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Capsaicin-sensitive vagal stimulation-induced gastric acid secretion in the rat: evidence for cholinergic vagal afferents

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1 The effects of electrical vagal stimulation on frequency-dependent gastric acid secretion were investigated in urethane-anaesthetized rats *in vivo*.

2 Stimulation at 4, 16 or 32 Hz was performed in rats treated with atropine ($1\,mg\,kg^{-1}$, i.v.), hexamethonium ($10\,mg\,kg^{-1}$, i.v. bolus and $1\,mg\,kg^{-1}\,min^{-1}$, i.v. infusion) or atropine and hexamethonium (doses as above); in some experiments pentagastrin ($1.2\,\mu g\,kg^{-1}\,h^{-1}$, i.v. infusion) was infused prior to stimulation.

3 Maximal acid secretion occurred at 16 Hz. This was significantly reduced but not abolished by atropine or hexamethonium and completely abolished after atropine and hexamethonium. In the presence of pentagastrin, the acid secretory response to 16 Hz stimulation was augmented, atropine or hexamethonium reduced stimulated secretion by about 70%, whereas atropine and hexamethonium completely abolished stimulated secretion.

4 In rats in which the vagus nerve was pretreated with capsaicin 10–14 days before experimentation there was a significant reduction (by about 40%) in stimulated acid secretion at 16 Hz, which was virtually abolished by atropine treatment. After acute treatment of the vagus nerve with capsaicin (at the time of experimentation) maximally stimulated acid secretion was significantly reduced by about 50%.

5 Taken together, these results indicate that capsaicin-sensitive afferent fibres contribute to the acid secretory response induced by electrical vagal stimulation in the rat. Based on pharmacological evidence, the capsaicin-sensitive afferent fibres may be cholinergic, since atropine and hexamethonium totally abolish vagal stimulation-induced acid secretion.

Keywords: gastric acid secretion; capsaicin; vagus nerve; atropine; hexamethonium

Introduction

Capsaicin has been widely used to study the structure and function of primary afferent neurones, since it was discovered that it induced the selective degeneration of a population of chemosensitive primary sensory nerves (Jancsó *et al.*, 1977) and see reviews by Buck & Burks (1986), Holzer (1988) and Maggi & Meli (1988). A number of studies have focused on the role of capsaicin-sensitive nerves in the stomach. A population of capsaicin-sensitive nerves, of spinal afferent origin, have been described in the gastric muscle and mucosa (Sharkey *et al.*, 1984; Sternini *et al.*, 1987; Su *et al.*, 1987; Green & Dockray, 1988). Ablation of primary afferent neurones by capsaicin treatment augmented mucosal damage produced by acid distension (Szolcsanyi & Bartho, 1981), pyloric ligation (Szolcsanyi & Bartho, 1981), indomethacin (Evangelista *et al.*, 1986; Holzer & Sametz, 1986), ethanol (Holzer & Sametz, 1986; Esplugues & Whittle, 1990) or platelet-activating factor (PAF) (Esplugues *et al.*, 1989) but was ineffective in augmenting ulcer formation produced by cold-restraint (Dugani & Glavin, 1986).

The effects of capsaicin on gastric acid secretion are unclear. Oral administration of capsaicin increased acid secretion in a dose-dependent manner (Limlomwongse *et al.*, 1979). Adult rats treated with capsaicin systemically ($300\,mg\,kg^{-1}$, s.c.; Alföldi *et al.*, 1986) or topically (on to the vagus, 1 mg; Raybould & Taché, 1989; Raybould *et al.*, 1990) had attenuated secretory responses to histamine (i.v.) and a thyrotropin releasing hormone (TRH) analogue (intracisternally), but not to pentagastrin, bethanechol or carbachol (i.v.). In contrast, adult rats treated with a low dose of capsaicin systemically ($65\,mg\,kg^{-1}$, s.c.) had a depressed response to pentagastrin (other secretagogues were not tested) (Dugani & Glavin, 1986). Finally, animals treated at birth with capsaicin showed no impairment in their responsiveness to any gastric secretagogue (Esplugues *et al.*, 1990).

