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Four motor effects of capsaicin on guinea-pig distal colon

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1 The motor effects of capsaicin on the guinea-pig distal colon have been investigated *in vivo* and *in vitro*.

2 Capsaicin (0.1–10 $\mu g\,kg^{-1}$ i.v.) produced a transient relaxation which was reduced by pretreatment with capsaicin itself, atropine, hexamethonium, phentolamine or guanethidine and almost abolished by tetrodotoxin (TTX). Topically applied capsaicin produced a transient inhibition of tone and spontaneous activity prevented by topically applied TTX.

3 In isolated preparations of distal colon, capsaicin produced a transient, TTX- and atropine-sensitive contraction which was followed by a depression of the contractile activity. The depressant effect was unaffected by atropine plus guanethidine but was greatly reduced by TTX, indicating activation of intramural non-adrenergic, non-cholinergic (NANC) mechanisms. The depressant effect on the first exposure to capsaicin (1 μM) was greater than that produced by a second, third or fourth exposure.

4 In preparations excised from capsaicin-pretreated animals, capsaicin (1 μM) only produced an inhibitory effect on spontaneous contractions. Desensitization did not occur to this inhibitory effect.

5 In preparations pre-exposed to capsaicin (1 μM, 1 h before), capsaicin (1–30 μM) produced a concentration-related inhibition of spontaneous contractions (IC_{50} = 19 μM) and of the high K^+-induced tonic contraction (IC_{50} = 23 μM). A similar effect on spontaneous motility was produced by capsaicin in colonic segments excised from capsaicin-pretreated guinea-pigs (IC_{50} = 16 μM) or guinea-pigs treated with TTX (IC_{50} = 20 μM).

6 It is concluded that, *in vivo*, capsaicin activates inhibitory reflexes, presumably due to stimulation of primary afferent fibres. This effect involves, at least in part, activation of sympathetic nerves to this organ. The contractile effect of capsaicin on the isolated colon involves activation of intramural cholinergic neurones, whereas the TTX-sensitive component of the inhibitory effect involves either release of an inhibitory transmitter through an axon reflex arrangement or activation of NANC neurones. In addition, at high concentrations capsaicin produces a direct depression of smooth muscle contraction.

Introduction

Distension of the guinea-pig isolated distal colon activates a local inhibitory reflex mediated by non-adrenergic, non-cholinergic (NANC) intramural inhibitory neurones (Costa & Furness 1976; Furness & Costa, 1977) and, in preparations with intact neural connections with sympathetic prevertebral ganglia, stimulation of mechano receptors also activates a sympathetic inhibitory colo-colonic reflex (Weems & Szurszewski 1976; Krier & Szurszewski, 1982; King & Szurszewski 1984). That both NANC and adrenergic inhibitory mechanisms may operate *in vivo* has been demonstrated recently in anaesthetized animals (Maggi *et al.*, 1985).

Thus these findings indicate that there are at least two distinct mechanisms which, following activation of colonic mechanoreceptors, produce a local 'receptive' relaxation. These are intrinsic and/or extrinsic reflex mechanisms, but both are presumably relevant to peristalsis.

Capsaicin, the pungent ingredient of many red peppers, exerts a selective excitatory effect on certain afferent nerve fibres leading to activation of a number

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of reflex responses at the cardiovascular, respiratory (Coleridge & Coleridge, 1984) intestinal (Cervero & McRitchie, 1982) and urinary level (Maggi *et al.*, 1984; 1986c). Its peculiar pharmacological profile makes capsaicin a useful tool for studying the physiology and pharmacology of the afferent branch of reflex responses, providing that, in each system, capsaicin selectivity for sensory structures is established.

Functional evidence for the involvement of capsaicin-sensitive fibres in inhibitory reflexes at the colonic level is lacking, although capsaicin induces release of substance P and stimulates neurotransmission in the inferior mesenteric ganglion of guinea-pigs (Gamse *et al.*, 1981; Tsunoo *et al.*, 1982; Dalsgaard *et al.*, 1983; Dun & Kiraly, 1984).

In view of the above, it appeared worthwhile to investigate whether or not capsaicin activates reflex responses in the distal colon of anaesthetized guinea-pigs and, if so, evaluate the mechanism(s) involved. In addition, since capsaicin produces contractions of the guinea-pig isolated taenia caeci (Szolcsányi & Barthò, 1979) we studied its effects in the isolated colon.

Evidence will be presented indicating that a direct and three indirect effects involving activation of cholinergic, adrenergic and, possibly, NANC nerves may account for the action of capsaicin in this preparation.

Methods

In vivo *experiments*

Male albino guinea-pigs weighing 240–300 g were anaesthetized with urethane 1.5 g kg^{-1}. The left jugular vein was cannulated for drug injection. A 1.0–1.5 cm long segment of the proximal part of the hypogastric loop of the distal colon (Elliot & Barclay-Smith, 1904) was cannulated with polyethylene tubing and prepared for intraluminal pressure recording (Maggi & Meli, 1984; Maggi *et al.*, 1985).

After a 10 min equilibration period at zero volume the segment was inflated with a small amount (0.1–0.2 ml) of saline to obtain a resting pressure of 8–14 mmHg. Warm saline-soaked cotton wool swabs were laid around the exteriorized segment to maintain its temperature and keep it moist.

The effects of intravenous capsaicin and other substances expected to modify colonic motility (dimethylphenylpiperazinium iodide 0.1 mg kg^{-1}, tyramine 10 mg kg^{-1}, noradrenaline 10 μg kg^{-1}) were investigated, after a 15–30 min equilibration period in controls or in preparations pretreated with various substances. Topical application of substances was made, as described previously (Maggi & Meli, 1982; 1984; Maggi *et al.*, 1985) by means of a Hamilton microsyringe in a volume of 0.1 ml 0.9% NaCl at 37°C. Topical application of the vehicle did not modify consistently the motor activity of the guinea-pig distal colon.

