Neurotransmitter Release and Vascular Reactivity in Spontaneously Hypertensive Rats

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This study was designed to investigate neurotransmitter release during the sympathetic nerve stimulation of perfused mesenteric arterial beds of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) at young and adult ages. The role of Ca in neurotransmitter release and vascular responsiveness was also examined by using a Ca-antagonist (verapamil). Pressor responses to electrical nerve stimulation and exogenous noradrenaline were greater in SHR than in WKY. Noradrenaline overflow by electrical nerve stimulation from mesenteric arterial beds was also significantly greater in young SHR than age-matched WKY. However, in adult SHR, the noradrenaline overflow was reduced compared with WKY. After verapamil infusion (5.0 x 10^{-7}M \sim 2.5 x 10^{-6}M), suppression of the pressor responses and noradrenaline overflow evoked by electrical nerve stimulation was greater in SHR than in WKY at both ages. The pressor responses to exogenous noradrenaline were also inhibited by verapamil more in young SHR than in young WKY. In adult SHR, the inhibition was similar to age-matched WKY. These results suggest that noradrenaline release from sympathetic nerve endings in SHR increase at a young age and decreases in adults, and depends at least partly on Ca-influx at both ages as dose vasoconstrictor reactivity. Therefore, Ca-dependency in SHR at both pre- and post-synaptic sites of neurotransmission may contribute to the pathogenesis of hypertension.

Altemation of the sympathetic nervous system might have a role in the pathogenesis, production and maintenance of hypertension. The involvement of both the central and peripheral sympathetic nervous systems has been proposed by several investigators. It has also been suggested that increased sympathetic nerve activity could contribute to the pathogenesis of hypertension in spontaneously hypertensive rats (SHR, Okamoto & Aoki). Changes in catecholamine turnover and in enzyme activities related to catecholamine synthesis in the brain have been reported in young hypertensive rats.1,2 In the peripheral sympathetic nervous system, enhanced activity has been demonstrated in splanchnic and renal nerves in SHR3,4. It has also been reported that plasma or urinary catecholamines are increased in hypertension, an indication of greater sympathetic nerve activity.5 Enhanced vascular responsiveness to various stimuli, such as noradrenaline, KCl or 5-hydroxytryptamine, has also been observed in SHR5. Thus, changes in both the sympathetic

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nervous system and in the responsiveness of vascular smooth muscles have been suggested as factors contributing to the hypertensive mechanism in SHR.

The present study was designed to measure neurotransmitter release from sympathetic nerve endings around the peripheral vascular beds of young and adult SHR and WKY. The vascular reactivity to exogenous noradrenaline was also determined. Ca-mediated adrenergic release and vascular reactivity were studied by using a Ca-antagonist.

MATERIAL AND METHODS

Male SHR (7–8 weeks-old and 20–22 weeks-old) and age-matched normotensive Wistar-Kyoto rats (WKY) were used in this study. Rats were anesthetized with pentobarbital (60 mg/kg of body weight, intraperitoneal injections). After excision, the anterior mesenteric artery was isolated and cannulated. The mesenteric loop preparation was prepared using the method described by Castellucci et al. Four main branches of the mesenteric artery and their dominating intestine were used. The preparation was perfused with modified Ringer-Locke solution (mmol/L): NaCl 120.7, KCl 5.9, CaCl₂ 2.5, MgCl₂ 1.3, NaHCO₃ 15.5, NaH₂PO₄ 1.2 and glucose 11.5. The solution was bubbled with 95% O₂, 5% CO₂ mixture and maintained at the flow rate of 0.8 ml/minute by a peristaltic pump (Harvard apparatus Model 1200). The perfusion pressure was recorded via a side arm with a pressure transducer connected to a polygraph (Nihon Kohden Model CP-620G). The platinum electrodes were placed around the periarterial plexus of the mesenteric artery. A 30-minute equilibration period was allowed before starting an experiment. The intramural sympathetic nerves were stimulated at 40 volts for 1 minute with biphasic rectangular pulses of 5 milliseconds-duration at various frequencies.
(5, 10, 15 Hz), using an electric stimulator (Nihon Kohden Model SEN-3201).

Exogenous L-noradrenaline (0.1 – 3.3 μg) was given as a single injection in 0.1 ml of buffer into the arterial cannula. Intervals between stimuli were always 12 minutes. The perfusate from the mesenteric-loop preparation was collected into tubes containing a mixture of EGTA (90 mg/L) and glutathione (60 mg/L) at a ratio of 20 μl/1 ml of perfusate for 3 minutes before and after stimulation. The latter collecting period actually consisted of the 1-minute stimulatory period and the following 2 minutes. Noradrenaline in the perfusate was absorbed on alumina and then assayed using high pressure liquid chromatography with an electrochemical detector (Bioanalytical System Inc. Model LC-4A)\(^8\)\(^9\). The noradrenaline overflow produced by nerve stimulation was defined as the difference in noradrenaline contents before and after stimulation.

