Noradrenergic Transmission in the Isolated Portal Vein of the Spontaneously Hypertensive Rat

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SUMMARY The effect of electrical field stimulation (1, 2, 5, 10 Hz for a total of 480 pulses at 15-minute intervals) on the release of $^3$H-norepinephrine from the superfused portal vein of spontaneously hypertensive rats (SHR) or Wistar-Kyoto rats (WKY) of various ages was studied. The ages of the animals were (in weeks) 5-6 (prehypertensive), 8-10 (young hypertensives), 16-18 (older hypertensives), and 28 (mature hypertensives). There was no difference in the release of $^3$H-norepinephrine or developed tension of the portal vein to any frequency of field stimulation of SHR or WKY at 5-6 weeks of age. However, there was a significantly greater release of $^3$H-norepinephrine and developed tension of veins of SHR in response to low (1 or 2 Hz) but not high frequencies (5 or 10 Hz) at 8-10, 16-18, and 28 weeks of age. Vessels from hypertensive animals also developed greater resting tension and spontaneous activity, which was reduced to that of WKY in the presence of an $\alpha$-adrenergic antagonist. The $\alpha_2$ selective adrenergic antagonist yohimbine produced the same degree of enhancement of release of $^3$H-norepinephrine to field stimulation of veins obtained from both SHR and WKY at 5-6, 8-10 and 16-18 weeks of age. However, the facility effect of yohimbine was significantly attenuated in portal veins obtained from SHR at 28 weeks of age compared to age-matched WKY. The $\beta$-adrenergic agonist isoproterenol produced a similar degree of enhancement of the field-stimulation-induced release of $^3$H-norepinephrine from vessels obtained from SHR and WKY at all ages. In contrast, the facility effect of angiotensin was significantly enhanced in vessels obtained from 10-, 12-, and 28-week-old SHR compared to age-matched WKY. Results demonstrate alterations in the in vitro evoked release of norepinephrine from blood vessels obtained from hypertensive animals. In addition, alterations were demonstrated in the functional activity of presynaptic receptors in peripheral noradrenergic neurons innervating the portal vein in hypertensive animals compared to age-matched controls. These alterations may be important in the development and maintenance of hypertension in the SHR. (Hypertension 6: 267-274, 1984)

KEY WORDS • prejunctional receptors • alpha adrenergic antagonists • angiotensin

Numerous studies now point to increased sympathetic nerve activity in the development and/or maintenance of hypertension in the spontaneously hypertensive rat (SHR). In SHR, depletion of central stores of catecholamines prevents, while peripheral sympathectomy attenuates, the hypertension. Changes in brain catecholamine turnover and enzymatic activity have also been observed, which is consistent with an increase in the sympathoexcitatory pathways in the central nervous system. An increase in sympathetic discharge following various types of stress or stimuli has also been reported in SHR compared to normotensive controls. More recently, several studies have examined the release of norepinephrine from peripheral noradrenergic neurons in SHR. Evidence suggests a greater release of norepinephrine in the kidney and mesenteric artery of SHR compared to vessels from normotensive control rats.

We have examined the effect of field stimulation on the release of $^3$H-norepinephrine from the portal vein of SHR and Wistar-Kyoto (WKY) rats at various ages, which correspond to times before and during the development of hypertension and after the SHR had been hypertensive for a considerable period of time. When greater release of norepinephrine from the portal vein of SHR was observed compared to age-matched WKY, possible alterations in prejunctional inhibitory and excitatory receptor function were also examined.
Methods

Blood Pressure Measurements

Male SHR and normotensive WKY rats were purchased from Taconic Farms, Germantown, New York, and were maintained under standard conditions with food and water ad libitum. At 1 or 2 days prior to sacrifice, systolic blood pressures were measured by tail-cuff plethysmography using an electrophysymomanometer and a pneumatic pulse transducer (Narco Biosystems, Houston, Texas) connected to a chart recorder. The average of five measurements per animal was taken as the blood pressure. The average systolic blood pressures of the animals at 6, 10, 18, and 28 weeks of age were 107 ± 5, 155 ± 7, 205 ± 10, and 200 ± 7 mm Hg for SHR and 102 ± 5, 105 ± 4, 105 ± 4, and 106 ± 4 mm Hg for WKY.

