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Heat loss reaction to capsaicin through a peripheral site of action

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1 The intravenous injection of 15 μg capsaicin produced an increase in the temperature of tail skin and paw pad and a fall in the colon temperature in conscious rats. These reactions reflect increased heat dissipation.

2 The increase in skin temperature induced by intravenous capsaicin was absent when the function of small diameter primary afferent neurones was impaired by treatment of the rats with capsaicin as neonates. Thus it appears that intravenous capsaicin triggered the thermoregulatory response predominantly by stimulation of peripheral heat receptors.

3 By means of local application of capsaicin to the nerves of the hind leg and by their chronic denervation, by treatment with phenoxybenzamine and guanethidine, evidence was obtained that reflex withdrawal of sympathetic vasoconstrictor tone mediates the heat loss reaction to intravenous capsaicin.

Introduction

Capsaicin given to rats, first stimulates central and peripheral heat receptors leading to a fall in body temperature accompanied by increased heat loss. Subsequently it desensitizes or damages the heat receptors leading to long-lasting hyperthermia and an inability of the rat to regulate its body temperature when exposed to increased ambient temperatures (Szolcsányi & Jancsó-Gábor, 1973; Szikszay, Obál & Obál, 1982). The main routes of heat dissipation in the rat are cutaneous vasodilatation in the tail and the paw pads, salivation and behavioural thermoregulation (Roberts & Mooney, 1974). Obál, Hajós, Benedek, Obál & Jancsó-Gábor (1981) showed that after capsaicin-desensitization the deficiency of the reactions leading to heat loss may be due to an impaired heat sensitivity caused by an effect of capsaicin on hypothalamic or other central and peripheral heat sensors. An effect of capsaicin on thermoregulation by stimulation and subsequent desensitization of hypothalamic heat sensors has been described on several occasions (Jancsó-Gábor, Szolcsányi & Jancsó, 1970b; Rabe, Buck, Moreno, Burks & Dafny, 1980; Cormarèche-Leydier, 1981; Hori, 1981; Hori & Tsuzuki, 1981; Dib, 1982). A peripheral site of action of capsaicin on polymodal nociceptors and heat receptors, which are first stimulated and subsequently inhibited, has also been demonstrated (Szolcsányi, 1977; Foster & Ramage, 1981; Kenins, 1982; Szolcsányi, 1982).

The present study was performed to investigate the immediate effect of capsaicin given intravenously to rats on heat dissipation mechanisms by measuring the temperature of the tail skin or the paw pad simultaneously with colon temperature. Systemic pretreatment with capsaicin, local application of capsaicin to the nerves of the hind leg and their chronic denervation, treatments with phenoxybenzamine and guanethidine were used to analyse the afferent and efferent pathways of the heat dissipation mechanism induced by capsaicin given intravenously.

Methods

Sprague-Dawley rats (strain OFA-SD, Himberg, Austria) of either sex weighing 250–350 g were used. Two days before the experiment the rats were anaesthetized with ether and a polyethylene cannula was implanted into a jugular vein under sterile conditions.

During the experiment the animals were kept singly in cages (36 × 21 × 15 cm) at an ambient temperature of 22–23°C. Diazepam (5 mg kg^{-1}, i.p.) was given to all rats 5 min before the experiments in order to reduce locomotion and to avoid heat production caused by locomotion.

A stock solution of capsaicin (10 mg ml^{-1}) was prepared in a mixture of ethanol: Tween 80: 0.9%

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NaCl 10: 10: 80; further dilutions were made in 0.9% NaCl to obtain a final concentration of 5 or 15 μg capsaicin in 0.3 ml. Thermoprobes (Digimed F5, F3a) were placed 6 cm deep in the colon and on the dorsal side of the tail 2 cm distal from its base or on the plantar side of each hind paw. Five min after the diazepam injection, 0.3 ml of the vehicle or of the capsaicin solution was injected into the jugular vein via the implanted cannula. Colon and tail temperature or colon and paw pad temperature were measured with a telethermometer (Digimed H11) before and after the intravenous injection at intervals of 2.5 min up to 30 min.

