Inhibition of Norepinephrine Release by Presynaptic 
Alpha\textsubscript{2}-adrenoceptors in Mesenteric Vasculature 
Preparations from Chronic DOCA-salt 
Hypertensive Rats

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SUMMARY

This study investigated norepinephrine release during electrical nerve stimulation and inhibitory characteristics of presynaptic \(\alpha_2\)-adrenoceptors in perfused mesenteric vasculature from deoxycorticosterone acetate (DOCA)-salt hypertensive rats (7–8 weeks after surgery). Electrical stimulation of sympathetic innervation caused a significantly greater release of endogenous norepinephrine into the mesenteric vasculature of DOCA-salt hypertensive rats than in age-matched normotensive controls. Pressor responses to electrical nerve stimulation were also enhanced in DOCA-salt hypertension. Yohimbine, a potent \(\alpha_2\)-adrenoceptor blocking agent, potentiated the stimulation-evoked release of norepinephrine into the vasculature in normotensive rats. This effect was blunted in DOCA-salt hypertension. These results suggest that increased norepinephrine release from the sympathetic nerve endings in DOCA-salt hypertension might partly reflect an impaired presynaptic \(\alpha_2\)-adrenoceptor-mediated inhibition, which could enhance vascular sympathetic tone in this model of hypertension.

Additional Indexing Words:
DOCA-salt hypertension Norepinephrine release Presynaptic \(\alpha_2\)-adrenoceptors Yohimbine Blood vessels

NOREPINEPHRINE release from sympathetic nerve endings is regulated by presynaptic receptors on the nerve terminals.\(^1\)–\(^3\) Presynaptic \(\alpha_2\)-adrenoceptors are particularly strong inhibitors of norepinephrine release from the sympathetic terminals, constituting an important autoregulatory system for sympathetic tone.\(^1\)–\(^3\) However, it is unclear whether presynaptic \(\alpha_2\)-adrenoceptor-mediated inhibition of norepinephrine release is altered in hypertension. Ekas et al observed that tramazoline (an \(\alpha_2\)-agonist) caused a...
dose-dependent inhibition of stimulus-induced norepinephrine release in the perfused kidney of 18-week old spontaneously hypertensive rats (SHR), suggesting a supersensitivity of presynaptic $\alpha_2$-adrenoceptors in this model of hypertension. In contrast, our previous reports showed that presynaptic $\alpha_2$-adrenoceptor-mediated inhibition of norepinephrine release was attenuated in the mesenteric vasculature of 7-8 week old SHR. Thus, the functional status of presynaptic $\alpha_2$-adrenoceptors in hypertension has not been clarified.

It has been recognized that enhanced sympathetic nerve activity may contribute to the maintenance of DOCA-salt hypertension. However, few studies have directly assessed the activity of presynaptic $\alpha_2$-adrenoceptors in DOCA-salt hypertension. This study measured norepinephrine release from the sympathetic nerve endings and the inhibitory characteristics of presynaptic $\alpha_2$-adrenoceptors in resistance vessels from DOCA-salt hypertensive rats.

**Materials and Methods**

Normotensive male Wistar rats (weighing 220-330 g) were used for preliminary investigations of the effects of yohimbine. DOCA-salt hypertension was induced in male Wistar rats (weighing 140-160 g). The rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneal injection) and left nephrectomy was performed. A 10 mg dose of DOCA was injected subcutaneously 3 days after the operation, and the injection was repeated once weekly throughout the experiment. The rats were given 1% sodium chloride as drinking water. Age-matched normotensive Wistar rats were used as the controls and given tap water. Experiments were carried out 7-8 weeks after the operation.

Rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneal injection), and perfused mesenteric vasculature preparations were obtained according to the method of Castellucci et al. The superior mesenteric artery was perfused through a cannula inserted at its origin from the aorta. In each preparation, only four branches from the superior mesenteric artery trunk were used; all other branches were ligated. The isolated preparation was placed in a chamber maintained at 37°C and placed gently on gauze to avoid traction on the cannulated artery. The preparation was perfused with modified Ringer-Locke solution (mmol/l: NaCl 120.7, KCl 5.9, CaCl$_2$ 2.5, MgSO$_4$ 1.3, NaHCO$_3$ 15.5, NaH$_2$PO$_4$ 1.2 and glucose 11.5, pH 7.4, 37°C), bubbled with a 95% O$_2$ and 5% CO$_2$ mixture. A constant flow rate was maintained with a peristaltic pump (Harvard apparatus, model 1200) at 0.8 ml/min. Changes in the perfusion pressure were monitored with a pressure transducer connected to a polygraph (Nihon Kohden, model CP-620 G).
Periarterial nerve stimulation was performed with bipolar platinum electrodes around the proximal end of the mesenteric artery. Stimulation was applied at a supramaximal voltage (40 V), with rectangular pulses of 5 msec duration for 1 min at 5, 10 and 15 Hz (electrical stimulator: Nihon Kohden, model SEN-3201). Pressor responses to the electrical nerve stimulation were determined as increases in the perfusion pressure. After perfusion of the mesenteric vascular beds was initiated, the basal perfusion stimulation was allowed to stabilize for 30 min prior to introducing experimental manipulations.

