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EFFECTS OF H_2-RECEPTOR ANTAGONISTS AND ANTICHOLINOCEPTOR DRUGS ON GASTRIC AND SALIVARY SECRETION INDUCED BY BETHANECHOL IN THE ANAESTHETIZED DOG

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1 The H_2-receptor antagonists, ranitidine and cimetidine, have been compared with atropine and pirenzepine for their effects on gastric acid output, and on salivary secretion from the left parotid gland in the anaesthetized dog. Gastric and salivary secretions were elicited by intravenous infusion of bethanechol.

2 Atropine (0.3–1 μg/kg) or pirenzepine (3–10 μg/kg) reduced both gastric and salivary secretions, pirenzepine showing little evidence of any selectivity for gastric secretion.

3 The H_2-receptor antagonists, ranitidine (30–1000 μg/kg) and cimetidine (100–3000 μg/kg), selectively inhibited gastric secretion and even at relatively high dose levels did not alter salivary volume.

Introduction

We have previously shown in the conscious Heidenhain pouch dog that the H_2-receptor antagonists, ranitidine and cimetidine, can inhibit gastric acid secretion elicited by histamine, pentagastrin or by the muscarinic agonist, bethanechol (Daly, Humphray & Stables, 1980). This inhibition of bethanechol-induced secretion is thought to reflect antagonism of the endogenous histamine which facilitates the gastric secretory response to bethanechol (Gardner, Jackson, Batzri & Jensen, 1978; Soll, 1978), rather than a cholinoceptor antagonist action of the H_2-receptor antagonists. Although ranitidine and cimetidine did not antagonize contractions of the guinea-pig isolated ileum preparation to bethanechol (Daly, Humphray & Stables, 1981), it was important to exclude the possibility that, *in vivo*, either H_2-receptor antagonist might possess an anticholinoceptor effect on salivary secretion. In addition, Bertaccini and colleagues recently claimed that ranitidine had some cholinoceptor agonist activity and potentiated salivary secretion elicited by bethanechol in the rat (Bertaccini & Coruzzi, 1981; Bertaccini, Molina, Bobbio & Foggi, 1981).

The use of cholinoceptor antagonists such as atropine in the treatment of peptic disease has been severely limited by their tendency to cause dry mouth, blurred vision and other side-effects (Lennard-Jones, 1973). Pirenzepine, a tricyclic compound recently introduced for the treatment of peptic ulcer disease, has anticholinoceptor activity on the guinea-pig atrium (Süsskand & Sewing, 1979) and guinea-pig ileum (Parsons, Bunce, Blakemore & Rasmussen, 1979). In clinical trials on pirenzepine, dry mouth has been reported as a side effect in some studies (Einig, 1977; Giger, Gonvers, Weber, *et al.*, 1979; Stockbrügger, Jaup, Hammer & Dotevall, 1979), but not in others (Schmid & Blaich, 1978; Jaup, Stockbrügger & Dotevall, 1979; Wegmann, Bliem, Loeffelmann & Lujf, 1979). We have now compared the H_2-antagonists, ranitidine and cimetidine with the cholinoceptor antagonists, atropine and pirenzepine, for their effects on gastric acid secretion and salivary secretion in the anaesthetized dog.

Methods

Beagle dogs of either sex weighing 6–10 kg were fasted overnight and anaesthetized with thiopentone (25 mg/kg i.v.) followed by chloralose (50 mg/kg i.v.) and urethane (500 mg/kg i.v.). Supplementary doses of chloralose and urethane were given as necessary to maintain anaesthesia. The trachea was intubated with a cuffed endotracheal tube and a femoral artery was cannulated to allow measurement of systemic blood pressure with a Hewlett Packard 1280 blood pressure transducer. A Hewlett Packard 7754A recorder was used to monitor blood pressure and heart rate (which was automatically derived from the blood pressure). Intravenous bolus doses of drugs or anaesthetic were administered via a cannula inserted into a femoral vein.