The effect of either capsaicin or other substances on the intraluminal pressure of the guinea-pig distal colon was calculated at the peak of their relaxant effect. To avoid interference from desensitization (see Szolcsányi, 1984), capsaicin was administered only once in each animal, unless otherwise stated. Capsaicin was administered at various times (3–10 min) after the administration of potential antagonists. Preliminary experiments indicated that, when resting intraluminal pressure was higher than 8 mmHg, the relaxant effects produced by the first dose of capsaicin are independent of the resting intraluminal pressure.

The effect of capsaicin or other substances was expressed as the difference (in mmHg) between maximal relaxation and the lowest value in intraluminal pressure recorded in the last 2–3 min before their administration. Duration of relaxation was calculated as the time required for return to the pre-drug values.

Some experiments were performed in animals pretreated with guanethidine s.c. which was given in two doses of 10 mg kg^{-1} each, administered 18 and 2 h before the experiment (Holzer, 1985).

In vitro *experiments*

Male albino guinea-pigs weighing 240–260 g were stunned and bled. A 1.0–1.5 cm long segment of the proximal part of the hypogastric loop of the distal colon was rapidly dissected out and placed in oxygenated (96% O_2 plus 4% CO_2) physiological salt solution (PSS), of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11. The segments were suspended, in an oxygenated solution contained in a 5 ml organ bath and connected to an isometric strain gauge and a Basile 7050 recorder in such a way as to register movements of the longitudinal muscle, as described by Costa & Furness (1979). Bath temperature was maintained at 37°C. An initial tension of 2 g was applied to the preparations which were then allowed to relax spontaneously.

After an equilibration period of 60 min, the effects of potential antagonists on capsaicin-induced contractions were evaluated after an incubation period of 10–15 min. Unless otherwise stated, each preparation was exposed to capsaicin only once, to prevent desensitization phenomena. In some experiments the K^+ concentration of the medium was increased by addition of KCl (100 mM). The effect of capsaicin on KCl-induced tonic contractions was studied by cumulative addition of various concentrations. This procedure was used because preliminary experiments indicated that the TTX-resistant depressant activity of capsaicin on the contractile activity did not show desensitization. In

some experiments the intramural NANC nerves were activated by field stimulation using a method similar to that described by Tonini *et al.* (1985).

Systemic capsaicin desensitization

Some experiments were performed in capsaicin-pretreated guinea-pigs. A total dose of 54.5 mg kg^{-1} (s.c.) capsaicin was administered over two days using the following schedule: 0.3 + 0.6 + 1.2 + 2.4 + 5 + 10 + 15 + 20. Injections were made at intervals of at least 2 h. Fifteen min before each injection of capsaicin the animals received atropine (1 mg kg^{-1} i.p.) plus terbutaline (0.1 mg kg^{-1} s.c.) and theophylline (2.5 mg kg^{-1} i.p.). Experiments were done 5 days after the last injection of capsaicin.

Statistical analysis

All data in the text, tables and figures are presented as the mean ± s.e.mean. Statistical analysis was made by means of Student's *t* test for paired or unpaired data or by means of the analysis of variance, when applicable. Regression analysis was performed by means of the least squares method. ED_{50} values and 95% confidence limites were calculated accordingly.

Drugs

Drugs used were: capsaicin (Serva), atropine HCl (Serva), hexamethonium bromide (Serva), dimethylphenylpiperazinium iodide (DMPP, Aldrich), tetrodotoxin (Sankyo), phentolamine mesylate (Regitin, Ciba), propranolol HCl (Sigma), noradrenaline HCl (Fluka) guanethidine sulphate (Ciba Geigy), theophylline (Serva), terbutaline sulphate (Terbasmin, Carlo Erba), verapamil HCl (Isoptin, Knoll). All substances were dissolved in 0.9% NaCl for *in vivo* administration (either intravenous or topical) or in PSS for *in vitro* experiments. For *in vivo* experiments a 3 mg ml^{-1} stock solution of capsaicin was made in absolute ethanol from which the appropriate dilutions in saline were made. For *in vitro* experiments a 10 mM capsaicin solution was made in absolute ethanol from which the appropriate dilutions were made in PSS.

Results

In vivo *experiments*

The saline-loaded distal colon of urethane-anaesthetized guinea-pigs exhibited a phasic contractile activity whose characteristic frequency and amplitude were 8–14 contractions per min and 1–4 mmHg, respectively. After an initial reduction following saline loading most preparations exhibited, for at least 20–30 min, fairly stable intraluminal pressure values (Maggi & Meli, 1984; Giotti *et al.*, 1985; Maggi *et al.*, 1985).

Effect of intravenous capsaicin Intravenously administered capsaicin (0.1–10 μg kg^{-1}) produced, within a few s, a sudden relaxation of the guinea-pig distal colon and disappearance or marked reduction in phasic contractile activity (Figure 1). Amplitude of the capsaicin-induced relaxations (3.6 ± 0.5 mmHg at 3 μg kg^{-1}, $n = 24$) was similar to that produced, on the same preparation, by DMPP or noradrenaline. A submaximally effective dose of capsaicin (3 μg kg^{-1} i.v.) was selected for further experiments since a higher dose (10 μg kg^{-1}) produced respiratory side-effects. The relaxant effect of a second dose of capsaicin (3 μg kg^{-1} i.v.) was reproducible after a 15–20 min interval (Figure 1). However, a third or fourth challenge with this same dose of capsaicin at 15–20 min intervals produced responses of progressively decreasing amplitude, indicating desensitization ($n = 4$). Amplitude of the capsaicin (3 μg kg^{-1} i.v.)-induced relaxation was independent ($n = 24$, $r = 0.03337$, NS) of the resting pressure, in the range of intraluminal pressures studied (8–16 mmHg).

Effect of intravenous DMPP, tyramine and noradrenaline DMPP (0.1 mg kg^{-1} i.v.) produced complex motor effects consisting of: (a) an initial transient contraction followed by a more sustained relaxation or (b) a small relaxation followed by a transient contraction and a further, more sustained relaxation (Figure 1), or (c) a sustained relaxation. In all instances recovery was complete within 60–120 s after DMPP administration. A distinct contraction could be observed in 9 out of 24 preparations. Mean amplitude of DMPP-induced contraction and relaxation were 4 ± 0.6 ($n = 9$) and 3.7 ± 0.2 mmHg ($n = 24$), respectively.

