Role of Dopamine in the Development of Spontaneous Hypertension

MAARTEN VAN DEN BUUSE, DIRK H.G. VERSTEEG, AND WYBREN DE JONG

SUMMARY To investigate the role of brain catecholamines in the development of spontaneous hypertension, rats were treated with different doses of the neurotoxins 6-hydroxydopamine (6-OHDA) or DSP-4 (N-[2-chloroethyl]-N-ethyl-2-bromobenzylamine hydrochloride). Intracerebroventricular (i.c.v.) 6-OHDA attenuated the development of hypertension in spontaneously hypertensive rats (SHR) and also lowered the systolic blood pressure (BP) in Wistar-Kyoto (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP). Norepinephrine was markedly and dose-dependently depleted in brain areas of all three substrains. Dopamine was affected also, although to a lesser extent. Pretreatment with the norepinephrine-uptake inhibitor desmethylimipramine (DMI) did not influence the effect of 6-OHDA on the development of hypertension in SHR. DMI largely antagonized the 6-OHDA-induced depletion of brain norepinephrine, while dopamine depletion was not affected. Specific depletion of brain norepinephrine by treatment with DSP-4 did not alter the rise in BP in SHR. These results suggest that the effect of 6-OHDA on the development of hypertension in SHR may not be mediated through destruction of brain norepinephrine neurons, but that interruption of brain dopaminergic mechanisms is a possibility in this respect. (Hypertension 6: 899-905, 1984)

KEY WORDS • 6-hydroxydopamine • DSP-4 • catecholamines • desmethylimipramine • stroke-prone spontaneously hypertensive rat • Wistar-Kyoto rat •

We undertook the present experiments to further analyze the effect of i.c.v. 6-OHDA on the development of spontaneous hypertension. To separate the roles of dopamine and norepinephrine, we used 6-OHDA in combination with the norepinephrine uptake inhibitor desmethylimipramine (DMI), which selectively affects dopamine systems, while we used the norepinephrine neurotoxin DSP-4 (N-[2 chloroethyl]-N-ethyl-2-bromobenzylamine hydrochloride) to affect only norepinephrine neurons. The results suggest a possible role of brain dopamine in the development of hypertension in the SHR.

Materials and Methods
We used male Wistar-Kyoto (WKY) normotensive control rats, SHR, and rats of the stroke-prone SHR substrain (SHRSP). All rats were derived from breeding stocks at our institute (SHR-Cpb and WKY-Cpb from TNO, Zeist, The Netherlands, were used for breeding of SHR and WKY). The animals were weaned at 4 weeks and kept in our laboratory under a constant light-dark regime with free access to standard pellet food and tap water.

To permit intracerebroventricular (i.c.v.) injections, the animals were operated on at the age of 4 weeks under Hypnorm anesthesia (fentanyl 10 mg and fentanyl 0.2 mg per ml, Duphar, Amsterdam, The Netherlands). To avoid unilateral depletions, poly-


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ethylene cannulas were placed bilaterally in the lateral cerebral ventricles. After a recovery period of 5 to 7
days, the rats were injected consciously either with 6-
OHDA, 50 or 200 µg dissolved as a base per 10 µl
0.9% saline with ascorbic acid (0.2 mg/ml), or injected
with the vehicle only. Solutions were prepared freshly
before the injections and were kept on ice. The first 10
µl injection was given through the right ventricular
cannula, and 48 hours later the second injection was
administered through the left ventricular cannula.
Again, 48 hours later, the third dose was given as two
bilateral 5/µl injections. Thus, rats injected i.c.v. with
6-OHDA received a total dose of 150 or 600 µg of the
base. After the third injection, the rats were allowed to
recover for 3 days, and then they underwent training
procedures to enable indirect BP measurement.

