Br. J. Pharmacol. (1994), **112**, 93–96

Comparative analysis of the vagal stimulation of gastric acid secretion in rodent isolated stomach preparations

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1 Electrical field stimulation produced a tetrodotoxin-sensitive, frequency-dependent, release of acid from isolated, lumen-perfused, stomach preparations from mouse, immature rat and guinea-pig.

2 In the guinea-pig and mouse preparations, the frequency-dependent response was abolished by hexamethonium, acetylcholine (ACh) muscarinic (M) and histamine H_2-receptor blockade, consistent with the hypothesis that the vagal ACh acts indirectly by stimulating the release of endogenous histamine.

3 In contrast, in the rat preparation the frequency-dependent response was partially refractory to all of these inhibitors. However, a combination of H_2- and ACh M-receptor blockade did abolish the effect.

4 We conclude that vagal-stimulated acid secretion in the rat, unlike the other two species, behaves as though there is a direct innervation of the oxyntic cells by either cholinergic or noncholinergic neurones.

Keywords: Receptor, muscarinic; gastric acid secretion; vagus nerve; histamine; rodent isolated stomach preparations

Introduction

The role of the vagus in the regulation of gastric acid secretion is complex. *In vivo*, the response to vagal stimulation may be inhibited by atropine, histamine H_2-receptor antagonists (Grossman & Konturek, 1974) or by antrectomy (Olbe, 1964), implicating a role for histamine and gastrin as well as acetylcholine (ACh) acting at ACh muscarinic (M)-receptors.

Previously, the frequency-dependent acid secretion obtained by electrical field stimulation of the isolated, lumen-perfused, stomach preparation from the mouse was concluded to be due to preganglionic stimulation of the vagus nerve resulting in the postganglionic release of ACh as judged by the inhibition produced by tetrodotoxin, hexamethonium and atropine (Angus & Black, 1982). Similar results were found (Baird & Main, 1978) in a gastric mucosal sheet preparation from the rat, although in that assay the stimulation was apparently post-ganglionic because it was refractory to hexamethonium. Subsequently, we presented preliminary data in the mouse stomach preparation showing that the frequency-response was abolished by histamine H_2-receptor blockade (Black & Shankley, 1987), although the response to a stable, efficacious, ACh M-receptor agonist, 5-methylfurmethide, was relatively refractory (Black & Shankley, 1985a). These results in the mouse were explained by proposing that neurally-released ACh, perhaps restricted by cholinesterase activity and localized release, acted predominantly to stimulate histamine secretion, whereas 5-methylfurmethide was able to gain access to the M-receptors on the oxyntic cell as well as those on the histamine cell. In contrast, Main & Pearce (1978) reported that the histamine H_2-receptor antagonist, metiamide, was ineffective against both methacholine and electrical field stimulation of the vagus in their rat isolated gastric mucosal sheet preparation.

In an attempt to resolve these conflicting results, we have investigated the response to electrical field stimulation in a comparative study in isolated, lumen-perfused, stomach preparations from mouse, immature rat and guinea-pig.

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Methods

Isolated, lumen-perfused, stomach preparations

Mouse and rat Gastric acid secretion was measured in isolated, lumen-perfused, stomach preparations essentially as described previously for the mouse (Black & Shankley, 1985b). Young adult male mice (Charles River 22–26 g), fasted for 18 h prior to experimentation but with free access to water, and pre-weaned rat pups (Wistar 32–38 g corresponding to age range 10–23 days) were used. Animals were killed by cervical dislocation, the abdomen opened and the oesophagus ligated close to the stomach. A polythene cannula (2 mm internal diameter) was inserted into the pylorus via the duodenal bulb, and a small incision made in the fundus through which the stomach contents were gently washed. A second cannula was tied into this incision. The stomachs were then transferred into a 40 ml organ bath containing buffered serosal solution (mM: NaCl 118, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.14, $NaHPO_4$ 15.9, $CaCl_2$ 0.65 and glucose 31.6) maintained at 37°C and gassed with 95% O_2 and 5% CO_2. The preparations were continuously perfused from the fundic to the pyloric cannulae with warmed unbuffered mucosal solution (mM: NaCl 135, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.3 and glucose 31.6) gassed with 100% O_2, and the perfusate passed over a pH-electrode system adjusted to provide 12 cmH_2O intragastric pressure.