Tyramine (10 mg kg^{-1} i.v., $n = 24$) produced a transient contraction (5.0 ± 0.7 mmHg) followed by a more sustained (90–150 s) relaxation (2.8 ± 0.3 mmHg). Noradrenaline (10 μg kg^{-1} i.v. $n = 24$) produced a transient (60–120 s) relaxation (3.7 ± 0.4 mmHg) (Figure 1).

Capsaicin (3 μg kg^{-1} i.v., $n = 4$) did not affect colonic motility of capsaicin-pretreated (54.5 mg kg^{-1} s.c. 5 days before, Figure 2) animals, while the effects of DMPP (0.1 mg kg^{-1}, i.v.), tyramine (10 mg kg^{-1}, i.v.) and noradrenaline (10 μg kg^{-1}, i.v.) were similar, both qualitatively and quantitatively, to those observed in controls.

Effect of atropine, hexamethonium and tetrodotoxin on capsaicin-induced relaxation Atropine-pretreatment (5 mg kg^{-1}, i.v., $n = 10$) significantly reduced

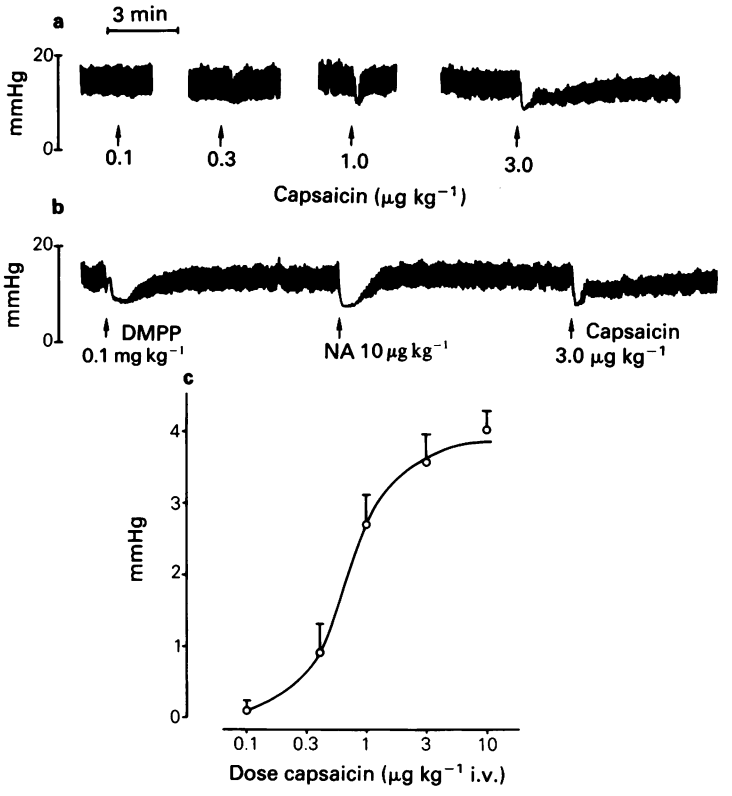

Figure 1 (a and b) Tracings showing the effects of i.v. capsaicin, noradrenaline (NA, 10 μg kg^{-1}) and dimethylphenylpiperazinium (DMPP, 0.1 mg kg^{-1}) on motility of the distal colon in urethane-anaesthetized guinea-pigs. Records were obtained in the same animal with a 15–25 min interval between doses. (c) Dose-response curve for the inhibitory effect of i.v. capsaicin on the resting tone of the guinea-pig distal colon.

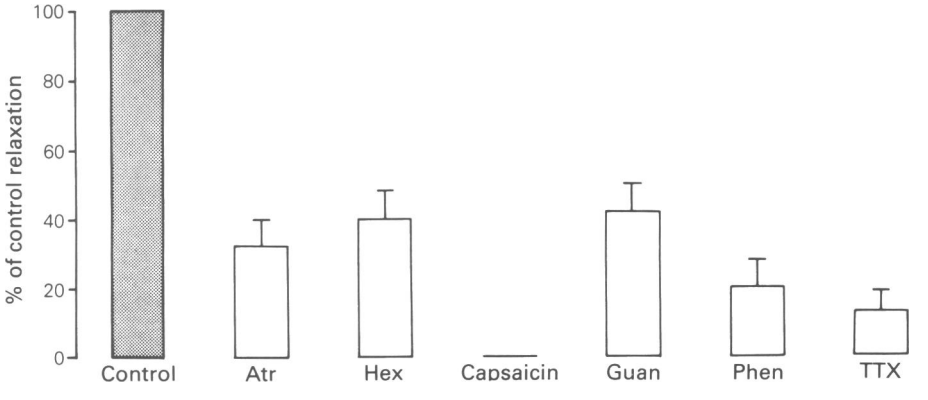

Figure 2 Effect of atropine (Atr, 5 mg kg^{-1} i.v.)-, hexamethonium (Hex, 40 mg kg^{-1} i.v.)-, capsaicin (54.5 mg kg^{-1} s.c., 5 days before)-, guanethidine (Guan, 20 mg kg^{-1} s.c. in two doses of 10 mg kg^{-1} each, 2 and 18 h before)-, phentolamine (Phen, 0.5 mg kg^{-1} i.v.)- and tetrodotoxin (TTX 20 μg in 0.1 ml, topical)- pretreatment on the colonic relaxation induced by intravenous capsaicin (3 μg kg^{-1} i.v.). All groups were significantly different from controls $P < 0.01$.

(about 60%) the capsaicin-induced relaxation (Figure 2) and almost suppressed the DMPP- or tyramine-induced contractions of the guinea-pig distal colon.