Preparation of Tissue

Animals were killed by decapitation at 5–6, 8–10, 16–18, and 28 weeks of age, and the portal vein was rapidly removed and placed in 1.9 ml of a Krebs-Henseleit buffer at 37°C for 30 minutes under an atmosphere of 95% O₂ + 5% CO₂. The composition of the buffer (in mmolar concentrations) was: NaCl, 113; NaHCO₃, 25; KCl, 4.75; KH₂PO₄, 1.18; CaCl₂, 2.5; MgSO₄, 1.19; glucose, 11.1; disodium EDTA, 0.029; and ascorbic acid, 0.289. After a 30-minute period of equilibration, the veins were then incubated for an additional 30 minutes in the presence of 5 µCi ¹H-7 norepinephrine (specific activity 22.7 Ci/mM, final concentration 2 × 10⁻³ M).

Individual tissues were then placed in specially prepared glass chambers jacketed with water at 37°C. The veins were positioned between two platinum ring electrodes placed 1 cm apart; one end of the vein was tied to an acrylic holder and the other connected to a Grass Force Displacement Transducer (FT03C, Grass Instrument Company, Quincy, Massachusetts) coupled to a Brush recorder (Brush Instrument Company, Cleveland, Ohio). Tension response curves were determined for vessels obtained from each age group, and the maximum basal tension was placed on the appropriate vessels accordingly. This generally ranged from 0.25 to 0.75 g.

Tissues were then superfused with Krebs-Henseleit buffer at a constant flow rate of 3 ml/min using a Gilson minipulse pump. Desipramine (1 µM) and methanephrine (80 µM) were added to the superfusion buffer to block neuronal and extraneuronal uptake of norepinephrine, respectively.

Application of Drugs and Field Stimulation

Frequency Response Curves

Following a washout of 20 minutes, tissues were electrically stimulated at 2 Hz for 300 pulses to clear extracellular and extraneuronal radioactivity. This was followed by superfusion for 60 minutes. Tissues were then electrically stimulated sequentially at 15-minute intervals for a total of 480 pulses at frequencies of 1, 2, 5, and 10 Hz (1 msec duration, supramaximal voltage). The waveform, intensity, and duration of the electrical pulses were continuously monitored by an Ev-70 Heath dual-trace oscilloscope (Heath Company, Benton Harbor, Michigan). Superfusate samples were continuously collected in 3-minute fractions by a fraction collector and then taken for analysis of ¹H-norepinephrine. Previous experiments have established that this type of electrical stimulation was completely dependent upon extracellular calcium, and release was blocked by tetrodotoxin. Preliminary experiments established that most of the ¹H remaining in the tissue after long periods of infusion was intact ¹H-norepinephrine. In addition, the major portion of ¹H released during field stimulation was in the form of ¹H-norepinephrine. Therefore, the evoked fractional release of total ¹H was interpreted as a reliable marker for the release of ³H-norepinephrine from adrenergic neurons in the present study. Tissues were removed at the end of the experiments, and, following extraction with Soluene 350 tissue solubilizer (Packard Instrument Company, Downers Grove, Illinois), assayed by liquid scintillation spectrometry. Release was expressed as a percentage of fractional release and represents the amount of ¹H released into the superfusate divided by the amount of ¹H present in the tissue at the time of field stimulation, × 100. The amount of ³H in the tissue at the start of any time interval was calculated by adding cumulatively the amount of norepinephrine released into the superfusate to the amount of ³H in the portal vein at the end of the experiment.

Influence of Yohimbine, Angiotensin, and Isoproterenol

The portal veins from animals of various ages were obtained, incubated with ¹H-norepinephrine, and superfused, as described above. Tissues were stimulated at a frequency of 1 Hz for a total of 28 minutes. To examine prejunctional α₂ adrenergic receptors, yohimbine at a concentration of 10⁻⁷ M was added to the superfusion buffer 10 minutes after the start of field stimulation and removed 10 minutes later.

