Presynaptic α- and β-Adrenoceptor Stimulation and Norepinephrine Release in the Spontaneously Hypertensive Rat

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SUMMARY The present study was designed to measure norepinephrine release during sympathetic nerve stimulation and evaluate the presynaptic inhibitory and facilitatory actions of α-adrenoceptor and β-adrenoceptor agonists respectively, in the isolated perfused kidney of normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Periarterial nerve stimulation (0.25 to 32 Hz) caused a significantly greater release of norepinephrine, which was measured as total tritium overflow, in the SHR. The vasoconstrictor responses to periarterial nerve stimulation as well as to norepinephrine, angiotensin II, and barium chloride were also significantly greater in the SHR. Presynaptic actions of tramazolene, an α2-adrenoceptor agonist, and salbutamol, a β2-adrenoceptor agonist, on norepinephrine release were determined during periarterial nerve stimulation at 2 Hz. Tramazolene (2 × 10⁻⁹ to 2 × 10⁻⁷ M) caused a concentration-dependent inhibition of stimulus-induced release of norepinephrine in SHR but not in WKY. While the highest concentration of tramazolene (2 × 10⁻⁷ M) exerted an inhibitory action in the WKY, this effect was of lesser magnitude than that seen in SHR. Salbutamol (10⁻¹⁰ to 10⁻⁶ M) produced an increase in norepinephrine release during periarterial nerve stimulation; however, this action of the β-adrenoceptor agonist was similar in both the WKY and SHR. These results demonstrate that norepinephrine release during sympathetic nerve stimulation is significantly greater in the SHR, and this phenomenon may contribute to the maintenance of hypertension. While presynaptic β-adrenoceptor function is similar in both the WKY and SHR, presynaptic α-adrenoceptors are supersensitive in the SHR. This supersensitivity may be of physiological importance in curtailing an already greater release of norepinephrine present in the SHR. (Hypertension 5: 198-204, 1983)

KEY WORDS • norepinephrine release • presynaptic receptors • tramazolene • salbutamol • hypertension

The release of the sympathetic neurotransmitter norepinephrine is reportedly controlled by the activity of various centers located within the central nervous system.¹,² Until recently, the post-ganglionic sympathetic nerve terminal was considered the site primarily involved in the synthesis, storage, and reuptake of norepinephrine. However, in recent years it has become increasingly clear that several mechanisms also exist at the nerve terminal level that can alter the amount of norepinephrine released during nerve stimulation.³⁻⁶ These studies suggest that, in addition to alterations in the activity of the central nervous system, norepinephrine release can also be altered by various substances that produce their actions by acting on presynaptic receptors located on the post-ganglionic sympathetic nerve terminal.⁴⁻⁵

The calcium-dependent release of norepinephrine can be modified by many endogenous substances that interact with presynaptic nerve terminal membrane via specific receptors. While activation of some of the receptors, including presynaptic α-adrenoceptors, causes inhibition of norepinephrine release, an augmentation of transmitter release is seen following stimulation of other receptors such as presynaptic β-adrenoceptors.⁵,⁶ Norepinephrine released during nerve activity can cause an inhibition of further transmitter release by activating presynaptic α-adrenoceptors, which is an important negative feedback mechanism involved in the modulation of neurotransmitter release.⁷,⁸ Although a physiological role of presynaptic

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\[\begin{align*}
\text{PRESYNAPTIC } \alpha - \text{AND } \beta - \text{ADRENOCEPTORS IN SHR/Ekas et al.} & \quad 199 \\
\end{align*}\]

\[\text{β-adrenoceptors has not yet been demonstrated, it has been shown that these receptors may be the target sites for circulating epinephrine.}^{5,9} \]