Intravenously administered hexamethonium (40 mg kg^{-1}, $n = 10$) produced a prompt and transient increase in tone (Giotti *et al.*, 1985; Maggi *et al.*, 1985) and prevented DMPP-induced motor effects. It halved the amplitude of capsaicin-induced relaxation (Figure 2), while the motor effects of tyramine or noradrenaline were unaffected.

Topical tetrodotoxin (20 μg in 0.1 ml, $n = 8$) significantly reduced the motor effects of capsaicin (Figure 2), DMPP and tyramine, while the amplitude of the noradrenaline-induced relaxation of the colon was barely affected.

Effect of guanethidine and phentolamine on capsaicin-induced relaxation Pretreatment with guanethidine (20 mg kg^{-1} s.c., $n = 20$) reduced the tyramine (10 mg kg^{-1} i.v.)-induced contraction by about 40–50% and almost suppressed the tyramine-induced relaxation, consistent with a functional impairment of noradrenergic nerves. The tyramine-induced contraction involves release of some substance(s) co-stored with noradrenaline in noradrenergic nerves, as described by Cheng & Shen (1986) in the rabbit isolated ileum. In guanethidine-pretreated animals the capsaicin-induced relaxation was about half that of controls (Figure 2) and in 6 out of 20 cases capsaicin did not affect colonic motility. The DMPP-induced contraction was significantly enhanced by guanethidine, but DMPP-induced relaxation was reduced compared to controls, thus confirming its partial dependence upon activation of sympathetic postganglionic elements (Maggi *et al.*, 1985).

Phentolamine (0.5 mg kg^{-1} i.v.) reduced significantly the capsaicin-induced relaxant responses (Figure 2) and in 5 out of 10 preparations completely prevented them. Phentolamine pretreatment nearly abolished the relaxant responses to noradrenaline and tyramine, slightly reduced the tyramine-induced contraction and DMPP-induced relaxation and potentiated the DMPP-induced contraction of guinea-pig distal colon.

Effect of topical capsaicin on resting tone and spontaneous activity of guinea-pig distal colon Topical capsaicin (0.1–1.0 μg in 0.1 ml saline) produced, within 5–15 s, a reduction in resting tone and, in some preparations a reduction in amplitude of phasic activity (Figure 3). Capsaicin-induced inhibition of colonic motility reached a peak within 20–60 s after its application and recovered thereafter. At all doses tested, the time to recovery varied from one preparation to another. In some preparations recovery of intraluminal pressure was slow and incomplete (Figure 3a). Peak relaxation induced by capsaicin was dose-related. The transient relaxations produced by topical application of capsaicin (0.3 μg in 0.1 ml) were not observed in colonic segments pretreated with topical TTX (20 μg in 0.1 ml) (Figure 3, $n = 6$).

In some experiments the capsaicin 'desensitization' phenomenon was explored by repeated administration (0.1–1.0 μg at 20 min intervals). A second dose of capsaicin produced a smaller transient inhibition of tone. Reduction of the inhibitory effect produced by a second application of capsaicin was roughly dose-related (cf. Maggi *et al.*, 1986a). A third application of capsaicin produced a smaller response than the second, i.e. repeated application of capsaicin led to desensitization.

In vitro *experiments*

In isolated segments of the distal colon of the guinea-pig, a train of pulses (5 Hz, 60 V, 0.5 ms for 1 s) applied at the peak of a spontaneous contraction produced a sudden transient relaxation, both in the absence (1.9 ± 0.3 g, $n = 6$) and the presence of atropine (3 μM) plus guanethidine (3 μM) (2.2 ± 0.4 g, $n = 5$). These relaxations were abolished by previous incubation with TTX (0.5 μM), indicating their dependence upon activation of intramural NANC nerves (see Maggi *et al.*, 1985).

Exposure to capsaicin (0.1–1 μM, $n = 15$) had a dual effect on motor activity, i.e. it at first produced a contraction lasting 1–2 min which was paralleled, in most preparations, by increased frequency of phasic activity (Figure 4). In most preparations, the amplitude of the contractions produced by a low concentration of capsaicin (0.1 μM) appeared to be less intense (0.2–0.4 g) and lasted less time than those produced by a higher concentration (1 μM) (1.4 ± 0.2 g, $n = 10$), but 3–10 μM capsaicin did not produce greater contractions than 1 μM. When the contractile effects of capsaicin had subsided, a decrease in amplitude of spontaneous phasic contractile activity was observed. This reduction reached a maximum ($43 \pm 6\%$ at 1 μM) within 4–10 min; the amplitude then recovered slowly to 80–90% of the original value in the continued presence of capsaicin (Figure 4). Removal of capsaicin by repeated washing resulted in a prompt recovery in the amplitude of the phasic contractions.

A second administration of capsaicin (1 μM) 1–2 h later did not produce excitatory effects, but a transient depression in spontaneous contractile activity (Figure 4). Inhibition ($13 \pm 2\%$, $n = 6$) produced by a second application of capsaicin was significantly ($P < 0.05$) lower, but similar to that generated by third and fourth challenges at intervals of 1 h (Table 1). The inhibitory effect produced by a second, third or fourth application of capsaicin (1 μM) reached its peak in 2–6 min