Guinea-pig Due to the large size of the guinea-pig stomachs, especially following distension because of the back pressure applied to the lumen-perfusate, 'half' stomach assays were prepared. Male guinea-pigs, weighing 180–220 g, were killed by cervical dislocation, the abdomen opened, the duodenal bulb removed and a polythene cannula inserted into the antral end of the stomach. A cut was made round the stomach from the lesser curvature at the level of the cardiac sphincter, retaining approximately half of the glandular portion, and the stomach contents were gently washed out. The tissue was removed and a further catheter inserted into the aperture created and tied to form a water-tight seal. The guinea-pig preparations were mounted in 40 ml organ baths which were maintained at 34°C because preliminary studies suggested that muscle contraction was reduced at this lower temperature.

Experimental design

Six preparations were used simultaneously and, after a 60 min stabilization period, any not showing stable basal responses were rejected (less than 5%). Thereafter, drugs were added to the serosal solution according to individual experimental protocols. The total vehicle volume did not exceed 1 ml. A randomized block design was used throughout for allocation of experimental treatments such that, as far as possible, each organ bath received each treatment within the course of an experiment.

Acid secretory responses were expressed as ΔpH, that is the difference between basal pH, measured immediately prior to experimental intervention, and stimulated pH. The stomach preparations were electrically stimulated with a pair of platinum, ring electrodes (ring diameter 2 mm, wire diameter 0.5 mm) placed either side of the stomach in the region of the fundic glands (Black & Shankley, 1986). The intensity of stimulation was standardized at 10 V with square wave pulses of 0.5 ms duration. Single cumulative frequency-effect curves were obtained over a frequency range of 1 to 30 Hz.

Data analysis

Where possible, the frequency-effect curve data from individual preparations were fitted by means of an iterative least squares minimization programme to a general logistic function to provide estimates of the midpoint location ($\log f_{50}$), midpoint slope parameter (p) and upper asymptote (α), as described previously (Black & Shankley, 1985b). For display purposes the individual computed parameter estimates for each treatment group were expressed as mean ± s.e.mean and single logistic curves simulated shown superimposed upon the experimental data.

Computed logistic curve-fitting parameters were compared using Student's t test. Values of $P<0.05$ were considered significant.

Drugs

Tiotidine (a gift from Zeneca Ltd.) and famotidine (a gift from Merck, Sharp and Dohme Ltd.) were dissolved in dilute HCl to give 0.2 mM stock solutions. Subsequent dilutions were made in distilled water. All other compounds were dissolved in distilled water and sources were: atropine sulphate, hexamethonium, tetrodotoxin (Sigma); CI-988 ([[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3,3,1,1,^{3,7}]dec-2-yl-oxy)carbonyl]amino]propyl]amino]-1-phenyl-ethyl]amino]-4-oxo butanoic acid (a gift from Parke Davis Ltd.), 5-methylfurmethide iodide (a gift from Wellcome Foundation Ltd.); and McN-A 343 (4-(N-[-3-chlorophenyl]carbamoyloxy)-2-butyryl-trimethylammonium chloride) (a gift from McNeil Laboratories USA Ltd.).

