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Intragastric capsaicin enhances rat gastric acid elimination and mucosal blood flow by afferent nerve stimulation

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1 This study investigated the effects of intragastric capsaicin on acid output, clearance of aniline, potential difference, and morphology of the mucosa in the rat stomach. The experiments were carried out on rats anaesthetized with urethane in which the stomachs were continuously perfused with saline.

2 When the stomach was perfused with normal saline (pH ~6), intragastric capsaicin (32–640 μM) had no effect on the output of titratable acid. In contrast, when acid output was stimulated by pentagastrin or when the stomach was perfused with acid saline (pH 3), capsaicin reduced acid output. Acid loss which occurred during perfusion with saline of pH 2 was not significantly increased by capsaicin. This suggests that capsaicin does not enhance acid back-diffusion but facilitates acid elimination by other means.

3 The gastric clearance of [^{14}C]-aniline, which is an indirect index of gastric mucosal blood flow, was estimated while the stomach was perfused with saline of pH 3. The clearance of aniline rose by 50–60% following intragastric administration of capsaicin (160 μM) whereas the mean arterial blood pressure was increased by about 2.5 mmHg only. Combined pretreatment of the rats with atropine, phentolamine, and propranolol did not alter the effect of capsaicin on the gastric clearance of aniline.

4 The gastric potential difference was not altered by capsaicin (160 μM) administered together with saline of pH 3. This and the finding that there were no signs of mucosal damage by light and scanning electron microscopy indicate that intragastric capsaicin does not irritate the gastric mucosa.

5 The effects of intragastric capsaicin on gastric acid output and aniline clearance and on blood pressure were absent in rats in which capsaicin-sensitive afferent neurones had been ablated by neonatal treatment with a neurotoxic dose of capasicin, which indicates that they result from stimulation of afferent nerve endings in the stomach. It is concluded that facilitation of acid elimination and mucosal blood flow may contribute to the previously reported protective action of capsaicin on the gastric mucosa.

Introduction

Administration of capsaicin into the rat stomach has been found to protect against the formation of gastric mucosal lesions caused by pylorus ligation (Szolcsányi & Barthó, 1981) or ethanol (Holzer & Lippe, 1988). Capsaicin is a selective stimulant of primary afferent neurones with Aδ- and C-fibres (Russell & Burchiel, 1984; Szolcsányi, 1984; Buck & Burks, 1986) and it seems as if the protective effect of capsaicin against ethanol-induced gastric damage is also the result of afferent nerve stimulation since it is absent following ablation of capsaicin-sensitive afferent neurones (Holzer & Lippe, 1988). Further analysis of capsaicin-induced gastric-protection indicated that capsaicin acted by a local mechanism in

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the stomach. As a first step in the analysis of this action, the influence of intragastric capsaicin on gastric acid output and aniline clearance, an indirect measure of mucosal blood flow, was examined in the present study. In addition, an investigation into whether capsaicin-induced changes in acid output and aniline clearance depended on the integrity of the sensory innervation of the stomach was carried out, in order to establish whether changes in these parameters have any bearing on afferent nerve-mediated protection of the gastric mucosa. In view of reports that crude preparations of capsaicin irritate the gastric mucosa (see Limlomwongse *et al.*, 1979; Szolcsányi & Barthó, 1981) the possibility has also to be considered that the gastric-protective influence of capsaicin is analogous to the well-documented protective action of a number of mild irritants (Robert *et al.*, 1983; Takeuchi *et al.*, 1987). Since irritation is associated with histologically visible damage to gastric surface cells and a fall in the gastric potential difference (Whittle, 1977; Takeuchi *et al.*, 1987) the effect of intragastric capsaicin on these indices of gastric barrier disruption was also examined. Some of the present findings have been published in abstract form (Lippe & Holzer, 1987).

Methods

Animals

Adult Sprague-Dawley rats (strain OFA-SD, Forschungsinstitut für Versuchstierzucht, Himberg, Austria) of either sex were used. The weight of the rats at the time of experimentation was 230–320 g. The animals were deprived of food for 20 h before the experiments but allowed free access to tap water.

Sensory denervation

On their second day of life, rats received a subcutaneous injection of capsaicin 0.16 mmol kg^{-1} (Gamse, 1982). This treatment is known to cause a permanent degeneration of unmyelinated afferent neurones (Russell & Burchiel, 1984; Buck & Burks, 1986). Control animals received equal volumes of vehicle (10% ethanol, 10% Tween 80, 80% saline, vol/vol/vol) only. All injections were performed under ether anaesthesia. The rats were then grown to adulthood and used for the experiments at the age of 4–5 months. The effectiveness of the treatment was checked by instilling a drop of a 0.33 mM solution of capsaicin in saline into one eye of the rats and counting the protective wiping movements (Gamse, 1982). This test was carried out one day before experimentation. Untreated or vehicle-treated rats responded instantly with wipings while capsaicin-treated rats did not react (Gamse, 1982). Animals treated with capsaicin that showed any wiping movements were excluded from the study. Whenever a rat had responded with such wipings the afflicted eye was immediately and thoroughly rinsed with water.

