Reflex Responses on Blood Pressure and Renal Nerve Activity to Local Intra-Arterial Injection of Capsaicin in Anesthetized Dogs

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Abstract The present study was performed to examine effects of intra-arterial and intravenous injection of capsaicin on efferent renal sympathetic nerve activity (RNA) in pentobarbital anesthetized dogs. In animals with intact baroreceptors, intra-arterial injection of capsaicin (4 ± 1 µg) produced significant increases in mean blood pressure (MBP) and heart rate (HR) by +43 ± 8 mmHg and +33 ± 15 beats/min at 20 s after the injection, respectively. There followed a recovery toward the control so that 60 s after the intra-arterial injection changes in MBP and HR were +8 ± 5 mmHg and +18 ± 8 beats/min, respectively. RNA showed a biphasic response, comprising of an initial increase (+52 ± 28% of the control at 20 s) followed by a decrease by −30 ± 8% of the control 60 s after the injection. Following bilateral cervical vagotomy the initial increase in RNA did not differ significantly from the value of RNA in animals with intact baroreceptors. However, the later decrease in RNA reversed above the control so that 60 s after the injection change in RNA was +5 ± 10% of the control. Complete baroreceptor denervation showed a similar response on RNA in the vagotomized animals (+49 ± 9 and +14 ± 10% of the control at 20 and 60 s after the injection, respectively). In contrast, intravenous injection of capsaicin (6 ± 1 µg/kg) resulted in significant decreases in MBP (−19 ± 11 mmHg) and HR (−9 ± 4 beats/min). RNA at 20 s after the injection showed a unidirectional decrease by −16 ± 7% of the control in animals with intact baroreceptors. These responses reversed above the control after cervical vagotomy. Thus, these data indicate that activation of C-fiber afferents in skeletal muscles by intra-arterial injection of capsaicin results in renal sympathoexcitation, and that the later sympathoinhibition is mediated by combined activation of systemic baroreceptors.

Received for publication December 20, 1989
Key words: capsaicin, renal nerve activity, systemic blood pressure, systemic baroreceptors, hind-limb.

Unilateral limb ischemia in human subjects (ALAM and SMIRK, 1937; LITTLE et al., 1984) and bilateral hindlimb ischemia in the rat (REDFERN et al., 1984) have been shown to result in increases in both blood pressure and heart rate. The excitatory effects of limb ischemia on the cardiovascular function has been considered to be mediated by thin unmyelinated C-fibers arising from the ischemic muscles (STONER and MARSHALL, 1984), and these C-fibers have been suggested to be activated by chemical substances such as capsaicin (STONER, 1986).

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), an extract of various species of Capsicum including the Mexican chile pepper and Hungarian red pepper (paprika), is a potent chemical stimulant of afferent free nerve endings, resulting in intense pain and irritation on injection in various species of animals (PORSZAZ et al., 1955). Capsaicin has been shown to preferentially excite C-fiber afferents from skeletal muscles (KAUFMAN et al., 1982). Intravenous injection of capsaicin elicits hypotension, bradycardia, and apnoea (PORSZAZ et al., 1955; TOH et al., 1955). The hypotension and apnoea have been attributed to vagally mediated reflexes because these responses were abolished after vagotomy (TOH et al., 1955) and vagal cooling (PORSZAZ et al., 1957).

On the other hand, it has been shown that injection of capsaicin into arteries supplying skeletal muscles causes increases in systemic blood pressure, heart rate, cardiac output, and respiratory minute volume (TOH et al., 1955; WEBB-PEPOOL et al., 1972; CRAYTON et al., 1981). Capsaicin also produces increases in vascular resistance in the hindlimb, gut, and kidney (WEBB-PEPOOL et al., 1972; CRAYTON et al., 1981). These cardiovascular responses to capsaicin are known to be reflex in nature because the responses were abolished after sectioning the afferent pathway from the skeletal muscles.