In the rat, a population of capsaicin-sensitive vagal afferent fibres have been described (Gamse *et al.*, 1981; Marsh *et al.*, 1987; Waddell & Lawson, 1989) and it has been demonstrated that these nerves partially mediate the increase in acid secretion in response to gastric distension (Raybould & Taché, 1989; Esplugues *et al.*, 1990). It is also well established that stimulation of the vagus nerve gives rise to atropine- and/or hexamethonium-resistant excitatory motor responses in the stomach (Delbro *et al.*, 1982; Fandriks & Delbro, 1983; Yano *et al.*, 1983; Okamoto *et al.*, 1986; Morishita & Guth, 1986; Beck *et al.*, 1988; Tsubomura *et al.*, 1987; 1988). Some of these effects have been attributed to the activation of unmyelinated vagal afferent fibres (Delbro *et al.*, 1982; Fandriks & Delbro, 1983; Tsubomura *et al.*, 1988). However, the potential contribution that capsaicin-sensitive vagal afferent fibres make to acid secretion induced by electrical stimulation is not known.

Hence in the present study we have investigated the effects of electrical vagal stimulation on frequency-dependent acid secretion in the presence and absence of atropine and/or hexamethonium and after perineural application of capsaicin on the vagus nerve in the anaesthetized rat. A preliminary account of this work has been published (Sharkey *et al.*, 1990).

Methods

Animals

Adult male Sprague-Dawley rats (Charles River) with a body weight of 312 ± 6 g (mean ± s.e.mean) were assigned to one of two groups to receive capsaicin or vehicle treatment 7–14 days before experimentation (pretreated group, $n = 16$) or topical application of capsaicin, vehicle or paraffin oil at the time of experimentation (acute group, $n = 53$).

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Capsaicin treatment

Pretreatment Animals were anaesthetized with Halothane (Fluothane, Ayerst; induced with 4% and maintained on 2%) in oxygen. Under aseptic conditions the left cervical vagus nerve was isolated with the aid of a dissecting microscope. A strip of parafilm (Fisher) was placed under the nerve and two small cotton balls were placed on either side of the nerve to prevent leakage of the capsaicin or vehicle over the surrounding tissues. Capsaicin (Sigma; 10 mg ml^{-1}, dissolved in 10% Tween 80 and 90% paraffin oil [vehicle], $n = 10$) or vehicle ($n = 6$) was applied to a 3–5 mm section of nerve for 30 min (approximate dose 1 mg/rat), after which the area was cleaned and thoroughly rinsed with sterile 0.9% saline (Jancsó & Such, 1983; Raybould & Taché, 1989). All animals received a single prophylactic injection of penicillin G (15,000 units, i.m.). The incision was closed and the animals were allowed to recover for 7–14 days before experimentation.

Acute treatment The acutely-treated group contained animals receiving (a) capsaicin ($n = 9$, dissolved as above), (b) vehicle alone ($n = 9$) or (c) paraffin oil alone ($n = 35$). The capsaicin or vehicle was applied to the nerve at the site of the stimulating electrode as described above.

Measurement of gastric acid secretion

Animals were prepared according to a modification of the method of Ghosh & Schild (1958). Rats were anaesthetized with urethane (1.5 g kg^{-1}, i.p.; Sigma), placed on a heating pad and maintained at 35° ± 1°C. A 3–5 mm section of the left cervical vagus nerve was isolated, the central end was cut or, in some experiments (acute capsaicin treatment) crushed and ligated and the peripheral portion was placed on a bipolar silver stimulating electrode and immersed in paraffin oil. The stomach was perfused through an orogastric tube (3 mm o.d.) with 0.9% sodium chloride at 37°C at rate of 1.3 ml min^{-1} by use of a roller pump (LKB). Effluent from the stomach was collected by a tube introduced through the pylorus and connected directly to a Radiometer Copenhagen Automatic Titration system enabling continuous monitoring of gastric acid output. The end point of all titrations was pre-set to the pH of the perfusate (about pH 6.5) the titrant used was 0.01 M sodium hydroxide calibrated daily with 0.01 M hydrochloric acid standard.

