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THE EFFECT OF ATROPINE ON ACID SECRETION STIMULATED BY ACETYLCHOLINE, HISTAMINE AND GASTRIN IN THE ISOLATED WHOLE STOMACH OF THE RAT

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1 An isolated stomach preparation from immature rats has been used to study the effect of atropine on gastric acid secretion.

2 The acid secretory response to acetylcholine was not inhibited by atropine at a concentration of 0.3 μM. Concentrations of atropine of 1 to 3 μM produced a measurable inhibition of acid secretion, and a concentration of atropine of 10 μM caused a complete block of acid secretion which could not be surmounted by high concentrations of acetylcholine.

3 The acid secretory response to histamine was not inhibited by concentrations of atropine of up to 1 mM.

4 Concentrations of atropine of 1 μM and 10 μM did not inhibit gastrin-stimulated acid secretion, although a significant inhibition of acid output was observed with atropine at concentrations of 0.1 mM and 1 mM.

5 These findings are discussed in relation to the role of cholinergic mechanisms in the control of gastric acid secretion.

Introduction

Cholinergic activity exerts a considerable influence on gastric acid secretion through the direct stimulation of parietal cells, the release of gastrin from the pyloric antrum, and by modifying the responsiveness of the parietal cells to gastrin and histamine. These cholinergic influences are controlled to some degree by long vago-vagal reflexes (Harper, Kidd & Scratcherd, 1959; Grossman, 1962; Debas, Konturek, Walsh & Grossman, 1974; Debas, Walsh & Grossman, 1975), and by local cholinergic reflexes which have been identified in the absence of vagal innervation (Grossman, 1961; Magee & Hu, 1975). A possible role for a local cholinergic mechanism in the control of acid secretion has been studied using atropine in animals in which the vagal innervation to the whole stomach or a fundic pouch has been severed, although it must be appreciated that atropine may also influence acid secretion by inhibiting gastrin release from the pyloric antrum; an effect which remains to be firmly established (Smith, Kewenter, Connell, Ardill, Hayes & Buchanan, 1975). In addition, gastrin has been shown to release acetylcholine from the myenteric plexus in guinea-pig ileum (Vizi, Bertaccini, Impicciatore & Knoll, 1972, 1973) and it is possible that the secretagogue action of gastrin on the stomach is partly mediated through the release of acetylcholine.

Atropine inhibits pentagastrin–and to a lesser extent histamine-stimulated acid secretion in the Heidenhain pouch dog (Code, Hightower & Hallenbeck, 1951; Janowitz & Hollander, 1956; Grossman & Konturek, 1974), pentagastrin-stimulated acid secretion in the Heidenhain pouch rat (Johansson, Lundell & Svensson, 1971), pentagastrin-stimulated acid secretion in the Heidenhain pouch cat (Svensson & Emås, 1974) and both pentagastrin- and histamine-stimulated acid secretion in vagotomized conscious cats provided with a gastric fistula (Emås, 1968). Konturek, Oleksy & Wysocki (1968) have also reported that atropine produced a slight inhibition of acid secretion stimulated by histamine or pentagastrin, in duodenal ulcer patients subjected to vagotomy and pyloroplasty.

The effect of atropine on acid secretion in isolated stomach preparations has not been extensively studied, although high concentrations of atropine (1–10 mM) have been used to inhibit histamine- and pentagastrin-stimulated acid secretion in isolated gastric mucosa preparations of amphibians (Thorpe & Durbin, 1969, 1972; Nakajima, Shoemaker, Hirschowitz & Sachs, 1970). In the present work the effect of atropine on acid secretion stimulated by

histamine, gastrin and acetylcholine has been investigated in the isolated whole stomach of the rat in which both neural and hormonal influences have been minimized.

Methods

Gastric acid secretion in the isolated stomach of the immature rat (35–45 g) was measured by the methods described previously by Bunce & Parsons (1976) and Bunce, Parsons & Rollings (1976). In brief, the rats were anaesthetized with pentobarbitone, the stomach exteriorized and the oesophagus ligated. An incision was made in the rumen of the stomach, and the contents washed out with warm Krebs-Henseleit solution. A second incision was made at the pyloric sphincter and polythene cannulae were inserted and tied into the stomach via these incisions. The stomach was rapidly dissected out and placed in Krebs-Henseleit solution at 37°C.

