Influence of capsaicin-sensitive afferent neurones on the acid secretory responses of the rat stomach *in vivo*

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- 1 The influence of capsaicin-sensitive afferent neurones in modulating acid-secretory responses has been investigated in the continuously perfused stomach of the anaesthetized rat.
- 2 Ablation of primary afferent neurones, after systemic neonatal pretreatment with high doses of capsaicin, did not modify acid responses to direct stimuli of the oxyntic cell with histamine (5 mg kg^{-1}), pentagastrin ($20 \mu\text{g kg}^{-1}$) or carbachol ($4 \mu\text{g kg}^{-1}$).
- 3 Acid responses to hypoglycaemia induced by insulin (0.3 iu kg^{-1}) were not influenced by systemic capsaicin pretreatment or by acute coeliac ganglionectomy. Vagotomy abolished this secretory response.
- 4 The increase in acid output induced by gastric distension ($20 \text{ cmH}_2\text{O}$) was abolished by systemic neonatal capsaicin pretreatment. Likewise, vagotomy and acute coeliac ganglionectomy eliminated this secretory response.
- 5 Acute intragastric infusion with high doses of capsaicin inhibited the acid responses to distension but failed to modify the increase in acid output induced by insulin.
- 6 Local application (7–14 days before) of capsaicin to the coeliac ganglion abolished the acid response to gastric distension. This lack of secretory response was not the result of a nonspecific destruction of the ganglion, since changes in intragastric pressure after electrical stimulation of the coeliac ganglion were unaffected by such treatment.
- 7 These observations indicate that peripheral capsaicin-sensitive sensory neurones, located both in the gastric mucosa and in the coeliac ganglion, play a physiological role in the acid secretory responses to gastric distension.

Introduction

Capsaicin, the pungent component of red peppers of the genus *Capsicum*, has become widely used as a pharmacological tool to assess the involvement of sensory neurones in biological functions (Buck & Burks, 1986; Holzer, 1988). When administered acutely, capsaicin induces a potent stimulation of small diameter unmyelinated sensory nerve fibres (C-fibres), followed by desensitization to the drug and by a prolonged blockade of transmission of stimuli. If administered to newborn rats, capsaicin induces a permanent and selective degeneration on unmyelinated primary afferent neurones. The presence of capsaicin-sensitive nerve fibres has been demonstrated in the gastric mucosa (Sharkey *et al.*, 1984; Green & Dockray, 1988) and there is growing evidence that these neurones participate in various aspects of the pathophysiology of the stomach such as changes in motility (Holzer *et al.*, 1986), blood flow regulation (Lippe *et al.*, 1989; Pique *et al.*, 1989) or the maintenance of the integrity of the gastric mucosa (Holzer & Sametz, 1986; Esplugues & Whittle, 1989a; 1990).

In the present study we have used capsaicin, administered either systemically or locally, as a tool to analyse indirectly the participation of capsaicin-sensitive afferent nerve fibres in the regulation of acid secretion in the anaesthetized rat. We have studied the influence of capsaicin desensitization on the effects of three direct stimuli of the oxyntic cell (pentagastrin, histamine and carbachol) and, furthermore, of CNS-mediated stimuli of gastric secretion, following hypoglycaemia induced by either insulin or gastric distension. In addition, we have investigated the effects of acute coeliac ganglionectomy and the local application of capsaicin to the caeliac ganglion on these responses.

Methods

Animals

Wistar rats of either sex, on their second day of life, received 50 mg kg^{-1} of capsaicin by subcutaneous injection in a volume of $10 \mu\text{l g}^{-1}$. This treatment has been shown to lead to functional ablation of primary afferent neurones (Buck & Burks, 1986; Holzer, 1988). Control animals received equal volumes of vehicle (10% ethanol, 10% Tween 80 and 80% saline, vol/vol/vol). All injections were performed under ether anaesthesia. The rats were then grown to adulthood and used for the experiments when weighing 230–260 g. Animals were fasted for 24 h before the experiments, but received drinking water *ad libitum*.