Surgical preparation

The rats were anaesthetized by a subcutaneous injection of urethane (1.5 g kg^{-1}). The body temperature was maintained at 36–37°C by means of a rectal thermometer and a heating lamp. A tracheostomy was performed and the trachea cannulated to ensure a patent airway. Blood pressure in a carotid artery was recorded via a cannula filled with heparinized saline and connected to a pressure transducer. A rubber connector positioned 4 cm away from the blood vessel and fitted with a syringe allowed withdrawal of blood samples from the carotid artery. A jugular vein was also cannulated to enable the infusion of pentagastrin or [^{14}C]-aniline at a flow rate of 1.5 ml h^{-1}. The gastric lumen was continuously perfused by the technique described by Main & Whittle (1973). Briefly, a soft catheter (inner diameter 0.8 mm) was inserted into the stomach through an incision in the cervical oesophagus and held in place by a ligature. This catheter was connected to a peristaltic pump, and saline maintained at 37°C was perfused through the gastric lumen at a rate of 0.7–0.8 ml min^{-1}. Gastric outflow was collected by a cannula (inner diameter 3.0 mm) which was inserted into the stomach via an incision in the duodenum and held in place by two ligatures around the duodenum. At the beginning of the experiment the stomach was flushed with 50 ml of saline (37°C) to remove any solid contents. After all surgical preparations had been completed a period of 1 h was allowed for equilibration.

Gastric acid output

Samples of the gastric perfusate were collected at intervals of 10 min (of 30 min in some experiments) and weighed. Their acid content was determined by titration to pH 7.0 with NaOH (0.01–0.001 N) using a digital burette (Brand, Wertheim, F.R.G.) and a digital pH-meter (WPI, New Haven, CT, U.S.A.). Gastric acid output was calculated by subtracting the acid contained in the perfusion medium before entering the stomach from the acid contained in the perfusate. Acid output was expressed as micro-equivalents secreted per minute.

Gastric [^{14}C]-aniline clearance

After the completion of surgery, [^{14}C]-aniline was injected intravenously in a loading dose of 6 $\mu g\,kg^{-1}$ (262 $kBq\,kg^{-1}$) followed by a continuous infusion of 0.1 $\mu g\,kg^{-1}\,min^{-1}$ (4.4 $kBq\,kg^{-1}\,min^{-1}$) to maintain a steady plasma concentration of aniline (Main & Whittle, 1973). The stomach was perfused with saline of pH 3. The estimation of [^{14}C]-aniline in blood and gastric perfusate necessitated the extraction of [^{14}C]-aniline and its separation from more polar metabolites. Blood samples (0.4 ml) were withdrawn 1 h after injection of the loading dose of aniline and at the end of the experiment, diluted to 4 ml with heparinized saline and centrifuged. A 3 ml aliquot of the clear supernatant was adjusted to pH 8, and [^{14}C]-aniline was extracted by adding 3 ml of benzene solvent (1.5% isoamyl alcohol in benzene, vol/vol). After centrifugation, a 2 ml aliquot of the organic phase was re-extracted with 2 ml 0.1 N HCl. Following centrifugation, a 1 ml sample of the acidic phase was added to 4 ml of scintillation fluid (Ready-Solv MP, Beckman, Galway, Ireland), and its radioactivity content determined by scintillation spectometry. Aliquots (3 ml) of the gastric perfusate collected at 10 min intervals were subjected to the same extraction procedure as described above. Clearance was calculated as the ratio of gastric output to blood concentration of [^{14}C]-aniline and expressed as $ml\,min^{-1}$ (Main & Whittle, 1973).

Gastric potential difference

The potential difference across the gastric mucosa was measured with two polyethylene catheters which were filled with saturated KCl in 3% agar (w/w) and which served as electrolyte bridges. One catheter was connected to the gastric outflow cannula, and the other one was connected to the cannula in a carotid artery. The opposite end of each catheter dipped into separate beakers which contained saturated KCl solution and accommodated a Ag/AgCl electrode (Ingold, Steinbach, F.R.G.). The potential difference between the two electrodes was recorded with a digital pH/mV-meter (WPI, New Haven, CT, U.S.A.).

Histology

At the end of a 30-min perfusion of the stomach with saline of pH 3 containing either vehicle or capsaicin (160 μM), the stomachs were rapidly removed, opened along the lesser curvature and rinsed with saline. Pieces from the glandular mucosa of the corpus were dissected and placed in fixative (2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer of pH 7.2). For scanning electron microscopy the tissues were left in the fixative for 3 h at room temperature and then postfixed in 2% OsO_4 for 1 h at room temperature. After dehydration in a graded series of ethanol the tissue samples were dried in a critical-point drying apparatus (Balzers CPD 020, Balzers, Liechtenstein) from liquid CO_2, mounted on aluminium stubs, and sputter-coated with gold palladium for examination on a scanning electron microscope (Zeiss DSM 950, Oberkochen, F.R.G.).