The dog was prepared for collection of gastric

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secretion from the whole stomach as follows: the stomach was exposed by laparotomy and a double purse-string suture inserted in the fundic region of the stomach. A Gregory duodenal cannula was then inserted through a small incision in the stomach wall and firmly secured by the purse-string sutures. The pylorus was ligated and the Gregory cannula anchored to the body wall by sutures. The incisions in the body wall and skin were closed around the barrel of the Gregory cannula with sutures or clips. The dog was then placed on its side and the stomach washed clean with water at body temperature, poured through a tube which had been passed down the oesophagus into the stomach. An incision was made in the throat and the oesophagus was ligated.

The dog was then prepared for measurement of salivary secretion as follows: the left parotid duct was cannulated by inserting a catheter into the duct opening which is located in the buccal cavity opposite the posterior margin of the fourth upper premolar. The dog was then placed in a hammock with a central hole for the Gregory cannula, and the hammock raised until the dog was in a standing position. A teflon insert was passed up the fistula to lift the dorsal stomach wall from the opening of the fistula to allow drainage of any gastric secretion. A plastic collection vessel was attached to the end of the gastric cannula and the parotid catheter was allowed to drain into a separate collection vessel.

Preliminary experiments were carried out to determine a dose of bethanechol which would produce submaximal stimulation of both gastric and parotid salivary secretions. Bethanechol was infused intravenously via a catheter inserted into a cephalic vein at doses of 1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$, each dose level being infused for 2 h. In all subsequent experiments gastric and salivary secretions were stimulated by an intravenous infusion of bethanechol at 3 $\mu g\,kg^{-1}\,min^{-1}$. Gastric and salivary collection vessels were changed every 15 min, the volume of each secretion measured, and the rate of secretion calculated in ml/min. The H^+ concentration in the gastric juice was measured by titrating an aliquot to pH 7 against 0.1 M NaOH using a Radiometer TTT2 Autotitrator. Acid output was then calculated in $\mu mol\ H^+$ per min. Once gastric acid and salivary secretions had reached constant levels the test drug was administered intravenously as a bolus dose.

The following drugs were administered: ranitidine 30–1000 $\mu g/kg$, cimetidine 100–3000 $\mu g/kg$, atropine 0.3–1 $\mu g/kg$ and pirenzepine 3–10 $\mu g/kg$. Each dose level was tested in at least 3 dogs.

Results were calculated as follows. In the preliminary experiments to determine a sub-maximal dose of bethanechol on gastric and salivary secretions the gastric acid output and salivary volume following each dose of bethanechol was calculated by taking the mean of the two highest consecutive values during each 2 h infusion period. Blood pressure and heart rate were measured at the end of infusion of each dose level of bethanechol. Results on the test drugs have been calculated as percentage change in gastric acid secretion and salivary volume by comparison of the mean of the four values preceding drug administration with the mean of the two values following administration of the test drug. Peak changes in blood pressure and heart rate were measured following injection of the drug. For each dose level of atropine and pirenzepine the percentage changes in gastric secretion have been compared with the changes in salivary volume using a Wilcoxon signed rank test (Siegal, 1956). ED_{50} values (with 95% confidence limits) for inhibition of secretion have been calculated by the method of least squares.

The drugs used were atropine sulphate (BDH), bethanechol chloride (Koch-Light Ltd), cimetidine (Smith, Kline & French Ltd), pirenzepine (kindly supplied by Boehringer Ingelheim Ltd) and ranitidine hydrochloride (Glaxo Group Research Ltd). Drug doses are expressed in terms of free base.

