The subtypes of postjunctional α-adrenoceptors activated by neuronally released and exogenous norepinephrine and the source of calcium used for vasoconstrictor responses were investigated in the feline mesenteric vascular bed. Under constant flow conditions, intra-arterial injections of phenylephrine and UK14304, α₁ and α₂-adrenoceptor agonists, increased mesenteric arterial perfusion pressure in a dose-related manner. Prazosin, an α₁-antagonist, reduced vasoconstrictor responses to phenylephrine without altering responses to UK14304. Yohimbine, an α₂-antagonist, reduced responses to UK14304 without altering responses to phenylephrine. The same pattern of blockade was observed in animals pretreated with 6-hydroxydopamine to destroy the integrity of adrenergic terminals. Responses to phenylephrine and UK14304 were reduced by nitrendipine, a calcium-entry blocking agent, and this agent decreased vasoconstrictor responses to sympathetic nerve stimulation by phenylephrine and norepinephrine. Responses to sympathetic nerve stimulation were selectively blocked by prazosin, but responses to norepinephrine were selectively blocked by yohimbine. Vasoconstrictor responses to tyramine were reduced by both prazosin and yohimbine. Nitrendipine also reduced responses to angiotensin II, U46619, a prostaglandin endoperoxide analogue, Bay K 8644, and potassium chloride. These data suggest the presence of α₁- and postjunctional α₂-adrenoceptors and support the hypothesis that norepinephrine released by nerve excitation acts mainly on α₁-receptors but that exogenous norepinephrine acts primarily on α₂-receptors. However, norepinephrine released by tyramine acts on both receptor subtypes. Nitrendipine inhibited responses to the α₁- and α₂-adrenoceptor agonists as well as those to nerve released and exogenous norepinephrine, the calcium agonist, Bay K 8644, and to other vasoconstrictor agents. These data suggest that in the feline mesenteric vascular bed, an extracellular source of calcium ions is required for vasoconstriction induced by a variety of mechanisms including activation of α₁- and postjunctional α₂-adrenoceptors.

(Circulation Research 1987;61:570-580)
Lippton et al  Blockade of Vasoconstrictor Responses

Materials and Methods

Cats of either sex (2.2 ± 0.1 kg, n = 102) were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Supplemental doses of anesthetic were given as needed to maintain a uniform level of anesthesia. The trachea was cannulated to ensure a patent airway, and catheters were inserted in the femoral artery for the measurement of systemic arterial pressure and in the external jugular vein for intravenous administration of drugs. For perfusion of the small intestine, an extracorporeal circuit was established so that the intestine could be perfused in situ under conditions of controlled blood flow. Briefly, the superior mesenteric artery was approached through a midline abdominal incision and cleared of surrounding connective tissue. After administration of heparin (1,000 U/kg i.v.), a carotid artery was cannulated and connected to the inlet side of the perfusion circuit. The outlet side of the perfusion circuit was connected to the cannulated superior mesenteric artery, and blood flow to the small intestine was maintained constant with a Sigmamotor pump (model T-8, Middleport, N.Y.). Mesenteric arterial perfusion pressure was monitored with a lateral tap on the perfusion circuit located between the pump and the superior mesenteric artery. Statham P23ID transducers (Gould Inc., Cleveland, Ohio) and a Grass polygraph (model 7, Grass Instruments, Quincy, Mass.) were used to record systemic arterial pressure and mesenteric perfusion pressure. The pumping rate was set so that perfusion pressure approximated systemic arterial pressure and was not altered through the course of the experiment. The flow rate averaged 18 ± 0.2 ml/min.

In experiments in which the sympathetic nerves were stimulated electrically, the nerve plexus of the superior mesenteric artery was carefully isolated and placed on a shielded Palmer electrode (Cincinnati, Ohio). The nerve was decentralized by crushing it proximally to the electrode and was stimulated with a Grass stimulator (SM6) in a random sequence with square wave pulses, 2 msec duration, 12 V, at 1, 3, and 10 Hz for periods of 30 seconds. Vasoconstrictor substances were injected into the perfusion circuit in small volumes (0.1–0.3 ml) close to the superior mesenteric artery, and blood flow through the course of the experiment. The flow rate averaged 18 ± 0.2 ml/min.

