Effects of Capsaicin and Bradykinin on Afferent Fibers with Endings in Skeletal Muscle

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SUMMARY Capsaicin, injected into the arterial supply of the skinned hindlimb of dogs, evokes reflex increases in cardiovascular function. Moreover, the cardiovascular reflexes evoked by capsaicin are very similar to those evoked by static exercise. The afferent fibers initiating these reflex increases have not been identified electrophysiologically, although their endings are believed to be located in skeletal muscle. We have, therefore, attempted to determine which afferent fibers are stimulated by capsaicin. In anesthetized dogs, we recorded impulses from afferent fibers with endings in either the gastrocnemius or gracilis muscles and injected capsaicin (10-30 µg/kg) into the abdominal aorta. Capsaicin stimulated 24 of 34 group IV (C fiber) endings, but only 5 of 19 group III (Aδ fiber) endings. By contrast, bradykinin (0.5-1.5 µg/kg) stimulated 17 of 33 group IV endings and 9 of 19 group III endings. Impulse activity for the 24 group IV afferents stimulated by capsaicin increased from 0.7 ± 0.1 to a peak of 9.3 ± 1.4 imp/sec. Firing started 6 ± 1 seconds after injection and remained above control levels for 24 ± 5 seconds. Capsaicin had no significant effect on the firing rate of 30 group I and II muscle afferents. Our results suggest that group IV muscle afferents are primarily responsible for causing the reflex increases in cardiovascular function evoked by injecting capsaicin into the arterial supply of the skinned hindlimb of dogs. Moreover, capsaicin is likely to be a useful pharmacological tool with which to determine the reflex autonomic effects caused by stimulation of group IV muscle afferents. Circ Res 50: 133-139, 1982

CAPSAICIN, when injected into the arterial supply of the skinned hindlimb of dogs, increases arterial blood pressure, heart rate, and cardiac contractility. These increases in cardiovascular function have been shown to be reflex in origin, because they are abolished or greatly attenuated by cutting the nerves supplying the hindlimb (Webb-Peploe et al., 1972; Crayton et al., 1981). The afferent fibers responsible for the reflex increases in cardiovascular function are believed to arise, at least in part, from endings located in skeletal muscle. For example, capsaicin, injected into the gracilis artery, reflexly evokes a pressor response, an effect which must be due to stimulation of afferent endings in the gracilis muscle (Crayton et al., 1981).

The afferent fibers responsible for evoking the reflex effects of capsaicin have yet to be identified electrophysiologically, although most investigators have speculated that thin fiber afferents are the candidates most likely to be stimulated by this substance (Webb-Peploe et al., 1972; Longhurst et al., 1980; Crayton et al., 1981). Such speculation appears quite reasonable for two reasons. First, capsaicin has been found to stimulate afferent vagal C fibers with chemosensitive endings in the heart, great vessels, and the lungs (Coleridge et al., 1964b; Coleridge et al., 1965; Coleridge et al., 1973; Coleridge and Coleridge, 1977). Second, thickly myelinated muscle afferents (i.e., group Ia, Ib, and II fibers) are believed to evoke little, if any, reflex cardiovascular effects (Coote and Perez-Gonzalez, 1970; McCluskey and Mitchell, 1972; McCluskey et al., 1972). The term “thin fiber muscle afferents,” however, refers not only to C fibers (group IV muscle afferents) but also to Aδ fibers (group III afferents), the latter conducting impulses between 2.5 and 24 m/sec (Matthews, 1972).

The possibility exists, therefore, that capsaicin exerts its reflex cardiovascular and ventilatory effects by stimulating group IV muscle afferents (Mense and Schmidt, 1974; Kumazawa and Mizumura, 1977).
Methods

General

Dogs (12-25 kg) were anesthetized with sodium pentobarbital (35 mg/kg, iv). The right common carotid artery and right external jugular vein were cannulated. The dogs were paralyzed with gallamine triethiodide (1-2 mg/kg, iv) and the lungs were ventilated by a Harvard pump through a cannula inserted into the trachea. The paralyzing agent was always allowed to wear off to permit assessment of the anesthesia level, which was maintained at surgical levels by injection of sodium pentobarbital. End-tidal CO$_2$ was monitored by a Beckman LB-2 gas analyzer and was kept between 4.0 and 5.0% by adjusting ventilation. Blood pressure in the aortic arch was recorded through the cannula inserted in the carotid artery, using a Statham P23 ID transducer. The gastrocnemius and gracilis muscles were exposed by incising the skin, and by removing the overlying fascia. The skin edges were dissected away from the sides of the muscles and then were tied to brass T-bars to form a pool, which was filled with warm (37°C) mineral oil to prevent the muscles from drying.

