Brain catecholamines in spontaneously hypertensive and DOCA-salt hypertensive rats.

Kazuyuki Fujino∗

*Okayama University,
Brain catecholamines in spontaneously hypertensive and DOCA-salt hypertensive rats.*

Kazuyuki Fujino

Abstract

The concentrations and alpha-methyl-p-tyrosine (alpha-MPT) induced disappearance of catecholamines, adrenaline, noradrenaline and dopamine, were measured in selected areas of the brainstem and hypothalamus of spontaneously hypertensive rats (SHR) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The catecholamine levels were measured by a sensitive radioenzymatic assay method combined with microdissection of the rat brain. The adrenaline concentration was higher in the area A1 of young SHR, but not in adult SHR, than in age-matched control rats. Noradrenaline concentrations and the alpha-MPT induced noradrenaline disappearance were less in the rostral part of the nucleus tractus solitarii (NTS) and the nucleus hypothalamic anterior of young SHR, and in the rostral part of the NTS of adult SHR. On the other hand in DOCA-salt hypertensive rats, the concentrations of adrenaline and noradrenaline were the same as in control rats in the examined areas. The alpha-MPT induced noradrenaline disappearance was less in the rostral part of the NTS of DOCA-salt hypertensive rats. Dopamine concentrations and the alpha-MPT induced dopamine disappearance were the same in the examined areas of SHR and DOCA-salt hypertensive rats. The results suggest that SHR have a change in adrenergic neural activity in the brainstem and a decrease in noradrenergic neural activity in the brainstem and hypothalamus while DOCA-salt hypertensive rats have a decrease in noradrenergic neural activity in the brainstem. Such changes in brain catecholaminergic neurons may have played an important role in the development of hypertension in these rats.

KEYWORDS: catecholamines, brainstem, hypothalamus, spontaneously hypertensive rats (SHR), DOCA-salt hypertensive rats

*PMID: 6149670 [PubMed - indexed for MEDLINE]
Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

BRAIN CATECHOLAMINES IN SPONTANEOUSLY HYPERTENSIVE AND DOCA-SALT HYPERTENSIVE RATS

Kazuyuki Fujino
Third Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan
(Director : Prof. Z. Ota)
Received February 25, 1984

Abstract. The concentrations and \(\alpha\)-methyl-\(p\)-tyrosine (\(\alpha\)-MPT) induced disappearance of catecholamines, adrenaline, noradrenaline and dopamine, were measured in selected areas of the brainstem and hypothalamus of spontaneously hypertensive rats (SHR) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The catecholamine levels were measured by a sensitive radioenzymatic assay method combined with microdissection of the rat brain. The adrenaline concentration was higher in the area A1 of young SHR, but not in adult SHR, than in age-matched control rats. Noradrenaline concentrations and the \(\alpha\)-MPT induced noradrenaline disappearance were less in the rostral part of the nucleus tractus solitarii (NTS) and the nucleus hypothalamic anterior of young SHR, and in the rostral part of the NTS of adult SHR. On the other hand in DOCA-salt hypertensive rats, the concentrations of adrenaline and noradrenaline were the same as in control rats in the examined areas. The \(\alpha\)-MPT induced noradrenaline disappearance was less in the rostral part of the NTS of DOCA-salt hypertensive rats. Dopamine concentrations and the \(\alpha\)-MPT induced dopamine disappearance were the same in the examined areas of SHR and DOCA-salt hypertensive rats. The results suggest that SHR have a change in adrenergic neural activity in the brainstem and a decrease in noradrenergetic neural activity in the brainstem and hypothalamus while DOCA-salt hypertensive rats have a decrease in noradrenergetic neural activity in the brainstem. Such changes in brain catecholaminergic neurons may have played an important role in the development of hypertension in these rats.

Key words : catecholamines, brainstem, hypothalamus, spontaneously hypertensive rats (SHR), DOCA-salt hypertensive rats.