There have been several studies describing the cardiovascular responses to injection of capsaicin into the arterial blood supply to skeletal muscles (TOH et al., 1955; WEBB-PEPOOL et al., 1972; CRAYTON et al., 1981) but none of them have investigated the sympathetic efferent response to this noxious chemical stimulant. Thus, the present study was performed in order to examine the renal sympathetic nerve response accompanying the cardiovascular response to intra-arterial injection of capsaicin. As intra-arterial and intravenous injection of capsaicin can elicit changes in systemic blood pressure, the role of the systemic baroreceptors in modifying the sympathetic and cardiovascular responses to intra-arterial and intravenous capsaicin was also investigated by severing these afferent nerves.

METHODS

General procedures. Eight adult mongrel dogs of either sex weighing between
5.5 and 9.5 kg were anesthetized with sodium pentobarbital (25 mg/kg, i.v.). The trachea was intubated with auffed endotracheal tube and the animals were mechanically ventilated with room air delivered from a Harvard large-animal respirator. A polyethylene cannula was placed in the lower abdominal aorta via the right femoral artery and connected to a pressure transducer (Gould, U.S.A.) for measurement of arterial blood pressure. Mean arterial blood pressure (MBP) was obtained by electrical averaging. Heart rate (HR) was measured by a cardiotachometer (San-ei 2140, Japan) triggered by Lead-II of the electrocardiogram. The right femoral vein was cannulated for the administration of fluids and drugs. Gallamine triethiodide (1 mg/kg) was administered intravenously to avoid cardiovascular effects secondary to muscle movement. Additional doses of pentobarbital and gallamine were administered when they were needed. The vagi and the carotid sinus nerves were isolated bilaterally for later denervation.

A cannula was introduced into the left femoral artery through one of its branches to administer capsaicin intra-arterially within 5 s. Capsaicin (Sigma, U.S.A.) was dissolved in physiological saline (50 μg/ml).

Recording and quantification of renal nerve activity. A left renal sympathetic nerve along the renal artery was exposed retroperitoneally via a left flank incision. The nerve was carefully isolated from the renal artery and the surrounding connective tissue. The crushed distal end of the nerve was placed on a bipolar silver electrode for recording renal nerve discharges and was immersed in mineral oil to avoid drying. Recorded discharges were amplified and displayed on a dual beam oscilloscope (Iwatsu Electric Co. Ltd. SS-5116, Japan) and monitored by an audiospeaker. The neurogram was rectified and integrated by an R-C integrator circuit (time constant of 2 s). Nerve discharges as well as other parameters were displayed on a multichannel recorder (San-ei, Recti-Horiz 85, Japan) and fed into magnetic tape for later analysis. The resting spontaneous nerve activity was normalized to 100% for each nerve preparation in each animal in order to compare nerve discharges between animals and preparations with different numbers of active fibers. Thus, changes in nerve activity are expressed as percentages of the resting spontaneous nerve activity in the control period preceding each injection. Background noise in the neural recordings was determined after crushing the nerves or after treating with a ganglionic blocking agent, hexamethonium bromide (0.5 mg/kg, i.v.) at the end of each experiment. This background noise was subtracted from the neural signal when the data were quantified.

Protocol. In each animal the response to both intra-arterial (4 ± 1 μg/kg) and intravenous (6 ± 1 μg/kg) injection of capsaicin was assessed in three groups: 1) intact baroreceptors, 2) after bilateral cervical vagotomy, and 3) after cervical vagotomy together with bilateral carotid sinus denervation. Dosage of capsaicin was fixed within the individual animal. Control injections consisted of an equal volume of saline, and were made both intra-arterially and intravenously in all animals.

