Vascular responses to stimulation of receptors in muscle by capsaicin

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Webb-Peploe, Michael M., David Brender, and John T. Shepherd. Vascular responses to stimulation of receptors in muscle by capsaicin. Am. J. Physiol. 222(1): 189-195, 1972. In dogs anesthetized with thiopentone and chloralose, and with both vagi and carotid sinus nerves sectioned, the injection of capsaicin into one iliac artery caused a reflex constriction of hindlimb, gut, and renal resistance vessels (constant-flow perfusion of contralateral iliac artery and superior mesenteric and renal arteries), constriction of splenic capacitance elements (venous pressure in isovolumetric spleen), and relaxation of cutaneous veins (constant-flow perfusion of saphenous vein). These responses were unaffected by ipsilateral lumbar sympathectomy or skimming of the limb, but were abolished by limb deafferentation. When one innervated carotid sinus was isolated, the opposite carotid sinus nerve cut, and the vagi sectioned, the capsaicin-induced reflex vasoconstriction was augmented at low and attenuated at high pressure in the isolated sinus. These vascular reflexes caused by injection of capsaicin were similar to those produced by electrical stimulation of afferent nerve fibers from hindlimb muscles. The receptors in the skeletal muscles that are stimulated by capsaicin may be those normally activated by muscular exercise to cause redistribution of blood flow.

cutaneous veins; muscle afferents; vascular reflexes; blood flow and exercise

capsaicin, a decylenic acid amide of vanillylamine, is known to stimulate mechanoreceptors in the pulmonary artery (6) and in the carotid sinus (4). When injected into the femoral arteries of anesthetized cats, it was found by Toh and co-workers (14) to cause hyperpnea, occasional twitching of the leg muscles, and a variable response in the femoral vein (outflow) pressures—was a measure of tone in the vein wall (18). The venous perfusate temperature was maintained at 37 °C, and perfusion rates of about 100 ml/min were used.

METHODS

Mongrel dogs, 15–25 kg in body weight, were anesthetized with intravenously administered thiopentone (20 mg/kg) and chloralose (80 mg/kg) and were artificially ventilated with oxygen using a Harvard respirator. Additional doses of chloralose were administered as required, and gallamine trichloride (Flaxedil, Davis and Geck) was used to prevent muscle movement.

The methods of measuring the responses of aortic pressure, of resistance vessels of the hindleg, gut, and kidney, of capacitance elements of the splanchic bed, and of the lateral saphenous vein have been described in detail elsewhere (11, 16, 18) but are summarized below.

Measurement of Responses

Aortic pressure. For this measurement, a catheter was inserted via the left brachial artery.

Resistance vessels. One or both iliac arteries, the superior mesenteric artery, and the left renal artery were cannulated and perfused at constant flow with blood from the terminal aorta by means of roller pumps with depulsators. Perfusion pressures were measured immediately upstream from the arterial cannulas, and the temperature of the perfusate was maintained at 37 °C by means of heat exchangers. Flows, initially adjusted to give perfusion pressures equal to mean aortic pressure, were kept constant throughout each study.

Capacitance elements of splanchic bed. Splenic vein pressure was measured through a fine catheter inserted into a left gastroepiploic vein and advanced until its tip lay in the splenic hilum. Except for the splenic artery and vein, all vessels entering or leaving the spleen were ligated or sectioned. During experiments, temporary arrest of splenic blood flow by occlusion of the splenic artery and vein ensured a constant splenic blood volume. Under these isovolumetric conditions, changes in splenic vein pressure were a measure of the reflex changes in tension of the smooth muscle of the splenic capsule and veins and represent the responses of the capacitance vessels throughout the splanchic bed (16).

