Changes in Central Catecholaminergic Neurons in the Spontaneously (Genetic) Hypertensive Rat

JUAN M. SAAVEDRA, HORST GROBECKER, AND JULIUS AXELROD

SUMMARY Catecholamines and catecholamine-synthesizing enzymes have been examined in specific brain areas during the development of spontaneously (genetic) hypertensive (SH) rats. Changes in catecholamine metabolism were localized to regions of the brain implicated in the regulation of blood pressure. Norepinephrine levels and dopamine-β-hydroxylase (DBH) activities were decreased in specific nuclei of the hypothalamus and in the nucleus interstitialis striae terminalis ventralis, in both young and adult rats. The decrease in the formation of norepinephrine can result in a reduced activation of central α-adrenergic receptors which may be related causally to the onset of hypertension. The activity of the epinephrine-forming enzyme, phenylethanolamine-N-methyltransferase (PNMT), was increased in the A1 and A2 areas of the brainstem in young SH rats, but it was normal in adult hypertensive animals. These results implicate adrenergic neurons in the brainstem and noradrenergic neurons in the hypothalamus in the development of spontaneous (genetic) hypertension in rats.

CATECHOLAMINES have been implicated in the pathophysiology of hypertension. The most compelling evidence of the involvement of catecholamines in hypertension is the demonstration that many drugs therapeutically useful in the treatment of hypertension also affect the adrenergic nervous system at a variety of sites, modifying the uptake, release, and storage of the neurotransmitter or acting directly on the adrenergic receptors.

It now is recognized that not only peripheral catecholaminergic nerves and the adrenal medulla play roles in the physiological regulation of blood pressure and in the expression of at least some forms of hypertension, but that central catecholaminergic neurons also are implicated.

Some of the catecholamine-rich areas in the brain that have been proposed to be involved in cardiovascular regulation are localized in the anterior hypothalamus and in the brainstem [areas A1 and A2]. These areas are densely supplied with noradrenergic nerves. Area A1 of the rat brain contains the cell bodies of the catecholamine neurons that send axons to the spinal cord, and area A2 corresponds in part to the nucleus of the tractus solitarius in which the majority of the fibers of the carotid sinus nerve terminate.

In addition to norepinephrine, the A1 and A2 areas of the brainstem have the highest levels of the epinephrine-forming enzyme, phenylethanolamine N-methyltransferase (PNMT) found in the brain. Epinephrine also has been detected in the A1 region and in the A2 area as well (Saavedra et al., unpublished results).

By the use of sensitive isotopic-enzymatic micromethods combined with microdissection of the rat brain, a study of catecholamine content and the enzymes involved in their formation in discrete brain areas associated with central autonomic control and blood pressure regulation is now possible.

An examination of norepinephrine content and the norepinephrine-forming enzyme in specific brain areas during the development of an animal model of hypertension is described. Spontaneously (genetic) hypertensive (SH) rats were chosen because they exhibited some essential aspects of human hypertension, and changes in the peripheral and central catecholamine metabolism have been reported in such rats.

Methods

Male SH rats and normotensive rats of the Wistar-Kyoto substrain from which SH rats were derived were maintained under identical conditions for 1 week after being received from Taconic Farms, Germantown, New York. The systolic blood pressure was measured in the tail, PE 300; Narco Biosystems, Inc.).

The rats were killed by decapitation at 9 a.m., the brains were quickly removed and immediately frozen on dry ice, and serial sections 300 μm thick were cut in a cryostat at −10°C. Specific areas and brain nuclei were located under a dissecting microscope and dissected by the use of a needle with an internal diameter of 0.5 mm, as described elsewhere. This dissection technique allows the precise localization and excision of specific brain nuclei and areas, with a high degree of reproducibility.

To assay norepinephrine, brain tissue from one rat was homogenized in 35 μl of 0.1 N perchloric acid, and 5 μl were removed for protein determination. After centrifugation, norepinephrine was measured in duplicate samples of the supernatant solution, as previously described. The assay was based on the incubation of norepinephrine in the presence of a methyl donor, [H]-methyl S-adenosyl-L-methionine, of high specific activity (New England
Nuclear: specific activity, 4.5 mCi/μmol and partially purified phenylethanolamine-N-methyltransferase. The radioactive normetanephrine formed in the reaction was absorbed on activated alumina, extracted by perchloric acid, and selectively extracted into an organic solvent. Under the conditions of the assay, as little as 25 pg of norepinephrine could be reliably estimated, with no interference from other catecholamines or catecholamine precursors.