For light microscopic examination the tissues were left in the fixative for 3 days at room temperature and, after dehydration in a graded series of ethanol, embedded in Historesin (LKB, Bromma, Sweden). Four-micrometer sections were cut and stained with a mixture of methylene blue-azure II and basic fuchsin.

Substances and solutions

[^{14}C]-aniline hydrogen sulphate (specific activity: 4 $GBq\,mmol^{-1}$) was obtained from Amersham International (Amersham, U.K.), atropine, benzene, ethanol, and isoamyl alcohol were obtained from Merck (Darmstadt, F.R.G.), capsaicin and urethane were purchased from Fluka (Buchs, Switzerland), and Tween 80 was from Merck-Schuchardt (München, F.R.G.). Pentagastrin and propranolol were gifts of ICI (Macclesfield, U.K.), and phentolamine was kindly provided by Ciba-Geigy (Basel, Switzerland).

Atropine, phentolamine, and propranolol were dissolved in saline (0.9% NaCl, wt/wt) and injected intravenously at a volume of 1 $ml\,kg^{-1}$. The dosage of atropine was 0.35 $\mu mol\,kg^{-1}$ (Yokotani & Osumi, 1986), the dosage of phentolamine 3.5 $\mu mol\,kg^{-1}$ (Ruwart *et al.*, 1980), and the dosage of propranolol 1.5 $\mu mol\,kg^{-1}$ (Farmer & Levy, 1968). The solutions of pentagastrin and [^{14}C]-aniline used for intravenous infusion were also prepared with saline. Stock solutions of capsaicin were made up in 10% ethanol, 10% Tween 80, and 80% saline (vol/vol/vol). The stock solutions, or the vehicle, were then diluted 100 fold with saline, and these solutions were used for gastric perfusion. Basal acid output or aniline clearance was determined while vehicle diluted in saline was perfused through the gastric lumen.

Statistics

All data are presented as means $\pm$ s.e.mean. The Mann-Whitney U test (two tailed) was used for statistical analysis of the blood pressure measurements. The acid output and aniline clearance data were statistically evaluated by the Quade test for several

related values (Theodorsson-Norheim, 1987). Probability values $P < 0.05$ were regarded as significant.

Results

Gastric acid output

When the stomachs were perfused with normal saline (pH ~6) the acid output amounted to $0.195 \pm 0.031\ \mu Eq\ min^{-1}$ ($n = 20$) 100 min after the start of perfusion. When the perfusion with normal saline was continued for another 90 min, acid output remained stable during this period ($n = 6$). Administration of capsaicin (160 or 640 μM, $n = 7$ for each concentration) during the period of 100–160 min failed to alter acid output significantly although some tendency towards a decrease was seen in some experiments.

In a second group of experiments (Figure 1), gastric acid output was stimulated by intravenous infusion of a submaximally effective dose of pentagastrin ($0.4\ nmol\ kg^{-1}\ min^{-1}$) (Main & Whittle, 1973). The infusion of pentagastrin was started 60 min after surgery and continued until the end of the experiment. Gastric acid output increased to peak levels within 40 min and then remained constant (Figure 1a). When 60 min after the start of pentagastrin infusion capsaicin was administered intragastrically, pentagastrin-stimulated acid output was reduced. This effect of capsaicin was concentration-related with respect to its onset, magnitude, and duration. At a concentration of 160 μM capsaicin, acid output fell significantly after a latency of 30 min but rapidly recovered following capsaicin withdrawal (Figure 1b). In contrast, with 640 μM capsaicin a significant inhibition of acid output was observed after only 10 min, and the acid output remained depressed even after capsaicin withdrawal and had recovered little within the subsequent 40 min (Figure 1c).

In a third series of experiments the stomachs were perfused with acid saline (pH 3). Also under these conditions acid output remained fairly stable during the experimental period of 190 min (Figure 2a). Addition of capsaicin (32 μM) to the perfusion medium did not significantly reduce acid output although such a tendency was clearly seen ($n = 6$; data not shown). At concentrations of 160–640 μM, capsaicin caused a significant reduction of acid output, and this action was concentration-dependent with regard to its onset, magnitude, and duration. With 160 μM capsaicin, acid output decreased significantly with a lag of 20 min but returned promptly to control values after withdrawal of capsaicin (Figure 2b). When 640 μM capsaicin was administered intragastrically, there was an immediate and significant fall in acid output which was sustained. No appreciable recovery of acid output was seen within 30 min following capsaicin withdrawal (Figure 2c).

