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The Site of Action of Capsaicin on the Guinea-Pig Isolated Ileum*

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Summary. The site and mode of action of capsaicin were analysed on the guinea-pig isolated ileum.

1. Capsaicin produced longitudinal contraction (EC_{50} 4.2×10^{-8} g/ml) followed by a specific, rapid and irreversible tachyphylaxis (IC_{50} 2.8×10^{-7} g/ml).

2. Capsaicin was ineffective in the presence of tetrodotoxin (2×10^{-7} g/ml) or on ilea kept for 24–48 h at 4° C, without an oxygen supply.

3. On ileal segments, the perivascular mesenteric nerves of which were transsected 5–8 days before the experiment, practically no response to capsaicin was obtained. Chronic abdominal bilateral vagotomy was without any effect.

4. Hyoscine ($1 \times 10^{-8} - 1 \times 10^{-6}$ g/ml) or morphine (2×10^{-6} g/ml) strongly inhibited contractions produced by capsaicin. Neither mecamylamine (1×10^{-5} g/ml), nor nicotine (5×10^{-5} g/ml) and dimethylphenylpiperazinium (5×10^{-6} g/ml) caused any change, while an increased response to capsaicin was obtained in the presence of hexamethonium (1×10^{-4} g/ml).

5. Unaltered contractions were produced by capsaicin on ileal segments made tachyphylactic to 5-HT, bradykinin or substance P. Histamine antagonists at H_1 and H_2 receptors (chloropyramine, burimamide), the prostaglandin synthesis inhibitor indomethacin, pretreatment with the adrenergic neuron blocking agent guanethidine, as well as in vivo reserpine pretreatment were also ineffective in this respect.

6. It is concluded that in the guinea-pig ileum capsaicin causes predominantly cholinergic contraction by stimulating terminals of extrinsic, non-parasympathetic nerves.

Key words: Capsaicin – Cholinergic mechanism – Periarterial mesenteric nerves – Sensory fibres – Ileum innervation.

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Introduction

Earlier experiments revealed that capsaicin, the pungent principle in the red pepper[1], is a potent and selective sensory stimulating agent acting on sensory receptors of C-fibres subserving chemogenic pain and warmth (Jancsó and Jancsó-Gábor, 1959; Szolcsányi, 1977). On the exteroceptive area, excitation of the capsaicin-sensitive nerve endings by orthodromic or antidromic stimuli results in a local efferent response, i.e. an increase in vascular permeability (Jancsó et al., 1967, 1968; Jancsó-Gábor and Szolcsányi, 1972; Garcia Leme and Hamamura, 1974).

It is an old observation that intake of capsicol[2] in a gelatine capsule is followed by an improved digestion and peristalsis (Högyes, 1878). The mechanism of this phenomenon, however, is still unknown. It has also been described that capsaicin produces contraction, followed by a specific tachyphylaxis on the guinea-pig isolated ileum (Toh et al., 1955; Molnár et al., 1969). However, the question whether the response is mediated through nervous structures or not, remained unanswered. In the light of our earlier findings the involvement of sensory nerve endings was suspected. Thus, as a first step of experiments, the role of neural elements in the capsaicin effect was analysed on the guinea-pig isolated ileum.

Methods

Adult guinea-pigs of either sex were killed by a blow to the head and bled. The ileum was excised and placed into Krebs solution of the following composition (g/l): NaCl 6.9, $CaCl_2 \times 2\,H_2O$ 0.37, KCl 0.35, KH_2PO_4 0.16, $MgSO_4 \times 7\,H_2O$ 0.29, $NaHCO_3$ 2.1, dextrose 1.0. About 3 cm segments from the middle region were suspended in a jacketed organ bath containing 5 ml Krebs solution at 37° C, bubbled with 5% CO_2 in O_2. Longitudinal contractions were recorded on smoked paper with an isotonic side-writing lever loaded with 0.5 g, with a magnification of 4 times.

1 For chemical formula of the compound see Toh et al. (1955).

2 (An oil-extract of the red pepper, Capsicum annuum).

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For denervation procedures, guinea-pigs were anaesthetized with pentobarbitone (50 mg/kg i.p.) and the abdomen was opened under semi-sterile conditions. For periarterial mesenteric denervation, one loop of middle ileum was pulled out gently in order to expose its mesenteric supply. The nerves were cut around the mesenteric vessels 2–3 cm apart from the ileal segment, under an operating microscope. Careful dissection did not cause damage of the vessels, so that on the day of the experiment the appearance of the denervated loops corresponded to that of the innervated ones. Segments showing signs of impaired circulation (edema, livid coloration) were discarded. Vagotomy was performed around the oesophagus below the diaphragm. In sham operated animals the area of denervation was exposed without dissection. After the operation the abdomen was closed with sutures and the animal was treated with penicillin, given i.m.