Central catecholaminergic neurons play an important role in the regulation of blood pressure and in the expression of some forms of hypertension (1-27). Intraventricular administration of noradrenaline causes a fall in systemic blood pressure and bradycardia in different animal species, and pretreatment with an intraventricular injection of an \(\alpha\)-blocker markedly diminishes these actions (4, 5). Pretreatment with 6-hydroxydopamine prevents the development of some forms of hypertension, including those of spontaneously hypertensive rats (SHR) (6) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats (7, 8). Changes in
catecholamine metabolism and activity have been studied in the brainstem and hypothalamus of hypertensive rats (1-3, 9-27). However, the studies have indicated both increased (10, 12, 17-19, 21, 27) and decreased (1, 2, 11, 16, 20, 24, 25) activity of catecholaminergic neurons by measurements of catecholamine levels, enzyme activity or catecholamine disappearance after pharmacological inhibition of synthesis. To eliminate these inconsistencies, the author studied the catecholamine concentrations and the DL-α-methyl-D-tyrosine methylester hydrochloride (α-MPT) induced catecholamine disappearance in selected areas of the brainstem and hypothalamus in an attempt to localize the dysfunctional sites leading to hypertension in SHR and DOCA-salt hypertensive rats. The catecholamine levels in discrete brain regions were measured by a sensitive radioenzymatic assay method combined with microdissection of the rat brain.

MATERIALS AND METHODS

Animals. Male SHR were used with normotensive age-matched Wistar-Kyoto (WKY) rats. The rats were purchased from Charles River Japan. Rats aged 4 weeks (young) and 12 weeks (adult) were habituated for one week in the laboratory. They were given chow and tap water ad libitum. The systolic blood pressure was measured in conscious rats by a tail sphygmographic method (Ueda Electronic Works, Model USM-105R).

Male Wistar rats aged 4 weeks were also purchased from Charles River Japan. DOCA-salt hypertension was produced by the method of Friedman et al. (28). The rats underwent a left nephrectomy by dorsal incision under ether anesthesia and were divided into two groups. The group of rats in which hypertension was induced received weekly subcutaneous injections of 10 mg of DOCA suspended in 0.2 ml of sesame oil and were given 1% NaCl in tap water ad libitum. The other group, the control rats, received weekly subcutaneous injections of sesame oil and were given tap water to drink. The systolic blood pressure was measured weekly in conscious rats by a tail sphygmographic method. The rats were sacrificed after 4 weeks of treatment 24 h after the last injection.

Catecholamine concentrations and α-MPT induced catecholamine disappearance in discrete brain regions. Some of the rats were killed by decapitation at 7 a.m. without treatment, and others were killed by decapitation at 11 a.m., 4 h after an intraperitoneal injection of α-MPT (24, 29) which inhibits tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine synthesis. The drug was diluted in physiologic saline and injected at a dose of 300 mg/kg (24, 29). The turnover of catecholamines was determined by the decline of endogenous catecholamine concentrations after inhibition of synthesis according to Brodie et al. (29).

The brains were taken out and frozen with powdered dry ice immediately after decapitation. Serial frontal sections of 400 μm thickness were cut with a freezing microtome at -10 °C. Specific brain areas were located under a stereoscope and punched out with a needle with an internal diameter of 0.4 – 1.1 mm, according to Palkovits (30). This technique allowed precise localization and excision of specific brain areas, with a high degree of reproducibility (31). The brainstem and hypothalamic areas were located according to the atlas of Palkovits et al. (32) and Pellegrino et al. (33), respectively.

Regions dissected were the area A4, the nucleus commissuralis (NCO), the area A3, the rostral part of the nucleus tractus solitarii (NTS) and the locus coeruleus (LC) of the brainstem, and the median eminence (ME), the nucleus paraventricularis (NPV) and the nucleus
Fujino: Brain catecholamines in spontaneously hypertensive and

Brain Catecholamines in Hypertensive Rats

Fig. 1. Drawings of frontal sections at different levels of the brainstem. The caudal (P) distance from the interauricular line is in μm. Punch numbers: 1, area A1; 2, nucleus commissuralis; 3, area A3; 4, rostral part of the nucleus tractus solitarii; 5, locus coeruleus.