Blood pressure was monitored from a femoral artery and used to check (a) the anaesthetic level of the animal, (b) the efficacy of the nerve stimulations (by observing bradycardia) and (c) the action of autonomic antagonists given to the animal. Drugs were administered intravenously through a femoral venous cannula which was also used for fluid replacement. Vagal stimulations (1 ms pulse, 4–32 Hz, 12–15 V) were delivered for 1 min from a Grass S48 stimulator connected to a stimulus isolator (Grass Instruments).

Drugs

The following drugs were used: atropine sulphate (Sigma; 1 mg kg^{-1}, i.v. bolus), hexamethonium bromide (Sigma; 10 mg kg^{-1}, i.v. bolus and 1 mg kg^{-1} min^{-1}, i.v. infusion) and pentagastrin (Peptavlon, Ayerst Laboratories, 1.2 μg kg^{-1} h^{-1}, i.v. infusion). All drugs were dissolved in 0.9% saline.

Experimental protocol

Animals were allowed to stabilize gastric acid output for a period of 30–45 min. When at least 10 min of stable acid secretion (μmol H$^+$ 5 min^{-1}) was measured (basal acid output) the vagus nerve was stimulated. Acid secretion was continuously monitored until it returned to basal levels, after which additional stimulations were performed in a random fashion. If basal secretion before and after stimulation was not the same, the mean of the pre- and post-stimulation basal values (defined as 10 min of stable secretion) were calculated and used as 'basal'. This procedure was repeated in the presence or absence of autonomic antagonists and in some experiments after pentagastrin infusion.

In one set of experiments the acute effects of capsaicin were studied. In these experiments the vagus nerve was stimulated in paraffin oil, vehicle and capsaicin (dissolved as above). The capsaicin was left on the nerve for 30 min, after which the nerve was put back in paraffin oil and re-stimulated.

Data analysis

The response to each stimulation was calculated as total acid output with basal subtracted for the period of the response. Data from capsaicin-treated or control animals were compared by analysis of variance for repeated measures with Newman-Keuls test for multiple comparisons, paired or unpaired Student's t tests as applicable. Statistical values reaching probabilities of $P < 0.05$ or less were considered significant.

Results

Basal acid secretion

The mean basal acid output in control (untreated) rats was 1.8 ± 0.2 μmol H$^+$ 5 min^{-1} (mean ± s.e.mean, $n = 39$ rats), in vehicle-treated rats was 1.8 ± 0.1 μmol H$^+$ 5 min^{-1} ($n = 10$) and in capsaicin pretreated rats was 1.8 ± 0.3 μmol H$^+$ 5 min^{-1} ($n = 13$).

Responses to electrical stimulation: untreated controls

Left cervical vagal stimulation in control (untreated) rats gave a graded increase in gastric acid secretion that peaked at a stimulation frequency of 16 Hz (Figures 1 and 2). The response at 32 Hz was lower than at 16 Hz ($P < 0.05$, ANOVA) and both 16 Hz and 32 Hz gave greater responses than 4 Hz stimulation. The maximum acid output (basal subtracted) to 16 Hz stimulation in control rats was 10.7 ± 1.0 μmol H$^+$ ($n = 17$). The time course of stimulation was similar at all frequencies examined; acid output increased 5 min after stimulation,

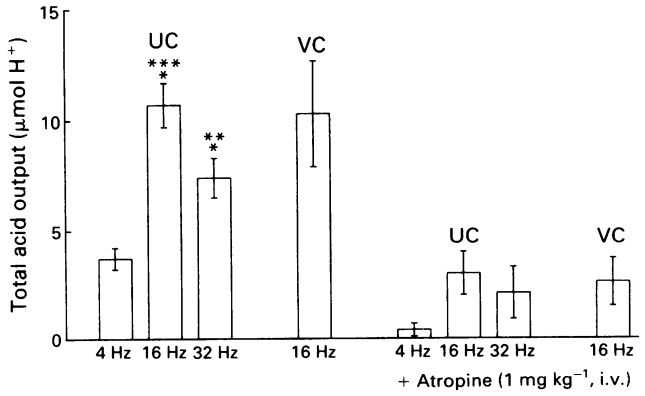

Figure 1 Effects of vagal stimulation at 4, 16 and 32 Hz on total gastric acid output (basal subtracted) from control rats in the presence or absence of atropine (1 mg kg^{-1}, i.v.). UC, untreated controls, VC, vehicle-treated controls (see text for details). *Significantly >4 Hz controls, $P < 0.05$ ANOVA; **significantly <16 Hz controls, $P < 0.05$ ANOVA; ***significantly >16 Hz capsaicin-treated rats (see Figure 3), $P < 0.05$ ANOVA. Atropine significantly reduced acid secretion at all frequencies tested.

