Agents and Actions, vol. 23, 1/2 (1988)

0065-4299/88/020004-08 $ 1.50+0.20/0

Antidromic vasodilatation and neurogenic inflammation

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Abstract

Antidromic stimulation of the lumbosacral dorsal roots of the rat 1.) evokes a long-lasting increase in cutaneous microcirculation of the paw as detected by the laser Doppler flowmeter, and 2.) induces plasma extravasation in the innervated skin areas and various pelvic organs. Both responses are strongly inhibited or abolished by systemic or local capsaicin desensitization. Cutaneous vasodilatation is evoked already by 1–2 pulses. Desensitization of the volar skin of the forearm abolishes the flare reaction both in the "afferent" and "efferent" side of the axon reflex. A dual sensory-efferent function for capsaicin-sensitive nociceptors is suggested. This local neuroregulatory mechanism mediates neurogenic inflammation, cutaneous vasodilatation and flare reaction not only when the receptors are activated by antidromic stimuli, but also when their orthodromic excitation by chemical means occurs.

Introduction

It was revealed in animal experiments twenty years ago [9] that antidromic stimulation of sensory nerves elicits not only vasodilatation but other characteristic signs of inflammation [11, 19–21]. This neurogenic inflammation is mediated by capsaicin-sensitive nerve endings and can be prevented by local or systemic pretreatment with the drug. The effect of capsaicin is neuroselective acting on primary afferent neurones, but not on efferent autonomic fibres [2, 8, 16, 20]. Substance P and other tachykinins – the putative cardinal mediators of neurogenic inflammation [13, 14, 19] – can be released and depleted from primary afferent neural elements but not from other types of neurones or their processes. Capsaicin pretreatment prevents also the development of neurogenic inflammation induced by orthodromic stimulation of nociceptive nerve terminals with pain producing substances. Non-neural inflammatory responses are not affected in this way [9, 10, 21].

Peripheral nerves contain admixed efferent fibres, which store and liberate not only biogenic amines but also vasoactive peptides [5]. Since all dorsal root fibres originate from sensory neurones of the spinal ganglia [7], therefore direct evidence for an efferent role of sensory nerve endings can be obtained when dorsal roots are stimulated instead of the peripheral nerves. The classical description of antidromic vasodilatation in the skin was also made in this way [1, 6, 12].

The present paper is the first approach of mapping which organs respond with inflammation to excitation of their sensory endings through stimulation of the spinal dorsal roots. Furthermore, utilization of the sensitive laser Doppler flowmetry enabled us to reveal new aspects of the classical antidromic vasodilatation response. Finally, the

concept of dual sensory-efferent function of capsaicin-sensitive nociceptors is summarized and new evidence for this theory [20] is presented in respect of the axon reflex response.

Methods

Vasodilatation in the plantar skin of the rat to antidromic stimulation of the dorsal roots

170–210 g Wistar rats of both sexes were anaesthetized by pentobarbitone (40 mg/kg i.p.) and pretreated with atropine sulphate (1 mg/kg i.p.). The animals were breathing spontaneously through a tracheal cannula and their rectal temperature was kept around 37 °C by a heating lamp. The lumbosacral dorsal roots were exposed after laminectomy and cut near to the spinal cord. In an oil pool formed from skin flaps the lumbar dorsal roots were stimulated (20–40 V, 0.5 ms, 1–8 Hz) by bipolar platinum hook electrodes. The hindleg was fixed to a supporting plate with modelling clay and the probe of a Periflux laser Doppler flow-meter (Perimed KB, Sweden) was placed to the middle region of the plantar skin of the paw. The output signal (V*) from the laser Doppler flow-meter was recorded on a pen recorder (MTA Kutesz).

Plasma extravasation, evoked by antidromic stimulation of the dorsal roots or trigeminal ganglion of the rat

Dorsal roots were stimulated as described above. Stimulation of the trigeminal ganglion was made with bipolar needle electrodes [9]. 50 mg/kg Evans blue was injected intravenously for detection the site of plasma extravasation. The dye was extracted from the tissues by formamide and determined quantitatively with a spectrophotometer (Beckman).