Fig. 2. Drawings of frontal sections at different levels of the hypothalamus. The rostral (A) distance from the interauricular line is in μm. Punch numbers: 6, median eminence; 7, nucleus paraventricularis; 8, nucleus hypothalmic anterior.
hypothalamic anterior (NHA) of the hypothalamus (Figs. 1, 2). The area A_4 corresponded to the catecholamine cell group A_4 of Dahlström and Fuxe (34). It was located in the rostral and lateral part of the nucleus reticularis lateralis of the medulla oblongata, between the pyramidal tract and the tractus spinalis nervi trigemini, lateral to the olivary complex and just under the ventral surface of the medulla oblongata. Areas containing the NTS were divided into three parts (35, 36). The NCO was the caudal part of the NTS, and was located in the midline just dorsal to the central canal. The area A_5 was the medial part of the NTS, and was located caudal to the obex, lateral to the central canal and ventrolateral to the area postrema, close to the bottom of the fourth ventricle. The rostral part of the NTS was located rostral to the obex and lateral to the bottom of the fourth ventricle. The LC corresponded to the catecholamine cell group A_8 of Dahlström and Fuxe (34), and was located rostral to the facial genu, between the medial edge of the pedunculus cerebellaris superioris and the fourth ventricle.

Biochemical assays. The punched out brain tissue was homogenized in 1 ml of 0.1 N perchloric acid, and 200 μl of the homogenate was used for protein determination by the method of Lowry et al. (37). The homogenates were subsequently centrifuged for 15 min at 10,000 × g. Adrenaline, noradrenaline and dopamine were measured in the supernatants by radioenzymatic assays, using a modification of the method described by Feuler and Johnson (38). Aliquots of 100 μl of the supernatant were transferred to incubation tubes. An additional 20 μl of 0.1 N perchloric acid was added to each tube. Internal standards consisting of 100 pg of adrenaline, noradrenaline and dopamine in 20 μl of 0.1 N perchloric acid were added to another set of tubes. Blanks consisted of 120 μl of 0.1 N perchloric acid. The reaction was initiated by addition of 40 μl of a mixture containing the following components: 200 μg of dithiothreitol; 0.2 μM of MgCl_2; 0.016 μM of benzoxamine; 56 μM of Tris·HCl buffer, pH 9.6; 10 μl of catechol-O-methyltransferase (COMT); and 2 μCi of S-adenosyl-L-[methyl-3H]-methionine.

COMT was partially purified from rat liver by the method of Coyle and Henry (39). Enzyme activity was measured with the catecholamine standards (100 to 1,000 pg) in aliquots of the enzyme preparation. The enzyme solution was diluted with 1 mM Tris·HCl buffer containing 0.1 mM dithiothreitol (pH 7.4) to insure maximal activity with a 10 μl aliquot of enzyme. The dopa decarboxylase activity in a COMT preparation was inhibited by benzoxamine (40, 41).

The reaction mixture was incubated for 90 min at 37 °C. The reaction was stopped by the addition of 50 μl of a solution consisting of 40 μM boric acid, 4 μM EDTA·Na_2, and 0.2 μM each of metanephrine, normetanephrine and 3-methoxytyramine in 1 N NaOH. The O-methylated radioactive products were extracted into 3 ml of toluene/isoamylalcohol (3:2, V/V). After shaking for 3 min in a mechanical shaker and centrifuging for 3 min at 800 × g to separate the phases, the aqueous phase was frozen in an acetone-dry ice bath. The organic phase was decanted into another tube containing 100 μl of 0.1 N acetic acid, and the O-methylated catecholamines were extracted into the aqueous phase. After shaking and centrifuging for 3 min at 800 × g, the aqueous phase was frozen in an acetone-dry ice bath, and the organic phase was aspirated and discarded. The aqueous phase was washed once with 2 ml of the toluene/isoamylalcohol mixture, and the organic phase was discarded.