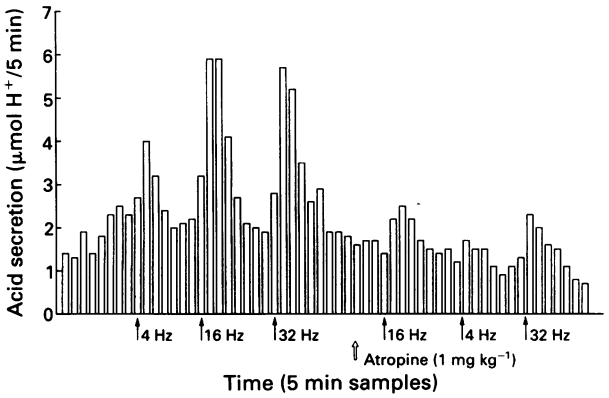

Figure 2 Stimulation of acid secretion in a single experiment from a control rat in the presence and absence of atropine (1 mg kg^{-1}, i.v.). Note that basal output is not subtracted. Each column represents acid output over 5 min. Stimulation was for 1 min at 4, 16 and 32 Hz (supramaximal voltage, 1 ms pulse).

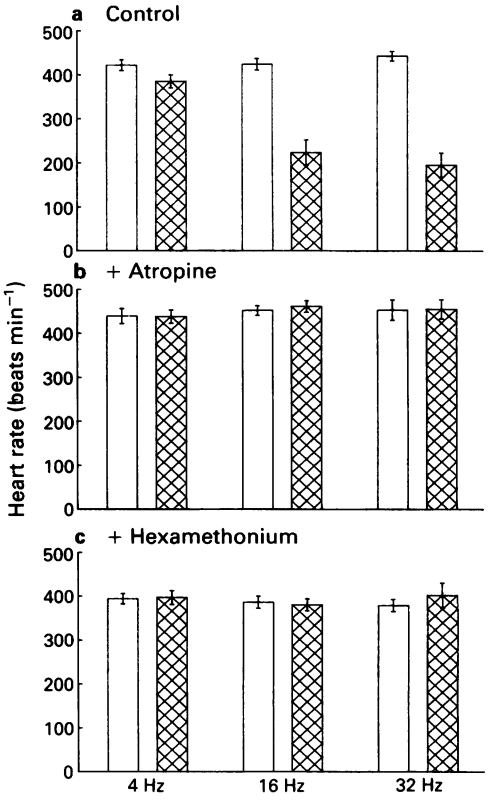

Figure 3 The effects of vagal stimulation at 4, 16 and 32 Hz on heart rate in normal rats (a) (4 Hz, $n = 13$; 16 Hz, $n = 16$; 32 Hz, $n = 14$), rats treated with atropine (b) (1 mg kg^{-1}, i.v.; 4 Hz, $n = 7$; 16 Hz, $n = 11$; 32 Hz, $n = 5$) or rats treated with hexamethonium (c) (10 mg kg^{-1}, i.v. bolus and 1 mg kg^{-1} min^{-1}, i.v. infusion; 4 Hz, $n = 3$; 16 Hz, $n = 6$; 32 Hz, $n = 4$). Open columns: unstimulated; cross-hatched columns: stimulated. Resting heart rate was lowered by hexamethonium (ANOVA, $P < 0.05$) and slightly raised by atropine. Note that bradycardia was abolished after treatment with either antagonist.

peaked at 10–15 min and returned to basal conditions within 30–40 min of stimulation (Figure 2). The frequency-response data is shown in Figures 1 and 2 and Table 1. During stimulation bradycardia was observed in all animals and in some animals this was quantified and is shown in Figure 3.