Pretreatments

Neonatal rats were anaesthetized with ether and injected with capsaicin (50 mg kg^{-1}, s.c.) according to Gamse, Holzer & Lembeck (1980). Guanethidine (20 mg kg^{-1}, s.c.) was injected 24 h before the experiment, a treatment known to destroy completely postganglionic sympathetic vasoconstrictor tone (Lembeck & Holzer, 1979). The α-adrenoceptor antagonist, phenoxybenzamine (5 mg kg^{-1} s.c.), was injected 24 h before the experiment and the β-adrenoceptor antagonist, (±)-propranolol (0.3 mg kg^{-1} s.c.), 15 min before the injection of capsaicin (Vapaatalo & Saynävälammi, 1980). The cholinoceptor antagonist, atropine (1.0 mg kg^{-1} s.c.), was injected 15 min before the experiment (Weiner, 1980).

In one set of experiments capsaicin was applied locally to the sciatic and the saphenous nerves of one hind leg 4 days before the experiment (for method see Gamse, Petsche, Lembeck & Jancsó, 1982). In another set of experiments the sciatic and saphenous nerves of one leg were ligated and cut distal to the ligation under ether anaesthesia 4 days before the experiment.

Drugs

Atropine sulphate and capsaicin (Merck, Darmstadt, F.R.G.), diazepam (Hoffmann-La Roche, Basle, Switzerland), guanethidine sulphate (Ciba-Geigy, Basle, Switzerland), phenoxybenzamine HCl (Röhm Pharma, Weiterstadt, F.R.G.) and (±)-propranolol HCl (ICI, Macclesfield, Great Britain) were used.

Results

Tail skin temperature

Control rats which received an intravenous injection of 0.3 ml of the vehicle showed a fall in colon temperature of -0.7 ± 0.1°C ($n = 6$) and a fall in tail skin temperature of -1.4 ± 0.1°C during the following

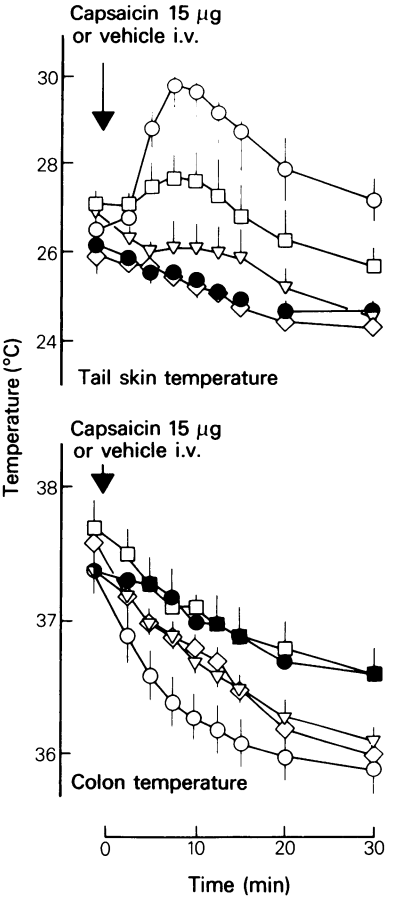

Figure 1 Tail skin and colon temperature in rats following the intravenous injection of 15 μg capsaicin or its vehicle into a jugular vein. All rats received diazepam (5 mg kg^{-1} i.p. 5 min prior to capsaicin or its vehicle i.v.). No pretreatment plus vehicle injection (●), no pretreatment plus capsaicin (15 μg)injection (○). Additional treatments: capsaicin, 50 mg kg^{-1} s.c. as neonates (◇); phenoxybenzamine, 5 mg kg^{-1} s.c. 24 h beforehand (▽); guanethidine, 20 mg kg^{-1} s.c. 24 h beforehand (□). Mean values, vertical lines indicate s.e.mean; $n = 6$.

30 min (Figure 1). The rats were awake but showed little movement due to the pretreatment with diazepam (5 mg kg^{-1} i.p.).

An intravenous injection of 5 μg capsaicin was followed by a rise in tail skin temperature of $+2.3 \pm 0.2°$ ($n = 6$) within 10 min and a fall in colon temperature of -0.4 ± 0.1°C. When 15 μg capsaicin was injected intravenously a larger rise in tail skin temperature occurred ($+4.5 \pm 0.3°$, $n = 6$, Figure 1). These temperature changes are calculated with reference to the fall in temperature which occurred after the injection of the vehicle alone. Repetition of the intravenous injection of 15 μg capsaicin in 6 animals

after one day produced similar changes in tail and colon temperature. This dose of capsaicin did not evoke any signs attributable to the stimulation of nociceptors.