Measurement of gastric acid secretion

The procedure followed was that described by Ghosh & Schild (1958). In brief, animals were anaesthetized with urethane (1 g kg^{-1} , i.p.) and the trachea intubated. A soft catheter was inserted into the stomach through an incision in the cervical oesophagus and held in place by a ligature. After a laparotomy, a second polyethylene cannula was inserted into the stomach via an incision in the duodenum, held in place by two ligatures, and led outside the abdominal wall. At the beginning of the experiment, the stomach was flushed with 50–100 ml of saline to remove any solid content. Once the surgical preparation had been completed a period of 1 h was allowed for stabilization. Thereafter, the stomach cavity was perfused via the oesophageal cannulae with saline at room temperature at a rate of 0.9 ml min^{-1} . The gastric effluent was collected continuously and separated in 20 min collections.

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H⁺ output was determined by automatic titration (Radiometer, Copenhagen, Denmark) of the perfusate to pH 7 with 0.01 N NaOH and expressed as $\mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ (when referring to the total acid output of a particular period of 20 min) or $\mu\text{Eq } 100 \text{ g}^{-1} 120\text{--}160 \text{ min}^{-1}$ (when referring to the total H⁺ production over the time span examined, 120 or 160 min, after stimulation of acid secretion).

Stimulation of acid secretion

Once the gastric acid output had remained constant for 40 min it was considered as basal or unstimulated acid secretion. Thereafter, three types of experiments were performed according to the type of stimulus of gastric acid secretion. In the first series of studies, histamine (5 mg kg^{-1}), pentagastrin ($20 \mu\text{g kg}^{-1}$) or carbachol ($4 \mu\text{g kg}^{-1}$) were injected (i.p.) as a bolus. All three substances are direct stimulants of the oxyntic cell (Debas, 1987).

In the second series of experiments, gastric acid secretion was stimulated by the hypoglycaemia induced by the administration of insulin (0.3 iu kg^{-1} , i.p.).

In the last series of studies, gastric distension was used as the stimulant of acid output. Distension of the stomach was obtained by placing the tip of the antroduodenal cannula 20 cm above the perfused stomach, where it was maintained for the remainder of the experimental period.

To analyse further the involvement of sensory nerves in acid secretory responses, in some rats the vagus was cut bilaterally on the midcervical portion of the nerve, or the coeliac ganglion was carefully removed under a stereomicroscope. In addition, in one set of experiments a solution of capsaicin (1% ethanol, 99% saline; 0.1 mg ml^{-1}) or vehicle was perfused intragastrically for 10 min (0.9 ml min^{-1}) immediately before the stimulus of acid secretion. In all cases each preparation was used for a single response only, with treatment being allocated randomly between preparations.

Local treatment with capsaicin of the coeliac ganglion

After pretreatment with atropine (0.5 mg kg^{-1} , i.p.), rats were anaesthetized with sodium pentobarbitone (70 mg kg^{-1} , i.v.). The coeliac ganglion was exposed and a small piece of gauze soaked in capsaicin solution, or solvent (10% Tween 80, 90% olive oil) was left on the ganglion complex for 30 min and then removed. After carefully cleaning with sterile swabs, the abdominal laparotomy was closed and the animals received antibiotic protection (ampicillin, i.m.). Acid secretory responses to gastric distension were evaluated 7–15 days after surgery.

In order to assess the specificity of the changes on acid secretion that follow the local application of capsaicin to the coeliac ganglion, we have also analysed its consequences on the gastric contractility changes induced by the electrical stimulation of the coeliac ganglion. After ligating the oesophagus, 5 ml of saline was instilled into the gastric lumen via a 25-gauge needle inserted through the forestomach wall. As described before (Esplugues & Whittle, 1989b), changes in intraluminal gastric pressure were recorded from a catheter introduced into the stomach through the pyloric sphincter via the duodenum, and ligated in place. The pyloric catheter was connected to a pressure transducer connected to a chart-recorder. Experiments started once the basal intragastric pressure had remained constant for at least 15 min. Field electrical stimulation of the coeliac ganglion was provided by a Grass SD5 stimulator via two platinum electrodes, placed 0.5 cm apart. Three trains of 300 square-wave pulses of 0.5 ms duration at 8 Hz were applied at supramaximal voltage. A period of 15 min was allowed between stimulations. Results, expressed as the difference (in mmHg) between basal and stimulated gastric pressure, were the overall mean score value of the three stimulations for each experiment. The response to electrical stimulation after applying lidocaine (1%) to the ganglion complex was used to confirm that the effects were nerve-mediated.

