Specific Changes in Hypothalamic Alpha-Adrenoceptors in Young Spontaneously Hypertensive Rats

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SUMMARY Changes in the activity of hypothalamic and brain-stem adrenergic neurons have been reported in young spontaneously hypertensive rats (SHR) prior to the development of hypertension. We have measured central α- and β-adrenoceptor concentrations in 4-week-old SHR and Wistar-Kyoto (WKY) controls by direct radioligand binding studies using [3H]prazosin (α1), [3H]clonidine (α2), and [125I]iodohydroxybenzopindolol (β). The concentration of α1-adrenoceptors was significantly elevated in the hypothalamus of the SHR, 156.9 ± 10.4 compared with WKY, 119.4 ± 10.0 fmole/mg protein (n = 7, mean ± SEM, p < 0.0125). Alpha1-adrenoceptor concentrations in both the brain stem and cerebral cortex were similar in the two groups of animals. The increase in hypothalamic adrenoceptors was specific for α1-adrenoceptors, since similar concentrations of α2- and β-adrenoceptors were found in this region. (Hypertension 3: 516-520, 1981)

KEY WORDS • α-adrenoceptors • β-adrenoceptors • spontaneous hypertension • hypothalamus

A number of different observations indicate that the sympathetic nervous system is involved in the development of spontaneous hypertension in the rat. The young spontaneously hypertensive rat (SHR) has decreased epinephrine concentration and increased phenylethanolamine N-methyltransferase (PNMT) activity in the A1 and A2 regions of the brain stem, indicating altered activity of epinephrine-containing neurons in this region, which has been shown to be involved in blood pressure (BP) regulation. Changes in the activity of hypothalamic noradrenergic neurons have also been reported in young SHR. Wijnen et al. showed decreased norepinephrine turnover in discrete hypothalamic nuclei of SHR prior to the development of hypertension, but not in older animals. A reduction in norepinephrine content and dopamine β-hydroxylase activity in specific nuclei of the hypothalamus was demonstrated as early as 4 weeks after birth in the SHR. Thus, central neural mechanisms are implicated in the onset of hypertension in the spontaneously hypertensive rat.

The adrenergic receptor concentration in tissues is modulated by the degree of sympathetic stimulation under different physiological and pathological conditions. Thus, increased sympathetic stimulation was shown to cause a reduction in rat pineal β-adrenoceptor concentration, while chemical sympathectomy resulted in increased α- and β-adrenoceptor concentrations in the rat salivary gland. Alpha- and β-adrenoceptor concentrations in the hearts of rats made hypertensive by renal artery occlusion or DOCA-salt treatment are reduced. In both of these experimental models, increased cardiac sympathetic drive has been demonstrated.

This study was undertaken to determine whether changes in adrenergic receptor concentration can be detected in the hypothalamus and brain stem of the young SHR relative to Wistar-Kyoto (WKY) controls.

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Materials and Methods

Animals

The SHR together with appropriate WKY controls were obtained from colonies derived from the Kyoto-Wistar substrain. Animals from two sources, Iffa-Credo (France) or the Flinders Medical Centre (Australia) were used; SHR and WKY were obtained simultaneously from each supplier and maintained under identical conditions. Equal numbers of male and female 25- to 32-day-old WKY and SHR were used in each experiment. Systolic BP was measured in unanesthetized animals by tail plethysmography.

Drugs and Chemicals

Clonidine-[4-3H(N)]HCl, specific activity 22.2 Ci/mmol, and [3H] iodoxyhydroxybenzylpindolol ([3H]-HYP), specific activity 2200 Ci/mmol, were obtained from New England Nuclear; [3H]prazosin, specific activity 28 Ci/mmol, was purchased from the Sigma Chemical Company. Phentolamine mesylate was donated by Ciba Geigy. All other chemicals were analytical grade.

Membrane Preparation

Pooled brain regions from six to eight animals were used to prepare each membrane homogenate. The rats were killed by decapitation, the brains quickly removed, and the hypothalami, brain stems (medulla oblongata and pons), and cerebral cortices dissected on ice following the procedure described by Glowinski and Iversen. After washing in isotonic saline at 0°C, the brain regions were homogenized using a Polytron homogenizer in approximately 20 ml of 0.25 M sucrose containing 50 mM sodium phosphate, 4 mM MgSO4, pH 7.4 (for [3H]-HYP binding) or 5 mM Tris-HCl, 1 mM MgSO4, pH 7.4 (for [3H]prazosin and [3H]clonidine binding).

