Neuronally Released Norepinephrine Does Not Preferentially Activate Postjunctional $\alpha_1$-Adrenoceptors in Human Blood Vessels in Vitro

Maxine J. Stevens and Robert F.W. Moulds
From the University of Melbourne, Department of Medicine, The Royal Melbourne Hospital, Parkville, Victoria, Australia

SUMMARY. Human isolated digital arteries and metatarsal veins, obtained postmortem, have been used to compare the effects of $\alpha$-adrenoceptor antagonists on contractile responses to nerve stimulation. The antagonists used were prazosin, rauwolscine, BE 2254, and yohimbine. Rauwolscine ($\alpha_2$-antagonist), as well as prazosin and BE 2254 ($\alpha_1$-antagonists), in concentrations of $10^{-9}$, $10^{-8}$, and $10^{-7}$ mol/liter, potently antagonized the frequency-response curves to nerve stimulation. Yohimbine ($\alpha_2$-antagonist) was slightly less potent, failing to antagonize responses to nerve stimulation in arteries at the concentration of $10^{-9}$ mol/liter significantly, but producing potent antagonism of responses in both arteries and veins at higher concentrations ($10^{-8}$, $10^{-7}$ mol/liter).

All antagonists inhibited the contractile responses to nerve stimulation to a greater extent in veins than in arteries. Rauwolscine ($10^{-9}$, $10^{-8}$, and $10^{-7}$ mol/liter) and BE 2254 ($10^{-7}$ but not $10^{-8}$ mol/liter) significantly enhanced stimulation-induced tritium efflux in arteries and veins. These results suggest that $\alpha_1$-adrenoceptor antagonists, despite their prejunctional effects, are potent antagonists of contractile responses to nerve stimulation. Thus, in these human blood vessels, endogenously released norepinephrine does not preferentially activate postjunctional $\alpha_1$-adrenoceptors, but activates receptors with the properties of both the $\alpha_1$- and $\alpha_2$-adrenoceptor subtypes. (Circ Res 57: 399-405, 1985)

It has been suggested from in vivo studies in animals that vascular responses to endogenous and exogenous norepinephrine may be mediated by different $\alpha$-adrenoceptor subtypes (Yamaguchi and Kopin, 1980; Langer et al., 1980, 1981a, 1981b). Antagonists selective for the $\alpha_1$-adrenoceptor were found to be more effective against pressor responses to sympathetic nerve stimulation than responses to infused norepinephrine, whereas the opposite was found for $\alpha_2$-adrenoceptor antagonists. Thus, responses to neuronally released (endogenous) norepinephrine may be mediated predominantly by $\alpha_1$-adrenoceptors, located intrasynaptically, whereas responses to exogenously applied norepinephrine may be mediated predominantly by $\alpha_2$-adrenoceptors, located extrasynaptically. If this suggestion is also true in humans, it has obvious important implications in understanding the mechanism of action of drugs used clinically to influence the adrenergic system.

We have previously found that human isolated arteries and veins contain differing postjunctional $\alpha$-adrenoceptor populations (Stevens and Moulds, 1981). Although these differences could not be totally explained by varying proportions of $\alpha_1$- and $\alpha_2$- subtypes, it is possible that responses to endogenous norepinephrine in these tissues are mediated by different $\alpha$-adrenoceptor subtypes. We have investigated this possibility by assessing the effects of selective $\alpha$-adrenoceptor antagonists on neurotransmitter efflux and also on contractile responses produced by nerve stimulation.

**Methods**

**Preparation**

Blood vessels studied were dorsal metatarsal veins and palmar common digital arteries to the second and third fingers, obtained postmortem between 3 and 30 hours after death.

