Alterations in Catecholamine Release in the Central Nervous System of Spontaneously Hypertensive Rats

Kazushi Tsuda, M.D.,* Seiko Tsuda, M.D.,** Yoshiaki Masuyama, M.D.,* and Menek Goldstein, Ph.D.***

SUMMARY

The aim of the present study was to investigate alterations in catecholamine release in the central nervous system of spontaneously hypertensive rats. Slices of hypothalamus, medulla oblongata and striatum were prepared from spontaneously hypertensive rats (SHR: 9-10 weeks old) and age-matched Wistar Kyoto rats (WKY). The slices were incubated with (3H)norepinephrine (NE) or (3H)dopamine (DA), superfused with Krebs-solution in vitro, and the release of the catecholamines was compared between the two strains. The basal release of hypothalamic (3H)NE did not differ between SHR and WKY slices. However, stimulation (1 Hz)-evoked (3H)NE release was significantly greater in SHR than in WKY (percent fractional release of total tissue NE: WKY 0.494±0.019%, n=6, SHR 0.730±0.053%, n=6, p<0.05). The stimulation-evoked (3H)NE release from the medulla oblongata did not differ significantly between SHR and WKY slices.

Finally stimulation-evoked release of striatal (3H)DA was significantly depressed in SHR (percent fractional release of total tissue DA: WKY 2.048±0.024%, n=6, SHR 1.460±0.068%, n=6, p<0.05).

These results indicate that the release of hypothalamic NE and striatal DA are altered in SHR. It is suggested that enhanced hypothalamic noradrenergic activity and reduced striatal dopaminergic activity can increase sympathetic outflow to the periphery, which may play a role in the pathogenesis of this form of hypertension.

Key Words:
Spontaneously hypertensive rats  Wistar Kyoto rats  Norepinephrine release  Dopamine release  Hypothalamus  Medulla oblongata  Striatum  Central nervous system

From the *Division of Cardiology, Department of Medicine, **Third Department of Internal Medicine, Wakayama Medical College, Wakayama, and ***Neurochemistry Research Laboratories, New York University Medical Center, New York.

Mailing address: Kazushi Tsuda, M.D., Division of Cardiology, Department of Medicine, Wakayama Medical College, 27, 7-Bancho, Wakayama 640, Japan.

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Central catecholaminergic mechanisms have been implicated in the regulation of blood pressure. Haesler et al. showed that intracerebroventricular administration of the neurotoxin 6-hydroxy dopamine to young spontaneously hypertensive rats (SHR) prevented the development of hypertension. This treatment caused marked and widespread depletion of brain norepinephrine (NE). In agreement with this finding, Bunag and Eferakeya observed that posterior hypothalamic lesions lowered blood pressure in SHR and renal hypertensive rats. Moreover, it was reported that microinjections of NE into the posterior hypothalamus increased systemic blood pressure of rats. From these observations, it is strongly suggested that abnormalities in noradrenergic transmission in the hypothalamus might have a role in the pathogenesis of hypertension.

The nucleus tractus solitarii in the medulla oblongata is also believed to have a crucial role in central cardiovascular regulation. Injection of catecholamines into this area results in a depression of systemic blood pressure. In contrast, bilateral lesions of the nucleus produce fulminating hypertension in rats. Thus, it seems likely that there might be regional differences in the role of norepinephrine in the central control of blood pressure.

The involvement of central dopaminergic pathways in the regulation of blood pressure and heart rate has been also well documented. Zandberg et al. reported that the local injection of dopamine (DA) into the nucleus tractus solitarii decreased blood pressure. Le Fur et al. and Chiu et al. have described an increase in the frontal cortical content of DA and an increase in the number of DA-binding sites in striatum and frontal cortex of SHR. Furthermore, in a clinical study, Kolloch et al. reported that dopaminergic stimulation by bromocriptine reduced the sympathetic outflow and lowered blood pressure in patients with essential hypertension. This finding produced the hypothesis that reduced central dopaminergic activity might be a factor in the cause and maintenance of hypertension.

In order to gain further insight into the relationship between central amine metabolism and hypertension, this study investigated alterations in the release of NE and DA in hypothalamic, medulla oblongata and striatal slices from spontaneously hypertensive rats. These studies document altered NE release from the hypothalamus and DA release from the striatum.

Materials and Methods

Animals:
Male SHR (9–10 weeks old: Taconic Farms, German Town, NY, USA) and age-matched male Wistar Kyoto rats (WKY: Taconic Farms)
were used for these studies. The systolic blood pressure, measured by the tail-cuff method (Narco Biosystem Inc., model PE-300, Houston, Texas, USA) was $176.1 \pm 2.9\ mmHg$ ($n=6$) in SHR and $125.1 \pm 2.0 \ mmHg$ ($n=6$) in WKY, respectively.