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Nitrendipine and Bay K 8644 (Miles Laboratories, New Haven, Conn.) were used as the calcium-entry blocking agent and calcium agonist and were dissolved in a 1-ml solution of 1:4 polyoxyethylene castor oil/50 mM Tris HCl, pH 7.4. The suspension was warmed, and then while vortexing, 2 ml of polyethylene glycol 400 (Sigma) was added. The solutions were made up to 8 ml with Tris HCl, pH 7.4, and were stored in a brown bottle in a refrigerator. On the day of use, the stock solutions were diluted with saline solution (0.9%) to the appropriate concentration.

The selective α₁- and α₂-adrenoceptor antagonists used in the studies were prazosin hydrochloride (Pfizer Laboratories, New York) and yohimbine hydrochloride (Sigma). Prazosin was sonicated for 20–30 minutes until dissolved to a concentration of 50 μg/ml in deionized water, and yohimbine was dissolved in 0.9% saline solution. Both antagonist solutions were prepared daily. In animals in which 6-hydroxydopamine was used to destroy the integrity of adrenergic terminals, the animals were treated with 6-hydroxydopamine HCl (Sigma), 100 mg/kg i.p., for 3 days and were studied 3–6 days later. Phenoxybenzamine hydrochloride (Smith Kline & French) was dissolved in a solution of 5% ethanol and 30% propylene glycol in distilled water to a concentration of 5 mg/ml. Cocaine hydrochloride (Mallinckrodt, St. Louis, Mo.) was dissolved in 0.9% saline solution at a concentration of 5 mg/ml.

Data on vasoconstrictor responses were analyzed by the methods of Snedecor and Cochran for paired or group comparison, and baseline pressure data obtained over time were analyzed with Tukey's test for multiple range analysis. Values for vasoconstrictor responses represent peak changes and are expressed as mean ± SEM. A p value less than 0.05 was considered significant.

Results

Influence of α-Adrenoceptor Agonists and Antagonists

To determine whether α₁- and postjunctional α₂-receptors are present in the feline mesenteric vascular bed, responses to α-adrenoceptor agonists and the effects of the antagonists were investigated under conditions of controlled blood flow. Intra-arterial injections of phenylephrine and UK14304 caused dose-related increases in mesenteric perfusion pressure (Figure 1). The peak rise in pressure in response to UK14304 occurred 20–30 seconds after injection of the α₂-adrenoceptor agonist, and pressure returned toward basal value over a 90–120-second period. The rise in pressure in response to the α₁-adrenoceptor agonist phenylephrine reached a peak in 10–15 seconds, and pressure returned toward baseline value over a 40–60-second period. When the molecular weights of phenylephrine and UK14304
are taken into account, both agonists were found to have similar vasoconstrictor activity in the mesenteric vascular bed.

The effects of prazosin, an $\alpha_1$-adrenoceptor antagonist, and yohimbine, an $\alpha_2$-adrenoceptor antagonist, on responses to the $\alpha$-adrenoceptor agonists were investigated, and these data are shown in the left-hand panels of Figure 1. Prazosin, 0.1 mg/kg i.v., significantly reduced the increases in mesenteric arterial pressure in response to phenylephrine but had no significant effect on responses to UK14304 (Figure 1, left panels). The administration of yohimbine, 1 mg/kg i.v., to the same animals that had received prazosin significantly reduced the mesenteric vascular response to UK14304 but had no additional blocking effect on responses to phenylephrine (Figure 1, left panels).