Rats which had been treated neonatally with capsaicin did not show an increase in tail skin temperature in response to capsaicin injected intravenously (Figure 1). The decline in colon temperature following the injection of capsaicin in rats treated neonatally with capsaicin did not differ from that seen in rats treated neonatally with capsaicin and injected with the vehicle (latter result not shown).

Pretreatment of rats with atropine ($n = 4$) or propranolol ($n = 4$) did not influence the capsaicin-induced changes in tail and colon temperature. The tail skin temperature of rats pretreated with guanethidine was higher than that of controls (+ 0.8°C, $n = 6$, $P < 0.05$, two sample t test), their colon temperature was not changed. In animals pretreated with guanethidine or phenoxybenzamine, the intravenous injection of 15 μg capsaicin did not produce a significant change in tail skin or colon temperature when compared with changes seen after the intravenous injection of the vehicle only (Figure 1).

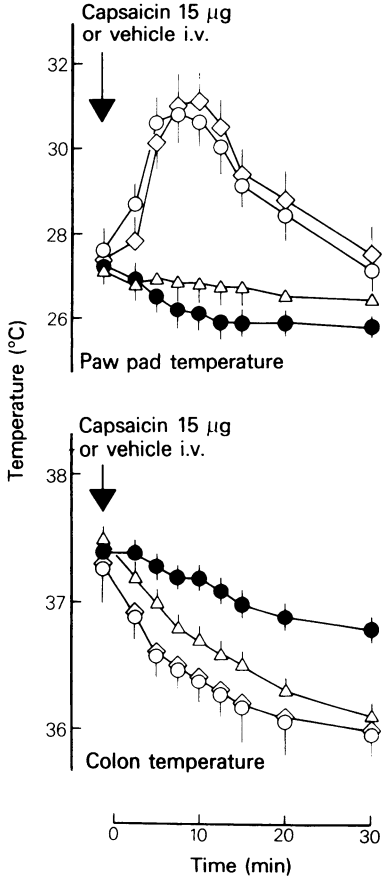

Figure 2 Paw pad and colon temperature in rats following the intravenous injection of 15 μg capsaicin or its vehicle into a jugular vein. All rats received diazepam (5 mg kg^{-1} i.p. 5 min prior to capsaicin or its vehicle i.v.). No pretreatment plus vehicle (●); no treatment plus capsaicin (15 μg) injection (○). Additional manipulations: capsaicin applied locally to the sciatic and saphenous nerve of one hind leg 4 days beforehand (◇); section of both nerves of one hind leg 4 days beforehand (△). Mean values, vertical lines show s.e.mean; $n = 6$.

Paw pad temperature

Control rats which received 0.3 ml of the vehicle intravenously showed a decline in their paw pad temperature of -1.4 ± 0.2°C ($n = 6$) during the following 30 min (Figure 2). After the intravenous injection of 15 μg capsaicin the paw pad temperature increased by $+4.6 \pm 0.5$°C within 7.5 min when compared with the controls (Figure 2).

Four days after the local application of capsaicin to the nerves of the hind leg the effect of intravenous capsaicin remained unchanged ($+4.8 \pm 0.5$°C, Figure 2). Four days after section of both nerves of the hind leg, the denervated paw pad did not respond to the intravenous injection of 15 μg capsaicin with a rise in temperature (Figure 2). The rise in temperature induced by intravenous capsaicin in the contralateral sham-operated paw was unchanged ($+4.7 \pm 0.5$°, after 7.5 min, not shown in the figure). The decrease in colon temperature following intravenous capsaicin also remained unaffected by the denervation of one hind leg (Figure 2).

