Evidence That Dopaminergic Innervation Is Not Involved in the Vasodilatation of Cat Superior Mesenteric Arterial Bed: The Role of β-Adrenoceptors and Circulating Catecholamines

Gyorgy Lonart, Lajos Gyorgy, Margit Doda, and E. Sylvester Vizi

In the presence of phentolamine, stimulation of the cat splanchnic nerve decreased the resistance of the superior mesenteric arterial bed. This effect was not significantly influenced by sulpiride. Sulpiride, in the presence of phentolamine, did not inhibit the decrease in resistance in animals pretreated with guanethidine but propranolol or ablation of the adrenal glands prevented the effect of stimulation. These results are not compatible with the assumption that dopamine released from dopaminergic nerves is involved in the vasodilatation but do provide evidence for the role of β-adrenoceptors and circulating catecholamines in the vasodilatation of mesenteric vessels. (Circulation Research 1988; 62:1134–1137)

Materials and Methods

Cats of either sex weighing 2.5–4.5 kg were used. Of these, 27 were pretreated orally with a single dose of guanethidine (50 mg/kg) 1 day before the experiment to interrupt transmission via noradrenergic neurons and induce possible mesenteric vasodilatation. The animals were anesthetized with chloralose and urethane (50 mg/kg and 400 mg/kg i.p.). The left femoral artery and vein were cannulated to record the blood pressure and to administer drugs, respectively. Blood flow in the superior mesenteric artery was measured by means of an electromagnetic probe (Hellege, Hiller system). The resistance of superior mesenteric artery was calculated from the blood pressure and the blood flow. The stimulation of the distal end of the sectioned splanchnic nerve was carried out with square-wave pulses of 1 msec duration at 6 V and at frequencies ranging from 2 to 64 Hz applied for 10-second periods at 4–5-minute intervals (EMG-Medicor Electrostimulator, Budapest). Drugs used were adrenaline (Rhone Poulenc, Paris), oxyphenonium bromide (CIBA, Basel, Switzerland), dopamine HCl (Fluka, Buchs, Switzerland), guanethidine (EGIS, Budapest), phentolamine methanesulphate (CIBA), propranolol HCl (ICI, Alderly Park, England), (±)-sulpiride (Delagrange, Paris). Drugs were dissolved in 0.9% NaCl solution. Results are expressed as mean ± SEM. Statistical difference between two means was calculated by analysis of variance (ANOVA). Statistical differences between mean and 0 values were determined by the Student’s t test. The level of significance was regarded as p<0.05 in all cases.

Results

Splanchnic nerve stimulation (32 Hz) of cats not pretreated with guanethidine increased mesenteric arterial resistance by 11,211 ± 4,719 dyne/sec/cm³ (n = 5). This effect was completely reversed by phentolamine (6 mg/kg), an α-adrenoceptor antagonist and even some decrease in resistance (− 1,946 ± 408 dyne/...
was observed that was not influenced by sulpiride (6 mg/kg) \((-2,186\pm469\text{~dyne/sec/cm}^2, n=5, p>0.05, \text{ANOVA, Figure 1})\). This finding excludes the possible involvement of DA in the vasodilatation. Since guanethidine treatment almost completely destroys noradrenergic neurons without affecting dopaminergic axons, in our next series of experiments, the cats were orally pretreated with guanethidine (50 mg/kg). In comparison with untreated cats, guanethidine did not change the mesenteric resistance: 6,310±907 dyne/sec/cm\(^{-2}\) in normal and 6,074±383 dyne/sec/cm\(^{-2}\) (\(n=5, n=27, p>0.05, \text{ANOVA}\)) in pretreated cats. In these cats, splanchnic nerve stimulation produced either no change or a slight increase in resistance (basal: 6,074±383 dyne/sec/cm\(^{-2}\); stimulated: 7,050±363 dyne/sec/cm\(^{-2}\); \(n=27, p>0.05, \text{ANOVA}\)). Therefore, in our further experiments intravenous phentolamine was added in doses of 3 mg/kg to exclude \(\alpha\)-adrenoceptors from responding to stimulation. In guanethidine-pretreated animals, phentolamine did not affect basal resistance (6,074±383 dyne/sec/cm\(^{-2}\) in the absence and 6,532±345 dyne/sec/cm\(^{-2}\) in the presence of phentolamine; \(n=27, p>0.05, \text{ANOVA}\)). After phentolamine administration, there was a consequent decrease in resistance in response to splanchnic nerve stimulation (Figure 2). The response could be elicited only with frequency of stimulation above 4 Hz. Resistance was decreased in a frequency-dependent manner with a maximal effect at 32 Hz. Stimulation with 32 Hz decreased the basal resistance from 6,532±345 dyne/sec/cm\(^{-2}\) to 5,381±280 dyne/sec/cm\(^{-2}\) (\(n=27, p<0.05, \text{ANOVA}\)), which represents an 18% decrease in basal resistance. The onset of decrease appeared by the fourth to fifth second of stimulation and lasted for 25 seconds at this frequency (Figure 2). The guanethidine