Lateral saphenous (cutaneous) vein. The left lateral saphenous vein was cannulated at the ankle and perfused at constant flow with blood from the median sacral artery. During experiments, arterial inflow to the leg was arrested by occlusion of the left iliac artery. With constant blood flow through the lateral saphenous vein, the driving pressure—that is, the difference between perfusion (inflow) and femoral vein (outflow) pressures—was a measure of tone in the vein wall (18). The venous perfusate temperature was maintained at 37 °C, and perfusion rates of about 100 ml/min were used.
All pressures were measured by means of strain-gauge transducers (Statham model P23 De) and were recorded on a Honeywell UV 1508 Visicorder.

**Denervation of Baroreceptors**

Both vagi were sectioned at the level of the cricoid cartilage, and both carotid sinus nerves were sectioned.

**Control of Carotid Sinus Pressure**

The right carotid sinus was isolated from its vascular connections (9) and perfused at constant flow with blood from the central end of the divided right common carotid artery. This blood was returned, by a cannula in the external carotid artery and a short length of Silastic tubing, to the right jugular vein. Pressure within the sinus was adjusted by means of a screw clamp on the tubing joining the external carotid artery and jugular vein and was measured through a catheter inserted via the lingual artery. The left carotid sinus was denervated, and both vagi were divided.

**Sympathetic Nerve Stimulation**

The lumbar sympathetic chain was sectioned at the level of the second or third vertebral body and dissected free down to the level of the sixth vertebral body; the sympathetic nerves accompanying the superior mesenteric and left renal arteries were dissected free and sectioned. The nerves were stimulated with shocks of 1 msec duration at increasing voltage and frequency using a Grass stimulator (model S4) and a platinum bipolar electrode. Frequencies and voltages of up to 20 cycles/sec and 10 v for the lumbar chain, 20 cycles/sec and 60 v for the superior mesenteric nerves, and 20 cycles/sec and 50 v for the renal nerves were used in order to achieve supramaximal stimulation of these nerves.

**Drugs**

**Capsaicin.** A solution was prepared by dissolving 20 mg of capsaicin (City Chemical Corp., N. Y.) in 0.2 ml of 95% ethyl alcohol and making this up to 20 ml with saline (0.9 g NaCl/100 ml). This stock solution was then further diluted in saline to give a solution containing 0.1 mg/ml. The dose of capsaicin injected ranged from 0.05 to 0.3 mg. Control injections consisted of an equal volume of an identical solution of ethyl alcohol and saline without the capsaicin.

**N-nonanoylvanillylamine.** A solution of this synthetic analog of capsaicin (kindly supplied by H. M. Coleridge) was prepared in the same way as the capsaicin. Since it produced responses that were identical to those obtained with capsaicin, the results of experiments using either compound are reported together.

**Norepinephrine.** Norepinephrine (Levophed, Winthrop Laboratories) was injected in a few experiments in a dose of 10 μg (base).

**RESULTS**

**Vascular Responses to Injection of Capsaicin Into One Iliac Artery**

Carotid sinus and vagal nerves sectioned. Thirteen dogs were studied; capsaicin and control solutions were injected into the iliac artery perfusate upstream from the roller pump to ensure adequate mixing.

In two of the dogs, both iliac arteries were perfused at constant flow by separate roller pumps, and the perfusion pressure was measured together with the venous pressure in the isovolumetric spleen. The injection of capsaicin (0.1 mg) into one iliac artery caused the perfusion pressure in that artery to increase markedly; this was accompanied by a smaller increase in perfusion pressure in the opposite iliac artery and an increase in aortic and splenic vein pressures. The increase in these latter three pressures com-

![Figure 1](attachment://figure1.png)

**FIG. 1.** Vascular responses to injection of capsaicin into one or both iliac arteries (both vagi and carotid sinus nerves sectioned). Both iliac arteries were perfused at constant flow. RIA and LIA = right and left iliac artery perfusion pressures, respectively. A = aortic pressure. SV = splenic venous pressure (circulation to spleen was arrested temporarily). Scale to left of figure shows right and left iliac artery perfusion pressures and aortic pressures. Scale to right shows pressure in isovolumetric spleen.
VASCULAR RESPONSES TO CAPSAICIN

menced a few seconds after the onset of the pressure increase in the leg receiving the capsaicin. When capsaicin was injected simultaneously into the arterial supply to both legs (0.1 mg to each leg), the increases in aortic and splenic vein pressures were greater (Fig. 1). The injection of the control solution was without effect.

