Vascular Effects of Procaine Amide in the Dog

PREDOMINANCE OF THE INHIBITORY EFFECT ON GANGLIONIC TRANSMISSION

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ABSTRACT

The mechanism of the vasodilator effect of procaine amide in dogs was investigated in isolated gracilis muscle and hindpaw perfused at constant flows with arterial blood. Inhibition of sympathetic ganglionic transmission contributed predominantly to the vasodilation in muscle that accompanied intravenous administration of procaine amide (20 mg/kg). In contrast, comparable amounts of procaine amide administered locally had no detectable effects on resistance vessels; in both muscle and paw, no decreases in base-line perfusion pressures and no inhibition of constrictor responses to phenylephrine, angiotensin, and 5-hydroxytryptamine occurred. Also, procaine amide had no detectable effects on constrictor responses to stimulation of sympathetic postganglionic nerves. Procaine amide reduced the reflex vasoconstrictor response to carotid hypotension, but this reduction was comparable to the inhibition of sympathetic ganglionic transmission. The absence of direct inhibitory effects on baroreceptors and central reflex pathways was supported by the failure of intracarotid administration of procaine amide to alter detectably in gracilis muscle base-line perfusion pressures and reflex constrictor responses to carotid hypotension. Procaine amide reduced reflex responses to carotid chemoreceptor stimulation with nicotine but not those to stimulation with cyanide, suggesting a direct selective inhibitory action on the carotid body but not on the central pathways of the reflex. We conclude that the vasodilator effect of procaine amide results from inhibition of ganglionic transmission. A similar hexamethoniumlike effect may account for the inhibition of carotid chemoreceptor stimulation by nicotine.

KEY WORDS antiarrhythmic drugs ganglionic blockade nicotine phenylephrine angiotensin II 5-hydroxytryptamine cyanide hexamethonium carotid baroreceptors carotid chemoreceptors gracilis muscle hindpaw sympathetic nerve stimulation resistance vessels pre- and postganglionic stimulation

The hypotension associated with the administration of procaine amide might be caused by vasodilation as well as by decreases in cardiac output (1). Several mechanisms could contribute to this vasodilation, but the relative importance of these is uncertain. A direct relaxant effect of procaine amide on vascular smooth muscle has been proposed (2). Vasodilation might also be related to blockade of alpha receptors; we have recently reported that another antiarrhythmic drug, quinidine, blocks responses to adrenergic constrictor stimuli (3). Transmission at the neuroeffector junction might be impaired, since procaine amide is a weak local anesthetic (4). Neurogenic transmission across ganglia might be impaired, since procaine amide is known to inhibit transmission of impulses through the superior cervical ganglion of the cat (5). The information that is available does not permit definitive conclusions about the relative contribution of these mechanisms to the vasodilator effects of procaine amide.

The present experiments were done to examine systematically the mechanisms of the vasodilator effects of procaine amide.

Methods

Dogs weighing 17-26 kg were anesthetized by intravenously administering chloralose (60 mg/kg) and urethane (600 mg/kg), treated with decamethonium bromide (0.3 mg/kg), and ventilated...
through an endotracheal tube using a respirator. Heparin (500 USP units/kg, iv) was also administered.

Effects of procaine amide were evaluated in two vascular beds, the gracilis muscle and the hindpaw. The gracilis artery and the cranial tibial artery to the hindpaw were cannulated and perfused at constant flow rates using separate Holter roller pumps (model RL 174). Other arteries to these beds were ligated so that there was little or no collateral flow, as indicated by the absence of backflow of blood from the distal ends of transected vessels and by the abrupt decreases in perfusion pressures to 10-20 mm Hg when the pumps were stopped. During constant flow, increases in perfusion pressure indicate vasoconstriction and decreases indicate vasodilatation. Flow to the muscle averaged 10 ml/min and flow to the hindpaw averaged 30 ml/min. Pressures were measured in the aorta and in the perfusion tubing just proximal to the muscle and the paw using Statham strain-gauge pressure transducers (P23AA).

Several groups of dogs were studied to determine the effects of procaine amide on (1) base-line vascular resistances in the gracilis muscle and the hindpaw, (2) constrictor responses to a variety of adrenergic and nonadrenergic stimuli, (3) responses to postganglionic and preganglionic sympathetic nerve stimulation, and (4) responses to the activation of baroreceptor and chemoreceptor reflexes.