Involvement of the sympathetic nervous system in the pathogenesis of hypertension in spontaneously hypertensive rats (SHR) has been extensively studied.\(^{10-13}\) It is suggested that an increase in the activity of sympathetic nervous system resulting from changes in the central noradrenergic control mechanisms,\(^{10,11}\) as well as alterations in specific receptor sensitivities,\(^{12,13}\) may be responsible for the hypertension in the SHR. Recent reports indicate that a greater release of neurotransmitter during sympathetic nerve stimulation due to alterations at the postganglionic sympathetic nerve terminal level may also contribute importantly to the hypertension in the SHR.\(^{14,15}\) Increased norepinephrine release in the SHR has been observed in isolated perfused mesentery\(^{14}\) and kidney\(^{15}\) during periarterial nerve stimulation. While the mechanisms responsible for this have not been determined, it is likely that alterations in presynaptic receptor mechanisms may lead to such a change in norepinephrine release in the SHR. More specifically, if the presynaptic α-adrenoceptors had become subsensitive in SHR, then norepinephrine will not be as effective in causing negative feedback inhibition of its own release and, for a given stimulus, a greater amount of transmitter will be released in the SHR. Another possible presynaptic receptor mechanism involved in this phenomenon could be the β-adrenoceptor-mediated facilitation of norepinephrine release. A supersensitivity of these receptors may also be a causative factor in the greater release of norepinephrine observed in the SHR.

The objectives of the present study were to measure the norepinephrine released during periarterial nerve stimulation in the isolated perfused kidney of SHR and to study presynaptic α-adrenoceptor-mediated inhibition and presynaptic β-adrenoceptor-mediated facilitation of norepinephrine release during nerve stimulation. In addition, we also evaluated vascular reactivity to several vasoconstrictor stimuli in the SHR.

**Methods**

Male Wistar-Kyoto (WKY) rats and SHR (aged 14 weeks) were obtained from Taconic Farms, Inc. Body weight, systolic blood pressure, and heart rate were measured weekly; when the animals were 18 weeks old, experiments were begun using the isolated perfused kidney preparation as previously described.\(^{16}\) Briefly, the right kidney was isolated under pentobarbital sodium (50 mg/kg, i.p.), and a cannula was placed into the mesenteric artery and advanced into the abdominal aorta next to the renal artery. The kidney was removed from the animal and perfused at a rate of 6 ml/min with Krebs-Ringer bicarbonate solution. After a stabilization period of 20 to 30 minutes, the neuronal norepinephrine storage sites were labelled with [7,8 \(^{3}\)H]-norepinephrine ([\(^{3}\)H-NE]). Then 10 μCi \(^{3}\)H-NE (11 Ci/mmoles) was perfused through the kidney for 30 minutes followed by a 40-minute washout with \(^{3}\)H-NE free fluid.

The amount of \(^{3}\)H-NE accumulated by the kidney during the labelling of the NE neuronal storage sites was measured. The arteriovenous difference of total tritium during this loading procedure was used as a measure of the ability of the kidney to accumulate \(^{3}\)H-NE. Accumulation is reported as a percentage of the total radioactivity which was perfused through the kidney (10 μCi).

Bipolar platinum electrodes were placed around the renal artery for periarterial nerve stimulation (PNS). Stimulation parameters were 40 V, 1-msec duration and a 20-second stimulation period. In the first series of experiments, \(^{3}\)H-NE release was measured during a frequency-response curve of 0.25 to 32 Hz. Vascular responses were measured during the frequency-response curve as well as during dose-response curves to norepinephrine (0.98–2000 ng), angiotensin (0.25–256 ng), and barium chloride (0.98–8000 μg). These curves were generated from the same preparation in the order given. Injection volumes were 0.1 ml. To measure \(^{3}\)H-NE release, 20-second collections of the venous effluent were obtained before (pre), during, and after PNS. The during and after PNS collections (stm) were combined for the measurement of the total amount of \(^{3}\)H-NE released by PNS. The actual amount of \(^{3}\)H-NE released during PNS is expressed as the amount of \(^{3}\)H-NE in the stm collection corrected for the baseline efflux of \(^{3}\)H-NE in the pre-PNS collection.

