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# Capsaicin-sensitive mechanisms and experimentally induced duodenal ulcers in rats

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The incidence and degree of cysteamine- or dulcerozine-induced duodenal ulcers are increased by systemic capsaicin desensitization (50 mg $kg^{-1}$ s.c. 4 days before) in adult rats. Acute administration of capsaicin, but not neurokinins or CGRP, produced a small but distinct plasma extravasation (Evans blue leakage) in the rat proximal duodenum which was absent in capsaicin-pretreated rats. These findings indicate the existence of a capsaicin-sensitive 'duodenal defence mechanism' in rats.

In recent years evidence has been accumulated indicating the presence, in the gastrointestinal tract, of various species of a capsaicin-sensitive sensory innervation containing a variety of neuropeptides such as substance P, somatostatin and calcitonin gene-related peptide (CGRP) (Costa et al 1980, 1981; Gamse et al 1981; Sharkey et al 1984; Clague et al 1985; Sternini & Brecha 1985). Functional studies indicate that this capsaicin-sensitive innervation plays a role in determining and co-ordinating gastrointestinal motility (Cervero & McRitchie 1982; Maggi et al 1986; Holzer 1986) and might be involved in a 'gastric defence mechanism' which affords protection toward gastric ulcers induced by pylorus ligation, acid distension (Szolcsányi & Barthó 1981) indomethacin, ethanol or cysteamine (Holzer & Sametz 1986). By analogy, it is believed that this capsaicin-sensitive 'gastric defence mechanism' could have a trophic antiulcer influence in humans as well.

We now present evidence for the existence of a capsaicin-sensitive mechanism which affords protection toward dulcerozine- or cysteamine-induced ulceration of the rat proximal duodenum. In this same intestinal segment capsaicin produces also a moderate but clearly evident plasma extravasation (Evans blue leakage) indicating an increased vascular permeability, which may contribute to antiulcer protection at this level.

## *Materials and methods*

*Duodenal ulcers.* Female albino rats Sprague-Dawley Morini strain, 180–200 g, were housed in plastic cages with wire bottoms to minimize coprophagy. Dulcerozine was suspended in an aqueous vehicle containing NaCl 0·9%, polysorbate (Tween) 80 0·4% and carboxymethylcellulose 0·5%, while cysteamine was dissolved in saline. All substances were administered in a volume of 5 mL $kg^{-1}$.

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Cysteamine (900 or 1200 mg $kg^{-1}$) was administered by gavage to unfasted animals and duodenal ulcers determined 24 h later. Dulcerozine (300 mg $kg^{-1}$) was administered by gavage to 24 h fasted animals (Kurebayashi et al 1984) and duodenal ulcers determined 16 h later.

The degree of intestinal ulceration was graded according to Szabó et al (1979) as follows: 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer usually with transmural necrosis, 3 = perforated ulcer.

*Plasma protein extravasation.* Female albino rats Sprague-Dawley Morini strain, 180–200 g were anaesthetized with subcutaneous urethane (1·2 g $kg^{-1}$) and artificially ventilated. Plasma protein extravasation was determined as described by Saria & Lundberg (1983). Test substances were injected after at least 30 min from completion of surgical procedures. Briefly, Evans blue (20 mg $kg^{-1}$) was injected through a polyethylene tubing inserted into the left jugular vein 5 min before the intravenous administration of test substances (capsaicin, neurokinins, calcitonin gene-related peptide, histamine).

Five min after the i.v. administration of test substances, saline (50 mL in 30 s) was injected into the animals via the thoracic aorta.

Evans blue content was determined by fluorimetry (Perkin-Elmer 512 Double Beam spectrophotometer) as described by Saria & Lundberg (1983). A segment of the proximal duodenum (the first 2 cm caudal to the pyloric sphincter) was gently blotted three times on filter paper and weighed. Evans blue content of duodenal segments was determined by fluorimetry and expressed as ng $mg^{-1}$ of wet weight.

*Systemic capsaicin desensitization.* Experiments were performed in rats desensitized to capsaicin (50 mg $kg^{-1}$ s.c. 4 days before) as described previously (Maggi et al 1986). Control rats received the vehicle (polysorbate 80 10, ethanol 10 and saline 80%).