A slightly different protocol was used to examine the effects of angiotensin and isoproterenol on adrenergic neurotransmission. After the initial washout of 60 minutes, each tissue was electrically stimulated two times at 30-minute intervals. Each stimulation period consisted of a total stimulation of 6 minutes at 2 Hz, 1.1 msec duration, and supramaximal voltage. All drugs were added between the first stimulation (S₁) and second stimulation (S₂) (15 minutes before S₂) by switching the superfusion buffer to one containing the drug in question. Following the S₂ period, there was a return to the drug-free superfusion buffer. For evaluation, the percentage of fractional release was calculated (see above), and the effect of drugs was expressed as a ratio between the overflow of tritium evoked by S₂ and the overflow evoked by S₁.

For liquid scintillation spectrometry, 1 or 2 ml of sample was placed in 10 ml of a Triton-based solution containing 5.5 g of 2,5-diphenyloxazole, 150 mg of 1.4-bis (2-(5-phenyloxazolyl)) benzene, and a 2:1 mixture of toluene and Triton X-100. The samples were
counted in Packard Tri Carb 300C (Packard Instrument Company, Downers Grove, Illinois) or Beckman LS7500 liquid scintillation spectrometer (Beckman Instrument Company, Irvine, California). Counting efficiency was determined by internal standardization.

Uptake and Accumulation of \(^3\)H-Norepinephrine

Portal veins were dissected as described above for release studies. Following 30 minutes of preincubation in normal buffer, the vessels were placed in a buffer containing \(^3\)H-norepinephrine (final concentration \(10^{-7}\) M) for 2 or 10 minutes. At the end of each time period, the vessels were removed, washed twice with normal buffer, weighed, and then homogenized in 0.4 N perchloric acid and centrifuged. \(^3\)H-norepinephrine in the supernatant was separated from other \(^3\)H-products by amberlite and alumina column chromatography (Westfall et al., unpublished data, 1976). Acid eluates (0.2 N HCl) from alumina columns were counted by liquid scintillation spectrometry, as described above. Data are expressed as disintegration per minute (dpm) of \(^3\)H-norepinephrine per milligram (mg) of wet weight.

Drugs Used

The following drugs were used in the present study: d,l-isoproterenol hydrochloride (Winthrop Laboratories, New York, New York); l-norepinephrine-7-\(^3\)H (New England Nuclear, Boston, Massachusetts); yohimbine (Aldrich, Milwaukee, Wisconsin); angiotensin II (Sigma, St. Louis, Missouri); and saralasin (Beckman, Palo Alto, California).

Statistics

All statistical analysis for significance was carried out by using an unpaired Student’s \(t\) test or analysis of variance. The standard errors of the means are included in the figures and tables.

Results

Effect of Field Stimulation on Release of \(^3\)H-Norepinephrine and Contractile Force

Field stimulation at all frequencies (1–10 Hz) resulted in a similar release of \(^3\)H-norepinephrine from and contraction of the superfused rat portal vein obtained from prehypertensive 5 to 6-week-old SHR and age-matched WKY (Figures 1 and 2). In contrast, low frequency field stimulation (1 and 2 Hz) resulted in a significantly greater release of \(^3\)H-norepinephrine associated with the contractile response of the isolated rat portal vein obtained from SHR at 8–10, 16–18, and 28 weeks of age compared to vessels obtained from age-matched WKY. Figure 1 depicts results obtained when the portal vein was stimulated at 1 Hz for a total of 480 pulses. The greatest difference in release and contractile force between SHR and WKY was seen in vessels obtained from animals 8–10 weeks of age, although there was still a significant difference between SHR and WKY of 16–18 and 28 weeks of age. At higher frequencies of stimulation (5 and 10 Hz for 480 pulses), no difference was observed in the release of \(^3\)H-norepinephrine and contraction of the portal vein between SHR and WKY (Figure 2).
Effect of Field Stimulation before, during, and after the Addition of Yohimbine

Table 1: Uptake and Accumulation of \(^3\)H-Norepinephrine (NE) into the Portal Vein of SHR and WKY

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Time after (^3)H-NE (min)</th>
<th>SHR</th>
<th>WKY</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2</td>
<td>2469 ± 559</td>
<td>2123 ± 499</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5485 ± 1586</td>
<td>4177 ± 715</td>
<td>NS</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
<td>2083 ± 455</td>
<td>3001 ± 338</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8639 ± 1807</td>
<td>5035 ± 326</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 3. Effect of field stimulation (1 Hz) before, during, and after the addition of yohimbine (10\(^{-7}\) M) on the percentage of fractional overflow of \(^3\)H-norepinephrine from the superfused rat portal vein obtained from SHR and WKY at 6 weeks of age. Data are plotted as means ± SEM of the fractional overflow vs superfusion time in minutes.

