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DIRECT EVIDENCE FOR NEUROGENIC INFLAMMATION AND ITS PREVENTION BY DENERVATION AND BY PRETREATMENT WITH CAPSAICIN

BY

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The question of the role played by the sensory nerves in the mechanism of the inflammatory response arose at the beginning of this century. Bruce (1910, 1913) found, for the conjunctiva of rabbits and the skin of cats, and Breslauer (1919), for human skin, that after transection of the sensory nerve the hyperaemia and oedema caused by mustard oil failed to occur if the nerve had degenerated. The above experiments, as well as the classical investigations of Bayliss (1901, 1923), Lewis (1927) and Krogh (1929) led to the assumption that neurogenic inflammatory reactions are based on the axon reflexes taking place in the end branchings of the sensory nerves. The flare component of the triple response of human skin was also explained by an axon reflex (Lewis & Grant, 1924). Later, several authors who examined various inflammatory tests in animal experiments could not confirm that the nervous system played a role in the induction of the inflammation (Shimura, 1924 ; Jasmin, 1956 ; Heite & Höland, 1956 ; Hertting & Stoklaska, 1958). Thus the problem of neurogenic inflammation, which was once in the foreground of interest, was almost entirely neglected in modern research and, in recent years, experimental work has been confined mainly to the study of axon reflex flare in human skin (Chapman, Ramos, Goodell & Wolff, 1961 ; Chapman & Goodell, 1964).

Our investigations (Jancsó, 1960 ; Jancsó & Jancsó-Gábor, 1959, 1963) concerning capsaicin, the pungent principle of red pepper, drew attention again to the problem of neurogenic inflammation. By repeated administration of capsaicin the pain receptors can be desensitized to chemical stimuli. It is interesting that in such desensitized animals irritants like mustard oil, xylene, capsaicin and related acyl amides fail to cause pain and parallel with it fail to induce an inflammatory response. Jancsó (1960) established that the above inflammation-producing agents exert their effect by the neurogenic route (cf Keele & Armstrong, 1964). Recently Fearn, Karády & West (1965) also have shown that this is true for xylene.

In the present paper further results concerning the mechanism of neurogenic inflammation are reported.

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METHODS

Methods for the evaluation of the inflammatory reactions in rats

Two methods were used. In one of them we determined the vascular permeability quantitatively. For this purpose the well-known Evans blue test was combined with an extraction technique elaborated in our laboratory. The dye which escapes from the vascular system in the course of the inflammatory reaction was extracted from the tissues with methanol containing 1% suramin (Bayer 205). Extraction was complete in about 2 weeks and the quantity of the extracted dye was determined colorimetrically (Jancsó-Gábor, Szolcsányi & Jancsó, 1967).

By the other method the localization of the inflammatory reaction was rendered visible in histological preparations (Jancsó, 1960, 1961). Immediately before applying the inflammatory stimuli, a 1% colloidal silver solution was injected into the tail vein of the rat (10 mg/100 g). Thereafter, the parts of the body involved in the inflammation process turned brown. Whereas Evans blue and other dyes used in inflammation research produce only diffuse patches of colour, with colloidal silver a good microscopic picture can be obtained which reveals the finest details of localization.

In both methods the skin and mucous membranes were removed after killing the animals by bleeding.

Stimulation of the saphenous and trigeminal nerves in rats

The effect of antidromic stimulation of the saphenous and trigeminal nerves was investigated. Under pentobarbitone anaesthesia (40 mg/kg) the saphenous nerve was exposed and cut high in the thigh. The peripheral end was stimulated with rectangular pulses by means of a bipolar platinum electrode. A wet chamber was used to avoid drying of the nerve. Animals were kept on a warmed plate and, when needed, an infra-red lamp was used so that the ambient temperatures could be kept at 36° C. In the case of the trigeminal nerve, its ophthalmic branch, and sometimes the maxillary branch too, were stimulated intracranially by means of a stereotaxic apparatus. The stimulating electrode was bipolar and consisted of two sharp stainless steel needles with a diameter of 0.5 mm placed with their tips 1.9 mm apart. They were insulated except for about 1.5 mm at their tips. Under pentobarbitone or buthalitone anaesthesia the top of the skull was trepanned and through the hole the bipolar electrode was inserted vertically so that the nerve was situated between the tips of the needles. The coordinates of the top of the electrode were: posterior = 1–2.5, lateral = 2–2.2, vertical = 11–12.5 mm related to the centre of intersection of the sagittal and coronary sutures. The nerve was stimulated for 20 min by rectangular pulses, in most cases with a voltage of 3 V, a duration of 0.8 msec and a frequency of 200/sec. Animals were kept warm by means of an infra-red lamp. After each experiment the skull was opened and the location of the electrodes was established.

Inflammatory reactions induced by chemical irritants in rats

In the case of mucous membranes and the skin, inflammation was induced with substances which, according to previous experiments, exert their effects by the neurogenic route (Jancsó, 1960). Thus, the skin of the paws, muzzle or snout of rats was painted with 5% mustard oil in liquid paraffin, xylene or a solution of 2% ω-chloracetophenone in acetone; into the eye a saturated aqueous solution of mustard oil or solutions of capsaicin (1 mg/ml.), ω-chloracetophenone (1 mg/ml.), or nicotine (2 or 4 mg/ml.) were instilled. According to the Evans blue test—using a dose of 50–100 mg/kg of the dye—the inflammatory reaction reached its maximum after 10–15 min; at this point the animals were killed by bleeding and the extent of the inflammatory reaction was measured.