In the second group of cats in this series, the order of administration of prazosin and yohimbine was reversed. Yohimbine, 1.0 mg/kg i.v., had no significant effect on mesenteric vasoconstrictor responses to phenylephrine; however, responses to UK14304 were reduced significantly (Figure 1, right panels). Subsequent administration of prazosin, 0.1 mg/kg i.v., to the same animals that had received yohimbine resulted in a significant decrease in the mesenteric vasoconstrictor responses to phenylephrine but resulted in no additional effect on responses to UK14304 (Figure 1, right panels). Vasoconstrictor responses to phenylephrine and UK14304 were not changed over the period of time in which experiments were carried out or by administration of the vehicles for the antagonists (data not shown). Prazosin significantly reduced baseline mesenteric and aortic pressures (control 139 ± 7 and 115 ± 4, prazosin 100 ± 8 and 79 ± 3 mm Hg). However, the addition of yohimbine had little additional effect on baseline mesenteric or aortic pressure. More over, when yohimbine was administered first in the second group of cats, this agent had no significant effect on mesenteric and systemic arterial pressures. However, subsequent administration of prazosin significantly reduced mesenteric and systemic arterial pressures.

Effects of 6-Hydroxydopamine

The results from experiments with phenylephrine and UK14304 suggest the presence of $\alpha_1$- and postjunctional $\alpha_2$-adrenoceptors in the feline mesenteric vascular bed. Since experimental evidence suggests that $\alpha_2$-adrenoceptors are located at both prejunctional and postjunctional sites, it is important to preclude a prejunctional effect of the $\alpha_1$-agonist by pretreating the animals with 6-hydroxydopamine. Pretreatment with 6-hydroxydopamine significantly reduced the mesenteric vasoconstrictor response to tyramine and enhanced the response to norepinephrine (data not shown). Following treatment with 6-hydroxydopamine, phenylephrine and UK14304 produced similar increases in mesenteric perfusion pressure as observed in untreated animals (Figures 1 and 2). In 6-hydroxydopamine–treated animals, prazosin, 0.1 mg/kg i.v., significantly decreased vasoconstrictor responses to phenylephrine, but responses to UK14304 were unchanged (Figure 2, left panels). Subsequent administration of yohimbine to these same animals significantly decreased vasoconstrictor responses to UK14304 with no further change observed in the vasoconstrictor response to UK14304 (Figure 2, left panels). The effects of reversing the order of administration of the $\alpha_2$-adrenoceptor blocking agents on vasoconstrictor responses were investigated in another group of cats pretreated with 6-hydroxydopamine. When yohimbine was given first, vasoconstrictor
Influence of Calcium-Entry Blockade on Responses to \( \alpha \)-Adrenoceptor Agonists

Recent studies have provided evidence that vasoconstrictor responses elicited by \( \alpha_2 \)-adrenoceptor agonists may be dependent on the influx of extracellular calcium ions but that \( \alpha_1 \)-mediated responses are not sensitive to calcium-entry blockade. To test this hypothesis in the feline mesenteric vascular bed, the effects of nitrendipine, a dihydropyridine calcium-entry blocking agent, on vasoconstrictor responses to phenylephrine and UK14304 were investigated. As in previous experiments, intra-arterial injections of phenylephrine and UK14304 increased mesenteric arterial perfusion pressure in a dose-related manner (Figure 3). During the infusion of nitrendipine, 1.0 \( \mu \)g/min, into the mesenteric vascular bed, vasoconstrictor responses to phenylephrine and to UK14304 were decreased significantly (Figure 3). Moreover, 60 minutes after the end of the nitrendipine infusion, vasoconstrictor responses to phenylephrine and UK14304 returned to control values (Figure 3). Nitrendipine caused a significant reduction in mesenteric and systemic arterial pressures, but infusion of the nitrendipine vehicle had no significant effect on vascular pressures (Table 1). In addition, the nitrendipine vehicle had no significant effect on vasoconstrictor responses in the mesenteric vascular bed (data not shown).

