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 x 10$^{-8}$ g/ml) followed by a specific, rapid and irreversible tachyphylaxis (IC$_{50}$ 2.8 x 10$^{-7}$ g/ml).

2. Capsaicin was ineffective in the presence of tetrodotoxin (2 x 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 transected 5-8 days before the experiment, practically no response to capsaicin was obtained. Chronic abdominal bilateral vagotomy was without any effect.

4. Hyoscine (1 x 10$^{-8}$ - 1 x 10$^{-6}$ g/ml) or morphine (2 x 10$^{-6}$ g/ml) strongly inhibited contractions produced by capsaicin. Neither mecamylamine (1 x 10$^{-5}$ g/ml) nor nicotine (5 x 10$^{-5}$ g/ml) and dimethylphenylpiperazinium (5 x 10$^{-6}$ g/ml) caused any change, while an increased response to capsaicin was obtained in the presence of hexamethonium (1 x 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; García 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$_2$ x 2 H$_2$O 0.37, KCl 0.35, KH$_2$PO$_4$ 0.16, MgSO$_4$ x 7 H$_2$O 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).
For denervation procedures, guinea-pigs were anaesthetized with pentobarbitone (50 mg/kg i.p.) and the abdomen was opened under semi-sterile conditions. For periarial 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), chloropropamine hydrochloride (Suprastin® ampoules, EGYT), dimethylphenylpiperazinium iodide (Aldrich), guanethidine sulphate (CIIBA), hexamethonium chloride (Fluka), hyoscine hydrobromide (Burroughs Wellcome and Co.), indomethacin (Chinoin), mecamylamine hydrochloride (Sigma), morphine hydrochloride, nicotine tartarate, pentobarbitone sodium (May and Baker), reserpine (Rausedy® 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. 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 ml. 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 $5 \times 10^{-9}$ to $1 \times 10^{-5}$ 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 $2 \times 10^{-6}$ 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.

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 $1 \times 10^{-8}$, $3 \times 10^{-8}$, $1 \times 10^{-7}$ and $1 \times 10^{-6}$ these intervals were $8.3 \pm 0.6, 5.3 \pm 0.9, 2.4 \pm 0.2$ and $1.7 \pm 0.1$ 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 ($2 \times 10^{-7}$) 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 x $10^{-6}$) fully developed in the presence of tetrodotoxin, i.e. the second dose of capsaicin (1 x $10^{-6}$) 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 x $10^{-6}$) was completely ineffective under such conditions whereas acetylcholine caused contraction and even a small fraction of the nicotinic response still persisted (Table 1).