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The effects of atropine and secoverine on gastric secretion and motility in the mouse isolated stomach

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1 The isolated perfused stomach of the mouse was used to study the effect of atropine and secoverine on bethanechol-induced gastric acid secretion and gastric motility.

2 Both atropine and secoverine inhibited cholinergically induced gastric acid secretion and gastric motility.

3 Inhibition of gastric acid secretion by atropine and secoverine occurred at a similar dose-range (10^{-9} and 2×10^{-9} M).

4 Secoverine inhibited bethanechol-induced hypermotility at doses (10^{-11} M and above) that were lower than those of atropine (2×10^{-9} M and above) required to produce this effect.

5 Secoverine, unlike atropine markedly inhibited gastric motility at lower doses than those which affected secretion.

Introduction

Recent studies suggest that secoverine (1-cyclohexyl-4-C [ethyl(*p*-methoxy-α-methylphenethyl) amino]-1-butanone hydrochloride, Figure 1) inhibits the increase in gastrointestinal motility, induced by cholinomimetic agents (Zwagemakers & Claasen, 1980; 1981; Sanger & Bennett, 1981) at doses that have no effect on salivary or gastric secretion. These data support the possible existence of different subclasses of muscarinic receptor within the gastrointestinal tract (Barlow, Berry, Glenton, Nikolau & Sah, 1976; Barlow, Burston & Vis, 1980). However, most experiments which support the selectivity of secoverine, were carried out in different organ preparations, often in different species, so that it is possible that apparent differences in selectivity may reflect variations in drug penetration in different preparations or even species differences. We have investigated the inhibitory effects of secoverine on both secretion and motility in an isolated preparation of mouse stomach. Increases in gastric secretion and gastric motility were induced by bethanechol and the results with secoverine were compared with atropine.

Methods

The effects of atropine and secoverine on bethanechol-induced gastric acid secretion and bethanechol-induced motility were investigated *in vitro*, using stomachs, removed from adult ASH/TO albino mice of either sex, weighing between 15 to 25 g. The technique was based on that described by Bunce & Parsons, (1976) and Wan, (1977). All experiments were carried out on stomachs from two mice, one to measure secretion and the other motility.

Animals were killed by a blow on the head and crushing the neck with artery forceps to destroy the spinal cord. The stomach was then exposed by a midline abdominal incision and a transverse incision was made over the pyloric sphincter area. A polythene cannula (2 mm o.d.) was inserted into the stomach and was secured in place by two strong surgical ligatures. The pyloric region was then separated completely from the duodenum. The stomach was slightly distended with warm oxygenated unbuffered mucosal solution (NaCl 135, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.3 and glucose 31.5 mmol l^{-1}),

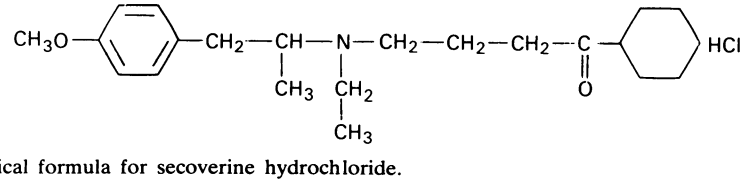

Figure 1 Chemical formula for secoverine hydrochloride.

which was continuously gassed with 100% O_2 and warmed to 37°C by passage through a convoluted glass tube in a water bath. With the stomach distended, the oesophagus was rapidly ligated close to the stomach and transected proximal to the ligature. An incision was then made at the fundus and after washing the stomach with warm mucosal solution, another polythene cannula (3 mm o.d.) was inserted and secured firmly. The stomach was washed again with mucosal solution, and then cut free from the surrounding mesentery. Then the whole isolated stomach was slightly distended and rapidly transferred to a 25 ml perspex organ bath containing buffered serosal solution (NaCl 118, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 0.14, Na_2HPO_4 15.9, $CaCl_2$ 0.65 and glucose 31.6 mmol l^{-1}; pH 7.4; osmolality 290 mosm/l), kept at 37°C by means of an automatic water circulator pump and heater and gassed vigorously with a 95% O_2 and 5% CO_2 mixture.