Influence of \( \alpha \)-Adrenoceptor Blocking Agents on Responses to Sympathetic Nerve Excitation, Norepinephrine, and Tyramine

It has been postulated that responses to sympathetic nerve stimulation result mainly from activation of
Table 1. Influence of Infusion of Nitrendipine and Nitrendipine Vehicle on Mean Vascular Pressures in the Cat

<table>
<thead>
<tr>
<th>Agent</th>
<th>Control</th>
<th>Minutes after onset of infusion</th>
<th>60 minutes after infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrendipine 1 μg/min i.a.</td>
<td>162±11</td>
<td>118±7*</td>
<td>110±9*</td>
</tr>
<tr>
<td>Nitrendipine vehicle 0.2 ml/min i.a.</td>
<td>141±6</td>
<td>157±11</td>
<td>148±6</td>
</tr>
</tbody>
</table>

Aortic pressure (mm Hg)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Control</th>
<th>30 minutes after onset of infusion</th>
<th>60 minutes after infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrendipine 1 μg/min i.a.</td>
<td>126±9</td>
<td>88±8*</td>
<td>111±7</td>
</tr>
<tr>
<td>Nitrendipine vehicle 0.2 ml/min i.a.</td>
<td>143±5</td>
<td>130±6</td>
<td>124±10</td>
</tr>
</tbody>
</table>

*p<0.05 when compared with corresponding control; analysis of variance with repeated measures and Tukey's multiple range test.

α₁-adrenoceptors but that responses to exogenous norepinephrine are due mainly to an effect on post-junctional α₁-receptors. To determine which receptor subtypes that the transmitter released by electrical excitation of the sympathetic nerves and exogenous norepinephrine act on, the effects of prazosin and yohimbine were investigated in 2 groups of cats, and these data are presented in Figure 4. In the first group of cats, responses to nerve stimulation were reduced significantly after administration of prazosin, 0.1 mg/kg i.v., but responses to exogenously administered norepinephrine were not changed significantly (Figure 4, left panels). Subsequent administration of yohimbine, 1 mg/kg i.v., to these same animals resulted in a significant decrease in mesenteric vasoconstrictor responses to norepinephrine, but no additional blocking effect was observed on responses to sympathetic nerve stimulation (Figure 4, left panels). In a second group of cats, administration of yohimbine, 1 mg/kg i.v., to these same animals resulted in a significant decrease in mesenteric vasoconstrictor responses to norepinephrine, but responses to electrical stimulation of the nerves were not changed significantly (Figure 4, right panels). Administration of prazosin, 0.1 mg/kg i.v., to these same animals significantly decreased mesenteric vasoconstrictor responses to electrical excitation of the nerves. Prazosin significantly reduced mesenteric and systemic arterial pressures, but yohimbine had no significant effect. The release of norepinephrine from sympathetic nerve terminals by electrical excitation of the nerves can be modified by agonists and antagonists that act on presynaptic α₁-receptors. However, norepinephrine released by tyramine is not subject to autoreceptor (presynaptic α₁) regulation. Therefore, the effects of yohimbine on responses to tyramine were investigated in the mesenteric vascular bed in another series of animals. Intra-arterial injections of tyramine caused dose-dependent increases in mesenteric perfusion pressure. Vasoconstrictor responses to tyramine were decreased significantly by yohimbine, 1 mg/kg i.v., and were decreased to an even greater extent by the administration of prazosin, 0.1 mg/kg i.v., in these same animals (Table 2). Moreover, when prazosin was administered first in other animals, responses to tyra-
Table 2. Influence of Selective \( \alpha \)-Adrenoceptor Antagonists on Mesenteric Vasoconstrictor Responses to Tyramine in the Cat

<table>
<thead>
<tr>
<th>Agent (i.a.)</th>
<th>Control</th>
<th>Prazosin</th>
<th>Yohimbine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 ( \mu )g</td>
<td>38 \pm 5</td>
<td>21 \pm 2*</td>
<td>7 \pm 3</td>
</tr>
<tr>
<td>300 ( \mu )g</td>
<td>61 \pm 5</td>
<td>35 \pm 5*</td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Responses to tyramine were determined 5–10 minutes after administration of the first blocking agent and again 5–10 minutes after administration of the second blocking agent.