Materials. The drugs used were acetylcholine chloride (VEB Berlin-Chemie), bradykinin triacetate (Sigma), burimamide (Smith, Kline and French), capsaicin (State Farm, Szatymaz), chloropyramine hydrochloride (Suprastin® ampoules, EGYT), dimethylphenylpiperazinium iodide (Aldrich), guanethidine sulphate (CIBA), hexamethonium chloride (Fluka), hyoscine hydrobromide (Burroughs Wellcome and Co.), indomethacin (Chinoin), mecamylamine hydrochloride (Sigma), morphine hydrochloride, nicotine tartarate, pentobarbitone sodium (May and Baker), reserpine (Rausedyl® ampoules, Richter), substance *P* bovine synthetic (Beckman), serotonin creatinine sulphate (Sandoz), tetrodotoxin (Sankyo).

Capsaicin was dissolved in ethanol and further diluted with an equal volume of distilled water to give a stock solution of 10 mg/ml. Stock solution for indomethacin (10 mg/ml) was prepared with pure ethanol. All stock solutions were kept in the refrigerator. Further dilutions were made on the day of the experiment in saline, except for indomethacin that was diluted with ethanol and administered in volumes of 1–10 µl. All concentrations correspond to g/ml of salts.

Statistics. All values presented are means ± S.E.M. Statistical significance of differences between means was estimated using Student's *t*-test.

Results

Contraction and Tachyphylaxis Induced by Capsaicin

Capsaicin at concentrations of 5×10^{-9} to 1×10^{-5} caused contraction of the isolated ileum. Repeated exposures, however, resulted in a rapid and long-lasting tachyphylaxis (Fig. 1). The response to the first dose of capsaicin was dose-related. If the height of contractions was expressed in per cent of the maximal response elicited by 2×10^{-6} acetylcholine a clear-cut dose-response curve was obtained (Fig. 2). The characteristics of the tachyphylaxis were as follows:

1. In accordance with the finding of Molnár et al. (1969) the tachyphylaxis was specific. No decrease in contraction to nicotine was observed on ilea made completely tachyphylactic to capsaicin (Fig. 1).

2. The degree of tachyphylaxis was directly related to the concentration applied (Fig. 2) and to the contact time.

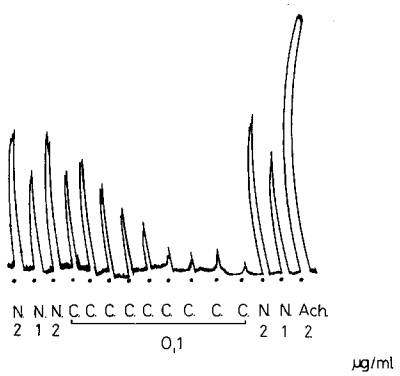

Fig. 1. Contractions of guinea-pig isolated ileum to capsaicin (*C*) and nicotine (*N*). Contact time 20 s; 10 min cycle; four changes of bath fluid after each contraction. The effect of acetylcholine (*Ach*) in a bath concentration of 2 µg/ml represents maximal contraction

3. In the presence of the first dose of capsaicin the higher the concentration, the shorter was the time required for the tone to return to the control level. At concentrations of 1×10^{-8}, 3×10^{-8}, 1×10^{-7} and 1×10^{-6} these intervals were 8.3 ± 0.6, 5.3 ± 0.9, 2.4 ± 0.2 and 1.7 ± 0.1 min, respectively.

4. After tachyphylaxis had ensued no recovery of the responses was found although the organ bath was repeatedly washed out during a period of 3–4 h.

Owing to this marked tachyphylaxis the effects of drugs and other procedures on the response to the first capsaicin exposure was measured and results obtained from other untreated ileal segments served as controls.

Tetrodotoxin

When the preparation was treated for 10 min with tetrodotoxin (2×10^{-7}) contractions due to capsaicin or nicotine were prevented (Table 1), suggesting that the site of action of these compounds is on neural elements. It is noteworthy that tachyphylaxis to capsaicin (1×10^{-6}) fully developed in the presence of tetrodotoxin, i.e. the second dose of capsaicin (1×10^{-6}) was almost completely ineffective in preparations of which the response to nicotine had returned completely owing to the elimination of tetrodotoxin by repeated washing.