Impulses from either sciatic or gracilis nerves were recorded (see below) and counted by ratemeter (Rate/Interval Analyzer, Frederick Haer & Co.) whose window discriminator (Amplitude Analyzer, Frederick Haer & Co.) was set to accept action potentials of a particular amplitude. The output of the ratemeter and arterial blood pressure were recorded on a Gould direct-writing recorder (model 2200). In addition, the action potentials and arterial blood pressure were recorded on a Gould electrostatic recorder (model ES 1000).

Recording of Afferent Impulse Activity

We recorded afferent impulse activity from fine filaments dissected from either the left sciatic or right gracilis nerves. When recording activity from the sciatic nerve, the dogs were placed in the prone position, whereas when recording from the gracilis nerve, the dogs were supine. In both cases, the ankle was clamped, fixing the leg in one position. For filaments containing either spontaneously active or silent fibers, we probed the appropriate skeletal muscles in order to stimulate their receptive fields. We discarded all fibers whose endings (receptive fields) we could not locate in skeletal muscle. To measure the conduction velocities of all fibers having endings in skeletal muscle, we electrically stimulated the appropriate nerve through a pair of electrodes fixed in a shielded assembly and computed the conduction velocity by dividing conduction distance between stimulating and recording electrodes by the conduction time.

Chemicals

We injected chemicals into the abdominal aorta through a catheter inserted into the femoral artery contralateral to the nerve from which impulses were being recorded. Capsaicin was dissolved as previously described by Coleridge et al. (1964a). Bradykinin trisuccinate was dissolved in saline. Capsaicin (10-30 µg/kg) and bradykinin (0.5-1.5 µg/kg) were injected in 1 ml of saline and flushed in with 2-3 ml of saline. The injection required 3-5 seconds; onset latencies were measured from the beginning of injection. None of the fibers were stimulated by the vehicles in which bradykinin and capsaicin were dissolved. We injected doses of capsaicin and bradykinin shown previously to stimulate afferent vagal C fibers with chemosensitive endings in the heart and lungs (Coleridge et al., 1964a, 1965; Kaufman et al., 1980a; 1980b).

Control firing rates were averaged over the 30 seconds before capsaicin and bradykinin were injected. Peak firing rates were averaged over 5 seconds if the fiber was stimulated by either substance. Control and peak firing rates are expressed as impulses per second. Control heart rates were calculated by counting the arterial pressure pulses over a 30-second period, whereas peak heart rates were calculated over a 5-second period. All values are expressed as the mean ± SEM. We used paired t-tests (one-tailed) and a $\chi^2$ test to determine statistical significance.

Results

Group IV Fibers

We recorded the impulse activity of 34 C-fibers (conduction velocity = 1.1 ± 0.1 m/sec; range = 0.6-2.1 m/sec) with endings in the gastrocnemius or gracilis muscles. None of the endings were stimulated by manually stretching the appropriate tendon, although no attempt was made to quantify the force applied to it. The receptive fields of 25 of the endings could not be easily stimulated by mechanical manipulation. We had to either pinch the muscle between a thumb and forefinger or vigorously press the muscle with a blunt glass rod to stimulate the endings. When applied to the investigators, both stimuli were perceived as being noxious. The receptive fields of the remaining nine endings were stimulated by gently stroking the muscle with a blunt glass rod.

Capsaicin (10-30 µg/kg) stimulated 15 of 21 C-fiber endings in the gastrocnemius muscle and 9 of 13 endings in the gracilis muscle (Fig. 1A, 2A). The responses of the C fibers in the two muscles were similar and, therefore, have been combined. For all 34 C fibers tested with capsaicin, average activity increased from 0.6 ± 0.1 to a peak of 6.7 ± 1.2 imp/sec ($P < 0.001$). For the 24 C fibers stimulated by capsaicin, activity increased from 0.7 ± 0.1 to a peak of 9.3 ± 1.4 imp/sec beginning 6 ± 1 seconds after injection and remaining above control levels for 24 ± 5 sec. In addition, we examined the phenomenon of tachyphylaxis in 4 C fibers. When the interval between injections varied between 3 and 12
CAPSAICIN STIMULATION OF GROUP IV AFFERENTS/Kaufman et al.