Drugs

Capsaicin was purchased from Fluka Chemic AG. Histamine, carbachol and Tween 80 were obtained from Sigma Chemical Co. Pentagastrin (Peptavlon, ICI) and insulin (Humulina NPH, Lilly) were obtained from clinically available preparations. All drugs, except capsaicin, were dissolved in saline and administered in a volume of 1 ml kg^{-1} .

Statistical analysis

All data are expressed as mean $\pm$ s.e.mean. Student's *t* test was used to analyse the results statistically. *P* values of less than 0.05 were taken as significant.

Results

Basal acid secretion

There were no significant differences in the rates of basal acid secretion between control animals ($4.2 \pm 0.7 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$, $n = 40$) and animals which had been treated with capsaicin as neonates ($3.7 \pm 0.5 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$, $n = 22$). Furthermore, neither bilateral vagotomy ($3.4 \pm 0.6 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$, $n = 12$) nor acute coeliac ganglionectomy ($3.2 \pm 0.5 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$, $n = 11$) exerted any significant influence on the levels of basal acid output when compared to those present in the control rats.

Acid responses to histamine, pentagastrin or carbachol

The intraperitoneal injection of histamine (5 mg kg^{-1}), pentagastrin ($20 \mu\text{g kg}^{-1}$) and carbachol ($4 \mu\text{g kg}^{-1}$) induced rapid increases in acid output by the stomach which peaked 40 min (pentagastrin and carbachol) or 60 min (histamine) later (Figures 1 and 2). Thereafter, acid secretion decreased progressively, and by the end of each experimental period, the levels of acid production were not significantly different from those of basal conditions. The values of total acid output and of peak secretory response for histamine ($91.5 \pm 12.5 \mu\text{Eq } 100 \text{ g}^{-1} 120 \text{ min}^{-1}$ and $26.7 \pm 4.8 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ respectively, $n = 9$) were not significantly different from those obtained after administration of pentagastrin ($113 \pm 16.9 \mu\text{Eq } 100 \text{ g}^{-1} 120 \text{ min}^{-1}$ and $32.5 \pm 4.6 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ respectively, $n = 10$). The total acid output after the administration of carbachol was $149.4 \pm 25 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ ($n = 7$) whereas the peak secretory response was $35.7 \pm 4 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$.

Figure 1 Stimulation of acid secretion elicited by a bolus injection (i.p.) of (a) histamine (5 mg kg^{-1}), or (b) pentagastrin ($20 \mu\text{g kg}^{-1}$) in the continuously perfused stomach of the anaesthetized rat: control (○) and neonatal capsaicin pretreatment (●). Each point represents the mean of 3 to 10 experiments and the vertical lines show s.e.mean.

Figure 2 Stimulation of acid secretion elicited by a bolus injection (i.p.) of carbachol ($4 \mu\text{g kg}^{-1}$) in the continuously perfused stomach of the anaesthetized rat: control (○), neonatal capsaicin pretreatment (●), and coeliac ganglionectomy (□). Each point represents the mean of 3 to 7 experiments and the vertical lines show s.e.mean.

Systemic neonatal treatment with capsaicin did not modify the responses elicited by any of the three direct secretagogues employed in our experiments. The values of total acid output and of peak secretory response were, respectively, $100.1 \pm 26.4 \mu\text{Eq } 100 \text{ g}^{-1} 120 \text{ min}^{-1}$ and $27.7 \pm 3.5 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ for histamine ($n = 3$); $73.8 \pm 27.8 \mu\text{Eq } 100 \text{ g}^{-1} 120 \text{ min}^{-1}$ and $24.5 \pm 4.4 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ for pentagastrin ($n = 3$); and $107.7 \pm 17 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ and $35.5 \pm 6 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ for carbachol ($n = 3$). The removal of the coeliac ganglion ($n = 3$) in control, non-capsaicin treated, animals did not significantly influence the values of total acid output ($163.8 \pm 22 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$) or of peak secretory response $30 \pm 1.8 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ induced by carbachol (Figure 2).

Acid responses to insulin

As is shown in Figure 3, the secretory effects of insulin (0.3 iu kg^{-1}) were only significantly evident 60 min after its i.p. administration, and a period of 100 min was needed to obtain the peak acid response. Although acid production decreased thereafter, at the end of the experimental period (160 min) the levels of acid output were still significantly ($P < 0.05$) higher than those present in basal, non-stimulated, conditions.

