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Short Communication

Direct Evidence for an Axonal Site of Action of Capsaicin

G. Jancsó[1], Elizabeth Király[1], and Aurelia Jancsó-Gábor[2]

[1] Department of Anatomy, University Medical School, P.O.B. 512, H-6701 Szeged
[2] Department of Physiology, University Medical School, Dóm tér 10, H-6720 Szeged, Hungary

SUMMARY

1. Local application of capsaicin to the sciatic nerve of rats induced a long-lasting increase in the nociceptive threshold as tested by the hot-plate method, and prevented neurogenic inflammation in the lateral part of the dorsal skin of the rat's paw.
2. Application of capsaicin to the saphenous nerve prevented the neurogenic inflammatory response, induced either by antidromic electrical stimulation of the saphenous nerve or by painting the skin with mustard oil, in the medial part of the dorsal skin of the paw.
3. The functional impairment induced by local capsaicin treatment of saphenous or sciatic nerves was strictly confined to the skin area supplied by the corresponding nerve.
4. It is suggested that local capsaicin treatment of peripheral nerves selectively damages the chemosensitive nerve fibres presumably by depleting their substance P content.

KEY WORDS: Capsaicin - chemosensitive pain fibres - neurogenic inflammation

INTRODUCTION

Capsaicin applied locally or parenterally renders sensory nerve endings insensitive to all kinds of chemical pain stimuli for a long time and inhibits neurogenic inflammation in adult rats and guinea pigs (Jancsó and Jancsó-Gábor, 1959; Jancsó, 1968; Jancsó et al., 1967). This functional impairment ensues at the level of the primary sensory neurone (Jancsó et al., 1967; Jancsó and Knyihár, 1975) without causing its degeneration (Joó et al., 1969); only mitochondrial swelling was observed in one type of sensory ganglion cells (Joó et al., 1969) and in some nerve endings of the rat cornea (Szolcsányi et al., 1975). However, a possible direct axonal site of action of capsaicin has not been considered.

On the other hand, neonatal capsaicin treatment has been shown to induce in rats the selective degeneration of chemosensitive primary sensory neurones involved in the mediation of chemogenic pain and in neurogenic inflammatory responses, as well (Jancsó et al., 1977; Jancsó and Király, 1980). In these animals a marked reduction in the number of unmyelinated afferent C fibres of peripheral nerves has been established (Jancsó et al., 1977, 1980).

We now report that capsaicin applied locally on peripheral nerves induces a long-lasting impairment in pain sensitivity and in neurogenic plasma extravasation.

METHODS

Sprague-Dawley rats of CFY strain of either sex were used. In one group of animals the sciatic nerve of both sides was exposed in the midthigh under ether anaesthesia and a piece of gelfoam (Spongostan, Ferrosan, Denmark) moistened with 0.1 ml of a 1% capsaicin solution was placed around the nerve. The gelfoam was removed after 15 min and the wound was closed with Michel clips. Another group of rats was treated in a similar way with only the solvent of capsaicin.

In another series of experiments the saphenous nerve of both sides was exposed high in the thigh and a piece of gelfoam moistened either with 1% capsaicin solution or with the solvent was placed on the nerves for 15 min. After removal of the gelfoam piece the wound was closed.

The nociceptive threshold was assessed by the hot-plate test. Rats were placed on a metal plate with a constant temperature of 56 °C and the reaction time (licking of the hind paw) was measured with a stop-watch. If no response ensued within 60 sec the rats were removed from the hot-plate to prevent tissue damage.

Neurogenic inflammation was brought about either by antidromic electrical stimulation of the saphenous nerve as described by Jancsó et al., (1967), or by painting the dorsal skin of the hind

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paw with mustard oil which, according to Jancsó (1960, 1968) and Jancsó et al., (1980), exerts its effect exclusively by the neurogenic route. Five min after i.v. injection of 50 mg/kg Evans blue dye the cut peripheral end of the saphenous nerve was stimulated antidromically with rectangular pulses by means of a bipolar platinum electrode for 10 min (10 V/1 ms/2 Hz), or the skin of the paw was painted with 5% mustard oil in liquid paraffin. At the end of electrical stimulation of the nerve, or 10 min after mustard oil painting of the paw the rats were killed by bleeding, the skin area was excised in which stimulation-induced extravasation could be expected and the exuded dye was extracted and measured spectrophotometrically according to the method of Jancsó-Gábor et al., (1967).