The homogenates were centrifuged for 1 minute at 10,000 g in a Sorvall RC2B centrifuge at 4°C. The supernatant was centrifuged at 38,000 g for 15 minutes and the pellet washed before resuspension in ice-cold 50 mM sodium phosphate, 4 mM MgSO4, 0.1% ascorbic acid, 1 mM pyrocatechol, 100 μM phentolamine, pH 7.4 for [3H]-HYP binding or 50 mM Tris-HCl, 10 mM MgSO4, 0.1% ascorbic acid, pH 7.4 for [3H]prazosin and [3H]clonidine binding.

Protein concentration of the final membrane suspension as measured by the method of Lowry et al. was 1-2 mg per ml for [3H]-HYP binding, and 3-4 μg per ml for [3H]prazosin and [3H]clonidine binding.

Adrenergic Receptor Analysis

Alpha1- and Alpha2-Adrenoceptors

Alpha1- and α1-adrenoceptors were measured using [3H]prazosin and [3H]clonidine respectively, as previously described. In brief, brain membranes (0.2-0.3 mg protein) were incubated with [3H]prazosin (0.1-0.4 nM) or [3H]clonidine (0.4-15 nM) for 30 minutes at 25°C in a total volume of 0.6 ml containing 50 mM Tris HCl, 10 mM MgSO4, 0.1% ascorbic acid, pH 7.4. Incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters. After washing with 15 ml of ice-cold incubation buffer and drying, the radioactivity on the filters was counted by liquid scintillation spectrometry. Specific binding was defined as the difference between total binding and binding that was not inhibited by 10 μM phentolamine.

As previously described, binding sites of [3H]prazosin and of [3H]clonidine on rat brain membranes were stereospecific and exhibited the expected pharmacological specificities of α1- and α2-adrenoceptors respectively. Thus, the relative order of potencies for inhibition of [3H]prazosin binding by adrenergic agonists was; 1-epinephrine > clonidine > l-norepinephrine > α-methylnorepinephrine > d-epinephrine > d-norepinephrine. The α1-selective antagonists, prazosin and WB4101, were potent inhibitors of [3H]prazosin binding, in contrast to the α2-antagonist, yohimbine. [3H]Clonidine binding was inhibited by the following α-agonsists in decreasing order of potency; clonidine > α-methylnorepinephrine > l-epinephrine > l-norepinephrine > d-epinephrine > d-norepinephrine. [3H] Clonidine binding was strongly inhibited by the α2-antagonist, yohimbine, while WB4101 and prazosin competed for binding only at high concentrations.

Beta-Adrenoceptors

Beta-adrenoceptors were measured using [3H]-HYP as previously described. Brain membranes (0.01-0.1 mg protein) were incubated with [3H]-HYP (0.02-0.3 nM) for 45 minutes at 37°C in 50 mM sodium phosphate, 4 mM MgSO4, 0.1% ascorbic acid, 1 mM pyrocatechol, 100 μM phentolamine, pH 7.4 in a total volume of 0.15 ml. Incubations were terminated by rapid filtration as above, except that filters were washed with 20 ml of 20 mM sodium phosphate, 4 mM MgSO4, pH 7.4 at 37°C. The filters were dried and counted in a Packard Autogamma Scintillation Spectrometer. Specific binding was defined as the difference between total binding and binding in the presence of 10 μM l-isoproterenol.

The order of potency of various β-adrenergic agonists in inhibiting [3H]-HYP binding to rat brain membranes was as follows: l-isoproterenol > l-epinephrine > l-norepinephrine > d-epinephrine. The l-stereoisomer of the antagonist, propranolol, was 100 times more potent in competing for binding than the d-isomer.

Results of all binding studies were analyzed according to the method of Scatchard. Values are expressed as mean ± SEM, and statistical significance was evaluated using the Student t test for unpaired samples.
Results

Systolic Blood Pressure and Heart Weight

Table 1 compares the systolic BPs, ventricle weights, and ventricle-to-body-weight ratios of 4-week-old WKY and SHR. As previously described in young animals, a slight elevation in systolic BP was seen in SHR (93.6 ± 1.9 mm Hg) compared to WKY (87.1 ± 1.6; n = 29, p < 0.01). Cardiac hypertrophy has been reported early in the development of spontaneous hypertension, and in this study a significant elevation in ventricle weight was also observed in the 4-week-old SHR.