In the presence of atropine, there was a reduction, though not abolition, of the response to electrical stimulation in control animals (Figures 1 and 2 and Table 1). At 16 Hz, atropine reduced acid secretion by about 70%, whereas at 4 Hz there was an almost total abolition of the response to stimulation. Bradycardia was not observed in animals treated with atropine (Figure 3). Mean arterial blood pressure in the atropine-treated rats was lower than in control rats before atropine treatment (75 ± 3 mmHg compared to 85 ± 4 mmHg, $P < 0.01$, paired t test).

In the presence of a dose of hexamethonium sufficient to block all cardiac effects of electrical vagal stimulation (Figure 3), stimulated acid secretion was not completely abolished in control rats (Table 1). At 16 Hz there was a 90% reduction in acid output. However, mean arterial blood pressure was reduced in hexamethonium-treated rats (49 ± 4 mmHg compared to 85 ± 4 mmHg, $P < 0.01$, paired t test). Combination of hexamethonium and atropine completely blocked all cardiac effects of electrical vagal stimulation and gastric acid secretion. The mean arterial blood pressure in these rats was also lower than in controls (38 ± 3 mmHg, $P < 0.01$, paired t test).

In the presence of a continuous infusion of pentagastrin (1.2 μg kg^{-1} h^{-1}) acid output was approximately doubled in control rats from about 1.8 to 3.3 ± 0.6 μmol H$^+$ 5 min^{-1} ($n = 18$). Stimulation of the vagus nerve at 16 Hz in these

Table 1 Gastric acid secretion in rats in response to electrical stimulation: effects of atropine, hexamethonium, pentagastrin and capsaicin

Stimulation frequency		*+ Atropine*	*+ Hexamethonium*	*+ Atropine + hexamethonium*
Untreated controls				
4 Hz	3.7 ± 0.5 (17)	0.4 ± 0.3 (5)	0.4 ± 0.2 (5)	0 (4)
16 Hz	10.7 ± 1.0 (17)*.**.***	3.0 ± 1.0 (6)	1.1 ± 0.3 (6)	0 (5)
32 Hz	7.4 ± 0.9 (17)*	2.1 ± 1.2 (3)	0.8 ± 0.3 (6)	0 (5)
+ Pentagastrin				
16 Hz	19.1 ± 3.0 (18)†	5.6 ± 1.8 (6)	5.0 ± 1.6 (7)‡	0.4 ± 0.3 (5)
Capsaicin pretreated				
4 Hz	3.6 ± 1.2 (9)	0 (2)	0 (3)	
16 Hz	6.3 ± 1.1 (10)*	0.6 ± 0.3 (4)	0.5 (2)	
32 Hz	5.6 ± 1.2 (10)*	0.3 ± 0.3 (3)	0 (3)	

Values (μmol H$^+$) are: mean ± s.e.mean (n).
* Significantly > 4 Hz within group (ANOVA, $P < 0.05$); **significantly > 32 Hz within group (ANOVA, $P < 0.05$); ***significantly > 16 Hz compared to capsaicin-treated group (ANOVA, $P < 0.05$).
† Significantly > 16 Hz between all groups (t test, $P < 0.01$).
‡ Significantly > untreated controls (t test, $P < 0.01$).
Note that atropine and hexamethonium significantly reduced acid secretion at all frequences in all groups.

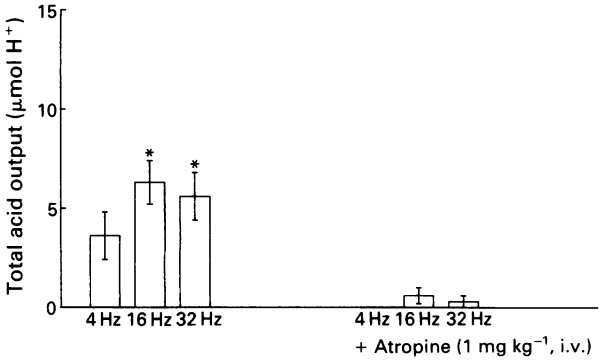

Figure 4 Effects of vagal stimulation at 4, 16 and 32 Hz on total gastric acid output (basal subtracted) from capsaicin-treated rats in the presence or absence of atropine (1 mg kg^{-1}, i.v., see text for details). *Significantly >4 Hz controls, $P < 0.05$ ANOVA. Atropine significantly reduced acid secretion at all frequencies tested.