The second series of experiments was conducted to evaluate the sensitivity of presynaptic α-, β-adrenoceptors by determining the inhibitory action of tramazoline on stimulus-induced release of \(^{3}\)H-NE. An initial stimulation at 4 Hz was performed to ensure that the electrodes were properly placed and the preparation was viable. All subsequent stimulations were performed at 2 Hz, and each stimulation was separated by a period of 10 minutes. Two stimulations were performed in the absence of tramazoline to obtain a control release of \(^{3}\)H-NE. After the second control stimulation, \(2 \times 10^{-9} \) M tramazoline was perfused through the kidney during the 10-minute interval between stimulations. Periarterial nerve stimulation was performed in the presence of tramazoline and three additional stimulations were obtained with increasing concentrations of tramazoline (\(2 \times 10^{-8}, 5 \times 10^{-8}, \) and \(2 \times 10^{-7} \) M). The kidney was subsequently perfused with tramazoline-free solution and two additional measurements of \(^{3}\)H-NE release obtained.

A third series of experiments was performed to evaluate effect of the β-adrenoceptor agonist, salbutamol, on stimulus-induced release of \(^{3}\)H-NE. The experimental protocol was similar to that described for tramazoline except during comparable stimulation periods salbutamol in the concentrations of \(1 \times 10^{-10} \) to \(1 \times 10^{-6} \) M was perfused.

The percentage of the \(^{3}\)H-NE and the metabolites, \(^{3}\)H-normetanephrine (\(^{3}\)H-NMN), \(^{3}\)H-dihydroxyamphetamine (\(^{3}\)H-DOMA), and \(^{3}\)H-dihydroxyphenylglycol (\(^{3}\)H-DOPEG), in the effluent before and during
periarterial nerve stimulation were calculated after chromatographic separation using the method of Graefe et al.\textsuperscript{17}

Values are reported as mean ± SEM. The data were analyzed using Student's \( t \) test and analysis of variance; \( p < 0.05 \) was taken as the level of significance.

**Results**

**Norepinephrine Release and Vascular Reactivity in the SHR**

These experiments were performed on animals aged 18 to 22 weeks. The systolic blood pressure was 116 ± 2 mm Hg in the WKY and 221 ± 5 mm Hg in the SHR. Vasoconstrictor responsiveness to periarterial nerve stimulation (0.25–32 Hz) and exogenous norepinephrine (0.98–2000 ng) in the WKY and SHR is illustrated in figure 1. The responses to both of these stimuli were significantly increased in the SHR, in that the maximum responses were greater and the threshold stimuli to elicit these responses were lower in the SHR. Periarterial nerve stimulation at 0.25 Hz caused only a 0.7 ± 0.5 mm Hg increase in perfusion pressure in the WKY, whereas in the SHR it caused an increase in the perfusion pressure of 8.3 ± 2 mm Hg. The maximum response at 32 Hz was 187 ± 20 mm Hg for WKY and 286 ± 17 mm Hg for SHR. The lowest dose of norepinephrine (0.98 ng) did not cause vasoconstriction in WKY, while in the SHR a response of 8 ± 3 mm Hg was observed. The maximum responses were 222 ± 10 mm Hg in WKY and 286 ± 6 mm Hg in SHR (fig. 1). The \(^3\)H-NE overflow was measured throughout the entire range of the frequency-response curve, to determine whether the greater vasoconstrictor responsiveness observed in SHR during periarterial nerve stimulation was the result of only an increase in vascular reactivity caused by postsynaptic changes, or also due to a greater release of neurotransmitter.

The results (table 1) showed that \(^3\)H-NE overflow during periarterial nerve stimulation was significantly greater in SHR at all frequencies of stimulation, by 1.5 to twofold in comparison to WKY. The spontaneous efflux of \(^3\)H-NE was not different between WKY and SHR. The spontaneous baseline efflux prior to 0.25 Hz stimulation was 1139 ± 88 cpm for WKY and 1186 ± 301 cpm in SHR, and the baseline efflux before the last stimulation at 32 Hz was 933 ± 92 cpm for WKY and 966 ± 169 cpm for SHR.