Denervation of areas innervated by saphenous or trigeminal nerves

The saphenous nerve of rats was cut high in the thigh under ether anaesthesia and the skin incision was sutured.

The trigeminal nerve of rats was subjected to diathermic cauterization, by means of a steel needle with a diameter of 1 mm which was, except for its tip, insulated with a thin polythene layer. Under ether anaesthesia a hole was made on the frontal bone of the skull at the level of the

interpupillary line, 1 mm lateral from the sagittal suture. The needle was inserted *via* this hole at an angle of 45° to the top of the skull until its tip reached the bone groove of the ophthalmic branch of the trigeminal nerve, or that of the Gasserian ganglion. The needle was inserted into the nerve or ganglion, which were then cauterized with a diathermic current.

In 60% of the animals cauterization produced complete denervation, about 20% perished, and in 20% the denervation was not complete. Complete denervation resulted in loss of the corneal reflex, and loss of sensitivity in an area extending to the tip of the nose and including the skin of the muzzle. Drying of the cornea was prevented by closing the palpebral fissure with a suture and cleaning the eye of accumulated secretions four times daily with cotton wool soaked in isotonic saline.

Recording of nerve action potentials

To study the mechanism of capsaicin desensitization action potentials were recorded from the peripheral end of the severed saphenous nerve. In these experiments the spinal cord of the rats were cut, under ether anaesthesia, between the VII cervical and I thoracic, as well as between the IV and V lumbar vertebrae, and the wounds were tamponed with cotton wool. During the experiment the animals were completely awake, breathed quietly and evenly, and were kept warm. The action potentials were recorded by means of bipolar platinum electrodes from the nerve exposed high in the thigh. The action potentials were recorded on a tape recorder and simultaneously observed on an oscilloscope. The recorders were played back from the tape to the oscilloscope, and photographed after the experiment (Pórszász & Szabó, 1959).

Desensitization with capsaicin

Rats can be desensitized against chemical pain with repeated incremental doses of capsaicin (Jancsó & Jancsó-Gábor, 1959; Jancsó, 1960, 1964), or, as we have found recently, by means of a single larger dose.

Capsaicin (trans-N[4′-hydroxy-3′-methoxyl-benzyl]-8-methylnon-6-enamide) is practically insoluble in water, so the 1% solution used for desensitization was prepared with Tween 80 as follows: 0.1 g crystalline capsaicin was dissolved in 2–3 drops of ethanol, to which 15 drops (about 0.6 g) of Tween 80 were added. The final volume was made up to 10 ml. with physiological saline. Dilutions used for testing were prepared from this stock solution.

Rats weighing 140–200 g were desensitized over a period of 24 hr; each rat was injected subcutaneously with 4, 8 and then 15 mg at 6-hr intervals, or with a single dose of 10–20 mg. With this treatment a degree of desensitization can be attained such that the animal is insensitive to a 0.1% capsaicin solution instilled into its eye. Usually we tested the animals 1 or 2 days following the last capsaicin injection. A stronger desensitization was achieved by repeating the last subcutaneous dose, or by giving a smaller dose—for example, 5 mg—intraperitoneally. Since capsaicin causes violent pain, the first injection was always given under light ether anaesthesia. In spite of the anaesthesia sometimes a reflex apnoea occurred and artificial respiration was necessary to save the rat. In all cases the first dose of capsaicin induced vasodilatation and a sudden and profound fall in body temperature.

The insensitivity to chemogenic pain is most pronounced in the early weeks, then gradually decreases, but the effect can still be demonstrated several months later.

If an area of the skin or mucous membrane (human face skin, rat tongue or cornea) is repeatedly treated with a 0.1–1.0% capsaicin solution, a local desensitization against chemical stimuli can be induced (Jancsó, 1955, 1960). This kind of desensitization lasts just a short time—some days only.

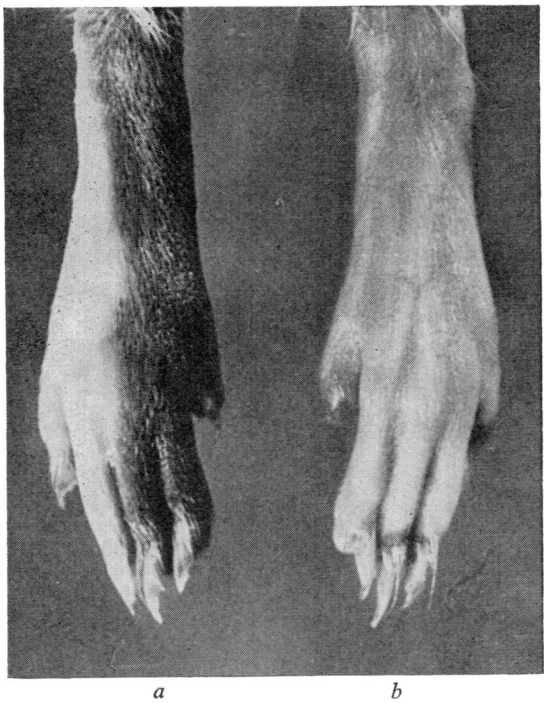
Drugs used

These were capsaicin (extracted from red pepper and crystallized in our laboratory), ω-chloracetophenone, mustard oil, nicotine hydrochloride, xylene, Evans blue, colloidal silver, pentobarbitone sodium, buthalitone sodium, suramin, atropine sulphate, physostigmine salicylate, hexamethonium bromide, phentolamine hydrochloride, propranolol hydrochloride, promethazine, chloropyramine hydrochloride, methysergide bimaleate, dibenamine.