\[\text{FIGURE 1. Effects of phentolamine (6 mg/kg i.v.) and phentolamine (6 mg/kg i.v.) combined with sulpiride (6 mg/kg i.v.) on the change in mesenteric resistance in response to splanchnic nerve stimulation. Cats were not pretreated with guanethidine. Asterisk compares phentolamine treated animals with untreated ones (*p<0.05, ANOVA). Data are presented as mean ± SEM.}\]
intra-adrenal and phentolamine-treated animals were divided into four groups so that the effects of a muscarinic receptor antagonist, a DA-receptor antagonist, a β-adrenoceptor antagonist, and the ablation of the adrenal glands could be investigated. At a dose of 500 μg/kg, oxyphenonium, a muscarinic receptor antagonist, did not significantly influence the effect of stimulation, intra-adrenal DA, or intravenous adrenaline (Table 1). A DA-receptor antagonist, sulpiride (6 mg/kg), had no effect on the response to electrical stimulation but blocked the effect of intravenous DA (Figure 2 and Table 1). This latter result is consistent with the observations of Gyorgy and Doda. The DA-induced increase in resistance (373 ±251 dyne/sec/cm², n = 5) observed after sulpiride treatment was not significantly different from 0 (p>0.05, Student’s t test). At a dose of 600 μg/kg, the β-adrenoceptor antagonist propranolol prevented the fall in resistance in response to both splanchnic nerve stimulation and adrenaline administration (Figure 2 and Table 1). The positive resistance changes (stimulation: 162 ±77 dyne/sec/cm², n = 6; intravenous adrenaline: 160 ±50 dyne/sec/cm², n = 5) were not significantly greater than 0 (p>0.05, Student’s t test). After acute ablation of the adrenal glands, splanchnic nerve stimulation was ineffective (Table 1). Adrenalectomy did not significantly effect (p>0.05, ANOVA) mesenteric arterial resistance. Values were 6,074 ±383 dyne/sec/cm² (n = 27) before and 6,398 ±927 dyne/sec/cm² (n = 6) after ablation of the adrenal glands.

The effects of drugs and adrenalectomy did not depend on the frequency of electrical stimulus applied (4, 8, 16, 32, and 64 Hz); therefore, the data concerning 32 Hz, which was the most representative, have been given.

### Table 1. Effects of Sulpiride, Propranolol, Oxymeniconium, and Ablation of Adrenal Glands on Change in Resistance of Superior Mesenteric Artery Caused by Splanchnic Nerve Stimulation or Intravenous Dopamine or Adrenaline