Vascular reactivity to angiotensin II (0.25–256 ng) and barium chloride (0.98–8000 \( \mu \)g) was also evaluated. There were increases in the maximum responses, and threshold doses were lower in the SHR for both of these vasoconstrictor agents (fig. 2). The responses to 0.25 ng angiotensin II in WKY and SHR were 0 and 9 ± 3 mm Hg respectively. The maximum response was 167 ± 3 mm Hg in WKY and 210 ± 8 mm Hg in SHR (fig. 2 A). The response to 1.95 \( \mu \)g barium chloride was 1 ± 0.5 mm Hg in WKY and 9 ± 3 mm Hg in SHR. The maximum response was 237 ± 7 mm Hg in WKY and 298 ± 12 mm Hg in SHR (fig. 2 B).

**Presynaptic Inhibition of Norepinephrine Release by Tramazoline in SHR**

These experiments were performed to determine whether changes in presynaptic \( \alpha \)-adrenoceptor-mediated inhibition of norepinephrine release may have been one of the causes of the greater \(^3\)H-NE overflow seen during periarterial nerve stimulation in the SHR. The overflow of \(^3\)H-NE was measured at a stimulating frequency of 2 Hz, and the influence of tramazoline, an \( \alpha_2 \)-adrenoceptor agonist, on stimulus-induced release was determined. As observed in the previous

**Table 1. Effect of Periarterial Nerve Stimulation on \(^3\)H-Norepinephrine Overflow in the Isolated Perfused Kidneys of WKY and SHR (Mean ± SEM; \( N = 6 \) per Group)**

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>WKY (^3)H-norepinephrine overflow (cpm)</th>
<th>SHR (^3)H-norepinephrine overflow (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>404 ± 125</td>
<td>708 ± 99*</td>
</tr>
<tr>
<td>0.50</td>
<td>934 ± 181</td>
<td>1,632 ± 297*</td>
</tr>
<tr>
<td>1.0</td>
<td>1,798 ± 231</td>
<td>3,348 ± 425*</td>
</tr>
<tr>
<td>2.0</td>
<td>3,396 ± 583</td>
<td>6,446 ± 829*</td>
</tr>
<tr>
<td>4.0</td>
<td>8,190 ± 1,236</td>
<td>14,760 ± 2,091*</td>
</tr>
<tr>
<td>8.0</td>
<td>18,658 ± 2,910</td>
<td>30,952 ± 3,992*</td>
</tr>
<tr>
<td>16.0</td>
<td>35,458 ± 6,962</td>
<td>55,516 ± 1,857*</td>
</tr>
<tr>
<td>32.0</td>
<td>37,248 ± 8,185</td>
<td>59,987 ± 1,057*</td>
</tr>
</tbody>
</table>

*Significantly different from corresponding WKY value at \( p < 0.05 \).
series of experiments, \(^3\)H-NE overflow during periarterial nerve stimulation was significantly greater in SHR than in WKY (table 2). There were no differences in the accumulation of \(^3\)H-NE (table 2). The first two stimulations were performed in the absence of tramazoline to obtain a control release ratio. Release ratio is calculated by dividing the amount of \(^3\)H-NE released during each stimulation period by the amount of \(^3\)H-NE released during the first control stimulation period (i.e., first control ratio = \(S_{2}\text{cpm}/S_{1}\text{cpm}\)). Control release ratios were similar in both the WKY and SHR (table 2). The influence of tramazoline on stimulus-induced release was determined by calculating release ratios. As shown in table 2, while the lowest concentration of tramazoline (2 \(\times\) 10\(^{-9}\) M) did not alter \(^3\)H-NE overflow during periarterial nerve stimulation in either the WKY or SHR, higher concentrations of tramazoline (2 \(\times\) 10\(^{-8}\), 5 \(\times\) 10\(^{-8}\), and 2 \(\times\) 10\(^{-7}\) M) caused dose-dependent decreases in stimulus-induced release of \(^3\)H-NE in SHR (table 2), which is reflected as decreases in release ratios to 0.87 \(\pm\) 0.04, 0.80 \(\pm\) 0.07 and 0.60 \(\pm\) 0.04 respectively. However, in the WKY only the highest concentration of tramazoline was effective in decreasing stimulus-induced release of \(^3\)H-NE to a ratio of 0.75 \(\pm\) 0.04 (table 2). This inhibitory effect in WKY was still significantly smaller than that seen in the SHR with the same concentration of tramazoline (2 \(\times\) 10\(^{-7}\) M). The release ratio for WKY was 0.75 \(\pm\) 0.04, whereas for SHR it was 0.60 \(\pm\) 0.04 (\(p < 0.05\)). The inhibition of stimulus-induced release of \(^3\)H-NE seen with tramazoline was reversible since perfusion with tramazoline-free Krebs-Ringer fluid restored the release ratio to control values (table 2).

