the binding data expressed as the ratio of [125I]HYP bound (mol/mg protein) to [125I]HYP free (mol/liter) plotted against increasing [3H]-DHE bound (fmol/mg protein).

TABLE 2. Concentrations and Affinities of Adrenergic Receptors in Heart, Kidney, and Lung Membranes of Hypertensive and Control Rats

<table>
<thead>
<tr>
<th>Adrenergic receptors</th>
<th>Hypertensive rats</th>
<th>Control rats</th>
<th>p value†</th>
<th>Number of experiments‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (fmol/mg)</td>
<td>Affinity (nmol/liter)</td>
<td>Concentration (fmol/mg)</td>
<td>Affinity (nmol/liter)</td>
</tr>
<tr>
<td>Cardiac β receptors</td>
<td>41 ± 9*</td>
<td>0.10 ± 0.02</td>
<td>89 ± 16</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Cardiac α receptors</td>
<td>52 ± 2</td>
<td>4.0 ± 0.8</td>
<td>80 ± 6</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Renal β receptors</td>
<td>13 ± 2</td>
<td>7.1 ± 1.5</td>
<td>10 ± 2</td>
<td>6.7 ± 1.4</td>
</tr>
<tr>
<td>Pulmonary β receptors</td>
<td>272 ± 28</td>
<td>2.2 ± 0.13</td>
<td>307 ± 27</td>
<td>2.2 ± 0.15</td>
</tr>
</tbody>
</table>

*Results are expressed as mean values = 1 SEM.
†Determined using an unpaired Student's t test. n.s. = not significant.
‡In each experiment the Scatchard analysis was compiled from six points.
Plasma Membrane Marker Enzyme Activities in Cardiac Membranes from Hypertensive Rats and Controls

The observed changes in myocardial adrenergic receptors may be part of a generalized decrease in plasma membrane components in hypertrophied hearts. Accordingly, plasma membrane marker enzyme activities were measured in membranes prepared from both normal and hypertrophied hearts. Activities of ouabain-sensitive phosphatase, 5'-nucleotidase, and basal adenylate cyclase are shown in Table 3. The activities of each of these enzymes were similar in membranes prepared from control and hypertensive animals. The results suggest that the diminished number of α and β receptors from hypertrophied myocardium is a specific deletion effect and not the result of nonspecific dilution by cardiac hypertrophy.

Discussion

The myocardium of DOCA-salt hypertensive, renal hypertensive, and spontaneously hypertensive rats has a reduced concentration of β-adrenergic receptors.7-10 The results of our experiments show that the number of α receptors is also reduced in hypertrophied hearts from rats with renal hypertension. Both α and β receptors were reduced by 40%-50%, a reduction similar to that reported previously for cardiac β receptors in DOCA-salt hypertensive rats6-9 and spontaneously hypertensive rats.10

Beta-receptor concentration can be regulated in vitro and in vivo by the concentration of β-adrenergic agonist. Increased agonist stimulation produces a decrease in receptors.11 Similar reduction in receptor concentration can be produced in vivo by increasing the sympathetic stimulation of particular tissues.22 Less is known about the control of α receptors, but preliminary evidence suggests that α-receptor concentration can also be reduced after exposure to agonists. Stittmatter et al.23 have shown reduced α receptors on parotid cells following incubation with epinephrine, and Cooper et al.24 have shown specific reductions in platelet α receptors when cells are incubated with epinephrine.

Adrenergic receptors can be modulated by other hormones as well as by adrenergic agonists. In general, other hormones have been reported to produce differential effects on the α- and β-receptor populations. Thus, estrogen treatment increases α receptors but not β receptors in rabbit uterus,25 and cortisol reduces liver β receptors but not α receptors in adrenalectomized rats.26 Thyroid hormones affect cardiac α and β receptors in reciprocal fashion; thyroxine treatment causes an increase in β receptors and a decrease in α receptors.27, 28

The similarity in the magnitude of the reductions in α and β receptors, however, suggests a common causative mechanism in renal hypertension where cardiac α- and β-receptor concentrations decreased to a similar degree. It is likely that the receptor reduction is associated with increased stimulation by adrenergic agonists. It is of interest that α and β receptors of the rat salivary gland were found to increase to a similar extent after chemical sympathectomy.29 The lack of any detectable change in renal and pulmonary β receptors suggest that the specific changes observed solely in the heart are not caused by raised circulating plasma catecholamine levels in renal hypertensive rats,31 but are more likely associated with the increased cardiac sympathetic drive reported in experimental renal hypertension.13

The failure to detect any differences in the hypertrophied hearts, in the activities of three different enzymes commonly associated predominantly with the plasma membrane, suggests that the deletion of adrenergic receptors is a specific effect and rules out the possibility that the decrease is a dilutional effect from cardiac hypertrophy or an artifact of membrane preparation from hypertrophied hearts. Experiments are being conducted to determine whether the changes in adrenergic receptors precede or parallel the development of cardiac hypertrophy.

Since retention of sodium has been shown to occur in one-kidney, one clip Goldblatt hypertension,30 an alternative explanation might be that the receptor changes are mediated by increased sodium. We have previously reported similarly decreased cardiac β receptors in DOCA-salt and one-kidney, one clip hypertensive rats, further suggesting a role for sodium.8, 9 The control unilaterally nephrectomized rats used in those experiments, however, had similar cardiac β-receptor concentrations even though the DOCA-salt controls were sodium-loaded. This suggests that increased sodium is not a direct cause of the observed receptor changes.

Since the physiological role of cardiac α receptors is uncertain, it is difficult to assess the significance of a decrease in their concentration. According to Langer32 and Guicheney et al.,33 approximately 30%-40% of rat

<table>
<thead>
<tr>
<th>Rat</th>
<th>S'-nucleotidase (nmol/min/mg)</th>
<th>Ouabain-sensitive phosphatase (nmol/min/mg)</th>
<th>Adenylate cyclase (pmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-kidney control</td>
<td>12 ± 2*</td>
<td>0.65 ± 0.32</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>One-kidney, one clip hypertensive</td>
<td>11.3 ± 2</td>
<td>0.66 ± 0.3</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

*Data are expressed as mean values ± 1 SEM.
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heart α receptors are presynaptic. The observed decrease in cardiac α receptors may thus involve either the presynaptic or postsynaptic receptors or both. It is not known whether presynaptic receptor numbers can also be modulated by agonist concentration. A recent report by Crewes and Smith, however, shows that the responsiveness of cardiac presynaptic α receptors is decreased by chronic treatment with desipramine, which increases synaptic norepinephrine concentration. Since the presynaptic and postsynaptic α receptors have different sensitivities to adrenergic drugs, it is possible to distinguish between them by direct binding methods. We are currently investigating the possibility of changes in the numbers of pre- and postsynaptic alpha receptors in cardiac membranes from hypertensive rats.

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References

Changes in tissue alpha- and beta-adrenergic receptors in renal hypertension in the rat.

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