In four dogs, capsaicin was injected into right iliac artery and left saphenous vein perfusates; a representative experiment is shown in Fig. 3. Intra-arterial injection caused an increase in aortic pressure and in venous pressure in the isovolumetric spleen. An injection into the left saphenous vein caused the perfusion pressure in that vein to increase, but there was no increase in aortic, iliac artery perfusion, or splenic venous pressure. In two of these dogs, increasing doses of capsaicin, from 0.05 to 0.3 mg, were injected into the right iliac artery perfusate and produced responses in aortic and splenic venous pressure that increased in magnitude up to a dose of 0.15–0.2 mg, thus establishing that 0.3 mg of capsaicin was a “supramaximal” dose. This quantity was then injected into the arterial supply to the right leg before and after right lumbar sympathectomy which involved the removal of the sympathetic chain between the levels of the second and sixth lumbar vertebral bodies. Figure 4 depicts the results in one of the two dogs; similar results were obtained in the other dog. Right lumbar sympathectomy had no effect on the response to capsaicin. In the other two dogs, comparison of the effects of 10 μg of norepinephrine and 0.3 mg of capsaicin injected into the right iliac artery perfusate showed that, although norepinephrine produced an increase in iliac artery perfusion pressure comparable to that seen with capsaicin, it did not have the same rapid effects on aortic, splenic venous, and saphenous perfusion pressures. These pressures increased only after norepinephrine had had time to enter the general circulation.

In two dogs, 0.3 mg of capsaicin was injected into the right iliac artery perfusate before and after the right hind-
The voltage at which the muscle just began to twitch, and with single shocks of 1 msec duration and increasing voltage was dissected out and stimulated in the efferent direction nerve fibers of muscular origin. After denervation of the reproductive capsaicin responses by stimulation of afferent injection, as shown by the low perfusion pressure.

In the last two dogs in this series, an attempt was made to reproduce the capsaicin responses by stimulation of afferent nerve fibers of muscular origin. After denervation of the baroreceptors, the nerve to the right hamstring muscles was dissected out and stimulated in the efferent direction with single shocks of 1 msec duration and increasing voltage. The voltage at which the muscle just began to twitch, and thus the voltage threshold of the alpha-efferent fibers, was established. The dogs were then paralyzed with gallamine triethiodide, the nerve was cut distally, and the polarity established. The dogs were then paralyzed with gallamine triethiodide, the nerve to the right hamstring muscles (both vagi and carotid sinus nerves divided). IA = right iliac artery perfused at constant flow; A = aortic pressure; SV = venous pressure in saphenous vein at constant flow. After limb was skin, capsaicin still caused increase in aortic and splenic venous pressure and decrease in saphenous perfusion pressure.

Leg was skin. The resulting increase in aortic pressure and in venous pressure in the isovolumetric spleen still occurred after skinning (Fig. 5). The perfusion pressure of the left saphenous vein decreased markedly after the capsaicin injection. This vasoconstriction occurred only when the vein was constricted prior to the administration of the drug. Thus, it was absent in the experiments shown in Figs. 3 and 4, in which the vein was relaxed before the injection, as shown by the low perfusion pressure.

In the remaining three dogs, the right iliac artery, superior mesenteric artery, and left renal artery were perfused at constant flow, and the three perfusion pressure-together with the aortic pressure were measured. Capsaicin in doses of 0.1-0.3 mg was injected into the right iliac artery. At the lower carotid sinus pressures, the capsaicin injection caused large reflex increases in perfusion pressure in the superior mesenteric and renal artery and in aortic pressure; as the sinus pressure was increased, these responses were attenuated (Fig. 7). The increase in perfusion pressure in the right iliac artery, caused by the direct effect of capsaicin, was not attenuated as the carotid sinus pressure was increased.