Discussion

The present investigation deals with the immediate increase in skin temperature and fall in colon temperature following a single intravenous injection of 15 μg capsaicin. Similar responses were observed when capsaicin (23 μg) was injected intraventricularly (Dib, 1982). Although capsaicin given intravenously is readily taken up into the brain (Saria, Skofitsch & Lembeck, 1982) it can be calculated that the quantities used in the present experiments were too small to reach effective brain concentrations. Thus it is likely that the reactions observed after the intravenous injection of capsaicin were due to the stimulation of peripheral heat sensors, similar to the reactions elicited by the subcutaneous administration of small quantities of capsaicin (Szolcsányi, 1977). Using electrophysiological methods it has been shown that capsaicin, when applied topically to nerve endings in the skin can excite polymodal nociceptors (Szolcsányi, 1977; Foster & Ramage, 1981; Kenins, 1982).

That a single intravenous injection of 15 μg capsaicin did not cause capsaicin desensitization (which includes interference with thermoregulation: Jancsó-Gábor, Szolcsányi & Jancsó, 1970a; Obál *et al.*, 1981; Sziksay *et al.*, 1982) is demonstrated by the fact that repetition of the intravenous injection of 15 μg capsaicin after 24 h produced effects comparable to those following the first injection.

Capsaicin was shown initially to stimulate polymodal nociceptors and heat receptors and to release substance P and somatostatin from primary sensory neurones. Systemic capsaicin treatment abolished the capsaicin-evoked release of substance P and somatostatin from primary sensory neurones (for literature see Lembeck & Gamse, 1982). Capsaicin treatment of neonatal rats causes depletion of the peptides and functional impairment of primary sensory neurones, but fails to affect preoptic neurones involved in the process of thermoregulation (Jancsó & Jancsó-Gábor, 1980). The observation that rats which had been treated with capsaicin on the 2nd day of life did not respond to intravenous injection of capsaicin in later life with cutaneous vasodilatation suggests that thermosensitive primary neurones form the afferent part of the vasodilator reflex response to intravenous capsaicin.

Local application of capsaicin to the femoral and sciatic nerves was shown to impair the function of the substance P containing afferent fibres in the hind leg (Gamse *et al.*, 1982). In these rats the vasodilatation in the treated paw remained unchanged following intravenous capsaicin. It is therefore not the result of stimulation of sensory, substance P-containing fibres leading to the local release of substance P from their peripheral terminals because the function of these nerves is eliminated by local capsaicin treatment. In addition it can be concluded that the vasodilatation in the treated paw is a reflex response to sensory nerve stimulation by intravenous capsaicin in other parts of the body which were not influenced by the local application of capsaicin. Atropine and propranolol did not influence the vasodilatation by intravenous capsaicin. Muscarinic or β-adrenoceptors vasodilator mechanisms in the efferent pathway of vasodilatation in the tail skin can therefore be excluded. The abolition of the vasodilator response to intravenous capsaicin by guanethidine and phenoxybenzamine indicates that the efferent pathway is adrenergic and consists of a reflex withdrawal of the sympathetic vasoconstrictor tone induced by the afferent stimulation of capsaicin-sensitive fibres. Reduction in sympathetic vasoconstrictor tone was shown to account for the increase in peripheral blood flow in response to the application of heat (for literature see Euler, 1961). Although an increase in the tail skin temperature for some time after adrenolytic treatment would be expected, only a slightly increased tail skin temperature was seen one day after guanethidine treatment. Cooper, Fewings, Hodge & Whelan (1963) also showed that 20 h after administration of guanethidine, the blood flow in the human hand had fallen to near-control values. Thus it seems that so far unknown mechanisms lead to a normalization of the blood flow, despite the continuing absence of a sympathetic vasoconstrictor tone.

Section of the femoral and sciatic nerves completely abolished the vasodilatation in the paw following intravenous capsaicin. This shows again that the vasodilatation in the paw is neurogenic as withdrawal of the sympathetic vasoconstrictor tone is no longer possible (Figure 2).

The immediate heat loss response to a small dose of capsaicin may be reflected in the eating of capsaicin containing 'hot' meals in hot climates. Sufficient amounts of capsaicin are likely to be absorbed from the gastrointestinal tract to facilitate heat loss reactions such as cutaneous vasodilatation and sweating.

Recently it has been found that patients with familial dysautonomia (Riley-Day syndrome) who exhibit reduced pain and temperature sensitivity, have a reduced population of sensory neurones and a depletion of substance P immunoreactivity in the substantia gelatinosa of the spinal cord (Pearson, Brandeis & Cuello, 1982). Thus it appears likely that substance P-containing neurones are involved in the afferent part of temperature regulation.

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