To measure gastric acid secretion, the stomach was perfused continuously via the fundic tube with mucosal solution at a rate of 1 ml min^{-1} by means of a peristaltic pump. The perfusate from the pyloric cannula was allowed to pass through a flow cell containing a miniature pH electrode (8CMWL, Russel pH Ltd), connected to a pH meter (Electronic Instruments-type 7020) and a Devices M2 pen recorder. The glass flow cell was raised to 18 cm above the level of the stomach to keep the preparation distended to approx. 2 cm long × 1 cm diameter. After an initial stabilization period of 40 min, a submaximal dose of bethanechol (5×10^{-7} M) was administered and the preparation left until a new steady baseline had been achieved for at least 10 min. Thereafter a cumulative dose-response curve was generated, using increasing concentrations of either secoverine (10^{-10} to 5×10^{-9} M) or atropine (5×10^{-10} to 2×10^{-9} M). A steady baseline was achieved for at least 10 min with each dose before the next dose was added. All drugs were injected into the organ bath and the concentrations refer to the final bath concentration.

Gastric intraluminal pressure was monitored in a separate preparation by means of a fluid-filled tube, inserted into the stomach via the oesophagus. This tube was connected to a pressure transducer (Bioscience Pressure Monitor), the output of which was amplified and displayed on a Devices MX2 Chart Recorder. During the measurement of gastric motility the polythene cannulae, which attached the isolated stomach to the perfusion pump, were clamped to enable motility recording without any loss of pressure, and throughout the experiment the stomach pressure remained stable. In order to prevent muscle fatigue, the motility response induced by a submaximal dose of bethanechol (5×10^{-7} M), was allowed to reach a peak and then the drug was immediately

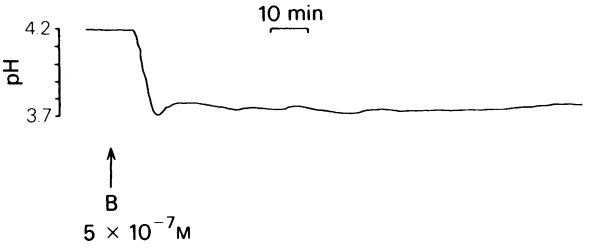

Figure 2 Effect of bethanechol (B) 5×10^{-7} M on the gastric acid secretion in the mouse isolated stomach with the trace illustrating a maintained secretory response.

washed out by exchanging the serosal fluid three times. The antagonist was then added and after a period of 15 min, the same dose of bethanecol was added. The procedure was repeated with increasing concentrations of atropine or secoverine as previously described.

Statistical analysis

The degree of significance between different groups of actions was assessed by applying Student's unpaired *t* test to the original absolute data.

Results

Preliminary experiments established that bethanechol at doses of 5×10^{-7}, 10^{-6} and 2×10^{-6} M increased basal acid secretion and induced gastric motility in the mouse isolated stomach, and that 5×10^{-7} M bethanechol induced submaximal responses. The increase in gastric secretion, induced by 5×10^{-7} M bethanechol was maintained over the experimental period (Figure 2). The delayed onset of the bethanechol-induced gastric secretion can be attributed to the dead space between the stomach and pH electrode.

Effect of atropine

The increases in gastric acid secretion and gastric motility were both inhibited by similar doses of atropine. Gastric acid secretion induced by bethanechol (5×10^{-7} M) was significantly inhibited at a dose of 10^{-9} M ($P < 0.05$) (Figure 3). Gastric motility was significantly inhibited at doses of 2×10^{-9} and 3×10^{-9} M atropine ($P < 0.001$) (Figure 4).

Effect of secoverine

Secoverine also inhibited both bethanechol-induced gastric acid secretion and motility, although the dose-