In case of pretreatment with DMI, 30 minutes before
every i.c.v. injection the rats received an intraperitone-
al (i.p.) injection of either DMI (25 mg/kg in distilled
water, 1 ml/kg)20 or the same volume of vehicle. Thus,
in the DMI experiment, four animal groups were used:
the control group received i.p. vehicle and i.c.v. vehi-
cle, the DMI group received i.p. DMI and i.c.v. vehi-
cle, the 6-OHDA group received i.p. vehicle and i.c.v.
6-OHDA; and the combined treatment group received
both DMI and 6-OHDA. DSP-4 was injected i.p. in a
dose of 50 mg/kg in 0.9% saline. This dose has been
described to be maximally effective.21

Three days after the injections, training procedures
began for the BP measurements. Systolic BP and heart
rate (HR) were measured on conscious animals with a
tail-cuff method, as previously described.22 Before the
actual measurements were performed, the animals
were trained daily to get used to the restraining cage
and the tail cuff. Actual measurements of BP were
performed daily during the 1st week and thereafter at
least three times a week to minimize any stress-in-
duced influences on the data. In general, the develop-
ment of hypertension was followed for at least 3
weeks, after which the rats were decapitated, and their
brains were taken out for dissection and catecholamine
assay. Postdecapitation reflexes were assessed as the
time during which limb movements occurred after
decapitation. Thus, the time between decapitation and
the actual occurrence of reflexes was not included in
the present data. Frontal cortex, hypothalamus, hippo-
campus, and medulla/pons were obtained according to
the dissection method of Gispen et al.23 The hearts
were briefly rinsed in cold saline, and then all tissue
parts were quickly weighed and frozen on dry ice.

Before catecholamine assay, the brain tissue was
homogenized with a Heidolph homogenizer (Heidolph
Elektro KG, Kelheim, FRG), and the heart tissue was
cut with a Polytron homogenizer, both in a volume of
0.1 N HClO4 appropriate to meet with the standard
curve of the assays. Norepinephrine, dopamine, and
in some experiments epinephrine were assayed in 20 /µl
samples of the homogenates with a radioenzymatic
assay according to the method of Van der Gugten et
al.24 Control and treatment groups of one experiment
were always measured within one assay. In this way,
interassay variations could not influence eventual
treatment effects. Catecholamine concentrations are
expressed as ng/100 mg tissue wet weight. Data are
expressed as means ± standard error of the mean
(SEM). For statistical comparison, Student’s t test was
used, in appropriate cases preceded by a one-way anal-
ysis of variance (ANOVA). A p value less than 0.05
(two-tailed) was considered to indicate a statistically
significant difference.

Results

In 5-week-old WKY, SHR, and SHRSP, the effects of three i.c.v. injections of 50 or 200 /µg 6-OHDA on
the BP are shown in Figure 1. The development of hypertension was markedly attenuated by 3 x 200 /µg
6-OHDA, as shown by the significantly lower systolic BP when compared to vehicle-treated control rats
(after 2 weeks, 171 ± 3 mm Hg for 6-OHDA-treated SHR vs 182 ± 4 mm Hg for vehicle-treated SHR; and
after 4 weeks, 160 ± 4 mm Hg vs 201 ± 4 mm Hg, respectively). Injection of 3 x 50/µg 6-OHDA result-
ed in significantly lower systolic BP, but only after 4
weeks (187 ± 3 vs 201 ± 4 mm Hg). Treatment with
3 x 200 /µg 6-OHDA in WKY also resulted in lower systolic BP, but the difference was less than that found
in SHR (after 2 weeks, 128 ± 2 mm Hg in 6-OHDA-
treated WKY vs 138 ± 2 mm Hg in vehicle-treated
WKY; after 4 weeks, 124 ± 2 vs 147 ± 4 mm Hg, respectively). Injection of 3 X 50/µg 6-OHDA did not
significantly affect BP in WKY. In SHRSP, only the 3
X 200 /µg dose was studied. Significantly lower sys-
tolic BP was found at both time points (after 2 weeks,
178 ± 3 mm Hg in 6-OHDA-treated SHRSP vs 192 ±
6 mm Hg in vehicle-treated rats; after 4 weeks, 178 ±
4 vs 210 ± 4 mm Hg, respectively). Thus, treatment
with 6-OHDA appeared to have marked effects on the
development of hypertension in SHR and SHRSP,
with only a small effect on the BP of WKY.