The lumen of the stomach was perfused at a rate of 1 ml/min with a modified Krebs-Henseleit solution from which the buffers ($NaHCO_3$ and KH_2PO_4) were omitted. The hydrogen ion concentration of the effluent perfusate from the stomach was continuously recorded, and the rate of acid secretion expressed as nmol per minute. After setting up the stomach preparation the basal H^+ output was allowed to stabilize, under control conditions and in the presence of atropine, before the secretory responses to an agonist were investigated. The response to a single dose of an agonist was calculated as the amount of acid secreted at peak response minus the preceding basal level. Fresh solutions of histamine, acetylcholine and atropine were made up each day in Krebs-Henseleit solution. A stock solution of gastrin at 2.5 mg/ml was prepared in 0.05 M NH_4HCO_3. This solution was stored at −5°C and diluted with Krebs-Henseleit solution as required.

Drugs

The following drugs were used: histamine acid phosphate, acetylcholine chloride and atropine sulphate (BDH Ltd.), gastrin (synthetic human gastrin I, Research Plus Laboratories Inc.), pentobarbitone (Sagatal, May & Baker Ltd.).

Analysis of results

Results are expressed as mean ± s.e. mean. An analysis of variance was used to test the effect of atropine on acid secretion, and potency ratios were calculated according to Finney (1964). Dose-ratio (DR) is the reciprocal of potency ratio. A P value of less than 0.05 was considered to be significant.

Results

The design of the present experiments was essentially the same as for those described previously in which the effects of metiamide on acid secretion were investigated in the same preparation (Bunce & Parsons, 1976; Bunce *et al.*, 1976). Suitable doses of both histamine and acetylcholine were chosen for carrying out 2 + 2 assays which were used to determine the effects of atropine. With gastrin, two doses of the agonist were used under control conditions followed by three doses of gastrin in the presence of atropine. Since in the present investigation atropine (1 mM) did not inhibit histamine-stimulated acid secretion the appropriate control experiments described by Bunce & Parsons (1976) were not repeated. However, atropine did inhibit acid secretion stimulated by both acetylcholine and gastrin and it was therefore considered necessary to repeat in this study the corresponding control experiments in the absence of an antagonist. As reported previously (Bunce *et al.*, 1976) no time-related changes in the sensitivity of the rat isolated stomach to either gastrin or acetylcholine were observed. As in previous experiments (Bunce *et al.*, 1976) 'priming' doses of gastrin and acetylcholine at 0.1 μM and 0.3 mM respectively were used before constructing dose-response curves. These 'priming' doses were not required when histamine was used as the secretagogue.

Basal acid secretion

The basal level of acid secretion was calculated immediately before an agonist dose was administered both under control conditions and in the presence of atropine. The results are shown in Table 1. Concentrations of atropine up to 10 μM did not produce a significant effect, but concentrations of atropine of 0.1 mM and 1 mM did significantly inhibit basal acid secretion.

Acetylcholine

The dose-response curve to acetylcholine is linear over the range 0.3 mM to 1 mM (Bunce *et al.*, 1976). A control 2-point dose-response curve was established on each stomach in the absence of atropine with concentrations of acetylcholine of 0.3 mM and 0.7 mM. The stomach was then equilibrated in Krebs-Henseleit solution containing the appropriate concentration of atropine for approximately 1 h, and the second curve constructed. Atropine was used at concentrations of 0.3, 1, 2, 3 and 10 μM. Preliminary experiments showed that no inhibition of acetylcholine-stimulated acid secretion was observed in the presence of 0.3 μM atropine. Atropine in the concentration range 1–3 μM produced an inhibition of acid secretion which could

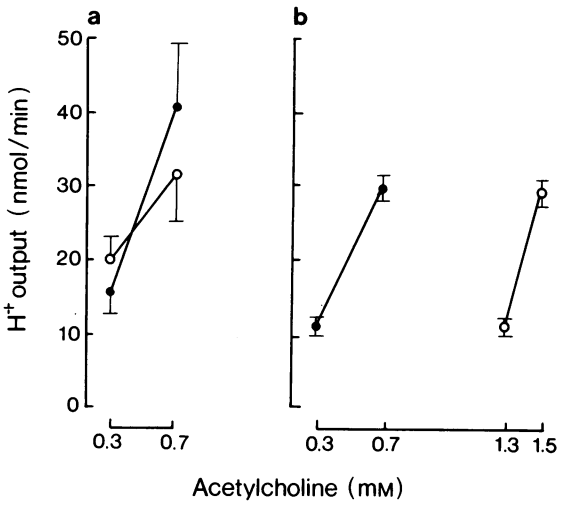

Figure 1 Dose-response curves to acetylcholine in the presence and absence of atropine. Vertical lines show s.e. mean. Analyses of variance on these data provided the following information. (a) Control ($n=8$); the second curve (O) is not significantly displaced from the first curve (●); the slopes of the two lines are not significantly different; (b) 2 μM atropine ($n=8$), the slope of the line in the presence of atropine (O) is not significantly different from that obtained with acetylcholine alone (●); the dose-response curve to acetylcholine in the presence of atropine is significantly displaced from the control curve.

