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Inhibition of acetylcholine-stimulated secretion in the isolated whole stomach of the rat

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The rat isolated whole stomach preparation has been used to investigate acetylcholine-stimulated pepsin secretion. Acetylcholine stimulated pepsin output in a dose dependent manner over the range 3×10^{-4} to 3×10^{-3} M. Metiamide (10^{-3} M) had no significant effect on the pepsin response to acetylcholine suggesting that histamine H_2-receptors are not involved in this response. Atropine (2×10^{-8}, 2×10^{-7} and 2×10^{-6} M) produced a dose-related inhibition of the pepsin output to 7×10^{-4} M acetylcholine, indicating that this response is mediated by muscarinic receptors. Pirenzepine also produced a dose-related inhibition of acetylcholine (7×10^{-4} M) stimulated pepsin output at 2×10^{-6}, 2×10^{-5} and 2×10^{-4} M. Increasing the concentration of acetylcholine to 3×10^{-3} M completely reversed the effect of atropine (2×10^{-6} M) but pirenzepine (2×10^{-4} M) still produced a significant inhibition of acetylcholine-stimulated pepsin secretion. In each series of experiments acid and pepsin secretion responded in a similar manner to the effects of inhibitors, providing no evidence for a separation of these responses.

It is well established that cholinomimetic drugs can stimulate pepsin secretion in-vivo in the rat, dog and man (Konturek et al 1974; Vatn et al 1975; Bunce et al 1979; Parsons et al 1979). However, there have been few studies on the cholinergic control of pepsin secretion in-vitro (Villareal 1953; Kapadia & Donaldson 1978). Recently the rat isolated whole stomach preparation has been shown to be suitable for the study of pepsin secretion (Bunce et al 1981), and therefore the receptors involved in the control of pepsin secretion can be investigated in the absence of the vascular, hormonal and neural influences that occur in the whole animal. The aim of the present study was to investigate the effects of three secretory inhibitors on acetylcholine-stimulated pepsin secretion in the rat isolated stomach and to compare their effects on pepsin and acid secretion. This paper describes the effect of atropine, pirenzepine, a tricyclic compound which has been shown to have anticholinergic properties (Hammer et al 1980; Parsons et al 1979), and the histamine H_2-antagonist metiamide on acetylcholine-stimulated secretion.

MATERIALS AND METHODS

The isolated stomach was set up as described by Bunce & Parsons (1976). 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 polyethylene cannulae were inserted and tied into the stomach via these incisions. The stomach was rapidly dissected out and placed in a 10 ml organ bath containing Krebs-Henseleit solution at 37 °C.

The lumen of the stomach was perfused at a rate of 1 ml min^{-1} with a modified Krebs-Henseleit solution from which the buffers ($NaHCO_3$ and KH_2PO_4) were omitted (Bunce & Parsons 1976). The hydrogen ion concentration of the effluent perfusate from the stomach was continuously recorded and the rate of acid secretion expressed as mol $\times 10^{-8}$ min^{-1}. After setting up the stomach the preparation was left for approximately 2 h before starting the experiment. Two experimental protocols were used:

(i) Basal secretion was collected for 30 min, the agonist was then added to the serosal solution and secretion was collected for a further 45 min.

(ii) Basal secretion was collected for 30 min after addition of an antagonist. At 30 min the agonist was added to the serosal solution and secretion collected for 45 min.

(iii) Basal secretion was collected for 75 min.

Pepsin assay

Pepsin output was measured by a modification of the method of Chiang et al (1966) as described by Bunce et al (1981). The products of peptic digestion of acidified haemoglobin substrate were indirectly quantified by spectrophotometric measurement of the amounts of tyrosine-like substances released. A calibration curve was constructed daily using porcine pepsin as a standard.

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Expression of results

Since there is a large between-preparation variability in pepsin output the results were normalized as described by Bunce et al (1981). The rate of secretion in a single stomach just before drug administration was expressed as 100% and all other secretory rates measured during the experiment were expressed as a percentage of this value. Basal secretion was expressed as pepsin units per 5 min.