Capsaicin pretreatment of rats

For *systemic capsaicin* desensitization 30 + 60 mg/kg capsaicin was injected subcutaneously under aether anaesthesia in two consecutive days. The experiment was made 4–5 days after the last injection. *Perineural* treatment of the sciatic nerve was made by putting Fibrospum sponge cuff soaked with 1% capsaicin solution (10% ethanol, 10% Tween 80 and 80% NaCl) around the nerve in a length of 5 mm. Parafilm coating prevented the diffusion of the drug to the surrounding tissue. The sciatic nerve of the other leg was treated with the solvent and served as control. In both sides the exposures lasted for 30 min, then the wounds were sutured. The experiment was made 2–3 days after the operation. For topical desensitization of the eye 1% capsaicin solution was instilled into the eye three times within a day. The experiment was made 24 hours later.

Axon reflex flare induced in the human skin

Substance P (5–10 ng in 10 µl 0.9% NaCl) or capsaicin (100 ng in 10 µl) was injected intradermally in the volar forearm skin of 5 male volunteers (19–46 years) of the laboratory staff. The borders of flare and wheal reactions were marked with ink and transferred to transparent plastic.

Capsaicin pretreatment of the human skin

A square area of about 4 × 4 cm or a 3–4 cm long strip of the volar surface of the forearm was painted with 1% capsaicin solution in 95% ethanol. Within a period of 24–48 hours 5 times painting was performed. 50% dimethylsulfoxide was applied to the area for 1 min before the first three application to achieve deeper penetration of the drug into the skin. The corresponding skin area on the other forearm served as control and was treated with the solvents alone.

Results

Antidromic vasodilatation in the plantar skin of the rat

Antidromic stimulation of the spinal dorsal roots (L_4–L_6) with few impulses resulted in a remarkable long lasting enhancement in cutaneous microcirculation. 4 out of 5 rats responded already to 1–2 pulses (Fig. 1A). Note that 4–16 pulses at 2 Hz evoked vasodilatation lasting for several minutes. The possible role of current spread was excluded since no muscular contractions were observed in the non-paralysed animals. Systemic (Fig. 1B) or perineural (Fig. 1C) capsaicin pretreatment almost completely abolished the effect and a delayed small response was

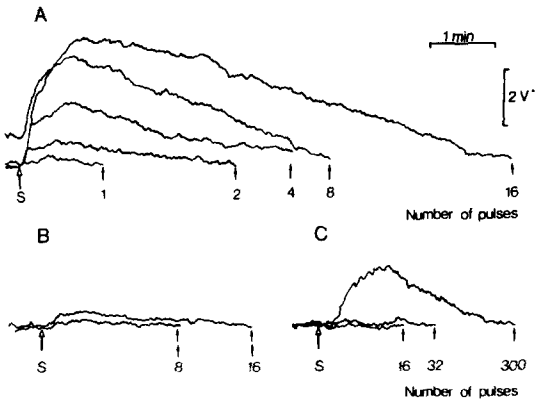

Figure 1
Laser Doppler recordings of microvascular blood flux of the glabrous skin of the paw of three (A, B, C) rats. Antidromic stimulations of the ipsilateral dorsal roots (L_4–L_6) start at the arrow S. Stimulation parameters: 20 V, 2 Hz, 0.5 ms, number of pulses as indicated. A: recordings from a control rat; B: recordings from a rat pretreated with systemic capsaicin injections (30+60 mg/kg s.c. 4 days before the experiment); C: recordings 3 days after perineural capsaicin treatment of the sciatic nerve. Calibration for laser blood flux signal and time are shown upper right of the figure. Note, the long duration of the increased microcirculation and the few number of pulses (unpublished data from ref. 24).

obtained only with long train of impulses (Fig. 1 C). The latency of the reactions in untreated animals was 5.3 ± 0.2 s. ($\bar{x} \pm$ S.E. $n=24$) at stimulation frequencies of 2–8 Hz. Solvent treatment of the nerves did not inhibit the responses ($n=4$).