For the assay of dopamine-β-hydroxylase (DBH), brain nuclei from two rats were removed, pooled, and homogenized in 75 μl of ice-cold 5 mM Tris-HCl buffer, pH 7.4, containing 0.1% of Triton X-100 (vol/vol). Five microliters were removed for protein determination, and the homogenates were centrifuged at 5000 g for 10 minutes. DBH was then measured by a modification of the method of Molinoff et al.¹³ The results were corrected by the use of internal standards of partially purified DBH added to identical samples of the supernatant extract of all regions studied.¹⁴ Phenylethanolamine N-methyltransferase (PNMT) was assayed by a modification of the method previously described.¹⁵ Brain tissue from one rat was homogenized in 75 μl of 0.05 M sodium phosphate buffer, pH 7.9, containing 0.1% of Triton X-100, and 5 μl were removed for protein determination.¹⁶ After centrifugation, the enzyme activity was measured in duplicate samples of the supernatant extract. The assay was based on the incubation of the enzyme in the presence of saturated concentrations of phenylethanolamine and a methyl donor of high specific activity, ⁴H-methyl-S-adenosyl-L-methionine. The radioactive product formed, ⁴H-N-methyl phenylethanolamine, was selectively extracted into toluene containing 3% of isoamylalcohol (vol/vol). The organic solvent was evaporated under a stream of air, and the remaining radioactivity was counted. The results were corrected by the use of internal standards of partially purified PNMT.¹⁰ The methyl donor of high specific activity and a drying procedure to eliminate a volatile radioactive contaminant allowed the precise measurement of as little as 50 fmol of enzyme activity (Saavedra, in preparation).

### Results

#### Norepinephrine

The norepinephrine content was examined in 18 separate nuclei of the hypothalamus of 4-week-old genetically hypertensive rats. At this age, rats had a slightly but significantly elevated blood pressure, compared to their normal controls (see Table 1). A significant reduction of the norepinephrine content in certain forebrain areas (nucleus interstitialis striae terminals ventralis, nuclei hypothalamic anterior, periventricularis, and paraventricularis) was found in young (4-week-old) rats (Table 1). These changes were localized to the areas mentioned above, because other nearby nuclei, such as the medial forebrain bundle, nucleus suprachiasmatis, area reticularis, and median eminence, did not show changes in the amine content. In addition to the forebrain areas, the nucleus dorsomedialis of the hypothalamus was the only nucleus to show significant decreases in norepinephrine content (Table 1; Fig. 1).

The reduction in norepinephrine levels not only was present in young rats, but persisted in the same discrete hypothalamic nuclei in adult rats (Table 1).

Both in young and in adult SH rats, these changes were localized only to the hypothalamus and closely related areas, but not to other norepinephrine-containing regions such as the locus coeruleus, an area of the brain containing noradrenergic cell bodies. The cerebellum, which receives its noradrenergic innervation from the locus coeruleus, and other brainstem areas, such as the areas A₁, A₂, area postrema, the nucleus raphe magnus, the inferior hypotalamic nuclei in adult rats (Table 1).
showing decreases in norepinephrine content. Thus, in 4-week-old SH rats, DBH activity was low in the nucleus interstitialis striae terminalis ventralis and the nucleus hypothalamic periventricularis (Table 2). The nucleus dorsomedialis showed a smaller but not significant decrease in enzyme activity.

In adult SH rats, reductions in DBH activity were noted in the nucleus interstitialis striae terminalis, the nucleus hypothalamic anterior and periventricularis, and the nucleus ventromedialis. Smaller, not significantly different, changes were also found in the nucleus dorsomedialis.

**Phenylethanolamine N-methyltransferase**

Elevation in PNMT activity in the A1 and A2 areas of the brainstem was detected in young SH rats (Fig. 2). These changes in PNMT activity were no longer present after the age of 6 weeks in the A1 area, and after 10 weeks of age in the A2 area. Thus adult 14-week-old SH rats, although they had a marked hypertension, did not show changes in enzyme activity when compared with age-matched Wistar-Kyoto controls (Table 3).

The PNMT activity of the area postrema was examined at 4, 10, and 14 weeks of age. Although increases in enzyme activity were noted, these results were not significantly different from data for age-matched controls (Table 3).

Neither the hypothalamic areas rich in PNMT, the nucleus paraventricularis and the median eminence, nor the nucleus locus coeruleus showed differences in enzyme activity when compared to the Wistar-Kyoto controls at any time during the development of hypertension.

**Discussion**

The SH rats were obtained by selective inbreeding of Wistar rats from the Wistar-Kyoto substrain. Thus, the Wistar-Kyoto rats represent the best control for comparison with the SH rats, since they are the closest, genetically. The use of different strains of Wistar rats as controls for the SH rats could result in differences in catecholamine metabolism arising solely from genetic factors which may not be related to the pathogenesis of the hypertension.