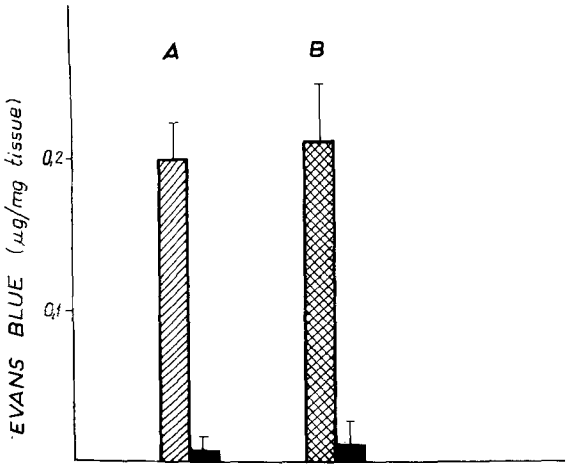

Fig. 1. A: Evans blue dye accumulation in the skin of the paw of rats in response to antidromic electrical stimulation of the saphenous nerve for 10 min (10 V/1 ms/2 HZ); ▨ = solvent-treated saphenous nerve, ■ = capsaicin-treated saphenous nerve. B: Plasma extravasation induced by mustard oil in the skin of the paw of rats on the saphenous nerve of which capsaicin was applied two weeks before; ▩ = skin area innervated by the sciatic nerve, ■ = skin area innervated by the saphenous nerve. Values are mean ± SEM of ten experiments

RESULTS

Local treatment of the sciatic nerves with capsaicin did not induce any apparent change in motor performance of the rat. Atrophy of the legs or other phenomena characteristic of section of the sciatic nerve have not been observed. On the other hand, a dramatic increase in the latency of the nociceptive response was observed as measured by the hot plate test. As shown in Table 1 this reduced pain sensitivity was evident already one day after local capsaicin treatment and persisted over the observation period.

TABLE 1. Latency of nociceptive response (sec) before (zero time) and at different intervals after local application of capsaicin to both sciatic nerves of rats

No rat	Time elapsed after the treatment (days)				
	0	1	7	16	33
1.	10		>60	>60	>60
2.	11		>60	>60	>60
3.	13		>60	>60	>60
4.	12		37	>60	>60
5.	7		36	30	38
6.	6		>60	>60	>60
7.	9	>60		>60	>60
8.	11	>60		>60	>60
9.	7	>60		>60	>60
10.	9	>60		>60	>60
11.	12	>60		>60	>60
12.	6	>60		>60	45

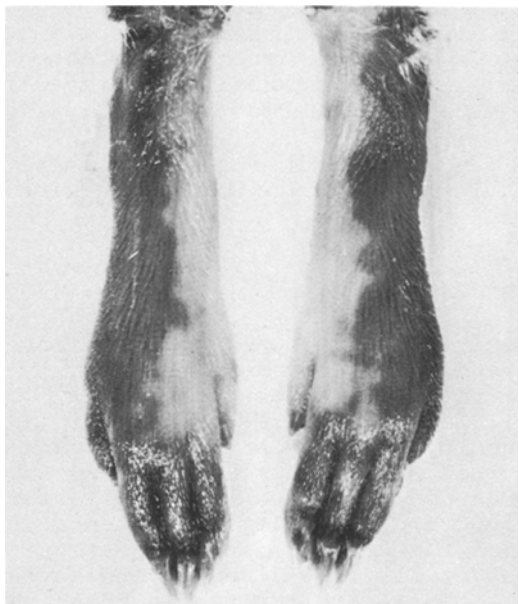

Fig. 2. Effect of local capsaicin treatment of the saphenous nerve on the plasma extravasation induced by mustard oil. Both saphenous nerves were treated with capsaicin 4 weeks before the experiment. Note that in the area innervated by the saphenous nerve no dye accumulation ensued

The nociceptive threshold of animals the sciatic nerves of which were treated with the solvent of capsaicin remained unchanged. Similarly, local capsaicin treatment of both saphenous nerves, which supply only the dorsomedial part of skin of the paw, did not increase the latency of the nociceptive response in the hot plate test.