The HR was also affected by 6-OHDA treatment. As
shown in Figure 1, the 3 X 200 /µg dose resulted in
significantly lower HR values in WKY, SHR, and
SHRSP, although the extent of the difference was
greater in both hypertensive strains. Only in WKY was
the HR reduced after three 50 /µg injections.

Concentrations of norepinephrine in the frontal cor-
tex, hypothalamus, and hippocampus of 6-OHDA-
treated rats were markedly and significantly lower as
compared to vehicle-treated control rats (Table 1). This
effect was dose-dependent. As little as 3 x 50 /µg
6-OHDA resulted in more than a 50% depletion of
norepinephrine in all brain regions studied. In general,
depletion of dopamine was less pronounced than that
of norepinephrine.

Final body weight was slightly, although signifi-
cantly, lower in rats of all three substrains after treat-
ment with 3 x 200 /µg 6-OHDA (Table 1). This effect
can be attributed to a transient decrease in body weight
caused by the first injection. Thereafter, body weight
gain was similar in all groups. The lower dose of 6-
OHDA did not affect body weight.
FIGURE I. Effect of 3 X 50 fg (stippled bars) and 3 X 200 ng (black bars) 6-hydroxydopamine (6-OHDA) on the systolic blood pressure (left panels) and heart rate (right panels) in Wistar-Kyoto (WKY), spontaneously hypertensive rats (SHR), and rats of the stroke-prone SHR-substrain (SHRSP), at 2 weeks (top panels) and at 4 weeks (bottom panels) after the first 6-OHDA injection. For the number of animals, see Table I. * refers to a statistically significant difference (p < 0.05, Student t test after ANOVA) as compared to control values from vehicle-treated rats (open bars).

After 6-OHDA treatment, SHR had markedly lower HR as compared to control rats treated with vehicle alone (Figure 2). This effect was not found in the SHR of the combined treatment group. At all time points, a significantly lower HR was found in 6-OHDA-treated SHR when compared to SHR treated with 6-OHDA and DMI.

The effect of combined treatment with 6-OHDA and DMI on the time course of the development of hypertension in SHR is shown in Figure 2. Data are only available for after Day 17 after the first injection, because of the recovery time and training procedure time. The effect of combined treatment did not differ from that of 6-OHDA treatment alone. In both groups, systolic BP was significantly lower than that found in the respective control groups, while a significant difference between 6-OHDA-treated SHR and combined-treated SHR was found only on Days 25 and 42.

After 6-OHDA treatment, SHR had markedly lower HR as compared to control rats treated with vehicle alone (Figure 2). This effect was not found in the SHR of the combined treatment group. At all time points, a significantly lower HR was found in 6-OHDA-treated SHR when compared to SHR treated with 6-OHDA and DMI.