### Table 2  Dopamine-β-hydroxylase Activity in Brain Nuclei of SH and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Nucleus interstitialis striae terminalis ventralis</th>
<th>Wistar-Kyoto</th>
<th>SHR</th>
<th>4 Weeks</th>
<th>14 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2 ± 0.3</td>
<td>1.5 ± 0.4*</td>
<td>5.1 ± 0.6</td>
<td>2.5 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>1.7 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>2.2 ± 0.1</td>
<td>1.4 ± 0.2†</td>
<td></td>
</tr>
<tr>
<td>3.7 ± 0.2</td>
<td>2.9 ± 0.1*</td>
<td>4.7 ± 0.2</td>
<td>3.0 ± 0.3*</td>
<td></td>
</tr>
<tr>
<td>2.0 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>2.9 ± 0.2</td>
<td>2.1 ± 0.2†</td>
<td></td>
</tr>
<tr>
<td>2.8 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>4.2 ± 0.4</td>
<td>3.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>22.9 ± 3.8</td>
<td>30.4 ± 4.8</td>
<td>23.7 ± 3.3</td>
<td>28.7 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>Area A1</td>
<td>3.4 ± 0.4</td>
<td>3.5 ± 0.4</td>
<td>1.3 ± 0.5</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Area A2</td>
<td>4.6 ± 0.8</td>
<td>4.2 ± 0.5</td>
<td>2.6 ± 0.5</td>
<td>3.4 ± 0.6</td>
</tr>
</tbody>
</table>

Results are expressed as nmol/mg of protein per hour (mean ± SEM), for groups of 10 individual rats. Statistically significant (by Student's t-test):
* P < 0.01.
† P < 0.05.
Since PNMT catalyzes the formation of epinephrine, and 

vations of increases in PNMT activity in the A, and A2 

areas of the brainstem of young (4-week-old) SH rats. 

regions, since other PNMT-rich areas in the brain did not 

(Saavedra et al., unpublished observations), the possibility 

TABLE 3 

were no longer present in adult, 14-week-old rats. 

show differences in enzyme activity. The elevations in 

of an increased formation of epinephrine in these regions 

with the increased sympathetic activity, as measured by 

creases are due to inhibition of enzyme activity, to a 

was reduced in the same brain areas and to approximately 

PNMT inhibitors could result in a normalization of the 

Hypertension.6 

Norepinephrine levels were decreased in specific nuclei 

the forebrain of young SH rats, and these changes persisted in adult rats. Some of the nuclei with reduced norepinephrine (nucleus interstitialis striae terminalis ventralis, nucleus hypothalamic anterior, and periventricularis) were located in close proximity to each other, in a small area ventral to the anterior commissura at the anterior hypothalamic level. Others, such as the nucleus dorsomedialis and the nucleus ventromedialis, were located in the posterior hypothalamus. 

The decrease in norepinephrine levels probably is due to a diminished synthesis, since the activity of DBH also was reduced in the same brain areas and to approximately the same extent as norepinephrine. Whether these decreases are due to inhibition of enzyme activity, to a reduced number of enzyme molecules, or to a decrease in the number of noradrenergic nerve terminals has not been established. 

The nuclei with reduced norepinephrine and DBH do not receive their innervation from the locus coeruleus, but probably from other brainstem areas.28, 29 Recent findings suggest that part of their noradrenergic innervation could come from the A2 area in the brainstem (Saavedra et al., unpublished observations). There were no changes in norepinephrine in the nucleus locus coeruleus or areas 

plasma19, 23, 24 and with the decreased epinephrine synthesis in the adrenal gland,24 also described at the onset of hypertension in SH rats. 

Elevated PNMT activity in the A1 and A2 areas also has been detected in another form of hypertension, the deoxycorticosterone acetate (DOCA)-salt hypertensive rats6 (Saavedra et al., unpublished observations); this indicates the possibility of a common mechanism in different forms of hypertension. 

The participation of adrenergic mechanisms in the development of the spontaneous hypertension and DOCA-salt hypertension in the rat suggested that the use of PNMT inhibitors could result in a normalization of the hypertension.6 

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\[ P \text{ 7.0 mm} \]

\[ P \text{ 7.4 mm} \]

FIGURE 2 Localization of changes in PNMT activity in specific nuclei of 4-week-old SH rats. Frontal sections are numbered in the lower righthand corner with the coordinates posterior to the frontal zero plane, as indicated by the Konig and Klippel stereotaxic atlas.20 Abbreviations: ap, area postrema; nts, nucleus tractus solitarius; rl, nucleus reticularis lateralis; P, tractus corticospinalis. The solid circles (A1, lower part of the sections; A2, upper part of the sections) represent the areas with significant increases in PNMT activity.