For postjunctional studies, spiral strips from these vessels were prepared and suspended in tissue baths according to previously described methods (Jauernig and Moulds, 1978). Parallel platinum wire electrodes, 13 mm long, were positioned on either side of the spiral strip, 3.5 mm apart. After an equilibration period of about 1 hour, the resting tension was adjusted to 1 g in the arteries and 0.3 g in the veins, which gives the maximum contractile responses in these tissues. A Grass S48 stimulator was used to apply rectangular wave pulses, 1 msec long. Isometric tension was measured with Grass FT03C or Hewlett-Packard FTA 100-1 transducers and Grass Polygraph 79D or Hewlett-Packard 7758A and 7702B recorders.

Prejunctional studies were performed with rauwolscine and BE 2254, according to the methods previously described for studying adrenergic transmitter release in human digital arteries (Rittinghausen and Moulds, 1980). In brief, cannulated segments were perfused for 30 minutes before being removed and incubated in [7,8-3H]norepinephrine hydrochloride for 60 minutes. The vessels then were remounted and perfused for another 60 minutes.
Perivascular field stimulation (1 msec in duration, 2 Hz, 60 V) was applied for 4 minutes at 30-minute intervals. Total tritium content in the perfusate and superfusate was measured by collecting 5-minute fractions (2.5 ml).

**Assessment of Drug Effects**

The antagonist effects of the 'selective' \( \alpha \)-adrenoceptor antagonists BE 2254 (a1), prazosin (a1), rauwolscine (a2), and yohimbine (a2) were tested against the contractile responses and tritium efflux produced by nerve stimulation.

**Postjunctional**

In all experiments, paired untreated and antagonist-treated spiral strip preparations from adjacent segments of an artery or vein were used. To compare responses to nerve stimulation and to exogenous norepinephrine and also to ensure optimal norepinephrine content of the nerves, we first established cumulative concentration-effect curves to norepinephrine for each spiral strip preparation and the maximum response to norepinephrine (Emax) was recorded. The minimum voltage required to produce the maximal response to electrical stimulation of each strip was then estimated by means of a "voltage-response" curve, at a set frequency of 4 Hz, in which the instrument voltage was increased in steps of 10 V until the maximum response was reached. A voltmeter was used to measure the applied voltage to this electrode system. Uncorrected instrument (stimulator) readings of 60 and 30 V corresponded to actual peak voltages of 12.5 and 6.2 V, respectively. Frequency-response curves (0.5-16 Hz), in which the instrument voltage was increased in steps of 10 V until the maximum response was reached, were established for each strip, using the voltage for stimulation previously established as being the minimum required to obtain the maximum response at 4 Hz. If the frequency required to produce a contraction equal to 50% of the contraction produced at 16 Hz was greater than 8 Hz, the vessel was considered to be insensitive, and discarded. After two frequency-response curves had been established (the second curve was taken as the control curve for that strip), they were repeated at 1-hour intervals in the presence of increasing concentrations of antagonist, and finally in the presence of tetrodotoxin (1 \( \mu \)g/ml). A contact time of 20 minutes was allowed for antagonists and 1 hour for tetrodotoxin. All responses were corrected by subtracting at each frequency any response obtained in the presence of tetrodotoxin. Antagonist-induced reductions of responses were corrected by taking into account any reductions of responses which occurred in the paired (non-antagonist-treated) strip.

For experiments examining the effect of propranolol on the inhibition of response produced by prazosin, four spiral strip preparations from adjacent segments of each vein (two with and two without propranolol) were used.

**Results**

**Responses of Arteries and Veins to Transmural Stimulation**

**General Observations**

Responses to transmural stimulation were obtained in 48 out of the 56 venous preparations and all of the 57 arterial preparations tested. Six of the arterial and six of the venous preparations which did respond to stimulation were discarded because they were too insensitive. An example of the response of a vein to norepinephrine and to transmural stimulation is shown in Figure 1.

The average minimum "apparent" voltage required to produce the maximal response to stimulation was 52 V in arteries and 42 V in veins.