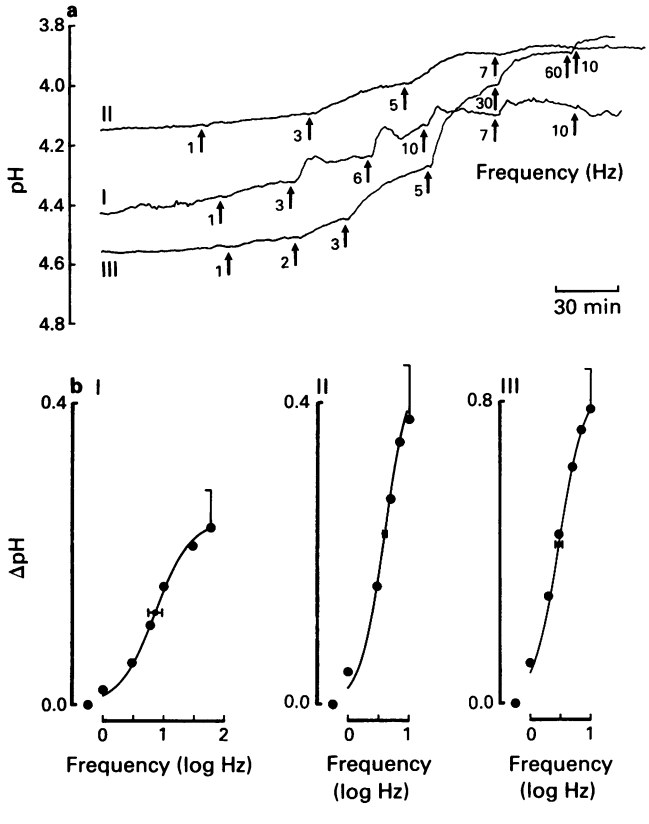

Figure 1 (a) Examples of experimental traces showing cumulative frequency-dependent changes in the pH of the lumen perfusate during electrical field stimulation of isolated, lumen-perfused, stomach preparations from (I) mouse and immature (II) guinea-pig and (III) rat. (b) Frequency-effect curve data corresponding to (a). The effect is expressed as the change in pH (ΔpH) of the lumen perfusate from the basal unstimulated level and is plotted as a function of the frequency of electrical field stimulation. The curves shown superimposed on the mean data points ($n = 4/5 \pm$ s.e.mean) were obtained using the logistic curve fitting parameters shown in Table 1 according to the methods described in the text.

Table 1 Logistic curve-fitting parameters (midpoint location, $\log f_{50}$; midpoint slope parameter, p and upper asymptote, α ± s.e.mean) of control frequency-effect curves obtained in isolated, lumen-perfused, stomach preparations from mouse, immature guinea-pig and rat

	n	$\log f_{50}$	α(ΔpH)	p
Mouse	5	0.86 ± 0.12	0.25 ± 0.05	1.50 ± 0.16
Guinea-pig	4	0.60 ± 0.03	0.45 ± 0.07	2.17 ± 0.48
Rat	5	0.48 ± 0.06	0.85 ± 0.11	2.04 ± 0.26

Results

Electrical field stimulation produced a frequency-dependent release of acid from all three preparations (Figure 1a) which was abolished by 10 μM tetrodotoxin (TTX) indicating neural origin. The inhibition in the presence of TTX of the maximum frequency-dependent response in the guinea-pig, mouse and rat preparations was 100 ± 13, 96 ± 5, 94 ± 3%, respectively. The responses in each assay were stable after 30 min, although an initial peak was frequently observed in the mouse assay (see Figure 1).

The frequency-dependent-response data could be fitted by the logistic function (Figure 1b) and the parameter estimates (Table 1) indicated that the curve obtained in the mouse stomach assay had a lower midpoint slope than that obtained in the immature guinea-pig and rat assays.

In the guinea-pig and mouse preparations, the frequency-dependent responses were also abolished by blocking ganglia (hexamethonium) and ACh M-receptors (atropine). The inhibition in the presence of hexamethonium, at a concentration (100 μM) shown previously to produce selective ganglionic blockade in the mouse stomach preparation by Angus & Black (1982), of the maximum frequency-dependent response in the guinea-pig and mouse preparations was 95 ± 5 and 96 ± 5%, respectively. The inhibition in the presence of atropine at concentrations approximately 1000 fold (20 μM) and 100 fold (2 μM) higher than the K_B values estimated in guinea-pig and mouse preparations (Welsh et al., 1992), of the maximum frequency-dependent response in the guinea-pig and mouse preparations was 95 ± 13 and 93 ± 9%, respectively. Similarly, histamine H_2-receptor blockade, achieved with concentrations of famotidine (20 μM) or tiotidine (100 μM) which are approximately 1000 fold their K_B values in these assays (Welsh et al., 1992), also abolished the responses in the guinea-pig and mouse preparations (Figure 2a,b).

In the rat assay, fully-defined frequency-effect curves could still be obtained in the presence of any of these three antagonists (Figures 2c and 3) although the curve maxima were significantly reduced (28 ± 6, 36 ± 6, 47 ± 11% inhibition with hexamethonium, atropine and tiotidine, respectively). However, in the presence of both 100 μM tiotidine and 20 μM atropine the response in the rat was completely inhibited (Figure 4).