Cold Storage

Guinea-pig ileum kept for 24–48 h at 4–5° C without an oxygen supply is irreversibly unresponsive to neurogenic stimuli while agonists having a site of action on the smooth muscle still produce contraction (Kosterlitz and Lees, 1964). Capsaicin (1×10^{-6}) was completely ineffective under such conditions whereas acetylcholine caused contraction and even a small fraction of the nicotinic response still persisted (Table 1).

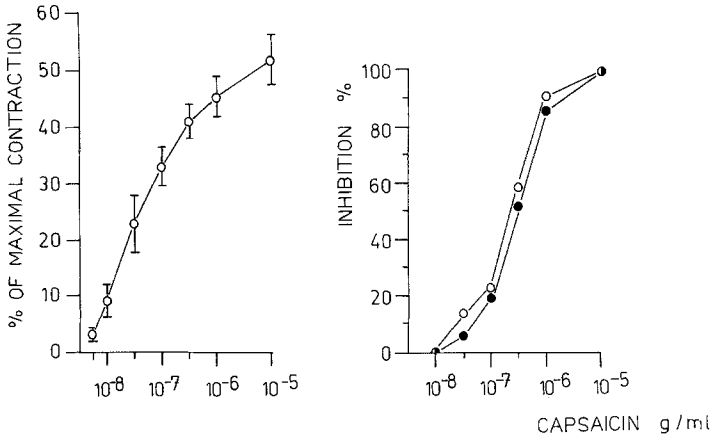

Fig. 2
A Dose response curve to capsaicin on the guinea-pig isolated ileum. Contractions induced by the first exposure of the drug to each segment. The values represent the mean of 5–8 experiments with the S.E.M. E_{max} = maximal contraction of the gut induced by 2×10^{-6} g/ml of acetylcholine. Contact time = 3 min.
B Decrease in response to the second exposure of the same dose (○——○) or of a constant 1×10^{-5} g/ml concentration of capsaicin (●——●) as compared with the average height of contraction evoked by the first dose. Abscissa: bath concentration of the first dose of capsaicin. Ordinate: inhibition in percent of the effect of the second dose. Contact time = 3 min; interval = 10–20 min; five changes of bath fluid after the first exposure. Each value is the mean of 5–8 experiments

Table 1. The effect of tetrodotoxin, cold storage and denervation on the contraction of the guinea-pig ileum to capsaicin or nicotine

Agonist	Conc. (g/ml)	Pretreatment	No. of exp.	Contraction % of E_{max} to acetylcholine
Capsaicin	1×10^{-6}	–	7	45.6 ± 3.4
Capsaicin	1×10^{-6}	Tetrodotoxin (2×10^{-7})	7	2.9 ± 0.7[a]
Capsaicin	1×10^{-6}	Cold storage	6	0[a]
Capsaicin	1×10^{-7}	Sham operation	7	25.9 ± 4.9
Capsaicin	1×10^{-6}	Sham operation	6	41.2 ± 3.1
Capsaicin	1×10^{-5}	Sham operation	6	44.2 ± 2.6
Capsaicin	1×10^{-7}	Mesenteric denervation	9	3.1 ± 1.1[b]
Capsaicin	1×10^{-6}	Mesenteric denervation	8	2.5 ± 0.9[b]
Capsaicin	1×10^{-5}	Mesenteric denervation	7	4.9 ± 3.2[b]
Capsaicin	1×10^{-6}	Vagotomy	5	47.2 ± 2.4
Nicotine	2×10^{-6}	–	38	55.1 ± 2.0
Nicotine	2×10^{-6}	Tetrodotoxin (2×10^{-7})	5	5.8 ± 1.6[a]
Nicotine	2×10^{-6}	Cold storage	6	18.2 ± 4.3[a]
Nicotine	2×10^{-6}	Sham operation	11	53.0 ± 3.4
Nicotine	2×10^{-6}	Mesenteric denervation	12	58.8 ± 1.9
Nicotine	2×10^{-6}	Vagotomy	6	54.8 ± 2.4

[a] or [b]Statistically significant differences as compared to untreated ([a]) or sham operated ([b]) segments: $P < 0.001$.
In the case of mesenteric denervation and sham operation data were obtained from 9, in the case of subdiaphragmal vagotomy from 4 animals

Perivascular Mesenteric Denervation

Segments of ileum the mesenteric nerves of which were dissected 5–8 days before the experiment, as well as innervated loops obtained from the same nine animals were tested. Mesenteric denervation almost completely abolished the response to capsaicin (Table 1). On the other hand, contraction due to the ganglionic stimulating agent nicotine was slightly enhanced. Twitch responses to field stimulation of cholinergic, postganglionic neural elements (Paton, 1955) were also reproducible on these denervated segments. The following stimulation parameters were used: 0.1 ms, 0.1 Hz, at a supramaximal voltage (30 V). These findings indicate that ganglion cells did not degenerate following the operation.