In these experiments the amount of intact \(^3\)H-NE in the perfusate and certain metabolites was also measured. Table 3 gives the percentage of \(^3\)H-NE and three of the metabolites during spontaneous efflux (pre) and during periarterial nerve stimulation (stm). The major percentage of radioactivity released during stimulation was intact \(^3\)H-NE. The percentage of \(^3\)H-NE present in the perfusate collected during stimulation was similar in both the WKY and SHR (table 3). While the percentage of \(^3\)H-DOMA, \(^3\)H-DOPEG, and \(^3\)H-NMN decreased during stimulation, there was a much greater reduction in the percentage of \(^3\)H-NMN in the perfu-

**TABLE 2.** Effect of Tramazoline on \(^3\)H-Norepinephrine Overflow Elicited During Periarterial Nerve Stimulation (2 Hz, 20-Second Stimulation Period) from the Isolated Perfused Kidney of WKY and SHR

<table>
<thead>
<tr>
<th>Rat</th>
<th>%(^3)H-NE* Accum.</th>
<th>Initial control release cpm ((S_{1}))</th>
<th>Release ratios†</th>
<th>Tramazoline concentrations (M)</th>
<th>Control</th>
<th>Control</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>15.4</td>
<td>4608</td>
<td>1.09</td>
<td>1.10</td>
<td>1.08</td>
<td>0.94</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>(\pm 1.6)</td>
<td>(\pm 380)</td>
<td>(\pm 0.05)</td>
<td>(\pm 0.05)</td>
<td>(\pm 0.09)</td>
<td>(\pm 0.06)</td>
<td>(\pm 0.04|</td>
</tr>
<tr>
<td>SHR</td>
<td>17.7</td>
<td>6193</td>
<td>1.00</td>
<td>0.93</td>
<td>0.87</td>
<td>0.80</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>(\pm 411|$</td>
<td>(\pm 0.04)</td>
<td>(\pm 0.02)</td>
<td>(\pm 0.04|</td>
<td>(\pm 0.07|$</td>
<td>(\pm 0.04|</td>
</tr>
</tbody>
</table>

*%\(^3\)H-NE accumulation is the amount of total tritium retained by the kidney after the 30-minute perfusion with 10 \(\mu\)Ci of \(\lbrack 7,8\rbrack\)H-norepinephrine followed by a 30-minute perfusion with radioactive free perfusion media.
†Release ratio is defined as the amount of \(^3\)H-norepinephrine released above baseline during each stimulation period divided by the amount of \(^3\)H-norepinephrine released above baseline during the first control stimulation period (1st control ratio = \(S_{2}\text{cpm}/S_{1}\text{cpm}\)).
‡Statistically significant difference from corresponding control value at the level of \(p < 0.05\).
§Statistically significant difference from WKY at the level of \(p < 0.05\).
Presynaptic Facilitation of Norepinephrine Release by Salbutamol in SHR