Table 1 The effect of atropine on basal acid secretion in isolated whole stomach of the rat

Acid output (H^+ nmol/min ± s.e. mean)		
Control	*Atropine*	
	1 μM	
30.8 (±3.8, $n=16$)	31.9 (±4.6, $n=16$)	$t=0.19$, $P>0.05$
	3 μM	
35.1 (±3.9, $n=20$)	26.7 (±2.4, $n=20$)	$t=1.82$, $P>0.05$
	10 μM	
42.8 (±7.2, $n=10$)	29.5 (±3.5, $n=10$)	$t=1.66$, $P>0.05$
	0.1 mM	
32.2 (±1.7, $n=12$)	22.9 (±2.3, $n=12$)	$t=3.23$, $P<0.01$
	1 mM	
31.9 (±3.5, $n=20$)	21.8 (±2.2, $n=20$)	$t=2.43$, $P<0.05$

be measured, but in the presence of 10 μM atropine concentrations of acetylcholine of 3 mM and 10 mM failed to stimulate acid secretion. The mean dose-response curves obtained under control conditions and in the presence of 2 μM atropine are shown in Figure 1, and details of this result together with those obtained using atropine at concentrations of 1 μM and 3 μM are recorded in Table 2. The dose-ratios obtained with 1 μM and 2 μM atropine were significantly different, but 3 μM atropine did not give a dose-ratio which was significantly greater than that obtained with 2 μM atropine. Although these results (Table 2) show that atropine at high concentrations is an effective inhibitor of acetylcholine-stimulated acid secretion in the isolated stomach, the plot of $\log_{10}$ (DR−1) against $\log_{10}$ [atropine] (Arunlakshana & Schild, 1959) using these data predictably showed a significant deviation from linearity and hence did not provide evidence of competition between acetylcholine and atropine.

Histamine

The histamine dose-response curve is linear over the range 10 μM to 0.1 mM histamine (Bunce & Parsons, 1976), and concentrations of histamine of 30 μM and 0.1 mM were therefore used for the construction of 2-point dose-response curves. The effect of atropine was investigated by the same experimental procedure as described for acetylcholine. Atropine was used at a concentration of 1 mM and the mean result from 8 experiments is shown in Figure 2. High concentrations of atropine (1 mM) failed to inhibit histamine-stimulated acid secretion.

Gastrin

The gastrin dose-response curve is linear over the range 30 nM to 0.3 μM (Bunce *et al.*, 1976), and concentrations of gastrin of 0.1 μM and 0.3 μM were used for the construction of the control 2-point dose-response curves. It has been shown previously (Bunce *et al.*, 1976) that, in the presence of metiamide, gastrin at 0.5 μM gave a smaller acid secretory response than 0.3 μM gastrin, and for this reason in the present experiments the concentration of gastrin was increased to 0.5 μM in the presence of atropine to

Table 2 The effect of atropine on acetylcholine-stimulated acid secretion in isolated whole stomach of the rat

Atropine (μM)	*Dose-ratio (95% confidence limits)*
1	2.7 (1.7–4.2)
2	7.9 (5.2–11.6)
3	8.3 (5.6–12.2)

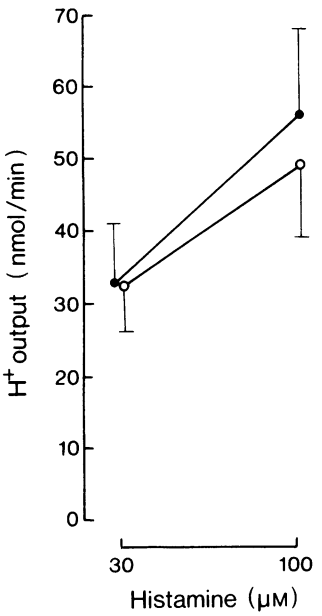

Figure 2 The effect of atropine (1 mM) on the acid secretory response to histamine. Control (●), atropine (○). Each point is the mean of 8 observations. Vertical lines show s.e. mean. An analysis of variance on these data provided the following information. The dose-response curve to histamine in the presence of atropine is not significantly displaced from the control curve; the slopes of the two lines are not significantly different.

determine whether a similar result was obtained. The experimental protocol was as described for acetylcholine.