The effects of a Ca-antagonist (verapamil) were assessed by studying changes in the pressor responses and noradrenaline overflow produced by 15 Hz electrical stimulation, and changes in the pressor responses induced by 3.3 μg of exogenous noradrenaline (the dose which caused almost maximal vasoconstriction). The drug was added to the perfusion fluid to achieve a final concentration of \(5.0 \times 10^{-7} \text{M} - 2.5 \times 10^{-6} \text{M}\). Whenever the perfusate drug concentration was changed, a 15-minute equilibration period was allowed before nerve stimulation or noradrenaline addition. The results were expressed as a percent of the results in preparations without verapamil.

Values were expressed as the average mean ± S.E.M. Statistical significance was determined by the Student’s t-test. A difference of \(p < 0.05\) was considered significant.

Verapamil was kindly donated by Eisai Co. Ltd. and L-noradrenaline was obtained from Sigma Chemical Company, U.S.A.

RESULTS

The pressor responses and noradrenaline overflow produced by electrical stimulation were completely blocked by the addition of guanethidine \((1.0 - 2.0 \times 10^{-6} \text{M})\) to the perfusate, whereas the pressor responses to exogenous noradrenaline were not affected (Fig. 1). Prazosin, an \(\alpha_1\)-blocking agent, dose-dependently inhibited the pressor responses to both electrical nerve stimulation and exogenous noradrenaline but had no significant effect on noradrenaline overflow. The time courses of pressor response and noradrenaline overflow are shown in Fig. 2. No significant reduction in the responses to either electrical stimulation or exogenous noradrenaline were found in 7 repeated stimuli, and all experiments were performed within this time course.

The systolic blood pressure was 168.2 ± 6.4 mmHg in young SHR and 126.2 ± 2.9 mmHg in age-matched WKY. Adult systolic pressures were 209.1 ± 11.6 mmHg in SHR and 144.4 ± 5.4 mmHg in WKY.

Vascular Reactivity and Noradrenaline Overflow in SHR

The initial perfusion pressures were 24.8 ± 1.9 mmHg (mean ± SEM, \(n = 11\)) and 17.2 ± 0.3 mmHg (\(n = 7\)) in young and adult SHR, respectively. There were no significant differences between SHR and age-matched WKY (young WKY \(22.2 ± 5.1\) mmHg, \(n = 13\), adult WKY \(18.7 ± 4.4\) mmHg, \(n = 10\)). No significant

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*Fig. 3. Vasoconstrictor responses to electrical nerve stimulation in the isolated mesenteric preparations of SHR and WKY at young and adult ages.*
Fig.4. Vasoconstrictor responses to exogenous noradrenaline in the isolated mesenteric preparations of SHR and WKY at young and adult ages.

The overflow of noradrenaline by periarterial nerve stimulation in isolated mesenteric preparations of SHR and WKY. Values are expressed as mean ± S.E.M. of noradrenaline (ng/g of wet tissue weight). * = p < 0.05; ** = p < 0.005.

<table>
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<th></th>
<th>5Hz</th>
<th>10Hz</th>
<th>15Hz</th>
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<tr>
<td>Young SHR (n = 7)</td>
<td>0.61 ± 0.13</td>
<td>1.17 ± 0.15**</td>
<td>1.43 ± 0.24*</td>
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<tr>
<td>Young WKY (n = 6)</td>
<td>0.34 ± 0.12</td>
<td>0.51 ± 0.10</td>
<td>0.65 ± 0.22</td>
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<td>Adult SHR (n = 6)</td>
<td>0.32 ± 0.12</td>
<td>0.67 ± 0.05</td>
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<td>Adult WKY (n = 6)</td>
<td>0.54 ± 0.14</td>
<td>0.78 ± 0.12</td>
<td>1.51 ± 0.20</td>
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The overflow of noradrenaline by periarterial nerve stimulation in isolated mesenteric preparations of SHR and WKY. Values are expressed as mean ± S.E.M. of noradrenaline (ng/g of wet tissue weight). * = p < 0.05; ** = p < 0.005.

and 125.0 ± 6.7 mmHg for young and adult SHR, while in WKY the responses were 36.6 ± 7.4 mmHg and 93.3 ± 3.8 mmHg, respectively.

The noradrenaline overflow from mesenteric vasculature during periarterial nerve stimulation was greater in young SHR than in WKY. On the contrary, in adult SHR, the noradrenaline overflow was reduced compared with WKY (Table I).