The experimental protocol was begun at least 1 h after the completion of the
initial surgery. Firstly the responses to intra-arterial and intravenous capsaicin were assessed in animals with intact baroreceptors. At least 10 min was allowed between each injection of capsaicin, and the order of the injections was randomized. After cervical vagotomy 30 min were allowed for stabilization in each parameter and the responses to intra-arterial and intravenous capsaicin were reassessed. Then, complete denervation of the systemic baroreceptors was achieved by additional sectioning of the bilateral carotid sinus nerves. After a further stabilization period of 20–30 min, complete baroreceptor denervation was confirmed by the lack of a reflex change in heart rate and renal nerve activity to the intravenous administration of phenylephrine (2–4 μg/kg, i.v.) and sodium nitroprusside (5 μg/kg, i.v.). Both intra-arterial and intravenous capsaicin injections were then carried out as described above.

Statistical analysis. All values in this text are reported as mean ± S.E. Percent values of nerve activity were converted to arcsine square root percentages for statistical analysis. Statistical comparisons were made using unpaired t-tests. A p-value of 0.05 or less was considered statistically significant.

RESULTS

Responses to intra-arterial capsaicin

Figure 1 shows a typical example of the response to intra-arterial capsaicin in animals with intact baroreceptors. Injection of capsaicin into the femoral artery resulted in concomitant increases in MBP and HR. However, renal nerve activity (RNA) showed a biphasic response; initial increase followed by decrease. As shown in Fig. 2, changes in MBP and HR 20 s after intra-arterial capsaicin injection were +43 ± 8 mmHg and +33 ± 14 beats/min, respectively. There followed by a recovery to the control so that 60 s after the injection changes in MBP and HR were +8 ± 5 mmHg and −4 ± 4 beats/min, respectively. Changes in RNA were +52 ± 28% of the control 20 s and −30 ± 8% of the control 60 s after intra-arterial injection of capsaicin.

After vagotomy the response to intra-arterial injection of capsaicin was characterized by similar increases in MBP (+44 ± 13 mmHg) and HR (+26 ± 11 beats/min) to those observed in animals with intact baroreceptors. However, the renal nerve response to intra-arterial injection of capsaicin differed significantly from that seen in animals with intact baroreceptors. The initial increase in RNA did not differ significantly between before (+52 ± 28% of the control) and after vagotomy (+71 ± 27% of the control). But the late decrease in RNA observed after intra-arterial injection of capsaicin in animals with intact baroreceptors (−30 ± 8% of the control) was reversed to an increase, which value of RNA 60 s after injection was +5 ± 10% of the control (Fig. 2). After complete baroreceptor deafferentation intra-arterial injection of capsaicin resulted in a significant elevation of MBP (+31 ± 10 mmHg), which was similar in magnitude to that observed in animals with intact baroreceptors. However, this pressor response was prolonged,
Fig. 1. An example of the response to intra-arterial injection of capsaicin in animals with intact baroreceptors. HR, heart rate; SBP, systemic blood pressure; MBP, mean blood pressure; RNA, renal nerve activity.

resulting in a significantly higher change in MBP (+25±8 mmHg) even at 60 s after intra-arterial injection of capsaicin when compared with the value before the injection. RNA was significantly elevated (+49±9% of the control) at 20 s and was +14±10% of the control 60 s after intra-arterial injection of capsaicin.

Responses to intravenous capsaicin

As shown in Fig. 3, intravenous capsaicin, which was injected through the femoral vein in animals with intact baroreceptors, resulted in significant falls in MBP (−19±11 mmHg at 20 s). RNA was reduced significantly by −16±7% of the control at 20 s after the injection. After vagotomy, intravenous injection of capsaicin reversed to increase MBP (+8±7 mmHg at 20 s) and HR (+4±2 beats/min at 20 s). In addition, the decrease in RNA, which was observed after intravenous injection of capsaicin in animals with intact baroreceptors, did
Fig. 2. Changes in mean blood pressure (MBP), heart rate (HR), and renal nerve activity (RNA) after intra-arterial injection of capsaicin in baroreceptor intact, vagotomized, and vagotomized and carotid sinus denervated (CSD) animals. Open bars indicate values of changes in MBP, HR, and RNA at 20 s and dotted bars values at 60 s after the injection. Asterisks indicate a significant difference ($p < 0.05$) from baseline values. Solid circles indicate a significant difference ($p < 0.05$) compared to values obtained in animals with intact baroreceptors within each time interval.