Concentrations of atropine of 1 μM and 10 μM did not inhibit gastrin-stimulated acid secretion, although a significant inhibition of acid output was observed with atropine at concentrations of 0.1 mM and 1 mM (Figure 3). The results recorded in Figure 3 also show that in the presence of atropine (0.1 mM and 1 mM) the mean acid secretory response to 0.5 μM gastrin is greater than the response to 0.3 μM gastrin (cf. the comparable data obtained in the presence of metiamide by Bunce *et al.* (1976)).

Discussion

The present investigation shows that acetylcholine-stimulated acid secretion is inhibited by concentrations of atropine of 1 to 3 μM. The latter concentrations are relatively high when compared with the concentration of atropine used to block muscarinic receptors ($pA_2 = 8.8$, equivalent to a concentration of 1.6 nM, Arunlakshana & Schild, 1959). Similar results have been obtained by other workers using concentrations of atropine ranging from 0.1 μM to 30 μM to inhibit acid secretion stimulated by cholinomimetic compounds in isolated gastric mucosa preparations of amphibians (Thorpe & Durbin, 1969, 1972; Nakajima *et al.*, 1970; Shoemaker, Makhlouf & Sachs, 1970; Goto & Watanabe, 1975). Arunlakshana & Schild (1959) have reported that a pA_{10} value for atropine of 4.2 (equivalent to a pA_2 value of 5.15) was obtained on the frog rectus which was considered to possess nicotinic receptors. Therefore although bethanechol, a predominantly muscarinic agonist (Koelle, 1975), does stimulate acid secretion in the rat isolated stomach (Bunce & Parsons, unpublished observations) the possibility remains that nicotinic receptors are partly involved in the acid secretory response to acetylcholine. However, dimethylphenylpiperazine (DMPP), an agonist at nicotinic receptors (Chen, Portman & Wickel, 1951), does not stimulate acid secretion in anaesthetized rats (Doss & van Zwieten, 1972) or conscious dogs (Odori & Magee, 1969).

Atropine has previously been shown to be a competitive antagonist at muscarinic receptors (Arunlakshana & Schild, 1959) and evidence was therefore sought for competition against acetylcholine in this preparation. Although atropine produced a parallel shift to the right of the acetylcholine dose-response curve (Figure 1) additional studies provided no evidence to support the supposition that atropine was inhibiting acetylcholine-stimulated acid secretion in a competitive manner. Firstly, the inhibition of acid secretion by 10 μM atropine was not surmounted by concentrations of acetylcholine up to 10 mM. However, concentrations of acetylcholine greater than 1 mM also failed to stimulate acid secretion under control conditions (Bunce *et al.*, 1976), and it is therefore possible that these large concentrations of acetylcholine inhibit acid secretion by a mechanism unrelated to their interaction with cholinergic receptors. Secondly, the plot of $\log_{10}(DR-1)$ against $\log_{10}$ [antagonist] according to the equation for competitive inhibition was not linear. Comparable studies on mammalian isolated stomach or gastric mucosa preparations have not been reported, but it has been suggested by Thorpe & Durbin (1972) that atropine is a competitive inhibitor of acetylcholine in the amphibian isolated gastric mucosa preparation.

The inhibition of gastrin-stimulated acid secretion in the present study only occurred at high concentrations of atropine (0.1 mM and 1 mM) and the result must be interpreted with caution. This inhibition cannot be ascribed to a non-specific effect on the acid secretory mechanism since histamine-stimulated acid secretion was not inhibited by 1 mM atropine, although a non-specific effect at the gastrin receptor cannot be excluded. In the guinea-pig ileum, contraction of the smooth muscle in response to gastrin, which is thought

