Reactivity of Norepinephrine Receptors in the Cardiovascular System of Hypertensive Rats

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SUMMARY
Cardiovascular (CV) reactivity to norepinephrine (NE) after cocainization, and to methoxamine was determined in spontaneously hypertensive rats (SHR), and in rats made hypertensive by clipping the renal artery and contralateral nephrectomy, or by the administration of deoxycorticosterone and salt. Cocaine inhibits the uptake of NE at the nerve terminals. Methoxamine is a sympathomimetic amine, not taken up by the nerve endings. The full dose-response curves were obtained without anesthesia after pithing, decerebration, and vagotomy. CV reactivity to NE was essentially unchanged after cocainization in the 3 types of hypertension. Reactivity to methoxamine was also unchanged in hypertension. The results indicate that neither differences in the mechanism of uptake nor sensitivity of the receptor sites is responsible for the elevated blood pressure (BP) in these types of hypertension. NE must be available in a larger amount at the receptor sites in the CV system to maintain the higher BP.

Additional Indexing Words: Cardiovascular reactivity Cocaine Methoxamine Sympathetic nervous system Uptake block

DETERMINATION of cardiovascular (CV) reactivity to norepinephrine (NE), the chemical transmitter of the sympathetic nervous system, in the hypertensive state after eliminating the sympathetic neural innervation would furnish basic information on the role of the nervous and CV systems in the mechanism of blood pressure elevation.1,2)

To obtain a reliable result it was necessary to solve several methodological problems. We have devised a preparation of the rat which meets the requirements by pithing, decerebration, and vagotomy.1) CV reactivity to NE in the spontaneously hypertensive rats (SHR), and in the rats made hypertensive by clipping the renal artery and contralateral nephrectomy, or by deoxycorticosterone (DOC) and salt administration, was essentially unchanged.

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Received for publication June 1, 1972.
A preliminary report of these studies was presented at the VII Annual Meeting of SHR Council and the First International Symposium on SHR in Kyoto, October, 1971.

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A possibility remains that uptake of NE by the sympathetic nerve endings might participate in the observed CV reactivity, since exogenous NE acts on both the receptor sites and the so-called transfer sites in the nerve terminals. Since post-ganglionic neurons remain intact in our preparation, overall reactivity to NE was determined by both sensitivity of the receptors and degree of the uptake. Therefore, CV reactivity was reexamined after blocking the uptake process with cocaine; or using methoxamine, a directly acting sympathomimetic amine, predominantly of α-type, which is not taken up by adrenergic nerve endings.3)

**METHODS**

SHR,4) renal hypertension induced by clipping, and DOC-salt hypertension were studied. Female rats were used throughout the experiments. The SHR were F21, 17 to 20 weeks of age, weighing 200 to 250 Gm., supplied by the Iyakushigen Institute for Medical Research. Systolic blood pressure (BP) determined by the plethysmographic tail method ranged from 160 to 200 mm.Hg. Wistar strain rats of the Animal Center Laboratory, Kyoto University Faculty of Medicine, from which the SHR had been separated, served as controls. They were matched in age and body weight with the SHR. Their BP was 110 to 140 mm.Hg. Renal hypertension was induced by clipping the left renal artery with a silver ribbon and removing the right kidney a week later in rats of the Donryu strain, 10 to 12 weeks of age, weighing 170 to 220 Gm. They were used 6 to 10 weeks after clipping. Body weight ranged from 200 to 280 Gm., and systolic BP, from 150 to 230 mm.Hg. DOC hypertension was induced by injecting deoxycorticosterone acetate (5 mg./wk., s.c.) in the Donryu strain, nephrectomized unilaterally 3 or 4 days previously and given 1% saline instead of drinking water. They were 10 to 12 weeks of age, weighing 160 to 210 Gm. After treatment for 6 to 10 weeks, the rats weighing 200 to 280 Gm. were used for the study. BP ranged from 170 to 210 mm.Hg. Normal rats of the Donryu strain, matched in age and body weight, were used as controls. Their BP ranged from 100 to 130 mm.Hg.

