Sympathetic Vasoconstriction Sensitive to a₂-Adrenergic Receptor Blockade

No Evidence for Preferential Innervation of c^⁻-Adrenergic Receptors in the Canine Femoral Bed

DIETMAR ELSNER, MAYTHEM SAEED, OLAF SOMMER, JÜRGEN HOLTZ, EBERHARD BASSENGE

SUMMARY In the canine femoral bed, we studied the involvement of vascular a₂-adrenergic receptors in vasoconstriction by stimulating the sympathetic nerve during different degrees of activation of metabolic counter-regulation (constant pressure and constant flow perfusion). In chloralose-anesthetized, despinalized dogs under ß-blockade (2 mg/kg nadolol) and under a constant femoral perfusion pressure, cumulative doses of rauwolscine (0.03, 0.3, and 3.0 mg/kg i.v., n = 8) or of prazosin (0.012, 0.12, and 1.2 mg/kg i.v., n = 8) caused dose-dependent shifts to the right of the dose-response curve of intraarterial norepinephrine (NE) infusions. These cumulative doses also caused attenuations of the vasoconstriction initiated by lumbar sympathetic stimulation (0.1, 0.3, 1.0, and 3.0 Hz). Sham treatment (n = 8) had no such results. In experiments with constant flow perfusion (n = 6 for each antagonist), rauwolscine shifted the NE dose-response curve significantly more compared to the experiments with constant pressure perfusion, while prazosin was similarly effective under both conditions. Under constant flow perfusion, both antagonists dose-dependently attenuated the vasoconstrictions caused by sympathetic stimulation. While prazosin and sham treatment did not modify the overflow of NE from the femoral bed during stimulation, this overflow was augmented by rauwolscine during stimulation at 3 Hz, which indicated presynaptic modulation of NE release. Under both experimental conditions, no significant difference could be observed in the attenuation of low-frequency sympathetic vasoconstriction by the two antagonists, when dosages were compared on the basis of their action against infused NE. It is concluded that both a a²-sensitive component and a prazosin-sensitive component are involved in the competition between sympathetic vasoconstriction and metabolic dilation. The vascular a-adrenergic receptors activated by these two components have a similar postsynaptic localization relative to the nerve endings.

(Hypertension 6: 915-925, 1984)

KEY WORDS prazosin rauwolscine constant flow perfusion constant pressure perfusion intrasynaptic a-adrenergic receptors

THERE is general agreement that more than one type of postsynaptic vascular a-adrenergic receptor exists in laboratory mammalian species and in humans. Norepinephrine (NE) induces vasoconstriction by activation of both a₁- and a₂-adrenergic receptors, which have been classified according to the effects of selective pharmacological agonists and antagonists. Recently, it has been shown that a₂-mediated vasoconstriction in vivo is more attenuated by calcium antagonists than vasoconstriction induced by a₁-activation. It was hypothesized that in antihypertensive therapy the calcium antagonists may selectively depress the contribution of a₂-adrenergic receptors to vascular tone. This would imply that a₂-adrenergic receptors are involved in blood pressure control. At the present time, however, the physiological role of these adrenergic receptors is not yet clear. It is still controversial whether the postsynaptic a₂-adrenergic receptors are activated by (NE) released from the sympathetic nerve endings, that is, whether they are "intrasynaptic" (innervated receptors) or "extrasynaptic" (humoral receptors). It is also unknown to what extent a₂-mediated constrictions are affected by mediators of metabolic vasodilation. So far, a₂-mediated
vasoconstriction in vivo has been documented only as an increase in perfusion pressure in preparations with constant (or roughly constant) flow, which thus reduces the physiological competition between adrenergic vasoconstriction and metabolic dilation.

Therefore, we analyzed whether both types of \( a \)-adrenergic receptors are involved in the flow reduction by sympathetic nerve stimulation under constant pressure perfusion. We compared the effect of the \( a_{1} \)-selective blocker prazosin and the \( a_{2} \)-selective blocker rauwolscine on vasoconstriction induced by NE infusion and by nerve stimulation. This was done in the canine femoral bed, in which both types of vascular receptors are believed to exist, as suggested by studies of the hemodynamic effects of selective drugs. Our experiments demonstrated a substantial role for innervated, vascular \( a_{2} \)-adrenergic receptors in the physiological competition between sympathetic vasoconstriction and metabolic dilation.