Mode of Procaine Amide Administration and Effects on Base-line Arterial Blood Pressure and Vascular Resistances.—Base-line variables were observed after procaine amide had been administered either intravenously or intra-arterially into the gracilis muscle and the hindpaw or intra-arterially into the common carotid arteries. The intravenous dose of procaine amide was 20 mg/kg. This dose was diluted in 10 ml of normal saline and infused in 6 minutes using a Harvard infusion pump. Intra-arterial doses were adjusted to give approximately the same local concentrations of drug as might have resulted from the intravenous dose; these doses were calculated as the fraction of the systemic dose which equals the cardiac output fraction to these beds. For example, flow to the gracilis muscle and hindpaw totaled 40 ml/min, which represents approximately 1.3% of an assumed cardiac output of 3 liters/min. Therefore, 1.3% of an intravenous dose of procaine amide of 40 mg/kg—530 μg/kg—was infused intra-arterially into the muscle and hindpaw over a period of 6 minutes. In a therapeutic context, this dose is high (1, 6). In an effort to demonstrate vasodilatation, the entire dose (530 μg/kg) was given directly to innervated gracilis muscle in one series of experiments. Similar considerations were involved in selecting doses of procaine amide for intracarotid infusions, except that these intra-arterial doses were calculated on the basis of an intravenous dose of 20 mg/kg. After the initial 6-minute intracarotid infusion, a sustained infusion intended to produce a local plasma concentration of 10-15 μg/ml was continued for the duration of the protocol. In separate groups of dogs, corresponding observations were made while infusions of the vehicle alone were administered; the vehicle consisted of 0.9% (w/v) benzyl alcohol, 0.05% sodium bisulphite, and HCl or NaOH to give a pH of 5. Base-line values before and at the termination of procaine amide infusions were compared using Student’s t-test for paired samples. A similar analysis was used to assess effects of the vehicle alone.

Effects of Procaine Amide on Vasoconstrictor Responses to Drugs and Postganglionic Sympathetic Nerve Stimulation.—Constrictor responses to adrenergic and nonadrenergic drugs were observed in denervated gracilis muscle and hindpaw. Constrictor drugs were: phenylephrine hydrochloride (Neosynephrine, Winthrop), 2 and 8 μg, angiotensin II amide (Hypertensin, Ciba), 0.5 and 2 μg, and 5-hydroxytryptamine, 2 and 8 μg. These drugs were injected into the perfusion tubing proximal to the pumps in volumes of 50 and 200 μl so that similar concentrations of a drug were delivered to each bed. Saline alone in these volumes did not alter perfusion pressures. In addition to drugs, constrictor responses to stimulation of the obturator and sciatic nerves at supramaximal voltage for 15 seconds using pulses 10 msec in duration and 1.5 and 6 Hz in frequency were observed. Stimulation of the obturator nerve and the sciatic nerve activated postganglionic sympathetic fibers to the gracilis muscle and the hindpaw, respectively. Administration of the neuromuscular blocking agent, decamethonium bromide, eliminated contraction of skeletal muscle that otherwise might have resulted from the stimulation of the motor nerves. Atropine was administered intra-arterially into muscle to block neurogenic cholinergic responses to nerve stimulation. The order of presenting these four interventions (three drugs and nerve stimulation) was varied using a Latin square.

Responses to drugs and nerve stimulation were observed in one group of eight dogs after the intra-arterial administration of procaine amide directly to muscle and paw. Corresponding observations were made in a second group of eight dogs after intra-arterial administration of vehicle. Responses after procaine amide administration and after vehicle administration were compared using analysis of variance and a parallel line bioassay (7).

Effects of Procaine Amide on Vasoconstrictor Responses to Preganglionic Sympathetic Nerve Stimulation.—Constrictor responses to preganglionic sympathetic nerve stimulation were observed in the gracilis muscle. The lumbar sympathetic chain was exposed through an incision in the flank, and a bipolar electrode was placed on the chain in the region of the second to fourth lumbar vertebrae (8). The chain was crushed cephalad to the electrode.

In six dogs constrictor responses to lumbar sympathetic chain stimulation were observed before and after intravenous administration of pro-
caine amide (20 mg/kg) and again after intravenous administration of the ganglionic blocking agent hexamethonium bromide (5 mg base/kg). The lumbar sympathetic chain contains some postganglionic nerves; responses after hexamethonium administration were observed to identify the magnitude of this component. The lumbar sympathetic chain was stimulated electrically for 15 seconds at supramaximal voltage using squarewave pulses 2 msec in duration and 4, 8, and 16 Hz in frequency. In the same experiments, responses to obturator nerve stimulation were examined before and after intravenous administration of procaine amide and again after administration of hexamethonium. The same protocol was carried out in six additional dogs except that vehicle was administered instead of procaine amide. Analysis of variance and a parallel line bioassay (7) were used to compare responses before and after procaine amide or vehicle administration in these studies.

Effects of Procaine Amide on Reflex Vasconstrictor Responses.—Both gracilis muscles were perfused. Reflex vasoconstriction was observed in the innervated gracilis muscle. Constrictor responses to stimulation of postganglionic sympathetic nerves were observed in the contralateral muscle; these responses were obtained in a manner similar to that already described except that three frequencies, 1.5, 3, and 6 Hz, were used. Reflex responses were obtained by bilaterally occluding the carotid arteries for 10 and 30 seconds to activate baroreceptor reflexes and by injecting nicotine (2 and 8 μg/kg) into the right common carotid artery to activate chemoreceptor reflexes. This latter response can be abolished by denervating either the carotid body or the gracilis muscle (9), which indicates that it is a neurogenic response. The right and left vagus nerves were sectioned in the midneck region to minimize the influence of cardiac and aortic baroreceptors on reflex responses.