Drugs

The following drugs were used in this study; acetylcholine chloride and atropine sulphate (BDH Ltd), pirenzepine dihydrochloride (Gastrozepin, Dr Karl Thomae GMBH), porcine pepsin (Sigma Ltd). Metiamide was synthesized in our own laboratories.

Analysis of results

Results are expressed as means ± s.e. of mean. Application of the Rankit test (Colquhoun 1971) to a sample of the normalized experimental data did not provide evidence of normal distribution, and therefore in the present work the difference between two samples was examined statistically by the Mann-Whitney U test as described by Siegel (1956). A two-tailed test was used. A P value of less than 0·05 was considered to be significant.

RESULTS

Basal secretion

Pepsin secretion was stable over the 75 min experimental period and fluctuated between a mean of 14·8 ± 3·0 and 17·6 ± 3·6 pepsin units per 5 min (n = 6).

Acetylcholine-stimulated secretion

Acetylcholine stimulated pepsin output in a dose-dependent manner, and the results are shown in Fig. 1. Concentrations of acetylcholine 3×10^{-4}, 7×10^{-4}, 10^{-3} and 3×10^{-3} M produced peak pepsin responses of 161·7 ± 15·2% (n = 5), 206·9 ± 29·5% (n = 7), 328·9 ± 29·1% (n = 6) and 364·6 ± 46·5% (n = 6) of the control value respectively. Acid output was dose related in the range 3×10^{-4} to 10^{-3} M with a maximum peak response of 328·7 ± 41·3% (n = 6) of control at 10^{-3} M. Acetylcholine (3×10^{-3} M) produced a submaximal response of 240·5 ± 51·0% (n = 6) of control.

Acetylcholine produced an almost parallel stimulation of pepsin and acid output at each dose. A dose of 7×10^{-4} M acetylcholine produced submaximal responses of both pepsin and acid output and was used in subsequent experiments to examine the effects of secretory inhibitors.

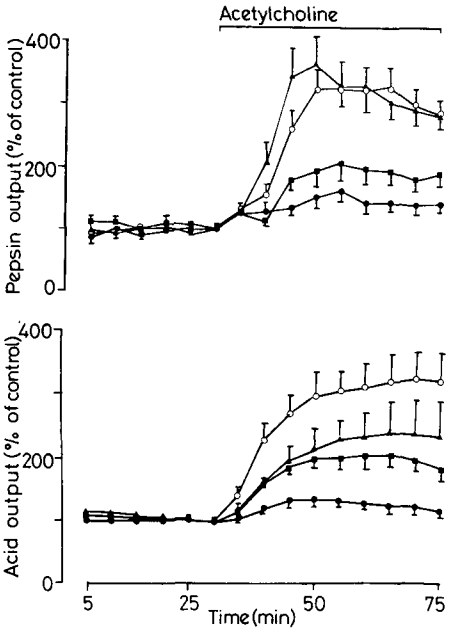

FIG. 1. The gastric secretory response in the isolated stomach of the rat to acetylcholine at concentrations of 3×10^{-4} M (●, n = 5), 7×10^{-4} M (■, n = 7), 10^{-3} M (▲, n = 6) and 3×10^{-3} M (○, n = 6). Vertical bars are s.e. mean.

The effects of metiamide on acetylcholine-stimulated secretion

Metiamide (10^{-3} M) had no significant effect on acetylcholine (7×10^{-4} M)-stimulated secretion except at 35 min during the pepsin response, and results are shown in Fig. 2. In the absence of metiamide the peak pepsin response to acetylcholine was 206·9 ± 29·5% (n = 7) of control and in the presence of metiamide the peak pepsin response was 181·0 ± 21·0% (n = 4) of control. Metiamide was also without effect on acetylcholine-stimulated acid secretion, as shown in Fig. 2.