Dose-response (DR) curves to NE were obtained in a preparation in which the central nervous system was eliminated by pithing, decerebration, and vagotomy, as reported previously.1) Since artificial respiration influenced the basal blood pressure level, the output volume and frequency of the positive-pressure respirator (Natume KN-56) were fixed at 30 ml. and 30 strokes/min. with a trachea cannula having a side opening of constant size. Systolic BP determined by the plethysmographic tail method were used as the original level before destruction of the central nervous system (CNS), since the values correlate well with those obtained by direct cannulation.

A series of NE or methoxamine solutions diluted at a constant ratio, were injected intravenously (0.5 ml./Kg.) in an ascending order in concentration. Cocaine (5 mg./Kg., i.v.) given 20 min. before obtaining the DR curves. 1-NE hydrochloride, methoxamine hydrochloride, and cocaine hydrochloride were used. Doses were referred to the salts. 150 μg./Kg. 1-NE HCl corresponds to 123 μg./Kg. 1-NE base.
RESULTS

CV reactivity to NE (Figs. 1 and 2)

DR curves of SHR and normal Wistar rats to NE at 9 dose levels (0.015–150 μg./Kg.) with the original and base blood pressure levels before and after destruction of the central nervous system (CNS) are shown in Fig. 1. Results in hypertensive rats induced by clipping or DOC and in normal rats of the Donryu strain are given in Fig. 2.

DR curves to NE of the hypertensive did not differ from the normotensive, although reaching higher levels at the 3 largest doses in the hypertensive

![Graph showing CV reactivity to NE in SHR and Wistar rats.](image-url)

Fig. 1. CV reactivity to NE in the spontaneously hypertensive (SHR) and normotensive Wistar rat. Vertical bars represent standard error (SE) of the mean; figures in parenthesis, the number of rats. Level indicates the basal BP level after CNS destruction. The original BP level before CNS destruction is plotted on the DR curve.
Doses of NE which elicited the original BP levels before CNS destruction were larger in hypertensive rats. The data have been presented in previous reports. The present results confirmed those of the previous studies, and served as controls for the present studies.

**CV reactivity to NE after cocainization (Figs. 3 and 4)**

Pretreatment with cocaine shifted the DR curves of NE to the left in both normotensive and hypertensive animals. The maximum responses were unchanged by cocaine. It is generally accepted that supersensitivity to NE after cocainization is mainly due to the inhibition of the uptake of NE at the
nerve terminals, although other mechanisms have also to be considered.5)–7) The failure to enhance magnitude of the maximum responses after cocaine in our preparations may be accounted for an evidence of uptake block as the main cause of supersensitivity.

DR curves of SHR and normal Wistar rats to NE after cocainization are shown in Fig. 3. The results in rats rendered hypertensive by clipping or DOC, as compared to controls are given in Fig. 4. Potency ratios of SHR and normal. Wistar rats were 2.5 and 2.4 at a BP level of 69.5 mm.Hg, which corresponds to the 50 per cent effective dose (ED 50) of NE in the normal controls. The ratios were 3.1 and 3.3 at a level of 92.4 mm.Hg, which cor-
responds to ED 50 in SHR. The reason that the DR curves became steeper after cocainization is unknown. Potency ratios of rats rendered hypertensive by clipping, or DOC, and the normal Donryu strain were 4.4, 3.8, and 4.2 at a BP level of 72.7 mm Hg, corresponding to ED 50 in the normal controls; and 5.1, 5.0, and 4.2 at 100 mm Hg, corresponding to ED 50 in the hypertensive rats, respectively.

DR curves of hypertensive rats were shifted to the left as much as those of normal controls. Relationships between the DR curves of hypertensive and normotensive controls were unchanged after cocainization, indicating that
Fig. 5. CV reactivity to methoxamine in the SHR and in the normotensive Wistar rat.

the uptake mechanism of NE is the same in the hypertensive rats, and consequently that the receptor sites are not modified.

CV reactivity to methoxamine (Figs. 5 and 6)

Pretreatment with cocaine did not affect DR curves to methoxamine at 8 dose levels (5–640 µg./Kg.) determined in each of 10 normotensive Donryu rats, confirming that it is not taken up by adrenergic nerve endings.3)

DR curves of SHR and normal Wistar rats to methoxamine are shown in Fig. 5. The results in hypertensive rats induced by clipping or DOC, and the controls are given in Fig. 6. The responses to methoxamine of the hypertensive rats did not differ from the normal controls, although reaching higher
levels at the 2 largest doses in hypertensive rats than did the controls. Doses of methoxamine required to attain the original BP levels before CNS destruction were larger in hypertensive rats. The results indicate again that neither the uptake mechanism nor sensitivity of the receptor sites is responsible for the elevated BP in these types of hypertension.