Antidromic plasma extravasation in the rat

In agreement with earlier findings [9, 21] local or systemic capsaicin pretreatment prevents the plasma extravasation in the eyelids (Fig. 2 B, C upper row) elicited by antidromic stimulation of the trigeminal ganglion. Note, that local desensitization of the eye did not inhibit the blueing response in the muzzle (Fig. 2 B lower row).
5–5 min stimulation of the lumbosacral dorsal roots by putting 2–3 roots successively to the electrodes induced unilateral cutaneous dye accumulation in the lower abdominal, pelvic areas of the trunk and in the scrotum, tail and hind leg. The blueing response to stimulation of the right L_3, L_4 dorsal roots can be seen on Fig. 3, which shows

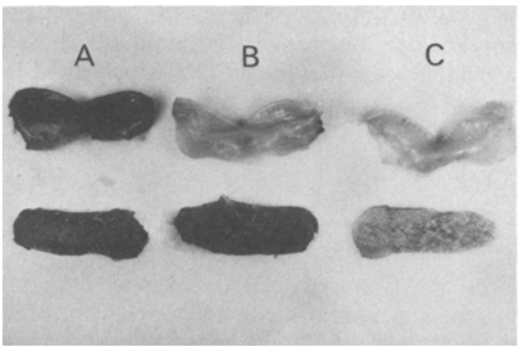

Figure 2
Evans blue accumulation in the eyelid (upper row) and muzzle (lower row) of three rats (A, B, C) in response to antidromic stimulation (20 V, 2 Hz, 1 ms for 10 min). A: tissues from a control rat; B: tissues from a rat in which the eye was desensitized by capsaicin instillations; C: tissues from a rat pretreated with systemic capsaicin injections as in Fig. 1.

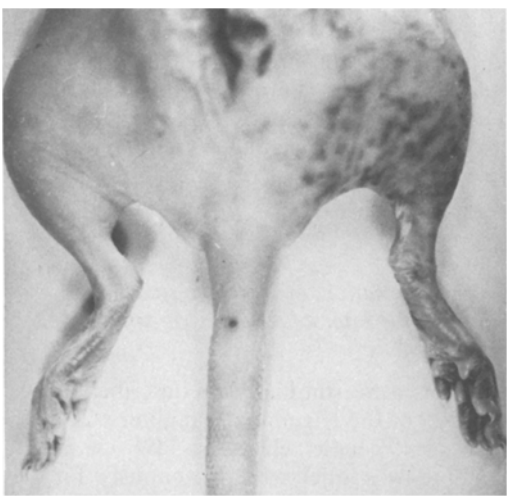

Figure 3
Plasma extravasation in the right hindleg evoked by antidromic stimulation (20 V, 5 Hz, 0.5 ms for 5 min) of the L_3 and L_4 lumbar dorsal roots.

the patchy blueing of the thigh, the marked response in the toes, but minimal dye accumulation in the middle part of the plantar skin of the paw. The latter is mainly innervated by the L_5 root. An unusual aberrant blueing spot appeared in this particular rat also in the root of the tail. Some quantitative data of cutaneous dye accumulation are depicted on Fig. 4.