not occur following vagotomy. Change in RNA at 20 s after intravenous injection was $+22 \pm 19\%$ of the control. After complete baroreceptor deafferentation, intravenous injection of capsaicin did not produce any significant change in MBP and HR, but caused significant increase in RNA ($+19 \pm 7\%$ of the control) when compared with animals with intact baroreceptors (Fig. 3). Figure 4 shows an example

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Fig. 3. Changes in mean blood pressure (MBP), heart rate (HR), and renal nerve activity (RNA) after intravenous injection of capsaicin in animals with intact baroreceptors (open bars), vagotomized (filled bars), and vagotomized and carotid sinus denervated (CSD) animals (dotted bars). Asterisks indicate a significant difference ($p < 0.05$) from baseline values. Solid circles indicate a significant difference ($p < 0.05$) compared to values obtained in animals with intact baroreceptors.

from this group. No significant changes in central venous pressure were observed after intravenous injection of capsaicin in all animals studied. Neither intra-arterial nor intravenous injection of the vehicle solution produced any significant change in all parameters.

DISCUSSION

When capsaicin was injected into an artery supplying the hindlimb in dogs with intact baroreceptors, there was a consistent significant increase in mean arterial
pressure and heart rate. These findings confirm previous investigations using both the dog (WEBB-PEPLOE et al., 1972; CRAYTON et al., 1981) and the cat (TOH et al., 1955). These previous studies showed that intra-arterial injection of capsaicin induced cardiovascular responses reflexly because these responses were abolished by sectioning the nerve supplying to the limb. The present study showed that capsaicin into the femoral artery produced a biphasic response in renal nerve activity; an increase followed by a decrease. This late decrease in renal nerve activity may be due to a stimulating effect of capsaicin on the cardiopulmonary receptors, resulting from leakage of capsaicin into the systemic circulation. The variable effects of intra-arterial injection of capsaicin on blood pressure observed by TOH et al. (1955) have been attributed to stimulation of cardiopulmonary receptors by capsaicin that leaked into the systemic circulation (CRAYTON et al., 1981). The doses of capsaicin used in the present study were similar to the range of doses used by the previous

*Japanese Journal of Physiology*
reports (TOH et al., 1955; WEBB-PEPLOE et al., 1972; CRAYTON et al., 1981). Intravenous injection of capsaicin has been shown to elicit bradycardia, hypotension, and apnoea by stimulating pulmonary receptors (BEVAN, 1962; COLERIDGE et al., 1964; PORSZAZ et al., 1957; TOH et al., 1955). The late sympathoinhibition observed in the present study could also be a baroreceptor mediated response triggered by an increase in arterial blood pressure. To test these possibilities, we injected capsaicin intravenously in the intact animals in order to stimulate the systemic baroreceptors. This study showed that intravenous injection of capsaicin caused bradycardia and hypotension as previously reported by other investigators (TOH et al., 1955; PORSZAZ et al., 1957; BEVAN, 1962; COLERIDGE et al., 1964). Additionally, the present study showed that the hypotension observed after intravenous injection of capsaicin was accompanied by a significant decrease in renal nerve activity. These responses were reversed to increase after sectioning the vagal nerves. Thus the present findings suggest that capsaicin injected intravenously stimulates cardiopulmonary receptors leading to a vagally mediated reflex response.