*Statistical analysis.* The percent incidence of ulcers and mortality were analysed according to the Chi square method. Data relative to degree of ulcers were analysed by means of Smirnov's test. Statistical analysis of parametric data was by means of the Student's *t*-test for unpaired data.

*Materials*. Drugs used were: capsaicin (Sigma); cysteamine (Sigma), dulcerozine (Daiichi), substance P (Peninsula), neurokinin A (Peninsula), rat calcitonin gene-related peptide (CGRP, Peninsula), kassinin (Peninsula), histamine dihydrochloride (Fluka), Evans blue (Serva). Neurokinin B was kindly supplied by Prof. D. Regoli, Department of Physiology and Pharmacology, Sherbrooke University, Sherbrooke, Canada.

*Results*

Both cysteamine (900–1200 mg $kg^{-1}$) and dulcerozine (300 mg $kg^{-1}$) produced duodenal ulcers which were almost restricted to the first 2 cm caudal to the pyloric sphincter. Compared with controls, systemic capsaicin desensitization significantly increased: (a) the degree of ulcers induced by a low dose of cysteamine, (b) the mortality induced by a high dose of cysteamine and (c) the incidence and degree of ulcers induced by dulcerozine (Table 1).

Intravenous capsaicin (5 μmol $kg^{-1}$) produced a clearly detectable blueing of the rat duodenum which was more evident in the first 2 cm caudal to the pyloric sphincter and faded away after the choledocoduodenal junction. Quantitative analysis of Evans blue content in this segment indicated that capsaicin produced a significant plasma extravasation (Fig. 1). This response to capsaicin amounted to about 70 and 30% of that induced by 9 and 30 μmol $kg^{-1}$ of intravenous histamine, respectively (Fig. 1). In capsaicin-desensitized animals, intravenous capsaicin (5 μmol $kg^{-1}$) did not induce any plasma extravasation while intravenous histamine (9 μmol $kg^{-1}$) still produced a significant response (Fig. 1).

Neither intravenous substance P, neurokinin A, neurokinin B, kassinin (each in a dose of 3·7 nmol $kg^{-1}$) or CGRP (2·6 nmol $kg^{-1}$) produced any significant extravasation of Evans blue in the rat proximal duodenum. These doses of neurokinins and CGRP produced consistent changes in motility of the duodenum and the urinary bladder (neurokinins) and lowered blood pressure (CGRP, neurokinins) (Maggi et al 1985, 1986). The same dose of intravenously administered neurokinins produced a distinct blueing in various areas

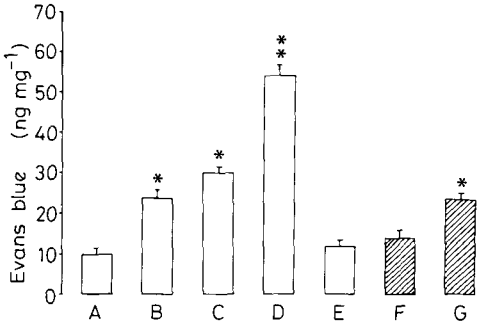


FIG. 1. Effect of intravenous capsaicin or other substance on Evans blue content of the rat proximal duodenum in control- or capsaicin-desensitized (50 mg $kg^{-1}$ s.c. 4 days before) animals. Each value is mean ± s.e. of at least 6 experiments. Key: A, controls; B, capsaicin 5 μmol $kg^{-1}$; C, histamine 9 μmol $kg^{-1}$; D, histamine 30 μmol $kg^{-1}$; E, substance P 4 μmol $kg^{-1}$; F, capsaicin 5 μmol $kg^{-1}$; G, histamine 9 μmol $kg^{-1}$. Significantly different from controls $^{*}P < 0.05$, $^{**}P < 0.01$. Open columns = controls, hatched columns = capsaicin-pretreated rats.

of the skin and viscera (Saria et al 1983; Lundberg et al 1984; Abelli, unpublished data): for instance, the Evans blue content of the urinary bladder increased from 7 ± 2 ng $mg^{-1}$ (vehicle-treated, n = 6) to 64 ± 9 and 92 ± 8 ng $mg^{-1}$ following intravenous substance P or kassinin, respectively (3·7 nmol $kg^{-1}$, n = 4 and 5, respectively). Moreover, preliminary experiments (n = 3 for each neutokinin) indicate that even a higher dose of intravenously administered substance P or neurokinin A (37 nmol $kg^{-1}$) failed to affect the Evans blue content of the rat proximal duodenum significantly.