Measurement of norepinephrine release during electrical nerve stimulation in the mesenteric vasculatures:

For the measurement of norepinephrine release from sympathetic nerve endings, the perfusate through the mesenteric preparation was collected in a tube containing the mixture solution of EGTA (90 mg/ml) and glutathione (60 mg/ml) (50 μl in each tube). The collecting periods were 3 min before the nerve stimulation (A) and a period during and 2 min after cessation of stimulation (B). Since the stimulation period lasted 1 min, the total period was 3 min. The norepinephrine release evoked by the electrical nerve stimulation was defined as the norepinephrine overflow, which was the difference of the norepinephrine contents between (A) and (B), and was normalized as nanogram per gram of wet tissue weight for each preparation.

Norepinephrine in the perfusate was adsorbed on alumina, extracted in 200 μl of perchloric acid (0.1 mol/l), and assayed by high performance liquid chromatography with an electrochemical detector (Bioanalytical system, model LC-4A, carbon electrode, 700 mV). The HPLC unit was equipped with a delivery system (Waters Assoc., model 510, flow rate 1.0 ml/min), an automatic injector (Waters Assoc., model 710 B, injected volume 100 μl) and Biophase ODS 5 μm column. The solvent for the separation of norepinephrine was 0.1 mol/l of monochloroacetic acid with 2 mmol/l of EDTA, 300 mg/l of sodium octyl sulfate and 9% (v/v) of acetonitrile (pH 3.02 at room temperature).

Previously, we showed that the pressor responses and norepinephrine overflow were completely blocked by adding guanethidine in the perfusion medium, which provided evidence that periarterial stimulation was neuronal in nature. Further, these responses were stable during at least seven repeated stimuli in the same preparation. We performed the experimental manipulations during this stable period.

First, we examined the pressor responses and norepinephrine overflow during electrical nerve stimulation in the DOCA-salt hypertensive rats and the normotensive control rats. In the second series of the experiments, we
investigated the effects of yohimbine, an \( \alpha_2 \)-adrenoceptor blocking agent, on these responses in the DOCA-salt hypertension and normotensive controls. Yohimbine was added into the perfusion medium 9 min prior to the next electrical stimulation. The effects of yohimbine on stimulation-evoked norepinephrine overflow and pressor responses were evaluated as the percentage of corresponding control values without yohimbine in the DOCA-salt hypertension and the normotensive controls, respectively.

Data are expressed as the means±SEM. Statistical significances were determined by Student's t-test, and a value of \( p<0.05 \) was considered significant.

Yohimbine was purchased from Sigma Chemical Co., Ltd. (USA).

**RESULTS**

1. Pressor responses and norepinephrine overflow in the mesenteric vasculatures of DOCA-salt hypertensive rats

   The systolic blood pressure, measured by the tail-cuff method (programmed electrophygmonanometer, Narco Biosystem Inc., model PE-300), was 186.7±9.4 mmHg (n=9) in DOCA-salt hypertension and 124.5±4.0 mmHg (n=8) in age-matched normotensive controls. The basal perfusion pressure and norepinephrine content in the perfusate during the unstimulated period (spontaneous output of norepinephrine from the vascular beds for 3 min) were not significantly different between the DOCA-salt hypertensive rats and the normotensive control rats (basal perfusion pressure: DOCA-salt hypertension 24.1±0.2 mmHg, n=9, normotensive controls 25.5±0.8 mmHg, n=8; basal norepinephrine output: DOCA-salt hypertension 0.24±0.03 ng/g of wet tissue weight, n=9, normotensive controls 0.27±0.05 ng/g of wet tissue weight, n=8). Figure 1 illustrates typical pressor responses to electrical nerve stimulation in the DOCA-salt hypertension rats and the normotensive controls. Stimulation-evoked pressor responses were greater in the DOCA-salt hypertension group (Table I). The stimulation-evoked norepinephrine release was also higher in the DOCA-salt hypertension rats (Table I).