Effect of Yohimbine on \(^3\)H-Norepinephrine Release in SHR and WKY

Figure 3 shows that there was no difference in the increase in release of \(^3\)H-norepinephrine from the portal vein of 5 to 6-week-old SHR or WKY in response to field stimulation at a frequency of 1 Hz. Similarly, the prejunctional \(\alpha\) antagonist yohimbine produced the same increase in the field stimulation-induced release of \(^3\)H-norepinephrine from tissues obtained from SHR and WKY. In contrast to what was observed in veins from prehypertensive animals, there was a significantly greater increase in the release of \(^3\)H-norepinephrine in veins obtained from hypertensive animals (15–16 weeks of age) when stimulated at a frequency of 1 Hz (Figure 4). However, the addition of yohimbine produced the same degree of enhancement in veins obtained from SHR or WKY. Figure 5 shows results obtained in the portal vein of 28-week-old animals. As with 15 to 16-week-old animals, a low frequency of field stimulation (1 Hz) resulted in a significantly greater release of \(^3\)H-norepinephrine from the SHR portal vein compared to the WKY vein. Upon the addition of yohimbine, however, the drug-induced enhancement of release during field stimulation was significantly attenuated in the veins of SHR compared to WKY.

Data presented in Figures 3–5 are summarized in Figure 6 and depict the further increase in \(^3\)H-norepinephrine release produced when yohimbine was added during field stimulation of the superfused portal veins from rats of different ages. There was an age-dependent increase in the yohimbine response observed in vessels from both WKY and SHR, which reached a maximum at 16–18 weeks of age in the WKY. The effect of yohimbine in enhancing the field stimulation-induced release of \(^3\)H-norepinephrine was markedly attenuated in 28-week-old SHR.

Effect of Angiotensin and Isoproterenol on \(^3\)H-Norepinephrine Release

The effect of angiotensin in enhancing the field stimulation-induced release of \(^3\)H-norepinephrine from the
Figure 5. Effect of field stimulation before, during, and after the addition of yohimbine (10^{-7} M) on the percentage of fractional overflow of \textsuperscript{3}H-norepinephrine from the superfused portal vein obtained from SHR and WKY at 28 weeks of age. Data are plotted as means ± SEM of the fractional overflow vs superfusion time in minutes.

Figure 6. Summary of the accumulated data plotted in Figures 3-5. Data represent the further increase in overflow of \textsuperscript{3}H-norepinephrine due to yohimbine when added during field stimulation of the portal vein vs the age of SHR and WKY in weeks.

Figure 7. Effect of field stimulation on the release of \textsuperscript{3}H-norepinephrine from the rat portal vein in the absence (S_1) or presence (S_2) of angiotensin (10^{-7} M). Data are plotted as a S_2/S_1 ratio of the fractional release vs age of SHR and WKY in weeks. Each bar is the mean ± SEM of 5 to 7 vessels.

Figure 8. Effect of field stimulation on the release of \textsuperscript{3}H-norepinephrine from the rat portal vein in the absence (S_1) or presence (S_2) of isoproterenol (10^{-7} M). Data are plotted as a S_2/S_1 ratio of the fractional release vs age of SHR and WKY in weeks. Each bar is the mean ± SEM of 5 to 7 vessels.
Discussion

