Arterial Neuroeffector Responses in Early and Mature Spontaneously Hypertensive Rats

Nicola Stephens, Stuart J. Bund, Carol Jagger, and Anthony M. Heagerty

Intramural sympathetic neuroeffector responses and presynaptic regulation of neurotransmission by amine uptake and $\alpha_2$-adrenergic receptors were examined in young (5-week-old) and mature (12-week-old) spontaneously hypertensive rats (SHR) and were compared with those of age-matched Wistar-Kyoto (WKY) control rats. Electrical field stimulation (20 V, 0.2-msec pulse width, 3-second pulse train each minute, 5–100 Hz) elicited contractile responses from isolated mesenteric arteries mounted in a myograph. There was a significant difference between the sensitivity of arteries to electrical field stimulation in the two age groups, with arteries from 12-week-old rats being more sensitive than arteries from 5-week-old animals. Also, there was a significant age-strain interaction: the sensitivity of arteries from SHR to electrical field stimulation increased dramatically with age compared with that of WKY rat arteries. Cocaine significantly increased the sensitivity to electrical field stimulation after inhibition of presynaptic $\alpha_2$-adrenergic receptors, and had a significantly greater effect in arteries from 5-week-old SHR compared with WKY controls. This would reflect an overactive neuronal amine uptake mechanism in young SHR. At 12 weeks there was no significant interstrain difference in the effect of cocaine. Yohimbine increased the sensitivity to electrical field stimulation both before and after inhibition of neuronal amine uptake, but there was no difference in its effect with age or strain. Therefore, although sensitivity to sympathetic nerve stimulation varies with age in the SHR, there is no evidence that this can be ascribed to $\alpha_2$-adrenergic receptor function. (Hypertension 1991;18:674–682)

In hypertension the factors that initiate the rise in blood pressure are unknown, but there is a large body of evidence to suggest that overactivity of the sympathetic nervous system may play a major role. Evidence from histofluorescence and morphometric studies suggests that there is an enhanced sympathetic innervation in arteries from the spontaneously hypertensive rat (SHR) compared with arteries from the Wistar-Kyoto (WKY) normotensive rat strain, and this may be apparent from as early as 2 weeks of age before the full development of hypertension.

Although the sensitivity of mesenteric arteries to exogenous norepinephrine is similar in SHR and WKY rats while neuronal amine uptake is functional, investigation of the neuroeffector responses of these arteries has revealed an increased sensitivity to sympathetic nerve stimulation in mature SHR. After vascular sympathetic stimulation, the absolute release of norepinephrine is greater in the SHR compared with WKY rats. When norepinephrine overflow is normalized for the tissue concentration of the amine, a greater fractional release occurs in the mesentery of young SHR with normal release in older rats, which may contribute to the development of hypertension. However, there is also evidence to indicate that fractional release of norepinephrine is only elevated in chronically hypertensive SHR.

The net amount of norepinephrine in the synaptic cleft is determined by the extent of sympathetic innervation and also by the presynaptic regulating mechanisms at the neuroeffector junction, including neuronal amine uptake and $\alpha_2$-autoinhibitory adrenergic receptors. The role of $\alpha_2$-adrenergic receptors in SHR mesenteric arteries is unclear with reports of normal activity, impaired function in young rats alone, and impaired function in chronically hypertensive rats alone. Furthermore, although neuronal reuptake of exogenously applied norepinephrine is shown to be enhanced in the SHR, which effectively normalizes vascular sensitivity to norepinephrine, the functional importance of this during endogenous sympathetic nerve stimulation has not been defined.
As with many studies in hypertension, these abnormalities are confusing with regard to whether they are causal in nature or consequent on the hypertension process itself. This contention is supported by a recent study where the hypertensive F₂ hybrids of SHR/WKY matings failed to show evidence of an enhanced autonomic neuronal amine reuptake mechanism. Accordingly, it was decided to investigate the sympathetic neuroeffector junction in the SHR at two time points, before and after the blood pressure had become elevated. These studies were carried out on arteries less than 300 μm in diameter because these vessels are considered to be involved in the maintenance of peripheral vascular resistance. Regulation of the neuroeffector responses by neuronal amine uptake and by presynaptic α₂-mediated autoinhibition were investigated, and the functional interaction of these two systems examined.