The possibility that gastrin was mediating part of the frequency-dependent response in the rat assay was investigated by use of the gastrin/CCK_B receptor antagonist, CI-988 (Horwell *et al.*, 1991). This ligand had no effect on the frequency-effect curve (curve maxima: 0.68 ± 0.08 and 0.81 ± 0.05ΔpH, in the absence and presence of CI-988, respectively) at a concentration (10 μM) greater than 300 fold its reported K_B value (23 nM) at gastrin/CCK_B-receptors in an isolated preparation of rat gastric mucosa (Patel & Spraggs, 1992).

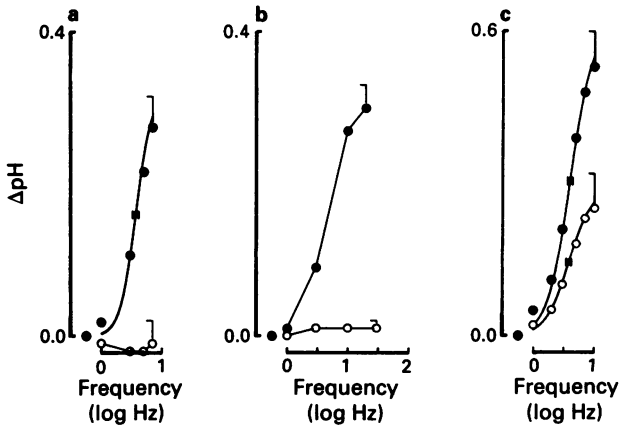

Figure 2 Frequency-effect curves obtained by electrical field stimulation in isolated stomach assays from (a) immature guinea-pig, (b) mouse and (c) immature rat in the absence (●) and presence (○) of histamine H_2-receptor block (100 μM tiotidine or 20 μM famotidine). The curves shown superimposed on the mean data points (n = 3/9 ± s.e.mean) were obtained by logistic curve fitting as described in the text.

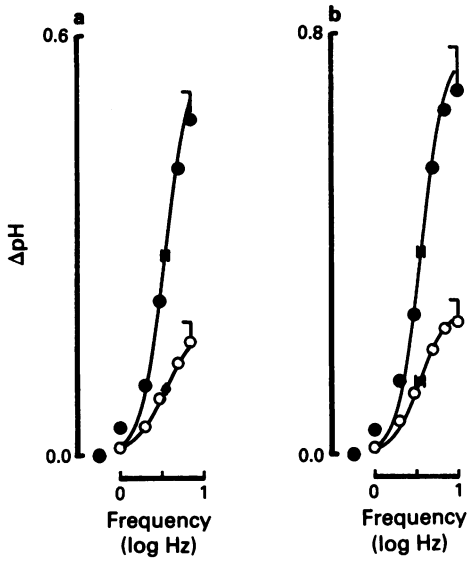

Figure 3 Frequency-effect curves obtained by electrical field stimulation of isolated stomach preparations from immature rats in the absence (●) and presence (○) of (a) hexamethonium (100 μM) and (b) atropine (20 μM). The curves shown superimposed on the mean data points (n = 7/8 ± s.e.mean) were obtained by logistic curve fitting.

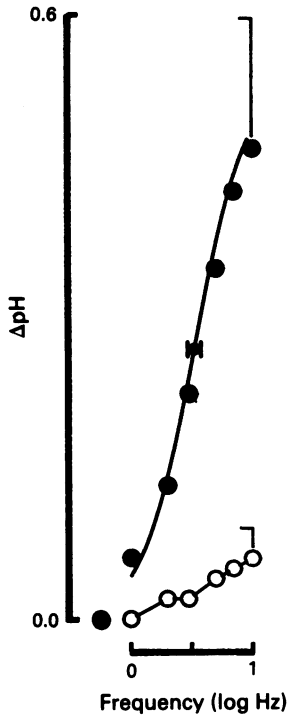

Figure 4 Frequency-effect curves obtained by electrical field stimulation in rat isolated stomach preparations in the absence (●) and presence (○) of both atropine (20 μM) and tiotidine (100 μM). The curves shown superimposed on the mean data points (n = 5/6 ± s.e.mean) were obtained by logistic curve fitting.

Discussion

Isolated, lumen-perfused, stomach preparations were chosen for this comparative analysis because they retain the gastric mucosal architecture so that the relationship between the oxyntic, neural and histamine-secreting cells is maintained during neural stimulation (Black & Shankley, 1985b). Electrical field stimulation gave stable and reproducible acid secretion responses in each of the assays using the stimulation parameters previously optimised in the mouse stomach (Angus & Black, 1982; Black & Shankley, 1986). The effects of electrical field stimulation were totally blocked by TTX in all preparations implying that the effects were mediated by neural activity.