**Experimental Procedure:**

The rats were killed by decapitation, and the hypothalamus, medulla oblongata and striatum were rapidly dissected on ice. Tissues were sliced at 0.3 mm thickness with a Brinkman tissue chopper (Brinkman Instrs., Inc., Westburney, NY, USA). For the hypothalamus and medulla oblongata, tissues were rotated 90° and sliced again (0.3×0.3 mm). The sliced tissues were washed 3 times with 2 ml Krebs-Ringer bicarbonate buffer (mmol/l: NaCl 118.0, KCl 4.80, CaCl$_2$ 1.20, KH$_2$PO$_4$ 1.15, MgSO$_4$ 1.20, NaHCO$_3$ 25.0, glucose 11.1, ascorbic acid 0.11, and disodium EDTA 0.04 saturated with a 95% O$_2$ and 5% CO$_2$ mixture at 37°C, pH 7.4). The slices of the hypothalamus and medulla oblongata were incubated with 3 ml of fresh buffer containing 0.1 μM of (3H)NE (specific activity 40.8 Ci/mmol: New England Nuclear Research Products, Boston, MA, USA) for 20 min at 37°C. The slices of striatum were incubated with 0.1 μM of (3H)DA (specific activity 37.0 Ci/mmol: New England Nuclear Research Products) according to the same procedure as described above. After the slices (5–7 mg) were rinsed with fresh buffer, they were transferred to a superfusion chamber (200 μl), jacketed with 37°C water, and suspended between two platinum electrodes (25 mm apart, 2.0 mm long). The slices were then superfused with Krebs-Ringer bicarbonate buffer at a rate of 0.7 ml/min. Sample collection began after 60 min of the superfusion when basal outflow of tritium had stabilized to a constant level. The electrical stimulation, applied 67 min after the beginning of the superfusion with a Grass stimulator (Model S4K, Quincy, MA, USA), consisted of trains of unipolar, rectangular pulses (1 Hz, 20 mA, 2 msec for 2 min). Samples of the perfusate were collected at 7-min intervals. At the end of the experiment the slices were sonicated for 20 sec. Radioactivity in collected samples and tissues was determined by liquid scintillation counting (Tri-carb Liquid Scintillation Spectrometer 3255, Packard Instr. Co., Inc., Sterling, VA, USA).

The amount of radioactivity in each sample was evaluated by dividing the total tritium collected in each sample by the total tritium present in the tissue at the time of sample collection (the tritium released into superfusate after that point plus the tritium remaining in the tissue at the end of experiment: percent fractional release). The stimulation-evoked release was calculated by subtracting the basal outflow during the 7 min prestimulation period (60–66 min) from the values in samples collected during 2 min and
after 5 min of electrical stimulation (67–73 min).

**Statistics:**

Values are presented as means±SEM. Significant differences were determined by the Wilcoxon rank-sum test. A p value less than 0.05 was accepted as the level of significance.

**RESULTS**

**Basal and Stimulation-Evoked Tritiated Norepinephrine Release from Slices of Hypothalamus and Medulla Oblongata of Spontaneously Hypertensive Rats and Wistar Kyoto Rats:**

The basal release of (3H)NE from the hypothalamus of SHR is shown in Table I. The basal release of (3H)NE was slightly increased in SHR, but the difference was not significant between SHR and WKY. On the other hand, as shown in Fig. 1, the electrical stimulation (1 Hz)-evoked release of (3H)NE was significantly increased in hypothalamic slices of SHR compared with WKY (percent fractional release, WKY, 0.494±0.019%, n=6, SHR 0.730±0.053%, n=6, +48%, p<0.05).

The basal (3H)NE release from the medulla oblongata did not differ between SHR and WKY (Table I). Figure 2 illustrates the stimulation (1 Hz)-evoked (3H)NE release in medulla oblongata of SHR and WKY; there was no significant difference in release between the two strains (percent fractional release during stimulation, WKY 1.436±0.078%, n=6, SHR 1.352±0.101%, n=6).

**Basal and Stimulation-Evoked Tritiated Dopamine Release from Slices of Striatum of Spontaneously Hypertensive Rats and Wistar Kyoto Rats:**

The basal release of (3H)DA from striatum of SHR was similar to that

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<th>WKY</th>
<th>SHR</th>
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<tr>
<td>Basal (3H)norepinephrine release from hypothalamus</td>
<td>1.622±0.029% (n=6)</td>
<td>1.719±0.034% (n=6)</td>
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<tr>
<td>Basal (3H)norepinephrine release from medulla oblongata</td>
<td>2.558±0.050% (n=6)</td>
<td>2.460±0.056% (n=6)</td>
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<tr>
<td>Basal (3H)dopamine release from striatum</td>
<td>6.380±0.098% (n=6)</td>
<td>6.247±0.166% (n=6)</td>
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Values are means±SEM. Percent (%) fractional release is expressed as the tritium released spontaneously from the tissue (during 60–66 min from the beginning of the superfusion) divided by the total tissue tritium at the time of sample collection (see Materials and Methods section in the text).
Fig. 1. Stimulation (1 Hz)-evoked release of (3H)norepinephrine from hypothalamic slices of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY). Percent fractional release is expressed as the tritium released during stimulatory period (67–73 min from the beginning of the superfusion) divided by the total tissue tritium at the time of sample collection (see text).