The effect of 6-OHDA treatment on body weight did not differ with or without pretreatment with DMI (Table 2). Thus, in both treatment groups, an initial decrease in body weight occurred after the first injection desmethylimipramine (DMI) on the development of hypertension (left panel) and heart rate (right panel) in spontaneously hypertensive rats. The abscissa shows the number of days after the first injection of 6-OHDA. For details concerning treatment groups, see Materials and Methods. For the number of animals per group, see Table 2. Blood pressure (BP) values in the 6-OHDA-treated group were significantly different from those of control rats on all days. The BP values in the combined-treatment group were significantly different from the values found in DMI-treated rats on all days except for Day 21 and significantly different from values found in the 6-OHDA-treated group only on Days 25 and 42. Heart rate (HR) values in the 6-OHDA-treated group were significantly different from those of the control group on all days. The HR in the combined-treatment group was significantly different from that in the DMI-treated rats on Day 32 only and significantly different from that in 6-OHDA-treated rats on all days.
TABLE 1. Effect of 3 X 50 fig or 3 x 200 fig 6-Hydroxydopamine (6-OHDA) on the Final Body Weight and Brain Catecholamine Concentrations of Wistar-Kyoto Rats, Spontaneously Hypertensive Rats, and Stroke-Prone Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Control vehicle</th>
<th>6-OHDA (3 x 50 Mg)</th>
<th>6-OHDA (3 x 200 Mg)</th>
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<tr>
<td>Wistar-Kyoto (n)</td>
<td>8</td>
<td>7</td>
<td>11</td>
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<tr>
<td>Body weight (g)</td>
<td>205 ± 5</td>
<td>197 ± 6</td>
<td>181 ± 6*</td>
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<tr>
<td>Norepinephrine (ng/100 mg tissue wet weight)</td>
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<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>35.2 ± 1.8</td>
<td>9.5 ± 0.8*</td>
<td>0.8 ± 0.1*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>239.6 ± 8.8</td>
<td>111.1 ± 12.7*</td>
<td>37.9 ± 6.0*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>60.7 ± 3.8</td>
<td>8.9 ± 1.9*</td>
<td>1.6 ± 0.3*</td>
</tr>
<tr>
<td>Dopamine (ng/100 mg tissue wet weight)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>30.3 ± 1.9</td>
<td>34.7 ± 5.4</td>
<td>16.7 ± 1.8*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>51.8 ± 1.8</td>
<td>44.5 ± 3.4</td>
<td>33.8 ± 5.2*</td>
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<td>Hippocampus</td>
<td>7.2 ± 1.5</td>
<td>2.7 ± 0.2*</td>
<td>3.1 ± 0.2*</td>
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<tr>
<td>Spontaneously hypertensive (n)</td>
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<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>228 ± 5</td>
<td>219 ± 5*</td>
<td>189 ± 5*</td>
</tr>
<tr>
<td>Norepinephrine (ng/100 mg tissue wet weight)</td>
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<tr>
<td>Frontal cortex</td>
<td>38.0 ± 2.2</td>
<td>10.5 ± 1.3*</td>
<td>2.8 ± 0.4*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>268.9 ± 9.1</td>
<td>106.3 ± 11.1*</td>
<td>61.1 ± 5.6*</td>
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<td>Hippocampus</td>
<td>25.1 ± 2.4</td>
<td>4.8 ± 0.7*</td>
<td>2.5 ± 0.5*</td>
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<tr>
<td>Dopamine (ng/100 mg tissue wet weight)</td>
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<tr>
<td>Frontal cortex</td>
<td>93.2 ± 15.5</td>
<td>82.5 ± 13.3</td>
<td>23.8 ± 4.4*</td>
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<tr>
<td>Hypothalamus</td>
<td>47.6 ± 4.5</td>
<td>46.3 ± 6.3</td>
<td>48 ± 3.3</td>
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<tr>
<td>Hippocampus</td>
<td>5.9 ± 0.7</td>
<td>3.7 ± 0.5*</td>
<td>4.3 ± 0.6</td>
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<tr>
<td>Stroke-prone spontaneously hypertensive (n)</td>
<td>5</td>
<td>13</td>
<td></td>
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<tr>
<td>Body weight (g)</td>
<td>214 ± 6</td>
<td>179 ± 4*</td>
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<tr>
<td>Norepinephrine (ng/100 mg tissue wet weight)</td>
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<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>31.5 ± 2.1</td>
<td>2.4 ± 0.4*</td>
<td></td>
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<tr>
<td>Hypothalamus</td>
<td>226.4 ± 12.2</td>
<td>56.6 ± 6.6*</td>
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<tr>
<td>Hippocampus</td>
<td>36.9 ± 3.5</td>
<td>17 ± 0.2*</td>
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<tr>
<td>Dopamine (ng/100 mg tissue wet weight)</td>
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<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>58.3 ± 8.0</td>
<td>25.9 ± 2.6*</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>49.7 ± 3.5</td>
<td>41.9 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9.1 ± 0.3</td>
<td>4.5 ± 0.4*</td>
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</table>