In the mouse and guinea-pig preparations we conclude that, (i) as the effects were annulled by hexamethonium then the frequency effects were mediated by preganglionic nerve stimulation, (ii) as atropine also suppressed the frequency effects then ACh was the postganglionic transmitter, (iii) as histamine H_2-receptor antagonism also blocked the frequency effects, then neurally-released ACh acted predominantly to release histamine. We have argued previously (Black & Shankley, 1987) that the ACh was restricted to the region of the histamine-secreting cells by neural configuration and by cholinesterase activity. This view was confirmed, in the mouse, by the finding that inhibition of cholinesterase produced tiotidine-refractory frequency effects as though ACh could now diffuse to the location of the oxyntic cells (Black & Shankley, 1987). We were unable to confirm this result in the guinea-pig because cholinesterase inhibition by physostigmine produced contractions of the stomach muscle sufficiently powerful to interfere with lumen-perfusion. Histamine-storing mast-cell-like cells in the subepithelium and enterochromaffin-like-cells (ECL cells) have been described (Hakanson, 1970; Hakanson & Sundler, 1991) although their relationship to cholinergic neurones in the gastric mucosa is apparently not described. However, discrete

innervation of mucosal histamine-containing cells in the small intestine has been reported (Newson *et al.*, 1993).

The present results obtained in the mouse stomach preparation are consistent with those previously reported by Angus & Black (1982). In the rat, however, hexamethonium only partially inhibited the frequency effects. If hexamethonium acts by blocking ganglionic nicotinic receptors this indicates that both pre- and postganglionic fibres were being stimulated or that there was an element of non-nicotinic receptor-mediated ganglionic transmission. Either way, on this evidence, we cannot know whether different nerve pathways are involved. Previously, using a mucosal sheet preparation from rat, Baird & Main (1978) found that responses to electrical field stimulation were completely resistant to hexamethonium. The difference between their results and ours may be due to destruction of the autonomic ganglia during the stripping of the smooth muscle, leaving only the postganglionic fibres intact (Angus & Black, 1982).

The rat also differed from the guinea-pig and mouse in that frequency effects were partially atropine-resistant. Atropine and hexamethonium produced almost identical effects on the frequency-effect curves. In both cases there was a decrease in amplitude of the frequency-effect curves of about 60% without changes in location or slope. This suggests that the effects which are resistant to both hexamethonium and atropine may be mediated by the same nerve fibres which would, therefore, be different from those mediating the drug-sensitive effects. The congruence of hexamethonium and atropine effects in the rat may imply that, as in other species, the preganglionic pathways connect to the cholinergic neurones.

In the rat preparation, histamine H_2-receptor blockade only partially inhibited the frequency effects. As with hexamethonium and atropine, tiotidine did not alter the location and slope parameters of the frequency-effect curves but decreased their amplitude by about 60%. However, in the presence of a combination of both atropine and tiotidine, the frequency effects were more or less abolished. Therefore, it seems that the histamine-dependent portion of the response is not the atropine-sensitive portion; that is, it is not, or not entirely, due to ACh M-receptor stimulated histamine release. It is as though atropine removes the component refractory to H_2-receptor blockade and tiotidine removes the component refractory to ACh M-receptor blockade, leading to the conclusion that there is both direct cholinergic innervation plus a non-cholinergic innervation which either releases histamine by releasing an unknown transmitter or, more economically, the transmitter is histamine. Histamine and gastrin containing nerves in the stomach submucosa have been described (Uvnas-Wallenstein *et al.*, 1977). Gastrin does not appear to be a candidate because the atropine-resistant frequency effects were not blocked by the gastrin/CCK_B-receptor antagonist, CI-988.

In the rat, the partial inhibitory effects of H_2-receptor blockade indicate that, unlike other species, direct innervation of oxyntic cells by cholinergic or non-cholinergic neurones is involved in addition to neural release of histamine. These indications raise the question of whether, in the rat, in addition to direct cholinergic and direct histaminergic mechanisms, there is also an indirect cholinergic release of histamine as in the other species.

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(Received November 9, 1993
Revised January 14, 1994
Accepted January 19, 1994)