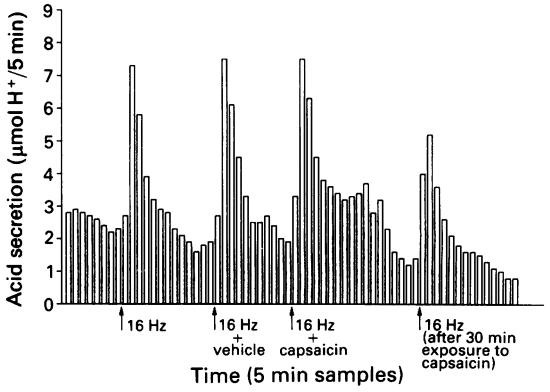

Figure 7 Results of an individual experiment in which vagal stimulation at 16 Hz was performed in the presence of paraffin oil (unlabelled), vehicle (10% Tween 80, 90% paraffin oil) or capsaicin (10 mg ml^{-1} dissolved in vehicle), see text for details. Note that basal secretion is not subtracted. Also that this animal had an elevated basal secretion at the start of the experiment which 'ran down' during the experiment.

animals gave an augmented acid response (Table 1), the total acid output was 19.1 ± 3.0 μmol H$^+$ 5 min^{-1}, which was greater than for untreated control rats ($P < 0.05$, t test, $n = 18$). In the presence of atropine, acid output was reduced by 70% as before, however, the response in the presence of hexamethonium was also only reduced by 70% (cf. 90% in the previous experiment, see Table 1) in the face of the reduced blood pressure. Combination of atropine and hexamethonium completely abolished the response to vagal stimulation in the presence of pentagastrin.

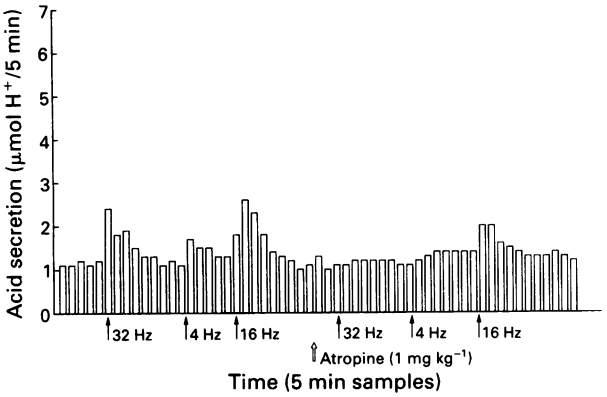

Figure 5 Simulation of acid secretion in a single experiment from a capsaicin-treated rat in the presence and absence of atropine (1 mg kg^{-1}, i.v.). Note that basal output is not subtracted. Each column represents acid output over 5 min. Stimulation was for 1 min at 4, 16 and 32 Hz (supramaximal voltage, 1 ms pulse).

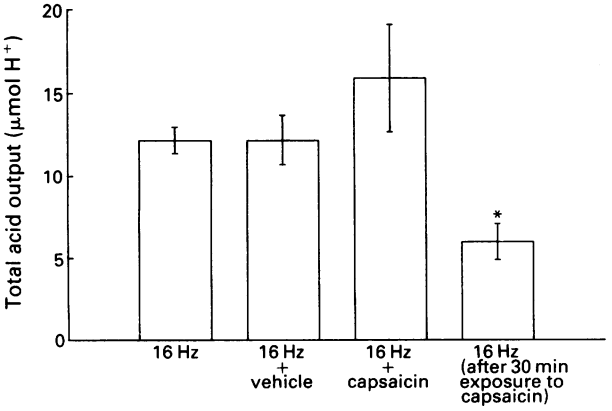

Figure 6 Effects of vagal stimulation at 16 Hz in the presence of paraffin oil (unlabelled), vehicle (10% Tween 80, 90% paraffin oil) or capsaicin (10 mg ml^{-1} dissolved in vehicle), see text for details. *Significantly <control, $P < 0.01$ paired t test.

