Enhanced 5-Hydroxytryptamine Release from Vascular Adrenergic Nerves in Spontaneously Hypertensive Rats

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With the technical assistance of Keizo Masumoto

SUMMARY The release of 5-hydroxytryptamine from the vascular adrenergic nerve by periarterial nerve stimulation in spontaneously hypertensive rats (SHR) was compared with that in normotensive Wistar-Kyoto rats (WKY). The isolated mesenteric vascular bed was perfused at a constant flow rate of 5 ml/min. Vasoconstrictor responses to periarterial nerve stimulation (4, 8, 12, and 16 Hz for 30 seconds) and 5-hydroxytryptamine (1 μM), but not norepinephrine (1 nmol), were significantly greater in SHR than in WKY. After treatment with 5-hydroxytryptamine (1 μM) for 15 minutes, vasoconstrictor responses to periarterial nerve stimulation previously reduced by prazosin (50 nM) were restored and a frequency-dependent pressor response reappeared. However, 5-HT treatment did not significantly affect the pressor response to exogenously administered norepinephrine (1 nmol), which was previously inhibited by prazosin. The degree of the restoration in SHR was significantly greater than that in WKY at all frequencies used. The restoration of the pressor response to periarterial nerve stimulation after 5-hydroxytryptamine treatment did not occur in the presence of the selective 5-hydroxytryptamine 2 receptor antagonists ketanserin (10 nM) or LY53857 (10 nM). In the perfused mesenteric vascular bed of both WKY and SHR prelabeled with [3H]5-hydroxytryptamine, periarterial nerve stimulation (4–16 Hz) evoked a frequency-dependent increase in tritium efflux that was abolished by Ca2+-free Krebs-Ringer solution or tetrodotoxin (100 nM) and treatment with 6-hydroxydopamine. The tritium efflux evoked by periarterial nerve stimulation was significantly greater in SHR than in WKY at all frequencies used. These results suggest that the release of 5-hydroxytryptamine from adrenergic nerve endings by periarterial nerve stimulation is enhanced in the mesenteric vascular bed of the SHR. (Hypertension 10: 321–327, 1987)

KEY WORDS • 5-hydroxytryptamine • vascular adrenergic nerve • spontaneously hypertensive rats

SPONTANEOUSLY hypertensive rats (SHR) have been used as a model for studying human essential hypertension. The increased total vascular resistance resulting from enhanced sympathetic adrenergic tone is postulated to be the cause of this hypertension. However, actual mechanisms underlying the development and maintenance of the hypertension remain unresolved. Several lines of evidence suggest that 5-hydroxytryptamine (5-HT) may be involved in the hypertension of SHR. In fact, the vasoconstrictor response to 5-HT has been shown to be exaggerated in SHR compared with normotensive Wistar-Kyoto rats (WKY). Moreover, the 5-HT receptor antagonist ketanserin reduces blood pressure in SHR as well as in hypertensive humans.

The accumulation of 5-HT in adrenergic nerve endings has been demonstrated in various tissues, including the vas deferens and intestine of the guinea pig, the pineal gland of the rat, and the canine saphenous vein and cerebral artery. The 5-HT taken up and accumulated in vascular adrenergic nerve endings is released by nerve stimulation in a calcium-dependent manner. Additionally, the 5-HT released from adrenergic nerve endings produces a vasoconstrictor response that is mediated by postsynaptic 5-HT receptors. Therefore, the present study was designed to compare the release of 5-HT that is taken up and accumulated in vascular adrenergic nerves between WKY and SHR.

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Materials and Methods

The study used 46 male SHR (14–15 weeks old) and 45 age-matched WKY (Charles River Japan, Atsugi, Japan), weighing 309.8 ± 2.1 and 294.9 ± 2.2 g, respectively. The animals were given food and water ad libitum and were housed in an air-conditioned room (20 ± 1°C) with a 12-hour light/dark cycle (light on at 0730).

Blood Pressure Measurement

The animals were anesthetized with pentobarbital Na (50 mg/kg i.p.). Before the operation, the left carotid artery was cannulated and mean arterial pressure was recorded on a polygraph (Model RM-6000; Nihon Kohden, Tokyo, Japan) with a pressure transducer (Model P23ID; Gould Statham Instruments, Hato Rey, Puerto Rico).