**Effect of Tetrodotoxin**

Stimulation frequency-response curves obtained in eight veins and nine arteries before and after the addition of tetrodotoxin are shown in Figure 2. It can be seen that tetrodotoxin markedly inhibited
responses to stimulation in both arteries and veins. However, the inhibitory effect on responses was significantly greater in veins than in arteries. It is assumed that the tetrodotoxin-sensitive portion of

the response is due to nerve stimulation and the subsequent release of endogenous norepinephrine. Therefore, the tetrodotoxin-resistant responses were subtracted from responses to each stimulation frequency for each frequency-response curve.

**Relationship to Norepinephrine Response**

Mean frequency-response curves, corrected for tetrodotoxin-resistant responses, in which the force of the contractile response is represented as a percentage of norepinephrine $E_{\text{max}}$, are shown for arteries and veins (Fig. 3). The frequency response curve of the veins is positioned to the left of the curve for the arteries, and at each stimulation frequency, the response is significantly greater in veins than in arteries. When responses were not corrected for tetrodotoxin resistance, but were expressed as a percentage of the norepinephrine $E_{\text{max}}$, they were not significantly different in arteries and veins, at any stimulation frequency. Norepinephrine $E_{\text{max}}$ values were not significantly different in the arteries (3.23 ± 0.19 g; $n = 51$) and the veins (3.19 ± 0.22 g; $n = 42$).

**Control Responses**

The degree of tachyphylaxis obtained when four successive frequency-response curves were established in non-antagonist-treated segments of artery or vein is shown in Figure 4. Each response has been corrected for tetrodotoxin resistance and is represented as a percentage of the control response at 16 Hz.

**Effects of $\alpha$-Adrenoceptor Antagonists on Responses to Nerve Stimulation**

The effects of rauwolscine, prazosin, BE 2254, and yohimbine at concentrations of $10^{-9}$, $10^{-8}$, and $10^{-7}$ mol/liter were assessed on nerve stimulation.
FIGURE 5. Effect of rauwolscine (panel A), prazosin (panel B), yohim-bine (panel C), and BE 2254 (panel D) on nerve stimulation "frequency-response" curves in arteries and veins (n = 8), = control; O = 10^{-3} mol/liter; = 10^{-4} mol/liter; D = 10^{-7} mol/liter. Each point represents the mean contractile response, corrected for tetrodotoxin-resistance and calculated as a percentage of the control response at 16 Hz. Vertical bars indicate SEM.

FIGURE 6. Effect of a combination of prazosin and rauwolscine on nerve stimulation "frequency-response" curves in arteries (n = 3). Panel A: • = control; O = prazosin 10^{-5} mol/liter; = prazosin 10^{-7} mol/liter; D = prazosin 10^{-8} mol/liter + rauwolscine 10^{-4} mol/liter. Panel B: • = control; O = rauwolscine 10^{-5} mol/liter; = rauwolscine 10^{-7} mol/liter; D = rauwolscine 10^{-8} mol/liter + prazosin 10^{-8} mol/liter. Each point represents the mean contractile response, corrected for tetrodotoxin-resistance and calculated as a percentage of the control response at 16 Hz. Vertical bars indicate SEM.

frequency-response curves in both arteries and veins (Fig. 5). It can be seen that the frequency-response curves in the presence of increasing concentrations of these antagonists were typically flat, presumably because the frequency cannot be increased sufficiently to overcome the α-receptor blockade completely and achieve the maximum response.

Prazosin (10^{-5} mol/liter) produced significant shifts of the frequency response curves in both arteries and veins. However, subsequent increases in the concentration of prazosin produced very little further shift in the arteries. Rauwolscine and BE 2254 produced shifts of the frequency-response curves which were similar to those produced by prazosin. Although rauwolscine was slightly more effective than BE 2254 at the lowest concentration, the highest concentration of BE 2254 almost eliminated responses to stimulation in veins. At lower concentrations, yohimbine was less potent than rauwolscine, very little shift of the curve being produced by yohimbine (10^{-9} mol/liter) in arteries.