Methods

Male SHR and normotensive WKY control rats were obtained from the stock colony bred at the University of Leicester at 5 and 12 weeks of age. Systolic blood pressure was determined under light ether anesthesia using tail-cuff plethysmography at least 24 hours before use. Rats were stunned and killed by cervical dislocation, and a section of jejunum with associated vasculature was dissected free and placed in physiological salt solution (PSS) with the following composition (mM): NaCl 119, KC1 4.7, CaCl₂ 2.5, MgSO₄ 1.17, NaHCO₃ 25, KH₂PO₄ 1.18, K₂EDTA 0.025, D-glucose 5.5. Two segments (2-mm-long) of second order branches of the superior mesenteric artery from each rat were dissected and mounted in a myograph (J.P. Trading, Aarhus, Denmark) for isometric tension measurements. Vessels were incubated in PSS for 30 minutes, during which time they were warmed to 37°C and gassed with O₂ containing 5% CO₂. The resting tension/internal circumference ratio was determined and each vessel set was normalized to a standardized internal circumference of L₁₀₀ where L₀ = 0.9 L₁₀₀ and L₁₀₀ is the internal circumference that the vessel would have under a transmural pressure of 100 mm Hg. Effective normalized lumen diameter, L₀, was calculated as L₀ = L₁₀₀/π.

Arteries were exposed to five activating solutions in a standard start procedure, each for a period of 2 minutes with a 4-minute relaxation period between each activation. Vessels were activated twice with 10 μM norepinephrine in K-PSS, where K-PSS is PSS containing 5% CO₂. The resting tension/internal circumference ratio was determined and each vessel set was normalized to a standardized internal circumference of L₁₀₀ where L₀ = 0.9 L₁₀₀ and L₁₀₀ is the internal circumference that the vessel would have under a transmural pressure of 100 mm Hg. Effective normalized lumen diameter, L₀, was calculated as L₀ = L₁₀₀/π.

Platinum foil electrodes (Goodfellow, Cambridge, UK) were secured in the myograph mounting heads on either side of the vessel. They were connected to an electrical stimulator and train programmer (Harvard Apparatus Ltd, Kent, UK) via stainless steel wire (Goodfellow). Frequency-response curves to electrical field stimulation (EFS), based on the parameters used by Angus et al., were obtained over a range of 5–100 Hz (20 V, 0.2-msec pulse width) with 3-second pulse trains at 1-minute intervals. The neurogenic component of the responses to EFS was investigated after incubation of some arteries in tetrodotoxin (0.1 μM) for 20 minutes.

In all experiments, arteries were exposed to drug solutions for 10 minutes before each frequency-response curve. During this time they received a 2-minute exposure to 10 μM norepinephrine. The role of α₁-adrenergic receptors and P₂X-purinergic receptors in the vascular EFS response was investigated using prazosin (0.1 μM) and α₁β₂-methylene ATP (3 μM). Arteries from two rats of each strain, at both ages, were studied. In the major study, the effects of cocaine (3 μM), yohimbine (0.1 μM), and a combination of the two on multiple frequency-response curves were investigated on arteries from a further eight rats of each strain, at both ages. In experiments on all arteries, the order of drug application for consecutive frequency-response curves was considered. The statistical package GLIM was used to fit straight lines to the log of the 

\[ \Delta NE-pD₂ = NE-pD₂ (yohimbine) - NE-pD₂ (control) \]

where NE is norepinephrine. The response at each frequency of electrical stimulation was expressed as a percentage of the maximum elicited during the frequency-response curve. The mean response of two vessels was taken when both vessels were viable. The statistical package GLIM was used to fit straight lines to the log of the
percentage maximum response and the log of the frequency for each individual frequency–response curve, the $E_{50}$, then being calculated as $\exp(-a/b)$ where $a$ was the intercept and $b$ the slope of the fitted line. The $E_{50}$'s were subsequently analyzed by repeated-measures analysis of variance with age and strain being between-subject factors and drug treatment a within-subject factor. Multivariate test statistics were used to test effects of these factors. Significant drug effects were then investigated by testing specific contrasts between control and cocaine, control and yohimbine, cocaine and cocaine plus yohimbine, and yohimbine and cocaine plus yohimbine. Exact probability values are stated. Mann-Whitney $U$ analysis was used to compare the maximum response to EFS, expressed as a percentage of the response to exogenous norepinephrine, and to compare NE-pD$_2$ values.