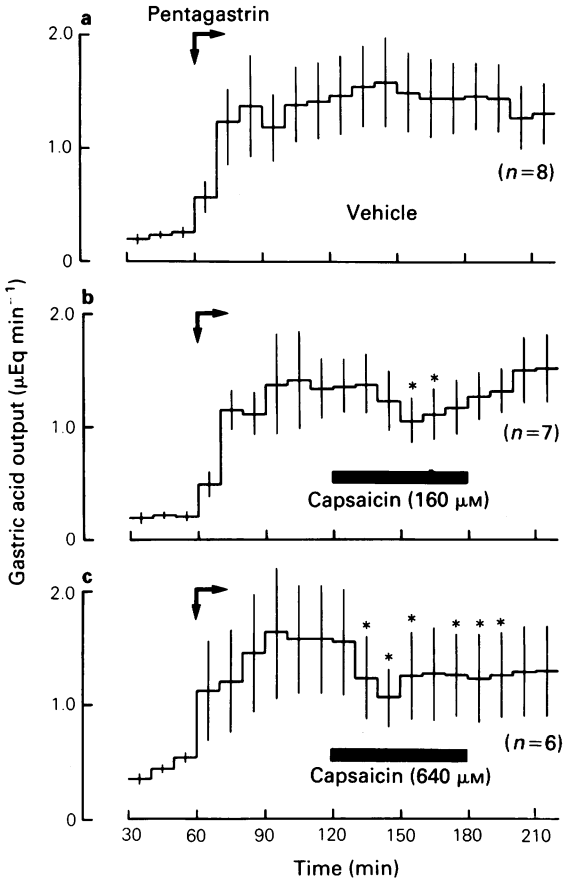

Figure 1 Gastric acid output in urethane-anaesthetized rats with stomachs perfused with normal saline (pH ~6) containing capsaicin (b and c) or an equivalent concentration of its vehicle (a). Pentagastrin ($0.4\ nmol\ kg^{-1}\ min^{-1}$) was administered by intravenous infusion which was started at 60 min and then maintained throughout the experiment. Gastric acid output was calculated by subtracting the acid contained in the perfusion medium before entering the stomach from the acid contained in the perfusate. Abscissa scale: time after completion of surgery. Means of n animals; Vertical lines show s.e.mean; * $P < 0.05$ versus the value obtained immediately before capsaicin administration.

In a fourth group of experiments, the stomachs were perfused with acid saline adjusted to pH 2. Under these conditions acid output was negative i.e. there was a continuous loss of acid from the gastric perfusate. In the period of 70–100 min after the start of perfusion, acid loss stabilized at a value of $0.191 \pm 0.024\ \mu Eq\ min^{-1}$ ($n = 10$). Capsaicin

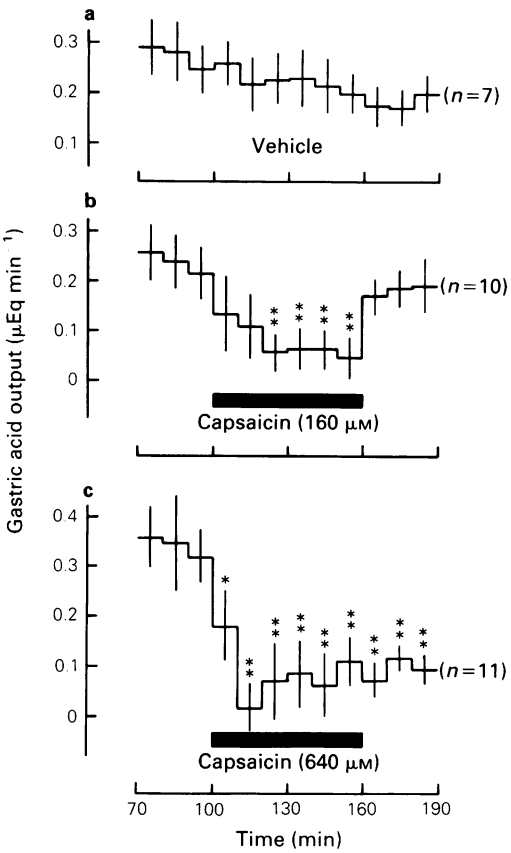

Figure 2 Gastric acid output in urethane-anaesthetized rats with stomachs perfused with acid saline (pH 3) containing capsaicin (b and c) or an equivalent concentration of its vehicle (a). Gastric acid output was calculated by subtracting the acid contained in the perfusion medium before entering the stomach from the acid contained in the perfusate. Abscissa scale: time after completion of surgery. Means of n animals; Vertical lines show s.e.mean. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the value obtained just before capsaicin administration.