**Calcium-mediated Changes of Neurotransmission in SHR**

The pressor responses both to electrical nerve stimulation and exogenous noradrenaline were inhibited dose-dependently by the addition of verapamil, although no changes were found in the resting perfusion pressure. The inhibitory effects on pressor responses to electrical stimulation were greater in SHR than WKY for the same concentration of verapamil (Table IIa). The differences between SHR and WKY were greater in young rats. Verapamil also produced a greater inhibition in the vasoconstrictor responses to exogenous noradrenaline in young SHR than in age-matched WKY. However, the amount of inhibition was similar in both groups of adult rats (Table IIb).

The influence of verapamil on electrically-stimulated noradrenaline overflow is shown in Table IIc. Suppression of noradrenaline overflow was greater in SHR than WKY at both young and adult ages.
## Table II

<table>
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<tr>
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<th>Verapamil concentrations</th>
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<tr>
<td></td>
<td>5.0 x 10^{-6}M</td>
<td>1.25 x 10^{-6}M</td>
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<td><strong>IIa</strong></td>
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<tr>
<td>Young SHR</td>
<td>37.6 ± 7.8**</td>
<td>20.1 ± 6.7</td>
<td>7.7 ± 3.0**</td>
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<tr>
<td>Young WKY</td>
<td>84.3 ± 10.3</td>
<td>45.8 ± 7.5</td>
<td>22.1 ± 2.9</td>
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<tr>
<td>Adult SHR</td>
<td>61.0 ± 2.9</td>
<td>33.1 ± 4.4</td>
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<tr>
<td>Adult WKY</td>
<td>85.3 ± 4.9</td>
<td>49.5 ± 4.8</td>
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<tr>
<td><strong>IIb</strong></td>
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<tr>
<td>Young SHR</td>
<td>56.6 ± 8.6***</td>
<td>44.9 ± 4.9***</td>
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<tr>
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<td>84.5 ± 2.7</td>
<td>88.8 ± 6.8</td>
<td>67.5 ± 5.1</td>
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<td>Adult SHR</td>
<td>92.4 ± 3.3</td>
<td>69.3 ± 8.8</td>
<td>55.9 ± 8.5</td>
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<tr>
<td>Adult WKY</td>
<td>94.0 ± 3.3</td>
<td>77.1 ± 7.6</td>
<td>61.8 ± 10.0</td>
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<tr>
<td><strong>IIc</strong></td>
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<tr>
<td>Young SHR</td>
<td>42.2 ± 9.8</td>
<td>36.9 ± 13.9</td>
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<tr>
<td>Young WKY</td>
<td>78.4 ± 19.4</td>
<td>62.1 ± 11.1</td>
<td>76.4 ± 12.4</td>
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<tr>
<td>Adult SHR</td>
<td>42.4 ± 4.2***</td>
<td>17.9 ± 11.1</td>
<td>18.1 ± 7.0</td>
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<tr>
<td>Adult WKY</td>
<td>83.2 ± 6.4</td>
<td>43.3 ± 10.3</td>
<td>20.2 ± 5.1</td>
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Effects of verapamil on vascular responsiveness to electrical nerve stimulation (IIa) and to exogenous noradrenaline (IIb), and on noradrenaline overflow produced by electrical stimulation (IIc) in isolated mesenteric preparations of SHR and WKY. Values are expressed as % of the results in controls without verapamil (mean±S.E.M.).

|        |  = p < 0.05; ** = p < 0.02; *** = p < 0.005; **** = p < 0.001. |

### Discussion

The results show large differences in both vascular reactivity of the mesenteric vasculature and noradrenaline overflow from the nerve endings between SHR and WKY rats. The pressor responses to either electrical nerve stimulation or exogenous noradrenaline were greater in SHR than in WKY at both young and adult ages. Direct measurement of endogenous noradrenaline overflow from mesenteric vascular beds revealed enhanced neurotransmitter release in young SHR compared with age-matched WKY, whereas in adult SHR the noradrenaline overflow was similar to or slightly less than in the normotensive controls. An alteration in adrenergic transmitter release has been demonstrated or suggested by several investigators but with somewhat inconsistent results. Collis et al. reported that neurotransmitter release and vasoconstrictor responses were greater in young (46 days old) SHR than in normotensive controls in the perfused kidney. Periarterial renal nerve stimulation in mature SHR (aged 18 weeks), caused a significantly greater release of noradrenaline than in age-matched WKY. Galloway et al. observed an enhanced release of adrenergic transmitter in the coccyeal arteries in young and adult SHR compared with the normotensive WKY. Similar results were reported by Zoster et al., who demonstrated that in 7–9 weeks-old SHR, transmural stimulation of the tail artery released twice as much 3H-labeled adrenergic transmitter as in WKY. The contrary, renal nerve stimulation caused a smaller increase in the overflow of intact tritiated transmitter from the kidneys of adult SHR (6 months of age) than from normotensive controls.