<table>
<thead>
<tr>
<th>Change in resistance (dyne/sec/cm²)</th>
<th>Electrical stimulus (32 Hz)</th>
<th>Dopamine (10 μg/kg i.v.)</th>
<th>Adrenaline (10 μg/kg i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-1,016±290</td>
<td>-1,253±177</td>
<td>-960±142</td>
</tr>
<tr>
<td>n = 8</td>
<td></td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Sulpiride (6 mg/kg i.v.)</td>
<td>-833±266</td>
<td>373±251*</td>
<td>-933±174</td>
</tr>
<tr>
<td>n = 8</td>
<td></td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Control</td>
<td>-933±319</td>
<td>-1,466±188</td>
<td>-981±159</td>
</tr>
<tr>
<td>n = 6</td>
<td></td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Propranolol (600 μg/kg i.v.)</td>
<td>162±77*</td>
<td>-2,159±779</td>
<td>160±50*</td>
</tr>
<tr>
<td>n = 6</td>
<td></td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Control</td>
<td>-1,390±286</td>
<td>-1,399±176</td>
<td>-1,166±99</td>
</tr>
<tr>
<td>n = 7</td>
<td></td>
<td>n = 4</td>
<td>n = 4</td>
</tr>
<tr>
<td>Oxymeniconium (500 μg/kg i.v.)</td>
<td>-1,200±235</td>
<td>-1,499±148</td>
<td>-1,033±114</td>
</tr>
<tr>
<td>n = 7</td>
<td></td>
<td>n = 4</td>
<td>n = 4</td>
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<tr>
<td>Control</td>
<td>-966±280</td>
<td>-1,419±163</td>
<td>-881±127</td>
</tr>
<tr>
<td>n = 6</td>
<td></td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Ablation of adrenal glands</td>
<td>0±0*</td>
<td>-1,333±133</td>
<td>-720±258</td>
</tr>
<tr>
<td>n = 6</td>
<td></td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
</tbody>
</table>

Cats were pretreated orally with guanethidine (50 mg/kg), and experiments were conducted after administration of phentolamine (3 mg/kg i.v.). Results are expressed as mean±SEM.

*Significant differences from the control (ANOVA), p<0.05. n = number of cats used.

### Discussion

The existence of mesenteric vasodilator nerves was proposed in the early 1970s when Ross described a noncholinergic and nonadrenergic vasodilatation in the mesenteric vessels of the cat pretreated with bretylium or 6-hydroxydopamine. In his paper, prostaglandin E1, vasodilator substance, and adenosine triphosphate were suggested to be responsible for the vasodilatation produced by periarterial mesenteric nerve stimulation. Armstead and coworkers showed that the vasodilatation in the feline mesenteric vascular bed under conditions of nonequilibrium α-adrenoceptor blockade is independent of the products of the cyclooxygenase pathway. Clark and Menninger suggested that DA might be responsible for the vasodilatation. They found that after guanethidine or phenoxybenzamine treatment, periarterial or preganglionic nerve stimulation induced a frequency-dependent increase of blood flow in canine superior mesenteric artery. These changes were resistant to atropine and propranolol but were inhibited by haloperidol.

In our experiments, the splanchnic nerve stimulation in cats pretreated with guanethidine and phentolamine resulted in a vasodilatation. Since propranolol completely inhibited this effect and sulpiride did not, it seems very likely that the vasodilatation was mediated via activation of β-adrenoceptors. These findings argue against a role for VIP and ATP as mediators of the decrease in mesenteric arterial resistance. Since DA is known to be a weak agonist (10–50 times less potent than noradrenaline and adrenaline) at both vasoconstrictor α-adrenoceptor and vasodilator β-adrenoceptors, it seems likely that the vasodilatation in response to splanchnic nerve stimulation is due to the effect of released DA on β-adrenoceptors. However, the finding that vasodilatation evoked by splanchnic nerve stimulation was blocked by propranolol while vasodilator response to exogenous DA was not attenuated by this β-adrenoceptor antagonist excludes the possibility that DA acted on β-adrenoceptors. Moreover, ablation of the adrenal glands prevented the effect of splanchnic nerve stimulation. These results suggest that vasodilatation is mediated via the release of nondopamine catecholamines from the adrenal glands.

In our experiments, evidence has been obtained that sulpiride-sensitive receptors are present in mesenteric vascular smooth muscle. Activation of these receptors by exogenous DA results in a vasodilatation. Nevertheless, the current study clearly demonstrated that these receptors are not innervated by the splanchnic nerve.

In summary, our data provide further evidence that in the cat the mesenteric vasodilatation is caused by activation of β-adrenoceptors most likely by circulating adrenal catecholamines. DA does not have a phys-
iological role in the control of resistance in the superior mesenteric arterial bed of this species.

References

KEY WORDS • cat superior mesenteric artery • β-adrenoceptors • dopamine receptors
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