Methods

For the experiments we used 52 mongrel dogs of either sex that weighed 19 to 37 kg. We studied 42 of them while they were under chloralose anesthesia. We divided 24 randomly into three groups of eight dogs for constant pressure experiments, and divided 18 dogs into three groups of six dogs for constant flow experiments. The remaining 10 dogs (two groups of five dogs) were used in constant flow experiments under pentobarbital anesthesia.

Preparation of Dogs

Forty-two dogs were premedicated with 0.5 mg scopolamine subcutaneously (s.c.), with 0.01 mg/kg fentanyl intramuscularly (i.m.), and 0.5 mg/kg droperidol i.m. They were anesthetized intravenously (i.v.) with chloralose 80 mg/kg initially and then 7 mg/kg/hr, and in addition with 0.06 mg/kg/hr fentanyl i.v. during the preparation period. They were relaxed with pancuronium (0.2 mg/kg i.v. initially and then 0.1 mg/kg/hr i.v.). We chose chloralose anesthesia because it has less depressive effects on cardiovascular neural control than other anesthetic agents, and we used chloralose-borax instead of chloralose-urethane to avoid the effects of urethane on \( a_{2} \)-adrenergic receptors. Ten dogs did not receive any premedication and were anesthetized with pentobarbital, 34 mg/kg i.v. initially and then 8 mg/kg/hr i.v. during the preparation period. No muscle relaxant was given to these 10 dogs.

The animals were intubated and ventilated artificially with air; a positive end-expiratory pressure of 5 cm H\( _{2} \)O was maintained. Arterial blood gases and pH were measured repeatedly throughout the experiments and kept at 35 to 41 mm Hg (pCO\( _{2} \)), 80 to 92 mm Hg (pO\( _{2} \)), and 7.36 to 7.49 (pH) by adjusting the ventilation or by i.v. infusion of 8.4% of sodium bicarbonate, if necessary. Rectal temperature was maintained at 37.5\( ^{\circ} \)C to 38.5\( ^{\circ} \)C by a heating pad. The dogs were vagotomized, desphalinated at C-2, and given long-lasting, unselective \( \beta \)-blockade (2 mg/kg nadolol i.v.). Both jugular veins were cannulated, one for injection of drugs and one for continuous infusion of anesthesia. Catheters were inserted into both carotid arteries, one for monitoring blood pressure (BP) and heart rate, the other one for collecting blood samples or balancing systemic BP by appropriate withdrawal of blood during sympathetic stimulation or NE infusions. The left lumbar sympathetic chain was exposed through a left flank incision and decentralized. Postganglionic and intemodal fibers between L4 and L5 were placed on a bipolar electrode. The left femoral artery and vein were carefully exposed 2 to 3 cm below the inguinal ligament. The dogs received 500 U/kg and 250 U/kg/hr heparin i.v. The femoral vein was cannulated through a small side branch for collection of venous blood samples.

<table>
<thead>
<tr>
<th>TABLE 1. Protocols of the Constant Pressure Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (duration in min)</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>I  (100-120)</td>
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<td></td>
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<td></td>
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<td>II (70-90)</td>
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<td></td>
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<tr>
<td>III (110-130)</td>
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<td></td>
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<td></td>
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<tr>
<td>IV (110-130)</td>
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Experimental Protocols

Constant Pressure Perfusion

The perfusion experiments under constant pressure, which were performed in 24 dogs anesthetized with chloralose, are summarized in Table 1. In this preparation, a snugly fitting Statham blood flow transducer and, distally from it, an occlusion cuff were placed...
TABLE 2. Baseline Data from the Constant Pressure Experiments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A: chloralose + rauwolfscine (n = 8)</th>
<th>Group B: chloralose + prazosin (n = 8)</th>
<th>Group C: chloralose + sham (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>I 76±7</td>
<td>II 80±6</td>
<td>III 77±8</td>
</tr>
<tr>
<td></td>
<td>II 75±6</td>
<td>III 78±6</td>
<td>IV 67±7</td>
</tr>
<tr>
<td>Femoral blood flow (ml/min)</td>
<td>I 88±13</td>
<td>II 78±15</td>
<td>III 82±12</td>
</tr>
<tr>
<td></td>
<td>II 91±8</td>
<td>III 75±9</td>
<td>IV 77±11</td>
</tr>
<tr>
<td>Basal arterial plasma NE concentration (10^-9 M)</td>
<td>I 0.97±0.12</td>
<td>II 1.34±0.15</td>
<td>III 0.89±0.15</td>
</tr>
<tr>
<td></td>
<td>I 1.19±0.12</td>
<td>II 1.40±0.30</td>
<td>IV 0.92±0.10</td>
</tr>
<tr>
<td></td>
<td>III 1.01±0.24</td>
<td>IV 1.21±0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV 0.83±0.21</td>
<td></td>
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</tr>
</tbody>
</table>