Ethanol (100 μl) was added to the acetic acid extract, and the acetic acid/ethanol solution was applied to silica-gel TLC plates. The development of the plates was accomplished by the use of a solvent containing t-amylalcohol/toluene/40% methylamine solution (6:2:3, V/V/V). The plates were dried, and three zones were visualized under UV light. The top zone contained 3-methoxytyramine, the middle zone metanephrine and the bottom zone norme-
Brain Catecholamines in Hypertensive Rats

Fujino: Brain catecholamines in spontaneously hypertensive and
taneprine.

For the assay of dopamine, the zone containing its O-methylated metabolite 3-methoxy-
tyramine was scraped into scintillation vials. To each vial was added 1 ml of 0.05 M ammo-
nium hydroxide, and the product was eluted with shaking. Ten milliliters of toluene/isoamyl-
 alcohol (7:3, V/V) was added, and 3-methoxytyramine was extracted by vigorous shaking.
After adding 0.5 ml of Liquifluor, the radioactivity was counted in a liquid scintillation counter.

For the assay of adrenaline and noradrenaline, the zone containing radioactive metan-
ephrine and normetanephrine was scraped into separate scintillation vials. To each vial was
added 1 ml of 0.05 M ammonium hydroxide to elute the catecholamine derivatives from the
silica-gel. Fifty microliters of 4% sodium periodate was added, and the cleavage reaction
was stopped 5 min later by the addition of 50 μl of 10% glycerol. The solution was then a-
cidified with 1 ml of 0.1 N acetic acid, and the radioactive products were extracted into 10 ml
of toluene containing 0.5 ml of Liquifluor by vigorous shaking. The radioactivity was count-
ed in a liquid scintillation counter.

The sensitivity of these assays (i.e., twice blank) was 3 pg for adrenaline and noradren-
aline, and 15 pg for dopamine for 100 μl samples. The assay procedures were linear from
less than 30 to at least 5000 pg. The coefficient of variation (CV) within assays of three dif-
f erent concentrations was 5.5 – 9.7 % in adrenaline assays, 6.4 – 8.4 % in noradrenaline as-
says, and 7.5 – 11.0 % in dopamine assays. The CV between assays was 6.3 – 11.2 % in
adrenaline assays, 5.7 – 10.0 % in noradrenaline assays, and 7.9 – 12.9 % in dopamine as-
says. Catecholamine concentrations were expressed as ng catecholamine per mg protein.

Statistical analysis. The data were analyzed using the unpaired Student’s t-test, with a pro-
bability of less than 0.05 being considered significant.

Drugs and chemicals. DOCA, α-MPT, dithiothreitol, benzylxamine, adrenaline, noradren-
aline, dopamine, metanephrine, normetanephrine and 3-methoxytyramine were obtained from
Sigma Chemical Co. S-adenosyl-L-[methyl-3H]-methionine (specific activity 15 Ci/mmol) was
obtained from the Radiochemical Centre, Amersham. Liquifluor was purchased from New
England Nuclear Co.

RESULTS

Animal data. The data on systolic blood pressure and body weight of SHR
and WKY rats are shown in Table 1. Table 2 shows the data on DOCA-salt
hypertensive rats and control rats. There was no significant difference in systolic
blood pressure among the groups at 4 weeks of age and before treatment. The
blood pressure of the control groups was fairly constant with age and treatment.

| Table 1. Blood pressure and body weight of WKY rats and SHR at 4 and 12 weeks of age |
|---------------------------------|---------------------------------|
|                                 | 4 weeks                        | 12 weeks                       |
|                                 | WKY                            | SHR                            | WKY                            | SHR                            |
| Blood pressure (mmHg)           | 104.3±3.0                      | 115.2±4.0                      | 123.8±4.1                      | 186.7±4.2*                     |
| Body weight (g)                 | 58.5±2.1                       | 61.7±1.7                       | 206.0±5.8                      | 214.6±6.6                      |

The values are expressed as the mean±SEM for groups of ten rats.

* p < 0.05, between SHR and WKY rats.

Produced by The Berkeley Electronic Press, 1984
TABLE 2. BLOOD PRESSURE AND BODY WEIGHT OF CONTROL AND DOCA-SALT HYPERTENSIVE RATS BEFORE AND AFTER TREATMENT

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>4 weeks after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DOCA-salt</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>104.6 ± 4.6</td>
<td>109.2 ± 3.0</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>76.5 ± 1.3</td>
<td>75.5 ± 2.4</td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± SEM for groups of ten rats.
* p < 0.05, between DOCA-salt hypertensive and control rats.