The values of total acid output and of peak secretory response induced by insulin ($123.1 \pm 31 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ and $23.4 \pm 7.1 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ respectively, $n = 7$) were neither significantly affected by neonatal treatment with capsaicin ($155 \pm 57 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ and $32.6 \pm 6.9 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$, $n = 5$) nor by acute coeliac ganglionectomy ($105.9 \pm 26 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ and $25.2 \pm 5 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$, $n = 5$). Cervical vagotomy ($n = 4$), however, abolished the secretory response induced by insulin with a total acid output of $23.7 \pm 4.2 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ ($P < 0.01$) and rates of acid secretion in all 20 min^{-1} periods measured not significantly different from those present in basal, pre-stimulated, conditions.

Acid secretion stimulated by gastric distension

Distension of the rat stomach *in vivo* ($n = 7$) with an intragastric pressure of $20 \text{ cmH}_2\text{O}$ produced a progressive increase in acid secretion, which peaked 140 min later with a H^+ pro-

Figure 3 Stimulation of acid secretion elicited by a bolus injection of insulin (0.3 iu kg^{-1} , i.p.) in the perfused stomach of the anaesthetized rat: control (○), neonatal capsaicin pretreatment (●), coeliac ganglionectomy (□) and bilateral vagotomy (■). Each point represents the mean of 4–7 animals and the vertical lines show s.e.mean. The level of significance of the difference from control is shown * $P < 0.05$; ** $P < 0.001$.

duction of $23.9 \pm 5.7 \mu\text{Eq } 100 \text{ g}^{-1} 20 \text{ min}^{-1}$. The total acid output during these experiments was $114.9 \pm 28.2 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ (Figure 4).

Neonatal pretreatment with capsaicin, acute coeliac ganglionectomy and bilateral vagotomy abolished ($P < 0.01$) the acid secretory responses to gastric distension, with values of total acid output of $19.7 \pm 7.4 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ ($n = 5$), $22 \pm 5.4 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ ($n = 3$) and $24.6 \pm 5.6 \mu\text{Eq } 100 \text{ g}^{-1} 160 \text{ min}^{-1}$ ($n = 5$) respectively. In the three cases, the rates of acid output during all the 20 min periods studied were not significantly different from those present in basal, non-stimulated, conditions.

Effects of the local application of capsaicin to the coeliac ganglion

As shown in Figure 5, the local application of capsaicin to the coeliac ganglion resulted in the abolition of the secretory response to gastric distension when studied 7–14 days later. The total acid output for the 160 min period of distension was

Figure 4 Stimulation of acid secretion elicited by the gastric distension ($20 \text{ cmH}_2\text{O}$) of the perfused stomach of the anaesthetized rat: control (○), neonatal capsaicin pretreatment (●), coeliac ganglionectomy (□) and bilateral vagotomy (■). Each point represents the mean of 3 to 7 animals and the vertical lines show s.e.mean. For reasons of clarity * $P < 0.05$; ** $P < 0.001$ versus the other three groups.

Figure 5 Effects of the local application of the coeliac ganglia of capsaicin (hatched columns), or vehicle (open columns), on (a) the acid response to intragastric distension (20 cmH₂O); and (b) the changes in intragastric pressure after the electrical stimulation of the coeliac ganglia. Each column represents mean with s.e.mean shown by vertical bars, and level of statistical difference from vehicle-treated group is shown by * $P < 0.05$. Numbers inside columns represent the number of animals used.

$110.8 \pm 25 \mu\text{Eq } 100 \text{ g}^{-1}$ ($n = 8$) in vehicle treated rats compared to $26.1 \pm 12.3 \mu\text{Eq } 100 \text{ g}^{-1}$ in animals receiving topical capsaicin ($n = 3$, $P < 0.05$).

In rats receiving a local application of capsaicin to the coeliac ganglion, the values of total acid production induced by distension were not significantly different from those in animals that had undergone vagotomy, acute coeliac ganglionectomy or systemic neonatal capsaicin administration.