In our early studies, the hypotension that accompanied the intravenous administration of procaine amide could have attenuated the decreases in carotid pressures and the magnitude of the reflex stimulation resulting from carotid occlusion. Therefore, in another group of dogs, carotid

![Figure 1](attachment:image.png)

Intravenous administration of procaine amide (PCA) (20 mg/kg) to this dog caused a decrease in mean arterial blood pressure in association with a decrease in perfusion pressure in the innervated gracilis muscle but did not affect perfusion pressures in the denervated muscle or the carotid sinuses. Responses to constrictor stimuli in denervated muscle were obtained by stimulating postganglionic sympathetic fibers in the obturator nerve, whereas responses in innervated muscle were obtained by activating carotid baroreceptor reflexes with 10 and 30 seconds of carotid hypotension. Reflex increases in muscle perfusion pressure during carotid hypotension were reduced 13–18 minutes after the intravenous administration of procaine amide and because of reductions in muscle perfusion pressure in response to stimulation of postganglionic sympathetic fibers were not altered. BCO = carotid hypotension produced by stopping perfusion pumps for 10 and 30 seconds.
pressures were maintained constant except during the activation of the baroreceptor reflexes. Both carotid bifurcations were exposed; the internal carotid arteries and the branches of the external carotid arteries were ligated. The blood supply to each carotid chemoreceptor was preserved by ligating the occipital arteries 1-2 cm downstream from their origins from the external carotid arteries. The common carotid and the external carotid arteries were cannulated. Arterial blood was pumped at a constant flow rate (70-110 ml/min) into each common carotid artery separately, and perfusion pressures were measured. Effluent blood flowed through the external carotid arteries and through Starling resistors to the jugular veins. Carotid perfusion pressures were adjusted initially by regulating the perfusion pumps and then maintained constant throughout the experiments by the Starling resistors. Stopping the perfusion pumps caused reproducible carotid hypotension (Fig. 1). As in previous experiments, bilateral vagotomies were performed to minimize the influence of cardiac and aortic baroreceptors on reflex responses.

Reflex vasoconstrictor responses in gracilis muscle were observed during 10 and 30 seconds of carotid hypotension before and after the intravenous infusion of procaine amide (20 mg/kg). Infusions were identical to those in the earlier studies of reflex responses. In four other dogs, corresponding observations were made before and after the intravenous administration of the vehicle alone. Reflex responses to carotid injections of nicotine (1 and 2 μg/kg) also were observed before and after administering either procaine amide or the vehicle. Reflex responses before and after

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP (mm Hg)</th>
<th>Innervated (mm Hg)</th>
<th>Denervated (mm Hg)</th>
<th>Denervated paw PP (mm Hg)</th>
<th>Carotid PP (mm Hg)</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>115 ± 7</td>
<td>191 ± 17</td>
<td>177 ± 25</td>
<td></td>
</tr>
<tr>
<td>PCA iv</td>
<td>9</td>
<td>87 ± 7∗</td>
<td>146 ± 12†</td>
<td>178 ± 25</td>
<td></td>
</tr>
<tr>
<td>B</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>92 ± 5</td>
<td>198 ± 17</td>
<td>137 ± 16</td>
<td></td>
</tr>
<tr>
<td>Vehicle iv</td>
<td>8</td>
<td>88 ± 4</td>
<td>198 ± 18</td>
<td>142 ± 16</td>
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</tr>
<tr>
<td>C</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>6</td>
<td>119 ± 6</td>
<td>135 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA iv</td>
<td>6</td>
<td>96 ± 6∗</td>
<td>110 ± 6∗</td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>130 ± 5</td>
<td>112 ± 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle iv</td>
<td>6</td>
<td>133 ± 5</td>
<td>113 ± 17</td>
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<tr>
<td>E</td>
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<tr>
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<td>8</td>
<td>75 ± 4</td>
<td>145 ± 23</td>
<td>107 ± 3</td>
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</tr>
<tr>
<td>PCA iv</td>
<td>8</td>
<td>50 ± 4∗</td>
<td>112 ± 15∗</td>
<td>107 ± 4</td>
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</tr>
<tr>
<td>F</td>
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</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>77 ± 8</td>
<td>86 ± 11</td>
<td>110 ± 4</td>
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<td>Vehicle iv</td>
<td>4</td>
<td>76 ± 4</td>
<td>88 ± 12</td>
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<tr>
<td>Control</td>
<td>6</td>
<td>119 ± 12</td>
<td>135 ± 10</td>
<td></td>
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</tr>
<tr>
<td>PCA ia</td>
<td>6</td>
<td>127 ± 15</td>
<td>135 ± 10</td>
<td></td>
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<td>H</td>
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<tr>
<td>Vehicle ia</td>
<td>8</td>
<td>130 ± 5</td>
<td>144 ± 13</td>
<td>113 ± 10</td>
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</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PCA ia</td>
<td>8</td>
<td>127 ± 15</td>
<td>144 ± 16</td>
<td>125 ± 8</td>
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</tr>
</tbody>
</table>

Values are means ± SE. MABP = mean arterial blood pressure, PP = perfusion pressure. Control = observations before any drug was administered. PCA iv = observations after intravenous administration of procaine amide (20 mg/kg), Vehicle iv = corresponding observations after intravenous administration of the vehicle alone, and PCA ia and Vehicle ia = observations after intra-arterial administration of these agents directly to muscle and paw. Means of observations before and after an intervention were compared using paired t-tests. Means for groups H and I were compared using the t-test for nonpaired samples. ∗p < 0.05. †p < 0.01.
procaine amide or vehicle administration were compared using analysis of variance and a parallel line bioassay (7).