Values are means ± SEM.
*p < 0.05, 6-OHDA-treated vs vehicle-treated rats.

of 6-OHDA, while body weight gain thereafter paralleled that of both control groups. Postdecapitation reflex time of 6-OHDA-treated SHR was shorter than that of vehicle-treated controls (Table 2). However, the duration of the post-decapitation reflexes was significantly longer in SHR treated with 6-OHDA and DMI when compared to 6-OHDA-treated SHR.

Norepinephrine concentrations in the frontal cortex, hypothalamus, hippocampus, and medulla pons were markedly lower in 6-OHDA-treated SHR as compared to control rats (Table 2). Dopamine concentrations were lower in the frontal cortex and hypothalamus. DMI pretreatment largely prevented the 6-OHDA-induced depletion of norepinephrine. The effect on dopamine level in the frontal cortex was not influenced, but in the hypothalamus no significant depletion was found after the combined treatment. Neither treatment had significant effects on epinephrine concentrations in the hypothalamus or medulla pons, although tendencies were sometimes observed.

The development of hypertension in SHR treated with DSP-4 was similar to that in vehicle-treated control rats. At none of the time points was a significant difference found between the systolic BP of SHR treated with DSP-4 or vehicle (at 5 weeks after injection, the BP in DSP-4-treated SHR was 205 ± 4 mm Hg and
in vehicle-treated SHR was 208 ± 3 mm Hg). Heart rate was slightly, though significantly, lower in SHR treated with DSP-4 (430 ± 7 bpm) as compared to control (468 ± 8 bpm) at 5 weeks after injection only. Final body weights were lower after treatment with DSP-4 (5 weeks after injection, 227 ± 3 g in treated SHR vs 242 ± 5 g in control). Postdecapitation reflex time in SHR treated with DSP-4 was significantly shorter than that found in vehicle-treated control rats (Table 3). Except in the heart, norepinephrine concentration was significantly lower in all regions studied. Dopamine and epinephrine concentrations were not affected except for dopamine in the medulla pons (Table 3).

Discussion

The present experiments were performed to further analyze the role of central catecholamines in the development of hypertension in the SHR. From the results, a role for dopamine in this process is suggested. Treatment with 6-OHDA dose-dependently affected both BP and HR in SHR as well as in WKY and SHRSP. The treatment resulted in a dose-dependent depletion of norepinephrine and, to a lesser extent, of dopamine. Pretreatment with DMI in SHR did not substantially influence 6-OHDA-induced attenuation of the development of hypertension, but a marked protection of norepinephrine against the action of 6-OHDA was found. Dopamine depletion, however, was not influenced. Depletion of norepinephrine alone by DSP-4 did not significantly affect the BP.

The attenuating effect of high doses of i.c.v. 6-OHDA on the development of hypertension in SHR has been reported previously.\textsuperscript{14,15} In the present experiments, WKY also displayed lower BP after 6-OHDA treatment, although less marked. A difference in the magnitude of the effect of several stimuli on the BP in SHR versus WKY has been described.\textsuperscript{16} In SHRSR, 6-OHDA resulted in markedly lower BP values, which suggests that catecholamines influence the development of hypertension in this substrain, as well. Administration of lower doses of 6-OHDA to SHR has not been reported earlier. Although in some areas of the brain a more than 70% depletion of norepinephrine was found, only a small effect on the rise in BP occurred (at 4 weeks, -7% difference vs — 20% after 3 X 200 /kg 6-OHDA). This could suggest that only extensive depletion (80%-90%, or more) of norepinephrine may result in a substantial effect on the development of hypertension in SHR. An alternative explanation could be that only the high dose of 6-OHDA affects other, less sensitive, neurotransmitter systems in the brain. Thus, it was only after 3 X 200 /kg 6-OHDA that dopamine concentrations in the frontal cortex were depleted. A greater sensitivity of norepinephrine neurons to the action of 6-OHDA, as compared to dopamine neurons, has been described.\textsuperscript{12,16}