RESULTS

Effect of electrical stimulation of sensory nerves on vascular permeability

Within a few minutes of stimulating the saphenous nerve, the skin area supplied by the nerve became visibly red. This was not simply a so-called "antidromic vasodilatation" because the dilatation of the capillaries was associated with an increased permeability as evidenced by an oedematous swelling of the foot. If, 10 min before the stimulation, Evans blue (50 mg/kg) in isotonic saline were administered intravenously, the area supplied by the saphenous nerve became blue. Usually 2–3 min after beginning stimulation a pale blue discolouration appeared which then turned gradually to a deeper blue. The skin area supplied by the saphenous nerve was indicated by a sharply demarcated area of intense blue dye accumulation (Fig. 1).



a *b*

Fig. 1. The effect of sensory nerve stimulation on vascular permeability. Evans blue dye accumulation in the saphenous skin area after electrical stimulation of the right saphenous nerve. Stimulation for 20 min with rectangular pulses of 1 V, 8 msec at 25/sec. *a*=stimulated side, *b*=non-stimulated side. Evans blue dose 50 mg/kg intravenously.

Stimulation with different parameters of stimulation gave a similar result. Table 1 shows the amount of Evans blue contained in the skin supplied by the saphenous nerve following stimulation, compared with that extracted from the corresponding skin area of the control foot. It may be seen that on the stimulated side the quantity of the dye was about 10–20 times more than that of the control side.

An inflammatory reaction could also be elicited by stimulation of the maxillary and ophthalmic branches of the trigeminal nerve in rats. When Evans blue (50 mg/kg) was

injected intravenously, the conjunctiva, skin and mucous membrane supplied by the second and third branches of the tregeminal nerve turned blue after a few minutes and a blue secretion was discharged from the nose on the stimulated side.

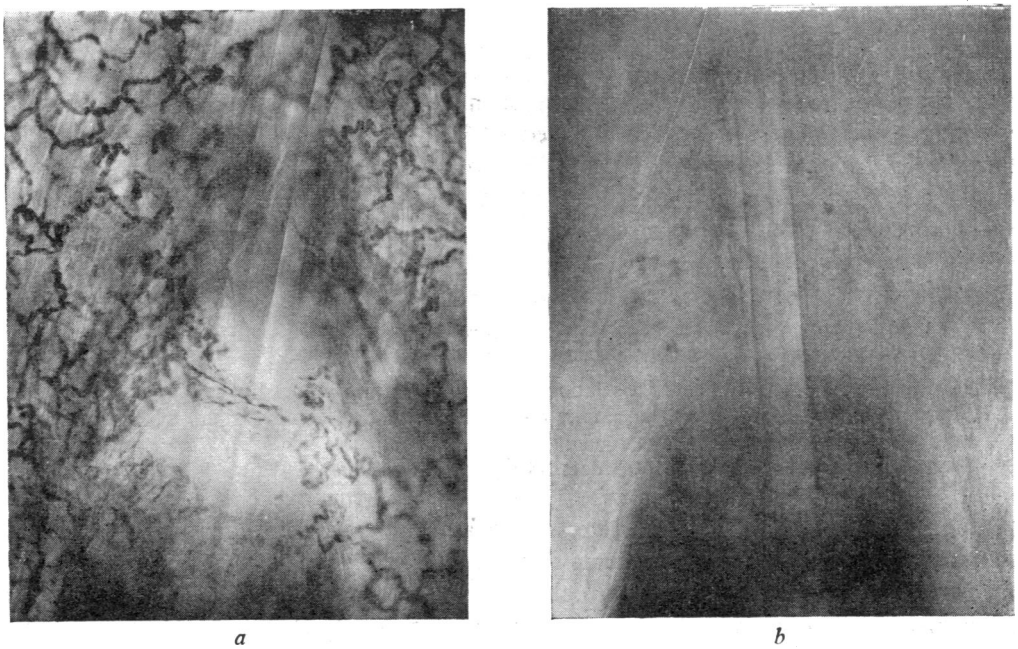
TABLE 1

EVANS BLUE DYE CONTENT OF THE SAPHENOUS SKIN AREA FOLLOWING ELECTRICAL STIMULATION OF THE NERVE

Evans blue dose 50 mg/kg intravenously. The excess dye values were obtained by subtracting the dye content of control sides from those of the stimulated sides.