FIGURE 1 Stimulation by capsaicin of two muscle afferents with endings in skeletal muscle. A: Injection of capsaicin (20 μg/kg) into the abdominal aorta at the bar stimulated a group IV muscle afferent (conduction velocity = 0.8 m/sec) whose ending was in the gracilis muscle. B: Injection of capsaicin (25 μg/kg) into the abdominal aorta at the bar stimulated a group III muscle afferent (conduction velocity = 3.2 m/sec) whose ending was in the gastrocnemius muscle. Above each panel, 1 second is indicated by the interval between ticks.

minutes (mean = 7 min), tachyphylaxis did not occur (Fig. 3).

Bradykinin stimulated 12 of 21 C fiber endings in the gastrocnemius muscle and 5 of 12 endings in the gracilis muscle (Fig. 2B). The effects of bradykinin on the firing of these 33 C fibers have been combined. For all 33 C fibers, average activity increased from 0.7 ± 0.1 to a peak of 2.6 ± 0.6 imp/sec (P < 0.01). For the 17 C fibers stimulated, activity increased from 0.7 ± 0.1 imp/sec to a peak of 4.4 ± 1.1 imp/sec, beginning 16 ± 2 seconds after injection and remaining above control levels for 33 ± 4 sec. By contrast to the C fibers stimulated by capsaicin, the C fibers stimulated by bradykinin displayed a low frequency discharge pattern, which was longer in onset and longer in duration (Fig. 2, A and B). The mechanical sensitivity of the endings of the C fibers did not predict their response to either capsaicin or to bradykinin.

Group III Fibers

We recorded impulses from 19 group III fibers (conduction velocity = 10.6 ± 1.1 m/sec; range = 3.2 – 19.2 m/sec), 17 of which had endings in the gastrocnemius muscle and two in the gracilis muscle. The receptive fields of 13 of the endings were very sensitive to mechanical deformation of the muscle, being stimulated by very gentle stroking with a wooden stick tipped with cotton wool. This stimulus was barely perceived by the experimenters. The endings of the remaining 6 group III fibers were stimulated only by forceful mechanical deformation of their receptive fields. To stimulate these fibers, we had to press or pinch the muscle vigorously.

Capsaicin (10–30 μg/kg) stimulated 5 of the 19 group III fibers (Fig. 1B). The responses of the 5 fibers, all of which had endings in the gastrocnemius muscle, consisted of a short lasting, high frequency, burst of impulses (Fig. 1B). The conduction velocities of the fibers stimulated by capsaicin were not significantly different from the conduction velocities of those not stimulated by this substance (9.8 ± 1.9 vs. 10.8 ± 1.3 m/sec, respectively). In addition, one group III fiber, not stimulated by 20 μg/kg of capsaicin, also was not stimulated by a higher dose, 42 μg/kg. Likewise, five other group III fibers were not stimulated by either 20 μg/kg or by 88–110 μg/kg of capsaicin. Capsaicin caused the average firing rate of all 19 group III fibers tested to increase from 0.4 ± 0.2 imp/sec to a peak of 3.1 ± 1.4 imp/sec (P < 0.05). Furthermore, a $\chi^2$ analysis revealed that capsaicin stimulated a significantly larger proportion of group IV than group III muscle afferents (P < 0.05).

FIGURE 2 Stimulation by capsaicin and by bradykinin of a group IV muscle afferent (conduction velocity = 1.4 m/sec) whose ending was in the gracilis muscle. A: Capsaicin (15 μg/kg) was injected at the arrow into the abdominal aorta. B: Bradykinin (0.9 μg/kg) was injected at the arrow into the abdominal aorta.
FIGURE 3 Effect of repeated injections of capsaicin upon the firing of a group IV muscle afferent (conduction velocity = 1.4 m/sec) whose ending was in the gracilis muscle. In A, B, and C, capsaicin (18 μg/kg) was injected at the bar into the abdominal aorta. The interval between A and B was 5 minutes and that between B and C was 3 minutes. Above each panel, 1 second is indicated by the interval between ticks.

