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THE EFFECT OF METIAMIDE ON ACID SECRETION STIMULATED BY GASTRIN, ACETYLCHOLINE AND DIBUTYRYL CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE IN THE ISOLATED WHOLE STOMACH OF THE RAT

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1 An isolated stomach preparation from immature rats is described. The lumen of the stomach was perfused and the hydrogen ion activity of the perfusate recorded continuously.

2 The preparation gave dose-dependent responses to gastrin, acetylcholine and dibutyryl cyclic adenosine 3′,5′-monophosphate and these responses were readily reversed on washing out the agonist.

3 The acid secretory response to gastrin was inhibited by metiamide at concentrations of 10^{-5} M and 3×10^{-5}M.

4 The acid secretory responses to acetylcholine and dibutyryl cyclic adenosine 3′,5′-monophosphate were not inhibited by concentrations of metiamide up to 10^{-3} M.

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

Introduction

The interrelationship of the three physiological gastric secretagogues, histamine, gastrin and acetylcholine in the stimulation of gastric secretion remains unresolved. Several lines of evidence implicate histamine as a mediator of gastrin-stimulated acid secretion. In the rat, the activity of gastric mucosal histidine decarboxylase (EC. 4.1.1.22) has been shown to be closely correlated with the serum gastrin level (Håkanson, Kroesen, Liedberg, Oscarson, Rehfeld & Stadil, 1974), and administration of gastrin diminishes the gastric mucosal histamine content (Haverback, Tecimer, Dyce, Cohen, Stubrin & Santa Ana, 1964; Kahlson, Rosengren, Svahn & Thunberg, 1964). Furthermore the histamine H_2-antagonists, metiamide and cimetidine, are potent inhibitors of pentagastrin-stimulated gastric acid secretion in several species (Wyllie & Hesselbo, 1973; Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975; Burland, Duncan, Haggie, Hesselbo, Mills, Sharpe & Wyllie, 1975). The role of endogenous histamine in cholinergically-stimulated gastric acid secretion is less clear. Although the H_2-antagonists do inhibit cholinergically-stimulated secretion (Brimblecombe *et al.* 1975; Parsons, 1975), higher dose levels of the antagonists are required than for the inhibition of histamine- and pentagastrin-stimulated secretion and the interpretation of these studies is complicated by the release of endogenous gastrin. Also, experiments in the rat have led to disagreement about the direct cholinergic effect of vagal excitation on the mobilization of gastric mucosal histamine (Rosengren & Svensson, 1969; Håkanson & Liedberg, 1972). In recent years the role of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in the control of gastric function has stimulated considerable interest, and the subject has been extensively reviewed (Scratcherd & Case, 1973; Kimberg, 1974).

In the present study the isolated perfused stomach of the rat has been used for a quantitative investigation of the inhibitory effect of metiamide against gastrin-, acetylcholine- and dibutyryl cyclic adenosine 3′,5′-monophosphate- (db cyclic AMP) stimulated acid secretion in the absence of neural and indirect hormonal influences.

Methods

Fed immature rats of either sex weighing between 35–45 g were anaesthetized with pentobarbitone (3 mg i.p.). The abdomen was opened and the oesophagus ligated close to the stomach. An incision was made in the rumen of the stomach and the contents washed out with warm Krebs-Henseleit