It is generally accepted that plasma noradrenaline is higher in SHR than WKY during the pre- or early phase of hypertension, whereas in the chronic or established phase the value is similar to that of WKY. Our observations suggest that, in the early phase of the hypertensive process, facilitation of adrenergic transmitter release, together with an increase in vascular reactivity to the released noradrenaline, could be the pathogenesis of hypertension. The results also suggest that impaired neurotransmitter release could be compensated by increased vascular reactivity or elevated vascular tone in the chronic phase of hypertension, when vascular wall-thickening with hypertrophy of the smooth muscle cells and narrowing of the intra-
vascular lumen may be involved.15

As our results showed an alteration in nor-
adrenaline overflow and vascular reactivity in
SHR, we undertook additional studies to clarify
the mechanisms of this.

Abnormality in Ca-handling by the cell
membrane has recently been suggested as one of
the causes of hypertension. Increases in fluxes of
Na+, K+ and Ca++ into vessels have been reported
in hypertension.16,17 Postnov found that the
Ca-binding ability of the erythrocyte membrane
was reduced in patients with essential hyper-
tension and SHR, and suggested that membrane-
bound calcium might determine the membrane
permeability of monovalent cations.18 In clinical
and experimental studies, Ca-antagonists have
been shown to have antihypertensive effects.19
There is much evidence to suggest that Ca-
antagonists can block the transmembrane Ca-flux
into the myocardial fibers or vascular smooth
muscle cells.20 It is also generally accepted
that calcium facilitates the secretion of neuro-
transmitter substances, and a Ca-hypothesis of
stimulus-secretion coupling has been proposed.21
Huković et al. showed that noradrenaline release
from cardiac sympathetic neurons was reduced
when Ca-concentration in the bathing medium
was reduced.22 Mulvany et al. observed an
increased calcium sensitivity of resistance vessels
in young and adult SHR.23 All of the preceding
observations are consistent with the finding that
calcium could play an important role in the
mechanisms of both neurotransmitter secretion
and vascular responsiveness.

We have previously reported that verapamil,
a Ca-antagonist, affected both pre- and post-
synaptic sites of resistance vessels and caused
a decrease in electrically-stimulated noradren-
aline overflow from sympathetic nerve endings
distributed in rat mesenteric arteries, in addition
to the direct effects on the reactivity of vascular
smooth muscles.24,25

On the basis of these findings, Ca-mediated
adrenergic neurotransmitter release from nerve
endings and vascular reactivity were investiga-
ted using verapamil, and Ca-dependency of
neurotransmission in SHR was studied. In
our preparations, after infusion of verapamil,
the suppression of pressor responses and nor-
adrenaline overflow evoked by electrical nerve
stimulation was more evident in SHR that in
WKY at both young and adult ages. Pressor
responses to exogenous noradrenaline were
also inhibited more in young SHR than in
age-matched WKY. These results suggest that
noradrenaline release from the sympathetic
nerve endings in SHR depends at least partly
on Ca-influx as well as contractile responsiveness
of vascular smooth muscles, especially at a young
age.

An increase in Ca-sensitivity could cause a
greater release of noradrenaline in young SHR,
though we cannot explain reduced noradrenaline
release in the chronic stage of SHR by increased
sensitivity to calcium. Recent studies have
revealed that noradrenaline release from the
sympathetic nerve endings is modulated by other
feed-back mechanisms. Many pre-junctional
receptors, such as a2-adrenergic, dopaminergic,
purinergic receptors and those affected by pros-
taglandins, inhibit neurotransmitter release.26,27
An impairment of pre-synaptic a2-receptor-
mediated control of transmitter release in SHR
has been reported by several investigators.11,12
Reduced noradrenaline overflow in adult SHR
in our study might be due to changes in inhibitory
feed-back mechanisms exerted on nerve ter-
inals. However, details still need to be resolved.

In summary, the results of our study showed
that noradrenaline release from sympathetic
nerve endings in SHR is enhanced at a young
age, and similar or reduced in adults, compared
with age-matched WKY. Vasoconstrictor res-
ponsiveness is increased in both ages in SHR.
Ca-dependency, studied using a Ca-antagonist,
is more marked at both pre- and post-synaptic
sites of neurotransmission in SHR than WKY.
These results suggest that an alteration in Ca-
handling by the cell membrane may contribute to
the pathogenesis of hypertension in SHR.

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