The effect of salbutamol, a β2-adrenoceptor agonist, on the stimulus-induced release of 3H-NE was evaluated in SHR. As seen in the previous experiments, the control 3H-NE overflow at the stimulation frequency of 2 Hz was greater in SHR than in WKY (table 4). The effect of increasing concentrations of salbutamol on stimulus-induced release of 3H-NE was determined by calculating release ratios as previously described. The control release ratios were similar for both the WKY and SHR. While the two lower concentrations of salbutamol did not cause any alterations in 3H-NE overflow in either the WKY or SHR, subsequently increasing concentrations of this β2-adrenoceptor agonist caused a dose-dependent increase in the stimulus-induced release of 3H-NE as reflected in the increases in release ratios (table 4). However, this facilitatory effect of salbutamol on stimulus-induced release was similar in both the WKY and SHR (table 4). The action of salbutamol was reversible since perfusion with salbutamol-free Krebs-Ringer fluid restored the release ratio to control level.

Discussion

The present study shows that a greater amount of the neurotransmitter, norepinephrine, is released during sympathetic nerve stimulation in the SHR. Such a facilitation of transmitter release was observed over a wide range of stimulation frequencies, suggesting that presynaptic changes have occurred that lead to a greater increase in the amount of norepinephrine released at any given frequency of stimulation in the SHR. The greater norepinephrine release observed in the SHR was also associated with increased vasoconstrictor responsiveness to periarterial nerve stimulation. While vascular reactivity to exogenous norepinephrine and other vasoconstrictor stimuli was also enhanced in the SHR, the results in figure 1 indicate that responses to periarterial nerve stimulation were increased to a greater degree than those to exogenous norepinephrine. A similar phenomenon has been reported in the isolated perfused mesentery of SHR. 19 This observation, when viewed collectively with the greater transmitter release seen in the SHR, suggests that the vasoconstrictor hyperreactivity noted during periarterial nerve stimulation results from an increased release of norepinephrine as well as an increase in the vascular reactivity to the released neurotransmitter. While it has been shown that postsynaptic changes resulting from nonspecific structural alterations and/or specific changes in receptor sensitivity may cause an increase in vascular reactivity to vasoconstrictor stimuli in SHR,12, 13, 19, 20 recently studies have also been performed to determine whether presynaptic alterations leading to an increased norepinephrine release may also contribute to this phenomenon of vasoconstrictor

**Table 3.** Percentage of 3H-Norepinephrine and 3H-Metabolites in the Effluent Collected Before (pre) and During (stm) Periarterial Nerve Stimulation from the Isolated Perfused Kidney of WKY and SHR

<table>
<thead>
<tr>
<th>Rat</th>
<th>No.</th>
<th>pre</th>
<th>stm</th>
<th>3H-DOPEG</th>
<th>3H-NE</th>
<th>3H-DOMA</th>
<th>3H-NMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>6.4</td>
<td>3.0</td>
<td>4.7</td>
<td>0.5</td>
<td>30.8</td>
<td>87.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.8</td>
<td>±0.5</td>
<td>±2.0</td>
<td>±0.2</td>
<td>±5.4</td>
<td>±0.8</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>8.3</td>
<td>3.5</td>
<td>11.2</td>
<td>2.4</td>
<td>31.2</td>
<td>84.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.7</td>
<td>±0.3</td>
<td>±4.0</td>
<td>±0.7</td>
<td>±4.0</td>
<td>±1.1</td>
</tr>
</tbody>
</table>

3H-DOPEG = 3, 4 dihydroxymandelic acid; 3H-DOMA = 3, 4 dihydroxyphenylglycol; 3H-NE = norepinephrine; 3H-NMN = normetanephrine; pre is the 20-second effluent collection before periarterial nerve stimulation; stm is the combined 20-second effluent collection during plus the 20-second effluent collection after periarterial nerve stimulation. Stimulation period = 2 Hz, 40 V, 20 seconds.