To evaluate the possibility that a non-selective destruction of the coeliac ganglion by the local application of capsaicin could account for the differences in the acid response to distension, changes in intragastric pressure during electrical stimulation of the coeliac ganglion were determined. Basal intragastric pressure was similar in animals receiving the topical application of vehicle ($4.3 \pm 0.2 \text{ mmHg}$, $n = 4$) or capsaicin ($4.1 \pm 0.3 \text{ mmHg}$, $n = 4$). Electrical stimulation to the ganglion caused a rapid, frequency-dependent and reproducible diminution in intragastric pressure that lasted for 45–60 s, slowly returning to basal levels thereafter. The highest frequency used (8 Hz) induced similar reductions of intragastric pressure after local application of capsaicin ($0.7 \pm 0.2 \text{ mmHg}$, $n = 4$) or vehicle ($0.8 \pm 0.1 \text{ mmHg}$, $n = 4$) (Figure 5). In all cases, the topical application of lidocaine to the ganglion caused a sustained rise in intragastric pressure and completely blocked the relaxation induced by the electrical stimulation of the coeliac ganglion, thus confirming that such relaxation was nerve-mediated.

Effects of intraluminal capsaicin on acid secretion

The intraluminal infusion of high doses (0.1 mg ml^{-1} , 0.9 ml min^{-1}) of capsaicin for 10 min abolished the acid responses to gastric distension (Figure 6). This was not due to a nonspecific effect of capsaicin on H⁺ secretion since acid responses to insulin were not affected by such treatment.

Discussion

We have analysed the changes induced by pretreatment with capsaicin, both systemically and locally, on the acid secretory responses of the rat stomach *in vivo*. In our study basal gastric acid secretion and acid responses to direct stimulation of the oxyntic cell (Debas, 1987) with histamine, pentagastrin and carbachol were not affected by neonatal treatment with high

doses of capsaicin. Such treatment has been shown to lead to the functional ablation of primary afferent neurones (Buck & Burks, 1986; Holzer, 1988). Previous studies on the effects of systemic capsaicin pretreatment on gastric acid secretion have shown a similar lack of effect on basal acid secretion, although, in contrast, the acid secretory responses to histamine (Alfondi *et al.*, 1986) or pentagastrin (Dugani & Glavin, 1986) were greatly reduced. Differences in the experimental protocol, anaesthesia and particularly, in the doses employed of the different secretagogues may account for the discrepancies.

Systemic capsaicin crosses the blood-brain barrier (Buck & Burks, 1986; Holzer, 1988). Thus it could be possible that central processes of sensory neurones involved in acid secretory responses were modified. In the present investigation two different stimuli of gastric acid secretion, through a CNS action have been employed, insulin-hypoglycaemia and gastric distension (Tache, 1987). The influence of neonatal systemic capsaicin pretreatment on the responses to both stimuli has been contradictory. Whereas acid-secretory responses to insulin administration were unaffected, responses to gastric distension were completely absent in capsaicin-pretreated rats. Differences in the nervous centres involved and its sensitivity to capsaicin treatment cannot be excluded. However, another explanation for such divergence could be based in the nature of the stimulus involved. Insulin administration induces a diminution of the level of glucose in plasma which, subsequently, is directly identified by the brain and leads to a vagally mediated increase in acid secretion. Distension of the stomach is detected by gastric mechanoreceptors which then, possibly through capsaicin-sensitive afferent sensory neurones, transmit the information to the CNS and trigger a vagal stimulus of acid secretion. The destruction by capsaicin of peripheral afferent neurones, rather than a central effect, could therefore explain the inhibition of the acid secretory response to gastric distension.

The recent finding that systemic capsaicin pretreatment decreased the acid-secretory responses induced by 2-deoxy-D-glucose (Evangelista *et al.*, 1989) could contradict our results with insulin. The actions of 2-deoxy-D-glucose on gastric acid secretion have been compared with those of insulin, since this analogue of glucose cannot be metabolized and stimulates the release of endogenous insulin by the pancreas (Debas, 1987; Tache, 1987). An involvement of capsaicin-sensitive neurones in the mechanisms leading to insulin release cannot be excluded. However, the effects of 2-deoxy-D-glucose are more complex and a direct action on hypothalamic, diencephalic and medullary sites in the CNS, as well as hepatic sites in the periphery have also been described (Debas, 1987; Tache, 1987). It is therefore feasible that any of these specific actions of 2-deoxy-D-glucose, either central or peripheral, could be significantly influenced by capsaicin pretreatment, thus eliminating the acid secretory response.