round the femoral artery. Three subgroups of eight dogs each were formed, and experiments were carried out in four periods. Cumulative dosages of rauwolfscine (0.03-3.0 mg/kg i.v.) were given 15 minutes prior to Periods II, III, and IV in Group A dogs, and of prazosin (0.012-1.2 mg/kg i.v.) prior to Periods II, III, and IV in Group B dogs. In Group C dogs, sham treatment consisted of gradual volume withdrawal to induce a slightly more pronounced systemic hypotension compared with the other two groups.

In one experimental period, which lasted for 70 to 130 minutes, vasoconstrictions were induced by four trains of 3 minutes’ duration each, of lumbar sympathetic stimulations (0.1, 0.3, 1.0, and 3.0 Hz, 2 msec impulse duration, supramaximal voltage, Grass Sd-9 stimulator) and by four or five NE infusions (0.5 ml/min) into the femoral artery. For each dose of infused NE, the NE concentration in the femoral arterial plasma was calculated from the plasma flow during the steady-state phase of the stimulus (femoral blood flow = 100 - hematocrit/100) and from the NE infusion rate. Between consecutive vasoconstrictive stimuli, pauses of up to 15 minutes were observed to allow femoral blood flow and plasma NE levels to return to basal values. For assay of plasma NE by radioenzymatic technique, arterial blood samples were collected repeatedly prior to sympathetic stimulation and NE infusions. Plasma concentrations were always found to be below 3-10^-9 M (see Table 2) and therefore were neglected in calculating the arterial NE concentrations during infusions. During nerve stimulations or intraarterial (i.a.) NE infusions, mean arterial pressure (MAP) was kept constant by appropriate withdrawal of arterial blood through the carotid artery catheter. After termination of the constrictive stimulus, the withdrawn blood was reinfused. During Period II (for the lowest dose of blockers, see Table 1), the sympathetic stimulation was not applied, to shorten the duration of the complete experiment.

Constant Flow Perfusion

These experiments were performed in 18 dogs under chloralose anesthesia and in 10 dogs under pentobarbital anesthesia. The protocols are summarized in Table 3. The left femoral artery was cannulated and perfused by a roller pump with blood from the right femoral artery. The left hindlimb vascular bed was isolated from the systemic circulation by ligating the arteria femoralis profunda, the caudal artery, and the left internal iliac artery. A drop in perfusion pressure to below 20 mm Hg during a temporary stop in perfusion was taken as an indication of sufficient vascular isolation. The perfusion rate to the hindlimb was set to maintain a perfusion pressure of 90 to 100 mm Hg throughout the experiment. Vasoconstrictions were quantified as rises in perfusion pressure during the steady-state phase of the stimulus. Stimuli were applied as described for the experiments with constant pressure perfusion.

In addition to determining basal arterial plasma NE levels (see Table 4), we analyzed the NE release from the femoral bed during nerve stimulation in Groups D, E, and F. Femoral venous and arterial blood samples (2 ml each) were collected prior to and during the 3rd minute of stimulation at 0.1 and 3.0 Hz.