*\( p < 0.05 \) when compared with corresponding control; \( p < 0.05 \) when compared with control and with first blocking agent; analysis of variance with repeated measures and Tukey's multiple range test.

Table 3. Influence of Cocaine on Mesenteric Vasoconstrictor Responses to Tyramine, Phenylephrine, and UK14304 in the Cat

<table>
<thead>
<tr>
<th>Agent (i.a.)</th>
<th>Control</th>
<th>Cocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 ( \mu )g</td>
<td>45 \pm 3</td>
<td>2 \pm 1*</td>
</tr>
<tr>
<td>300 ( \mu )g</td>
<td>76 \pm 3</td>
<td>9 \pm 2*</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 ( \mu )g</td>
<td>47 \pm 6</td>
<td>44 \pm 4</td>
</tr>
<tr>
<td>UK14304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 ( \mu )g</td>
<td>40 \pm 4</td>
<td>32 \pm 4</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Responses to the agonists were determined 5–10 minutes after administration of cocaine.

*\( p < 0.05 \) when compared to corresponding control; analysis of variance with repeated measures and Tukey's multiple range test.

Sympathetic nerve stimulation and norepinephrine, the effects of nitrendipine, a calcium-entry blocking agent, were investigated. In these experiments, a dose-response curve for norepinephrine and a frequency-response curve for electrical stimulation of the sympathetic nerves were obtained before, during, and 60 minutes after termination of the nitrendipine infusion. n, number of animals, *significantly different from control.
not modified by phenoxylbenzamine in a dose of 5 mg/kg, i.e., which reversed the mesenteric vasoconstrictor response to norepinephrine (Figure 7). Although the nonselective \( \alpha \)-blocking agent had no significant effect on the response to potassium chloride, subsequent infusion of nitrendipine, 1 \( \mu \)g/min, into the mesenteric vascular bed in the \( \alpha \)-blocked animal significantly reduced the response to potassium chloride (Figure 7, upper panel).

The effects of nitrendipine on responses to Bay K 8644, a nifedipine analogue that promotes calcium entry, were investigated in another group of cats, and these data are presented in Table 4. Intra-arterial injections of Bay K 8644 in doses of 0.1, 0.3, and 1 \( \mu \)g elicited dose-related increases in mesenteric arterial perfusion pressure. The increases in mesenteric arterial pressure were reduced markedly during infusion of nitrendipine, 1 \( \mu \)g/min (Table 4). Moreover, vasoconstrictor responses to the calcium agonist returned to control values 60 minutes after the end of the nitrendipine infusion.