Direct application of capsaicin leads to a selective and prolonged blockade of the sensitive afferent fibres located in a peripheral tissue or nerve (Buck & Burks, 1986; Holzer, 1988). Therefore, it provides a useful tool for analysing the anatomical pathways by which the activation by gastric distension of capsaicin sensitive afferent neurones leads to the stimulus of acid secretion. The local application of capsaicin to the vagus nerve has been shown to reduce by 40% the acid secretory responses to acute (6 min) gastric distension. Bilateral vagotomy, which will sever both afferent and efferent neurones, equally reduced acid-responses to distension to a level not significantly different from those present in rats treated with topical capsaicin on the vagus. On this basis, Raybould & Tache (1989) have proposed that capsaicin-sensitive vagal afferent fibres mediate the vagal portion of the secretory response to gastric distension. Our findings with a longer period of distension do not exclude this idea, but suggest that capsaicin-sensitive afferent fibres mediating acid responses to distension are also located in the coeliac ganglion. Local application of capsaicin to the coeliac ganglion or acute gangli-

onectomy, abolished acid secretory responses to distension. Acid responses to insulin were not influenced by these two procedures, therefore implying a specific involvement of the coeliac ganglion in the acid responses to distension. The possibility of a non-specific destruction of the whole of the nervous tissue located in the ganglion has been excluded, since changes in intragastric pressure after the electrical stimulation of the ganglion were not altered by the topical application of capsaicin. It is also relevant that local treatment with capsaicin of the coeliac ganglion, as well as acute ganglionectomy, has been shown to abolish reflex cardiovascular changes after the application of warm fluids to the mucosal surfaces of the rat stomach, jejunum or ileum (Rozsa *et al.*, 1988). The degree of inhibition of the acid response after the local application of capsaicin to the coeliac ganglion was not different from that obtained after systemic treatment. This observation thus indicates that most capsaicin-sensitive neurones involved in the secretory response to distension are located in the coeliac ganglion or, at least, that the integrity of those present is necessary for a correct response to distension.

The hypothesis that a peripheral effect, rather than a central action, of capsaicin is mainly responsible for its inhibitory effect on the acid responses to gastric distension is further reinforced by the fact that intragastric infusion with high doses of capsaicin prevents the increase in acid output induced by distension. This was not due to a nonspecific inhibitory effect of capsaicin on the gastric mucosa, since acid responses to insulin and carbachol were not affected by such treatment (data not shown). Furthermore, it would suggest that gastric distension is detected by mucosal receptors, and would represent a pharmacological proof of the presence of capsaicin-sensitive afferent fibres in the gastric mucosa. In keeping with this idea, recent studies by Lippe *et al.* (1989) have shown that intragastric perfusion with lower doses of capsaicin could facilitate the elimination of the acid present in the stomach, independently of its endogenous or exogenous origin, by mechanisms still unknown. This effect requires the integrity of afferent neurones, since it was absent after neonatal treatment of the rats with high doses of capsaicin. These functional studies thus confirm previous investigations, using immunohistochemical techniques for neuropeptides, which localized such neurones in close association with blood vessels of the gastric mucosa of the rat (Sharkey *et al.*, 1984; Green & Dockray, 1988).

In conclusion, the present findings suggest that capsaicin-sensitive afferent neurones mediate acid responses to gastric distension, but not to hypoglycaemia or direct stimulation of the oxyntic cell with histamine, pentagastrin or carbachol.

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Figure 6 Effects of the intragastric infusion with capsaicin (0.1 mg min^{-1} , 10 min, hatched columns) or vehicle (open columns) on the stimulation of acid secretion in the anaesthetized rat by gastric distension ($20 \text{ cmH}_2\text{O}$) or insulin (0.3 iu kg^{-1} , i.p.). Each column represents mean with s.e.mean shown by vertical bars, and the level of statistical difference from vehicle-treated group is shown by $** P < 0.01$. Numbers inside the columns represent the number of animals used.

Such neurones would be located in the gastric mucosa and the coeliac ganglion and would be susceptible to the action of local capsaicin. The present observations also imply that ingestion of food rich in capsaicin, if the amount were high enough, could modify the acid secretory responses of the stomach. This may be a relevant observation when it is considered that such food has for long been included (Soll, 1989) among the aetiological factors in different gastrointestinal disorders such as dyspepsia and peptic ulcer disease.

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