*Discussion*

Capsaicin-sensitive sensory fibres might be involved in a 'gastric defence mechanism' (Szolcsányi & Barthó 1981; Holzer 1985; Holzer & Sametz 1986; Evangelista et al 1986). The sensory fibres, activated by chemical stimuli (such as HCl) would have released their stored neuropeptide(s) leading to an increase in mucosal blood

Table 1. Effect of capsaicin-desensitization (50 mg $kg^{-1}$ s.c., 4 days before) on cysteamine- or dulcerozine-induced duodenal ulcers.

| Treatment | Ulcerogen (mg $kg^{-1}$ p.o.) | n | Duodenal ulcers | | Mortality |
|---|---|---|---|---|---|
| | | | Incidence | Degree* | |
| Controls | cysteamine 900 | 17 | 70·6 | 1·00 | 17·6 |
| Capsaicin | cysteamine 900 | 16 | 87·5 | 1·75† | 18·7 |
| Controls | cysteamine 1200 | 21 | 95·2 | 1·90 | 38·0 |
| Capsaicin | cysteamine 1200 | 18 | 94·4 | 2·23 | 77·7† |
| Controls | dulcerozine 300 | 24 | 33·3 | 0·85 | — |
| Capsaicin | dulcerozine 300 | 11 | 91·3* | 1·63† | — |

* Expressed as mean score.
† $P < 0.05$ compared with respective control group (Smirnov test).

flow and/or vascular permeability resulting in a trophic effect on the gastric mucosa (Szolcsányi & Barthó 1981; Szolcsányi 1984; Holzer & Sametz 1986).

Our findings support the view that a similar 'defence mechanism' operates in the rat proximal duodenum, since systemic capsaicin desensitization led to an exacerbation of either cysteamine- or dulcerozine-induced duodenal ulcers.

Inhibition of gastric motility (enterogastric reflex) (Cervero & McRitchie 1982), activation of duodenal motility (Maggi et al 1986) and increase in vascular permeability (this study) produced by chemical activation (HCl or capsaicin itself) of the capsaicin-sensitive fibres (Hoyes & Barber 1981) may contribute to protect duodenal mucosa from the ulcerogenic effect due to prolonged exposure to acid. The observation that the capsaicin-induced changes in vascular permeability are confined to the first few cm caudal to the pyloric sphincter suggests that the resulting trophic effect on the duodenal mucosa may serve to counteract the irritation produced by the sudden drop in duodenal pH following gastric emptying. Removal of these protective mechanisms by systemic capsaicin desensitization leads to an increase in incidence and degree of duodenal ulcers induced by cysteamine and dulcerozine whose effects are ascribable to an increase in gastric acid secretion (Szabó et al 1979; Kurebayashi et al 1984; Kim et al 1985).

Although the capsaicin-induced changes in vascular permeability in various organs and tissues seem to be ascribable to the action of released neurokinins and/or CGRP (Saria & Lundberg 1983; Lundberg et al 1984; Saria et al 1983) neither of them affected vascular permeability of the rat proximal duodenum. We cannot exclude that endogenous neurokinins and/or CGRP released from sensory fibres in the rat duodenum may have affected vascular permeability indirectly, e.g. by releasing histamine or other mediators from mast cells. Indeed substance P is a potent activator of mast cell degranulation (see Piotrowski & Foreman 1985). However, if this were the mechanism involved, it is unclear why exogenous substance P was unable to induce any significant blueing of the duodenum, even at a dose as high as 37 nmol $kg^{-1}$. Other neuropeptides have been described to be present in capsaicin-sensitive sensory neurons (Jancsó et al 1981; Decker et al 1985). The identity of the sensory transmitters responsible for changes in vascular permeability at duodenal level and their possible involvement in the corresponding 'duodenal defence mechanism' remains to be elucidated.

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