The effect of atropine on acetylcholine-stimulated secretion

Atropine produced a dose-related inhibition of pepsin output and the results are shown in Fig. 3. The peak pepsin response to 7×10^{-4} M acetylcholine was 206·9 ± 29·5% (n = 7) of control. Atropine (2×10^{-8}, 2×10^{-7} and 2×10^{-6} M) reduced the peak pepsin response to acetylcholine to 173·5 ± 28·9% (n = 4), 130·2 ± 7·2% (n = 6) and 109·2 ± 5·9% (n = 7) of control respectively.

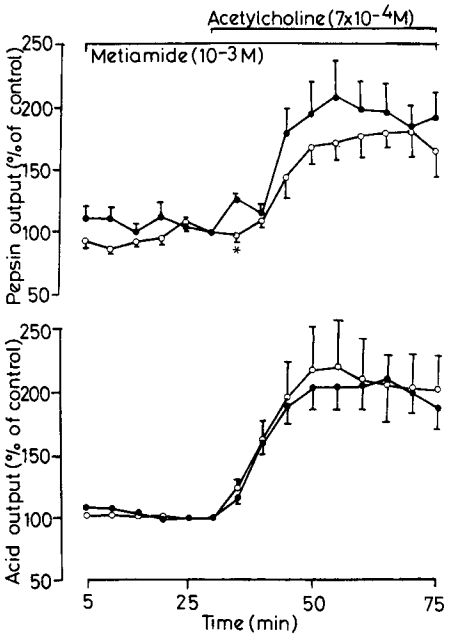

FIG. 2. The gastric secretory response in the isolated stomach to 7×10^{-4} M acetylcholine (●, n = 7), and the effect of metiamide 10^{-3} M (○, n = 4) on this response. * $P < 0.05$. Vertical bars are s.e. mean.

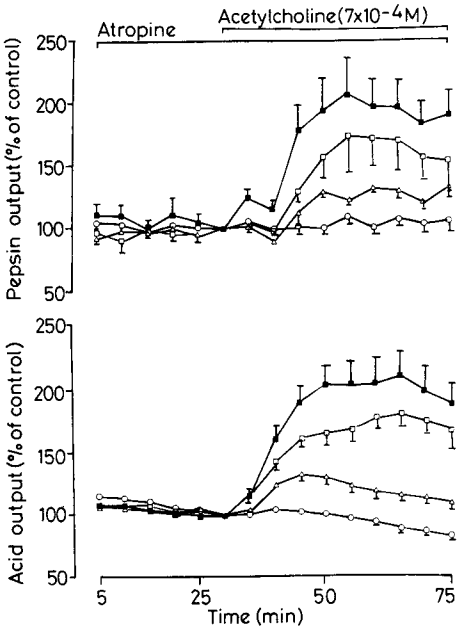

FIG. 3. The gastric secretory response in the rat isolated stomach to 7×10^{-4} M acetylcholine (■, n = 7) and the effect of atropine 2×10^{-8} M (□, n = 4) 2×10^{-7} M (△, n = 6) and 2×10^{-6} M (○, n = 7) on this response. Vertical bars are s.e. mean.

At the doses of atropine used acetylcholine-stimulated acid output was inhibited in a parallel manner, as shown in Fig. 3.

The effect of pirenzepine on acetylcholine-stimulated secretion

Pirenzepine also produced a dose-dependent inhibition of secretion although higher concentrations were required than atropine. The results are shown in Fig. 4. Pirenzepine (2×10^{-7} M) had no significant effect on pepsin or acid output stimulated by acetylcholine (7×10^{-4} M). Pirenzepine (2×10^{-6}, 2×10^{-5} and 2×10^{-4} M) reduced the peak pepsin output to 7×10^{-4} M acetylcholine from 206·9 ± 29·5% of control to 148·3 ± 15·6% (n = 5), 123·7 ± 5·0% (n = 6) and 109·8 ± 5·5% (n = 3) of the control value respectively.

Acetylcholine-stimulated acid secretion was inhibited in a similar manner to pepsin secretion by pirenzepine as shown in Fig. 4.