solution. A second incision was then 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 immediately in a 10 ml organ bath containing Krebs-Henseleit solution at 37°C. The lumen of the the stomach was perfused by use of an H.R. Flow-Inducer (Watson-Marlow Ltd.), at a rate of 1 ml/min with a modified Krebs-Henseleit solution at 37°C. Both salt solutions were gassed with 95% O_2 and 5% CO_2. The effluent perfusate from the stomach was passed over a micro dual electrode (Russell pH Ltd.) clamped 20 cm higher than the stomach. Changes in pH were converted to a function of hydrogen ion activity by an antilog function generator (Research Institute Workshop, SK&F Laboratories Ltd.) and continuously recorded on a potentiometric pen recorder (Servoscribe). The rate of acid secretion was then expressed as $[H^+]$ mol $\times 10^{-8}$ per minute. The Krebs-Henseleit solution in the organ bath contained (mM) 119.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 1.03 KH_2PO_4 and 5.6 glucose. The modified lumen perfusate had a similar composition but with the $NaHCO_3$ and KH_2PO_4 omitted. All drugs were added in a volume not exceeding 0.5 ml, to the Krebs-Henseleit solution bathing the serosal surface of the stomach. After setting up the stomach preparation the basal H^+ output was allowed to stabilize, under control conditions and in the presence of metiamide, before the secretary 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 pentagastrin, acetylcholine and metiamide were made each day in Krebs-Henseleit solution. A stock solution of db cyclic AMP at 10^{-2} M was prepared in Krebs-Henseleit solution and a stock solution of gastrin at 200 μg/ml was prepared in 0.05M NH_4HCO_3. Both stock solutions were stored at 4°C and diluted with Krebs-Henseleit solution as required.

Drugs

The following drugs were used: gastrin (synthetic human gastrin I) and pentagastrin (Peptavlon, ICI Ltd.), acetylcholine chloride (BDH Ltd.), dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP, Sigma Chemical Co. Ltd.), pentobarbitone (Nembutal, Abbott Laboratories Ltd.), metiamide (SK&F 92058) was synthesized in our own laboratories.

Analysis of results

Results are expressed as mean ± s.e. mean. An analysis of variance was used to test the effect of metiamide on acid secretion stimulated by each agonist. A P-value of less than 0.05 was considered to be significant.

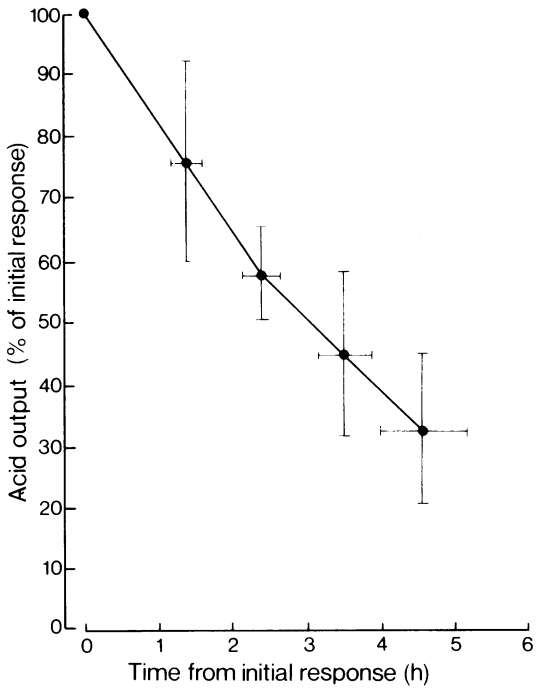

Figure 1 Tachyphylaxis of the acid secretory response to repeated doses of pentagastrin at 10^{-7} M. The first response of each stomach was expressed as 100%, and the subsequent responses were recorded as a percentage of this first response. The figure records the mean times at which the doses of pentagastrin were administered. Each point is the mean of 4 to 5 observations. The vertical and horizontal bars show s.e. mean.

Results

For gastrin and acetylcholine, sequential dose-response curves have been constructed in order to determine the dose-range over which a linear dose-response relationship was obtained. Two suitable concentrations of each agonist were then chosen for carrying out the 2 + 2 assays, which were used to investigate the effect of metiamide on acid secretion. A prerequisite for this study was to establish that the preparation gave reproducible responses to a particular agonist. Therefore two control 2-point dose-response curves were constructed on eight stomach preparations for each agonist. These preliminary experiments were then used to determine whether