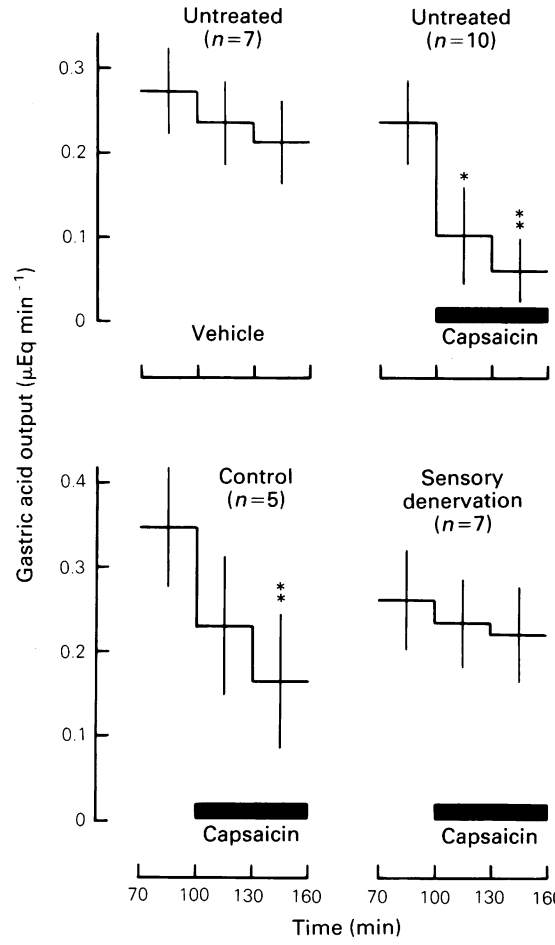

Figure 3 Gastric acid output in urethane-anaesthetized rats with stomachs perfused with acid saline (pH 3) containing capsaicin (160 μM) or an equivalent concentration of its vehicle. The experiments were performed either on untreated rats or on rats treated with capsaicin (0.16 mmol kg^{-1}; sensory denervation) or its vehicle (control) as neonates. Gastric acid output was calculated by subtracting the acid contained in the perfusion medium before entering the stomach from the acid contained in the perfusate. Abscissa scale: time after completion of surgery. Means of n animals; Vertical lines show s.e.mean. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the value obtained just before capsaicin administration.

(160 μM), present in the perfusion medium from 100 min onwards, failed to alter significantly the loss of acid from the perfusate although some tendency towards a transient increase was observed. In the period of 100–130 min, acid loss was 0.258 ± 0.049 μEq min^{-1} ($n = 10$), and in the period of 130–160 min acid loss amounted to 0.187 ± 0.031 μEq min^{-1} ($n = 10$).

In a fifth group of experiments, the effect of intragastric capsaicin (160 μM) on gastric acid output in untreated rats was compared with that in rats which had been treated with a high dose of systemic capsaicin or its vehicle as neonates (Figure 3). The stomachs were again perfused with acid saline (pH 3). Addition of capsaicin to the gastric perfusion medium reduced acid output in untreated rats and in rats treated with the capsaicin vehicle to a similar extent. In contrast, in rats treated with systemic capsaicin as neonates, intragastric capsaicin failed to affect acid output. The basal rate of acid output was

not altered by the neonatal treatment with capsaicin or its vehicle (Figure 3).

Gastric clearance of aniline

The effect of intragastric capsaicin (160 μM) on the clearance of [^{14}C]-aniline into the gastric perfusate was measured in order to obtain an indirect estimate of the effect of capsaicin on gastric mucosal blood flow. In all these experiments acid saline (pH 3) was used for perfusion of the stomach. Addition of capsaicin to the perfusion medium resulted in a prompt rise in the gastric aniline clearance which, after a peak increase by 50–60%, started returning to control values while capsaicin was still present (Figure 4a). This effect of capsaicin remained essentially unchanged when the rats had been treated with a combination of atropine, phentolamine and propranolol 45 min before the administration of capsaicin (Figure 4b). The basal rate of aniline clearance also appeared unaltered by this treatment.

In a second series of experiments the effect of intragastric capsaicin (160 μM) on the gastric clearance of aniline was examined in rats that had been treated with a high dose of systemic capsaicin or its vehicle as neonates (Figure 5). While in vehicle-treated rats intragastric capsaicin enhanced aniline clearance by some 60%, an effect very similar to that observed in untreated rats (compare Figures 4a and 5a), capsaicin failed to change the clearance of aniline in capsaicin-treated rats (Figure 5b). The basal rate of aniline clearance was not altered in capsaicin-treated animals as compared with vehicle-treated rats.

Gastric potential difference

Following a 60-min period of gastric perfusion with saline adjusted to pH 3 the potential difference across the gastric mucosa was -37.1 ± 0.8 mV ($n = 5$). Intragastric administration of 160 μM capsaicin for 30 min did not alter the potential difference which was -37.0 ± 0.8 mV ($n = 5$) 10 min and -36.4 ± 1.0 mV ($n = 5$) 30 min after the start of capsaicin perfusion. The validity of the method was ascertained by showing that perfusion of the stomach with 25% (w/w) ethanol in normal saline led, within 5 min, to a fall of the potential difference from -36.9 ± 2.9 mV to -17.0 ± 2.6 mV ($n = 5$; $P < 0.05$).