Perfusion of the Mesenteric Vascular Bed

The mesenteric vascular bed was prepared for perfusion by the method of McGregor, as reported previously. The isolated mesenteric vascular bed was placed on a 10-ml water-jacketed organ bath maintained at 37°C and perfused with modified Krebs-Ringer bicarbonate solution at a constant flow rate of 5 ml/min by means of a peristaltic pump (Model SJ-1215; ATTO, Tokyo, Japan). The preparation was also superfused with the same solution at a rate of 0.5 ml/min to prevent drying. The Krebs-Ringer solution had the following composition (in mM): NaCl, 120; KCl, 5.0; CaCl_2, 2.4; MgSO_4, 1.2; NaHCO_3, 25; KH_2PO_4, 1.2; disodium EDTA, 0.027; and dextrose, 11.0 (pH = 7.4). Calcium-free Krebs-Ringer solution was prepared by omission of CaCl_2 when required. The Krebs-Ringer solution was aerated with a mixture of 95% O_2, 5% CO_2 before passage through a warming coil maintained at 37°C. Changes in the perfusion pressure were measured with a pressure transducer (Model MPU-0.5A; Nihon Kohden) and recorded on a polygraph (Model RM-25; Nihon Kohden).

Basal perfusion pressure was allowed to stabilize, and the perfused mesenteric vascular bed was then subjected to periarterial nerve stimulation (PNS) and infusion of norepinephrine (NE). PNS was performed for 30 seconds at 5-minute intervals using an automatic timer with bipolar platinum ring electrodes placed around the superior mesenteric artery. Square-wave pulses 2 milliseconds long and supramaximal voltage were delivered at 4, 8, 12, and 16 Hz by an electronic stimulator (Model SEN 1101; Nihon Kohden). The neural basis of the pressor response mediated by the stimulation of the periarterial adrenergic nerve was previously confirmed by abolition of the response after perfusion of guanethidine (100 mM) and tetrodotoxin (100 nM) and two treatments with 6-hydroxydopamine (6-OHDA, 50 mg/kg/day i.p.). NE (1 nmol) was infused directly into the perfusate proximal to the arterial cannula with an infusion pump (Model 975; Harvard Apparatus, South Natick, MA, USA) for 10 seconds in a volume of 0.05 ml.

Experimental Protocols

After initial pressor responses to PNS (4–16 Hz) and NE (1 nmol) infusion were obtained, both PNS and NE infusion were performed in the presence of prazosin (50 nM). Thereafter, 5-HT was perfused in the presence of prazosin for 15 minutes. After discontinuation of 5-HT perfusion, the preparation was perfused for at least 30 minutes with Krebs-Ringer solution containing prazosin and then both PNS and NE infusion were performed in the presence of prazosin. In the experiments using 5-HT receptor antagonists, the perfusion of Krebs-Ringer solution containing the antagonist and prazosin was initiated 20 minutes after the discontinuation of the 5-HT perfusion and continued through the rest of the experiment.

Measurement of Tritium Efflux

The isolated mesenteric vascular bed was perfused with Krebs-Ringer solution at a constant flow rate of 5 ml/min and superfused at 0.5 ml/min by means of the peristaltic pump. After a 30-minute perfusion to stabilize the perfusion pressure, the vascular bed was perfused and labeled with 100 nM tritiated [1,2-^3H(N)]5-HT (specific activity, 24.2 Ci/mmol) for 20 minutes. The bed then was rinsed for 90 minutes, and the perfusate was collected in 1-minute fractions. PNS was carried out for 30 seconds at 10-minute intervals using an automatic timer. Rectangular pulses lasting 2 milliseconds at 4, 8, 12, and 16 Hz and supramaximal voltage were given through the platinum ring electrodes. Aliquots of 0.5 ml were added to 4.0 ml of scintillation fluid (Aquazol-2; New England Nuclear, Boston, MA, USA), and counted for 10 minutes with a scintillation counter (Model LSC-730; Aloka, Tokyo, Japan). The perfusion of Krebs-Ringer solution containing tetrodotoxin or Ca^{2+}-free Krebs-Ringer solution was begun 20 minutes before the first PNS (4 Hz) and continued through the rest of the experiment. For the chemical sympathectomy of the mesenteric artery, the animals were pretreated with 6-OHDA at a dose of 50 mg/kg, which was dissolved in 0.9% saline containing 0.1% ascorbic acid and administered intraperitoneally on Day 1 and Day 2 (24 hours apart). The preparation was made on Day 3, 24 hours after the last administration. The control vehicle (0.9% saline containing 0.1% ascorbic acid, 1 ml/kg i.p.) was administered according to the same schedule as 6-OHDA administration. After completion of the experiment, the mesenteric vasculature was weighed, digested with tissue solubilizer (Soluene-100; Packard Instrument, Downers Grove, IL, USA), and counted for 10 minutes. The total tritium efflux was expressed as the percentage of the amount of tritium in the tissue. The net efflux of tritium induced by PNS was determined by tritium efflux of two 1-minute samples (during and after PNS) minus the spontaneous efflux just before PNS.