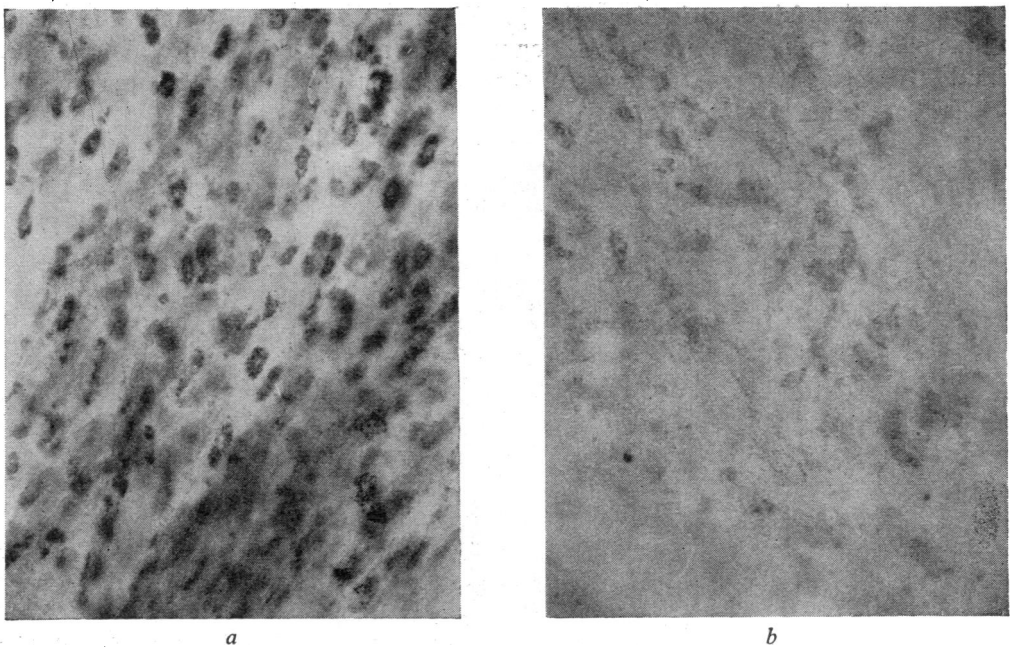
Parameters	Rats (No.)	Dye content (μg)		Excess dye (μg) in stimulated skin
		Stimulated side	Control side	
1 V, 8 msec, 25/sec, 20 min	21	20·1	1·3	18·8
1 V, 20 msec, 25/sec, 12 min	5	34·4	1·7	32·7
8 V, 8 msec, 25/sec, 10 min	4	22·1	1·1	21·0

When a colloidal silver solution was injected instead of Evans blue the inflammatory reaction could be visualized histologically too. Previous investigations (Jancsó, 1960, 1961) have shown that the colloidal silver accumulated in the inflammatory tissues within a few minutes. First the silver formed brown-coloured coatings on the internal surface of small vessels especially on the postcapillary venules. By 1–2 hr, not only the sharply outlined vascular system, but the histiocytes of the surrounding tissue were also filled with silver (Figs. 2 and 3). Thus, the substance released from the nerve endings by electrical stimulation increased the permeability to such an extent that the colloidal silver particles which have a diameter of 200 Å escaped from the vessels and were stored by the histiocytes.



a *b*

Fig. 2. Colloidal silver fixation in the wall of the small vessels of the rat's muzzle (*a*) after electrical stimulation of the trigeminal nerve with 3 V, 0.8 msec at 200/sec for 30 min. A dose of 1 ml./100 g of 1% colloidal silver solution was given intravenously 5 min after the beginning of the stimulation. On the non-stimulated side (*b*) no silver deposition can be seen.



a *b*

Fig. 3. Colloidal silver accumulation in the histiocytes of the conjunctival connective tissue 2 hr after an injection of colloidal silver and stimulation of the ophthalmic branch of the trigeminal nerve, *a* = stimulated side ; *b* = control side.

In order to provide information about the chemical nature of the permeability factor involved in the neurogenic inflammatory response we carried out some experiments with blocking-agents. These drugs were injected 20–30 min before the Evans blue (50 mg/kg), then the saphenous nerve was stimulated for 20 min with rectangular impulses of 1 V, 8 msec at 25/sec. No inhibition of the effect of nerve stimulation was seen in rats injected subcutaneously with atropine (10 mg/kg), physostigmine (0.5 mg/kg), hexamethonium (90 mg/kg), phentolamine (20 mg/kg), propranolol (2 mg/kg), promethazine (20 mg/kg), chloropyramine (10 mg/kg), methysergide (3 mg/kg), or dibenamine (20 mg/kg ; given intravenously 1 hr before stimulation). Atropine and methysergide both slightly enhanced the effect.

Effect of denervation on the course of inflammatory responses

When 0.5 ml./100 g 1% Evans blue solution was injected intravenously and subsequently a normally innervated skin area was painted with xylene, 5% mustard oil, or 2% chloracetophenone in acetone, the treated area turned dark blue within 10–15 min. After application of the irritant, the animals showed signs of pain—they licked their paws and rubbed their snouts. Instillation of capsaicin, chloracetophenone, aqueous mustard oil or nicotine into the eye induced inflammation and blue colouration of the conjunctiva. Pain reactions were observed (blepharospasm, lachrymation, rubbing of the eye).

When an inflammatory stimulus was applied to an area of skin or to the eye, the sensory nerve of which had been transected at least one day before the experiment, the

blue colouration failed to develop (Fig. 4). When rats were tested within a few hours of the operation, the inflammatory reaction was quite normal and the affected area turned blue. The change in responsiveness of the skin set in rather abruptly, approximately 20 hr after the nerve transection.