2. Effects of yohimbine, an \( \alpha_2 \)-adrenoceptor blocking agent, on norepinephrine overflow in DOCA-salt hypertension

   In a previous study using the mesenteric vasculature of normotensive Wistar rats,\(^6\) low concentrations of yohimbine \((5.0\times10^{-8} \text{ M} - 1.0\times10^{-7} \text{ M})\) markedly potentiated stimulation-evoked norepinephrine overflow. However, this effect was attenuated at higher concentrations of yohimbine. A high concentration of yohimbine may have actions other than blockade of
Fig. 1. Typical pressor responses to electrical nerve stimulation in the mesenteric vasculatures of DOCA-salt hypertensive rats and normotensive control rats.

Table I. Pressor Responses and Norepinephrine Overflow during Electrical Nerve Stimulation in the Mesenteric Vasculature Preparations from DOCA-salt Hypertensive Rats (DOCA-salt HT) and Normotensive Control Rats (NT-controls)

<table>
<thead>
<tr>
<th>Pressor responses</th>
<th>10 Hz</th>
<th>15 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-salt HT</td>
<td>76.4±6.6 mmHg*</td>
<td>148.0±12.7 mmHg*</td>
</tr>
<tr>
<td></td>
<td>(n=9)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>NT-controls</td>
<td>35.4±4.2 mmHg</td>
<td>44.6±2.1 mmHg</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td>(n=8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Norepinephrine overflow</th>
<th>10 Hz</th>
<th>15 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-salt HT</td>
<td>1.37±0.12 ng/g*</td>
<td>1.63±0.10 ng/g*</td>
</tr>
<tr>
<td></td>
<td>(n=9)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>NT-controls</td>
<td>0.34±0.12 ng/g</td>
<td>0.75±0.08 ng/g</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

Pressor responses are expressed as the increase in the perfusion pressure of preparations. Norepinephrine overflow was determined as the difference between the norepinephrine content of the vascular perfusate before and during electrical nerve stimulation. It is normalized as ng/g of wet tissue weight for each preparation (mean±SEM, * p<0.005, statistical significance between DOCA-salt HT and NT-controls).
Therefore, we selected $5.0 \times 10^{-8}$ M and $1.0 \times 10^{-7}$ M doses of yohimbine for these experiments.

Table II shows the effects of yohimbine on norepinephrine overflow from the mesenteric vasculature during electrical stimulation (10 Hz) of sympathetic innervation in DOCA-salt hypertension and the normotensive control groups. Yohimbine dramatically increased the stimulation-evoked norepinephrine overflow in the normotensive control rats. This facilitation of norepinephrine overflow was absent in preparations from the DOCA-salt hypertension group. The effects of yohimbine on pressor responses to electrical nerve stimulation were also blunted in the DOCA-salt hypertension group (Table II).

**DISCUSSION**

Norepinephrine release from sympathetic nerve endings is modulated by several mechanisms. One prominent mechanism is negative feedback regulation by presynaptic $\alpha_2$-adrenoceptors. Additionally, it is well known that the blockade of presynaptic $\alpha_2$-adrenoceptors by yohimbine, a selective $\alpha_2$-adrenoceptor antagonist, induces an increase in norepinephrine release both in vitro and in vivo. In this study, we used yohimbine to investigate whether this presynaptic $\alpha_2$-adrenoceptor-mediated inhibition of norepinephrine re-

### Table II. Effects of Yohimbine on Pressor Responses and Norepinephrine Overflow during Electrical Nerve Stimulation of Mesenteric Vasculature

<table>
<thead>
<tr>
<th></th>
<th>DOCA-salt HT</th>
<th>Normotensive controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pressor responses</strong> (10 Hz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yohimbine</td>
<td>5.0 $\times 10^{-8}$ M</td>
<td>1.0 $\times 10^{-7}$ M</td>
</tr>
<tr>
<td>DOCA-salt HT</td>
<td>93.5±2.9 %</td>
<td>64.1±2.7 %</td>
</tr>
<tr>
<td></td>
<td>(n=4)  §</td>
<td>(n=4) §</td>
</tr>
<tr>
<td>NT-controls</td>
<td>153.3±11.7 % *</td>
<td>141.9±14.6 %</td>
</tr>
<tr>
<td></td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td><strong>Norepinephrine overflow</strong> (10 Hz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOCA-salt HT</td>
<td>94.7±3.8 %</td>
<td>78.4±3.6 %</td>
</tr>
<tr>
<td></td>
<td>(n=4) §</td>
<td>(n=4) §</td>
</tr>
<tr>
<td>NT-controls</td>
<td>189.2±13.7 % *</td>
<td>187.2±19.9 %*</td>
</tr>
<tr>
<td></td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
</tbody>
</table>