In some experiments, the mesenteric vascular bed was labeled by perfusion of 100 nM [^3H]5-HT for 20 minutes. After a 90-minute rinse, the vascular bed was weighed, digested with Soluene-100, and counted for...
10 minutes. Data are expressed as disintegrations per minutes of tritium per milligram of wet weight.

**Statistical Analysis**

Data are expressed as means ± SEM and were statistically analyzed using Student's t-test for group mean comparison. A p value less than 0.05 was considered a statistically significant difference.

**Drugs**

The following drugs were used: [1,2-3H(N)]5-HT creatinine sulfate (New England Nuclear); 5-HT hydrochloride (Sigma Chemical Corp., St. Louis, MO, USA); ketanserin tartrate (gift of Janssen Pharmaceutica, Beerse, Belgium); 6-OHDA hydrobromide (Sigma); LY53857, [4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl) 4,6,6A,7,8,9,10,10A-octahydroindolo(4,3-F G) quinoline maleate] (gift of Eli Lilly, Indianapolis, IN, USA); r-NE hydrochloride (Sigma); prazosin hydrochloride (gift of Taito Pfizer, Tokyo, Japan); and tetrodotoxin (Sigma). Both 5-HT and NE were dissolved in 0.9% saline containing 0.1% ascorbic acid and stored in a freezer. On the day of the experiments, final dilutions of 5-HT and NE were made with Krebs-Ringer solution just before being perfused or infused. All other drugs were dissolved in distilled water and diluted in Krebs-Ringer solution aerated with a mixture of 95% O2, 5% CO2 before perfusion.

**Results**

Table 1 summarizes systemic arterial pressure, mean mesenteric basal perfusion pressure, and pressor responses to PNS, NE infusion, and 5-HT perfusion in WKY and SHR. The mean carotid blood pressure of SHR was significantly greater than that of WKY. A significant elevation of mean basal perfusion pressure was also observed in the SHR preparation. Vasoconstrictor responses to PNS and 5-HT, but not to NE, were also significantly greater in SHR than in WKY. Treatment with 6-OHDA caused a significant decrease in mean blood pressure of both WKY and SHR compared with vehicle-treated controls and abolished the vasoconstrictor response to PNS (Table 2).

As shown in Figure 1A, PNS (4–16 Hz) induced a frequency-dependent increase of perfusion pressure in the perfused mesenteric vascular bed of the SHR. The infusion of NE (1 nmoL) also increased the perfusion pressure (see Figure 1A). In the presence of prazosin (50 nM), pressor responses to both PNS and NE infusion were markedly reduced by approximately 90 to 95% compared with control response (see Figure 1B). When the preparation was treated with 5-HT (1 μM) for 15 minutes, the reduced pressor response to PNS in the presence of prazosin was restored and a frequency-dependent pressor response to PNS reappeared (see Figure 1C). However, the reduced pressor responses to exogenous NE in the presence of prazosin was not altered by 5-HT treatment (see Figure 1C, Figure 2A).

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**Table 1.** Systemic Arterial Pressure, Mean Perfusion Pressure, and Pressor Response to Periarterial Nerve Stimulation, Norepinephrine, 5-Hydroxytryptamine in the Perfused Mesenteric Vascular Bed of WKY and SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
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<tbody>
<tr>
<td>Pressure or pressor response (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean carotid arterial pressure</td>
<td>94.4 ± 2.3 (34)</td>
<td>159.6 ± 2.6* (34)</td>
</tr>
<tr>
<td>Mean mesenteric basal perfusion pressure</td>
<td>29.1 ± 0.7 (34)</td>
<td>34.2 ± 0.9* (34)</td>
</tr>
<tr>
<td>Pressor response to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNS</td>
<td></td>
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<tr>
<td>4 Hz</td>
<td>0.6 ± 0.2 (11)</td>
<td>1.4 ± 0.2† (14)</td>
</tr>
<tr>
<td>8 Hz</td>
<td>1.5 ± 0.5 (11)</td>
<td>6.2 ± 1.2 (14)</td>
</tr>
<tr>
<td>12 Hz</td>
<td>5.9 ± 1.2 (11)</td>
<td>37.2 ± 7.3 (14)</td>
</tr>
<tr>
<td>16 Hz</td>
<td>19.5 ± 2.5 (11)</td>
<td>81.6 ± 10.1* (14)</td>
</tr>
<tr>
<td>NE (1 nmol)</td>
<td>14.2 ± 5.1 (11)</td>
<td>32.6 ± 9.0 (14)</td>
</tr>
<tr>
<td>5-HT (1 μM)</td>
<td>42.8 ± 12.2 (11)</td>
<td>87.3 ± 14.4† (14)</td>
</tr>
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</table>

Values are means ± SEM. Numbers in parentheses represent number of experiments.