Calculations and Drugs Used

Mean values ± SEM are presented. For comparisons within a group, analysis of variance (ANOVA) for repeated measurements was performed. The following drugs were used (dosages refer to the base of the salts): scopolamine bromide (Eifelfango, Bad Neu-
TABLE 4. Baseline Data from the Constant Flow Experiments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period</th>
<th>Group D: chloralose + rauwolscine (n = 6)</th>
<th>Group E: chloralose + prazosin (n = 6)</th>
<th>Group F: chloralose + sham (n = 6)</th>
<th>Group G: pentobarbital + rauwolscine (n = 5)</th>
<th>Group H: pentobarbital + prazosin (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>I</td>
<td>98 ± 8</td>
<td>95 ± 6</td>
<td>94 ± 7</td>
<td>89 ± 8</td>
<td>95 ± 3</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>90 ± 5</td>
<td>78 ± 5</td>
<td>64 ± 6</td>
<td>74 ± 5</td>
<td>92 ± 7</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>75 ± 9</td>
<td>79 ± 5</td>
<td>50 ± 8</td>
<td>63 ± 4</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Femoral blood flow (ml/min)</td>
<td>I</td>
<td>97 ± 6</td>
<td>116 ± 20</td>
<td>117 ± 12</td>
<td>154 ± 8</td>
<td>139 ± 14</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>95 ± 6</td>
<td>114 ± 21</td>
<td>122 ± 10</td>
<td>151 ± 9</td>
<td>163 ± 7</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>91 ± 10</td>
<td>116 ± 20</td>
<td>112 ± 15</td>
<td>142 ± 11</td>
<td>163 ± 8</td>
</tr>
<tr>
<td>Basal arterial plasma NE</td>
<td>I</td>
<td>1.09 ± 0.17</td>
<td>1.06 ± 0.21</td>
<td>1.21 ± 0.19</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>concentration (nM)</td>
<td>II</td>
<td>1.32 ± 0.17</td>
<td>1.34 ± 0.26</td>
<td>0.98 ± 0.22</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1.38 ± 0.24</td>
<td>1.69 ± 0.34</td>
<td>1.29 ± 0.30</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Since the dogs in the different groups were not matched for body weight, the femoral blood flow under control conditions differed among groups. In Groups G and H, plasma norepinephrine (NE) was not measured.
Period I caused a decline in flow from 39% ± 5% at 0.1 Hz to 67% ± 3% at 3 Hz in Group A, from 41% ± 5% to 71% ± 5% in Group B, and from 42% ± 5% to 75% ± 4% in Group C, respectively (Figure 3). In Groups A and B, α2- and α1-blockade, respectively, caused significant attenuations of these constrictions (Figure 3). In the sham-treated dogs of Group C, the small attenuation of the constrictive responses during Periods III and IV did not reach a level of significance. In four dogs of Group B, after completion of the experiments in Period IV (with 1.2 mg/kg prazosin), 0.3 mg/kg i.v. rauwolscine was added. Thereafter, the constrictions by nerve stimulation were attenuated further, but not abolished. The reduction in flow in these four dogs after prazosin and rauwolscine administration were 21% ± 4% at 0.3 Hz, 22% ± 7% at 1.0 Hz, and 40% ± 10% at 3.0 Hz.

Constant Flow Perfusion

The baseline data obtained during the three experimental periods in Groups D-H are shown in Table 4. Again, both rauwolscine and prazosin caused systemic hypotension, but no significant alterations in basal arterial NE concentrations (Groups D and E). In the sham-treated dogs of Group F, the systemic hypotension induced by volume withdrawal was again slightly more pronounced than in the other groups (Table 4). The dose-response curves of the normalized increase in perfusion pressure during the steady-state phase of the NE infusions demonstrated an approximately parallel shift to the right caused by rauwolscine and by prazosin, but not by sham treatment (Figure 4). The EC50 in these experiments under constant flow perfusion is the arterial plasma NE concentration that caused an increase in the perfusion pressure of 50% of the value induced by 5·10−8 M NE in the control Period I of the individual animal. The dose of 5·10−6 M was...
arbitrarily chosen, since this dose caused maximal effects well within the plateau of the dose-response plot in all experiments under constant pressure perfusion (Figure 2). Both rauwolscine and prazosin caused a significant, dose-dependent decline of the negative logarithm of this EC\(^{-5}\) under chloralose anesthesia, as well as under pentobarbital anesthesia (Table 6).

The increases in perfusion pressure during the steady-state phase of nerve stimulation were significantly attenuated by both \(\alpha\)-blockers, but not by sham treatment (Figure 5). However, in dogs under pentobarbital anesthesia and under the lower dose of rauwolscine, the vasoconstrictions induced by stimulations at higher frequencies were not attenuated (Figure 5). In dogs under chloralose anesthesia, a stimulation-induced NE release from the femoral bed could not be demonstrated at 0.1 Hz, but it could be at 3.0 Hz (Figure 6). This increase was significantly augmented by rauwolscine, but it was not affected by prazosin or by sham treatment.