TABLE 3. CATECHOLAMINE CONCENTRATIONS IN DISCRETE BRAIN REGIONS OF WKY RATS AND SHR AT 4 AND 12 WEEKS OF AGE

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Adrenaline</th>
<th>Noradrenaline</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td></td>
</tr>
<tr>
<td>4 weeks of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area A₁</td>
<td>0.52 ± 0.07</td>
<td>0.83 ± 0.07</td>
<td>15.9 ± 2.24</td>
</tr>
<tr>
<td>Nucleus commissuralis</td>
<td>0.86 ± 0.17</td>
<td>1.17 ± 0.20</td>
<td>28.1 ± 4.62</td>
</tr>
<tr>
<td>Area A₂</td>
<td>1.22 ± 0.10</td>
<td>1.41 ± 0.34</td>
<td>30.6 ± 3.14</td>
</tr>
<tr>
<td>Rostral part of nucleus tractus solitarii</td>
<td>0.88 ± 0.27</td>
<td>1.22 ± 0.40</td>
<td>26.5 ± 4.81</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>0.50 ± 0.04</td>
<td>0.46 ± 0.13</td>
<td>15.5 ± 3.27</td>
</tr>
<tr>
<td>Median eminence</td>
<td>1.83 ± 0.28</td>
<td>1.94 ± 0.30</td>
<td>21.5 ± 3.23</td>
</tr>
<tr>
<td>Nucleus paraventricularis</td>
<td>2.58 ± 0.55</td>
<td>3.35 ± 0.30</td>
<td>54.3 ± 8.67</td>
</tr>
<tr>
<td>Nucleus hypothalamic anterior</td>
<td>1.19 ± 0.12</td>
<td>1.02 ± 0.19</td>
<td>33.2 ± 3.48</td>
</tr>
<tr>
<td>12 weeks of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area A₁</td>
<td>0.80 ± 0.25</td>
<td>0.78 ± 0.12</td>
<td>28.5 ± 3.44</td>
</tr>
<tr>
<td>Nucleus commissuralis</td>
<td>0.70 ± 0.12</td>
<td>0.76 ± 0.42</td>
<td>28.9 ± 5.95</td>
</tr>
<tr>
<td>Area A₂</td>
<td>0.80 ± 0.09</td>
<td>0.97 ± 0.18</td>
<td>30.2 ± 4.95</td>
</tr>
<tr>
<td>Rostral part of nucleus tractus solitarii</td>
<td>0.96 ± 0.26</td>
<td>1.64 ± 0.31</td>
<td>22.9 ± 3.87</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>0.38 ± 0.05</td>
<td>0.39 ± 0.15</td>
<td>14.0 ± 5.57</td>
</tr>
<tr>
<td>Median eminence</td>
<td>1.10 ± 0.46</td>
<td>1.85 ± 0.28</td>
<td>15.9 ± 2.35</td>
</tr>
<tr>
<td>Nucleus paraventricularis</td>
<td>3.21 ± 0.47</td>
<td>4.16 ± 1.31</td>
<td>69.8 ± 9.55</td>
</tr>
<tr>
<td>Nucleus hypothalamic anterior</td>
<td>1.49 ± 0.63</td>
<td>1.12 ± 0.48</td>
<td>22.9 ± 2.80</td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± SEM for groups of five rats (ng/mg protein).
* p < 0.05, between SHR and WKY rats.

On the other hand, there was a significant increase in blood pressure of SHR and DOCA-salt hypertensive rats.

Catecholamine concentrations and α-MPT induced catecholamine disappearance in discrete brain regions.

SHR and WKY rats (Table 3). The adrenaline concentration was higher in
the area $A_1$ of young SHR than in age-matched WKY rats. In adult rats the difference disappeared.