PNS = periarterial nerve stimulation; NE = norepinephrine; 5-HT = 5-hydroxytryptamine.

* p<0.001, † p<0.05, ‡ p<0.01, compared with values for WKY.

The restored pressor response to PNS (4–16 Hz) after 5-HT treatment was significantly greater in the SHR preparation than in the WKY preparation at all frequencies used (see Figure 2A).

In both WKY and SHR, the reduced pressor response to PNS in the presence of prazosin (50 nM) was not altered by 5-HT treatment (1 μM) when the selective 5-HT2 receptor antagonist, ketanserin (10 nM) (see Figures 1D and 2B) or LY53857 (10 nM), was present in the perfusate. Additionally, the pressor responses to 5-HT was markedly inhibited by either ketanserin (see Figure 1D) or LY53857.

In both WKY and SHR, PNS (4–16 Hz) of the perfused mesenteric vascular bed labeled with [3H]5-HT produced a frequency-dependent increase in tritium efflux in the perfusate (Figure 3A). The net tritium efflux induced by PNS at 4 to 16 Hz was significantly greater in SHR than in WKY (see Figure 3B). The PNS-evoked tritium efflux was abolished by Ca2+-free Krebs-Ringer solution and tetrodotoxin (100 nM) and by treatment with 6-OHDA (Figure 4) in both WKY and SHR.

There was no significant difference in the accumulation of [3H]5-HT in the mesenteric vascular bed from WKY or SHR (1.39 ± 0.10 vs 1.33 ± 0.19 104 dpm/mg wet weight; n = 6 of each strain).

**Discussion**

The present study demonstrated that the vasoconstrictor responses to PNS previously abolished in the presence of prazosin was restored by treatment with 5-HT, especially in the SHR, but did not affect the pressor response to NE previously abolished by prazosin. The restoration of the pressor response to PNS after 5-HT treatment did not occur when the selective 5-HT2 receptor antagonist, ketanserin, or the 5-HT2 receptor antagonist, LY53857, was present in the perfusate.
TABLE 2. Effects of Vehicle and 6-Hydroxydopamine Treatment on Systemic Arterial Pressure, Mean Perfusion Pressure, and Pressor Response to Periarterial Nerve Stimulation in the Isolated Perfused Mesenteric Vascular Bed of WKY and SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n = 5)</td>
<td>6-OHDA (n = 6)</td>
</tr>
<tr>
<td>Mean carotid arterial pressure</td>
<td>89.3±4.8</td>
<td>68.2±1.7*</td>
</tr>
<tr>
<td>Mean mesenteric basal perfusion pressure</td>
<td>25.7±1.1</td>
<td>27.1±0.8</td>
</tr>
<tr>
<td>Pressor response to PNS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Hz</td>
<td>3.0±0.2</td>
<td>0.5±0.18</td>
</tr>
<tr>
<td>8 Hz</td>
<td>7.7±2.2</td>
<td>1.0±0.1§</td>
</tr>
<tr>
<td>12 Hz</td>
<td>25.4±6.5</td>
<td>1.6±0.2*</td>
</tr>
<tr>
<td>16 Hz</td>
<td>49.8±10.9</td>
<td>2.3±0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. n = number of experiments. 6-OHDA = 6-hydroxydopamine; PNS = periarterial nerve stimulation.

Treatment: 6-OHDA, 50 mg/kg/day i.p., twice; vehicle, 0.1% ascorbic acid 1 ml/kg/day i.p., twice.

*p < 0.001, §p < 0.01, ||p < 0.05, compared with values for WKY.