| TABLE 2. Effect of 3 X 200 fig 6-Hydroxydopamine (6-OHDA) With or Without Pretreatment With Desmethyli mipramine (DMI) on Body Weight, Postdecapitation Reflexes, and Brain Catecholamine Concentrations of Spontaneously Hypertensive Rats |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Controls (n = 18) | DMI-treated (n = 18) | 6-OHDA-treated (n = 10) | 6-OHDA + DMI-treated (n = 9) |
| Body weight (g) | 254±7           | 244±5           | 223±8*           | 213 ±10*          |
| Postdecapitation reflexes (sec) | 21±1           | 21±1           | 4±2*             | 15±2*             |
| Norepinephrine (ng/100 mg tissue wet weight) | | | | |
| Frontal cortex | 37.3 ± 2.5       | 36.7±2.3       | 2.4±0.6*         | 20.3± 3.3*         |
| Hypothalamus    | 163±9.1          | 175.8 ± 8.6 | 64.3± 12.6*   | 159.0±22.6t        |
| Hippocampus     | 35.2±3.0         | 37.5 ± 2.6   | 2.0±0.4*        | 13.4±3.3*         |
| Medulla pons    | 42.6±2.9         | 45.8±2.6     | 18.2±3.2*       | 35.1±2.9*         |
| Dopamine (ng/100 mg tissue wet weight) | | | | |
| Frontal cortex | 52.9±14.7        | 45.4±11.9     | 16.5±5.4*       | 10.1±2.4*         |
| Hypothalamus    | 45.3±2.7         | 42.7±2.4     | 32.4±2.5*       | 45.1±3.6t         |
| Hippocampus     | 3.0±0.4          | 2.4 ± 0.2    | 2.4±0.4         | 1.7±0.3           |
| Medulla pons    | 4.2±0.6          | 4.2±0.4      | 3.8±0.5         | 4.4±0.4           |
| Epinephrine (ng/100 mg tissue wet weight)* | | | | |
| Hypothalamus    | 6.5±1.1          | 6.5±0.8      | 2.8±0.5         | 6.7±2.2           |
| Medulla pons    | 1.0±0.2          | 1.3±0.3      | 2.2±0.6         | 0.9±0.1           |