The noradrenaline concentrations were lower in the rostral part of the NTS and the NHA of young SHR and in the rostral part of the NTS of adult SHR, when compared with age-matched WKY rats. The same tendency existed in the area $A_2$ of young SHR, and in the NPV of adult SHR. The $\alpha$-MPT induced noradrenaline disappearance was less in the rostral part of the NTS and the NHA of young SHR (Fig. 3). This parameter was also observed to be less in
Fig. 4. Noradrenaline concentrations before and 4 h after \(\alpha\)-MPT administration in discrete brain regions of WKY rats (○) and SHR (●) at 12 weeks of age. Vertical bars represent one standard error of the mean for groups of five rats. \(*p<0.05\), difference between SHR and WKY rats. For abbreviations, see the text.

the rostral part of the NTS of adult SHR (Fig. 4).

In contrast, the dopamine concentrations and the \(\alpha\)-MPT induced dopamine disappearance did not differ from control values in the examined areas of young and adult rats.

When compared with young rats from the same strain, the adrenaline concentration in the area \(A_2\) and the noradrenaline concentration in the NHA were lower in adult WKY rats, and the noradrenaline concentration in the rostral part of the NTS was lower in adult SHR. The noradrenaline concentration in the
Fujino: Brain catecholamines in spontaneously hypertensive and

Brain Catecholamines in Hypertensive Rats

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Adrenaline</th>
<th>Noradrenaline</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (ng/mg)</td>
<td>DOCA-salt (ng/mg)</td>
<td>Control (ng/mg)</td>
</tr>
<tr>
<td>Area A₁</td>
<td>0.91±0.39</td>
<td>1.24±0.33</td>
<td>18.3±2.29</td>
</tr>
<tr>
<td>Nucleus commissuralis</td>
<td>0.93±0.27</td>
<td>1.31±0.39</td>
<td>39.0±4.80</td>
</tr>
<tr>
<td>Area A₂</td>
<td>2.39±0.35</td>
<td>2.20±0.21</td>
<td>42.2±3.04</td>
</tr>
<tr>
<td>Rostral part of nucleus tractus solitarii</td>
<td>1.18±0.43</td>
<td>1.41±0.17</td>
<td>20.7±6.26</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>0.49±0.18</td>
<td>0.51±0.30</td>
<td>16.1±2.93</td>
</tr>
<tr>
<td>Median eminence</td>
<td>2.05±0.39</td>
<td>2.45±0.25</td>
<td>23.9±5.25</td>
</tr>
<tr>
<td>Nucleus paraventricularis</td>
<td>3.89±0.68</td>
<td>3.20±0.42</td>
<td>62.2±9.31</td>
</tr>
<tr>
<td>Nucleus hypothalamic anterior</td>
<td>1.44±0.32</td>
<td>1.52±0.26</td>
<td>32.5±5.58</td>
</tr>
</tbody>
</table>

The values are expressed as the mean±SEM for groups of five rats (ng/mg protein).

area A₁ was higher in adult WKY rats and SHR, and the dopamine concentrations in the rostral part of the NTS and NPV were higher in adult WKY rats than in young rats.

**DOCA-salt hypertensive rats** (Table 4). The concentrations of adrenaline, noradrenaline and dopamine did not differ between DOCA-salt hypertensive rats and control rats in the examined areas. The α-MPT induced noradrenaline disappearance was less in the rostral part of the NTS of DOCA-salt hypertensive rats, when compared with control rats (Fig. 5). In the other areas, there were no differences in this parameter as well as the α-MPT induced dopamine disappearance between the two groups.

When compared with young and adult WKY rats, the adrenaline and noradrenaline concentrations in the area A₂ and the dopamine concentration in the ME were higher in Wistar control rats. The dopamine concentrations in the area A₂ and NPV were higher in Wistar control rats than in young WKY rats. The noradrenaline concentration in the area A₁ was lower in Wistar control rats than in adult WKY rats.

**DISCUSSION**

Since SHR were derived from WKY rats, they are genetically close to each other (42, 43), and WKY rats are the best controls for comparison with SHR. The use of different strains of Wistar rats as controls for SHR could result in differences in catecholamine metabolism arising solely from genetic factors which might not be related to the pathogenesis of hypertension. In the present study, Wistar control rats exhibited different values of catecholamine concentrations in some brain areas compared with WKY rats.