**Table 4.** Effect of Salbutamol on 3H-Norepinephrine Overflow Elicited During Periarterial Nerve Stimulation (2 Hz, 20-Second Stimulation Period) from the Isolated Perfused Kidney of WKY and SHR

<table>
<thead>
<tr>
<th>Rat</th>
<th>No.</th>
<th>Control release cpm (S₀)</th>
<th>1 x 10⁻¹⁰</th>
<th>1 x 10⁻⁸</th>
<th>1 x 10⁻⁶</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>6</td>
<td>4501</td>
<td>±638</td>
<td>±0.05</td>
<td>±0.03</td>
<td>±0.07+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.96</td>
<td>0.94</td>
<td>0.95</td>
<td>±0.03</td>
<td>±0.04+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x 10⁻¹⁰</td>
<td>1 x 10⁻⁸</td>
<td>1 x 10⁻⁶</td>
<td>1 x 10⁻⁷</td>
<td>1 x 10⁻⁹</td>
</tr>
<tr>
<td>SHR</td>
<td>6</td>
<td>6651</td>
<td>±805†</td>
<td>±0.02</td>
<td>±0.04</td>
<td>±0.03†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.99</td>
<td>0.98</td>
<td>0.92</td>
<td>±0.02</td>
<td>±0.05†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x 10⁻¹⁰</td>
<td>1 x 10⁻⁸</td>
<td>1 x 10⁻⁶</td>
<td>1 x 10⁻⁷</td>
<td>1 x 10⁻⁹</td>
</tr>
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</table>

Release ratios* Salbutamol concentrations (M) Control

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<thead>
<tr>
<th>Rat</th>
<th>No.</th>
<th>Control release cpm (S₀)</th>
<th>1 x 10⁻¹⁰</th>
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<tr>
<td></td>
<td></td>
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<td>1 x 10⁻⁸</td>
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<td>1 x 10⁻⁹</td>
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</tbody>
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*Release ratio is defined as the amount of 3H-norepinephrine released above baseline during each stimulation period divided by the amount of 3H-norepinephrine released above baseline during the first control stimulation period (1st control ratio = S₀ cpm/S₀ cpm).

†Statistically significant difference from corresponding control value at the level of p < 0.05.
hyperresponsiveness in experimental hypertension. It has been previously reported by us that a greater release of neurotransmitter was present in isolated perfused mesentery of SHR. However, in this study only one frequency of nerve stimulation was employed. Similarly, in the isolated perfused kidney of young (6 weeks old) SHR, while an increased release of norepinephrine was observed during stimulation at two different frequencies, there were no alterations in the vascular reactivity to exogenous norepinephrine. The results of the present study show that in the mature SHR, a greater amount of norepinephrine is released over a wide range of stimulation frequencies, and in addition there is also an increase in vascular reactivity to exogenous norepinephrine. Therefore, it appears that in the mature SHR, both presynaptic as well as postsynaptic alterations contribute to the vasoconstrictor hyperresponsiveness noted during periarterial nerve stimulation.

Since a greater release of norepinephrine existed in the SHR, we performed additional studies to determine the mechanisms responsible for this phenomenon. Norepinephrine release during sympathetic nerve stimulation is modulated by the negative feedback presynaptic \( \alpha \)-adrenoceptor mechanism. We wanted to determine whether the greater increase in norepinephrine release, observed in the SHR, could have been due to alterations in the presynaptic \( \alpha \)-adrenoceptor-mediated inhibition of norepinephrine release during nerve stimulation. It was reasoned that a subsensitivity of presynaptic \( \alpha \)-adrenoceptors may cause a decrease in the effectiveness of norepinephrine in causing negative feedback inhibition of transmitter release, which consequently would lead to a greater transmitter release in the SHR. However, contrary to what would be expected if this mechanism was playing a causative role in the observed changes in norepinephrine release, the presynaptic \( \alpha \)-adrenoceptors were actually supersensitive in SHR. This was evident from the finding that tramazoline, a selective \( \alpha \)-adrenoceptor agonist, was more effective in inhibiting stimulus-induced release of norepinephrine in the SHR. These results appear to rule out the possibility of a subsensitive presynaptic \( \alpha \)-adrenoceptor mechanism as being one of the factors in causing a greater release of norepinephrine and in subsequent maintenance of hypertension in the SHR. However, our findings raise some important points regarding differences in the development of sensitivity changes at presynaptic receptors in comparison with postsynaptic receptors, and how this may influence neurotransmitter release and vascular responsiveness.