**Discussion**

Results of the present study in the cat show that phenylephrine and UK14304, \( \alpha_{1} \) and \( \alpha_{2} \)-adrenoceptor agonists, increase mesenteric arterial perfusion pressure in a dose-related manner. Inasmuch as blood flow to the intestinal vascular bed was maintained constant, the increases in perfusion pressure indicate that both types of agonists cause vasoconstriction. Vasoconstrictor responses to phenylephrine were blocked in a selective manner by prazosin, but responses to UK14304 were selectively blocked by yohimbine. Moreover, similar data were obtained in experiments in which 6-hydroxydopamine was used to destroy the integrity of adrenergic terminals and exclude a presynaptic action of the \( \alpha_{1} \)-adrenoceptor agonists and antagonists.\textsuperscript{7,26–28} In these experiments, treatment with 6-hydroxydopamine had no significant effect on pressor responses to phenylephrine and UK14304, but responses to norepinephrine were enhanced, and responses to tyramine were significantly reduced. These data are in agreement with the studies of Langer et al\textsuperscript{28} in the cat where increases in mean arterial pressure in response to phenylephrine and guanabenz, \( \alpha_{1} \) and \( \alpha_{2} \)-adrenoceptor agonists, were not altered by 6-hydroxydopamine treatment, but responses to norepinephrine were enhanced, and responses to tyramine were reduced significantly. The observation in the present study and in the studies of Langer et al\textsuperscript{28} that responses to phenylephrine were not enhanced at a time in which responses to norepinephrine were increased markedly suggest that neuronal uptake of phenylephrine is not a major inactivation mechanism in the systemic vascular bed of the cat. The present data suggest the presence of postjunctional receptors mediating vasoconstriction in the mesenteric vascular bed with pharmacologic characteristics similar to \( \alpha_{1} \) and \( \alpha_{2} \)-adrenoceptors.\textsuperscript{22,23} These data support previous work showing the presence of \( \alpha_{2} \)-adrenoceptors in the peripheral and pulmonary vascular beds and suggest that the magnitude of the \( \alpha_{2} \)-receptor-mediated vasoconstriction in the mesenteric vascular bed may be greater than observed in other organ systems.\textsuperscript{2,5,7,22,23} Although the present studies suggest that \( \alpha_{1} \) and \( \alpha_{2} \)-adrenoceptors coexist on resistance vessels in the feline mesenteric vascular bed, work on isolated mesenteric arteries from the same species does not support this concept.\textsuperscript{29} In isolated jejunal arteries, 0.4–0.6 mm o.d., the \( \alpha_{2} \)-agonist clonidine had no
Table 4. Influence of Nitrendipine Infusion on Mesenteric Vasoconstrictor Responses to Bay K 8644 in the Cat

<table>
<thead>
<tr>
<th>Agent (i.a.)</th>
<th>Control</th>
<th>During nitrendipine infusion (1 μg/min i.a.)</th>
<th>60 minutes after infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bay K 8644</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg</td>
<td>17±2</td>
<td>4±2*</td>
<td>23±6</td>
</tr>
<tr>
<td>0.3 μg</td>
<td>35±3</td>
<td>13±2*</td>
<td>50±13</td>
</tr>
<tr>
<td>1.0 μg</td>
<td>53±5</td>
<td>18±3*</td>
<td>69±9</td>
</tr>
</tbody>
</table>

*p<0.05 when compared to corresponding control; n = 6.

contractile activity, and studies with prazosin and yohimbine or rauwolscine suggest the presence of only one type of α₁-adrenoceptor. Moreover, studies in isolated rat mesenteric arteries have revealed receptor binding profiles that suggest a mixed α₁- and α₂-receptor population. However, in the isolated perfused mesenteric vascular bed of the rat, tramazoline, an α₁-adrenoceptor agonist, had no significant pressor activity, and in isolated resistance vessels from the rat intestine and in the perfused mesenteric vascular bed, responses to exogenous norepinephrine are mediated by α₁-adrenoceptors. These data suggest that there are species differences, differences in the relative populations of α₁- and postjunctional α₂-adrenoceptors and that results in isolated vessels may differ from those obtained in the mesenteric vascular bed in vivo.