Responses to capsaicin injected into right iliac artery and direct stimulation of sympathetic nerves. In five dogs, the baroreceptors were denervated and the left iliac artery, superior mesenteric artery, and left renal artery were perfused at constant flow. The responses of the three arterial perfusion pressures to 0.3 mg of capsaicin injected into the right iliac artery were then recorded. The left lumbar sympathetic chain and the sympathetic nerves accompanying the superior mesenteric and renal arteries were then dissected out, divided proximally, and stimulated with shocks of supramaximal voltage and frequency (Table 1). In the first dog, the left iliac artery perfusion pressures showed virtually no response to stimulation of the left lumbar sympathetic chain, which must have been damaged during the dissection; these results are not included in the Table. The average maximal perfusion pressures reached in the hindlimb, gut,
VASCULAR RESPONSES TO CAPSAICIN

193

Carotid Sinus Pressure (mm Hg): 100

135 180 90

Capsaicin into Right Iliac Artery
0.1 mg 0.1 mg

FIG. 7. Effect of changes in carotid sinus pressure on vascular responses to stimulation of receptors in muscle by capsaicin (one carotid sinus denervated and both vagi severed). Capsaicin was injected into right iliac artery at each carotid sinus pressure. Right iliac artery, left renal artery, and superior mesenteric artery were perfused at constant flow.

Renal Artery

Mean Aorta

Iliac Artery

SECONDS

TABLE I. Comparison of vascular responses to stimulation of muscle receptors by capsaicin with responses to direct stimulation of sympathetic nerves

<table>
<thead>
<tr>
<th>Artery Perfused</th>
<th>Dog</th>
<th>Baroreceptors Denervated</th>
<th>Nerve Stimulation</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Capsaicin, 0.3 mg</td>
</tr>
<tr>
<td>Iliac</td>
<td>1</td>
<td>250</td>
<td>260</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Mean</td>
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<td>269</td>
</tr>
<tr>
<td>Superior mesenteric</td>
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</tr>
<tr>
<td></td>
<td>Mean</td>
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<td>331</td>
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</table>

Arteries were perfused at constant flow. Capsaicin was injected into contralateral iliac artery. Vasomotor center was uninhibited by baroreceptors. Data are mean perfusion pressures in millimeters Hg.

The afferent fibers involved in the reflex responses were carried in the femoral and sciatic nerves of the hindlimb into which the drug was injected, since section of these nerves markedly attenuated or abolished the responses. Lumbar sympathectomy with interruption of the sympathetic nerve supply of the hindlimb into which the drug was injected had no effect on the capsaicin reflex. Cooper and Kerslake (7, 8) have suggested that afferent fibers from temperature receptors travel with the lumbar sympathetic nerve chains in man; if such fibers exist in the dog, they are not involved in the responses to capsaicin.

The reflex constriction of the splanchnic capacitance vessels and of the resistance vessels in the hindlimb, gut, and kidney was accompanied by a dilatation of the saphenous and kidney after the capsaicin injection were similar to those reached with maximal stimulation of the sympathetic nerves.

DISCUSSION

When either capsaicin or its synthetic analog was injected into the arterial supply to a hindlimb, the marked increase in perfusion pressure in the limb showed that a strong constriction of the resistance vessels of that leg was the immediate response to the arrival of the drug in the leg. Since this response persisted after section of the sciatic and femoral nerves to the limb and after the sympathetic nerves to that limb were interrupted surgically, it was due to a local action of this substance on the resistance vessels. When the carotid sinus nerves and vagi were severed, the injection of capsaicin was accompanied by an increase in aortic pressure, an increase in perfusion pressure in the opposite hindlimb, and an increase in venous pressure in the isovolumetric spleen. These increases occurred at the same time as or within a few seconds of the increase in pressure in the limb receiving the capsaicin. Therefore, they occurred too soon after the arrival of capsaicin in the injected leg to have been anything other than reflex in nature, a conclusion reinforced by the fact that the responses of the splenic capsule and veins could not have been due to direct drug action since the circulation of the spleen was temporarily arrested throughout each experiment. Since changes in splenic vein pressure, with the circulation to the spleen arrested, are representative of the response of the splanchnic capacitance vessels (16), presumably the capsaicin caused a reflex constriction of the capacitance vessels throughout the splanchnic bed.