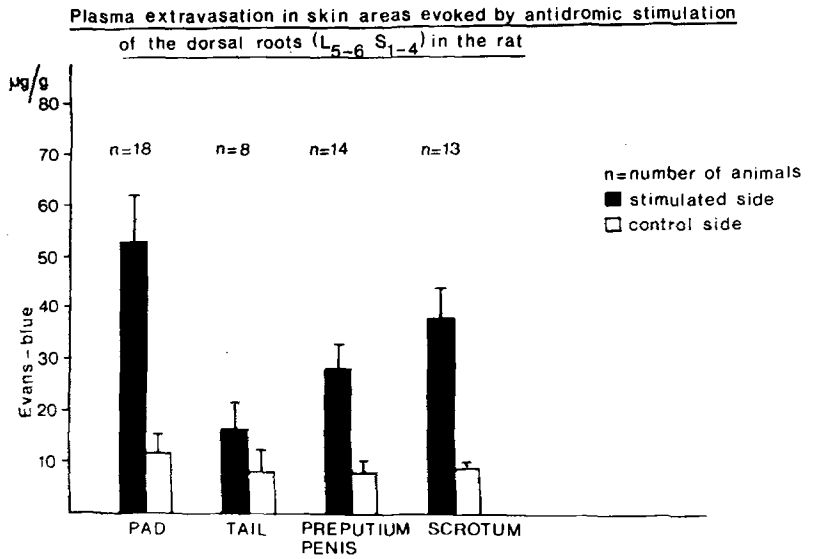

Figure 4
Plasma extravasation in skin areas evoked by antidromic stimulation of the dorsal roots (L_{5-6}, S_{1-4}) in the rat. Stimulus parameters: 20–40 V, 5 Hz, 0.5 ms for 5 min. Evans blue accumulation ($\bar{x} \pm$ S.E.).

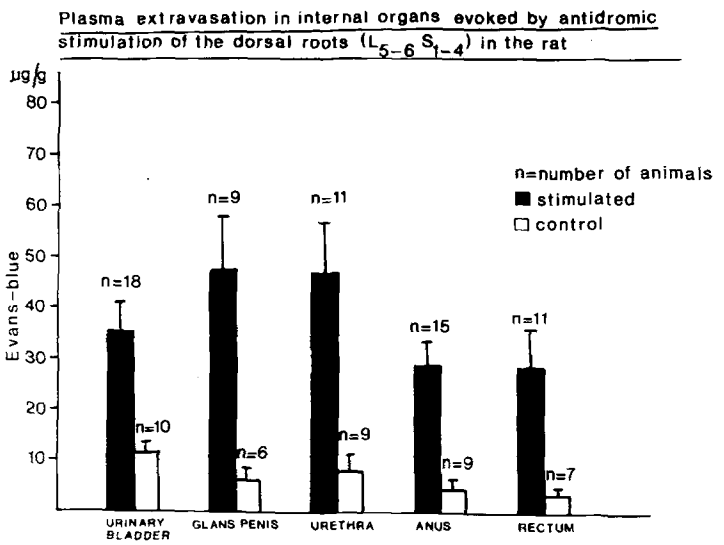

Figure 5
Plasma extravasation in pelvic organs evoked by antidromic stimulation of the dorsal roots as indicated on Fig. 4.

Plasma extravasation was observed also in the urogenital tract (urethra, urinary bladder, ureter, uterus, vagina, bulbus and glans penis, clitoris), in the rectum and anus, in the two caudal nipples, around the articular ligaments and subcutaneous tissues of the leg including the tendons and the fascia of the muscles. Evans blue content of some organs is shown on Fig. 5. It is important to note that in internal organs the dye accumulation was not so strictly unilateral, therefore control values

were obtained from a separate group of animals. No significant accumulation was observed in rats pretreated with 30+60 mg/kg capsaicin 4–5 days before the experiment ($n=4$) or in the vas deferens, testis, prostate gland, plantar and abdominal muscles of untreated animals.

Inhibition of the axon reflex flare in the human skin by topical capsaicin desensitization

The aim of these testings on five subjects was to analyse the capsaicin sensitivity of the hypothetical [15] "afferent" and "efferent" endings of the axon reflex in the flare reaction [3, 4, 23, 25]. The rate of recovery of the axon reflex flare was followed for several days by injecting substance P [5 ng] intracutaneously near to the edge of the capsaicin treated area.

Fig. 6 shows some results of a typical series of testings on one subject. The flare induced by substance P did not spread to the capsaicin treated area (first column on the figure) seven days after the treatment. This finding indicates a blockade of the "efferent" side of the response. Similar injection within the desensitized area (second column) prevented the development of flare not only inside, but also outside of the treated area. Thus, a blockade of the "afferent" side seemes also obvious. Furthermore, "jumping" of the flare reaction through the capsaicin treated strip (third column) shows that axonal conduction of the fibres is apparently not abolished under the treated area. Consequently, under the present experimental conditions the impaired function of the terminals of the axon reflex seems to be responsible for the blockade. The recovery of the flare response on the following days revealed that both the sensory and the mediator releasing sites regained their function in parallel (Fig. 6).