![FIGURE 4. Dose-response curves for norepinephrine (NE) infusions into the femoral artery in constant flow experiments in Groups D-F. Abscissa: calculated arterial plasma NE concentration. Ordinate: increase in femoral perfusion pressure, expressed as a percentage of the increase induced by \(5 \times 10^{-6}\) M NE during control Period I in the individual animal; 100% = 157 ± 19 mm Hg in Group D, 164 ± 23 mm Hg in Group E, and 144 ± 18 mm Hg in Group F. Data are from Period I (control, black symbols) and Period II (alpha-blocker, open symbols) for each group, demonstrating the roughly parallel displacement by both \(\alpha\)-blockers.](http://hyper.ahajournals.org/)

![FIGURE 3. Vasoconstrictions induced by lumbar sympathetic stimulation in constant pressure experiments in Groups A-C. Asterisks indicate significance of difference between flow reduction during control period (black symbols) and after \(\alpha\)-blockers (open symbols). (*\(p < 0.05\); **\(p < 0.01\); ***\(p < 0.001\).)](http://hyper.ahajournals.org/)

**TABLE 5. Constant Pressure Experiments: Effect of Alpha-Blockade on Norepinephrine-Induced Vasoconstriction**

<table>
<thead>
<tr>
<th>Period</th>
<th>Group A: chloralose + rauwolscine (n = 8)</th>
<th>Group B: chloralose + prazosin (n = 8)</th>
<th>Group C: chloralose + sham (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.37 ± 0.19</td>
<td>7.27 ± 0.11</td>
<td>7.31 ± 0.09</td>
</tr>
<tr>
<td>II</td>
<td>7.06 ± 0.05t</td>
<td>6.92 ± 0.07*</td>
<td>7.37 ± 0.08</td>
</tr>
<tr>
<td>III</td>
<td>6.47 ± 0.06t*</td>
<td>6.55 ± 0.10*H</td>
<td>7.14 ± 0.12</td>
</tr>
<tr>
<td>IV</td>
<td>5.51 ± 0.08*H</td>
<td>6.02 ± 0.18tH</td>
<td>7.18 ± 0.12</td>
</tr>
</tbody>
</table>

**VALUES ARE EXPRESSED AS - log EC\(^{-5}\) (NEGATIVE LOGARITHM OF THE INTRATUNIC NOREPINEPHRINE CONCENTRATION CAUSING A 50% REDUCTION IN FEMORAL BLOOD FLOW).**

Significance of differences from control (Period I): *\(p < 0.05\); t\(p < 0.01\); Xp < 0.001.

Significance of differences from preceding period: §\(p < 0.05\); \(\|p < 0.01\); \(||p < 0.001\).
TABLE 6. Constant Flow Experiments: Effect of Alpha-Blockade on Norepinephrine-Induced Vasoconstrictions

<table>
<thead>
<tr>
<th>Period</th>
<th>Group D: chloralose + rauwolscine (n = 6)</th>
<th>Group E: chloralose + prazosin (n = 6)</th>
<th>Group F: chloralose + sham (n = 6)</th>
<th>Group G: pentobarbital + rauwolscine (n = 5)</th>
<th>Group H: pentobarbital + prazosin (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.09±0.07</td>
<td>6.88±0.08</td>
<td>7.01±0.10</td>
<td>6.94±0.10</td>
<td>6.60±0.06</td>
</tr>
<tr>
<td>II</td>
<td>6.41±0.10$</td>
<td>6.39±0.09*</td>
<td>7.01±0.12</td>
<td>6.49±0.15t</td>
<td>6.37±0.07t</td>
</tr>
<tr>
<td>III</td>
<td>5.61±0.12t</td>
<td></td>
<td></td>
<td>6.19±0.12t</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as $-\log EC^5$ (negative logarithm of the intraarterial norepinephrine concentration causing 50% of the maximal increase in perfusion pressure).

Significance of differences from control (Period I): *p < 0.05; **p < 0.01; ***p < 0.001.
Significance of differences from preceding period: §p < 0.05; Up < 0.01; \p < 0.001.

FIGURE 5. Vasoconstrictions induced by lumbar sympathetic stimulation in constant flow experiments in Groups D—H. Ordinate: increase in femoral perfusion pressure expressed as a percentage of the increase induced by stimulation at 3 Hz in control Period I (black symbols) in the individual animal; 100% = 99 ± 19 mmHg in Group D, 92 ± 15 mmHg in Group E, 98 ± 15 mmHg in Group F, 108 ± 12 mmHg in Group G, and 98 ± 15 mmHg in Group H. Doses of a-blockers are given in mg/kg (open symbols). Asterisks indicate significance of difference between increase in perfusion pressure during control Period I and after a-blockers. *p < 0.05; **p < 0.01; ***p < 0.001.