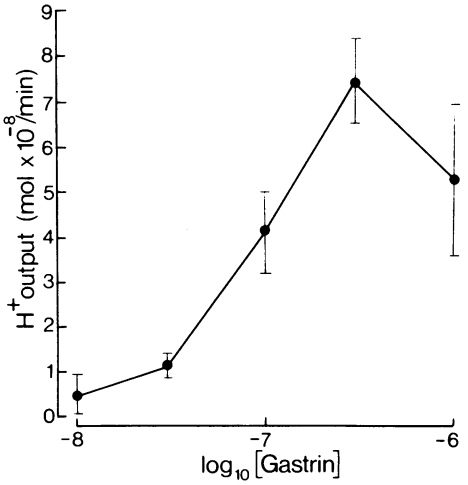

Figure 2 Sequential dose-response curve to gastrin. Each point is the mean of 4 observations. Vertical lines show s.e. mean.

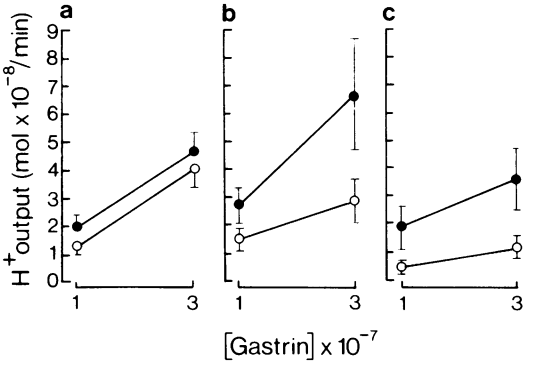

Figure 3 Dose-response curves to gastrin in the presence and absence of metiamide. Vertical lines show s.e. mean. Analyses of variance on these data provided the following information. (a) Control ($n=8$); the second curve (○) was not significantly displaced from the first curve (●); the slopes of the two lines were not significantly different; (b) 10^{-5} M metiamide ($n=9$); (c) 3×10^{-5} M metiamide ($n=7$). In each case the slope of the line in the presence of metiamide (○) was not significantly different from that obtained with gastrin alone (●). The dose-response curves to gastrin in the presence of metiamide (10^{-5} M and 3×10^{5} M) were significantly displaced from their respective control curves.

there were any time-related changes in the sensitivity of the preparation to an agonist, which might be unrelated to the presence of metiamide.

Pentagastrin

Preliminary experiments showed that the rat isolated stomach did not give reproducible responses to repeated doses of pentagastrin. The acid secretory responses to repeated doses of pentagastrin at 10^{-7} M are shown in Figure 1. Over a period of 5 h, during which five doses of pentagastrin were administered, the acid secretory response diminished by approximately 70%. The tachyphylaxis to pentagastrin made this secretagogue unsuitable for the construction of dose-response curves, and therefore gastrin was next investigated.

Gastrin

Sequential dose-response curve. The sequential dose-response curve for gastrin is shown in Figure 2. The threshold concentration was 10^{-8} M and the plot was linear over the range 3×10^{-8} M to 3×10^{-7} M. A concentration of gastrin of 10^{-6} M gave a submaximal response. Doses of 10^{-7} M and 3×10^{-7} M were therefore used for the construction of 2-point dose-response curves, the doses being administered in a randomized order.

Reproducibility of response. Although the acid secretory response of the isolated stomach also showed some tachyphylaxis to gastrin, particularly between the first two responses to the hormone, this was less marked than with pentagastrin. For this reason the first dose of gastrin was considered to be a 'priming' dose, and if a dose of 10^{-7} M gastrin was given for this purpose the subsequent four responses were then sufficiently reproducible for the construction of the 2-point dose-response curves.