Fig. 5 compares the effects of atropine and pirenzepine on acetylcholine-stimulated pepsin and acid secretion. From the Figure it is evident that in the rat isolated stomach preparation atropine exerts its inhibitory effect at lower concentrations than pirenzepine against a submaximal concentration of acetylcholine (7×10^{-4} M). The Figure also shows

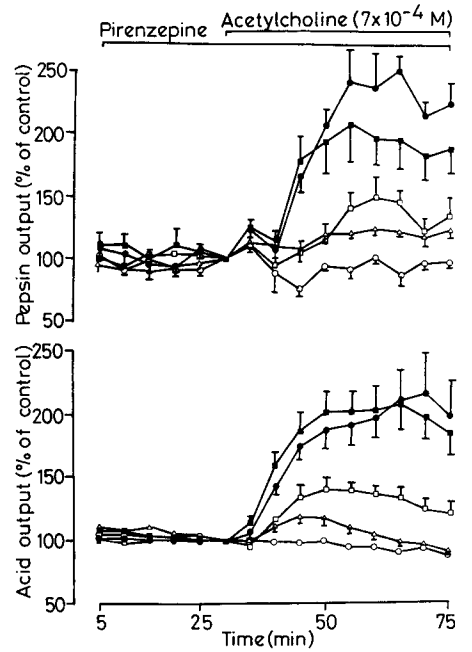

FIG. 4. The gastric secretory response in the rat isolated stomach to 7×10^{-4} M acetylcholine (■, n = 7) and the effect of pirenzepine, 2×10^{-7} M (●, n = 4), 2×10^{-6} M (□, n = 5), 2×10^{-5} M (△, n = 6) and 2×10^{-4} M (○, n = 4), on this response. Vertical bars are s.e. mean.

that inhibition of acid and pepsin responses closely parallel one another.

The effect of atropine (2×10^{-6} M) and pirenzepine (2×10^{-4} M) on a high concentration of acetylcholine (3×10^{-3} M)

Atropine (2×10^{-6} M) and pirenzepine (2×10^{-4} M) completely inhibited the responses to acetylcholine (7×10^{-4} M). The effects of increasing the concentration of acetylcholine to 3×10^{-3} M were investigated to determine whether the inhibitory actions of atropine and pirenzepine were reversible. The results are shown in Fig. 6. Atropine (2×10^{-6} M) had no significant inhibitory effect on either the pepsin or acid response to 3×10^{-3} M acetylcholine, except at 40 min during the pepsin response. The peak pepsin response in the absence of atropine was 364·4 ± 45·6% of control (n = 6) and in the presence of atropine was 326·4 ± 27·7% (n = 6) of control. The peak acid response in the absence and presence of atropine were 240·5 ± 51·0% (n = 6) and 296·8 ± 53·0% (n = 6) of the control value respectively.

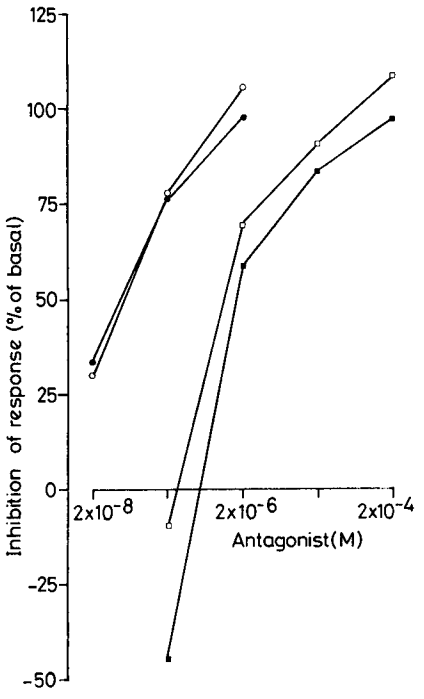

FIG. 5. Comparison of the inhibitory effects of atropine and pirenzepine on acetylcholine (7×10^{-4} M) stimulated pepsin and acid secretion. Mean of peak inhibition of acetylcholine secretory responses above basal are shown. Atropine ● pepsin, ○ acid. Pirenzepine ■ pepsin, □ acid.