In the present study, we showed that vagotomy abolished the late sympathoinhibition which occurred after intra-arterial injection of capsaicin in the intact animals. These results suggest a possibility that the late decrease in renal nerve activity is mediated by the stimulation of vagal afferent fibers. Capsaicin leakage into the cardiopulmonary circulation after the intra-arterial administration may stimulate cardiopulmonary receptors directly, because capsaicin did not cause a significant increase in central venous pressure, and therefore did not stimulate cardiopulmonary receptors by stretching of the atrial wall. Another possibility is that reflex activation of the systemic baroreceptors by an increase in arterial blood pressure may contribute to the late sympathoinhibitory response to the intra-arterial injection of capsaicin. Evidence to support this possibility is provided by the present findings that the pressor response and sympathoexcitation were more prolonged in dogs with complete baroreceptor denervation than in the intact animals when capsaicin was injected intra-arterially. Thus, it appears that intra-arterial injection of capsaicin into the femoral artery results in increases in blood pressure, heart rate, and renal nerve activity. This sympathoexcitatory response is modified and minimized primarily by the activation of cardiopulmonary vagal afferents and also by an activation of the arterial baroreflex by the increase in systemic blood pressure.

Furthermore, the present study is in agreement with the findings of WEBB-PEPLOE et al. (1972) and CRAYTON et al. (1981). They reported decreases in renal blood flow following intra-arterial injection of capsaicin. The effect of capsaicin on renal vasoconstriction, which results from renal sympathoexcitation as shown in this study, is consistent with the findings of a decrease in renal blood flow observed during the pressor response induced by stimulation of afferent somatic nerve (FELL, 1968; JOHANSSON, 1962; HILTON and MARSHALL, 1982). Activation of nociceptive afferent fibers by electrical stimulation (HILTON and MARSHALL, 1982) has been implicated in the development of the defense reaction, which is also characterized by a decrease in renal blood flow (HILTON, 1966; HILTON and REDFERN,
1986). Therefore, it seems likely that stimulation of capsaicin-sensitive nociceptors in skeletal muscles produces a similar response to stimulating somatic nerves electrically which itself activates nociceptive afferents.

Kaufman et al. (1982) showed that the unmyelinated C-fibers were primarily responsible for the cardiovascular changes evoked by intra-arterial injection of capsaicin. The free nerve endings of these C-fibers are located in skeletal muscles (Crayton et al., 1981). The rise in blood pressure and tachycardia in response to application of bilateral hind limb tourniquets in the rat (Redfern et al., 1984) is caused by afferent impulses originating in the ischemic muscles (Stoner, 1986). In support of this investigation, Alam and Smirk (1937) showed that the pressor response to unilateral limb ischemia in human subjects is related to the bulk of the ischemic muscles. The afferent impulses responsible for these changes during limb ischemia arise in polymodal receptors in the ischemic muscles (Mense, 1977) and ascend to the spinal cord via the thin unmyelinated C-fibers (Stoner and Marshall, 1982, 1984). Mense and Stahnke (1983) characterized the behavior of receptors in skeletal muscles and designated one type as nociceptive which transmit the pain of ischemic muscle contractions. Therefore, from these observations including our results, it seems likely that intra-arterial injection of capsaicin activates these nociceptive receptors in skeletal muscles to produce the pressor response and tachycardia. Additionally, the present study provides direct evidence that stimulation of receptors in skeletal muscles produces a sympathoexcitation. Such a sympathoexcitation may be of great importance in the cardiovascular response to nociceptive afferent impulses produced by somatic nerve stimulation and limb ischemia. The cardiovascular response to peripheral injuries in animals may be related to an activation of chemosensitive nerve endings in the damaged tissues. It is unclear from the present study what the chemical stimulus for the activation of these free nerve endings is, although potassium ions (Harpuder and Stein, 1943), metabolic products (Moore et al., 1934), and peptides such as bradykinin (Sicuteri et al., 1865; Dichièsi et al., 1975) have been implicated in this response. Further studies are required to establish more exactly the contribution of neural and humoral factors to the cardiovascular response during limb ischemia.

The authors wish to thank Ms. R. Kanzawa and Ms. R. Tsukada for their secretarial assistance. Richard O. Jones is a post-doctoral fellow supported by Japan Society for the Promotion of Science (1989 program).

All experiments reported here conformed to the Guiding Principles for the Care and Use of Animals of the Physiological Society of Japan.

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Japanese Journal of Physiology


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