The effect of metiamide. A study of the effect of H_2-receptor blockade on the response to gastrin was made with two concentrations of metiamide (10^{-5} M and 3×10^{-5} M). For this purpose two 2-point gastrin dose-response curves were constructed on each stomach preparation. A control dose-response curve was established and each stomach was then equilibrated in Krebs-Henseleit solution containing the appropriate concentration of metiamide for approximately 1 h, and then the second curve constructed. The mean dose-response curves obtained in these experiments are shown in Figure 3. The control data (Figure 3a) show that there were no significant time-related changes in the sensitivity of the preparation to gastrin, and this result indicates that the omission of the first 'priming' gastrin dose from the assay was expedient. The results presented in Figure 3 show that metiamide is an effective inhibitor of gastrin-stimulated acid secretion in this preparation, although the pattern of inhibition differs from that obtained when histamine was used as the agonist (Bunce &

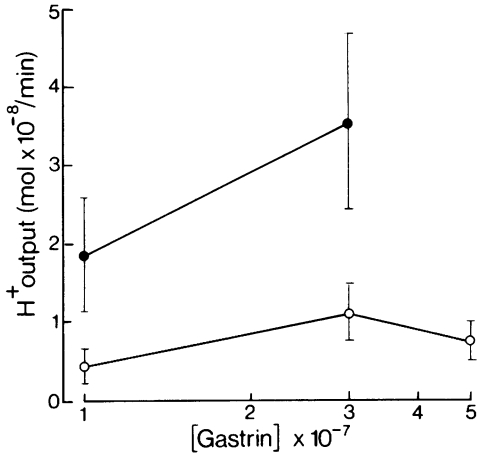

Figure 4 The acid secretory response to 5×10^{-7} M gastrin in the presence of metiamide (3×10^{-5} M). Control (●), metiamide (○). Each point is the mean of 7 observations. Vertical lines show s.e. mean.

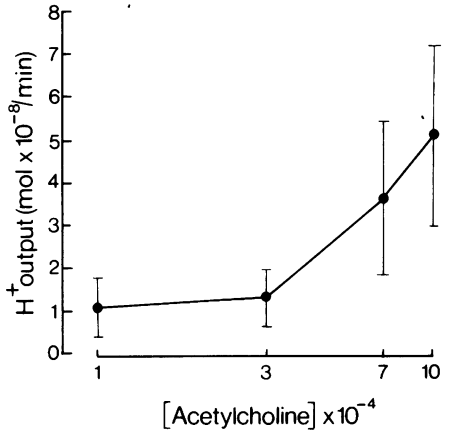

Figure 5 Sequential dose-response curve to acetylcholine. Each point is the mean of 4 observations. Vertical lines show s.e. mean.

Parsons, 1976). The latter authors showed that a parallel displacement of the histamine dose-response curve to the right was obtained which enabled dose-ratios to be calculated; in the present study the concentration of gastrin was increased to 5×10^{-7}M in the presence of metiamide in an effort to provide comparable data. However, as can be seen in Figure 4, the response to this latter concentration of gastrin was less than that obtained to a concentration of 3×10^{-7}M, and the resultant depression of the maximum response compared to the control suggests a non-competitive type of antagonism. In the control dose-response curves in the present experiments, concentrations of gastrin greater than 3×10^{-7}M were not used to determine whether a similar reduction in response was obtained. However, extrapolation from the sequential dose-response curve (Figure 2) does indicate that under control conditions also, a concentration of gastrin above 3×10^{-7}M gives a submaximal response. Calculation of dose-ratios for comparison of the present results with those obtained with histamine was therefore impossible.

Acetylcholine

Sequential dose-response curve. The sequential dose-response curve for acetylcholine is shown in Figure 5. The threshold concentration of acetylcholine was 10^{-4} M, and the plot was linear over the range 3×10^{-4} M to 10^{-3} M. A concentration of acetylcholine of 3×10^{-3} M not only failed to stimulate acid secretion, but also diminished the basal rate of acid output. Doses of 3×10^{-4} M and 7×10^{-4} M were therefore used for the construction of 2-point dose-response curves, and these doses were administered in a randomized order.

Reproducibility of response. As with gastrin, the first dose of acetylcholine produced a secretory response which was greater than the subsequent responses to similar doses of this compound. Therefore a 'priming' dose of 3×10^{-4} M acetylcholine was routinely used in these experiments. However, unlike gastrin, the responses subsequent to that produced by the 'priming' acetylcholine dose showed a slight tendency to increase during the course of an experiment.