Blood pressure

The mean arterial blood pressure was routinely monitored in all experiments of this study. Intragastric capsaicin caused a slight rise in blood pressure in a concentration-dependent manner, reaching a maximal effect ($+2.5 \pm 0.4$ mmHg, $n = 53$; $P < 0.01$) with capsaicin (160 μM). Peak increases in blood pressure were usually observed within 5 min of the start of capsaicin administration; thereafter blood pressure slowly returned to control values. Rats treated with capsaicin as neonates failed to respond to intragastric capsaicin (160 μM) with such a hypertensive reaction ($+0.3 \pm 0.9$ mmHg, $n = 10$) whereas

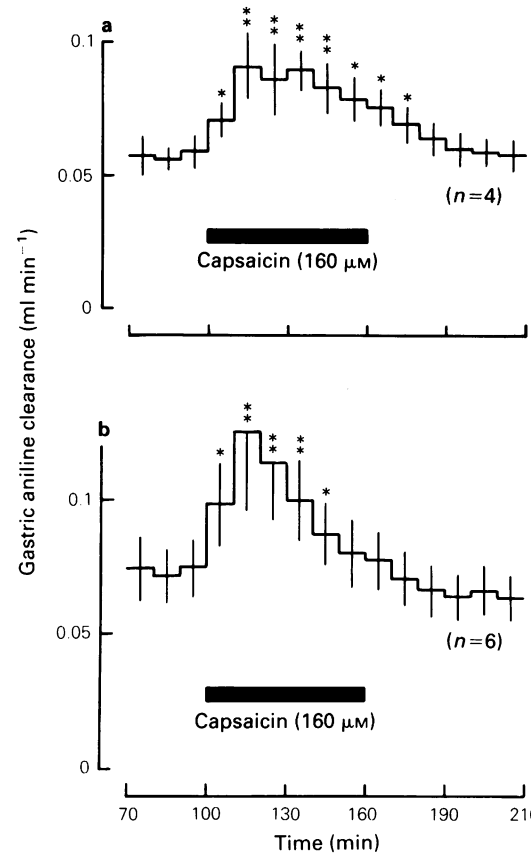

Figure 4 Gastric clearance of [^{14}C]-aniline in urethane-anaesthetized rats with stomachs perfused with acid saline (pH 3) containing capsaicin or an equivalent concentration of its vehicle. (a) Clearance in untreated rats; (b) clearance in rats which had been treated with a combination of atropine (0.35 μmol kg^{-1}), phentolamine (3.5 μmol kg^{-1}) and propranolol (1.5 μmol kg^{-1}) 45 min before capsaicin administration. Abscissa scale: time after completion of surgery. Means of n animals; Vertical lines show s.e.mean. * $P < 0.05$, ** $P < 0.01$ versus the value obtained just before capsaicin administration.

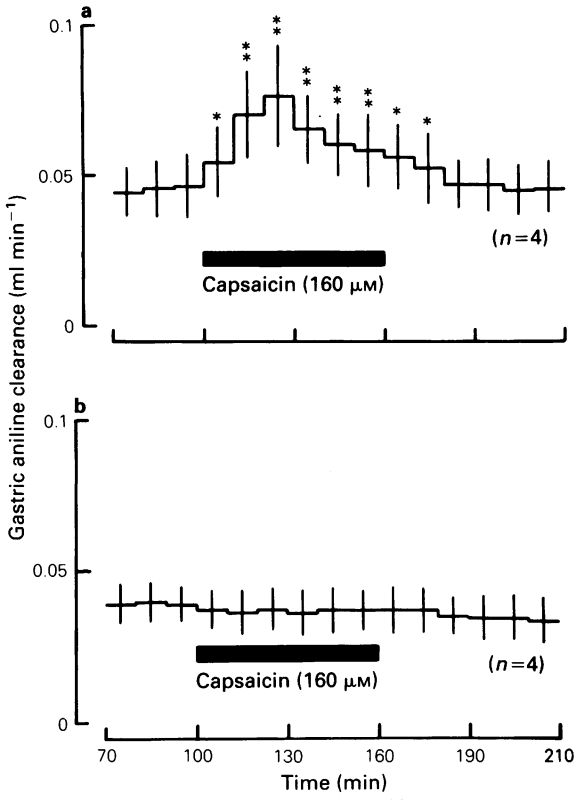

Figure 5 Gastric clearance of [^{14}C]-aniline in urethane-anaesthetized rats with stomachs perfused with acid saline (pH 3) containing capsaicin or an equivalent concentration of its solvent only. Clearance was studied in rats pretreated with 0.16 mmol kg^{-1} capsaicin (b) or its vehicle (a) as neonates. Abscissa scale: time after completion of surgery. Means of n animals; Vertical lines show s.e.mean. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the value obtained just before capsaicin administration.

vehicle-treated rats showed a similar increase in blood pressure to untreated rats ($+1.8 \pm 0.7$ mmHg; $n = 9$; $P < 0.05$). The blood pressure measured just before capsaicin administration did not significantly differ between untreated (88.2 ± 1.1 mmHg, $n = 92$), vehicle-treated (94.3 ± 3.9 mmHg, $n = 9$), and capsaicin-treated (90.9 ± 4.5 mmHg, $n = 10$) animals.