Additional studies were done to investigate possible direct effects of procaine amide on baroreceptors and chemoreceptors and on central reflex pathways. These experiments are described in Results.

**Results**

**Effects of Procaine Amide on Systemic Arterial Blood Pressure and Perfusion Pressure in Gracilis Muscle.—**An intravenous infusion of procaine amide (20 mg/kg) caused a reduction in mean arterial blood pressure and vasodilatation in the innervated gracilis muscle (Fig. 1 and Table 1, groups A, C, and E). These changes were most pronounced at the end of an infusion; thereafter, values tended to return to preinfusion levels. Neither hypotension nor vasodilatation was associated with intravenous administration of the vehicle alone to separate groups of dogs (Table 1, groups B, D, and F).

The intravenous administration of procaine amide did not alter perfusion pressures in denervated muscle (Table 1, group A). Neither were perfusion pressures altered in denervated muscle (or paw) during intrarterial infusions of vehicle or procaine amide (Table 1, groups H and I). The intrarterial administration of procaine amide (530 μg/kg) directly into innervated muscle also did not cause detectable alterations in perfusion pressure (Table 1, group G).

**Effects of Procaine Amide on Responses to Local Constrictor Stimuli.—**Intravenous administration of procaine amide (20 mg/kg) did not inhibit constrictor responses in the gracilis muscle. Rather, procaine amide caused a reduction in mean arterial blood pressure and vasodilatation (Fig. 1 and Table 1, groups A, C, and E). These changes were most pronounced at the end of an infusion; thereafter, values tended to return to preinfusion levels. Neither hypotension nor vasodilatation was associated with intravenous administration of the vehicle alone to separate groups of dogs (Table 1, groups B, D, and F).

**TABLE 2**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Relative potency</th>
<th>95% Confidence limits</th>
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</thead>
<tbody>
<tr>
<td>Sympathetic nerve stimulation</td>
<td>1.28</td>
<td>0.78-2.27</td>
</tr>
<tr>
<td>Preganglionic</td>
<td>0.48</td>
<td>0.17-0.93*</td>
</tr>
<tr>
<td>Reflex stimuli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotid hypotension</td>
<td>0.54</td>
<td>0.28-0.96*</td>
</tr>
<tr>
<td>Chemoreceptor stimulation with nicotine</td>
<td>0.12</td>
<td>0.00-0.44*</td>
</tr>
</tbody>
</table>

The data on responses to postganglionic and preganglionic sympathetic nerve stimulation are in Figures 2 and 3, respectively. The data on responses to carotid hypotension are in the text, and the data on responses to chemoreceptor stimulation are in Figure 5A. Relative potency is the ratio of a stimulus that produces a certain response in the control period to the stimulus required to produce the same response after procaine amide administration. These values were calculated using analysis of variance and a parallel line bioassay (7). A relative potency value of 0.12 means that only 0.12 as much nicotine would be required before procaine amide administration to produce the same response. Stated another way, a standard dose of nicotine is eight times less potent as a chemoreceptor stimulus after intravenous administration of procaine amide. Responses consisted of increases in muscle perfusion pressures. Parameters of nerve stimulation were 1.5, 3, and 6 Hz in the case of postganglionic fibers in the obturator nerve and 4, 8, and 16 Hz in the case of preganglionic fibers in the sympathetic chain. Responses to baroreceptor activation were obtained with 10 and 30 seconds of carotid hypotension; responses to chemoreceptor stimulation were obtained with 1- and 2-μg/kg doses of nicotine injected into the right carotid artery.

*The ratio 1.00 is not included within the 95% confidence limits, which indicates that procaine amide significantly (*P < 0.05) inhibited the vasoconstrictor responses to preganglionic sympathetic nerve stimulation and reflex stimuli.
effects of procaine amide (PCA) on vasoconstrictor responses to lumbar sympathetic chain and obturator nerve stimulation. See the legend to Figure 2 for explanations. Base-line data for these dogs are given in Table 1, groups C and D. Increases in perfusion pressure during lumbar chain stimulation after administration of hexamethonium (C) were subtracted from corresponding increases before (control) and after procaine amide (or vehicle) administration to obtain responses to preganglionic sympathetic nerve stimulation. These values were used in calculating analysis of variance and the relative potency (Table 2).

Effects of intra-arterially administered procaine amide on responses to constrictor stimuli (Table 3).