In addition to suggesting the existence of postjunctional α₁- and α₂-adrenoceptors, the present experiments demonstrate that vasoconstrictor responses to electrical excitation of the sympathetic nerves are blocked by prazosin but that responses to exogenous norepinephrine are blocked by yohimbine. These data support the hypothesis that responses to exogenous norepinephrine in the systemic vascular bed are mediated mainly by stimulation of postjunctional α₁-adrenoceptors but that responses to norepinephrine released by nerve impulses are due, for the most part, to activation of α₂-receptors. However, the receptor subtype stimulated by exogenous norepinephrine may not be similar in all organ systems. Recent studies have shown that vasoconstrictor responses to exogenous norepinephrine in the lung are due mainly to activation of α₁-adrenoceptors. Thus, in the cat pulmonary and mesenteric vascular bed, responses to norepinephrine are mediated differently. Tyramine also releases norepinephrine from adrenergic terminals. However, the effects of prazosin on vasoconstrictor responses to tyramine and to sympathetic nerve stimulation are different in the mesenteric vascular bed. Whereas prazosin, but not yohimbine, was effective in reducing responses to electrical excitation of the sympathetic nerves, prazosin and yohimbine had similar blocking effects on the response to tyramine. These data suggest that norepinephrine released by tyramine may act on both α₁- and postjunctional α₂-adrenoceptors, but transmitter released by nerve excitation only acts on α₁-receptors. The explanation for the differences in the effects of the blocking agents on responses to tyramine when compared with responses to nerve stimulation is uncertain, but similar results have been obtained in the rat vas deferens. It is possible that the transmitter pool displaced by tyramine may differ from the stores released by electrical stimulation and that the transmitter released by tyramine may diffuse to greater distances and thereby interact with postsynaptic α₁-adrenoceptors that are remote from the nerve terminal. Tyramine is an indirect acting amine that displaces norepinephrine from adrenergic terminals by a calcium-independent process. Although tyramine may possess some direct activity, vasoconstrictor responses to this agent are mainly indirect in the feline mesenteric vascular bed because they are almost entirely blocked by cocaine, an agent that blocks neuronal uptake of norepinephrine.

Although yohimbine reduced responses to tyramine by approximately 50%, the effects of this agent on responses to electrical stimulation of the sympathetic

Figure 7. Top panel: Influence of phenoxybenzamine and phenoxybenzamine + nitrendipine on vasoconstrictor responses to potassium chloride (KCl) injections in the mesenteric vascular bed. Lower panel: Influence of phenoxybenzamine and of phenoxybenzamine + nitrendipine on vasoconstrictor responses to norepinephrine (NE) in the mesenteric vascular bed. n, number of animals. *significantly different from control.
nerves are difficult to interpret since presynaptic and postsynaptic blocking actions of the antagonist would be expected to have opposing effects on the pressor response.25 The present studies suggest that the mesenteric vascular bed of the cat possesses \( \alpha \)- and postsynaptic \( \alpha \)-receptors mediating vasoconstriction. It has been postulated that \( \alpha \)-adrenoceptor-mediated vasoconstriction may be primarily dependent on the entry of extracellular calcium ions, but \( \alpha \)-mediated responses depend more on the release of calcium from intracellular stores. This hypothesis is based on studies showing that calcium-entry blocking agents have a differential effect on responses to \( \alpha \)- and \( \alpha \)-adrenoceptor agonists. Moreover, it has been suggested that \( \alpha \)-mediated responses may be distinguished by the source of calcium used for vasoconstriction. Since the mesenteric vascular bed has \( \alpha \)- as well as \( \alpha \)-vasoconstrictor mechanisms, this would be an appropriate model system to examine the sensitivity of \( \alpha \)- and \( \alpha \)-mediated responses to the inhibitory effects of a calcium-entry blocking agent. The results of these studies show that vasoconstrictor responses to \( \alpha \)- and to \( \alpha \)-adrenoceptor agonists are blocked to a similar extent and in a reversible manner by nifedipine, a dihydropyridine calcium-entry blocking agent. Similar results have also been obtained with other calcium-entry blocking agents in the feline mesenteric vascular bed. These data are not in agreement with results obtained in the pithed rat or cat and in the ganglion-blocked rabbit in which calcium-entry blocking agents had a selective blocking effect on \( \alpha \)-mediated responses.4,14-16 The reason for the different results in the feline mesenteric circulation and in previous work is uncertain but is not related to species because it has been previously reported that nifedipine has a greater inhibitory effect on increases in diastolic pressure elicited by BHT920 than by phenylephrine in the cat.4,14-16 The present studies are based on results obtained with one dihydropyridine calcium-entry blocking agent. It is possible that other types of calcium-entry antagonists, such as diltiazem and verapamil, could have a different spectrum of activity on adrenergic responses in the feline mesenteric vascular bed. Moreover, cardiac output and regional vascular responses were not measured in the pithed animals so that the effects of the \( \alpha \)-adrenoceptor agonists and their interactions with the calcium-entry blocking agents on vascular resistance are difficult to assess.4,14,15,16 The present data provide support for the alternate hypothesis that similar sources of calcium are required for vasoconstriction elicited by selective \( \alpha \)- and \( \alpha \)-adrenoceptor agonists in the feline mesenteric vascular bed.