Responses to electrical stimulation: vehicle-treated controls

Rats pretreated with the vehicle used to dissolve the capsaicin showed no significantly different responses from that of untreated control rats (Figure 1). Stimulation of the vagus caused bradycardia in these animals.

Responses to electrical stimulation

Capsaicin-pretreated animals Perineural application of capsaicin 7–14 days before vagal stimulation caused a reduction in the response at 16 Hz compared to controls ($P < 0.05$, ANOVA), a non-significant reduction at 32 Hz but almost no effect on the response at 4 Hz (Figures 4 and 5 and Table 1). Furthermore, capsaicin-treatment virtually abolished the differences between responses at the three different frequencies of stimulation. In the presence of either atropine or hexamethonium, the response to vagal stimulation was almost completely abolished at all frequencies (Table 1). Bradycardia was again observed in all animals during stimulation prior to treatment with an autonomic blocker.

Acute capsaicin treatment In this group of rats, stimulation of the left cervical vagus at 16 Hz gave a total acid output of 12.2 ± 0.8 μmol H$^+$ ($n = 8$), in the presence of the vehicle the response was 12.2 ± 1.5 μmol H$^+$ ($n = 5$) (Figures 6 and 7). Electrical stimulation during capsaicin treatment gave a slightly augmented response of 15.9 ± 3.2 μmol H$^+$ ($n = 6$), however, after 30 min of exposure to capsaicin the response significantly declined to 6.0 ± 1.1 μmol H$^+$ ($n = 8$, $P < 0.01$, paired t test). At all rates of stimulation bradycardia was again observed and in 2 animals it was quantified. The pre-stimulation heart rate was 430 beats per min, which was reduced to 147 beats per min by stimulation at 16 Hz in the presence of vehicle. In the presence of capsaicin, stimulation-induced bradycardia was 141 beats per min and stimulation after capsaicin was 175 beats per min. At the end of the

experiment the heart rate was unchanged at 430 beats per min.

Discussion

We have demonstrated that capsaicin-sensitive fibres in the vagus nerve contribute to vagal stimulation-induced gastric acid secretion in the anaesthetized rat *in vivo*. We also demonstrated that under appropriate conditions there is an atropine- or hexamethonium-resistant component of acid secretion.

Direct application of capsaicin to the vagus nerve leads to a prolonged and selective blockade in sensitive afferent fibres. Use of this allowed Raybould & Taché (1989) to demonstrate that capsaicin-sensitive vagal fibres partially mediate the acid secretory response to distension. These studies have been extended by Esplugues *et al.* (1990) who showed that local application of capsaicin to the coeliac ganglion also decreased the secretory response to gastric distension. In this context Esplugues *et al.* (1989) and Esplugues & Whittle (1990) also demonstrated that morphine or its analogues augment gastric damage induced by ethanol or PAF, through a mechanism that involves capsaicin-sensitive afferent fibres. In the present study, capsaicin was applied to the vagus under acute or chronic experimental paradigms. In both cases a similar result was observed; a reduction in maximal acid secretion by about 40 to 50%. Stimulation at low frequencies (4 Hz) produced comparable responses in control and capsaicin-treated rats, suggesting that fibres activated by high frequency stimulation are more susceptible to capsaicin. Because of the specificity of capsaicin this may be interpreted as preferential activation of efferent fibres at low frequencies of stimulation and afferent fibres at high frequencies.

The effects of electrical vagal stimulation at varying frequencies on gastric acid output have previously been investigated in the rat (Berthoud *et al.*, 1986), cat (Martinson, 1965) and ferret (Grundy & Scratcherd, 1982). In the rat, Berthoud *et al.* (1986) obtained a frequency-response curve similar to that found in the present study, with the reduction in secretion at higher stimulation frequencies, presumably due to conduction block. In that study the peak occurred at about 4 Hz, whereas in our study acid output peaked around 16 Hz. This difference may be due to different stimulation systems; Berthoud *et al.* (1986) used constant current stimulation whilst constant voltage was used in this study.