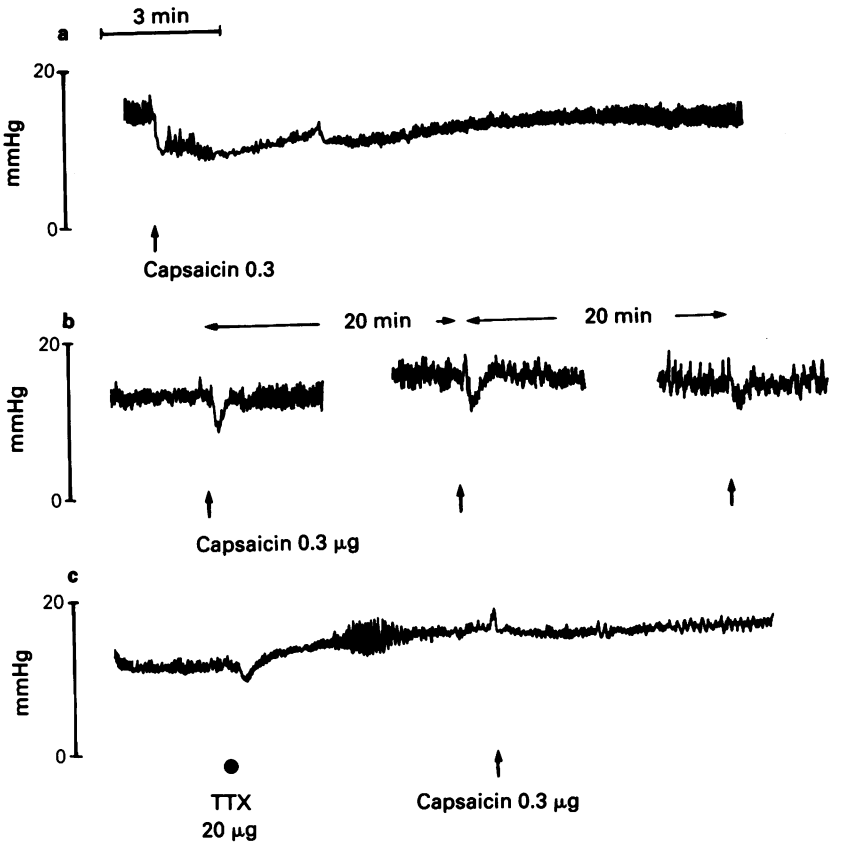

Figure 3 Tracings showing the inhibitory effect on colonic motility produced by topical application of capsaicin (0.3 μg in 0.1 ml saline) in control (a and b) or tetrodotoxin (TTX, 20 μg in 0.1 ml saline) pretreated (c) preparations. (a) This preparation shows a slow and incomplete tone recovery after topical application of capsaicin. (b) In this preparation capsaicin produced a transient relaxation. Repeated application of capsaicin produced a response of progressively decreasing amplitude, indicating desensitization.

and was maintained for at least 15 min without any sign of recovery. Washing out at any time after addition of capsaicin produced a prompt recovery in the amplitude of spontaneous contractions.

Effect of tetrodotoxin, atropine, adrenoceptor blockers and guanethidine In the presence of either TTX (0.5 μM) or atropine (3 μM), exposure to capsaicin (1 μM) produced a contraction in only 3 of 8 preparations in both groups. The amplitude was significantly lower than in controls (Table 1). In atropine-treated preparations the capsaicin-induced inhibition of spontaneous contractions was similar to that observed in controls (Table 1). On the other hand, in TTX-treated preparations, the capsaicin-induced inhibition of spontaneous contractions (about 20%) was significantly less than in controls (Table 1).

Neither phentolamine plus propranolol (0.2 μM each) nor guanethidine (3 μM) affected the inhibitory effects of capsaicin.

Capsaicin-desensitized animals Colonic segments from desensitized (54.5 mg kg^{-1} s.c., 5 days before) guinea-pigs ($n = 6$) exhibited spontaneous contractile activity (1.5 ± 0.2 min^{-1}, 4.8 ± 0.8 g) similar to controls (1.5 ± 0.1 min^{-1}, 5.4 ± 0.4 g, $n = 10$). In these preparations, field stimulation produced a relaxation similar in amplitude (2.2 ± 0.4 g) to controls. Further, TTX (1 μM)-sensitive relaxant responses elicited by field stimulation in the presence of atropine plus guanethidine (3 μM, $n = 6$) were similar in amplitude to those observed in controls (2.3 ± 0.2 g, $n = 6$).

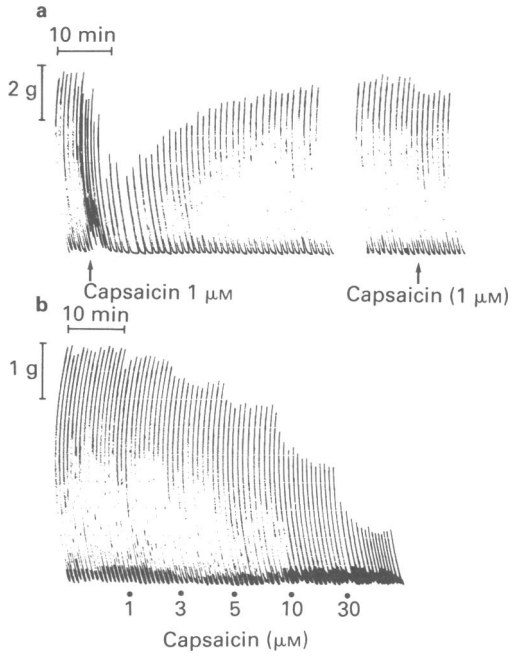

Figure 4 Tracings show the motor effects of capsaicin on the guinea-pig isolated distal colon. (a) Upon first application, capsaicin (1 μM) produced a transient contraction and acceleration of spontaneous activity; this excitatory phase was followed by a reduction in amplitude of about 40% of the spontaneous contraction. A second (right panel) application of capsaicin (1 μM) after 1 h produced only a 10–12% inhibition. (b) The direct depressant (e.g. on muscle cells) effect of increasing, cumulative concentrations of capsaicin on spontaneous contractions of the guinea-pig distal colon. The preparation was primed with capsaicin (1 μM, 1 h before) to avoid interference from neurogenic (tetrodotoxin-sensitive) phenomena.

In preparations from capsaicin-pretreated animals, capsaicin (1 μM, $n = 6$) failed to produce any contraction, but had a depressant effect (Table 1) on the amplitude of spontaneous contractions. This inhibitory effect was significantly less than that produced by the first exposure to capsaicin in controls (Table 1). Second or third applications of capsaicin at 1 h intervals produced an inhibitory effect not significantly different from that produced by the first dose (Table 1).