and kidney after the capsaicin injection were similar to those reached with maximal stimulation of the sympathetic nerves.
ous vein. This dilatation must have been reflex because 1) it occurred too early for the capsaicin to have entered the general circulation, passed through the venous pump circuit, and arrived at the vein, and 2) other experiments showed that the direct effect of the drug on venous smooth muscle was to cause constriction, not dilatation. These dilator responses of the lateral saphenous vein were only seen when the vein was reflexly constricted before capsaicin was administered.

The reflex responses obtained with capsaicin were not initiated by its local constrictor action on the hindlimb resistance vessels, since a comparable constriction of these vessels by norepinephrine did not elicit these responses. The receptors responsible for initiating the capsaicin reflex were not situated in the larger hindlimb veins; capsaicin injected into the lateral saphenous vein caused direct constriction of that vessel, but this did not initiate the vascular reflex noted with the injection of capsaicin into the arterial supply to that limb. These receptors were not situated in the skin, since the reflex changes persisted after skinning of the limb into which the drug was injected.

These results confirm those of Toh and associates (14), who injected capsaicin into the femoral arteries of cats anesthetized with chloralose and concluded that capsaicin acts on undetermined receptors in the skeletal musculature. They found that this drug causes hyperpnea and has a variable effect on the systemic blood pressure, and that these effects could be abolished by sectioning the nerve supply to the muscles. The lack of consistent response of aortic blood pressure in their experiments may have been due to the fact that the baroreceptors were intact. Our earlier experiments showed that the reflex responses were attenuated when the vagal and carotid sinus nerves were intact, in contrast to the marked response after denervation.

Experiments in which the carotid sinus pressure was controlled while the vagus nerves were sectioned and the other sinus nerve cut confirmed that the capsaicin induced constrictor response of the resistance vessels of the hindlimb, gut, and kidney and of the splanchnic capacitance elements were potentiated at low carotid sinus pressure controlled while the vagus nerves were sectioned and the sympathetic nerves were intact, in contrast to the marked response after denervation.

In man, muscular activity causes increased adrenergic activity leading to a constriction of the resistance vessels of the skin (2), nonexercising muscles (2), splanchnic region (13), and kidney region (5) which is proportional to the severity of the exercise. Little is known about the receptors and afferent pathways responsible for this reflex vasoconstriction with exercise, but there is some evidence in both man (1) and animals (10) that the reflex originates in the active muscles. The use of capsaicin in the present study has demonstrated that there are receptors in muscle capable of initiating constriction of the resistance vessels of the hindleg, gut, and kidney of magnitude similar to that obtained by maximal sympathetic stimulation. This constrictor reflex, which also involves the splanchnic capacitance elements, is capable of fine adjustment by the baroreceptors, and the capsaicin-sensitive receptors in muscle may well be those normally activated by muscular exercise to cause redistribution of blood flow. Evidence supporting this suggestion was obtained when it was shown that a response exactly similar to that obtained with capsaicin could be produced by stimulating the nerve to the hamstring muscles in an afferent direction with shocks of 40 cycles/sec at a voltage some 100 times the voltage threshold for the alpha-ef fenter fibers in the same nerve. Such high voltages and frequencies are known to cause activation of the slowly conducting myelinated group 4 afferents (12, 15), and we concluded that the endings stimulated by capsaicin were served by such group 4 fibers.

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