It has been reported that supersensitivity to postsynaptic adrenoceptors develops in most tissues following denervation of sympathetic nerves to that particular organ, and while the cellular mechanisms for such a change in receptor sensitivity have not been fully elucidated, it is suggested that the causative factor appears to be the loss of contact between the adrenergic receptor and the neurotransmitter, norepinephrine. Similarly, postsynaptic subsensitivity develops when there is an excessive amount of agonist in contact with the receptor. While there are only few studies where changes in presynaptic receptor sensitivities were studied, the findings from these studies are completely opposite to those reported for postsynaptic receptors. Recently it has been shown that interruption of ganglionic transmission, and subsequent blockade of sympathetic neurotransmitter release, caused a subsensitivity of presynaptic \( \alpha \)-adrenoceptors, which was in contrast to the supersensitivity developing at postsynaptic \( \alpha \)-adrenoceptors. The results of our study show that in the presence of a greater amount of norepinephrine released in SHR, supersensitivity of presynaptic \( \alpha \)-adrenoceptor had developed. While the mechanism for such a change remains to be determined, we suggest that this is probably a pathophysiological adaptation, and it would function to further minimize the presynaptic facilitation of transmitter release present in the SHR. It would be interesting to determine at what stage in the development of hypertension such a change occurs, and whether there is a temporal relationship between changes in the sensitivity at presynaptic \( \alpha \)-adrenoceptors and alterations in norepinephrine release observed in the SHR. It should be pointed out that in an earlier study in the isolated perfused mesentery, we did not observe any changes in the responsiveness of presynaptic \( \alpha \)-adrenoceptors when only one concentration of the \( \alpha \)-adrenoceptor antagonist, phenolamine, was used in the study. It is likely that we may have used a concentration of phenolamine that was very near to the top of the dose-response curve. Alternatively, the use of an antagonist may not allow us to detect subtle changes in sensitivity occurring at these receptors. The present results show that since agonist experiments allow the use of a wide range of different concentrations, they are more appropriate for studying changes occurring at presynaptic \( \alpha \)-adrenoceptors.

In addition to the presence of presynaptic \( \alpha \)-adrenoceptors, the postganglionic sympathetic nerve terminal is also endowed with a large number of other receptors. Activation of presynaptic \( \beta \)-adrenoceptors by appropriate agonists results in an increase in the stimulus-induced release of norepinephrine. Presynaptic \( \beta \)-adrenoceptors are reportedly located at sympathetic nerves innervating several different tissues, and we have recently demonstrated the presence of these receptors on sympathetic nerves to different tissues, and have demonstrated similar increases in the stimulus-induced release of \( \beta \)-NE in both WKY and SHR. One of the explanations for this finding could be that...
norepinephrine may not be the endogenous agonist at these receptors since presynaptic β-adrenoceptors are β2-type.3 5 Therefore, if presynaptic β-adrenoceptors played a role in hypertension, it could be due to an overactivity of epinephrine at these receptors, rather than any changes in sensitivity of these receptors. In this context, it should be noted that circulating levels of epinephrine are also reportedly increased in SHR6 and it is likely that an action of this amine on presynaptic β-adrenoceptors may lead to facilitation of norepinephrine release in SHR. Additional experiments in intact animals need to be performed to obtain evidence in support for this hypothesis.

In summary, the results of our study show that greater amounts of norepinephrine are released during sympathetic nerve stimulation in SHR, which contributes to the vasoconstrictor hyperresponsiveness noted during sympathetic nerve stimulation and subsequently to the hypertension. The presynaptic α-adrenoceptors are supersensitive in the SHR, suggesting that, while this is not a causative factor in hypertension, it may be a consequence of the greater norepinephrine release present in SHR and could serve an important adaptive pathophysiological mechanism to protect against further increases in norepinephrine release in SHR.

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