The effect of metiamide. Two 2-point dose-response curves to acetylcholine were constructed on each stomach preparation. The experimental procedure was as described for gastrin. Metiamide was used at concentrations of 3×10^{-5} M, 10^{-4} M and 10^{-3} M. The mean dose-response curves obtained in these experiments are recorded in Figure 6. The control curves obtained in the absence of metiamide (Figure 6a) show that there was a small but insignificant shift of the dose-response curve to the left. Similar changes were also seen in the presence of metiamide at concentrations of 3×10^{-5} M and 10^{-4} M (Figure 6b and c), and a further increase of the metiamide concentration to 10^{-3} M produced comparable results. At each concentration investigated, metiamide failed to produce a shift of the dose-response curve to the right, and this result indicates that metiamide is not an inhibitor of

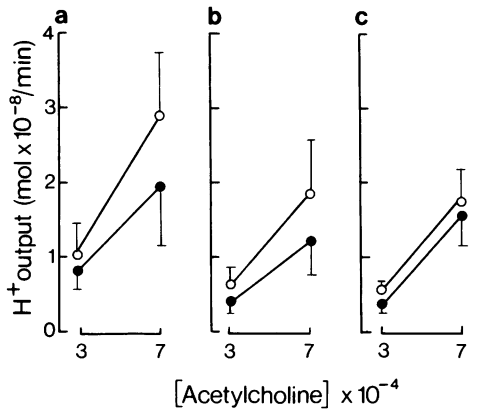

Figure 6 Dose-response curves to acetylcholine in the presence and absence of metiamide. Vertical lines show s.e. mean. Analyses of variance on these data provided the following information. (a) Control ($n=8$); the second curve (O) was not significantly displaced from the first curve (●); the slopes of the two lines were not significantly different; (b) 3×10^{-5} M metiamide ($n=7$); (c) 10^{-4} M metiamide ($n=8$). In each case the slope of the line in the presence of metiamide (O) was not significantly different from that obtained with acetylcholine alone (●). The dose-response curves to acetylcholine in the presence of metiamide (3×10^{-5} M and 10^{-4} M) were not significantly displaced from the respective control curves.

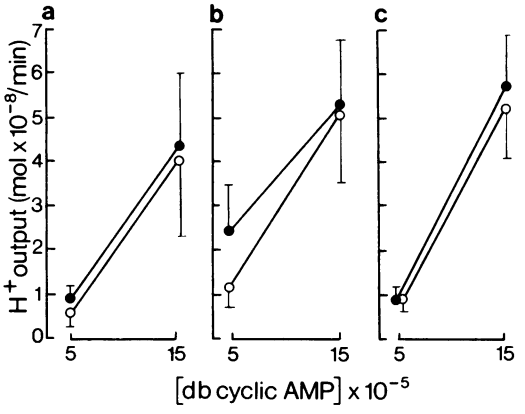

Figure 7 Dose-response curves to dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP) in the presence and absence of metiamide. Vertical lines show s.e. mean. Analyses of variance on these data provided the following information. (a) Control ($n=8$); the second curve (O) was not significantly displaced from the first curve (●); the slopes of the two lines were not significantly different; (b) 10^{-4} M metiamide ($n=9$); (c) 10^{-3} M metiamide ($n=7$). In each case the slope of the line in the presence of metiamide (O) was not significantly different from that obtained with db cyclic AMP alone (●). The dose-response curves to db cyclic AMP in the presence of metiamide (10^{-4} M and 10^{-3}M) were not significantly displaced from the respective control curves.

acetylcholine-stimulated secretion in the isolated stomach preparation.

Dibutyryl cyclic adenosine 3',5'-monophosphate

Preliminary experiments with db cyclic AMP showed that the threshold concentration was 5×10^{-6} M, and that concentrations of 5×10^{-5} M and 1.5×10^{-4} M gave dose-related responses which could be used to investigate the effect of metiamide.