Direct depressant effect of capsaicin In these experiments we investigated the depressant effect of capsaicin on phasic contractile activity. To prevent interference by contractile effects, preparations were previously exposed (1 h) to capsaicin (1 μM). Capsaicin (1–30 μM, $n = 6$) reduced, in a concentration-related manner, the amplitude of spontaneous phasic contractions (IC_{50} 19.4 μM, 14–30 μM, mean and 95% confidence limits (c.l.), Figure 4). The depressant effect of capsaicin was unaffected (IC_{50} 20.4 μM, 17–24 μM, mean and 95% c.l.) by TTX (0.5 μM, $n = 6$). In the colon from capsaicin-pretreated animals the inhibitory effect of capsaicin (IC_{50} 16.1 μM, 14–21 μM, mean and 95% c.l.) was not significantly different from controls. The direct nature of the effect was further demonstrated by the observation that there was no significant difference between the inhibitory effect of capsaicin (30 μM) administered during a cumulative dose-response curve (75 ± 6% inhibition, $n = 6$, Figure 4b) and that resulting from a single dose (81 ± 6%, $n = 4$). The inhibitory effect of the capsaicin vehicle (15 μl of absolute ethanol for capsaicin 30 μM) did not exceed 15%.

Addition of KCl (100 mM) to the bathing solution produced a sudden phasic contraction which declined leaving a sustained component. The latter reached steady values (3.7 ± 0.5 g, $n = 8$) after 15–30 min. Capsaicin (1–30 μM, $n = 4$) produced a concentration-related inhibition (IC_{50} 23 μM, 21–27 mM, mean and 95% c.l.) of the high K^+-induced tonic contraction. Under similar experimental conditions, verapamil (1 μM, $n = 4$) produced a 96 ± 2% inhibition of high K^+-induced tonic contractions.

Discussion

Taken as a whole our findings indicate that capsaicin exerts three indirect effects mediated by nervous pathways and a direct effect on motility in the guinea-pig distal colon (Table 2). The indirect effects include: (a) transient inhibition of colonic motility *in vivo* which is, at least in part, guanethidine-sensitive, indicating the involvement of the postganglionic sympathetic innervation; (b) a TTX-and atropine-sensitive contractile effect on the isolated preparation; (c) a TTX-sensitive but atropine- and guanethidine-resistant (NANC) inhibition of spontaneous activity.

Further, there was a depressant, TTX-insensitive inhibitory effect seen *in vitro* on exposing colonic segments to high concentrations ($IC_{50} > 10$ μM) of this substance (action (d), Table 2).

Effects (a), (b) and (c) exhibit desensitization (Table 2) and may thus be ascribed to an indirect effect of capsaicin (e.g. activation of sensory nerves) whereas effect (d) does not exhibit desensitization and may be due to a direct (unspecific) action of capsaicin on smooth muscle contractility.

Pharmacological analysis (Table 2) suggests that, *in vivo*, capsaicin produces transient relaxation of the

Table 1 Effect of capsaicin on the spontaneous motor activity of the guinea-pig isolated distal colon

Treatment	n	Effect of capsaicin	Response to capsaicin (1 μM) First challenge	Second challenge	Third challenge
Controls	8	Contraction (g)	1.4 ± 0.2	—	—
		Inhibition (%)	43.5 ± 7	13.2 ± 2[b]	12.5 ± 2[b]
Capsaicin-pretreated	5	Contraction (g)	—	—	—
		Inhibition (%)	13.2 ± 2[a]	13.5 ± 2	12.2 ± 0.7
Tetrodotoxin	8	Contraction (g)	0.2 ± 0.1[a]	—	—
		Inhibition (%)	20.0 ± 4[a]	14.0 ± 3[b]	14.2 ± 3[b]
Atropine	8	Contraction (g)	0.3 ± 0.1[a]	—	—
		Inhibition (%)	43.2 ± 8	13.5 ± 1[b]	12.5 ± 0.5[b]

Each value is mean ± s.e.mean. Experiments were performed in colonic segments excised from capsaicin-pretreated animals (54.5 mg kg^{-1} s.c. 5 days before) or exposed to tetrodotoxin (0.5 μM) or atropine (3 μM). Capsaicin (1 μM) was applied three times in each preparation at 1 h intervals. In controls, capsaicin produces a contraction (expressed in g) followed by an inhibition of the spontaneous contractions (expressed %).
[a]Significantly different from control, $P < 0.05$.
[b]Significantly different from the first response to capsaicin, $P < 0.01$.

distal colon by activating a hexamethonium-sensitive neurogenic mechanism(s) which transiently inhibits the cholinergic neural input elicited by saline distension (Maggi *et al.*, 1985). There is both sympathetic (Weems & Szurszewski 1976; Furness & Costa, 1980; Krier & Szurszewski 1982; King & Szurszewski 1984; Maggi *et al.*, 1985) and NANC inhibitory innervation in the guinea-pig distal colon (Costa & Furness, 1976; Furness & Costa, 1977; Maggi *et al.*, 1985). Recent findings indicate that capsaicin-sensitive fibres inhibit gastrointestinal motility by activating extrinsic sympathetic (Holzer, 1986; Holzer *et al.*, 1986) and intrinsic NANC mechanisms (Maggi *et al.*, 1986a,b).

Therefore, we might speculate that, *in vivo*, capsaicin activates a colo-colonic reflex which inhibits motility by stimulating intramural afferent fibres and, at least in part, the sympathetic supply to the colon. The existence of a capsaicin-sensitive mechanoreceptive input from the guinea-pig distal colon to the inferior mesenteric ganglion has been described recently (Kruelen & Peters, 1986). The possible contribution of a NANC intramural inhibitory mechanism to the capsaicin-induced relaxation *in vivo* (see below) cannot be excluded.

Effects of capsaicin on the isolated colon

Capsaicin produces a contraction of guinea-pig isolated ileum which is neurogenic in origin (Barthò & Holzer 1985) and depends upon the release, from intramural nerves, of substance P (and possibly other neuropeptides). The peptides produce a contraction by a direct action on muscle cells and by activating intramural cholinergic neurones.