Small amounts of adrenaline were detected in all examined brain areas. The
central origin of brain adrenalin was suggested by the presence in brain of the adrenaline forming enzyme, phenylethanolamine-N-methyltransferase (PNMT). The area A₁ and A₂ were virtually identical to the PNMT-positive cell groups, which are presumed the origin of adrenergic pathways to specific hypothalamic nuclei (44, 45).
In the area A₁ of young SHR, the adrenaline concentration was high even prior to the onset of hypertension. The result is in agreement with that of Wijnen et al. (15) who reported a high adrenaline concentration in the area A₁ of 2- and 4-week-old SHR, but is in contrast with that of Saavedra (12) who reported a low adrenaline concentration in the area A₁ of 4-week-old SHR. There were no differences in adrenaline concentrations in the hypothalamus of young and adult SHR, although Versteeg et al. (13-15) found high adrenaline concentrations in the hypothalamus of adult SHR. In the present study, the α-MPT induced adrenaline disappearance was not detected, because the quantity of adrenaline was near the limit of assay sensitivity. Therefore, it is not clear whether the elevated adrenaline level reflects increased or decreased neural activity. Saavedra et al. (11, 12) and Gianutsos et al. (19) found increased PNMT activity in the area A₁ and A₂ of 4-week-old SHR. This could be an indication of increased activity of adrenergic neurons. On the basis of these findings, it is suggested that the high adrenaline concentration in the medullary area may contribute to the initiation of hypertension in SHR.

Noradrenaline concentrations were lower in specific brain areas of SHR compared with age-matched WKY rats. These areas included the rostral part of the NTS of the medulla and the NHA of the hypothalamus of young rats, and the rostral part of the NTS of adult rats. The low noradrenaline levels were due to diminished synthesis, since the α-MPT induced noradrenaline disappearance was low in these areas of SHR. The results suggested a decrease in noradrenaline turnover (29). This is the first report of decreased noradrenaline turnover in the rostral part of the NTS of young and adult SHR, since significant differences have not been found in earlier reports. Saavedra (11, 12), Wijnen et al. (14, 16), Gianutsos et al. (19) and Fuxe et al. (20) reported that there were no differences in noradrenaline concentrations or noradrenergic neural activity in the medullary areas of young and adult SHR. Although Elghozzi et al. (22, 23) found low noradrenaline concentrations in some medullary areas of 4-week-old SHR, they did not study noradrenaline turnover. The changes in the NHA of young SHR are partially in agreement with earlier reports. Saavedra (11) reported low noradrenaline concentrations and dopamine-β-hydroxylase (DBH) activity in the rostral-hypothalamic nuclei of both 4- and 14-week-old SHR. Elghozzi et al. (22, 23) reported low noradrenaline concentrations in various hypothalamic nuclei of 4-week-old SHR, and found no differences in 12-week-old SHR. Wijnen et al. (16) reported a low noradrenaline concentration in the NHA of 10-week-old SHR and little α-MPT induced noradrenaline disappearance in the rostral hypothalamic nuclei of 3- and 10-week-old SHR. In contrast with our results, several reports have presented evidence which suggests increased activity of noradrenergic neurons of SHR. Nagaoka et al. (17) reported that the activity of TH and DBH in the medulla and hypothalamus was higher in 5- and 8-week-old SHR than in WKY rats. Nakamura et al. (18) found high DBH activity in the area A₂ and
NPV of 5-week-old SHR. In another study (21), high affinity noradrenaline uptake was found in the frontal cortex, cerebellum, hypothalamus and pons-medulla of 2- to 13-week-old SHR. Such discrepancies, which are found also in the adrenaline concentration, may be explained by colony differences, differences in the dissection procedures or differences in the assay procedures.