FIGURE 6. Norepinephrine release (NE) from the femoral bed (plasma flow x venous-arterial difference of NE concentration) during the steady-state phase of sympathetic stimulation in constant flow experiments in Groups D-F. Asterisks indicate significance of difference between NE release during the control period (black symbols) and after a-blockers (*p < 0.05; ***p < 0.001). Basal NE release prior to stimulation was 6 ± 2.1 ± 2, and 3 ± 4 ng/min during the control period and after 0.03 and 0.3 mg/kg rauwolscine, respectively, in Group D; 2 ± 4.0 ± 2, and 6 ± 5 ng/min during the control period and after 0.012 and 0.12 mg/kg prazosin, respectively, in Group E; 3 ± 2.1 ± 3 and 0 ± 3 ng/min during the control period and Periods I and III (sham treatment), respectively, in Group F.
Discussion

In all experiments with constant flow perfusion and with constant pressure perfusion, the dose-response curves for circulating NE were significantly shifted to the right by \(a_2\)- and by \(\alpha\text{-blockade (Tables 5 and 6)}, \) respectively, which indicated that circulating NE acts on two types of vascular \(\alpha\text{-adrenergic receptors. This is in agreement with conclusions drawn from other studies of this bed}^{20,21}\text{ and of other preparations.}^{11}

In experiments with chloralose anesthesia, \(\alpha\) blockade had a similar effect on NE-induced constrictions under constant flow and constant pressure conditions. The two doses of prazosin (0.012 and 0.12 mg/kg) studied under both conditions caused 2.24-fold and 5.25-fold elevations of EC\(_{50}\) in the constant pressure experiments (see the differences in Table 5) and 3.09- and 4.9-fold elevations in the constant flow experiments (Table 6). Alpha\(a_2\)-blockade, however, had a stronger effect on NE-induced constrictions under constant flow conditions (4.8- and 30.2-fold elevations of EC\(_{50}\) by 0.03 and 0.3 mg/kg rauwolscine, Table 6) than on constrictions under constant pressure conditions (2.0- and 7.9-fold elevations by these dosages, Table 5). These differences in the shift of the EC\(_{50}\) values between the two conditions were significant \((p < 0.05 \text{ with 0.03 mg/kg rauwolscine and } p < 0.01 \text{ with 0.3 mg/kg). It should be stressed, however, that a 50% reduction in flow under constant pressure and a 50% maximal increase in perfusion pressure under constant flow do not indicate an identical degree of vasoconstriction. Therefore, absolute EC\(_{50}\) values cannot be comparable under the two conditions.}

The weaker action of \(a_2\)-blockade on NE-induced constriction under constant pressure conditions might indicate that the \(a_2\)-mediated component of this constriction plays a minor role, when metabolic counter-regulation is activated by the constriction-induced decline in flow. In the pithed rat, pressor responses to some \(\alpha_2\)-selective agonists are strongly inhibited by acidosis.\(^9\) However, there is no uniform effect of acidosis on constrictions induced by different \(\alpha_2\)-agonists.\(^9,20\) Therefore, conclusions concerning the effect of acidosis on the \(a_2\)-mediated action of NE are not possible. Adenosine (probably one of the metabolites that accumulates during reductions in flow) induces an increase in \(\alpha_2\)-agonist-binding sites in the rat vas deferens.\(^21\) If this occurs in vascular tissue, it would mean that the \(a_2\)-agonist-binding component of the vasoconstriction should predominate during activated metabolic counterregulation. Our results suggest that such an induction is not important during the vasoconstrictions that lasted 3 minutes in our model. At present, we do not know the mechanism underlying the preferential attenuation of the rauwolscine effect, which was brought about by activated metabolic counterregulation. Yet, in spite of this attenuation, our results clearly show that the flow reduction by circulating NE was partially mediated by an arteriolar constriction sensitive to \(a_2\)-blockade.

The effects of \(a_2\)-blockade on vasoconstrictions induced by sympathetic stimulation are more difficult to understand because of the possible presynaptic effects of this blockade.\(^22\) Probably, the presynaptic \(a_2\)-mediated autoinhibition of the NE release induced by the action potential is operative under physiologic conditions.\(^24,25\) Thus, \(a_2\)-blockade should augment the stimulation-induced transmitter release, thereby partially counteracting the competitive postsynaptic \(a_2\)-blockade. This presynaptic autoinhibition plays a greater role under higher frequencies of stimulation\(^26\) and requires a certain amount of time to be activated.\(^27\) The significant augmentation of the NE overflow from the femoral bed by rauwolscine during stimulation at 3.0 Hz (Figure 6) strongly suggests that the autoinhibition is activated within the 3-minute stimulation periods at higher frequencies in our experiments. Thus, our experiments with rauwolscine underestimate the contribution of postsynaptic \(a_2\)-activation to the stimulation-induced vasoconstriction.