In contrast pirenzepine (2×10^{-4} M) still produced a significant inhibition of both pepsin and acid responses to 3×10^{-3} M acetylcholine. The peak pepsin response was reduced from 364·6 ± 46·5% (n = 6) of control to 162·6 ± 19·0% (n = 5) of control. The peak acid response was reduced from 240·5 ± 51·0% (n = 6) of control to 133·2 ± 12·3% (n = 5) of control.

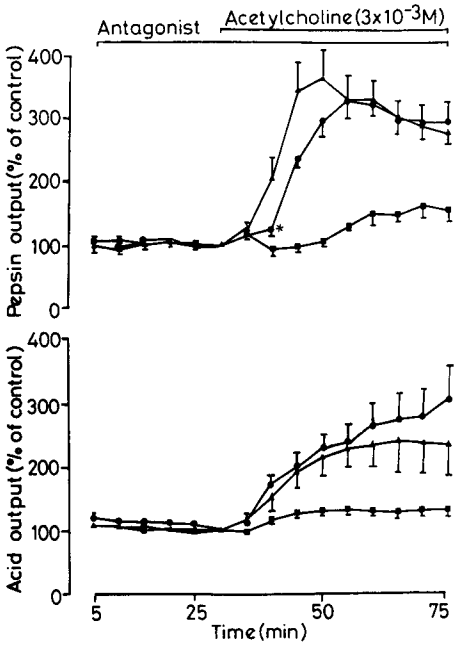

FIG. 6. The gastric secretory response in the rat isolated stomach to 3×10^{-3} M acetylcholine (▲, n = 6) and the effect of atropine 2×10^{-6} M (●, n = 6) and pirenzepine 2×10^{-4} M (■, n = 6) on this response. Vertical bars are s.e. mean.

DISCUSSION

Acetylcholine stimulated pepsin secretion in the rat isolated stomach in a dose dependent manner. This effect has also been described by Kapadia & Donaldson (1978) in rabbit isolated gastric mucosa maintained in organ culture and in an isolated tissue preparation of the mouse, using carbachol (Villareal 1953). A direct comparison of pepsin output in these studies is complicated by the use of different units to define peptic activity, however, from a comparison of dose ranges used in these studies it can be seen that the agonist concentrations required to stimulate pepsin secretion in the rabbit and mouse preparations were lower than those used in the rat isolated stomach. Kapadia & Donaldson (1978) showed that

acetylcholine produced a dose-dependent increase in pepsin secretion in the rabbit isolated mucosa in the range of $10^{-8} - 10^{-3}$ M with a maximum response at 10^{-4} M of approximately 200% of control. Carbachol produced a bell-shaped dose-response curve in the mouse stomach with a maximum response at 5×10^{-6} M (10^{-1} mg%) of approximately 300% of control (Villareal 1953). In the present study concentrations of $3 \times 10^{-4} - 3 \times 10^{-3}$ M acetylcholine were used, with a maximum pepsin response of 365% of control. Acid responses of a similar magnitude to those of pepsin were obtained in the range 3×10^{-4} to 10^{-3} M acetylcholine, and this effect is in contrast to histamine-stimulated gastric secretion where higher concentrations of histamine were required for the stimulation of pepsin secretion than acid secretion (Bunce et al 1981).