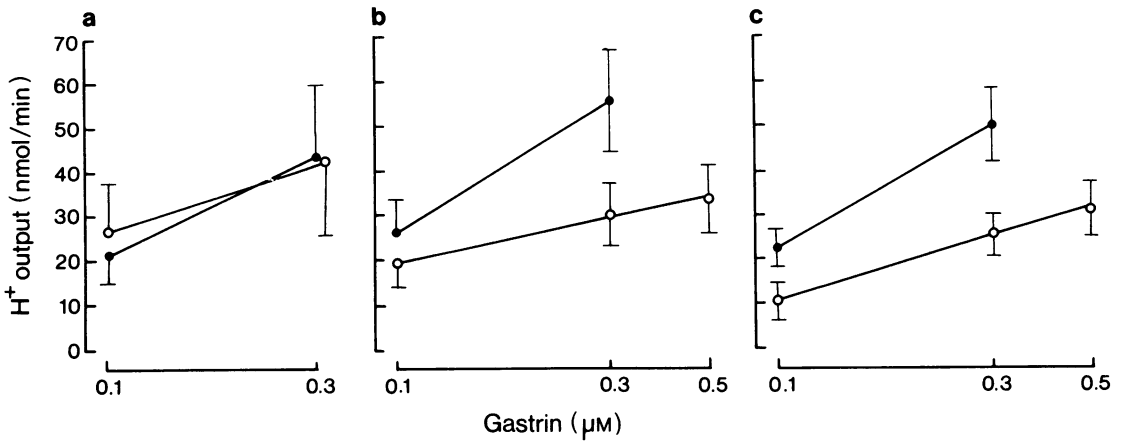

Figure 3 Dose-response curves to gastrin in the presence and absence of atropine. Vertical lines show s.e. mean. Analyses of variance on these data provided the following information. (a) Control ($n=5$); the second curve (○) is not significantly displaced from the first curve (●); the slopes of the two lines are not significantly different; (b) 0.1 mM atropine ($n=6$); (c) 1 mM atropine ($n=10$). In each case the slope of the line in the presence of atropine (○) is not significantly different from that obtained with gastrin alone (●). The dose-response curves to gastrin in the presence of atropine (0.1 mM and 1 mM) are significantly displaced from their respective control curves.

to be mediated via the release of acetylcholine (Vizi *et al.*, 1972, 1973), is inhibited by a dose of hyoscine (15 nM) which also inhibits contractions produced by acetylcholine (Bennett, 1965). Since gastrin-stimulated acid secretion in the isolated stomach of the rat is inhibited by concentrations of atropine (0.1 mM and 1 mM) which are approximately 100-fold greater than those required (1–3 μM) to inhibit acetylcholine-stimulated secretion, it appears unlikely that gastrin is stimulating acid secretion partly through a release of acetylcholine. For comparison, the present work is in agreement with some studies on the amphibian isolated gastric mucosa in which high concentrations of atropine at 2 mM (Thorpe & Durbin, 1972) and 10 mM (Nakajima *et al.*, 1970) were required to inhibit pentagastrin-stimulated acid secretion, although Goto & Watanabe (1975) have reported that tetragastrin-stimulated acid secretion is inhibited by 30 μM atropine in the isolated gastric mucosa of the frog.

Histamine-stimulated acid secretion was not inhibited by high concentrations of atropine (1 mM) in this investigation. This observation indicates that in this preparation a cholinergic mechanism is not involved directly in the acid secretory response to histamine, and the result is also incompatible with the hypothesis of receptor interaction proposed by Grossman & Konturek (1974). Goto & Watanabe (1975) have also reported that atropine failed to inhibit histamine-stimulated acid secretion in amphibian isolated gastric mucosa, although these workers used atropine at a concentration of only 30 μM. In contrast, Thorpe & Durbin (1972) found that 1 mM atropine did inhibit acid secretion in response to histamine in the frog isolated mucosa.

In the present study, doses of atropine which inhibited acetylcholine-stimulated acid secretion (i.e. 1–3 μM) did not affect acid secretion stimulated by either gastrin or histamine, and this result might indicate that the background cholinergic tone in the isolated perfused stomach of the rat, if it exists at all, is very low. This latter assumption conflicts with the observations of Johansson *et al.* (1971) who found that a dose of atropine (100 μg/kg i.v.) which inhibited methacholine-stimulated acid secretion also inhibited gastrin-stimulated acid secretion in the Heidenhain pouch rat. However, Albinus & Sewing (1969) have reported that in the anaesthetized lumen-perfused rat a dose of atropine (0.5 mg/kg i.v.) which inhibits bethanechol-stimulated acid secretion does not affect acid secretion stimulated by either gastrin or histamine. The proposition that the isolated stomach of the rat possesses a low background cholinergic tone would be consistent with the view that the latter mechanism is not responsible for the basal level of acid secretion and with the evidence that the basal acid secretion is not inhibited by atropine. Indeed, inhibition of the spontaneous acid output was only observed in the presence of a high concentration of atropine (0.1 mM–1 mM, Table 1).

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