Our results confirm and extend the preliminary observations of increases in PNMT activity in the A1 and A2 areas of the brainstem of young (4-week-old) SH rats. Since PNMT catalyzes the formation of epinephrine, and this amine has been identified in both A1 and A2 areas,20 our results confirm and extend the preliminary observations6 of increases in PNMT activity in the A1 and A2 areas of the brainstem of young (4-week-old) SH rats. 

The early increase in brain PNMT activity coincides with the increased sympathetic activity, as measured by increased norepinephrine levels and DBH activity in plasma19, 23, 24 and with the decreased epinephrine synthesis in the adrenal gland,24 also described at the onset of hypertension in SH rats. 

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receiving their noradrenergic innervation from this nucleus, such as the cerebellum, in any of the brainstem areas, including the areas A₁ and A₂ in SH rats, indicating that the changes in norepinephrine metabolism are confined to certain forebrain areas of the hypothalamus.

The changes in catecholamines in SH rats occur in hypothalamic areas related to the regulation of blood pressure. These hypothalamic regions may be a part of complex blood pressure regulatory pathways, and probably are connected with regions of the brainstem receiving the baroreceptor afferent fibers, such as the nucleus tractus solitarii (NTS). A number of hypothalamic brain stem connections have been described, and recently Saper et al. reported a direct anatomical connection between the hypothalamus and the NTS.

Early in the development of hypertension, there is a decreased norepinephrine content in selected brain areas, increased PNMT in the brainstem, and activation of the peripheral sympathetic system, as indicated by elevation of plasma norepinephrine and DBH levels (Grobecker et al., unpublished observations). There also is a decreased formation of catecholamines in the adrenal medulla (Table 4) which could be a compensatory mechanism related to increased peripheral sympathetic nerve activity and/or to changes in catecholamine neurons in the brain.

In adult, hypertensive rats, the changes in brain norepinephrine persist, but the increases in brain PNMT are no longer present. The signs of increased peripheral sympathetic activity subside, and catecholamine synthesis in the adrenal gland is increased mainly through induction of tyrosine hydroxylase (Table 4) (Grobecker et al., unpublished observations).

The existence of a selective deficiency of central norepinephrine neurons could explain the central hypotensive actions of antihypertensive drugs such as clonidine and \( \alpha \)-methyl dopa, as well as the hypotensive effect seen after treatment of patients with l-dopa. The reduction of norepinephrine in the anterior hypothalamus indicates the possibility of a deficiency of stimulation of \( \alpha \)-adrenergic receptors in this area of the brain in hypertension. Since it has been proposed that clonidine reduces blood pressure by acting centrally as an \( \alpha \)-adrenergic stimulating agent, our findings suggest that the anterior hypothalamus is one of the possible sites of action for clonidine. This is consistent with the findings of Struyker-Boudier and co-workers9 to the effect that decreases in blood pressure can be obtained after administration of clonidine or norepinephrine to areas caudoventral to the commissure, anterior and rostral to the fornix region of the brain, which we have found to have a reduced norepinephrine content. The action of another antihypertensive drug, \( \alpha \)-methyl dopa, might be explained by its ability to cross the blood-brain barrier and be subsequently converted to \( \alpha \)-methyl norepinephrine, which can stimulate the central \( \alpha \)-adrenergic receptors and thus decrease blood pressure. The hypotensive effects of l-dopa administration might also be explained through production of catecholamines in brain and \( \alpha \)-receptor stimulation.

### Table 4  Sequential Changes on Sympathetic Activity in SH Rats

<table>
<thead>
<tr>
<th></th>
<th>Young (4-week-old rats)</th>
<th>Adult (14-week-old rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral sympathetic nerves</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine in plasma</td>
<td>Decreased (19, 24)</td>
<td>Normal (34, 24)</td>
</tr>
<tr>
<td>DBH in plasma</td>
<td>Decreased (19, 23, 24)</td>
<td>Normal (34, 24)</td>
</tr>
<tr>
<td>Total catecholamines</td>
<td>Normal (19)</td>
<td>Increased (34)</td>
</tr>
<tr>
<td><strong>Adrenal medulla</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine hydroxylase</td>
<td>Decreased*</td>
<td>Increased (35)</td>
</tr>
<tr>
<td>DBH</td>
<td>Decreased*</td>
<td>Normal</td>
</tr>
<tr>
<td>PNMT</td>
<td>Decreased*</td>
<td>Normal</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Normal (19)</td>
<td>–</td>
</tr>
</tbody>
</table>


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