The effect of metiamide. The experimental procedure was as described for gastrin and acetylcholine. Metiamide was used at concentrations of 10^{-4} M and 10^{-3} M, and the mean dose-response curves obtained in these experiments are shown in Figure 7. Control dose-response curves in the absence of metiamide (Figure 7a) showed that there were no significant time-related changes in the sensitivity of the preparation to db cyclic AMP. Metiamide, at the high concentrations used, did not inhibit db cyclic AMP-stimulated acid secretion in this preparation.

Discussion

The present experiments on the rat isolated stomach show that the preparation is a useful tool for the elucidation of the control of gastric function. One problem associated with the preparation which was immediately apparent is the tachyphylaxis observed to repeated doses of pentagastrin. This phenomenon of tachyphylaxis to gastric secretagogues has also been recorded by other workers both *in vivo* and *in vitro*. Kasbekar (1972) found that the amphibian isolated gastric mucosa exhibited tachyphylaxis to repeated doses of pentagastrin. In the anaesthetized cat repeated injections of a gastrin extract also produced tachyphylaxis (Uvnäs, 1945; Emas, 1960; Blair, Harber, Lake & Reed, 1963), although in the anaesthetized lumen-perfused rat a progressive increase in the sensitivity of the preparation to gastrin has been demonstrated (Lai, 1964; Parsons, 1969). This latter observation is in direct contrast with the results obtained on the isolated stomach of the rat. In the present work, the tachyphylaxis to pentagastrin was more severe than that to gastrin; an observation which cannot be explained at the present time.

Some workers have reported the failure of pentagastrin to stimulate acid secretion from mammalian isolated gastric mucosa preparations, e.g. piglet gastric mucosa (Forte, Forte & Machen, 1975) and guinea-pig gastric mucosa (Spencer, 1974). However, acid secretion has previously been obtained in response to pentagastrin from *in vitro* rat gastric mucosa and stomach preparations. Brennan, Arbakov, Stefankiewicz & Groves (1975) obtained a maximum secretory rate of approximately 33 μEq/h above basal in response to 3×10^{-5} M pentagastrin and Wan, Assem & Schild (1974) recorded a maximum rate of approximately 12 μEq cm^{-2} $30\ min^{-1}$ above basal to 1.3×10^{-6} M pentagastrin. In this investigation the isolated stomach of the rat gave a maximum acid secretory response of 7.5×10^{-8} mol min^{-1} (equivalent to 4.5 μEq/h) in response to 3×10^{-7} M gastrin (Figure 2), and for comparison the surface area of the acid secreting mucosa in the immature rat was approximately 2 cm^2. However, the preparations of Wan *et al.* (1974) and Brennan *et al.* (1975) also gave higher acid secretory responses to histamine than the preparation described by Bunce & Parsons (1976). Possible explanations for these differences are that in this study immature animals were used, whereas both Wan *et al.* (1974) and Brennan *et al.* (1975) used mature rats. The techniques employed also differed considerably. The latter groups of workers both used static systems, whereas a perfusion technique was used in the present work. Also, of course, it may not be valid to compare the effects of pentagastrin and gastrin.

The reduction of the maximal response to gastrin in the presence of metiamide (Figure 4) is compatible with a non-competitive type of inhibition, and is similar to results obtained *in vivo* using pentagastrin in the Heidenhain pouch rat (Lundell, 1975), and by Black (1973) using the Heidenhain pouch dog. However metiamide has been shown to act like a competitive antagonist at H_2-receptors (Parsons, 1973; Bunce & Parsons, 1976), and this situation led Black (1973) to postulate that canine gastric mucosa possesses two types of gastrin receptor. One type of receptor, whose effects are mediated by H_2-receptors, is excitatory, and the other, which is not affected by metiamide, is inhibitory. Under these circumstances it is possible that metiamide, a competitive H_2-receptor antagonist, would depress the maximum secretory response to gastrin as seen in the present study.