Subdiaphragmal Vagotomy

Subdiaphragmal bilateral vagotomy performed 5–6 days before the experiment did not change the effect of capsaicin or nicotine as revealed on nine segments obtained from four animals (Table 1).

Hyoscine, Morphine, Ganglionic Blockade

Exposure to hyoscine ($1 \times 10^{-8} - 10^{-6}$ for 5 min) strongly inhibited the response to capsaicin (Table 2). However, a fraction (20–30%) of the response persisted even in the presence of a high concentration (1×10^{-6}) of the muscarinic receptor blocking agent. Morphine inhibits the release of acetylcholine from cholinergic nerve endings in the longitudinal muscle of

Table 2. The effect of drugs on the contraction of guinea-pig ileum to capsaicin (1×10^{-6})

Pretreatment	Conc. (g/ml)	Contact time (min)	No. of exp.	Contraction		P
				% of E_{max} to Ach	% of the control	
Control	–	–	7	45.6 ± 3.4	100	
Hyoscine	1×10^{-8}	5	5	18.8 ± 4.6[a]	41.2	0.001
Hyoscine	1×10^{-7}	5	5	17.6 ± 4.1[a]	38.6	0.001
Hyoscine	1×10^{-6}	5	6	11.7 ± 3.3[a]	25.7	0.001
Morphine	2×10^{-6}	5	5	16.6 ± 2.4[a]	36.4	0.001
Hexamethonium	1×10^{-4}	30	7	64.1 ± 7.5[b]	140.6	0.02
Mecamylamine	1×10^{-5}	10	6	49.2 ± 5.3	107.1	n.s.
DMPP	5×10^{-6}	20	5	43.0 ± 4.8	94.3	n.s.
Nicotine	5×10^{-5}	30	5	40.4 ± 3.3	88.6	n.s.
Bradykinin desensitization	1×10^{-6}	5	6	40.6 ± 4.2	89.0	n.s.
Substance P desensitization	1×10^{-7} to 1×10^{-6}	5	6	39.7 ± 4.1	87.1	n.s.
5-HT desens.	2×10^{-5}	15	5	42.6 ± 3.6	93.4	n.s.
Chloropyramine	1×10^{-6}	5	6	43.0 ± 6.8	94.3	n.s.
Burimamide	1×10^{-5}	10	6	47.8 ± 3.5	104.8	n.s.
Guanethidine	3×10^{-6}	30	6	43.3 ± 6.5	95.0	n.s.
Indomethacin	1×10^{-5}	15	6	38.7 ± 6.5	84.9	n.s.

n.s.: $P > 0.05$

the guinea-pig isolated ileum (Paton, 1957). In the presence of morphine (2×10^{-6} for 5 min) the response of the tissue to capsaicin was reduced nearly to the same extent as in the case of the highest (1×10^{-6}) concentration of hyoscine (Table 2).

In the case of ganglionic blocking agents like hexamethonium (1×10^{-4} for 30 min), mecamylamine (1×10^{-5} for 5 min), or ganglionic blocking concentrations of nicotine (5×10^{-5} for 30 min) and dimethylphenylpiperazinium (5×10^{-6} for 20 min) no reduction in the effect of capsaicin was noted. In the case of hexamethonium the contractions were even higher (Table 2). On the other hand, all these pretreatments completely abolished the response to nicotine ($2 \times 10^{-6} - 1 \times 10^{-5}$). These results suggest that, although the smooth muscle contraction due to capsaicin is predominantly cholinergic in nature, ganglionic transmission, at least through nicotinic receptors is not involved in the response. Since the results obtained on denervated segments suggested a site of action on endings of extrinsic nerves, the mediation of the response through non-nicotinic intramural ganglionic receptors remained still a possible way of excitation. In the following experiments capsaicin was therefore tested on segments made previously unresponsive to different putative neurotransmitters.