*p < 0.01, §p < 0.05, ||p < 0.001, compared with vehicle treatment.

receptor antagonist, ketanserin or LY53857, was present in the perfusion medium, suggesting that the response probably was mediated by 5-HT released from vascular adrenergic nerves. In the mesenteric vascular bed labeled with [3H]5-HT, PNS evoked an increase in tritium efflux in both WKY and SHR. The PNS-evoked tritium efflux was abolished by tetrodotoxin, elimination of calcium from the perfusion medium, and treatment with 6-OHDA. Because these drugs and the treatment are well known to interrupt the transmitter release (i.e., NE) from adrenergic nerve endings, it appears that the tritiated substance, probably [3H]5-HT, is released from vascular adrenergic nerve endings by PNS and that the release of 5-HT is carried out by the calcium-dependent exocytotic process. Taken together, these results indicate 1) that 5-HT is taken up

Figure 1. Typical effect of treatment with 1 μM 5-hydroxytryptamine (5-HT) on pressor responses to periarterial nerve stimulation (PNS; 4–16 Hz) and 1 nmol norepinephrine (NE) infusion in the presence of prazosin and effect of ketanserin in the perfused mesenteric vascular bed of the SHR. A. Pressor responses to PNS and NE. B. Pressor responses to PNS, NE, and 5-HT in the presence of 50 nM prazosin. C. Pressor responses to PNS and NE 30 minutes after 5-HT perfusion in the presence of prazosin. D. Pressor responses to PNS, NE, and 5-HT in the combined presence of prazosin and 10 nM ketanserin.
and accumulated in vascular adrenergic nerve endings,
2) that this 5-HT is then released by PNS, and 3) that
the released 5-HT produces a vasoconstrictor re-
sponse. These findings confirm our previous report.13

In the present experiments, the degree of restoration
of pressor response to PNS seen after 5-HT treatment
was greater in SHR than in WKY. Because the vaso-
constrictor response to perfusion of 5-HT was also
greater in SHR than in WKY, the increased sensitivity
to 5-HT in the SHR preparation may contribute, in
part, to a greater restoration of pressor response to PNS
after 5-HT treatment. However, the pressor response
to perfused 5-HT (1 μM) in SHR was twice that of
WKY (see Table 1), whereas the ketanserin-sensitive
pressor responses to PNS (12 and 16 Hz) in SHR were
eight and seven times greater than those in WKY (see
Figure 2A), indicating increased 5-HT release from
vascular adrenergic nerves of SHR. This possibility is
supported by the present results showing that, in the
preparation labeled with [3H]5-HT, a greater efflux of
tritium evoked by PNS was observed in the SHR as
compared with the WKY. Therefore, the release of 5-
HT from vascular adrenergic nerves by PNS probably
is enhanced in the SHR preparation.

A greater release of endogenous NE or [3H]NE
(both) from adrenergic nerve endings by nerve stimu-
lation has been demonstrated in various blood vessels
and organs of SHR, including mesenteric artery,16,17
caudal artery,18 kidney,19 and portal vein20 as com-
pared with those of WKY. Because 5-HT has been
shown to behave similarly to endogenous NE (i.e., it is
taken up into adrenergic nerve endings by a cocaine-
sensitive mechanism and incorporated into vesicles),12,13,21
these reports may further support the present
finding of the increased release of 5-HT from
adrenergic nerve endings in the SHR.
A number of studies have provided evidence of an increased accumulation (probably due to an increased uptake) of NE or [³H]NE (or both) in the mesenteric artery of SHR as compared with WKY. 22–24 Although Head et al.25 reported a similar neuronal uptake of [³H]NE between mesenteric arteries from WKY and SHR. Thus, the uptake of 5-HT, like NE, into the mesenteric artery may be enhanced in SHR. This speculation implies that the increased release of 5-HT from vascular adrenergic nerves of SHR may be due in part to an increased accumulation of 5-HT in adrenergic nerve endings. However, the present study showed that the uptake and accumulation of 5-HT in the mesenteric vascular bed of SHR were similar to those in WKY. Additionally, it has been reported that no significant difference exists between WKY and SHR in the uptake of 5-HT into the blood platelets, which has been used as an ideal model for central serotoninergic nerve functioning.26 Further study is needed to clarify the uptake of 5-HT into the vascular adrenergic nerves of SHR.

In conclusion, the present results suggest that 1) 5-HT is taken up into adrenergic nerves of the mesenteric artery and released by nerve stimulation, 2) the release of 5-HT from the vascular adrenergic nerve is enhanced in SHR, and 3) the 5-HT released from adrenergic nerves produces a vasoconstrictor response. 5-HT has been shown to amplify the effect of vasoactive substances such as NE.3 Therefore, the enhanced 5-HT release from vascular adrenergic nerves may contribute to the maintenance of hypertension in SHR.

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