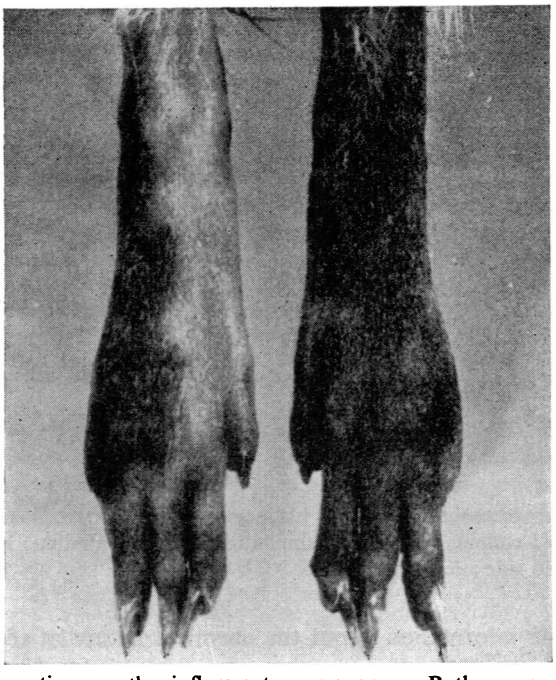


Fig. 4. Effect of denervation on the inflammatory response. Both paws were painted with 5% mustard oil. The right saphenous nerve was transected 5 days before the experiment. Evans blue dose 50 mg/kg intravenously.

TABLE 2

EFFECT OF DENERVATION ON THE INFLAMMATORY RESPONSES ELICITED BY PAINTING THE SKIN OF RATS WITH XYLENE OR 5% MUSTARD OIL

Evans blue dose 50 mg/kg intravenously. In experiments 1, 2, 4 and 5 the right trigeminal nerve, and in experiments 7, 8 and 9 the right saphenous nerve was severed. In experiments 3, 6 and 10 normal controls were used.

Expt.	Skin region	Time after denervation (days)	Irritant	Evans blue content μg/100 mg skin		Quotient normal/ denervated
				Left	Right	
1	Cheek	10	Xylene	4·9	1·5	3·3
2	Cheek	12	Xylene	6·3	1·5	4·2
3	Cheek	—	—	2·1	2·1	—
4	Nose	10	Xylene	6·7	3·5	1·9
5	Nose	12	Xylene	8·4	4·5	1·9
6	Nose	–	–	5·6	4·1	—
7	Paw	5	Mustard oil	10·7	1·4	7·7
8	Paw	5	Mustard oil	10·1	1·6	6·3
9	Paw	5	Mustard oil	9·2	1·9	4·8
10	Paw	—	—	1·5	1·5	—

Table 2 shows the relation of the dye content of some normal and some denervated skin areas following painting with mustard oil or xylene. An inflammatory reaction could not be elicited on the denervated skin areas, the dye content being the same as that of the untreated controls. The dye contents of normal and denervated eye-lids 10 min after the instillation of an irritant into the conjunctival sac are shown in Table 3. The difference between the normal and the denervated eye-lids became apparent by 20 hr after the transection of the nerve.

TABLE 3

EFFECT OF DENERVATION ON INFLAMMATORY RESPONSES OF THE CONJUNCTIVA CAUSED BY DIFFERENT IRRITANT SUBSTANCES IN RATS

Evans blue dose 100 mg/kg intravenously

Expt.	Time after denervation (hr)	Instilled irritant (mg/ml.)	Evans blue in the eyelids μg/100 mg tissue		Quotient normal/ denervated
			Normal	denervated	
1	8	Capsaicin 1	9·8	11·3	0·9
2	20	Capsaicin 1	11·0	18·2	0·6
3	20	Capsaicin 1	19·9	15·3	1·3
4	20	Capsaicin 1	20·1	10·8	1·9
5	20	Capsaicin 1	12·1	3·3	3·7
6	42	Capsaicin 1	10·9	3·2	3·3
7	42	Capsaicin 1	9·8	3·9	2·5
8	42	Chloracetophenone 1	7·0	2·0	3·5
9	42	Chloracetophenone 1	10·0	6·3	1·6
10	42	Chloracetophenone 1	10·8	3·1	3·5
11	42	Mustard oil, aqueous	9·3	4·3	2·2
12	42	Nicotine 2	5·5	3·7	1·5
13	43	Nicotine 4	7·1	4·1	1·7
14	43	Nicotine 4	5·2	3·3	1·6
15	42	—	3·1	3·5	—
16	42	—	3·5	4·0	—

The unresponsiveness of the denervated saphenous skin area did not persist for very long. If the animals were examined several months after severing the nerve, only small islets of skin failed to respond to irritation with mustard oil or xylene. A gradual recovery of the pain sensitivity was observed in the previously insensitive skin, in parallel with the reappearance of the dye accumulation.

The role of pain receptors in the induction of certain inflammatory responses was also supported by the observation that in animals of phylogenetically lower species (frog, pigeon or hen) in which capsaicin did not evoke pain the inflammatory response also failed to occur. In these species chemosis did not develop in the eye after capsaicin administration and when capsaicin was injected under the skin of pigeons no increase in permeability was observed.