$\alpha$, $\beta$, Methylene ATP, cocaine hydrochloride, (±)-norepinephrine hydrochloride, prazosin hydrochloride, (d,l)-propranolol hydrochloride, tetrodotoxin, and yohimbine hydrochloride were obtained from Sigma Chemical Co. Ltd., Dorset, UK.

**Results**

At 5 weeks of age, body weight and mean indirect systolic blood pressure were not significantly different in the SHR and WKY rats (Table 1). The mean normalized lumen diameter was slightly reduced in the resistance arteries from the SHR, but this did not attain statistical significance (Table 1). The effective active pressure induced by 10 $\mu$M norepinephrine occurred at yohimbine concentrations greater than 0.3 $\mu$M (Figure 1). There was no significant difference in NE-pD$_2$ in the absence or presence of yohimbine (0.1 $\mu$M) for arteries from 5-week-old SHR and WKY rats. Similarly, there was no significant difference in NE-pD$_2$ in the absence and presence of yohimbine for arteries from 12-week-old SHR and WKY rats. The sensitivity shifts produced by yohimbine ($\Delta$NE-pD$_2$) are shown in Table 2.

**Preliminary Action of Yohimbine**

Preliminary experiments indicated that antagonism of the contractile responses to exogenous norepinephrine occurred at yohimbine concentrations greater than 0.3 $\mu$M (Figure 1). There was no significant difference in NE-pD$_2$ in the absence or presence of yohimbine (0.1 $\mu$M) for arteries from 5-week-old SHR and WKY rats. Similarly, there was no significant difference in NE-pD$_2$ in the absence and presence of yohimbine for arteries from 12-week-old SHR and WKY rats. The sensitivity shifts produced by yohimbine ($\Delta$NE-pD$_2$) are shown in Table 2.

**Field Stimulation Studies**

The contractile response of a mesenteric artery to EFS, over a range of 5–100 Hz, is shown in Figure 2. The stimulation parameters used elicited highly consistent responses over the course of each experiment. Responses elicited over a range of 5–25 Hz were comparable to those obtained by Angus et al.¹⁶ Maximum responses to EFS occurred invariably at 60

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**Table 1. Characteristics of 5- and 12-week-old Spontaneously Hypertensive Rats and Wistar-Kyoto Rats and Isolated Mesenteric Arteries**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WKY (5 wk)</th>
<th>SHR (5 wk)</th>
<th>WKY (12 wk)</th>
<th>SHR (12 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>79.3±2.6</td>
<td>90.3±4.1</td>
<td>213.0±6.4</td>
<td>280.0±8.8*</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>105±4.9</td>
<td>102±5.6</td>
<td>127±5.6</td>
<td>158±1.1*</td>
</tr>
<tr>
<td>$l_0$ ($\mu$m)</td>
<td>218±9.2</td>
<td>198±5.6</td>
<td>257±9.2</td>
<td>242±7.6</td>
</tr>
<tr>
<td>$\Delta$P$_{NE}$ (kPa)</td>
<td>19.3±1.1</td>
<td>27.5±0.7†</td>
<td>21.9±1.9</td>
<td>33.4±2.1*</td>
</tr>
</tbody>
</table>

Values given are mean±SEM for $n$=8 rats unless shown otherwise. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. ANE-pD$_2$, shift in sensitivity caused by yohimbine; NE-pD$_2$, effective active pressure elicited by 10 $\mu$M exogenous norepinephrine.

*p<0.01, †p<0.001 indicate significantly different interstrain differences within age groups.