Our findings indicate that a similar mechanism may occur in the isolated colon since, in agreement with

Table 2 Characteristics of the four motor effects of capsaicin on guinea-pig distal colon

Effect on motility	Capsaicin desensitization	Sensitivity to: Atropine	Guanethidine	Tetrodotoxin
a Inhibition	Yes	Yes	Yes	Yes
b Contraction	Yes	Yes	No	Yes
c Inhibition	Yes	No	No	Yes
d Inhibition	No	No	No	No

Effect (a) was observed in *in vivo* conditions. Effects (b), (c) and (d) were observed *in vitro* but may also be involved in *in vivo* conditions. Effect (d) was evident only at high concentrations ($EC_{50} > 10$ μM).

previous findings (Szolcsányi & Barthò, 1979), a muscarinic receptor antagonist markedly reduced the capsaicin-induced contractions. Whether the release of sensory transmitters acting on intramural cholinergic neurones, as described for the ileum (Barthò & Holzer, 1985) is involved, remains to be established.

In addition, in the isolated colon, the first exposure to capsaicin produced a marked inhibitory response (about 40% inhibition at 1 μM). Although a component of this seems to be due to a non-desensitizing effect on muscle cell contractility (see below), a part could be due to activation of intramural sensory nerves leading to a transient neurogenic inhibition of motility. Intramural NANC inhibitory neurones (Costa & Furness, 1976; Furness & Costa, 1977; Maggi *et al.*, 1985) may be involved, as described for the capsaicin-induced relaxation of the rat isolated duodenum (Maggi *et al.*, 1986a). However, we cannot exclude the possibility that sensory transmitters are released by capsaicin through an axon reflex arrangement which inhibits colonic motility without involving intramural inhibitory neurones.

In preparations desensitized to capsaicin, the amplitude of the neurogenic NANC relaxations induced by field stimulation was similar to that of controls, consistent with the notion that capsaicin-desensitization spares non-sensory innervation (Szolcsányi & Barthò, 1979; Szolcsányi, 1984).

Direct depressent effect of capsaicin on contractility

Capsaicin may have direct effects on either cardiac or smooth muscle (Lundberg *et al.*, 1984; Zernig *et al.*, 1984). The effects on isolated cardiac preparations from guinea-pigs and on the rat isolated uterus were attributed to interference with transmembrane Ca^{2+} fluxes, possibly through a membrane-stabilizing action (Zernig *et al.*, 1984).

However, further studies indicated that at least some of the cardiac effects of capsaicin are ascribable to the release of previously unrecognized neuropeptides from sensory nerves (Lundberg *et al.*, 1984; Franco-Cereceda *et al.*, 1985). The effect of capsaicin on cardiac chrono- and inotropism exhibited marked desensitization (Zernig *et al.*, 1984) and was absent in preparations excised from capsaicin-pretreated animals (Franco-Cereceda *et al.*, 1985). Desensitization is typical of the 'specific' action of capsaicin on sensory nerves and can be observed even at very low concentrations (Szolcsányi, 1984). We may thus propose that those visceromotor effects of capsaicin showing desensitization are due to a specific action on sensory nerves, while those which did not show desensitization may be direct actions on the muscle cells.

We observed a depressant effect of capsaicin on spontaneous and KCl-induced contractions, which was also observed in preparations pre-exposed to capsaicin without any sign of desensitization. It may thus be ascribed to a direct action on myocytes. Both spontaneous and KCl-stimulated contractions of the guinea-pig isolated distal colon are likely to be dependent upon a trans-membrane influx of Ca^{2+}, since they are promptly inhibited by low concentrations of Ca^{2+} entry blockers or by exposure to a high K^+-Ca^{2+} free medium (Maggi, unpublished data). However, it appears unlikely that Ca^{2+} entry blockade is involved in the direct inhibitory action because capsaicin does not affect the plateau phase of the cardiac action potential (Zernig *et al.*, 1984).

References

BARTHÒ, L. & HOLZER, P. (1985). Search for a physiological role of substance P in gastrointestinal motility. *Neuroscience*, **16**, 1–32.

CERVERO, F. & MC RITCHIE, H.A. (1982). Neonatal capsaicin does not affect unmyelinated efferent fibers of the automatic nervous system: functional evidence. *Brain Res.*, **239**, 283–288.

CHENG, J.T. & SHEN, C.L. (1986). Tyramine-induced release of neuropeptide Y (NPY) in isolated rabbit intestine. *Eur. J. Pharmac.*, **123**, 303–306.

COLERIDGE, J.C.G. & COLERIDGE, H.M. (1984). Afferent vagal C-fibre innervation of the lungs and airways and its functional significance. *Rev. Physiol. Biochem. Pharmac.*, **99**, 1–110.

COSTA, M. & FURNESS, J.B. (1976). The peristaltic reflex: an analysis of the nerve pathways and their pharmacology. *Naunyn-Schmiedebergs Arch. Pharmac.*, **294**, 47–60.

COSTA, M. & FURNESS, J.B. (1979). The sites of action of 5-hydroxytryptamine in nerve-muscle preparation from the guinea-pig small intestine and colon. *Br. J. Pharmac.*, **65**, 237–248.

DALSGAARD, C.J., VINCENT, S.R., SCHULTZBERG, M., HOKFELT, T., ELFVIN, L.G., TERENIUS, L. & DOCKRAY, G.J. (1983). Capsaicin-induced depletion of substance P-like immunoreactivity in guinea pig sympathetic ganglia. *J. Autonom. Nervous System*, **9**, 595–606.

DUN, N.J. & KIRALY, M. (1984). Capsaicin causes release of a substance P-like peptide in the guinea pig inferior mesenteric ganglia. *J. Physiol.*, **340**, 107–120.

ELLIOTT, T.R. & BARCLAY-SMITH, E. (1904). Antiperistalsis and other muscular activities of the colon. *J. Physiol.*, **23**, 272–304.