Values are means ± SEM.
\*p < 0.05, between either 6-OHDA-treated rats and controls or between DMI + 6-OHDA-treated rats and DMI-treated rats.
\#p < 0.05, between DMI + 6-OHDA-treated rats and 6-OHDA-treated rats.
Markedly decreased epinephrine concentrations were found in the hypothalamus of 6-OHDA-treated rats. However, analysis of variance indicated no statistically significant difference. On the basis of Student’s (t) test, the difference between 6-OHDA-treated rats and controls would have been significant.
TABLE 3. Effect of DSP-4 (50 mg/kg) on Final Body Weight, Postdecapitation Reflexes, and Brain Catecholamine Concentrations of Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 17)</th>
<th>DSP-4 (n = 19)</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>242 ± 5</td>
<td>227 ± 3*</td>
</tr>
<tr>
<td>Postdecapitation reflexes (sec)</td>
<td>21 ± 1</td>
<td>9 ± 3*</td>
</tr>
<tr>
<td>Norepinephrine (ng/100 mg tissue wet weight)</td>
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<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>63.6 ± 9.4</td>
<td>19.8 ± 1.5*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>297.0 ± 50.3</td>
<td>206.7 ± 18.5*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>68.8 ± 11.0</td>
<td>13.2 ± 2.1*</td>
</tr>
<tr>
<td>Medulla pons</td>
<td>160.0 ± 11.2</td>
<td>76.4 ± 7.6*</td>
</tr>
<tr>
<td>Heart</td>
<td>130.0 ± 8.2</td>
<td>141.0 ± 7.1</td>
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<tr>
<td>Dopamine (ng/100 mg tissue wet weight)</td>
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<tr>
<td>Frontal cortex</td>
<td>60.2 ± 9.4</td>
<td>60.0 ± 8.2</td>
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<tr>
<td>Hypothalamus</td>
<td>56.7 ± 5.6</td>
<td>55.7 ± 2.6</td>
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<tr>
<td>Hippocampus</td>
<td>3.4 ± 0.2</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Medulla pons</td>
<td>8.8 ± 0.6</td>
<td>6.0 ± 0.5*</td>
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<tr>
<td>Epinephrine (ng/100 mg tissue wet weight)</td>
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<tr>
<td>Hypothalamus</td>
<td>6.0 ± 0.4</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>Medulla pons</td>
<td>1.6 ± 0.2</td>
<td>2.2 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SEM.
* p < 0.05 for difference between rats treated with DSP-4 and control vehicle.
† The number of rats in the epinephrine determinations was 9 and 7, respectively.

Recently, lesion studies have revealed that ascending noradrenergic pathways are not of major importance in the rise of BP in SHR.13,17 Thus, forebrain depletion of norepinephrine by knife-cut lesions or by 6-OHDA-microinjections in the ascending noradrenergic bundles does not interfere with the development of spontaneous hypertension. The present results, for which DMI was used to specifically block the action of 6-OHDA against noradrenergic neurons,18 support and extend these findings. The effect of DMI can probably be attributed to a blockade of the uptake of 6-OHDA into the neuron.12,13 A protecting action of DMI on norepinephrine neurons also in the spinal cord is suggested by the differences in the postdecapitation reflex time between the various treatment groups (Table 2).9 Recently, it has been shown that 6-OHDA-induced depletion of epinephrine can also be prevented by DMI.26 While the effects of 6-OHDA on the BP rise were not influenced by DMI pretreatment, it is noteworthy that dopamine depletion was not altered either. It is unlikely that the small remaining depletion of norepinephrine in the frontal cortex and hippocampus after combined treatment can account for the observed effects on BP, if we consider the even larger depletions of norepinephrine in these structures together with smaller effects on BP after 3 x 50 fig 6-OHDA treatment in the first experiment. The effect of 6-OHDA on HR, however, does seem to be dependent on depletion of norepinephrine, since DMI pretreatment almost completely returned the HR values to the level found in controls.

Treatment with DSP-4 did not significantly affect the development of hypertension and slightly decreased the HR in SHR. The DSP-4 has been introduced as a specific neurotoxin for norepinephrine neurons in the brain after peripheral administration.19,20 Although DSP-4 was injected i.p., no depletion of norepinephrine was found in the heart, which suggests that there was no interference of peripheral factors with the action of DSP-4. Norepinephrine levels in the brain were decreased, while there was little effect on dopamine and epinephrine. Postdecapitation reflex time was decreased in rats after treatment with DSP-4, which suggests that the spinal cord norepinephrine was also depleted.27 Although the depleting effects of DSP-4 do not seem as marked as after treatment with 3 x 200 fig 6-OHDA, the absence of an effect of the treatment on the development of hypertension, together with an almost complete absence of dopamine depletion, could be interpreted as additional support for the results of the experiments with DMI. Higher doses of DSP-4 do not result in higher depletion values of norepinephrine in the brain.9

In conclusion, the present results do not support a major role for norepinephrine in the development of hypertension in the SHR, but rather suggest an involvement of other systems, notably dopamine.

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