In a separate series of experiments, the residual responses to nerve stimulation in arteries in the presence of prazosin (10^{-7} mol/liter) were markedly reduced by the addition of rauwolscine (10^{-8} mol/liter) (Fig. 6A). Similarly, residual responses to nerve stimulation in the presence of rauwolscine (10^{-7} mol/liter) were markedly reduced by the addition of prazosin (10^{-8} mol/liter) (Fig. 6B).
TABLE 1
Percent Reduction of Contractile Response to Nerve Stimulation at 4 Hz by \(\alpha\)-Adrenoceptor Antagonists

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Concentration</th>
<th>Arteries</th>
<th>Veins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yohimbine</td>
<td>10(^{-9})</td>
<td>-3.7 ± 15.6</td>
<td>28.1 ± 5.3*</td>
</tr>
<tr>
<td></td>
<td>10(^{-8})</td>
<td>37.9 ± 7.6</td>
<td>59.3 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>10(^{-7})</td>
<td>73.1 ± 6.2</td>
<td>77.0 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>10(^{-6})</td>
<td>26.3 ± 11.6</td>
<td>49.3 ± 6.6*</td>
</tr>
<tr>
<td></td>
<td>10(^{-5})</td>
<td>51.8 ± 8.5</td>
<td>72.3 ± 4.7*</td>
</tr>
<tr>
<td></td>
<td>10(^{-7})</td>
<td>70.7 ± 8.8</td>
<td>77.3 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td>10(^{-6})</td>
<td>19.7 ± 12.3</td>
<td>48.7 ± 6.7*</td>
</tr>
<tr>
<td></td>
<td>10(^{-7})</td>
<td>36.3 ± 11.9</td>
<td>61.2 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>10(^{-8})</td>
<td>54.6 ± 7.5</td>
<td>72.4 ± 5.9*</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>BE 2254</td>
<td>10(^{-8})</td>
<td>14.7 ± 11.9</td>
<td>40.0 ± 1.8*</td>
</tr>
<tr>
<td></td>
<td>10(^{-9})</td>
<td>47.4 ± 9.7</td>
<td>86.6 ± 7.1*</td>
</tr>
<tr>
<td></td>
<td>10(^{-7})</td>
<td>61.0 ± 11.9</td>
<td>86.6 ± 3.8*</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM, and have been corrected for tetrodotoxin-resistant responses and tachyphylaxis (see text). Numbers in parentheses are number of experiments.

* Significant difference between arteries and veins (Mann-Whitney ranking test).

The reduction by the \(\alpha\)-adrenoceptor antagonists of contractile responses to nerve stimulation at 4 Hz was calculated as a percentage of the control (i.e., initial) response, corrected for tetrodotoxin-resistant responses and, also, for tachyphylaxis occurring in the control strip. The results are given in Table 1.

Prazosin, rauwolscine, and BE 2254 had similar effects on the response to nerve stimulation at 4 Hz (no significant differences between antagonists at each concentration) in both arteries and veins. Yohimbine (10\(^{-9}\) mol/liter) was less effective, especially in arteries. At the concentration of 10\(^{-9}\) mol/liter, all antagonists had a significantly greater effect in veins than in arteries.

To ensure that \(\beta\)-adrenoceptor-mediated relaxation was not contributing to the greater antagonism of contractile responses to nerve stimulation in veins than in arteries, the effect of propranolol on the inhibition produced by prazosin in veins was investigated in three further experiments. In these veins, the mean (±SEM) percentage inhibition by prazosin (10\(^{-3}\) mol/liter) of responses to nerve stimulation at 4 Hz was similar when experiments were carried out in the absence (47.4 ± 4.5%) or the presence (52.1 ± 7.2%) of 10\(^{-7}\) mol/liter propranolol (\(P > 0.2\), paired Student's \(t\)-test).

Contact times for the antagonists of more than 20 minutes did not produce any further blockade of responses, and repetition of the frequency-response curves in the same concentration of antagonist did not cause any further inhibition.