There are few reports on the inhibition of gastrin- or pentagastrin-stimulated acid secretion by an H_2-antagonist *in vitro*. Wan *et al.* (1974) reported that metiamide inhibited the acid secretory response to pentagastrin in the isolated stomach of the rat and Schofield, Tepperman & Tepperman (1975) found that metiamide (5×10^{-3} M) inhibited pentagastrin-stimulated acid secretion in the *in vitro* kitten fundic mucosa. Both of these groups of workers investigated the effect of metiamide on one dose level only of pentagastrin, and the test and control experiments were carried out on separate mucosa preparations. In this investigation two dose levels of gastrin were used, and both test and control dose-response curves were constructed on each stomach. However, the results obtained in the present work do confirm the observations of Wan *et al.* (1974) and Schofield *et al.* (1975), and provide additional evidence to that reported *in vivo* (Parsons, 1975) that the acid secretory response to gastrin is probably mediated at least in part through a histaminergic pathway.

There is little information available about acid secretion stimulated by db cyclic AMP and cholinomimetic compounds in mammalian isolated stomach and gastric mucosa preparations. In the present experiments both acetylcholine (10^{-3} M) and db cyclic AMP (1.5×10^{-4} M) stimulated an acid secretory response of approximately 5.0 mol $\times 10^{-8}$/min (equivalent to 3.0 μEq/h). A similar rate of acid secretion (2.63 μEq $cm^{-2}\ h^{-1}$) was obtained by Schofield *et al.* (1975) from the isolated kitten fundic mucosa in response to acetylcholine (10^{-4} M). Higher rates of acid secretion were obtained by Brennan *et al.* (1975) on the isolated rat stomach in response to both bethanechol (2.2×10^{-4} M) and db cyclic AMP (10^{-4} M), and also by Fromm, Schwartz & Quijano (1975) on the rabbit isolated gastric mucosa in response to db cyclic AMP (1.2×10^{-3} M). However, it is of interest that both the latter groups of workers used stomachs from mature animals, whereas Schofield *et al.* (1975) used stomachs from immature animals as in the present investigation.

In vivo, cholinergic excitation causes acid secretion by a direct action on the fundic mucosa, and also via the release of gastrin from the pyloric antrum (Csendes, Walsh & Grossman, 1972). *In vitro,* there is no information about the release of gastrin from the rat stomach although it is unlikely that sufficient amounts of gastrin would be released into the serosal solution for biological action, and on this basis the isolated stomach of the rat enables a study of the direct secretagogue effect of acetylcholine. The failure of metiamide to inhibit acetylcholine-stimulated acid secretion *in vitro* (Figure 6) has also been shown by Schofield *et al.* (1975) and agrees well with previous observations on guinea-pig ileum where metiamide has no specific interaction with muscarinic receptors (Black & Spencer, 1973), although the result is in contrast with the view held by Kowalewski & Kolodej (1974) that metiamide does exhibit some cholinoceptor blocking activity. Also, the ineffectiveness of metiamide against acetylcholine-stimulated acid secretion indicates that acetylcholine *per se* does not mobilize gastric mucosal histamine, but stimulates acid secretion through a direct cholinergic pathway.

Interaction of histamine with H_2-receptors

influences the level of cyclic AMP in rat gastric mucosa. Exogenous histamine increases the activity of gastric mucosal adenylate cyclase and elevates the level of cyclic AMP in rat gastric mucosa, and these changes can be prevented by H_2-antagonists (McNeill & Verma, 1974; Ruoff & Sewing, 1975). The latter experiments show that cyclic AMP operates at a position 'distal' to histamine in the chain of events which lead to the secretion of acid. This conclusion is supported by the results obtained in the present study in which metiamide failed to inhibit db cyclic AMP-stimulated acid secretion, and similar results have been obtained by other workers using isolated mammalian stomach and gastric mucosa preparations (Fromm *et al.* 1975; Hearn & Main, 1975; Wan, 1976).

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