Increased presynaptic $\alpha$-adrenoceptor-mediated regulation of noradrenaline release in the isolated perfused kidney of spontaneously hypertensive rats

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Summary

1. Release of $[^3]H$noradrenaline during periarterial nerve stimulation and its inhibition by the presynaptic $\alpha$-adrenoceptor mechanism were studied in the isolated perfused kidney from spontaneously hypertensive and Wistar-Kyoto rats.

2. A frequency related vasoconstriction as well as $[^3]H$noradrenaline release were observed over the stimulating range of 0.25–32 Hz in both the Wistar-Kyoto and spontaneously hypertensive rats. The spontaneously hypertensive rat kidneys exhibited both an increased vasoconstrictor response and a greater $[^3]H$noradrenaline release when compared with the Wistar-Kyoto rat kidneys.

3. Presynaptic inhibition of $[^3]H$noradrenaline release was evaluated at 2 Hz by using the $\alpha$-adrenoceptor agonist, tramazoline. Increasing concentrations of tramazoline from $2 \times 10^{-9}$ mol/l to $2 \times 10^{-7}$ mol/l caused a dose-dependent decrease in the stimulus-induced release of $[^3]H$noradrenaline in spontaneously hypertensive rats but not in Wistar-Kyoto rats. Only $2 \times 10^{-7}$ mol/l tramazoline had an inhibitory effect in the Wistar-Kyoto rats.

4. These data indicate that noradrenaline release during sympathetic nerve stimulation is greater in the spontaneously hypertensive rat. The supersensitivity of presynaptic $\alpha$-adrenoceptors observed in spontaneously hypertensive rats may be a consequence of the greater noradrenaline release present in these animals.

Key words: noradrenaline release, presynaptic $\alpha$-adrenoceptors, spontaneously hypertensive rats, tramazoline.

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Introduction

Noradrenaline release from sympathetic nerve terminals can be modified by various endogenous substances which produce their effects by interacting with the presynaptic nerve terminal membrane through specific receptors [1–3]. These presynaptic mechanisms have been demonstrated in a wide variety of tissues and, in the cardiovascular system, in the heart and blood vessels. However, of all presynaptic receptors studied, only the presynaptic $\alpha$-adrenoceptors have been reported to play a possible physiological role in the regulation of noradrenergic transmission [2].

Recent studies indicate that, in addition to postsynaptic vascular changes, presynaptic alterations, leading to a greater increase in noradrenaline release, may also contribute importantly to the pathogenesis of hypertension in the spontaneously hypertensive rat [4, 5]. Although the mechanisms responsible for this increased transmitter release have not been elucidated, it is likely that alterations in the presynaptic regulation of noradrenaline release may be a causative factor in this phenomenon. More specifically, it is possible that a subsensitivity of presynaptic $\alpha$-adrenoceptors could lead to a greater transmitter release from sympathetic nerves in spontaneously hypertensive rats.

Experiments were therefore performed to further study noradrenaline release and its modification by the presynaptic $\alpha$-adrenoceptor mechanism during periarterial nerve stimulation in the isolated perfused kidney of spontaneously hypertensive rats and normotensive Wistar-Kyoto rats.

Methods

The right kidney of male Wistar–Kyoto and spontaneously hypertensive rats of the Okamoto
strain was isolated under pentobarbitone sodium (50 mg/kg, intraperitoneally) anaesthesia as previously described [6]. The neuronal noradrenaline storage sites were labelled by perfusing the kidney with [1-3H]noradrenaline (10 μCi/180 ml; 5.1 × 10⁻⁹ mol/l; sp. radioactivity 11 Ci/mmol or 407 GBq/mmol) at 6 ml/min. Bipolar platinum electrodes were placed around the renal artery for periarterial nerve stimulation; stimulation characteristics: 40 V, 1 ms duration and a 20 s stimulation period. To measure [3H]noradrenaline release, 20 s collections of the venous effluent were taken before, during and after periarterial nerve stimulation. Collections during and after periarterial nerve stimulation were combined for use as a measure of the total amount of [3H]noradrenaline release during periarterial nerve stimulation. The actual amount of [3H]noradrenaline released during periarterial nerve stimulation is expressed as the amount of [3H]noradrenaline in the collection after stimulation corrected for the baseline efflux of [3H]noradrenaline in the collection before stimulation.

In the first series of experiments, vasoconstrictor responses as well as [3H]noradrenaline release were measured during periarterial nerve stimulation over the frequency range 0.25–32 Hz. The second series of experiments were conducted at a frequency of 2 Hz in order to evaluate the presynaptic α-adrenoceptor mechanism. The protocol and appropriate definitions are provided in the Results section. Since the amount of [3H]noradrenaline in all effluent samples was 82–88% of the total 3H measured during periarterial nerve stimulation, the total 3H values are used as a measure of [3H]noradrenaline release.

Values are reported as means ± SEM. Analysis of data was performed with Student’s t-test and analysis of variance. Level of significance was assigned as P < 0.05.

Table 1. Effect of tramazoline on [3H]noradrenaline release during periarterial nerve stimulation from the isolated perfused kidney of normotensive Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR).

<table>
<thead>
<tr>
<th>Conc. of tramazoline (mol/l)</th>
<th>Release ratio</th>
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<tbody>
<tr>
<td></td>
<td>2 × 10⁻⁸</td>
</tr>
<tr>
<td></td>
<td>WKY 6</td>
</tr>
<tr>
<td>Control</td>
<td>1.09 ± 0.05</td>
</tr>
<tr>
<td>2 × 10⁻⁸</td>
<td>1.08 ± 0.09</td>
</tr>
<tr>
<td>2 × 10⁻⁸</td>
<td>0.75 ± 0.04*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

Results

This study was conducted on animals 15–21 weeks old. The mean systolic blood pressure was 116 ± 2 mmHg for the Wistar–Kyoto rats and 221 ± 5 mmHg for the spontaneously hypertensive rats.