**Table 2. Norepinephrine Sensitivities of Isolated Arteries: Effect of Yohimbine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WKY (5 wk)</th>
<th>SHR (5 wk)</th>
<th>WKY (12 wk)</th>
<th>SHR (12 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE-pD$_2$</td>
<td>5.617±0.010</td>
<td>5.487±0.077</td>
<td>5.577±0.039</td>
<td>5.489±0.023</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>5.627±0.095</td>
<td>5.491±0.077</td>
<td>5.490±0.028</td>
<td>5.390±0.027</td>
</tr>
<tr>
<td>$\Delta$NE-pD$_2$</td>
<td>0.010±0.039</td>
<td>0.004±0.011</td>
<td>-0.087±0.020</td>
<td>-0.090±0.033</td>
</tr>
</tbody>
</table>

Values are mean±SEM, $n$=5 rats. Intrastrain norepinephrine sensitivities (NE-pD$_2$) at both ages were not significantly different in the absence or presence of yohimbine. $\Delta$NE-pD$_2$, shift in sensitivity caused by yohimbine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.
**TABLE 3. Maximum Electrical Field Stimulation Responses of Isolated Arteries: Effects of Cocaine, Yohimbine, and Cocaine and Yohimbine Combined**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WKY (5 wk)</th>
<th>SHR (5 wk)</th>
<th>WKY (12 wk)</th>
<th>SHR (12 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>47.4±4.1</td>
<td>47.3±3.1</td>
<td>47.3±2.4</td>
<td>56.4±3.0*</td>
</tr>
<tr>
<td>Cocaine</td>
<td>46.3±5.0</td>
<td>43.2±4.1</td>
<td>47.0±2.9</td>
<td>54.9±3.4</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>51.6±5.1</td>
<td>46.5±4.0</td>
<td>50.9±3.0</td>
<td>53.9±3.1</td>
</tr>
<tr>
<td>Cocaine and yohimbine</td>
<td>48.5±4.4</td>
<td>45.9±4.0</td>
<td>48.1±2.5</td>
<td>54.0±3.3</td>
</tr>
</tbody>
</table>

Responses (mean±SEM, n=8 rats) expressed as a percentage of the exogenous norepinephrine (10 μM) response. Cocaine, yohimbine, and the combination of the two had no significant effect on maximum responses to electrical field stimulation. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*p=0.04 indicates significant interstrain differences within age groups.

between age and strain (p=0.028) and drug treatment and strain (p=0.012). More detailed analysis of these effects are presented below.

**Basal Response to Electrical Field Stimulation**

The maximum responses to EFS, expressed as a percentage of the maximum norepinephrine response, of arteries from 5-week-old SHR, 5-week-old WKY rats, and 12-week-old WKY rats were not significantly different. However, arteries from 12-week-old SHR attained a significantly greater maximum response to field stimulation compared with arteries from 12-week-old WKY rats (p=0.04, Table 3).

Analysis of variance of the control EF_{50} values for SHR and WKY rat arteries at 5 and 12 weeks revealed significant differences with age (p=0.0008). The frequency required to produce the half-maximal response was reduced for 12-week-old arteries compared with 5-week-old arteries (Table 4), indicating an overall greater sensitivity to EFS in the older rats. An age–strain interaction (p=0.045) was also observed indicating a differing effect of age on EFS.
TABLE 4. Electrical Field Stimulation Sensitivity of Isolated Arteries Expressed as EFS. Effects of Cocaine, Yohimbine, and Cocaine and Yohimbine Combined