Histology

The glandular mucosa of the stomach was routinely inspected at the end of all experiments described here. No macroscopically visible lesions were observed following perfusion of the stomach with capsaicin. The stomachs from 4 rats, which had been perfused with saline of pH 3 containing 160 μM capsaicin for a period of 30 min, were also examined histologically and compared with stomachs perfused with vehicle-containing saline (pH 3) alone. No damage of the gastric mucosa was detectable in any of the sections examined with the light microscope (Figure 6). Scanning electron microscopy revealed that capsaicin was without injurious effect on the surface epithelium (Figure 6). In no instance did capsaicin disrupt the continuity of the epithelium or cause any overt damage to the surface epithelial

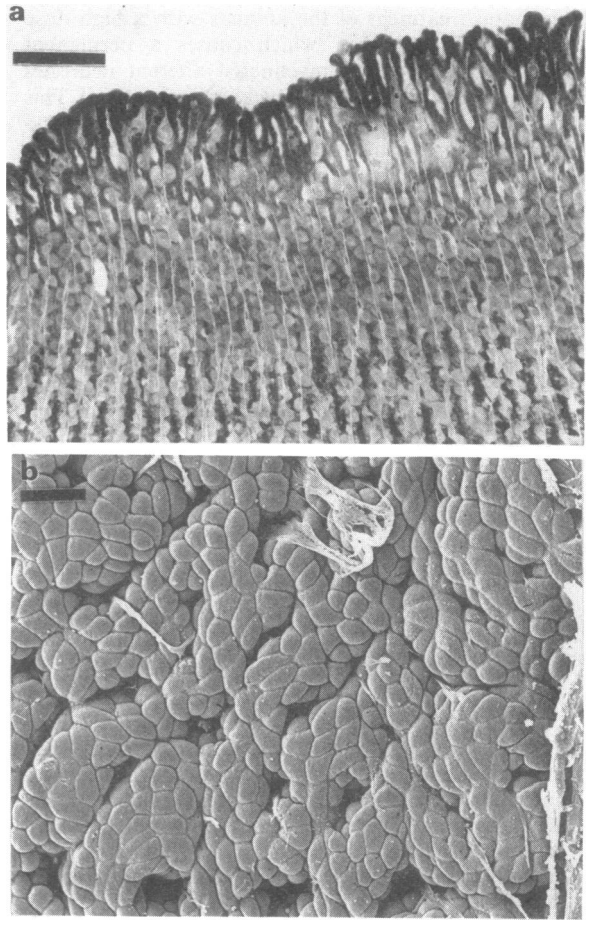

Figure 6 Light (a) and scanning electron (b) microscopic appearance of the mucosa from the rat gastric corpus taken after a 30-min perfusion of the stomach with saline of pH 3 containing 160 μM capsaicin. No differences from control sections were seen. The bar in (a) equals 100 μm, and that in (b) equals 20 μm.

cells. A few cells exhibited apical erosions but this type of injury was also seen in stomachs that had been perfused with saline (pH 3) alone.

Discussion

The present study has shown that intragastric capsaicin, at concentrations identical to those that protect against ethanol-induced haemorrhagic lesions of the gastric mucosa (Holzer & Lippe, 1988), affects gastric acid output, gastric aniline clearance, and blood pressure in the rat. Common to all these actions of intragastric capsaicin is that they are abolished after neonatal treatment of the animals with a high dose of systemic capsaicin which causes a permanent ablation of certain unmyelinated afferent neurones (Russell & Burchiel, 1984; Buck & Burks, 1986). This implies that the acute effects of intra-gastric capsaicin rely on the integrity of the sensory innervation of the stomach or, in other words, probably result from stimulation of afferent nerve endings.

Administration of capsaicin into the stomach reduced acid output only when acid output was stimulated by pentagastrin or when the stomach was perfused with acid saline (pH 3), a procedure which resulted in a similar degree of gastric acidity as that following pentagastrin infusion. Thus it seems as if the ability of capsaicin to reduce acid output depends on the presence of elevated levels of acid but is independent of whether the acid is of endogenous or exogenous origin. A reduction in gastric acidity may be due to a reduction in acid secretion, to an increase in the secretion of neutralizing factors such as bicarbonate (Flemström, 1987; Silen, 1987), or to an increase in other ways of acid disposal including acid back-diffusion (Whittle, 1977; Guth & Leung, 1987). The finding that capsaicin failed to alter acid output when the stomach was perfused with saline of low acidity (pH $\sim$6) may suggest that acid secretion is not inhibited and that hence the reduction of acid output in the presence of elevated levels of acid arises from facilitated elimination of acid. Augmented acid back-diffusion, however, is unlikely to account for capsaicin-induced acid disposal since acid loss at pH 2 was not significantly enhanced by capsaicin. Which other way of acid elimination is actually stimulated by capsaicin remains to be determined.