Values are expressed as a percentage of the control responses without yohimbine in DOCA-salt hypertensive rats (DOCA-salt HT) and normotensive rats (NT-controls), respectively. Means±SEM, * significant increase in response to yohimbine compared to the control value without the drug, p<0.05; § significant difference between DOCA-salt HT and NT-controls, p<0.05.
lease is altered in the blood vessels of rats with DOCA-salt hypertension. In the perfused mesenteric vasculature from normotensive control rats, yohimbine caused a marked increase in norepinephrine overflow during periarterial nerve stimulation. However, this facilitatory effect was blunted in the DOCA-salt hypertensive rats. Similarly, pressor responses to nerve stimulation did not increase after yohimbine application in DOCA-salt hypertension. These findings suggest that presynaptic α₂-adrenoceptor function is attenuated in the mesenteric vasculature of rats with DOCA-salt hypertension. This study also demonstrated that both the stimulation-evoked norepinephrine overflow and the pressor responses were enhanced in DOCA-salt hypertension. These results are compatible with previous reports of an increased plasma norepinephrine concentration in DOCA-salt hypertensive rats.7,16

It seems likely that exaggerated sympathetic nerve activity may depend, at least in part, on the dysfunction of negative feedback mechanisms by presynaptic α₂-adrenoceptors. Bouvier and de Champlain have also reported that yohimbine did not affect epinephrine secretion from adrenal medulla in response to carotid occlusion in DOCA-salt hypertensive rats17; they proposed that inhibitory α₂-adrenergic modulatory mechanisms of adrenal medullary epinephrine release are impaired in this model of hypertension. It is possible that abnormalities in presynaptic α₂-adrenoceptor function may induce both increased vascular sympathetic transmission and enhanced adrenomedullary tone in DOCA-salt hypertension.

The mechanisms responsible for this alteration in presynaptic α₂-adrenoceptor activity in DOCA-salt hypertension remain unclear. It has been reported that characteristics of platelet α₂-adrenoceptors are modified by sodium ions in vitro.18,19 In the central nervous system, Gavras has proposed that increased dietary sodium intake decreases the affinity of brainstem α₂-adrenergic receptors, suggesting that sodium-loading contributes to hypertension by decreasing the responsiveness of central α₂-adrenergic receptors.20 The results of other studies have also supported this hypothesis. Kohman et al have observed that saline infusion attenuates the pressor responses to clonidine, an α₂-adrenoceptor agonist, in rats.21 Greenberg et al have reported that increased sodium concentration inhibits the binding of α-adrenergic receptor agonists to α₂-adrenergic receptors in vitro.22 However, little is known of the precise mechanisms by which sodium can affect the sensitivity of α₂-adrenoceptors.

Several studies have examined presynaptic α₂-adrenoceptor function in other forms of hypertension. Ekas et al have reported a supersensitivity of presynaptic α₂-adrenoceptors in the perfused kidneys of SHR.4 In contrast, Westfall et al23 have observed that presynaptic α₂-adrenoceptor activity was
attenuated in isolated portal veins from adult, but not young SHR. Galloway and Westfall\(^{24}\) have also suggested that presynaptic \(\alpha_2\)-adrenergic function in coccygeal arteries was decreased in SHR, since there was less enhancement of norepinephrine release by yohimbine in adults. Finally, we have reported that norepinephrine release in the mesenteric arteries of SHR was relatively insensitive to yohimbine, suggesting a decreased sensitivity of presynaptic \(\alpha_2\)-adrenergic receptors in SHR.\(^{5,6}\) Thus, it seems likely that there are distinct differences in the function of presynaptic \(\alpha_2\)-adrenoceptors according to the tissues, ages or types of hypertension.

In summary, this study demonstrates that norepinephrine release and pressor responses were increased in the mesenteric vasculature preparations from DOCA-salt hypertensive rats. This change might reflect, at least in part, an impaired presynaptic \(\alpha_2\)-adrenoceptor-mediated inhibition of norepinephrine release from the vascular adrenergic neurons. Finally, impaired presynaptic \(\alpha_2\)-adrenoceptors may contribute to the development of DOCA-salt hypertension.

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