**Basal BP level after CNS destruction (Table I)**

The levels in SHR did not differ from those of the controls in the present studies, although they were significantly higher in the previous studies. The results in other types of hypertensive rats are in accord with the previous ones.
Table I. Basal Blood Pressure Levels after CNS Destruction in Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>No. of Rats</th>
<th>Mean BP after CNS Destr.</th>
<th>Systolic BP before CNS Destr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>20</td>
<td>46.5±1.2</td>
<td>127.0±2.1</td>
</tr>
<tr>
<td>SHR</td>
<td>16</td>
<td>46.6±0.8</td>
<td>165.6±1.8**</td>
</tr>
<tr>
<td>Donryu</td>
<td>20</td>
<td>49.8±1.2</td>
<td>115.5±1.7</td>
</tr>
<tr>
<td>Clipping</td>
<td>15</td>
<td>55.0±2.6*</td>
<td>188.7±5.6**</td>
</tr>
<tr>
<td>DOC</td>
<td>17</td>
<td>43.2±1.5**</td>
<td>190.0±4.0**</td>
</tr>
</tbody>
</table>

B. Cocaine Pretreatment

<table>
<thead>
<tr>
<th></th>
<th>No. of Rats</th>
<th>Mean BP after CNS Destr.</th>
<th>Systolic BP before CNS Destr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>10</td>
<td>51.2±1.6</td>
<td>125.0±2.2</td>
</tr>
<tr>
<td>SHR</td>
<td>9</td>
<td>51.4±2.0</td>
<td>172.2±4.0**</td>
</tr>
<tr>
<td>Donryu</td>
<td>10</td>
<td>61.5±2.6</td>
<td>114.0±1.6</td>
</tr>
<tr>
<td>Clipping</td>
<td>9</td>
<td>72.2±4.6</td>
<td>190.0±8.7**</td>
</tr>
<tr>
<td>DOC</td>
<td>9</td>
<td>49.4±2.2**</td>
<td>183.3±5.0**</td>
</tr>
</tbody>
</table>

* P<0.05     ** P<0.01

The BP levels after CNS destruction were dependent on the artificial respiration, which was, however, kept constant throughout the experiments. The relationship between BP levels and respiratory tidal volumes remains to be determined.

DISCUSSION

CV reactivity to NE after cocaine pretreatment and to methoxamine was determined in SHR and in the hypertensive rat made by clipping or by DOC and salt. CV reactivity to NE was essentially unchanged after cocaineization in the 3 types of hypertension. Reactivity to methoxamine was also unchanged in hypertension.

Cocaine inhibits the uptake process of NE at the nerve terminals. This was the main effect of cocaine in our preparation, judged by the shift of the DR curves to NE. The fact that cocaine pretreatment did not change relationship between DR curves to NE of hypertensive rats and those of the controls, indicates that the uptake process at the adrenergic terminals was unchanged in hypertensive rats. Since overall reactivity was the same, the receptor sites are not modified in hypertension.

Methoxamine is not taken up by adrenergic nerve endings. CV reactivity to methoxamine was unchanged in hypertensive rats. The results indicate again that neither uptake mechanism nor sensitivity of the receptor sites is responsible for the high BP in these types of hypertension.
The action of methoxamine is predominantly $\alpha$-type. DR curves to this drug is the results of its effect mostly on the peripheral vascular system, and the effect on the heart must be minimal. After the pithing, cardiac hemodynamic was altered in our preparation, but determination of CV reactivity to methoxamine can eliminate this cardiac factor.

Survey of literatures on CV reactivity to NE in experimental hypertensions indicated some inconsistency.\(^1\),\(^2\) Recent works in SHR and in preparation utilizing the pithing procedures were summarized in the previous paper.\(^2\) More works have been published since, showing that CV reactivity to NE was increased, decreased, or unchanged in hypertensive rats,\(^8\)\(\text{to}^{13}\) rabbits,\(^14\),\(^15\) or dogs.\(^16\)

**REFERENCES**