The stimulation of pepsin secretion by acetylcholine in the rat isolated stomach suggests a direct effect on the chief cells of the gastric mucosa. An alternative explanation has been suggested by the work of Johnson (1972) who provided evidence for a mechanism in the gastric mucosa sensitive to hydrogen ions which stimulates pepsin secretion through a local cholinergic reflex, and the parallelism between acid and pepsin responses at some doses of acetylcholine in the rat isolated stomach could be seen to support this hypothesis. However, Bunce et al (1981) showed that pepsin output was not stimulated if the mucosa was bathed with acid in a concentration range achieved by endogenous acid (pH 3·5–4·0) in the rat isolated stomach. Bathing the mucosa with higher acid concentrations (pH 2·0) resulted in overt damage to the tissue, and although pepsin output was increased this may have been a consequence of damage to the gastric mucosa. Support for a direct effect of cholinomimetics on pepsin secretion has been provided by Simpson et al (1980). In their study bethanechol stimulated pepsinogen secretion from the frog isolated oesophagus where there are no acid-secreting cells. In the anaesthetized rat Szelnyi & Engler (1980) showed that perfusion of the gastric lumen with acid produced a transient increase in pepsin secretion which was abolished by atropine, and that carbachol produced a further, larger increase in pepsin output than that caused by acid perfusion, an effect which was also inhibited by atropine. Therefore the possibility remains that the effects of acetylcholine seen in this study may be partly through a direct effect of chief cells and partly through a H^+ ion sensitive mechanism.

The histamine H_2-receptor antagonist metiamide has no significant effect on acetylcholine-stimulated pepsin secretion. Similarly, in this study and in the previous report of Bunce et al (1976), metiamide was without effect on acetylcholine-induced acid secretion. These results suggest that H_2-receptors are not involved in these responses in-vitro.

The contrasting effects of H_2-antagonists on cholinergically-induced acid secretion in-vitro and in-vivo are now documented, with responses in-vivo susceptible to inhibition by H_2-antagonists and responses in-vitro unaffected (Brimblecombe et al 1975; Bunce et al 1976). However, it is of interest that cholinergically-induced pepsin secretion appears to behave similarly in-vitro and in-vivo in the rat with respect to H_2-antagonism. Thus, as in the present study where metiamide was without effect on acetylcholine-stimulated pepsin secretion, in the Heidenhain pouch rat, a dose of metiamide which inhibited bethanechol-stimulated acid secretion had no effect on the corresponding output of pepsin. (Bunce et al 1979). Similar results have been reported in the Pavlov pouch rat (Lundell 1975).

In the present study atropine inhibited acetylcholine-stimulated pepsin secretion in a dose-dependent manner, a concentration of 2×10^{-6} M completely abolishing the response. This effect could be reversed by increasing the concentration of agonist. This result indicates that the pepsin response to acetylcholine is mediated by cholinergic receptors.

The concentrations of atropine used in this study were higher than would be predicted from its pA_2 value in other tissues. Recently Angus & Black (1979) and Angus et al (1980) have drawn attention to the anomalous pA_2 values obtained with atropine and metiamide for inhibition of acid secretion in the isolated whole mouse stomach, compared with other tissues such as heart or uterus for metiamide and guinea-pig ileum for atropine. They point out that there is an obvious difference between measurements of acid secretion and contraction of tissue since the flow of specific ions and molecules across the parietal cell membrane and their continuous removal by perfusion means that local concentrations of antagonists in the region of the basement membrane could not be in equilibrium. This hypothesis would presumably also apply to agonists and therefore may partly explain the high concentrations of both agonist and antagonists required in this study.

Pirenzepine also produced a dose-dependent inhibition of acetylcholine-stimulated pepsin and acid output, although higher concentrations were

required than atropine. Pirenzepine is a tricyclic antisecretory agent, and binding studies have suggested that it may be relatively selective for muscarinic receptors in the gastric mucosa (Hammer et al 1980). Our results suggest that in the rat isolated stomach pirenzepine is acting, at least in part, by an anticholinergic mechanism although it was not as active as atropine against acetylcholine-stimulated secretion. This finding is in agreement with reports in the anaesthetised rat and guinea-pig ileum where pirenzepine was shown to have approximately 1/100th the potency of atropine against carbachol stimulated-acid secretion and -contraction respectively (Parsons et al 1978).

The inhibitory effect of 2×10^{-4} M pirenzepine could only be slightly overcome by increasing the agonist concentration, in contrast to the results obtained with atropine. There are two possible explanations for this effect. Firstly, at high concentrations pirenzepine may have a toxic effect in the rat isolated stomach or alternatively, at this concentration the antagonism of acetylcholine-induced secretion by pirenzepine is of a different nature to that produced by atropine.

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