Effect of capsaicin desensitization

Jancsó (1955, 1960, 1964), Jancsó & Jancsó-Gábor (1959) and Jancsó, Jancsó-Gábor & Takáts (1961) have shown that the parenteral or local application of capsaicin and related acyl amides produces a selective insensitivity whereby the sensory nerve endings become completely insensitive to chemical stimuli, but remain sensitive to physical—for example, mechanical—stimuli. This was demonstrated by recording the action potentials of

sensory nerves, toto (Pórszász & Jancsó, 1959). In agreement with these findings it was observed that capsaicin desensitization abolished impulse generation in the cutaneous pain receptors by chemical stimuli, whereas the response to mechanical stimulation was unaffected (Fig. 5).

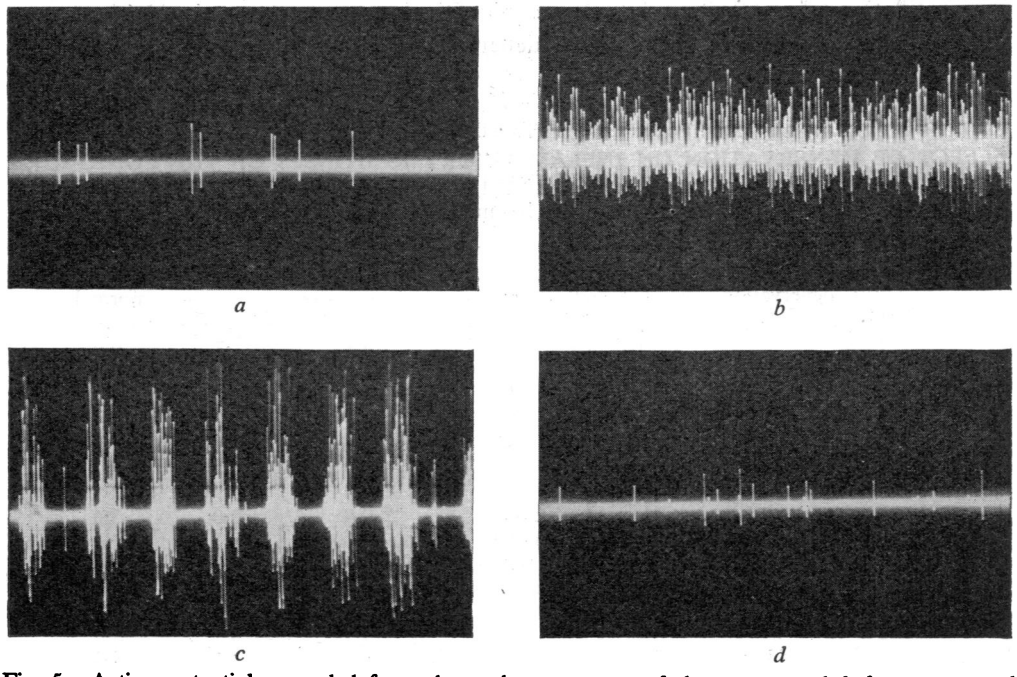
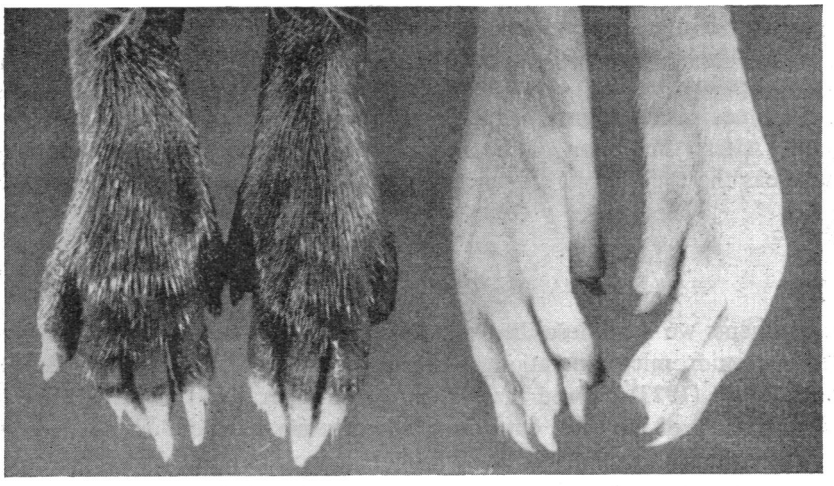


Fig. 5. Action potentials recorded from the saphenous nerve of the rat: *a* and *b* from a normal animal, *c* and *d* from a desensitized one. *a*=activity before the application of the irritant; *b*=effect of subcutaneous injection of capsaicin 1 μg/ml. (0.05 ml.). *c* When the skin of the desensitized animal was lightly stroked the discharges characteristic of touch occurred. *d* shows that application of capsaicin was almost totally ineffective in the desensitized animal. Each record corresponds to a 2-sec period.

Capsaicin desensitization suppressed not only the function of the chemosensitive pain receptors, but also prevented the neurogenic inflammatory responses. Figure 6 shows the inflammatory response of a normal rat and the total absence of dye accumulation in a desensitized one. Similarly, no chemosis or dye exudation was seen when a solution of capsaicin, mustard oil, chloracetophenone or nicotine was instilled into the conjunctival sac of a desensitized animal.