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WKY (5 wk)</th>
<th>SHR (5 wk)</th>
<th>WKY (12 wk)</th>
<th>SHR (12 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(23.27±1.48)</td>
<td>25.53±1.53</td>
<td>(21.17±1.01)</td>
<td>18.10±0.94</td>
</tr>
<tr>
<td>Cocaine</td>
<td>23.50±1.33</td>
<td>24.52±0.70</td>
<td>20.45±0.84</td>
<td>16.87±0.65</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>21.80±1.35</td>
<td>25.20±1.51</td>
<td>17.26±0.67</td>
<td>16.60±0.71</td>
</tr>
<tr>
<td>Cocaine and yohimbine</td>
<td>21.66±1.99</td>
<td>21.17±1.30</td>
<td>14.90±0.58</td>
<td>13.02±0.62</td>
</tr>
<tr>
<td>∆EFS&lt;sub&gt;o&lt;/sub&gt; (cocaine)</td>
<td>0.6%</td>
<td>16%</td>
<td>13.7%</td>
<td>21.6%</td>
</tr>
<tr>
<td>∆EFS&lt;sub&gt;o&lt;/sub&gt; (yohimbine)</td>
<td>7.8%</td>
<td>13.7%</td>
<td>21.7%</td>
<td>22.8%</td>
</tr>
</tbody>
</table>

EFS<sub>o</sub> values given as mean±SEM, n=8 rats. Statistical comparisons: (a) effect of age, p=0.0008; (b) age-strain interaction, p=0.045; (c) 5 weeks interstrain difference, not significant (NS); (d) 12 weeks, interstrain difference, NS. Overall effects of drugs: (e) cocaine, NS; (f) yohimbine, p=0.01; (g) cocaine in the presence of yohimbine, p=0.0001; (h) yohimbine in the presence of cocaine, p=0.0001. Interstrain differences in EFS<sub>o</sub> reduction caused by application of cocaine in the presence of yohimbine: (i) 5 weeks, p=0.02; (j) 12 weeks, NS; caused by application of yohimbine in the presence of cocaine: (k) 5 weeks, NS; (l) 12 weeks, NS.

Inhibition of Neuronal Amine Uptake Using Cocaine

The application of cocaine (3 μM) to block neuronal amine uptake, in the absence or presence of yohimbine, appeared to have no influence on the maximum force response attained by either strain at either age to EFS (Table 3).

Analysis of variance of EFS<sub>o</sub> values indicated that application of cocaine alone had no overall effect on the sensitivity of arteries to EFS (Table 4). However, after application of yohimbine to block presynaptic α<sub>2</sub>-adrenergic receptors, cocaine increased the overall sensitivity to EFS (p=0.0001) resulting in a reduction in EFS<sub>o</sub> values (Table 4) and a leftward shift in frequency–response curves (Figure 4). In addition, there was an interaction between drug treatment and strain at 5 weeks (p=0.02); the reduction in EFS<sub>o</sub> caused by cocaine was significantly greater for SHR arteries compared with those from WKY rats (SHR 16.0% and WKY rats 0.6%, Table 4). At 12 weeks, there was no significant difference in the reduction in EFS<sub>o</sub> between the strains (SHR 21.7% and WKY rats 13.7%, Table 4). Figure 4 shows these effects on the whole frequency–response curves for SHR and WKY rat arteries at both ages.

Inhibition of Presynaptic α<sub>2</sub>-Adrenergic Receptors With Yohimbine

The application of yohimbine (0.1 μM), alone or in the presence of cocaine, appeared to have no influence on the maximum force generated by either strain at either age (Table 3).

Analysis of variance indicated a significant overall effect (p=0.01) of yohimbine to increase the sensitivity to EFS and thus reduce EFS<sub>o</sub> values (Table 4). The reduction in EFS<sub>o</sub> values did not vary with age or strain. After pretreatment with cocaine to block amine uptake, the overall effect of yohimbine was to increase the sensitivity to EFS, thus reducing EFS<sub>o</sub> values, thus (Table 4, p=0.0001).

Again, the reduction in EFS<sub>o</sub> values was not significantly different between the ages or strains. Figure 5 shows the similar leftward shifts in frequency–
response curves produced by yohimbine in the presence of amine uptake blockade for SHR and WKY rat arteries at 5 and 12 weeks.