Prejunctional Effects of \(\alpha\)-Adrenoceptor Antagonists

The effects of prazosin and yohimbine on stimulation-induced tritium efflux (SIE) have been described previously (Stevens and Moulds, 1982). Rauwolscine (10\(^{-9}\), 10\(^{-8}\), and 10\(^{-7}\) mol/liter) significantly enhanced SIE in both arteries and veins, although the effect of the lowest concentration in arteries was not statistically significant. BE 2254 significantly enhanced SIE in arteries at 10\(^{-7}\) but not 10\(^{-8}\) mol/liter (Table 2). Neither antagonist altered the spontaneous tritium efflux.

**Discussion**

The results of this study indicate that neuronally released norepinephrine does not preferentially activate the \(\alpha_1\)-adrenoceptor subtype in the human digital artery or metatarsal vein. This is suggested...
by the ability of the \(\alpha_2\)-adrenoceptor antagonists; rauwolscine and yohimbine (Weitzeil et al., 1979), as well as the \(\alpha_1\)-adrenoceptor antagonists, prazosin (Cambridge et al., 1977) and BE 2254 (Gothert et al., 1981), to inhibit the contractile responses to nerve stimulation to similar extents.

Before any definite conclusions can be reached, however, the complicating factor of prejunctional \(\alpha\)-adrenoceptors must be considered. Unless single pulses or very low frequencies of nerve stimulation are used, the effects of \(\alpha\)-adrenoceptor antagonists on responses to sympathetic nerve stimulation may be complicated by enhancement of neurotransmitter release produced by blockade of inhibitory prejunctional \(\alpha\)-adrenoceptors (Langer, 1974; Starke, 1977). We have previously shown that yohimbine, but not prazosin, significantly enhances stimulation-induced tritium efflux in these vessels (Stevens and Moulds, 1982). The present study revealed that rauwolscine had an effect similar to that of yohimbine. The significant effect of BE 2254 is consistent with its reported high affinity to presynaptic \(\alpha\)-adrenoceptors in the rabbit pulmonary artery, despite its preferential activity at postsynaptic \(\alpha\)-adrenoceptors (Gothert et al., 1981).

It is of great importance, therefore, that the \(\alpha_2\)-adrenoceptor antagonists, rauwolscine and yohimbine, at concentrations which enhance neurotransmitter release, potently reduced contractile responses to nerve stimulation in these preparations. In particular, the finding that rauwolscine was as potent as prazosin in producing antagonism of postjunctional responses to nerve stimulation, despite the fact that prazosin has no prejunctional effects, indicates that rauwolscine may be having a greater effect than prazosin on the \(\alpha\)-adrenoceptors which mediate postjunctional responses to endogenously released norepinephrine. Thus, if anything, the effects of the \(\alpha_2\)-adrenoceptor antagonists on contractile responses of these preparations to nerve stimulation will be an underestimation of their antagonism at the postjunctional \(\alpha\)-adrenoceptors activated by transmitter norepinephrine.

The ability of rauwolscine to effectively antagonize prazosin-resistant responses to nerve stimulation, and vice versa, further demonstrates the involvement of both \(\alpha_1\)- and \(\alpha_2\)-adrenoceptor subtypes in responses to neuronally released norepinephrine in these vessels. The finding in this study that yohimbine and rauwolscine were as potent as, or even more potent than, prazosin when tested against contractile responses to nerve stimulation has also been found in other vascular preparations, e.g., rabbit isolated portal vein (Docherty and Starke, 1981; Starke and Docherty, 1982) and the vasculature of the anesthetized cat (Drew and Whiting, 1979). Other reports, however, have indicated that these antagonists do antagonize vasopressor responses to nerve stimulation, but are somewhat less effective than prazosin, and are often effective only at low stimulation frequencies, e.g., in the pithed rat and rabbit and the perfused hindlimb of the dog, cat and rabbit (Docherty and McGrath, 1980a, 1980b; Madjar et al., 1980; Hicks and Waldron 1981; Langer et al., 1981c; McGrath and McKean, 1981; Gardiner and Peters, 1982). The prejunctional effects of \(\alpha_2\)-adrenoceptor antagonists may not be responsible for their lesser effects in all cases, since rauwolscine was found to be less potent than prazosin against pressor responses to stimulation in the pithed rat when either trains of pulses, or single pulses, to preclude negative feedback, were used (Brown et al., 1981).