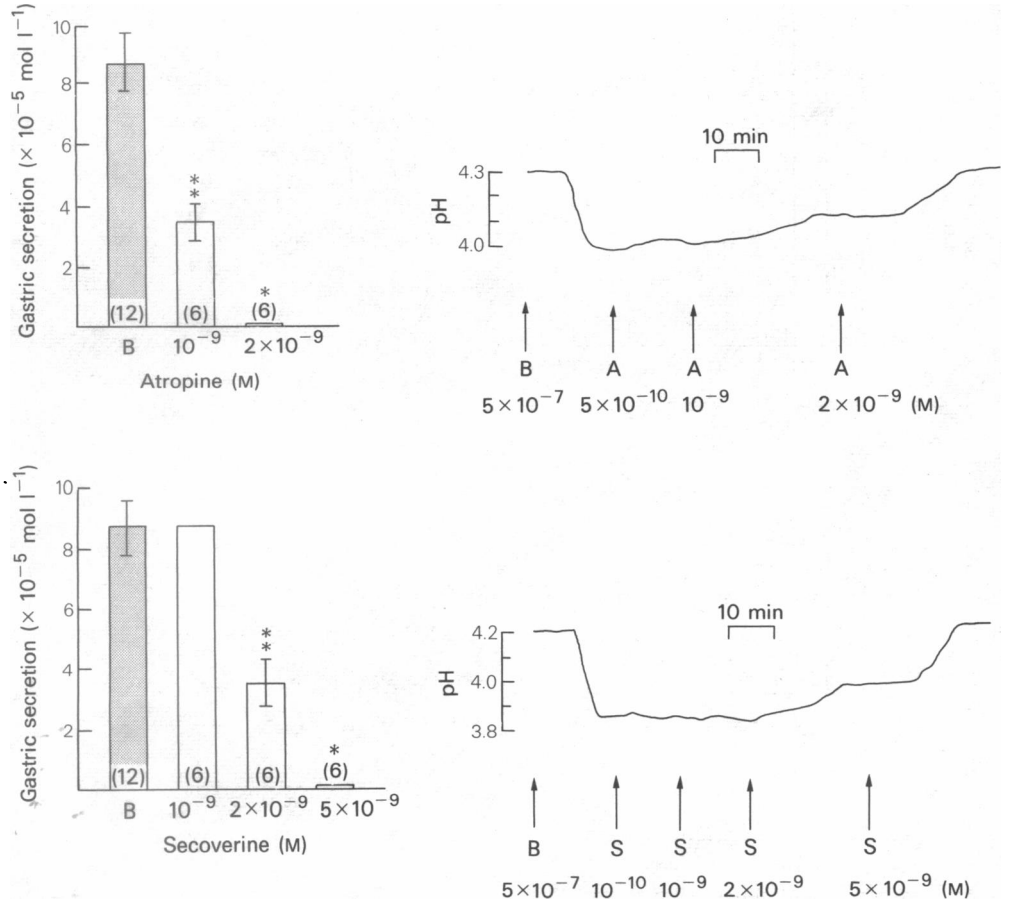

Figure 3 Effect of atropine (A) and secoverine (S) on the bethanechol-induced (B) gastric acid secretion in the mouse isolated stomach. Atropine 2×10^{-9} M and secoverine 5×10^{-9} M caused complete inhibition of the bethanechol-induced response. Gastric acid secretion is expressed as hydrogen ion concentration in mol l^{-1} * = $P < 0.001$; ** = $P < 0.005$. The numbers in parentheses refer to the number of individual experiments performed.

responses were different. Gastric motility was significantly inhibited by secoverine at doses of 10^{-11} M and above ($P < 0.001$) (Figure 4), whereas doses of 2×10^{-9} M and above were required to inhibit acid secretion ($P < 0.001$) (Figure 3). Similar doses of secoverine and atropine inhibited gastric secretion in this preparation, although secoverine was over 200 times more potent an inhibitor of gastric motility (see Figure 4).

Discussion

Our findings show that secoverine, unlike atropine, inhibits bethanechol-induced gastric motility at a dose 50 times below that required to inhibit gastric secretion. This evidence strongly supports the hypothesis that secoverine is a selective antagonist of muscarinic receptors as it shows selectivity of action in identical preparations taken from the same animal. Similar observations have been reported for the rat and ferret small intestine *in vivo* (Greenwood, Read, Hardcastle & Hardcastle, 1983). However, in the rat jejunum, we found that atropine also exhibited a selectivity of action, though at much lower doses.