<table>
<thead>
<tr>
<th>Nerve stimulation</th>
<th>Phenylephrine</th>
<th>Angiotensin</th>
<th>5-Hydroxytrypt-amine</th>
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</thead>
<tbody>
<tr>
<td>Group</td>
<td>1.5 Hz</td>
<td>6 Hz</td>
<td>2 µg</td>
</tr>
<tr>
<td>H</td>
<td>24 ± 4</td>
<td>79 ± 12</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>I</td>
<td>18 ± 4</td>
<td>64 ± 11</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>P</td>
<td>&gt; 0.05</td>
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ΔPP (mm Hg) in Denervated Gracilis Muscle

<table>
<thead>
<tr>
<th>Group</th>
<th>24 ± 4</th>
<th>79 ± 12</th>
<th>11 ± 2</th>
<th>38 ± 5</th>
<th>30 ± 6</th>
<th>77 ± 11</th>
</tr>
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<tbody>
<tr>
<td>H</td>
<td>22 ± 4</td>
<td>135 ± 27</td>
<td>32 ± 4</td>
<td>115 ± 27</td>
<td>43 ± 13</td>
<td>125 ± 24</td>
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<tr>
<td>I</td>
<td>37 ± 8</td>
<td>106 ± 22</td>
<td>36 ± 10</td>
<td>117 ± 25</td>
<td>43 ± 13</td>
<td>125 ± 24</td>
</tr>
<tr>
<td>P</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
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</table>

ΔPP (mm Hg) in Denervated Hindpaw

Each value is the mean ± SE of observations in eight dogs. Base-line values for these dogs are in Table 1, groups H and I. Increases in perfusion pressure (ΔPP) in response to vasoconstrictor stimuli in dogs given the vehicle were compared with corresponding increases in a separate group of dogs given procaine amide using a parallel line bioassay (7). Dogs given intra-arterial infusions of vehicle into the gracilis muscle (group H) and dogs given corresponding infusions of procaine amide (group I) did not differ significantly with respect to any of the responses.
chain at L₅-L₆ were decreased $(P < 0.05)$ after intravenous administration of procaine amide in six dogs (group C) but not after intravenous administration of the vehicle alone to six other dogs (group D) (Fig. 3 and Table 2). Subsequent administration of hexamethonium to both groups markedly decreased the vasoconstrictor responses to stimulation of the sympathetic chain, indicating that most of the responses observed in these experiments were caused by preganglionic stimulation. In the same dogs, vasoconstrictor responses to electrical stimulation of the obturator nerves (which exclusively activated postganglionic sympathetic fibers) were not reduced after intravenous administration of procaine amide and hexamethonium.

**Effects of Procaine Amide on Baroreceptor Reflexes.**—In nine dogs (group A) procaine amide caused a marked reduction in reflex vasoconstriction during bilateral occlusion of the common carotid arteries. Before any drugs were administered, increases in muscle perfusion pressure during 10 seconds and 30 seconds of carotid occlusion averaged 21 ± 4 (SE) mm Hg and 42 ± 6 mm Hg, respectively. Corresponding averages after the intravenous administration of procaine amide (20 mg/kg) were reduced $(P < 0.05)$ to 5 ± 1 mm Hg and 14 ± 3 mm Hg. Interpretation of these results is difficult, because the reduction in systemic arterial blood pressure associated with the intravenous administration of procaine amide would be expected to diminish the change in carotid sinus transmural pressure during carotid occlusion. Thus, hypotension could account for the reductions in reflex vasoconstriction.

Additional studies were done in which pressure in the carotid baroreceptors was maintained constant before and after administration of procaine amide (Fig. 1). In eight dogs (group E) the intravenous administration of procaine amide (20 mg/kg) significantly inhibited baroreceptor reflexes (Fig. 1 and Table 2). In the control state reflex increases in muscle perfusion pressure during 10 seconds and 30 seconds of carotid occlusion averaged 22 ± 4 mm Hg and 45 ± 6 mm Hg, respectively. After administration of procaine amide, the corresponding responses were reduced $(P < 0.05)$ to 18 ± 3 mm Hg and 30 ± 8 mm Hg.

Vehicle alone administered intravenously to four dogs (group F) did not reduce reflex vasoconstriction. Carotid hypotension for 10 seconds and 30 seconds initially caused increases in muscle perfusion pressure averaging 20 mm Hg and 54 mm Hg, respectively. After intravenous administration of vehicle, corresponding averages were 24 mm Hg and 60 mm Hg.

Inhibition of reflex responses to carotid hypotension and inhibition of constrictor responses to preganglionic sympathetic nerve stimulation were equivalent (Table 2).

To determine if procaine amide had additional effects on baroreceptors or central nervous system pathways that mediate the baroreceptor reflex, studies were carried out using three different experimental preparations. In these studies, the common carotid arteries were perfused separately at constant flow rates, and perfusion pressures were maintained constant using Starling resistors. Administration of procaine amide directly into the common carotid arteries would be expected to produce high concentrations of procaine amide locally but negligible concentrations in the systemic circulation. This expectation was confirmed in three dogs in which the concentration of procaine amide in blood from the carotid effluent and the brachial artery was measured. The concentration of procaine amide in the isolated carotid system averaged 17 µg/ml plasma during the sustained infusion, whereas the plasma concentration in systemic arterial blood averaged 2 µg/ml.