Results

The results of the preliminary experiments on the effects of different dose levels of bethanechol on gastric and salivary secretions, blood pressure and heart rate are shown in Table 1. Both gastric acid output and salivary secretion from the left parotid duct were stimulated in a dose-related manner by bethanechol over the range 1–10 $\mu g\,kg^{-1}\,min^{-1}$. At the highest dose of 10 $\mu g\,kg^{-1}\,min^{-1}$ the maximum levels of gastric and salivary secretions reached were not maintained, but began to decline after 30–45 min of the bethanechol infusion. Blood pressure and heart rate also gradually declined from this time. Consequently, as bethanechol at 10 $\mu g\,kg^{-1}\,min^{-1}$ was barely tolerated by the dog, higher dose levels were not tested. For all subsequent experiments a dose of bethanechol of 3 $\mu g\,kg^{-1}\,min^{-1}$ was used as this produced a sustained, sub-maximal stimulation of gastric and parotid salivary secretions without significant reduction in blood pressure or heart rate. Gastric secretion was slightly more sensitive to stimulation by bethanechol than was salivary secretion.

The effects of intravenous administration of ranitidine, cimetidine, atropine and pirenzepine on bethanechol-induced gastric acid and salivary secretions, and on cardiovascular parameters are shown in Table 2. Both ranitidine and cimetidine caused dose-related inhibition of acid output, ranitidine being approximately 7 times more potent than cimetidine, with inhibitory ED_{50} values (with 95% confidence

Table 1 Effect of bethanechol on gastric and salivary secretions, blood pressure and heart rate in the anaesthetized dog

Bethanechol ($\mu g\,kg^{-1}\,min^{-1}$)	*Gastric acid output* ($\mu Eq\,H^+/min$)	(% max*)	*Left parotid salivary volume* (µl/min)	(% max*)	*Blood pressure* (mmHg) *Systolic*	*Diastolic*	*Heart rate* (beats/min)
Control	–	–	–	–	218±10	152±11	218±16
1	37±17	23±11	14± 5	3±1	202± 7	129± 6	201± 9
3	137±20	76±12	184±44	42±8	210±11	130± 9	191± 8
10	187±27	100	426±72	100	189± 8	88± 9	114±10

*Calculated as a percentage of the response elicited by bethanechol at 10 $\mu g\,kg^{-1}\,min^{-1}$.
Each dose level was tested in three dogs. Mean values ± s.e.mean are given.

limits) of respectively 0.26 (0.16–0.39) and 1.9 (1.1–3.4) µmol/kg. Neither ranitidine nor cimetidine caused any significant changes in salivary secretion at dose levels up to approximately 10 times their ED_{50} values for inhibition of gastric secretion.

Atropine and pirenzepine were extremely potent inhibitors of bethanechol-induced gastric acid secretion with ED_{50} values in nmol/kg of 1.5 (0.8–2.2) and 11.0 (1.9–17.8) respectively. Both atropine and pirenzepine also inhibited salivary secretion, with respective ED_{50} values of 3.3 (2.2–11.9) and 16.5 (9.9–29.4) nmol/kg. There was little evidence for selectivity of either atropine or pirenzepine as an inhibitor of gastric secretion. Using a Wilcoxon signed rank test there was no significant difference ($P > 0.1$), following any particular dose of either drug, between the reduction in gastric secretion and the reduction in salivary volume.

There were no marked changes in blood pressure or heart rate with any of the antisecretory drugs.

Discussion

Ranitidine and cimetidine are both potent inhibitors of bethanechol-induced gastric acid secretion in the anaesthetized dog. However, neither drug had any inhibitory effects on salivary volume at dose levels approximately 10 times their ED_{50} values for inhibition of gastric secretion, suggesting that neither drug has significant cholinoceptor antagonist activity *in vivo*. The most likely explanation for the antisecretory activity of H_2-antagonists against bethanechol-induced gastric secretion is that endogenous histamine plays a facilitatory role in the gastric secretory reponse to bethanechol and H_2-antagonists inhibit

Table 2 Effects of ranitidine, cimetidine, atropine and pirenzepine on gastric and salivary secretions induced by bethanechol and cardiovascular parameters in the anaesthetized dog