Local capsaicin treatment of the saphenous or sciatic nerves resulted in a complete loss of the neurogenic inflammatory response in the skin areas innervated by the nerves, induced either by painting the skin with mustard oil or by antidromic electrical stimulation of the nerves (Fig. 1). Fig. 2 shows the effect of mustard oil on the skin of the paws of a rat whose saphenous nerves were treated locally with capsaicin 4 weeks before. There was no dye extravasation in the skin area innervated by the saphenous nerve.

DISCUSSION

The present experiments furnish direct evidence for an axonal site of action of capsaicin. In fact, local treatment of both sciatic nerves with capsaicin resulted in a long-lasting increase in the nociceptive threshold as tested by the hot-plate method, and parallel with this a complete abolition of the neurogenic inflammatory response ensued in the lateral part of the dorsal skin of the paw. This points to a marked impairment of the function of these nerves, since the sciatic nerve provides the sensory innervation of the plantar, and of the lateral part of the dorsal skin of the rat's paw (Devor et al., 1979). On the other hand, if capsaicin was applied to the saphenous nerve, which supplies the medial part of the dorsal skin of the rat paw, there was a complete abolition of the neurogenic inflammation induced either by antidromic electrical stimulation of the nerve or by painting the dorsal skin of the paw with mustard oil, in the area innervated by the nerve. The possibility that these phenomena were due to a systemic action of capsaicin can be excluded, since the functional impairment was strictly confined to the skin area supplied by the capsaicin-treated nerve.

These findings support the notion that the afferent (transmission of nociceptive impulses) and the efferent (release of a permeability increasing factor) functions of chemosensitive nerve fibres are intimately connected and are the expression of the two different functions of these same nerve fibres. Local capsaicin treatment of peripheral nerves presumably affects selectively the chemosensitive nerve fibres (Jancsó et al., 1977, 1980), since motor functions remained apparently intact. In addition, the impairment of unmyelinated adrenergic postganglionic nerves seems also unlikely, since following the ligation of the sciatic or saphenous nerves, fluorescence histochemistry revealed a similar accumulation of noradrenaline proximal to the ligature in both capsaicin treated and control nerves (unpublished observation).

As to the possible mechanism of the long-lasting functional impairment of chemosensitive nerve fibres induced by local capsaicin treatment of peripheral nerves, the following suggestions are to be considered.

Lembeck and his coworkers provided convincing evidence that substance P (SP) is the mediator of antidromic vasodilation and neurogenic plasma extravasation (Lembeck and Holzer, 1979; Gamse et al., 1980). Accordingly, SP has been proposed to be the transmitter released by chemosensitive primary sensory neurones (Lembeck and Holzer, 1979; Gamse et al., 1980; Nagy et al., 1980). Therfore, it is reasonable to assume that local capsaicin treatment of the nerve may result in a powerful depletion of SP from chemosensitive nerve fibres which in turn induces long-lasting functional impairment of these fibres. It is to be mentioned in this context that depletion of SP from spinal primary sensory afferents has been proposed as the mechanism of the prolonged thermal analgesia induced by capsaicin introduced directly into the subarachnoid space in the rat (Yaksh et al., 1979).

Another alternative would be that capsaicin applied topically may inhibit the axonal transport of SP which has been shown to take place in peripheral nerves (Hökfelt et al., 1975; Gamse et al., 1979). An inhibitory effect of capsaicin on the axoplasmic transport - similar to that of colchicine - has been suggested by Yaksh (cited by Virus and Gebhart, 1979). However, a general inhibitory effect of capsaicin on axoplasmic transport seems to be unlikely, since it failed to block the intraaxonal transport of noradrenaline. Therefore, in this case a highly selective action on SP-containing nerves should be assumed. Either the depletion of SP or the inhibition of its axonal transport may lead to a diminished SP content of the chemosensitive nerve endings and consequently this might be the reason why neurogenic inflammation cannot be induced in the skin areas supplied by the capsaicin treated nerves. Furthermore, if depletion of SP from chemosensitive primary sensory neurones actually takes place following local capsaicin treatment of

the peripheral nerves, one may argue that SP is involved in the transmission of nociceptive impulses. The demonstration of a decrease in SP content of the affected neuronal structures would afford further support to the involvement of SP in the function of these chemosensitive neurones as it has recently been suggested (Jessel et al., 1978; Lembeck and Holzer, 1979; Holzer et al., 1979; Gamse et al., 1980; Nagy et al., 1980). In addition, local capsaicin treatment may be helpful in studies concerning the role of different sensory nerve fibres in the transmission of nociceptive impulses. Finally, these findings may open new possibilities for the treatment of certain pathological pain conditions.