In the first series of experiments (Fig. 4A), baroreceptors were isolated from the cerebral circulation by ligating the internal carotid arteries and the branches of the external carotid arteries to eliminate collaterals to the brain. Neither the intracarotid administration of procaine amide nor the administration of vehicle altered $(P > 0.05)$ reflex vasoconstriction in muscle during carotid hypotension.

In the second series of experiments (Fig. 4B), the internal carotid arteries were not ligated, and procaine amide was infused into the common carotid arteries and the brain as well as into the baroreceptors. Perfusion pressures in the carotid arteries were maintained considerably higher than systemic arterial blood pressure to increase the area of
Intra-carotid administration of procaine amide (PCA) did not inhibit reflex increases in muscle perfusion pressure during carotid hypotension. Procaine amide was administered directly to the baroreceptors exclusively (A), the baroreceptors and the brain (B), and the brain exclusively (C). The dose of procaine amide was 600 µg/kg infused in 6 minutes followed by a continuous infusion of 1,500 µg/min. Carotid arteries were perfused at 100 ml/min. In three dogs, plasma concentrations of procaine amide in the carotid effluent were measured; they averaged 17 µg/ml. Thus, local concentrations of procaine amide in excess of accepted therapeutic levels had no detectable effects on the reflex responses illustrated in this figure. Solid lines indicate reflex responses in separate groups of dogs before (control) and after procaine amide administration. Broken lines indicate reflex responses before and after administration of vehicle alone. Points are means ± SE. The number of dogs is indicated in parentheses. Base-line values for systemic arterial blood pressure (MAP) and perfusion pressures in the gracilis muscle (PPm) or the carotid arteries (PPc) just before reflex constrictor responses were obtained are shown at the top of the figure. Note that procaine amide in these experiments did not influence base-line values significantly (P > 0.05 using t-test for paired samples).

In the third series of experiments (Fig. 4C), the internal carotid arteries downstream to the baroreceptors were ligated and cannulated so that procaine amide could be administered directly and exclusively to the brain. Under these conditions, procaine amide did not inhibit baroreceptor reflexes.

In three dogs, reflex responses to carotid hypotension were observed as described earlier before and after the infusion of procaine amide into the left vertebral artery. The dose of procaine amide was identical to the dose administered into the carotid circulation in earlier studies. Before procaine amide administration, reflex increases in muscle perfusion pressure averaged 21 ± 9 mm Hg and 34 ± 8 mm Hg in response to 10 seconds and 30 seconds, respectively, of carotid hypotension. After procaine amide administration, the corresponding averages were 17 ± 6 mm Hg and 29 ± 7 mm Hg. Responses before and after intravertebral artery infusions of pro-
caine amide were not statistically different. A similar tendency for responses to decrease slightly was observed after administration of the vehicle alone into the left vertebral artery of one dog. Before vehicle administration, the reflex increases were 15 mm Hg and 33 mm Hg, whereas the corresponding reflex increases after vehicle administration were 11 mm Hg and 25 mm Hg.

Effects of Procaine Amide on Chemoreceptor Reflexes.—Intravenous administration of procaine amide (20 mg/kg) to eight dogs in which baroreceptor reflexes were also tested (group E) caused a marked reduction in reflex vasoconstrictor responses to stimulation of the chemoreceptors with nicotine (Fig. 5A). Relative potency calculations indicated that there was an eightfold inhibition of chemoreceptor reflexes; this inhibition was more pronounced than either the twofold inhibition of baroreceptor reflexes or the inhibition of constrictor responses to preganglionic sympathetic nerve stimulation (Table 2). These results suggested that procaine amide affected the chemoreceptors directly. Further studies were done to investigate this possibility. In these studies, the common carotid arteries were perfused at constant flow and pressure, and the blood flow to the carotid bodies was preserved as described previously.

When procaine amide was administered into the common carotid artery, there was a significant inhibition ($P < 0.05$) of the reflex constrictor responses to chemoreceptor stimulation with nicotine (Fig. 5B). Intracarotid administration of the vehicle alone did not affect this reflex response (Fig. 5B).

Separate studies were done to determine if procaine amide also inhibited ventilatory responses to chemoreceptor stimulation. Dogs were anesthetized by the intravenous administration of chloralose (50 mg/kg) and urethane (500 mg/kg). They breathed spontaneously through an endotracheal tube. Air flow was recorded using a Fleisch pneumotachograph (Dynasciences) and was electrically integrated to measure minute volume. After control observations, procaine amide was administered into the carotid artery. The dose was 660 $\mu$g/kg over a period of 6 minutes followed by a sustained infusion of 82 $\mu$g/kg min$^{-1}$. In four dogs increases in minute volume in response to carotid injections of nicotine (10 $\mu$g) were reduced by procaine amide from 4.4 to 1.3 liters/min, 2.7 to 0.5 liters/min, 1.7 to 0.8 liters/min, and 3.4 to 2.4 liters/min. In contrast to the reduction in the ventilatory response to nicotine, the ventilatory response to chemoreceptor stimulation with intracarotid injections of sodium cyanide (1 mg) was not reduced by procaine amide. In the first two dogs in which response to nicotine had been tested, reflex increases in minute volume in response to cyanide were 2.6 liters/min and 2.7 liters/min before and 5.2 liters/min and 2.6 liters/min after the intracarotid administration of procaine amide.