Apart from providing information about the postjunctional \(\alpha\)-adrenoceptor subtypes in human arteries and veins, this study has demonstrated some other important differences in the characteristics of these vessels. The responses to stimulation, when corrected for tetrodotoxin-resistance and expressed as a percentage of the norepinephrine \(E_{\text{max}}\), were significantly greater in veins than in arteries at each frequency, indicating a greater sensitivity of veins to nerve stimulation. This could be the result of a number of factors, including differences between the two vessels in the distribution of innervation, and, hence, neuromuscular gap width (Bevan, 1978, 1979), and differences in the sensitivity of the postjunctional \(\alpha\)-adrenoceptor population to norepinephrine. The latter would be consistent with the greater sensitivity of veins than arteries to exogenous norepinephrine (in the absence and presence of neuronal and extraneuronal uptake and \(\beta\)-receptor blockade) which we have previously reported (Stevens and Moulds, 1980, 1981), and also, with the observation that canine arteries and veins contain differing proportions of postjunctional \(\alpha_1\)- and \(\alpha_2\)-like adrenoceptor subtypes (DeMey and Vanhoutte, 1981).

Responses of veins to nerve stimulation were also more sensitive to the effects of \(\alpha\)-adrenoceptor antagonists. Since this was observed with both \(\alpha_1\) and \(\alpha_2\)-adrenoceptor antagonists, it is unlikely that it is due to varying proportions of \(\alpha_1\)- and \(\alpha_2\)-adrenoceptor subtypes in the two tissues. The failure of propranolol to reduce the inhibitory effect of prazosin on responses to nerve stimulation in veins also indicates that this difference is not due to activation of relaxant \(\beta\)-receptors by neurally released norepinephrine and subsequent overestimation of \(\alpha\)-adrenoceptor antagonist potency.

In these vascular preparations, prazosin does not reduce responses to the non-adrenergic constrictor stimuli barium chloride and 5-hydroxytryptamine (Jauernig et al., 1978). We are also not aware of any evidence to indicate that the selective \(\alpha\)-adrenoceptor antagonists, rauwolscine, yohimbine, and BE2254, reduce non-adrenergic constrictor stimuli.

The observation that the maximum response to nerve stimulation (16 Hz) in either arteries or veins was less than the norepinephrine \(E_{\text{max}}\) is probably a consequence of the limited concentration of norepinephrine that can be achieved, through diffusion, in the vessel wall (Bevan 1979).
Stevens and Moulds / Human Vascular α-Receptors

In conclusion, the responses of the human isolated digital artery and metatarsal vein to transmural nerve stimulation, in contrast to observations in some other vascular beds, are not mediated predominantly by the α₁-adrenoceptor subtype, but by a population of receptors which has the properties of both the α₁- and α₂-adrenoceptor subtypes.

References

Hicks PE, Waldron C (1981) Antagonism by α-adrenoceptor antagonists of the responses to 2(N,N-dimethyl)-amino, 6, 7-dihydroxy-1, 2, 3, 4 tetrahydroxypalene in the guinea-pig ileum and the pithed rat preparation. Br J Pharmacol 74: 254P-255P
Weitzell R, Tanaka T, Starke K (1979) Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. Naunyn Schmiedebergs Arch Pharmacol 308: 127-136

INDEX TERMS: Postjunctional α₁- and α₂-adrenoceptors • Prejunctional α₂-adrenoceptors • Human isolated arteries and veins • Neuronally released norepinephrine • α₁- and α₂-adrenoceptor antagonists
Neuronally released norepinephrine does not preferentially activate postjunctional alpha 1-adrenoceptors in human blood vessels in vitro.

M J Stevens and R F Moulds

Circ Res. 1985;57:399-405
doi: 10.1161/01.RES.57.3.399

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/57/3/399