Data obtained in the present study show that low frequencies of field stimulation resulted in greater $^3$H-norepinephrine release from and contraction of the portal veins obtained from 10-, 16-, and 28-week-old SHR compared to age-matched WKY. When this comparison was made before the time that SHR became hypertensive, no differences in either release or contractile response were noted. Nor were differences noted in the release of $^3$H-norepinephrine from the portal vein of SHR and WKY at high frequencies of field stimulation (5 or 10 Hz). These data are consistent with the results of Eikenburg et al.,$^{21}$ who observed an increase in the field stimulation-induced (4 Hz) release of $^3$H-norepinephrine of the perfused mesenteric artery of 14- to 18-week-old SHR compared to normotensive controls.$^{21}$ Moreover, these results in the portal vein are similar to what we have observed in the caudal artery of the SHR, where an enhancement of the potassium-induced release of endogenous norepinephrine was also seen.$^{22-25}$ The similarity in results$^{22}$ obtained from both the portal vein (the present study) and caudal artery of SHR compared to WKY is of great interest. It suggests that these alterations are fairly general and involve both capacitance as well as resistance vessels. The finding of a similarly enhanced neurotransmission in both the arterial and venular circulation might imply that SHR would increase both the blood supply to the heart and peripheral resistance, and both of these effects could increase blood pressure.

Our results differ slightly from those of Eikenburg et al., who observed an enhancement of the field stimulation-induced release of $^3$H-norepinephrine at a frequency of 4 Hz, whereas our enhancement was seen at frequencies of 1 or 2 Hz and not at 5 or 10 Hz. These investigators did not report the results of the effect of other frequencies; therefore, a direct comparison cannot be made.$^{23}$

Our results are also slightly different than those reported by Vanhoutte and coworkers,$^{21-22}$ who reported an enhancement in the release of norepinephrine from the kidney of young hypertensive animals (6 weeks of age) but not in adult animals (6 months of age) when compared to normotensive controls.$^{21, 22}$ Our results cannot be directly compared with those of Vanhoutte et al.$^{21, 22}$ because there are numerous differences in the experimental protocols (i.e., superfusion in our case vs superfusion in the case of Vanhoutte; various frequencies of 1, 2, 5, and 10 Hz at a set number of pulses, 480 pulses in our case, vs frequencies of 2, 6, and 16 Hz for 2 or 4 minutes in the case of Vanhoutte). In addition, our adult animals were 2 months younger than those of Vanhoutte et al.$^{21, 22}$ However, it is possible that there are distinct differences in neurotransmission in various vascular beds during genetic hypertension.

Vessels from hypertensive animals developed significantly greater resting tension and spontaneous activity than vessels from normotensive animals. In addition, a further increase of tension developed in the vessels of hypertensive animals in response to low frequency nerve stimulation compared to veins obtained from age-matched normotensive animals. Therefore, the greater release of norepinephrine is coupled to greater contractile force and provides a functional correlate to the enhanced release. Others$^{26-28}$ have reported increased responsiveness of vascular smooth muscle of the SHR to nerve stimulation or vasoactive substances.

The fact that an increased transmitter release to field stimulation was observed only in animals after they have become hypertensive, especially the young hypertensive animals, suggests that such an activity may contribute to the production and maintenance of hypertension. This is of interest in view of the studies that have suggested an increase in sympathetic outflow from the central nervous system in young prehypertensive animals but not in animals after they had become hypertensive or following established hypertension. The central nervous system may be eminently involved in the initial development of hypertension, while alterations in peripheral noradrenergic neurons may be more involved in the maintenance of the hypertension.

Since our in vitro studies were primarily conducted in the presence of Uptake I and Uptake II blockers, it is possible that alterations in reuptake mechanism during hypertension could neutralize the effect of increased release. We measured the neuronal and extraneuronal uptake of $^3$H-norepinephrine into portal veins of SHR and age-matched WKY. No differences were noted in the uptake of $^3$H-norepinephrine into veins obtained from 11- to 12-week-old SHR compared to WKY. However, we noted that 28-week-old SHR had a slightly greater accumulation (10 minutes) in the portal vein compared with age-matched WKY. Therefore, we feel that alterations in uptake would not play a role in modulating the concentration of norepinephrine in the synaptic cleft of the portal vein in our studies.