To investigate further the possibility that procaine amide inhibits chemoreceptor responses to nicotine but not those to cyanide, reflex vasoconstrictor responses in the gracilis muscle to chemoreceptor stimulation with nicotine and cyanide were studied (Fig. 6A). In three dogs intracarotid administration of procaine amide reduced reflex vasoconstriction produced by intracarotid injec-

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A: Intravenous administration of procaine amide (PCA) (20 mg/kg) to eight dogs (solid lines) significantly reduced reflex increases in perfusion pressure in the gracilis muscle caused by activating the carotid chemoreceptors with carotid injections of nicotine. The vehicle administered to three dogs (broken lines) had no detectable effect on the reflex chemoreceptor response. B: Intracarotid administration of procaine amide to eight dogs (doses given in Fig. 4) caused a significant reduction in the reflex chemoreceptor response, whereas vehicle given to eight other dogs did not alter the responses significantly. Base-line values before constrictor responses in A are given in Table 1, groups E and F; base-line values before constrictor responses in B are given in Figure 4A.
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tions of nicotine but did not reduce corresponding responses to cyanide.

In seven dogs the effects of procaine amide on the central neural pathways of the chemoreceptor reflex were investigated. Carotid perfusion pressures were controlled, and reflex constrictor responses to chemoreceptor stimulation with cyanide were observed before and after the intracarotid administration of procaine amide and again during a recovery period 45-60 minutes after the drug had been stopped. Procaine amide, administered to the brain via patent internal carotid arteries as a result of carotid perfusion pressures considerably higher than systemic arterial blood pressures, produced a minimal and nonsignificant (P > 0.05) reduction in the chemoreceptor reflex (Fig. 6B). A recovery period of 30-45 minutes was associated with a further decline in this response.

Discussion

In these studies, the intravenous administration of procaine amide caused hypotension. Since vasodilatation in the innervated gracilis muscle also occurred, there must have been a reduction in neurogenic vasocostrictor tone. To determine more precisely the mechanism(s) of these changes, the following possible explanations for vasodilatation were considered: (1) a direct vasodilator effect of procaine amide on resistance vessels; (2) inhibition of circulating vasocostrictor stimuli; (3) inhibition of sympathetic postganglionic transmission; (4) inhibition of sympathetic ganglionic transmission; (5) alterations in the function of baroreceptors, and (6) alterations in central neural pathways mediating cardiovascular reflexes.

The results of these studies provide no evidence that procaine amide has direct vasodilator effects on resistance vessels. Intravenous doses of procaine amide that produced hypotension did not alter base-line vascular resistance in denervated gracilis muscle. However, the possibility could not be excluded that humoral factors released as a result of hypotension obscured a direct vasodilator effect. Therefore, additional studies were done and procaine amide was administered directly into the arterial inflow to denervated muscle and paw. Hypotension was avoided. Vasodilatation still was not detected, despite the administration of a relatively large dose of procaine amide locally (1, 6). Vasodilatation also was not detected in the innervated gracilis muscle during intraarterial administration of procaine amide, despite the facts that the innervated bed undoubtedly had greater vasocostrictor tone and, therefore, a greater sensitivity to vasodilator stimuli than the denervated beds and the dose of procaine amide was three times greater than it was in our earlier studies of denervated beds.

These results also provide no evidence that procaine amide inhibits responses to circulating vasocostrictor stimuli. The direct intraarterial administration of procaine amide

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to muscle and hindpaw did not significantly alter vasoconstrictor responses to adrenergic and nonadrenergic drugs. It should be emphasized that doses of procaine amide infused intra-arterially to muscle and paw were intended to reproduce approximately the fraction of an intravenous dose of 40 mg/kg which would be expected to reach these beds. Therefore, relatively large amounts of procaine amide failed to inhibit responses to circulating vasoconstrictor stimuli.

There is no evidence from these studies that procaine amide inhibits sympathetic postganglionic transmission. There were no significant changes in vasoconstrictor responses to stimulation of sympathetic postganglionic nerves in experiments involving the intravenous administration of procaine amide to separate groups of dogs (Figs. 2 and 3) and in other experiments involving the intra-arterial administration of procaine amide to muscle and hindpaw (Table 3).

Since procaine amide does not cause detectable vasodilatation in denervated and innervated vascular beds and does not inhibit constrictor responses to adrenergic stimuli, its vascular effects would appear to be quite different from those of quinidine. Recent observations in our own laboratory (3) have indicated that quinidine possesses a direct vasodilator action and also an inhibitory effect on stimuli that activate alpha receptors.