Table 1. Systolic Blood Pressure and Heart Weight in 4-Week-Old WKY and SHR

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Ventricle weight (g)</th>
<th>Ventricle/body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>87.1 ± 1.6</td>
<td>0.23 ± 0.01</td>
<td>0.46 ± 0.01</td>
</tr>
<tr>
<td>SHR</td>
<td>93.6 ± 1.9*</td>
<td>0.29 ± 0.01†</td>
<td>0.53 ± 0.01†</td>
</tr>
</tbody>
</table>

Hypothalamic Adrenergic Receptors

Similar concentrations of α1- and β-adrenoceptors were present in membranes prepared from hypothalami of 4-week-old WKY and SHR; however, an increased concentration of α2-adrenoceptors in SHR was observed in this region. Data from a typical [3H]clonidine binding experiment are shown in figure 1. Mean results of all experiments are shown in table 2. Similar results were obtained with rats from the two different sources (see Methods).

No differences were detected in the affinities of α1-, α2-, or β-adrenoceptors in the hypothalami of WKY and SHR, mean apparent dissociation constants (Kd) being 0.21 ± 0.04 nM for [3H]prazosin, 5.74 ± 0.77 nM for [3H]clonidine, and 0.14 ± 0.02 nM for [125I]-HYP sites.

Brain Stem and Cerebral Cortical Adrenergic Receptors

No differences were found in either the concentrations or the affinities of α1- and α2-adrenoceptors in the brain stems of WKY compared with SHR (table 2). The mean apparent dissociation constant for [3H]prazosin was 0.11 ± 0.01 nM, and for [3H]clonidine, 3.86 ± 0.47 nM. To determine whether this increase in α2-adrenoceptors was specific to the hypothalamus, α2-adrenoceptors were also measured in membranes prepared from the cerebral cortices of WKY and SHR. Similar concentrations of α2-adrenoceptors in WKY and SHR were present in this region (table 2), the mean apparent dissociation constant being 4.38 ± 0.51 nM.

Table 2. Adrenergic Receptor Concentrations* in Brain Regions of WKY and SHR

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Rat group</th>
<th>[3H]clonidine (α2)</th>
<th>[3H]prazosin (α1)</th>
<th>[125I]HYP (β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>WKY</td>
<td>119.4 ± 10.0†</td>
<td>101.5 ± 4.3</td>
<td>10.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>156.9 ± 10.4†</td>
<td>110.8 ± 5.8</td>
<td>10.4 ± 1.0†</td>
</tr>
<tr>
<td>Brain stem</td>
<td>WKY</td>
<td>87.6 ± 17.5</td>
<td>98.6 ± 5.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>84.2 ± 18.1</td>
<td>103.5 ± 5.3</td>
<td>—</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>WKY</td>
<td>101.5 ± 5.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>97.1 ± 12.1</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Receptor concentrations are expressed as fmole of ligand bound per mg of protein (mean ± SEM). Numbers in parentheses denote number of experiments.
†p < 0.01 by unpaired Student t test.
Discussion

Results of this study demonstrate a selective increase in the concentration of hypothalamic adrenergic receptors in 4-week-old SHR prior to the development of hypertension. This increase in receptor concentration was restricted to \( \alpha_2 \)-adrenoceptors, there being no change in \( \alpha_1 \)- or \( \beta \)-adrenoceptors in the same preparation. Moreover, the concentration of \( \alpha_2 \)-adrenoceptors was selectively increased in the hypothalamus of SHR. In two other brain regions studied, the brain stem and the cerebral cortex, no differences in \( \alpha_2 \)-adrenoceptor concentrations were detected. The observation that changes in \( \alpha \)-adrenoceptors are restricted to a discrete brain region may explain why other workers failed to find any difference in the concentration of \([\text{H}]\)clonidine binding sites in membranes from whole brains of young WKY and SHR.\(^{20}\) However, changes in \( \alpha \)-adrenoceptors in discrete brain regions have been reported. Gheyouche et al.\(^{21}\) have described an increase in \( \alpha_1 \)-adrenoceptor concentrations in the pons, midbrain, and hippocampus of the adult SHR, but no changes in the hypothalamus. In contrast, Cantor et al.\(^{22}\) have reported elevated \( \alpha_1 \)-adrenoceptor concentrations in the hypothalamus of SHR but no changes in the pons, midbrain, hippocampus, medulla, or striatum. The reason for the conflicting results reported in this and other studies is not clear, but may be due in part to methodological differences. Gheyouche et al.\(^{21}\) and Cantor et al.\(^{22}\) used only single concentration points for the majority of their receptor assays. All of the receptor concentrations reported in this study are the results of Scatchard analysis of binding at six different ligand concentrations.