Fig. 2. Stimulation (1 Hz)-evoked release of (3H)norepinephrine from slices of medulla oblongata of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY).
Fig. 3. Stimulation (1 Hz)-evoked release of (3H)dopamine from striatal slices of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY).

of WKY (Table I). However, the stimulation (1 Hz)-evoked (3H)DA release was depressed in SHR, as indicated by a significantly lower fractional release of (3H)DA (Fig. 3; percent fractional release, WKY 2.048±0.024%, n=6, SHR 1.460±0.068%, n=6, -29%, p<0.05).

DISCUSSION

It has been suggested that brain catecholamines actively participate in the central regulation of blood pressure. In this study, it was shown that the basal release of medullary and hypothalamic NE and striatal DA did not differ significantly between SHR and WKY. McKeon and Hendley reported that the NE level in hypothalamus and brain stem and the DA level in striatum were not significantly different between SHR and WKY. Previously, Ekas and Lokhandwala proposed that the retention and storage of NE of the tissue are similar when the basal release of (3H)NE is similar. Thus, it seems likely that the basal release, retention and storage of catecholamines do not differ significantly between SHR and WKY.

On the other hand, the stimulation-evoked (3H)NE was significantly elevated in the hypothalamus of SHR. The alterations in the stimulation-
evoked release of (3H)NE in SHR are suggestive of a specific change at the level of the nerve terminals. Bunag and Eferakeya\textsuperscript{2)} reported that bilateral posterior hypothalamic lesions had a greater hypotensive effect in SHR. Zawolski\textsuperscript{3)} also reported that microinjection of NE into the posterior hypothalamus increased blood pressure. Furthermore, DeQuattro et al\textsuperscript{14)} observed an elevated norepinephrine level in whole hypothalamus of animals with renovascular hypertension. These findings suggest that increased noradrenergic activity in the hypothalamus subserves an important role in the pathogenesis of hypertension. Thus, enhanced NE release in the hypothalamus of SHR could contribute to the elevated blood pressure in this animal model.

By contrast, the stimulation-evoked (3H)NE release in the medulla oblongata did not differ significantly between SHR and WKY. The mechanisms for the regional differences in NE release in the brain of SHR are still uncertain. Kubo et al\textsuperscript{15)} reported that presynaptic \(\alpha_2\)-adrenoceptor function, which has a negative feedback modulation on NE release from nerve endings, was significantly diminished in the hypothalamus, but that it was enhanced in the brain stem of SHR. This suggests that changes in presynaptic NE autoreceptors may be a factor in the regional differences in NE release in SHR. Furthermore, DeJong and Petty\textsuperscript{4)} observed that catecholamines injected into the nucleus tractus solitarii inhibited sympathetic outflow to the periphery and lowered systemic blood pressure, suggesting that the nucleus is a depressor center. Meldrum and Westfall\textsuperscript{16)} further suggested that this depressor component is attenuated in SHR, based upon their findings that NE release in brain stem tissues was significantly reduced in mature SHR. Although further studies are required to determine the precise mechanisms which could modulate NE release in the central nervous system of SHR, NE release in medulla oblongata in SHR appears incapable of counteracting the pressor effects induced by increased hypothalamic NE.

Recently, the involvement of central dopaminergic pathways in the central regulation of blood pressure has been described both in experimental and human hypertension.\textsuperscript{6)-8)} Kolloch et al\textsuperscript{9)} reported that bromocriptine, a dopaminergic agonist, significantly reduced the blood pressure in patients with essential hypertension, suggesting the significance of DA as an important depressor component in the central nervous system. In this study, we showed that the stimulation-evoked (3H)DA release was significantly decreased in the striatum of SHR, indicating a functional dopaminergic insufficiency in this form of hypertension. It is well known that prolactin, vasopressin and thyrotrophic hormone are mainly under dopaminergic control, with DA as an inhibitory factor, and that the plasma levels of these hormones have been
reported to be increased in SHR and in patients with essential hypertension. These findings support the hypothesis that central dopaminergic activity might be decreased in primary hypertension. Chiu et al observed that the in vitro (3H)spiroperidol binding to the SHR striatum was significantly increased in SHR. This result is suggestive of DA receptor supersensitivity and decreased DA content in synaptic junctions in hypertension, which is consistent with our present results. However, Van den Buuse et al proposed that the nigro-striatal dopaminergic system might have a role in elevating systemic blood pressure, because brain dopamine depletion by lesions in the substantia nigra attenuated the development of hypertension in SHR. Thus, the role of central dopamine in the regulation of blood pressure is still controversial, and further studies should be performed to evaluate the mechanisms of dopaminergic actions in the central nervous system of hypertension.

In summary, the results of the present study show that stimulation-evoked NE release was elevated in the hypothalamus, but unchanged in the medulla oblongata of SHR compared with age-matched WKY. In addition, the stimulation-evoked striatal DA release was significantly lower in SHR than in WKY. It is suggested that the enhanced noradrenergic activity in the hypothalamus and decreased dopaminergic activity in the striatum of SHR can produce an increased sympathetic outflow which could play a role in the pathogenesis of this form of hypertension.

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References