Ablation of capsaicin-sensitive afferent neurones abolished the effect of intragastric capsaicin on acid output in the presence of elevated acid levels but did not alter basal acid output. This latter observation is in agreement with previous reports (Szolcsányi & Barthó, 1981; Alföldi *et al.*, 1986; Dugani & Glavin, 1986). However, the present results contrast with those of Limlomwongse *et al.* (1979) who found that orally administered capsaicin enhanced pentagastrin-stimulated acid output. It is not unlikely that a number of differences in their experimental model, including the use of pentobarbitone as anaesthetic, the absence of a steady basal acid output, the repeated aspiration of the gastic contents, and different dosages of capsaicin and pentagastrin, could account for the conflicting observations between the two studies.

Consistent, though, with the findings of Limlomwongse *et al.* (1979), who observed that intragastric capsaicin increased the gastric clearance of aminopyrine, is the present result that the gastric clearance of [^{14}C]-aniline was augmented. The gastric clearance of aniline or aminopyrine is thought to provide an indirect estimate of gastric mucosal blood flow, although the validity of this technique is limited inasmuch as the clearance of weak organic bases may also reflect parietal cell secretion of acid and consequently overestimate changes of mucosal blood flow when acid output and mucosal blood flow change in parallel (Müller-Lissner *et al.*, 1981; Leung *et al.*, 1984; Guth & Leung, 1987). Since under the present experimental conditions, acid output decreased while the clearance of aniline increased in response to intragastric capsaicin, the capsaicin-induced increase in mucosal blood flow may have actually been underestimated by the increase in aniline clearance. At any rate, it seems safe to conclude that afferent nerve stimulation with capsaicin results in a pronounced increase in gastric mucosal blood flow although the absolute changes in mucosal blood flow remain to be determined by other techniques. The facilitation of aniline clearance is most likely due to a dilatation of submucosal blood vessels since it appears improbable that the very minor hypertension produced by intragastric capsaicin could to a significant degree account for this effect.

As crude capsaicin has been reported to be a gastric irritant (see Limlomwongse *et al.*, 1979; Szolcsányi & Barthó, 1981) the possibility arises that augmented acid loss is a consequence of capsaicin-induced mucosal damage and that acid entering the tissue may in turn enhance aniline clearance (Whittle, 1977). However, such an explanation is very unlikely for a number of reasons. (i) Irritation causes damage to gastric surface cells (Silen, 1987; Takeuchi *et al.*, 1987) but neither light nor scanning electron microscopic examination of the mucosa revealed any capsaicin-induced lesion of the gastric epithelium. (ii) Disruption of the gastric mucosal barrier is associated with a fall in the gastric potential difference (Whittle, 1977; Takeuchi *et al.*, 1987) yet capsaicin failed to alter this parameter. (iii) The

back-diffusion of acid was not enhanced by capsaicin as would be expected if capsaicin were to break the mucosal barrier (Whittle, 1977). (iv) Intragastric capsaicin also fails to enhance vascular permeability in the stomach (Holzer & Lippe, 1988), which is another consequence of mucosal injury (Szabo *et al.*, 1986). (v) Capsaicin-induced disruption of the gastric mucosa would most probably be independent of the sensory innervation, yet ablation of capsaicin-sensitive afferent neurones abolished the gastric effects of capsaicin measured here. Thus, there is no evidence that purified capsaicin is a gastric irritant; on the contrary, intragastric capsaicin can counteract ethanol-induced lesion formation (Holzer & Lippe, 1988). As this protective action also requires an intact sensory innervation (Holzer & Lippe, 1988) it is probable that facilitation of gastric acid elimination and aniline clearance may be among the mechanisms by which afferent nerve-mediated protection of the gastric mucosa is accomplished.

The increase in aniline clearance produced by intragastric capsaicin was not inhibited by a combination of atropine, phentolamine, and propranolol. It would appear therefore that, like afferent nerve-mediated gastric mucosal protection against ethanol (Holzer & Lippe, 1988), afferent nerve-mediated facilitation of aniline clearance involves neither the autonomic nervous system nor the adrenal glands. This parallel suggests that a rise in gastric mucosal blood flow, as deduced from the aniline clearance, could be of importance for afferent nerve-mediated gastric mucosal protection and that both processes could be brought about by a local release of vasodilator mediators from afferent nerve endings within the gastric mucosa and submucosa. This view is further supported by the observation that blood vessels in the submucosa of the rat stomach receive a particularly dense innervation by capsaicin-sensitive afferent neurones (Sharkey *et al.*, 1984). An increase in mucosal blood flow is thought to be an essential factor in the ability of gastric mucosa to protect itself against acute injury (Cheung, 1984; Pihan *et al.*, 1986; Guth & Leung, 1987). Enhanced mucosal blood flow leads to enhanced disposal of acid entering the tissue (Cheung, 1984; Guth & Leung, 1987), and the apparent elimination of acid caused by capsaicin may conceivably be a consequence of facilitated mucosal blood flow. Vasodilatation and a rise in mucosal blood flow may also counteract the circulatory stasis in submucosal blood vessels, which for example is a characteristic of ethanol injury (Pihan *et al.*, 1986), and thus explain the protective effect of intragastric capsaicin against ethanol-induced haemorrhagic lesions (Holzer & Lippe, 1988).

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