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REFERENCES

Devor, M., Schonfeld, D., Seltzer, Z., Wall, P.D.: Two modes of cutaneous reinnervation following peripheral nerve injury. J.comp.Neurol., 185, 211-220 (1979)

Gamse, R., Lembeck, F., Cuello, A.C.: Substance P in the vagus nerve. Immunochemical and immunohistochemical evidence for axoplasmic transport. Naunyn-Schmiedeberg's Arch.Pharmacol. 306, 37-44 (1979)

Gamse, R., Holzer, P., Lembeck, F.: Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. Br.J.Pharmac. 68, 207-213 (1980)

Holzer, P., Jurna, I., Gamse, R., Lembeck, F.: Nociceptive threshold after neonatal capsaicin treatment. European J.Pharmacol. 58, 511-514 (1979)

Hökfelt, T., Kellerth, J.-O., Nilsson, G., Pernow, B.: Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. Brain Res. 100, 235-252 (1975)

Jancsó, G., Knyihár, E.: Functional linkage between nociception and fluoride-resistant acid phosphatase activity in the Rolando substance. Neurobiology 5, 42-43 (1975)

Jancsó, G., Király, E.: Distribution of chemosensitive primary sensory afferents in the central nervous system of the rat. J.comp.Neurol. (1980, in press)

Jancsó, G., Király, E., Jancsó-Gábor, A.: Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature 270, 741-743 (1977)

Jancsó, G., Király, E., Jancsó-Gábor, A.: Chemosensitive pain fibres and inflammation. Int.J.Tiss.Reac. 2, 159-168 (1980)

Jancsó, N.: Role of nerve terminals in the mechanism of inflammatory reactions. Bull.Millard Fillmore Hosp. (Buffalo, N.Y.) 7, 53-76 (1960)

Jancsó, N.: Desensitization with capsaicin and related acylamides as a tool for studying the function of pain receptors. In: Pharmacology of Pain (R.K.S. Lim, ed.) pp. 33-55. Oxford-New York: Pergamon Press 1968

Jancsó, N., Jancsó-Gábor, A.: Daueraus-schaltung der chemischen Schmerzempfindlichkeit durch Capsaicin. Naunyn-Schmiedeberg's Arch.exp.Path.Pharmak. 236, 142-145 (1959)

Jancsó, N., Jancsó Gábor, A., Szolcsányi, J.: Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br.J.Pharmac. 31, 138-151 (1967)

Jancsó-Gábor, A., Szolcsányi, J., Jancsó, N.: A simple method for measuring the amount of azovan blue exuded into the skin in response to an inflammatory stimulus. J.Pharm.Pharmac. 19, 486-487 (1967)

Jessel, T.M., Iversen, L.L., Cuello, A.C.: Capsaicin-induced depletion of substance P from primary sensory neurons. Brain Res. 152, 183-188 (1978)

Joó, F., Szolcsányi, J., Jancsó-Gábor,A.: Mitochondrial alterations in spinal ganglion cells of the rat accompanying the long-lasting sensory distrubance induced by capsaicin. Life Sci. 8, 621-626 (1969)

Lembeck, F., Holzer, P.: Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn-Schmiedeberg's Arch.Pharmacol. 310, 175-183 (1979)

Nagy, J.I., Vincent, S.R., Staines,W.M.A., Fibiger, H.C., Reisine, T.D.:,Yamamura, H.I.: Neurotoxic action of capsaicin on spinal substance P neurons. Brain Res. 186, 435-444 (1980)

Szolcsányi, J., Jancsó-Gábor, A., Joó, F.: Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin. Naunyn--Schmiedeberg's Arch.Pharmacol. 287, 157-169 (1975)

Virus, R.M., Gebhart, G.F.: Pharmacologic actions of capsaicin: apparent involvement of substance P and serotonin. Life Sci. 25, 1273-1284 (1979)

Yaksh, T.L., Farb, D.H., Leeman, S.E., Jessel, T.M.: Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. Science 206, 481-483 (1979)

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