The present results suggest that inhibition of sympathetic ganglionic transmission contributes predominantly and possibly totally to the vasodilator action of procaine amide. Four considerations in addition to our previous evidence against other effects of procaine amide support this conclusion. (1) Procaine amide administered intravenously caused an inhibition of vasoconstrictor responses to stimulation of the lumbar sympathetic chain. (2) This inhibition involved mainly the preganglionic component of the lumbar chain. (3) Decreases in base-line vascular resistance in the gracilis muscle in response to procaine amide could not account for these results, because a decrease in base-line resistance should augment rather than inhibit vasoconstriction (3, 10–16). (4) Since inhibition was not associated with administration of the vehicle alone to separate groups of dogs, it must have been related specifically to the administration of procaine amide.

Although procaine amide inhibits vasoconstrictor responses in gracilis muscle that result from activation of baroreceptor reflexes, the inhibition appears to be largely the result of a reduction in ganglionic transmission. According to the calculated relative potency, baroreceptor reflexes were reduced by a factor of two; this degree of inhibition was equivalent to the reduction in responses to preganglionic sympathetic nerve stimulation and not the greater reduction that would be expected if independent inhibition of baroreceptors and central reflex pathways were involved. The absence of independent effects on baroreceptors and central pathways mediating baroreceptor reflexes is further supported by observations that direct intracarotid administration of procaine amide had no detectable influence on baseline mean arterial blood pressure and vascular resistance in innervated muscle and no detectable influence on reflex vasoconstrictor responses to carotid hypotension. It should be emphasized that procaine amide might affect baroreceptors or central pathways mediating baroreceptor reflexes when higher doses are employed but that plasma concentrations in the range of 17 µg/ml (achieved in the carotid circulation in these studies) had no detectable effects on baroreceptor reflexes. These plasma concentrations often are associated with toxic manifestations clinically (1, 6) so that the smaller plasma concentrations present in most clinical situations should not affect baroreceptors or central pathways.

The possibility that procaine amide did not reach the brainstem in these studies was considered. It seems unlikely however, since carotid perfusion pressure was approximately 60 mm Hg higher than systemic arterial blood pressure and collaterals to areas other than the brain were ligated so that perfusion of the carotid arteries would be expected to supply procaine amide to a large area of the brain. In three dogs procaine amide was administered directly into the left vertebral artery and reflex vasoconstrictor responses to carotid hypotension were observed. No significant changes were detected in these responses.

In the case of chemoreceptor reflexes, inhibition by the intravenous administration of procaine amide tended to exceed the inhibition of baroreceptor reflexes and sympa-
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thetic ganglionic transmission (Table 2). This finding suggested an independent action of procaine amide on chemoreceptors or neural pathways mediating the chemoreceptor reflex. An independent action on chemoreceptors was also indicated by observations that intracarotid administration of procaine amide exclusively to the carotid receptors and not to the brain inhibited both ventilatory and vasoconstrictor responses to chemoreceptor stimulation. However, procaine amide did not inhibit all chemoreceptor stimuli. Whereas chemoreceptor responses to carotid injections of nicotine were reduced considerably, corresponding responses to cyanide were not reduced. The results of these experiments which suggest that procaine amide is a specific inhibitor of nicotine-induced chemoreceptor stimulation are supported by work from other laboratories. Previous reports have indicated that hexamethonium, a ganglionic blocking agent with a mechanism of action similar to that of procaine amide (5), inhibits ventilatory responses to nicotine but not those to cyanide (17, 18). Thus, the inhibitory effects of procaine amide on chemoreceptors would appear to be related to mechanisms that may also account for inhibition of ganglionic transmission.

Since stimulation of chemoreceptors by cyanide was not affected by procaine amide, this stimulus was employed in the investigation of a possible effect of procaine amide on central neural pathways of the chemoreceptor reflex. Administration of procaine amide to the brain did not significantly inhibit the reflex vasoconstriction in muscle caused by carotid injections of cyanide. Thus, it appears that procaine amide is a specific inhibitor of chemoreceptor stimulation with nicotine and lacks other direct inhibitory effects on chemoreceptors and central pathways of the chemoreceptor reflex.

The major findings of the present study are as follows. (1) The only detectable effect of procaine amide on the sympathetic vasomotor system in this study involves inhibition of transmission across sympathetic ganglia, an action that mimics that of hexamethonium (5). (2) Procaine amide, even in relatively large doses, has no detectable direct vasodilator effects on resistance vessels in the gracilis muscle and the hindpaw, muscular and predominantly cutaneous vascular beds in the canine hind limb. (3) Procaine amide does not inhibit responses to circulating adrenergic and nonadrenergic constrictor stimuli. (4) Procaine amide does not impair postganglionic transmission in sympathetic nerves. (5) Procaine amide does not inhibit baroreceptors and central neural pathways mediating baroreceptor and chemoreceptor reflexes, but it does specifically inhibit chemoreceptor stimulation with nicotine.

The ganglionic blocking action of procaine amide which accounts totally for the vasodilator effects of the drug in this study could be unusually deleterious in patients who are dependent on compensatory increases in sympathetic vasomotor activity for the maintenance of normal systemic arterial blood pressure.

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