**Discussion**

This study investigated neuroeffector responses of resistance arteries from SHR and WKY rats using EFS in young (5-week-old) and mature (12-week-old) rats. The advantage of this technique is that arteries are stimulated by the electrically induced release of endogenous neurotransmitters from intramural nerve fibers. The neurogenic origin of the contractile response obtained to EFS was confirmed using tetrodotoxin. In addition to norepinephrine, further neurotransmitters may play a role in the vasoconstrictor responses elicited by EFS. Certainly, \( \alpha_1 \)-adrenergic receptor blockade incompletely inhibited the force response to EFS (Figure 2); the residual contraction was further slightly reduced by \( \alpha_2 \beta \)-methylene ATP suggesting the possible involvement of \( P_2 \)-purinergic receptors in the EFS response. Neuropeptide Y (NPY) is known to coexist with norepinephrine in a variety of species and vascular beds. Release of NPY may be dependent on the pattern and intensity of sympathetic stimulation. The residual contractile response to EFS in the present study may reflect release of NPY particularly at higher frequencies, although presently we are unable to confirm its role.

The timepoints chosen (5 weeks and 12 weeks) allowed vascular responses to be investigated in genetically hypertension-prone rats both before and after the high blood pressure had become established. As reported by previous authors, we found no interstrain difference between the sensitivities of SHR and WKY rat arteries to exogenous norepinephrine under conditions of functional neuronal uptake. However, analysis of frequency–response curves elicited by electrically stimulated release of endogenous neurotransmitters did reveal differences in sensitivities between the young and mature rats, and also within the strains as the rats matured. First, arteries from 12-week-old rats were more sensitive to EFS than arteries from 5-week-old animals, probably reflecting developmentally incomplete innervation in the immature rats. Of particular interest was the dramatic shift in the sensitivity of SHR arteries to EFS between 5 and 12 weeks of age,
Figure 5. Line graphs show frequency–response curves for spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats at 5 and 12 weeks in the absence (○) and presence (●) of yohimbine (0.1 μM). All responses are after prior inhibition of amine uptake. Yohimbine had a similar effect on SHR and WKY rat arteries at 5 and 12 weeks. EFS, electrical field stimulation.

indicated by the significant age-strain interaction. Figure 3 clearly shows this shift: at 5 weeks the SHR frequency–response curve was placed to the right of that for WKY rat arteries. By 12 weeks, arteries from both strains were more sensitive to EFS, but the SHR curve was now placed to the left of the WKY curve.

The present study investigated further the possible presynaptic mechanisms that may contribute to altered sensitivities to EFS with age and during the development and establishment phase of hypertension. It is known that neuronal amine uptake and presynaptic α2-adrenergic receptors regulate the net availability of neurotransmitter in the synapse in rat mesenteric arteries. We examined the function of these two systems using cocaine and yohimbine.

The selectivity of yohimbine for presynaptic α2-adrenergic receptors was investigated by comparing its effects on vasoconstrictor responses to exogenous norepinephrine and to electrically stimulated release of endogenous neurotransmitters. Yohimbine (0.1 μM) produced no significant shift in exogenous NE-pD2, whereas vasoconstrictor responses to EFS were significantly enhanced by yohimbine suggesting that the concentration used was selective for α2-adrenergic receptors, having little effect on postsynaptic α1-adrenergic receptors. Pharmacological studies have demonstrated that α1-adrenergic receptors predominantly mediate the vasoconstrictor responses to both exogenous and endogenous norepinephrine in this preparation. Furthermore, radioligand binding studies have demonstrated that the predominant type of postsynaptic α-adrenergic receptor in rat mesenteric arteries is the α1-subtype. Thus, antagonism of postsynaptic α2-adrenergic receptors is unlikely to complicate the neuroeffector response in these vessels. Although cocaine has a number of actions, the principle one appears to be blockade of neuronal amine reuptake.

Neuronal amine uptake and presynaptic α2-adrenergic receptor function are almost certainly not mutually exclusive processes. It is possible that an increase in synaptic norepinephrine concentration resulting from the inhibition of presynaptic α2-adrenergic receptors could initiate a subsequent increase in neuronal amine uptake to normalize synaptic norepinephrine concentrations. Conversely, the use of cocaine to inhibit the reuptake mechanism would increase synaptic norepinephrine concentration and facilitate autoinhibition of neurotransmitter release by activation of α2-receptors presynaptically. In the present study, the role of neuronal amine uptake was examined with cocaine alone and also after inhibition
of presynaptic α₂-adrenergic receptors. Similarly, α₂-adrenergic receptor function was investigated in the presence and absence of uptake inhibition.