At stimulation frequencies ranging from 0.25 to 32 Hz, the renal vasoconstrictor responses were significantly greater in the spontaneously hypertensive rats. Furthermore, the amount of [3H]noradrenaline released was also significantly greater in the spontaneously hypertensive rat during all frequencies of stimulation. The vasoconstrictor responses, at frequencies of 0.25, 0.5, 1, 2, 4, 8, 16 and 32 Hz, were 1 ± 1, 2 ± 1, 5 ± 1, 14 ± 5, 90 ± 28, 156 ± 27, 187 ± 20 and 187 ± 20 mmHg in the Wistar–Kyoto rats and 9 ± 2, 14 ± 4, 33 ± 9, 95 ± 25, 218 ± 14, 254 ± 8, 289 ± 12 and 286 ± 17 mmHg in the spontaneously hypertensive rats (n = 6 per group; P < 0.05). The respective results for [3H]noradrenaline release during these frequencies of stimulation were 404 ± 125; 934 ± 181, 1798 ± 21, 3396 ± 583, 8190 ± 1236, 18658 ± 2910, 35458 ± 6962 and 37248 ± 8185 c.p.m. in the Wistar–Kyoto rats and 708 ± 99, 1632 ± 197, 3348 ± 425, 6446 ± 829, 14760 ± 2091, 30952 ± 3992, 55516 ± 1857 and 59987 ± 1057 c.p.m. for the spontaneously hypertensive rats. There was no difference in the spontaneous baseline release of [3H]noradrenaline between the Wistar–Kyoto rats and spontaneously hypertensive rats.

Evaluation of the presynaptic α-adrenoceptor mechanism was performed to determine whether an alteration in this mechanism may be one of the causative factors for the greater release of [3H]noradrenaline in the spontaneously hypertensive rat. Release of [3H]noradrenaline was measured at a stimulating frequency of 2 Hz and the results are summarized in Table 1. Control
release was greater in spontaneous hypertensive rats than in the Wistar-Kyoto rats (6193 ± 411 vs 4608 ± 380 c.p.m.). There were no differences in the accumulation of \[^3H\]noradrenaline between spontaneously hypertensive rats and Wistar-Kyoto rats during the labelling of the kidneys with \[^3H\]noradrenaline. Initially, two control stimulations were performed and the release of \[^3H\]noradrenaline during these two stimulations was used to calculate a control (non-drug) release ratio as defined in Table 1. All subsequent release of \[^3H\]noradrenaline is reported as release ratios. After two control stimulations, increasing concentrations of tramazoline, a presynaptic \(\alpha\)-adrenoceptor agonist, were perfused through the kidney and its effects on \[^3H\]noradrenaline release were determined. As shown in Table 1, tramazoline caused a dose-dependent decrease in the stimulus-induced release of \[^3H\]noradrenaline in the spontaneously hypertensive rats. In the Wistar-Kyoto rats only the highest dose of tramazoline was effective in decreasing \[^3H\]noradrenaline release during periartrial nerve stimulation (Table 1).

Discussion

An increased noradrenaline release from sympathetic nerve terminals of spontaneously hypertensive rats may be one of the important factors contributing to the hypertension. In the present study, the involvement of this peripheral site in the increase in vascular reactivity and hypertension was investigated in the spontaneously hypertensive rat. Our results show a greater release of neurotransmitter during sympathetic nerve stimulation in the spontaneously hypertensive rat, which was accompanied by increased vasoconstrictor responses. This finding agrees with results from our laboratory for the isolated perfused mesenteric artery [4] and that of Collis et al. [5] for the isolated kidney from 6 week old spontaneously hypertensive rats. Since the present study was performed over a wide range of stimulation frequencies, these results suggest that presynaptic changes have occurred to increase the amount of neurotransmitter released at any given frequency in the spontaneously hypertensive rat.

Noradrenaline release during neuronal activity is regulated by the negative feedback presynaptic \(\alpha\)-adrenoceptor mechanism [1–3]. Since a greater noradrenaline release existed in the spontaneously hypertensive rat, we wanted to determine whether this abnormality was due to alterations in the presynaptic \(\alpha\)-adrenoceptor mediated inhibition of noradrenaline release. However, contrary to what would be expected if this mechanism was playing a causative role in the observed changes in neurotransmitter release, the presynaptic \(\alpha\)-adrenoceptors were supersensitive in spontaneously hypertensive rats, as evidenced in the finding that tramazoline was more effective in inhibiting noradrenaline release during sympathetic nerve stimulation in spontaneously hypertensive rats. Although the present results rule out the possibility of a subsensitive presynaptic \(\alpha\)-adrenoceptor mechanism as being the causative factor in the maintenance of hypertension, the observed supersensitivity may be of importance in curtailing the greater neurotransmitter release in spontaneously hypertensive rats.

In conclusion, the results of the present study show that: (a) noradrenaline release during sympathetic nerve stimulation is greater in the spontaneously hypertensive rat and such a presynaptic alteration may play an important role in the hypertension, and (b) the presynaptic \(\alpha\)-adrenoceptor is supersensitive, suggesting that whereas it is not a causative factor, it may be a consequence of the greater noradrenaline release in the spontaneously hypertensive rat.

Acknowledgments

This study was supported by NIH grant HL-22868 from National Heart, Lung and Blood Institute. The authors are grateful to Mike Woods for excellent technical assistance and to Mrs Mary Warren for the preparation of this manuscript.

References