These results are in agreement with those presented by Jancsó (1960), in which it was shown that capsaicin desensitization can serve as an experimental tool for the study of neurogenic inflammatory responses.

The response of desensitized animals to electrical stimulation of sensory nerves was also examined. In markedly desensitized animals the local dye accumulation usually seen on electrical stimulation of the saphenous nerve was completely absent. Table 4 shows the amount of dye in μg exuded following nerve stimulation in normal and in desensitized animals. The figures represent the means of several experiments. The degree of inhibition in desensitized animals was 92.0–92.9% and the normal/desensitized dye quotient was 12.5–14.0.



a *b*

Fig. 6. Effect of capsaicin desensitization on the Evans blue exudation in the skin after application of the irritants. The right paw was painted with xylene, the left paw with 5% mustard oil. *a*=normal rat, *b*=desensitized rat. There was a total absence of the inflammatory response in the desensitized animal. Evans blue dose 50 mg/kg intravenously.

TABLE 4

EVANS BLUE DYE ACCUMULATION IN THE SKIN OF NORMAL AND DESENSITIZED RATS AFTER ELECTRICAL STIMULATION OF THE SAPHENOUS NERVE WITH DIFFERENT PARAMETERS

The excess dye values were obtained by subtracting the dye content of control sides from those of the stimulated sides. Evans blue dose 50 mg/kg intravenously

	Normal		Desensitized			
Parameters	Rats (No.)	Excess dye (μg)	Rats (No.)	Excess dye (μg)	Inhibition (%)	Quotient normal/ des.
1 V, 8 msec, 25/sec, 20 min	21	18·8	10	1·5	92·0	12·5
1 V, 20 msec, 25/sec, 12 min	5	32·7	5	2·4	92·7	13·6
8 V, 8 msec, 25/sec, 10 min	4	21·0	4	1·5	92·9	14·0

The long-lasting inhibitory effect of capsaicin desensitization on the enhancement of the permeability induced by stimulation of the nerve is demonstrated in Table 5. Following the last capsaicin injection, 88.9% inhibition was still present even after a week.

TABLE 5

FAILURE OF EVANS BLUE DYE ACCUMULATION IN THE SKIN OF DESENSITIZED RATS AFTER ELECTRICAL STIMULATION OF THE SAPHENOUS NERVE AT DIFFERENT INTERVALS AFTER THE LAST CAPSAICIN INJECTION

The calculation of the rate of inhibition was based on the average of the "excess dye" values of the control rats. Evans blue dose 50 mg/kg intravenously. Stimulation parameters were 1 V, 8 msec 25/sec, 20 min

Rats (No.)	Time after desensitization	Excess dye (μg)	Inhibition (%)
4	6 hr	0·5	97·8
7	1–2 days	1·8	91·2
6	6–7 days	2·9	88·9

The development of the inflammation induced by stimulation of the nerve was also inhibited by local desensitization. This was demonstrated on the eye of rats by stimulation of the trigeminal nerve. Desensitization was achieved by instilling a 1% capsaicin solution six times over a period of 2 days, with the last instillation 4 hr before the experiment. After stimulation of the nerve, the dye exuded only into the skin of the muzzle whereas the eye-lid and conjunctiva remained uncoloured.

DISCUSSION

The experiments we have described provide evidence that inflammatory responses can be elicited by antidromic stimulation of sensory nerves. Stricker (1876), Bayliss (1901, 1923) and Langley (1923) showed that, when the peripheral ends of divided dorsal roots or the distal ends of severed cutaneous sensory nerves were stimulated, arteriolar dilatation ensued. This is the well-known phenomenon of " antidromic vasodilatation." However, as far as we know, it has never been proven experimentally whether or not vascular permeability changes, characteristic of inflammation, could also be evoked by antidromic sensory nerve stimulation. Our experiments show that in addition to vasodilatation, electrical antidromic stimulation of the sensory nerves is followed by an enhancement of the permeability, protein exudation, the fixation of injected colloidal silver at the surface of small vessels and later its storage in histiocytes. Presumably the nerve endings release under the influence of antidromic stimulation a permeability increasing factor, a " neurohumor," of considerable effectiveness. Possibly, this permeability factor is identical with the peripheral mediator of the " antidromic vasodilatation." According to other reports in the literature (cf. Chapman *et al.*, 1961), antidromic vasodilatation cannot be inhibited with atropine or antihistamines or potentiated with physostigmine. We now report that the same is true for the permeability-increasing effect of antidromic stimulation. In addition, this effect could not be inhibited by hexamethonium, methysergide, phentolamine or dibenamine. Thus, the neurohumor released by sensory nerve endings is not likely to be acetylcholine, histamine, 5-hydroxytryptamine, adrenaline or noradrenaline. At present there is no direct evidence to indicate the nature of this substance. According to Chapman *et al.* (1961), in the axon reflex flare induced by stimulation of dorsal roots the release of " neurokinin " from the nerve is involved. Keele & Armstrong (1964), however, are of the opinion that Chapman's " neurokinin " may be a mixture of plasma kinins, histamine and possibly SP and other substances. Our experiments show that stimulation of sensory nerves also increases vascular permeability and so it is difficult to decide whether the " neurokinin " activity is due to a substance released from the nerve or from the leaked plasma. As regards other substances proposed as mediators of antidromic vasodilatation (ATP, SP, kallikrein) the evidence is not satisfactory (cf. Chapman *et al.*, 1961).