There is considerable evidence available to indicate an involvement of central catecholaminergic mechanisms in various forms of experimental hypertension.\(^{5, 23-25}\) Early studies in the SHR suggested that in this model, central catecholaminergic neurons were probably involved more in the onset and development of the hypertension than in its maintenance. Haeusler et al.\(^{26, 27}\) showed that intraventricular 6-hydroxydopamine (6-OHDA) administration to adult SHR had no effect on BP; however, similar treatment in young SHR markedly attenuated the rise in BP. Neonatal 6-OHDA treatment of SHR was shown to prevent the development of hypertension.\(^{28}\)

Alterations in other indices of central catecholamine metabolism have been described in the young SHR. Significant reductions in norepinephrine content\(^4 \) and dopamine \( \beta \)-hydroxylase activity\(^\delta \) have been reported in discrete hypothalamic nuclei of 4-week-old SHR. Several laboratories have reported reduced levels of catecholamines in discrete brain regions of adult SHR,\(^5, 29\) while Versteeg et al.\(^{20}\) reported increased concentrations of catecholamines in selected brain-stem areas, and Wijnen et al.\(^1\) could detect little difference in steady-state norepinephrine concentration between WKY and SHR at 3, 7, and 10 weeks of age. However, some reports of age-dependent alterations in catecholamine metabolism exist: le Quan Bui et al.\(^4\) recently described reduced norepinephrine content in some medullary and hypothalamic nuclei in the SHR at 4 but not 12 weeks of age. The alterations in epinephrine concentration\(^\gamma \) and phenylethandamine N-methyltransferase (PNMT) activity\(^\delta \) in brain-stem areas described by Saavedra were restricted to younger, 4-week-old SHR.

Such discrepancies between laboratories may reflect methodological or strain differences. In any case, interpretation of changes in endogenous catecholamine levels is difficult and does not necessarily imply differences in sympathetic activity. The observation by Wijnen et al.\(^1\) of reduced norepinephrine turnover in discrete hypothalamic nuclei of 3-week-old SHR led these authors to suggest that decreased activity of norepinephrine neurons terminating in the anterior hypothalamus is related to the onset and early development of genetic hypertension. A recent report of augmented vasodepressor responsiveness to a centrally administered \( \alpha \)-adrenergic agonist in the SHR supplies further evidence for the involvement of central \( \alpha \)-adrenergic mechanisms in BP control in this hypertensive model.\(^{31}\)

Hypothalamic \( \alpha_2 \)-adrenoceptors have been implicated in the inhibition of central sympathetic outflow.\(^{40}\) Furthermore, the hypotensive actions of clonidine and \( \alpha \)-methyldopa have been explained in terms of their stimulation of central \( \alpha \)-adrenoceptors (Scriabine et al.\(^{32}\) Davies and Reid,\(^{44}\) Isaac\(^45\)).

The hypothalamus contains a functionally heterogeneous group of adrenergic receptors,\(^46\) and it is likely that the increase in total hypothalamic \( \alpha_2 \)-adrenoceptors we observed in young SHR reflects increases in certain critical areas of the hypothalamus. While the pre- or postsynaptic location of these \( \alpha_2 \)-adrenoceptors within the central nervous system is not known with any certainty, Haeusler and Finch\(^47\) reported that intracerebroventricular administration of 6-ODHA did not alter the hypotensive response to clonidine, suggesting an involvement of postsynaptic \( \alpha \)-adrenoceptors. Central \( \alpha_2 \)-adrenoceptors measured using \([\text{H}]\)clonidine have been shown to be largely postsynaptic on the basis of their persistence after surgical or chemical sympathectomy.\(^47, 48\)

Thus, in the hypothalamus, diminished norepinephrine activity and reduced stimulation of \( \alpha \)-adrenoceptors may be directly related to the rise in BP in the SHR. Our finding of increased \( \alpha_2 \)-adrenoceptors in the hypothalamus of 4-week-old SHR is further evidence for the understimulation of specific central \( \alpha \)-adrenergic receptors in these animals.

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