Capsaicin releases the mediator from the peripheral endings of the primary sensory neurone in a tetrodotoxin-resistant manner [20, 21]. Intracutaneous injection of capsaicin (100 ng) in the human skin evokes pain sensation lasting for few seconds and elicits a flare reaction. Pain sensation and the flare response, but not the reddening at the site of injection, was prevented by sc injection of lignocaine (1–2%, $n=4$–4). These vascular reactions were absent if capsaicin was applied to a skin area, the nerve supply of which had been degenerated [10].

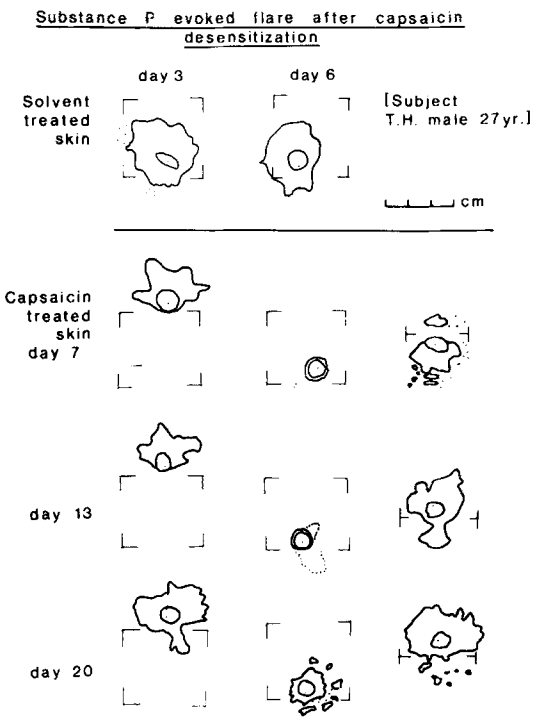

Figure 6
Flare and wheal reactions in the volar surface of the human forearm evoked by intracutan injection of substance P (5 ng). Injections were made near to the edge of skin areas (marked by faint lines) treated with capsaicin or with the solvent as indicated. For more details see text.

Discussion

Peripheral nerve terminals of capsaicin-sensitive sensory neurones are responsible for mediation of antidromic vasodilatation and neurogenic inflammation. There is no evidence that other type of neurones might evoke these responses. Among the cutaneous receptors mechano-heat sensitive nociceptors, particularly the C-polymodal nociceptors, are excited and can be desensitized by capsaicin [8, 16, 18, 22]. Antidromic stimulation of the fibres of these receptors elicits plasma extravasation in the skin around the receptive field [11].

Polymodal nociceptors – the most sensitive cutaneous endings to nociceptive chemicals – respond with few spikes to moderate mechanical stimuli (e.g. to pressing the skin with a tooth brush). These non-noxious stimulations do not

produce pain sensation in the human skin. Since 1–2 antidromic pulses already elicit cutaneous vasodilatation, this efferent function of these sensory neurones might be activated under physiological conditions and could participate in regulation of the microcirculation of the skin. Train of few more spikes induced long lasting responses and 30 pulses at 2 Hz is sufficient to produce manifest sign of inflammation [20]. Thus, in different kinds of inflammation, when chemically induced pain is present, neurogenic inflammatory response should participate in the tissue reaction. In inflammation evoked by pathological conditions or chemical agents the degree of neurogenic part depends on the activity of capsaicin-sensitive polymodal nociceptors, on one side, and the direct vasoactive effect of the mediators or irritant, on the other side. The role of neurogenic part in these *partial neurogenic responses* [21] can be revealed by capsaicin desensitization and sensory denervation.

It is remarkable that the present findings revealed, that antidromic stimulation of the dorsal roots induces plasma extravasation in a variety of internal organs. Detailed description of segmental innervation will be published elsewhere. It has already been shown that antidromic stimulation of the trigeminal, laryngeal, vagal, splanchnic, pudendal and pelvic nerves, the sciatic and brachial plexuses induces plasma extravasation in the mouth, respiratory airways, esophagus, bile duct, ureter, urinary bladder, penis and vagina [19–21]. Furthermore, topical or intraarterial capsaicin evoked also protein leakage from the vessels in different organs [19, 21]. In all these approaches, however, only indirect evidence support the notion that sensory nerve endings are responsible for the effect. Intervention of admixed autonomic fibres and reflex changes in microcirculation induced by the sensory stimulant effect of capsaicin can certainly modify or alter qualitatively the responses.