Tachyphylaxis to 5-HT, Bradykinin or Substance P

High concentration of 5-HT blocks responses to 5-HT not only at the receptors of smooth muscle but also at the receptors of the intramural nervous plexus in the guinea-pig ileum (Rocha e Silva et al., 1953; Brownlee and Johnson, 1963). In the presence of desensitizing concentration of 5-HT (2×10^{-5} for 15 min) when the contraction induced by the drug had already faded the response to capsaicin was still unaltered (Table 2).

Addition of bradykinin or substance P to the ileum in supramaximal concentrations results in a transient unresponsiveness to the respective peptide (Lembeck and Fischer, 1967). Bradykinin or substance P in high concentrations (1×10^{-6} and $1 \times 10^{-7} - 1 \times 10^{-6}$, respectively) were added to the tissue for 5 min. These concentrations were adjusted to be about 50 times higher than the EC_{50} value. Three washes followed the exposure. Within 2 min the respective peptide in a dose of the control EC_{50} was completely ineffective and 10–15 min were necessary for a complete recovery provided the organ bath was washed out 3 times in every 2 min. Capsaicin was added within the first 2-min period. No change in the response was observed during the tachyphylaxis to either bradykinin or substance-P (Table 2).

Different Antagonists

No inhibition in the response to capsaicin was observed on ileal segments pretreated with the H_1 or H_2 histamine antagonists chloropyramine (1×10^{-6}) or burimamide (1×10^{-5}), respectively. Similarly, no inhibition in the effect of capsaicin was found after a 30 min exposure of the ileum to the adrenergic blocking agent

guanethidine (3×10^{-6}), or on loops obtained from guinea-pigs pretreated with 10 mg/kg reserpine s.c., 24 h before the experiment.

Inhibition of prostaglandin synthesis by indomethacin (1×10^{-5} for 15 min) was also without any significant effect on the response to capsaicin.

Discussion

The present findings with capsaicin provide evidence for a new type of neural activation of the longitudinal muscle of the guinea-pig isolated ileum.

It has already been described that capsaicin gives rise to contraction followed by specific tachyphylaxis on the guinea-pig isolated ileum (Toh et al., 1955; Molnár et al., 1969). According to Molnár et al. (1969) specific blocking agents of acetylcholine, histamine or serotonin, even when applied together failed to prevent the response to capsaicin. Owing to the tachyphylaxis, however, no quantitative analysis of the responses was made and no suggestion was given about the site of action of the drug.

Our results provide evidence for the neurogenic nature of the smooth muscle response brought about by capsaicin on the guinea-pig ileum.

1. Degeneration of intramural neural elements after cold storage (Kosterlitz and Lees, 1964) completely prevented the effect of capsaicin although the tissue responded readily to acetylcholine.

2. Practically no contraction occurred in the presence of tetrodotoxin. This poison selectively abolishes axonal conduction and spike generation in neural tissues without affecting the depolarization and contraction of the smooth muscle (Kao, 1966).

3. Almost complete blockade was observed after perivascular mesenteric denervation.

Thus neural elements, more precisely nerve terminals of extrinsic fibres, mediate the response to capsaicin. Consequently cell bodies of neurones, the endings of which are stimulated by capsaicin, seem not to be situated in the submucosal or myenteric plexuses of the gut. On the other hand, these extrinsic fibres are not identical with the terminal arborizations of the vagus nerves, since full responses were obtained after chronic bilateral vagotomy. In accordance with this assumption after blocking the ganglionic nicotinic receptors by hexamethonium, mecamylamine, or by high doses of dimethylphenylpiperazinium or nicotine the responses to capsaicin were not inhibited although the contraction of the small intestine to nicotine or to stimulation of the vagus nerves were abolished in this way (Szolcsányi and Barthó, 1978). Hence, it can be concluded that the effect of capsaicin is not due to stimulation of the endings of preganglionic parasympathetic fibres.

Nevertheless, a marked reduction in the response was obtained in the presence of a low concentration of hyoscine. The blockade remained incomplete even in the presence of a hundred times higher concentration of the antimuscarinic agent. Similar inhibition occurred after pretreatment with morphine. This drug inhibits the release of acetylcholine from cholinergic nerve endings of the gut (Paton, 1957; Schaumann, 1957; Ehrenpreis et al., 1976). Both findings suggest a final cholinergic step in the response.