Several areas in the medulla rich in catecholamines have been described as the control center of blood pressure (9). In particular, attention has focused on the NTS, which is considered to be the primary synapse area of the afferents of the arterial baroreceptors (9, 46). Moreover, it was found that the noradrenergic pathway from the medulla terminated in the rostral hypothalamus (50), and Saper et al. (51) reported a direct anatomical connection between the hypothalamus and the NTS. Interestingly in the present study, a decrease in noradrenaline turnover was observed in these areas, especially in young SHR. The results are compatible with those of de Jong (49) who reported that a microinjection of noradrenaline in the NTS of Wistar rats resulted in decreased blood pressure, and those of Struyker Boudier et al. (50) who reported that a microinjection of noradrenaline and adrenaline in the rostral hypothalamus of normotensive rats resulted in decreased blood pressure. From these findings, it is suggested that decreased noradrenergic neural activity in the medullary and rostral hypothalamic areas may be one of the factors involved in the development of spontaneous hypertension of rats.

On the other hand, no differences from control values were observed in adrenaline and noradrenaline concentrations in brain areas of DOCA-salt hypertensive rats in the present study, and those by Wijnen et al. (14), Elghozi et al. (23) and Saavedra (27). The α-MPT induced noradrenaline disappearance was low only in the rostral part of the NTS (Fig. 5). In DOCA-salt hypertensive rats, decreased noradrenaline turnover in the brainstem has been observed by Nakamura et al. (2) in association with increased turnover in various peripheral organs. However, it was not clear whether the decrease in brainstem noradrenaline turnover was secondary to the elevation of blood pressure or whether this phenomenon indicated a primary dysfunction in the brainstem. De Champlain and van Ameringen (24, 25) demonstrated that decreased noradrenaline turnover in the brainstem of DOCA-salt hypertensive rats persisted even after the blood pressure was lowered to a normal level after spinal cord section. This result indicated that decreased noradrenaline turnover in the brainstem was independent of blood pressure variations and reflected a primary dysfunction in the vasomotor center of the brainstem. In the present study, the turnover was measured in specific areas of the brainstem by the determination of the α-MPT induced noradrenaline disappearance. Ionic disturbances in central nervous tissue induced by DOCA and salt administration might decrease noradrenaline turnover, especially in the rostral part of the NTS.

DOCA-salt hypertensive rats exhibited differences in brain catecholamines, when compared with SHR. That is, DOCA-salt hypertensive rats did not show
Brain Catecholamines in Hypertensive Rats

either high or low adrenaline and noradrenaline concentrations in the examined areas, and did not have low $\alpha$-MPT induced noradrenaline disappearance in the hypothalamus. The results might reflect differences in mechanisms underlying hypertension in the various models.

Dopamine concentrations and the $\alpha$-MPT induced dopamine disappearance were not different in all examined areas of SHR and DOCA-salt hypertensive rats. This is consistent with earlier reports (12, 14, 19, 22, 23). However, our results do not rule out the possibility of dopamine mechanisms in the development of hypertension. Elevated dopamine concentrations were observed in the frontal cortex, nucleus interstitialis striae terminalis and area A3 of SHR by Versteeg et al. (13). Decreased dopamine uptake was observed in the frontal cortex and striatum of SHR by Myers et al. (21). Treatment of SHR (51, 52), and human hypertensives (53, 54), with dopamine receptor agonists was shown to lower the blood pressure. The results of each report are consistent with decreased dopaminergic neural activity in SHR.

The present results suggest early changes in catecholaminergic neural activity in specific areas of the brainstem and hypothalamus of SHR, and more restricted changes in catecholaminergic neural activity of DOCA-salt hypertensive rats. These changes may play an important role in the development of spontaneous and DOCA-salt hypertension of rats. Further experiments are necessary to investigate more dynamic aspects of catecholamine metabolism in relation to the development of hypertension.

Acknowledgments. I wish to thank Prof. Zensuke Ota, Assist. Prof. Shinya Suzuki and Dr. Jiro Takahara for their helpful advice and critical reading of the manuscript, and Dr. Yoshiyuki Aoki for his excellent technical assistance.

REFERENCES


Produced by The Berkeley Electronic Press, 1984


Brain Catecholamines in Hypertensive Rats


340

K. Fijino