If calcium-entry blockers are effective in inhibiting both \( \alpha \)- and \( \alpha \)-mediated vasoconstrictor responses, then responses to nerve-released and exogenous norepinephrine should be reduced by calcium-entry blocking agents. The present results show that responses to electrical stimulation of the sympathetic nerves and to injected norepinephrine are blocked in a reversible manner by nifedipine. The finding that vasoconstrictor responses to electrical stimulation of the sympathetic nerves are reduced by calcium-entry blocking agents is similar to the findings of Holck and Gerold in the pithed rat. The present data extend the studies of Holck and Gerold by showing that responses to electrical stimulation, tyramine, and \( \alpha \)-agonists are attenuated by calcium-entry blocking agents. Since the release of norepinephrine from adrenergic nerves by tyramine is not dependent on the influx of calcium and since responses to nerve excitation and tyramine are reduced to a similar extent, these data suggest that the major effect of the calcium-entry blocking agent in resistance vessels of the intestine is postsynaptic in nature. Although the release of norepinephrine from adrenergic nerves is a calcium-dependent process, it is not impaired by concentrations of calcium-entry antagonists that have a marked effect on vascular smooth muscle. The inhibitory effect of nifedipine on vasoconstrictor responses in the mesenteric vascular bed is nonspecific in that responses to U46619, a thromboxane A2 mimic, angiotensin II, and potassium chloride were reduced. Moreover, pressor responses to potassium chloride and to U46619 and angiotensin II, which act on specific membrane receptors, were reduced to approximately the same extent. The observation that vasoconstrictor responses to Bay K 8644, a novel dihydropyridine that promotes calcium entry, are blocked by nifedipine suggests that calcium channels in the plasma membrane of resistance vessels in the small intestine are blocked.44 The present data suggest that vasoconstrictor responses to these pressor substances and to potassium chloride, which depolarizes smooth muscle, require extracellular calcium. The response to potassium chloride is not reduced by an \( \alpha \)-adrenergic blocking agent in doses that block or reverse responses to norepinephrine, suggesting that this response is not dependent on the release of norepinephrine from adrenergic terminals. The observation that responses to nerve-released and exogenous norepinephrine are blocked or reversed by \( \alpha \)-receptor blocking agents in the feline mesenteric vascular bed suggests that these receptors are different from the "junctural receptors" in guinea pig mesenteric arterioles in which responses were not blocked by phentolamine.13,17 The present data indicate that vasoconstrictor responses in the cat mesenteric vascular bed elicited by agents that stimulate receptors on the postsynaptic membrane or depolarize resistance vessels require an extracellular source of calcium and differ from studies in isolated vascular tissue.45-46 The present results suggest that the inhibitory effects of calcium-entry blocking agents on vasoconstrictor responses to both sympathetic nerve stimulation and exogenous vasoconstrictor hormones may be involved in the mechanism of the antihypertensive action of these drugs.

Acknowledgment

The authors wish to thank Ms. Janice Ignarro for editorial assistance.
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\textbf{Key Words} • calcium entry blockade • \(\alpha\)-adrenoceptors • vasoconstrictor mechanisms • mesenteric vascular bed
Influence of calcium-entry blockade on vasoconstrictor responses in feline mesenteric vascular bed.
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_Circ Res._ 1987;61:570-580
doi: 10.1161/01.RES.61.4.570

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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