The question arises, how a cholinergic transmission can participate in the effect of capsaicin if the stimulated extrinsic nerves are not parasympathetic in origin.

The first possibility would be that acetylcholine is released from capsaicin sensitive nerve terminals of non-vagal extrinsic mesenteric fibres. The second possible explanation is that an unknown transmitter is released from these extrinsic nerve terminals, which in turn excite intramural cholinergic neural elements through non-nicotinic receptors.

In the light of the following experimental results and considerations the second possibility is favoured.

1. Evidence for the release of a mediator substance from the excited capsaicin sensitive sensory nerve endings was obtained on exteroceptive skin and mucous membrane areas (Jancsó et al., 1967, 1968; Jancsó-Gábor and Szolcsányi, 1972). This mediator that triggers an increase in vascular permeability is not identical to acetylcholine (Jancsó et al., 1967; Garcia Leme and Hamamura, 1974).

2. In general, excitation-secretion coupling in presynaptic nerve endings is not inhibited by local anaesthetics or by tetrodotoxin (Katz and Miledi, 1967; Bell, 1968; Krauss et al., 1970). The same holds good for the neurogenic increase in vascular permeability induced by capsaicin (Jancsó et al., 1967). However, in the gut, the effect of capsaicin was abolished by tetrodotoxin indicating involvement of axonal conduction and spike generation in the development of the contraction.

3. A variant of the first possibility would be that acetylcholine was released from sympathetic adrenergic nerve terminals as a manifestation of a cholinergic link in adrenergic neurotransmission (Burn, 1971, 1977). However, tachyphylaxis to capsaicin on the ileum did not interfere with the effect of sympathetic nerve stimulation although it blocked the cholinergic contraction elicited by the stimulation of the perivascular mesenteric nerves in preparations pretreated with adrenergic neuron-blocking agents (Szolcsányi and Barthó, 1978).

Capsaicin is a selective sensory stimulating agent acting on sensory receptors of C-fibres subserving chemogenic pain and warmth (Jancsó and Jancsó-Gábor, 1959; Szolcsányi, 1977), as well as on C-fiber vascular interoceptors of the thoracic vagus nerves

(Coleridge and Coleridge, 1972). Capsaicin sensitive sensory fibres are also present in the mesenterial nervous plexus (Baraz et al., 1968).

In rats and guinea-pigs a long-lasting or irreversible impairment in function of these receptors develops after local or parenteral pretreatment with larger doses of the drug (Jancsó and Jancsó-Gábor, 1959; Szolcsányi and Jánossy, 1971; Szolcsányi et al., 1975). A similar selectively impaired sensitivity occurred also on the capsaicin treated human skin or tongue to chemical pain or warmth but not to mechanical pain, tactile coldness or gustatory stimuli (Jancsó and Jancsó-Gábor, 1959; Szolcsányi, 1977). In rats, selective ultrastructural changes in small type of primary sensory neurons as well as in some free sensory nerve endings of the corneae accompanied the loss of function (Szolcsányi et al., 1975). It has been suggested that capsaicin is a specific sensory neuron blocking agent which activates and subsequently blocks the physiological function in nerve terminals of one type of primary sensory neurons, resembling the action of guanethidine on adrenergic neurons (Szolcsányi et al., 1975).

Excitation of capsaicin sensitive exteroceptive nerve endings by chemical means or by antidromic electrical stimulation results in an efferent response as well (Jancsó et al., 1967, 1968). The transmitter released from these nerve endings is not identical to acetylcholine, adrenaline, noradrenaline, histamine, 5-HT, bradykinin or ATP and there is evidence against the mediating role of prostaglandins and substance P as well (Jancsó et al., 1967; Jancsó-Gábor and Szolcsányi, 1972; Garcia Leme and Hamamura, 1974).

According to the present findings on the gut, histamine antagonists at the H_1 and H_2 receptors, desensitization of 5-HT receptors (Rocha e Silva et al., 1953) at the smooth muscle and at the intramural ganglion cells (Brownlee and Johnson, 1963), or desensitization of the peptide receptors with bradykinin or substance P (Lembeck and Fischer, 1967) as well as inhibition of prostaglandin synthesis did not cause a significant change in the response to capsaicin. Consequently, in the nerve-mediated contraction produced by capsaicin no transmitting role can be attributed to histamine, 5-HT, prostaglandins, bradykinin, substance P and, owing to the crossed tachyphylaxis between peptides (Lembeck and Fischer, 1967), to kallidin, physalaemin or eledoisin.

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