Nevertheless, femoral vasoconstriction by nerve stimulation under both conditions was sensitive to \(a_2\)-blockade (Figures 3 and 5). Unspecific attenuation of responsiveness due to the deterioration of the preparation could not have caused the reduced vasoconstrictions following rauwolscine, since this reduction did not occur in the sham-treated dogs with an even more pronounced hypotension. Under chloralose anesthesia, the attenuation by rauwolscine was slightly less in the presence of metabolic counterregulation as compared to the constant flow condition, but this difference was not significant. Prazosin at the highest dose applied (1.2 mg/kg, Group B) did not completely abolish the flow reductions during nerve stimulation (Figure 3), but these prazosin-resistant flow reductions were further attenuated by 0.3 mg/kg rauwolscine. Similar additive effects of \(a_2\) and \(a_2\)-blockade on stimulation-induced pressure increases have been demonstrated in the canine femoral bed\(^27\) and in pithed rats and rabbits.\(^28\) These data suggest a contribution of vascular \(a_2\)-adrenergic receptors to sympathetic vasoconstriction both under conditions of constant flow as well as of activated metabolic counterregulation.

The contribution of vascular \(a_2\)-adrenergic receptors to sympathetic flow reduction does not exclude a "preferential innervation of \(\alpha_2\text{-adrenoceptors.}^{"\text{ To study this question, we analyzed in greater detail the effects of the two }a\text{-blockers on small vasoconstrictions. This was done for two reasons: 1) the disturbing presynaptic effects of }\alpha_2\text{-blockade should be minimal at the lowest frequency of stimulation; 2) if the vascular }\alpha_2\text{-adrenergic receptors were situated more closely to the nerve endings (intrasynaptically) than the }\alpha_2\text{-adrenergic receptors, then a lower synaptic transmitter concentration during low frequencies of stimulation should lead to less "spill over" of the transmitter to (and, thus, activation of) the more remote }\alpha_2\text{-adrenergic receptors than would a high-frequency NE release.}

Therefore, from the individual NE dose-response plot in each dog, we determined the intraarterial NE concentration that caused a flow reduction identical to that induced by nerve stimulation at 0.1 Hz in the same dog. The effects of the \(a\)-blockers on the flow reductions induced by these two equivalent stimuli are de-
picted in Figure 7. The vasoconstriction by nerve stimulation was not more attenuated by prazosin than by rauwolscine at doses that acted identically against the equivalent constrictions induced by circulating NE. Thus, these experiments on adrenergic flow reduction cannot support the hypothesis of innervated \( \alpha \)-adrenergic receptors and noninnervated, humoral \( \alpha \)-adrenergic receptors, which had been proposed (among others) on the basis of constant flow experiments in dogs under pentobarbital anesthesia.\(^9\)

Differences in the type of anesthesia might be relevant for this issue, if neuronal reuptake of catecholamines is affected by the anesthetics. In rodents, inhibition of neuronal reuptake was observed with droperidol\(^28\) and pancuronium,\(^29\) both applied in our dogs during chloralose anesthesia (although in lower doses). Inhibition of neuronal reuptake exposes the intrasynaptic vascular \( \alpha \)-adrenergic receptors more to the action of circulating NE (which is removed effectively from such innervated receptors by this uptake) and exposes the noninnervated, extrasympathetic vascular \( \alpha \)-adrenergic receptors more to the action of transmitter NE.\(^30\) Therefore, we performed experiments with constant-flow conditions under chloralose anesthesia and under the pentobarbital anesthesia used by Langer et al.\(^*\)