Bradykinin (0.5–1.5 μg/kg) stimulated 9 of the 19 group III fibers. The endings of all 9 fibers stimulated were in the gastrocnemius muscle (Fig. 4B). For all 19 fibers tested, average activity increased from 0.3 ± 0.1 imp/sec to a peak of 1.9 ± 0.5 imp/sec (P < 0.01). For the 9 fibers stimulated by bradykinin, activity increased from 0.5 ± 0.2 to a peak of 3.9 ± 0.7 imp/sec, beginning 22 ± 4 seconds after injection and remaining above control levels for 113 ± 13 seconds. Three of the five group III fibers stimulated by capsaicin were also stimulated by bradykinin. The mechanical sensitivity of the group III endings, like that of the group IV endings, did not predict their responses to either capsaicin or bradykinin.

Group I and II Fibers

We recorded impulses from 30 group I and II fibers (range of conduction velocities = 24–100 m/sec) with endings in, either the gastrocnemius (18) or in the gracilis muscles (12). All but one were stimulated by tendon stretch. Capsaicin (10–30 μg/kg) had diverse effects on the firing of these afferents, increasing activity by 1–6 imp/sec in 9, decreasing activity by 1–4 imp/sec in 5, and having no effect on 16 (Fig. 5A). Firing, for the group, changed insignificantly from 18.9 ± 3.0 imp/sec to a peak of 19.4 ± 3.1 imp/sec after injection of capsaicin.

Bradykinin (1.0 μg/kg) had little effect on the impulse activity of 12 group I and II fibers. Only one fiber changed its firing rate by more than 1 imp/sec. This fiber, whose ending was exquisitely sensitive to stretching the calcaneal tendon, fired during inflation of the lungs.

Because most of the group I and II fibers did not respond to either capsaicin or bradykinin, we were concerned about their accessibility to chemicals injected into their arterial supply. Therefore, we examined the responses to succinylcholine (500 μg) of 3 group I fibers and one group II fiber, which were not stimulated by either capsaicin or by bradykinin. Succinylcholine stimulated all 4 fibers, activity increasing 10–40 imp/sec above control levels (Fig. 5B).

Cardiovascular Responses to Capsaicin and to Bradykinin

In the 25 dogs used in this study, mean arterial blood pressure increased from 126 ± 4 to 148 ± 5 mm Hg (P < 0.001) after capsaicin was injected. Heart rate increased insignificantly from 146 ± 8 to 148 ± 9 beats/min. The increase in blood pressure started 8 ± 1 seconds after injection.

Mean arterial pressure decreased from 122 ± 4 to 82 ± 4 mm Hg (P < 0.001) after injection of bradykinin. The decrease in blood pressure started
Discussion

Capsaicin, when injected into the abdominal aorta, stimulated 24 of the 34 group IV (C fiber) afferents tested, but only 5 of the 19 group III afferents (Aδ fiber) tested. Therefore, our results suggest that the reflex increases in cardiovascular function, evoked by injecting capsaicin into the arterial supply of the skinned hindlimb (Webb-Peploe et al., 1972; Crayton et al., 1981) were caused, mainly by stimulation of group IV muscle afferents. The reflex relaxation of tracheal smooth muscle evoked by injecting this substance into the arterial supply of skeletal muscle (Coleridge et al., 1980; Ordway et al., 1981) also appears to be caused mostly by group IV fibers. Since 1 of every 4 group III fibers was stimulated by capsaicin, it is possible that these fibers contributed to the reflex effects we observed.

Previously, the responses of thin fiber (i.e., group III and IV) muscle afferents to many algesic substances have been examined. Until now, the results have been the same, regardless of the substance used. For example, Mense and Schmidt (1974) and Mense (1977) found, in cats, that bradykinin, serotonin, histamine, and KCl stimulated both group III and group IV muscle afferents. In fact, bradykinin stimulated a greater proportion of group III than group IV afferents. Similarly, Kumazawa and Mizumura (1977) found, in dogs, that many of these same algesic substances stimulated both group III and group IV afferents. Capsaicin, when compared with the algesic substances used by Mense and Schmidt (1974), Mense (1977), and Kumazawa and Mizumura (1977), appears unique because this substance stimulates a significantly greater proportion of group IV than group III afferents.

Capsaicin has been shown to be selective in its actions on vagal afferents innervating the heart, great vessels, and lungs. In the lungs, for example, capsaicin stimulates pulmonary and bronchial C fiber endings, but has little or no effect on slowly or rapidly adapting receptors supplied by A fibers (Coleridge et al., 1965; Armstrong and Luck, 1974; Coleridge and Coleridge, 1977). In addition, capsaicin stimulates chemosensitive C fiber endings in the heart and aorta, but this substance does not stimulate either A or C fiber mechanoreceptors in these structures (Coleridge et al., 1964b; Coleridge et al., 1973; Kaufman et al., 1980a).