A significant overall effect of cocaine to increase the sensitivity of arteries to EFS was only apparent after prior blockade of presynaptic α₂-adrenergic receptors, indicating the possible functional interaction between these mechanisms. In this respect, our data conflict directly with that recently published in which the neuronal uptake system appeared to play no role in the modulation of neurotransmission in rat mesenteric arteries, in either the absence or presence of α₂-adrenergic receptor blockade. The difference in findings may reflect the use of desipramine to inhibit neuronal amine uptake. Vascular inhibitory postsynaptic actions of desipramine are well documented: in our experience desipramine (0.1 μM) inhibits postsynaptic α-receptors in rat mesenteric arteries, which counteracts the enhancement of neuroeffector responses resulting from amine uptake blockade (unpublished observations from our laboratory).

Statistical analysis of the present data indicated that the effect of cocaine differed for the strains at the different ages. At 5 weeks, the increase in sensitivity caused by cocaine was significantly greater for SHR arteries than for WKY rat arteries: there was a reduction in EF₅₀ of 16.0% for SHR arteries compared with a negligible 0.6% for WKY rat arteries. This clearly indicates a more active amine uptake mechanism in the young SHR. However, at 12 weeks, although the increase in sensitivity for SHR arteries (EF₅₀ reduction, 21.6%) appeared to be greater than for WKY rat arteries (EF₅₀ reduction, 13.7%), the difference was not significant.

Application of yohimbine produced a significant overall increase in sensitivity to EFS both before and after blockade of neuronal amine uptake, although there were no differences in its effects with age or with strain. Therefore, these data provide no evidence to suggest impairment of presynaptic α₂-adrenergic receptor function, in either young or mature SHR. This would agree with the report by Nilsson and Sjöblom that the α₂-receptor antagonist idazoxan increased the sensitivity of isolated mesenteric arteries to EFS to a similar extent in adult SHR and normotensive Wistar rats. In contrast, Tsuda et al. provided evidence of impaired presynaptic α₂-adrenergic receptor function in the perfused mesentry of 7-8-week-old SHR compared with WKY rats, although this was not apparent for older rats. This study was performed in the absence of amine uptake blockade which, in view of the probable functional interaction between the two mechanisms, could contribute to the difference in findings.

The data presented demonstrate an interstrain difference with maturation between the enhancement of EFS sensitivity in arteries from SHR and normotensive WKY rats. Clearly, there is a greater increase in the EFS sensitivity of SHR arteries, compared with WKY rats, with age. At 5 weeks of age, the frequency–response curve for SHR arteries is placed to the right of that for WKY rat arteries; this may be a result of the overactive neuronal amine uptake in these vessels. However, by 12 weeks the sensitivity of SHR arteries has increased to the extent that the SHR curve is now placed to the left of the WKY curve. Evidence for an overactive amine uptake does not remain statistically significant at 12 weeks, and neuronal reuptake is obviously unable to downgrade sympathetic sensitivity as the SHR matures. In addition, our data suggest that impaired presynaptic α₂-adrenergic receptor function is unlikely to contribute to enhanced sensitivity to EFS as the SHR matures.

Therefore, the dramatic increase in the sensitivity to EFS in the SHR may reflect an enhanced fractional release of norepinephrine from sympathetic nerve fibers, as proposed by Galloway and Westfall, or a greater absolute release of norepinephrine, reflecting an enhanced innervation in the SHR. Measurement of tissue norepinephrine content, morphometric analysis of nerve fibers and histofluorescence techniques provide considerable evidence to suggest that there is increased innervation in mesenteric arteries from both young and mature SHR. In addition, our functional finding that the maximum response to EFS at 12 weeks of age is increased provides further corroborating evidence of an overactive sympathetic nervous system as the SHR ages.

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**References**


**KEY WORDS** • adrenergic receptors • neuronal uptake • spontaneously hypertensive rats • electric stimulation • vascular resistance • cocaine • yohimbine
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