According to the classical concept [15], the mediator of antidromic vasodilatation is released from a nerve ending which is specialized for release of a transmitter. It is distinct in this way from the sensory receptor, which is specialized for initiation of afferent impulses. Thus, the classical axon reflex arrangement consists of a branching axon having a sensory receptor specialized for accepting stimuli on one end and an effector terminal for innervation of the vessels on the other end (Fig. 7 A).

Antidromic vasodilatation in the present experiments and electrically evoked axon reflex in the human skin [26] start only after a delay of few seconds. These data do not favour a specialized neuroeffector junction, since tachykinins, the putative transmitters of these responses are fast acting peptides. On the other hand, if axon reflex flare is elicited by transcutaneous electrical stimulation [26], the onset of vasodilatation at a distance of 15 mm corresponded to that obtained by antidromic stimulation of the rat's dorsal roots. This finding argues against the slow spreading of the flare response. "Jumping" the flare through a desensitized strip of skin neither favour the "cascade theory of flare response" [14]. Therefore, it is assumed that axonal branching (Fig. 7 B) or axo-axonal transmission between two polymodal fibres (17, see Fig. 7 C) is necessary for the development of flare reaction. It has been suggested, however, that at both ends of the axon reflex arch are receptors which possess a dual sensory-efferent function [10, 20, 21, 23]. The released mediator gains access to the arterioles and venules not by neuroeffector junction, but by diffusion (Figs. 7, and 8). The release process, like the generator potential, is tetrodotoxin-resistant and can not be abolished by local anaesthetics either [10, 21]. The concept of a dual sensory-efferent func-

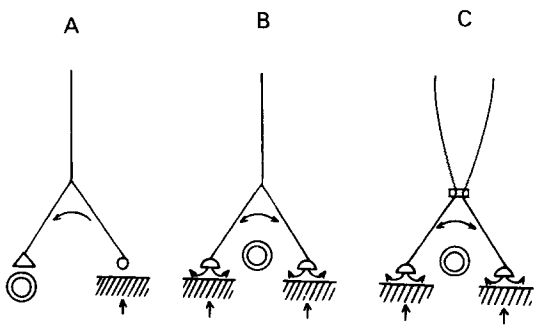

Figure 7
Schematic representations of different theories for the axon reflex arrangement. A: classical axon reflex theory of Lewis [15], according to which excitation of the receptor ending (○) activates a neuroeffector collateral ending (△) at the vessels through a unidirectional axon reflex; B and C: two versions of a bidirectional axon reflex arrangement with nerve endings having dual sensory-efferent function.

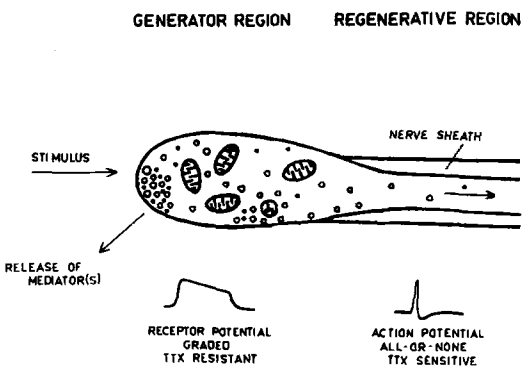

Figure 8
Theory of dual sensory-efferent function of capsaicin-sensitive nociceptors. For more details see text.

tion of peripheral terminals of capsaicin-sensitive primary afferent neurones [20] opens new horizons in local neuroregulatory mechanisms. Neurogenic responses including inflammatory reactions are evoked in this way at localized areas of various organs where capsaicin-sensitive endings are excited (local efferent function). On the other hand, activation of these endings evokes sensation and autonomic reflex responses which form the overall reaction of the organism triggered by the sensory impulses initiated from the same endings.

Acknowledgements.

This study was supported by grants OTKA No. 84 and ETT No. 911-11-92. I am grateful to Dr. Erika Pintér, Dr. Béla Sebök and Dr. Loránd Barthó for the valuable assistance in various parts of the experiments.

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