The permeability-increasing neurohumor can also be released by orthodromic stimulation of the pain sensitive nerve terminals. Thus, certain chemical irritants are able to activate the mechanism concerned in antidromic stimulation of the sensory nerves. Our denervation experiments furnish further evidence that many well-known irritants—for example, capsaicin, xylene, mustard oil, ω-chloracetophenone—really exert their inflammation-producing effect by stimulation of the sensory nerves. According to

previous experiments (Jancsó, 1960) on the denervated ear of the rat, inflammation could not be induced with the above mentioned irritants once degeneration of the nerve had ensued. The same holds good for our present experiments on the skin areas supplied by the saphenous and trigeminal nerves. The degeneration of the nerves usually requires 24–48 hr, and so during the early hours following the transection an inflammatory response is still obtainable. A few months later, the sensitivity of the denervated area of skin begins to be restored gradually. At the same time, the inflammation-producing effect of irritants is also restored. Presumably this is due in the first place to " collateral nerve sprouting," that is, to ingrowth of newly formed side branches of adjacent sensory fibres.

That the pain sensitive nerve terminals play a decisive role in the action of some inflammation-producing agents is also suggested by the fact that in certain species (frog, pigeon or hen) capsaicin does not cause pain, nor does it induce inflammation.

The experiments on animals which had been pretreated with capsaicin also showed that for the development of some inflammatory responses, the pain sensitive nerve terminals must be intact. Desensitized animals are insensitive to all chemogenic pain, but at the same time, they respond readily to physical pain and tactile stimuli (Jancsó, 1955, 1960 ; Jancsó & Jancsó-Gábor, 1959). By means of action potentials recorded from the sensory nerve it has been shown that the desensitization effect is caused by a lesion at the level of the pain receptors (Pórszász & Jancsó, 1959). Recently, Green & Tregear (1964) confirmed on excised cat eyes that, after repeated application of capsaicin, the action potential discharges evoked by different irritants are blocked without causing any change in the response to cooling or to tactile stimuli.

By blockade of the pain sensitive nerve terminals the inflammatory effect of mustard oil, xylene, ω-chloracetophenone, capsaicin and related acyl amides, can be completely inhibited just as it can be by denervation. These substances all produce severe pain in normal rats. Oedema-inducing agents which act by releasing vasoactive substances without causing pain (dextran, egg white), as well as substances with a direct vascular action (histamine, 5-HT), exert an unchanged inflammatory effect on desensitized rats. The permeability enhancing effect of these agents is also unchanged after denervation.

Capsaicin desensitization will inhibit the effect only of those substances which exert their action by the neurogenic route (Jancsó, 1960). The present investigations show that, with capsaicin desensitization, not only the increase of permeability elicited orthodromically by stimulation with chemical substances, but also that induced by electrical stimulation of the sensory nerve antidromically, can be inhibited. In all probability this is due to a failure of the nerve endings to release the neurohumor. Possibly the capsaicin desensitization interferes with the synthesis of this substance in the pain sensitive nerve terminals or neurones.

In view of the above, it can be established that there exists a pure neurogenic form of inflammation. This is supported by the finding that antidromic stimulation of the sensory nerve evokes an inflammatory response. Our experiments on rats which had been desensitized with capsaicin suggest that the permeability-increasing neurohumor is released from the pain sensitive nerve terminals. It has been demonstrated that a group of inflammation-producing agents exerts its action by a neurogenic route. The existence of neurogenic inflammation does not rule out Cohnheim's (1873) classic concept that

inflammation with all its characteristic signs can also develop independently of the nervous system, because a considerable number of substances exert their effect without the involvement of the nervous system.

According to our experiments the inflammation-producing substances can be arranged into two groups: (1) those exerting their effect by the neurogenic route (for example, capsaicin, mustard oil, ω-chloracetophenone, xylene); and (2) those not acting by the neurogenic route (for example, dextran, egg white, 5-HT, histamine). By sensory denervation as well as by capsaicin desensitization it is possible to classify inflammation-producing agents.

SUMMARY

1. By antidromic electrical stimulation of the sensory nerves (saphenous or trigeminal) of rats, the following signs of an inflammatory response could be elicited: arteriolar vasodilatation, enhancement of vascular permeability, protein exudation, fixation of injected colloidal silver onto the walls of venules and, later, their storage in histiocytes.

2. The inflammatory response induced by electrical stimulation could not be altered by parenterally administered atropine, physostigmine, hexamethonium, phentolamine, dibenamine, propranolol, promethazine, chloropyramine, or methysergide.

3. After the degeneration of the sensory nerve, capsaicin, xylene, mustard oil and ω-chloracetophenone did not evoke inflammation. Hence, these substances induce inflammation purely or dominantly through the involvement of sensory nerves.

4. Capsaicin desensitization inhibited the signs of inflammation induced both by antidromic stimulation of the sensory nerve and by orthodromic stimulation of pain sensitive nerve terminals with irritants.

5. The experiments suggest that a mediator substance, a neurohumor, is released by orthodromic or antidromic stimulation of pain sensitive nerve terminals and that this substance is responsible for the signs of inflammation produced by some substances.

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