Drug	*Intravenous dose* (µg/kg)	(mol/kg)	*Changes in gastric acid secretion* (%)	*Changes in salivary volume* (%)	*Changes in blood pressure* (mmHg) *Systolic*	*Diastolic*	*Changes in heart rate* (beats/min)
Ranitidine	30	(9.6×10^{-8})	−19.4±10.6	+13.7± 6.0	− 3±3	− 3±3	+ 1±1
	100	(3.2×10^{-7})	−66.0± 6.8	+ 7.9± 1.7	0±1	0±2	+ 1±1
	300	(9.6×10^{-7})	−72.8± 3.7	+12.3±11.9	+ 3±5	− 8±3	+ 3±1
	1000	(3.2×10^{-6})	−96.7± 2.4	− 4.3±12.4	+10±4	+ 3±7	+10±2
Cimetidine	100	(4.0×10^{-7})	−13.7± 8.1	+ 9.2±15.1	− 3±2	+ 2±2	0±0
	300	(1.2×10^{-6})	−44.2±10.1	+20.6±15.0	− 1±2	− 2±1	0±0
	1000	(4.0×10^{-6})	−65.0±12.0	+ 4.3± 3.9	− 2±3	− 6±2	+ 2±3
	3000	(1.2×10^{-5})	−80.8± 7.6	+13.4± 2.5	− 6±7	− 8±8	− 3±7
Atropine	0.3	(1.0×10^{-9})	−32.0±15.8	−19.1± 7.4	+16±3	+17±2	−12±3
	1	(3.5×10^{-9})	−87.3± 2.0	−51.1± 5.5	+ 9±2	+16±3	− 9±9
Pirenzepine	3	(8.7×10^{-9})	−43.7±10.2	−29.3± 2.1	+ 2±1	+ 3±1	− 3±1
	10	(2.9×10^{-8})	−77.5± 3.9	−68.4±10.5	+ 9±1	+ 7±1	0±4

Mean values ± s.e.mean are given. Each dose level was examined in at least three different dogs.

the effects of this endogenous histamine (Gardner *et al.*, 1978; Soll, 1978). Although Bertaccini *et al.* (1981) claimed that ranitidine potentiated bethanechol-induced salivary secretion in the rat, we were unable to show any such effect in our experiments in the dog.

Unlike the H_2-antagonists, atropine and pirenzepine inhibited both gastric and salivary secretions, results compatible with pirenzepine possessing a cholinoceptor antagonist action. Neither atropine nor pirenzepine showed any significant selectivity for inhibiting gastric secretion. In a recent double blind study in man (Jaup, Stockbrügger & Dotevall, 1980) L-hyoscyamine (the active isomer in atropine) and pirenzepine both reduced maximum salivary capacity, although there were fewer reports of dry mouth in the volunteers receiving pirenzepine. A more marked distinction reported between the two drugs was on the cholinergically innervated muscular system of the eye. There, L-hyoscyamine produced the expected relaxation of the ciliary muscle whereas pirenzepine had no effect (Jaup, *et al.*, 1980). This is consistent with the results in the mouse where Heathcote & Parry (1980) have shown that pirenzepine is less effective than other anti-cholinoceptor drugs in increasing pupil diameter.

In radioligand binding studies on broken cell preparations of dog stomach pirenzepine showed higher affinity for muscarinic receptors from fundic mucosa than those from smooth muscle (Hammer, 1980), suggesting a difference in muscarinic receptors in these two regions of the stomach. The fundic mucosa is mainly involved with acid and pepsinogen secretion whereas the smooth muscle regulates motor functions of the stomach. In the rat, radioligand binding studies have shown that pirenzepine has a high affinity for muscarinic receptors in the salivary glands and fundic mucosa and a low affinity for muscarinic receptors in the heart (Birdsall, Burgen, Hammer, Hulme & Stockton, 1980). These findings are in agreement with our results in the dog which suggest that pirenzepine has a similar affinity for muscarinic receptors in the salivary glands and parietal cells.

In conclusion, whilst there is evidence from the literature for some selectivity of the anti-muscarinic action of pirenzepine, our results suggest that this compound does not differentiate between muscarinic receptors of the salivary glands and parietal cells. In contrast, cimetidine and ranitidine inhibited gastric acid secretion (presumably through their histamine H_2-receptor blocking activity) without reducing salivary flow.

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