It is well known that receptor-mediated responses can be influenced by the level of activity operating on the receptor-effector system. It is also well known that noradrenergic neurotransmission can be influenced by a variety of prejunctional receptors, including inhibitory alpha and excitatory beta and angiotensin receptors.$^{29-32}$ Since the percentage of fractional release of $^3$H-norepinephrine at low frequencies of field stimulation was enhanced in SHR animals, we tested the possibility that the enhanced release could result in or contribute to decreased prejunctional inhibitory and/or increased excitatory adrenergic receptor activity. Since yohimbine owes its enhancing effect on noradrenergic transmission to blocking prejunctional $\alpha_2$ adrenergic receptors, it was reasoned that an increase in the enhancing effect would be compatible with increased activity of prejunctional $\alpha_2$ adrenergic receptors. A decrease in the enhancing effect, on the other hand, would be consistent with a subsensitivity of prejunctional $\alpha_2$ adrenergic receptors. Similar reasoning was developed for the use of angiotensin and isoproterenol, both of which are known to enhance adrenergic neurotransmission. It was observed that yohimbine caused an equal enhancement of the field stimulation-
induced release of \( ^{1}H \)-norepinephrine from the portal vein of SHR and age-matched WKY at 6, 10, and 16 weeks of age. On the other hand, the ability to enhance the release of \( ^{1}H \)-norepinephrine from the veins of 28-week-old SHR was significantly attenuated compared to the veins of normotensive controls.

This difference in the action of yohimbine is compatible with the idea that there is a decreased or subsensitive prejunctional \( \alpha_{1} \) adrenergic receptor activity in older SHR. A similar decrease in the enhancing effect of yohimbine was seen when norepinephrine release was examined from the caudal artery of the same SHR animals.\(^{24}\) Thus, the increased release of norepinephrine by neurons innervating vascular smooth muscle during early hypertension may lead to a refractory desensitization of the prejunctional adrenergic receptor and this could be a contributing factor in the maintenance of hypertension.

In addition to evidence for an alteration in prejunctional \( \alpha_{1} \) adrenergic receptor, our study demonstrated that there is an increase in the ability of angiotensin to enhance adrenergic neurotransmission. Interestingly, this was seen at an earlier age than the effect on \( \alpha_{1} \) adrenergic receptors. The increase in the angiotensin response was already apparent in animals 10 weeks of age, although the effect was even greater when examined in older animals. The specificity of this effect is supported by the observation that there was no increase in the field-stimulation-induced release of \( ^{1}H \)-norepinephrine by isoproterenol. A facilitory effect of angiotensin on adrenergic neurotransmission in the perfused mesenteric bed of the SHR has also been reported.\(^{11}\) Moreover, these investigators have also reported\(^{13}\) that there is a decrease in the inhibitory effect of purines on norepinephrine release in the SHR. Neither of these studies examined these effects in SHR of different ages so it is unknown if they occur only in animals with established hypertension. Nevertheless, it would appear that alterations in several prejunctional receptor-effector systems are present in SHR. Moreover, there have also been reports\(^{35-38}\) of alterations in central and peripheral adrenergic receptors as assessed by ligand binding studies.

The cause of the increased release of \( ^{1}H \)-norepinephrine to low frequencies of nerve stimulation in the superfused portal vein from hypertensive animals is unclear, although it appears to be present only when rats are hypertensive and is most dramatic in young hypertensive animals. The increased release of norepinephrine may contribute to the hypertension, since there was also a greater contraction of the vascular smooth muscle that parallels the release of norepinephrine. It is possible that increased sympathetic nerve traffic from the central nervous system coupled to the enhanced facilitory activity of angiotensin may contribute to the development of hypertension. The increased release of norepinephrine may ultimately lead to a decrease in the activity of presynaptic \( \alpha_{1} \) adrenergic receptors in chronic hypertensive animals. In chronic hypertensive animals, the decrease in the activity of prejunctional \( \alpha_{1} \) adrenergic receptors may also result in enhanced release of norepinephrine when the sympathetic nerves are activated in selected vascular beds. These may contribute to the maintenance of hypertension. Further studies are clearly needed to explore whether there are differences in selective vascular beds in young and older animals.

References

Noradrenergic transmission in the isolated portal vein of the spontaneously hypertensive rat.
T C Westfall, M J Meldrum, L Badino and J T Earnhardt

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