If neuronal reuptake inhibition should have falsely indicated an intrasynaptic localization of vascular \( \alpha \)-adrenergic receptors in our experiments under chloralose, then we should expect under pentobarbital (provided that neuronal reuptake is not inhibited) an identical effect of rauwolscine against circulating NE, and we should expect no effect, or a smaller one, with prazosin. This was not observed (Table 6). Pentobarbital attenuated the effect of rauwolscine against circulating NE. The NE dose-response curve was shifted by a factor of 4.8 and of 30.2, respectively, by 0.03 and by 0.3 mg/kg of rauwolscine under chloralose (Group D), but only by a factor of 2.8 and of 6.7, respectively, under pentobarbital (Group G); the difference was significant \((p < 0.05)\) for the higher dose. No significant differences in the shift of the NE dose-response curve induced by 0.012 and by 0.12 mg/kg prazosin were observed under both types of anesthesia: the factors were 3.09 and 4.9 under chloralose and 1.7 and 2.8 under pentobarbital (Table 6). Under both types of anesthesia, however, the lowest dose of prazosin (0.012 mg/kg) significantly affected the dose-response curve (Table 6).

In the study by Langer et al., a similar dose (0.01 mg/kg) also attenuated the duration of vasoconstrictions induced by i.v. bolus injections of NE, but these authors based their conclusions on peak effects, which were not attenuated in all cases. When we compared the effects of the lowest doses of the two \( \alpha \)-blockers on the vasoconstrictions induced by sympathetic stimulation at 0.1 Hz during constant flow perfusion (Figure 8), we did not find any stronger action of prazosin under both types of anesthesia nor did Langer et al.\(" in their experiments.

Thus, the differing conclusions in these two studies are more due to interpretations than to differences in data. Both studies agree to the role of vascular \( \alpha \)-adrenergic receptors in constrictions by blood-borne NE. Langer et al. concluded from indirect interpretation that a presynaptic effect of rauwolscine did not exist in their experiments. Consequently, they analyzed the strong vasoconstrictions and observed (as we did) more attenuation by prazosin than by rauwolscine. We assume a strong presynaptic effect of rauwolscine (Figure 6) and, therefore, preferentially analyzed the small constriction induced by low frequency stimulation. Although we did not completely analyze all of the
differences observed between the two types of anesthesia (attenuation of rauwolscine effects both against circulating and against released NE under pentobarbital in our series), we reached the same conclusion under both anesthetics. By using the action against circulating NE during the steady-state constrictions as the basis for comparison of the a-blockers (see Figure 7), we observed no preferential effect of prazosin against vasoconstriction by stimulation at low frequency.

In experiments in vivo, the identification of subtypes of a-adrenergic receptors must necessarily be indirect and depend on the selectivity and specificity of the drugs used. With this reservation in mind, we can conclude that both a prazosin-sensitive component and a rauwolscine-sensitive component are involved in the physiological competition between sympathetic constriction and metabolic dilation. The same seems to be true for vasoconstriction in the pitiled rat, in which a preferential a2-innervation had been proposed previously, although there have been disagreeing views.30–31 This conclusion does not exclude the possibility that different types of ganglionic transmission might act on different vascular a-adrenergic receptors.30–32 that circulating epinephrine might act preferentially on a2-adrenergic receptors due to differences in affinity,33 and that the adrenergic receptors identified by prazosin and rauwolscine can both be subdivided further.

According to the hypothesis by Van Meel et al.,3 the antihypertensive effect of calcium antagonists results, at least in part, from their preferential attenuation of a2-mediated vasoconstriction. In agreement with this hypothesis is the recent conclusion30 that a prazosin-resistant, rauwolscine-sensitive component of sympathetic vasoconstriction in the canine femoral bed is more attenuated by nifedipine than the rauwolscine-resistant, prazosin-sensitive component.

Our study has demonstrated in the same preparation that innervated, rauwolscine-sensitive a-adrenergic receptors are involved in vasoconstriction by experimental nerve stimulation. This involvement is a prerequisite for (but not proof of) an involvement of vascular a2-adrenergic receptors in moment-to-moment BP control and for their role in the antihypertensive effect of calcium antagonists.

Acknowledgments

We are indebted to Helmut Siegel, Gisela Kühne, Rosi Henn, and Margot Olsen for careful technical assistance. Drugs were generously supplied by Von Heyden, Munich (nadolol) and by Pfizer, Karlsruhe (prazosin). Mathem Saeed was a DAAD-scholar from Mosul University, Mosul, Iraq.

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Sympathetic vasoconstriction sensitive to alpha 2-adrenergic receptor blockade. No evidence for preferential innervation of alpha 1-adrenergic receptors in the canine femoral bed.

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_Hypertension_. 1984;6:915-925
doi: 10.1161/01.HYP.6.6.915

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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