FRANCO-CERECEDA, A. & LUNDBERG, J.M. (1985). Calcitonin gene-related peptide (CGRP) and capsaicin-induced stimulation of heart contractile rate and force. *Naunyn-Schmiedebergs Arch. Pharmac.*, **331**, 146–151.

FURNESS, J.B. & COSTA, M. (1977). The participation of enteric inhibitory nerves in accommodation of the intes-

tine to distension. *Clin. exp. Pharmac. Physiol.*, **4,** 37–41.

FURNESS, J.B. & COSTA, M. (1980). Type of nerves in the enteric nervous system. *Neuroscience*, **5,** 1–20.

GAMSE, R., WAX, A., ZIGMOND, R.E. & LEEMAN, S.E. (1981). Immunoreactive substance P in sympathetic ganglia: distribution and sensitivity toward capsaicin. *Neuroscience*, **6,** 437–441.

GIOTTI, A., LUZZI, S., MAGGI, C.A., SPAGNESI, S. & ZILLETTI, L. (1985). Modulatory activity of GABA B receptors on cholinergic tone in guinea-pig distal colon. *Br. J. Pharmac.*, **84,** 883–895.

HOLZER, P. (1985). Stimulation and inhibition of gastrointestinal propulsion induced by substance P and substance K in the rat. *Br. J. Pharmac.*, **86,** 305–312.

HOLZER, P. (1986). Capsaicin-sensitive afferent neurones and gastrointestinal propulsion in the rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **332,** 62–65.

HOLZER, P., LIPPE, I. TH. & HOLZER-PETSCHE, U. (1986). Inhibition of gastro-intestinal transit due to surgical trauma or peritoneal irritation is reduced in capsaicin-treated rats. *Gastroenterology*, **91,** 360–363.

KING, B.F. & SZURSZEWSKI, J.H. (1984). Mechanoreceptor pathways from the distal colon to the autonomic nervous system in the guinea pig. *J. Physiol.*, **350,** 93–107.

KREULEN, D.L. & PETERS, S. (1986). Non-cholinergic transmission in a sympathetic ganglion of the guinea pig elicited by colon distension. *J. Physiol.*, **374,** 315–334.

KRIER, J. & SZURSZEWKI, J.H. (1982). Effect of substance P on colonic mechano-receptors motility and sympathetic neurons. *Am. J. Physiol.*, **243,** G259–G267.

LUNDBERG, J.M., HUA, Y. & FREDHOLM, B.B. (1984). Capsaicin-induced stimulation of the guinea pig atrium. Involvement of a novel sensory transmitter or a direct action on myocites? *Naunyn-Schmiedebergs Arch. Pharmac.*, **325,** 176–182.

MAGGI, C.A., MANZINI, S., GIULIANI, S., SANTICIOLI, P. & MELI, A. (1986a). Extrinsic origin of the capsaicin-sensitive innervation of rat duodenum: possible involvement of calcitonin gene-related peptide (CGRP) in the capsaicin-induced activation of intramural non-adrenergic non-cholinergic neurons. *Naunyn-Schmiedebergs Arch. Pharmac.*, **331,** 172–180.

MAGGI, C.A. & MELI, A. (1982). An in vivo procedure for estimating spasmolytic activity in the rat by measuring smooth muscle contractions to topically applied acetylcholine. *J. Pharmac. Methods*, **8,** 39–46.

MAGGI, C.A. & MELI, A. (1984). Eserine-induced hypertone of the guinea pig distal colon "in vivo": a new pharmacological precedure for testing smooth muscle relaxants. *J. Pharmac. Methods*, **12,** 91–96.

MAGGI, C.A., SANTICIOLI, P., MANZINI, S. & MELI, A. (1985). Contribution of neurogenic and myogenic factors to the contractile activity of the guinea pig distal colon in vivo and in vitro. *J. Autonom. Pharmac.*, **5,** 177–187.

MAGGI, C.A., SANTICIOLI, P., MANZINI, S. & MELI, A. (1986b). Capsaicin activates neurogenic non-adrenergic non-cholinergic relaxations of the isolated rat duodenum. *Eur J. Pharmac.*, **120,** 367–370.

MAGGI, C.A., SANTICIOLI, P. & MELI, A. (1984). The effects of topical capsaicin on rat urinary bladder motility in vivo. *Eur. J. Pharmac.*, **103,** 41–50.

MAGGI, C.A., SANTICIOLI, P., BORSINI, F., GIULIANI, S. & MELI, A. (1986c). The role of the capsaicin-sensitive innervation of the rat urinary bladder in the activation of micturition reflex. *Naunyn-Schiedebergs Arch Pharmac.*, **332,** 275–283.

SZOLCSÁNYI, J. & BARTHÓ, L. (1979). Capsaicin-sensitive innervation of the guinea pig taenia caeci. *Naunyn-Schmiedebergs Arch. Pharmac.*, **309,** 77–82.

SZOLCSÁNYI, J. (1984). Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function. In *Antidromic Vasodilation and Neurogenic Inflammation.* ed. Chahl, L.A., Szolcsányi, J. & Lembeck, F. pp. 26–52. Budapest: Akademial Kiado.

TONINI, M., ONORI, L., PERUCCA, E., MANZO, L., DE PONTI, F. & CREMA, A. (1985). Depression by morphine of the excitability of intrinsic inhibitory neurons in the guinea pig colon. *Eur. J. Pharmac.*, **115,** 317–320.

TSUNOO, A., KONISHI, S., & OTSUKA, M. (1982). Substance P as an excitatory transmitter of primary afferent neurons in guinea pig sympathetic ganglia. *Neuroscience*, **7,** 2025–2037.

WEEMS, W.A. & SZURSZEWSKI, J.H. (1976). Modulation of colonic motility by peripheral neural inputs to neurons of the inferior mesenteric ganglion. *Gastroenterology*, **73,** 273–278.

ZERNIG, G., HOLZER, P. & LEMBECK, F. (1984). A study of the mode and site of action of capsaicin in guinea pig heart and rat uterus. *Naunyn-Schmiedebergs Arch. Pharmac.*, **326,** 58–63.

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