In this study we demonstrated an apparent atropine-resistant component of vagal stimulation-induced acid secretion. This has been observed previously in the rat (Stapelfeldt *et al.*, 1988), as has a vagally-stimulated atropine-resistant gastric vasodilatation (Yano *et al.*, 1983). The dose of atropine given ($1\,mg\,kg^{-1}$) was sufficient to block the cardiovascular effects of vagal stimulation, however, higher doses of atropine were not examined. That combined cholinergic blockade (atropine and hexamethonium) blocked acid secretion suggested that cholinergic nerves may be responsible for mediating vagal stimulation-induced acid secretion. The potential contribution of adrenergic mechanisms was not examined but Stapelfeldt *et al.* (1988) demonstrated a small adrenergic component in vagally-stimulated acid secretion. They also demonstrated that naloxone augmented acid secretion suggesting that endogenous opiates normally have an inhibitory action on vagally-mediated secretion.

The observation that cholinergic nerves are largely responsible for vagally-stimulated acid secretion is further supported by the results of stimulation in the presence of pentagastrin. In this case even when acid secretion was augmented, combined cholinergic blockade abolished stimulated acid secretion. The observation that pentagastrin potentiated the effects of vagal stimulation has been described previously and is reviewed by Debas (1987). The effects of hexamethonium on acid secretion are complicated by its profound hypotensive action. However, when hexamethonium was given in the presence of pentagastrin, even though blood pressure was significantly reduced, secretion was still maintained and was, in fact, comparable to that seen after atropine treatment.

Taken together, these results suggest that the capsaicin-sensitive component of vagal stimulation-induced gastric acid secretion is mediated by cholinergic neurones. One possible interpretation of these data is that vagal afferents are themselves cholinergic. There is a substantial body of evidence that demonstrates a sub-population of vagal afferent neurones in the rat (Helke *et al.*, 1983; Palouzier *et al.*, 1987; Ternaux *et al.*, 1989) and cat (Fujiwara & Kurahashi, 1976; Fujiwara *et al.*, 1978) are cholinergic and some evidence for gastric cholinergic vagal afferents (Tsubomura *et al.*, 1987; 1988). It is interesting to note that of the neuropeptides found in the nodose ganglia (notably substance P and calcitonin gene-related peptide) only a very small proportion were found to project to the stomach (Dockray & Sharkey, 1986; Green & Dockray, 1988). Thus, electrical vagal stimulation may result in activation of efferent preganglionic fibres and afferent fibres both of which are cholinergic. Recently, Tayo and coworkers (Tayo & Williams, 1988; Tayo & Helke, 1990) have provided strong evidence that some gastric vagal motor neurones are apparently non-cholinergic and may contain dopamine. However, we found no functional evidence for inhibitory actions of vagal stimulation in the rat *in vivo*, as has been described in the mouse stomach *in vitro* (Davison & Najafi-Farashah, 1987). Thus the simplest model that explains the data in this study is that the hexamethonium-resistant component of stimulated acid secretion is due to capsaicin-sensitive afferent fibres, which are atropine-sensitive. The atropine-resistant component may be partially afferent (non-cholinergic) or more likely may represent a vagal efferent non-cholinergic response. Since a vagal non-cholinergic gastrin secretion has already been described (Debas, 1987; Walsh, 1989) it is conceivable that this contributes to the atropine-resistant component of acid secretion.

In conclusion, these results indicate that capsaicin-sensitive afferent fibres contribute to the acid secretory response induced by electrical vagal stimulation in the rat. Based on pharmacological evidence, capsaicin-sensitive afferent fibres give rise to cholinergic actions, since atropine and hexamethonium (at the doses used in this study) totally abolished vagal stimulation-induced acid secretion. This observation is consistent with that of Limlomwongse *et al.* (1979) who demonstrated that intragastric capsaicin-stimulated acid secretion could be blocked by cholinoceptor antagonists. At this time it is not clear if cholinergic neurones are the final mediator of the acid secretory response or if they act to release another agent (e.g. a peptide). The physiological significance of cholinergic vagal afferent fibres remains to be elucidated, but its interesting to note that acute capsaicin administration was found to be protective in ulcer formation (Szolcsanyi & Bartho, 1981; Holzer & Lippe, 1988; Holzer *et al.*, 1989; 1990). This suggests that capsaicin-sensitive vagal afferents in the stomach may play a role in protective reflexes in that organ.

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