In preliminary work originating from this laboratory, capsaicin was found to stimulate both group III and IV muscle afferents in cats (Vance and Mitchell, 1977), whereas, in the present work, capsaicin was found to stimulate few group III afferents but many group IV afferent in dogs. Although Vance and Mitchell (1977) injected a relatively large amount of capsaicin close arterially, whereas we injected capsaicin into the abdominal aorta, this difference is unlikely to be the cause of the discrepancy because, in our study, increasing the dose of capsaicin still did not stimulate six group III endings. Possibly, the cause may be a species difference.

We do not know whether the thin fiber muscle afferents that we studied were nociceptors or ergoreceptors (Kniffki et al., 1978). Although many were stimulated by bradykinin, a potent algesic substance (Guzman et al., 1962), many of the same afferents, especially those with group III fibers, were also vigorously stimulated by very gentle probing of their receptive fields. Because of their extreme sensitivity to light touch, it is difficult to understand how these thin fiber afferents transduce the sensation of pain. Alternatively, these mechanically sensitive afferents may be stimulated by muscle contraction, such as that which is seen during static exercise.

Webb-Peploe et al. (1972) have suggested that the C fiber endings in skeletal muscle that are stimulated by capsaicin may be the same endings that are stimulated by static exercise. As of yet, there is no electrophysiological evidence to support such speculation. However, it is interesting to note...
that the magnitudes of the reflex increases in cardiovascular function evoked by injection of capsaicin into the arterial supply of the skinned hindlimb are very similar to the magnitudes of the reflex increases evoked by ventral root stimulation (Crayton et al., 1981), a maneuver which causes static exercise. Moreover, the reflex cardiovascular responses evoked by dynamic exercise are not blocked until the afferent pathway is cooled to 0 to 1°C (Tibes, 1977), a finding which suggests that C fibers play an important role in causing the reflex responses.

Although our preparation was well suited to determine the afferent fibers stimulated by capsaicin and bradykinin, it was of limited value for determining the reflex cardiovascular effects of stimulating thin fiber muscle afferents, because a hindlimb nerve was partially dissected, the dogs were anesthetized with a barbiturate, and the substances were injected into the abdominal aorta. Despite these limitations, mean arterial pressure increased after injecting capsaicin. This effect was likely to be caused by stimulation of afferent endings in the skin (Foster and Ramage, 1981) and in the abdominal viscera (Longhurst et al., 1980), as well as by stimulation of endings in skeletal muscle. In addition, injecting bradykinin into the abdominal aorta decreased mean arterial pressure and increased heart rate, the latter effect most probably being a manifestation of the baroreceptor reflex. The most likely explanation for the decrease in mean arterial pressure evoked by bradykinin is that this substance relaxed vascular smooth muscle (Nakano, 1965), an effect which overwhelmed any reflex vasoconstrictor response arising from muscle afferent stimulation.

Because bradykinin stimulates both group III and group IV muscle afferents, this substance might be expected to evoke cardiovascular and ventilatory reflexes when injected into the arterial supply of skeletal muscle. Recently, it has been shown that bradykinin, injected into the arterial supply of the hindlimb of conscious rabbits, reflexly evokes a biphasic change in blood pressure, consisting of a depressor response followed by a pressor response. However, when these rabbits were anesthetized with either chloralose-urethane or with sodium pentobarbital, only the depressor response was evoked by bradykinin (Tallarida et al., 1979). Whether similar reflex responses can be evoked by injecting bradykinin into the arterial supply of skeletal muscle in anesthetized dogs remains to be determined.

Capsaicin appears to be a fairly specific stimulus of group IV muscle afferents, at least in dogs. Furthermore, the response of the afferents to capsaicin is not tachyphylactic. Therefore, this substance may prove to be a useful pharmacological tool with which to determine the reflex autonomic effects of stimulating group IV muscle afferents. In addition, capsaicin may be useful in determining the connec-

tions of group IV muscle afferents to sites in the central nervous system that are involved in cardiovascular control.

Acknowledgments

We thank Gaye Ash and Musarrat Alavi for their technical assistance and Nelda Jean Holcombe and Diane Doach for typing the manuscript.

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Circ Res. 1982;50:133-139
doi: 10.1161/01.RES.50.1.133

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