The observation that secoverine and atropine were approximately equipotent inhibitors of bethanechol-induced acid secretion in the mouse isolated stomach differed from observations in the rat stomach *in vivo*, where atropine was over 100 times more potent

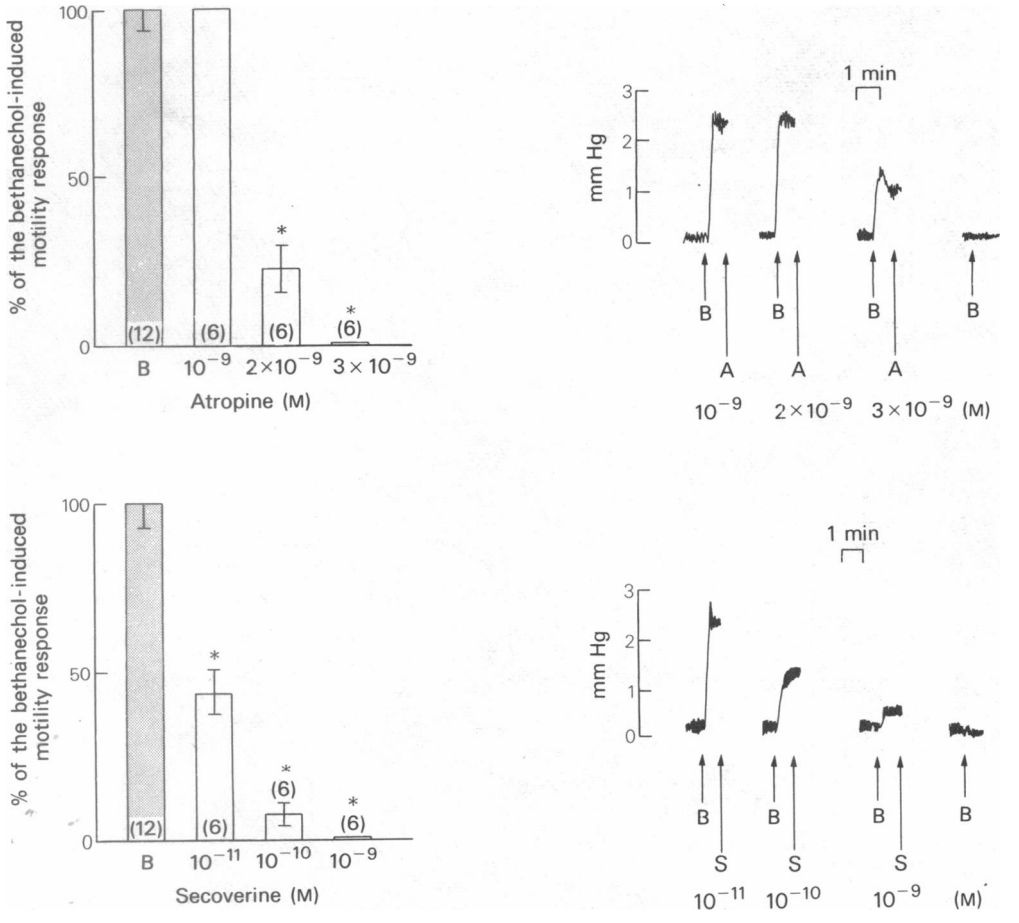

Figure 4 Effect of atropine (A) and secoverine (S) on bethanechol-induced (B) gastric motility in the mouse isolated stomach. Atropine at doses of 2×10^{-9} M and above, inhibited motility, whereas secoverine at doses of 10^{-11} M and above inhibited the response. * = $P < 0.001$.

(Zwagemakers & Claassen, 1980; 1981). Thus it would appear that the relative potency of these drugs can vary with the animal species.

The action of secoverine clearly differs from that of another putative selective muscarinic antagonist, pirenzepine, which inhibits acid secretion without causing typical antimuscarinic side effects (Hammer, Berrie, Birdsall & Hulme, 1980), or apparently influencing gastric motility. Thus it would appear that two major classes of muscarinic receptors exist in the gut, one mediating motor responses, and selectively inhibited by secoverine; the other mediating secretion and selectively inhibited by pirenzepine. Binding studies using pirenzepine have shown that muscarinic antagonists can have different affinities for binding sites within the same organ (Barlow *et al.*, 1976; 1980).

The muscarinic antagonists clearly differ from selective antagonists of histamine or adrenaline in that the range over which they are selective is relatively small. In other words, there is a difference in potency at different sites rather than an absolute selectivity. Nevertheless pirenzepine and secoverine may be the precursors of other compounds with greater selectivity, which may be used in clinical practice to inhibit acid secretion without affecting gastric emptying or colonic function or to inhibit colonic spasm or slow gastric motility without causing a dry mouth.

Reprint requests to B.G. in Sheffield, please.

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