Br. J. Pharmac. (1974), **51**, 217-223

CAN THE ACTIONS OF ADRENOCEPTOR AGONISTS AND ANTAGONISTS ON PENTAGASTRIN-INDUCED GASTRIC SECRETION BE DUE TO THEIR EFFECTS ON HISTAMINE FORMATION?

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1 The effects of some adrenoceptor agonists and antagonists which have been reported to affect histamine formation in leucocytes (Assem & Feigenbaum, 1972) have been investigated on gastric secretion in conscious dogs with Heidenhain pouches.

2 Submaximal secretion in response to pentagastrin was enhanced by propranolol (0.1-1.0 mg/kg i.v.) and phenylephrine (1.0 $\mu g\,kg^{-1}\,min^{-1}$ i.v. for 20 min), which increase histamine formation, and was decreased by phentolamine (2 mg/kg i.v.) and isoprenaline (0.05-0.2 $\mu g\,kg^{-1}\,min^{-1}$ i.v. for 30 min), which decrease histamine formation. Practolol (2 mg/kg i.v.), which has no effect on histamine formation, had no effect on secretion.

3 Acid secretion in response to histamine was either unaffected or affected in the opposite direction by these drugs.

4 The effects of the drugs on pentagastrin-induced secretion were not secondary to changes in mucosal blood flow (radioactive aniline clearance).

5 The results are consistent with the hypothesis that acid secretion in response to pentagastrin involves the formation of endogenous histamine.

Introduction

It has been known for some years that adrenoceptor agonists can depress mammalian gastric acid secretion (Harries, 1956; Pradhan & Wingate, 1962). More recently it has been reported that while both noradrenaline and isoprenaline inhibit canine gastric acid secretion in response to pentagastrin, they act by different mechanisms (Curwain & Holton, 1972a, b). Noradrenaline probably acts by vasoconstriction whereas isoprenaline directly inhibits the secretory apparatus. Other β-adrenoceptor agonists have been shown to act in a similar way to isoprenaline on canine gastric acid secretion (Curwain, Holton & Spencer, 1972).

It was reported by Harries (1957) and later confirmed (Pradhan & Wingate, 1962; Curwain, Endersby & Holton, 1971; Curwain & Holton, 1972b) that gastric acid secretion in response to histamine is less readily depressed by catecholamines than acid secretion in response to other stimuli. A possible explanation for this is that histamine stimulates the secretory cell directly at a site resistant to inhibition by isoprenaline whereas pentagastrin initiates a longer chain of events, one step of which is inhibited by isoprenaline, culminating in the secretory response. Acid secretion in response to pentagastrin would therefore be vulnerable to inhibition at several points in the chain but histamine-induced secretion could be inhibited at one point only.

In 1944 Emmelin & Kahlson suggested that the mechanism of action of gastrin involved histamine liberation and this view was endorsed by Code (1956) who proposed that histamine was the final secretagogue which stimulated the oxyntic cells. Although this hypothesis was not generally accepted (Grossman, 1967) it was supported by the work of Kahlson and his colleagues (reviewed by Kahlson, Rosengren & Svensson, 1973) who emphasized the importance of histamine-forming capacity (histidine decarboxylase activity) in the gastric mucosa of the rat. Recent evidence in favour of the involvement of histamine in gastrin-induced gastric secretion in the dog and man comes from the work of Black, Duncan, Durant, Ganellin & Parsons (1972) who showed that burimamide, a specific H_2-receptor antagonist, blocks the action of gastrin and pentagastrin as well as histamine.

A link between the inhibitory action of

isoprenaline on gastric secretion and the hypothesis that gastrin acts by liberating histamine was suggested to us by the work of Assem & Feigenbaum (1972) who found that both isoprenaline and phentolamine decreased histamine formation by human leucocytes whereas phenylephrine and propranolol increased histamine formation. However, the specific β_1-adrenoceptor blocking drug, practolol, did not increase histamine formation (Assem & Feigenbaum, personal communication). Isoprenaline also decreased histamine formation in the rat stomach (Svensson, personal communication). If increased histamine formation is involved in the secretory response to pentagastrin, propranolol, but not practolol, would be expected to enhance pentagastrin-induced gastric secretion whereas isoprenaline and phentolamine would be expected to decrease secretion. Phenylephrine would be expected to enhance secretion provided its direct action was not prevented by concomitant vasoconstriction. The drugs would not be expected to affect histamine-induced secretion. The results described in this study show that these drugs have the predicted effects.

Methods

Healthy mongrel bitches (11-22 kg) with well-established Heidenhain pouches were used. Food was withheld but water was allowed 18 h before each experiment. On the day of the experiment intravenous catheters were inserted with aseptic precautions. One catheter (0.63 mm outside diameter) was used for infusion of saline (0.9% w/v NaCl solution) at 1 ml/min throughout the experiment. Mucosal blood flow was estimated by ^{3}H-aniline clearance as previously described (Curwain & Holton, 1973). Drugs and aniline were added to the saline infusion. Blood samples were withdrawn every 30 min via a second vein catheter (0.9 mm outside diameter). Gastric juice was collected at 10 or 15 min intervals and aliquots were titrated against 0.1 N NaOH with phenolphthalein as indicator.

The following drugs were used: histamine acid phosphate (B.D.H.), pentagastrin (Peptavlon, I.C.I.), (±)-isoprenaline sulphate (Macarthys), phentolamine mesylate (Rogitine, Ciba), (±)-practolol (Eraldin, I.C.I.) and (±)-propranolol (Inderal, I.C.I.).

Experimental procedure and calculation of results

The secretagogue was administered by intravenous infusion in a dose which elicited 40-70% maximal response. When a stable plateau of secretion had been attained the test drug was administered according to the following schedules:

Propranolol. Doses of 0.1, 0.3, 0.6 and 1.0 mg/kg were administered by intravenous injection at 30-60 min intervals. Since propranolol is relatively slowly metabolized, the dose of propranolol is expressed as the sum of the doses received during the experiment. For example, when 0.3 mg/kg was administered 60 min after giving 0.1 mg/kg the total dose is 0.4 mg/kg.

Practolol, phenylephrine, phentolamine. Single dose levels of each of these drugs were used in several dogs.

Isoprenaline was given as a constant infusion at two dose levels ($0.05\ \mu g\ kg^{-1}\ min^{-1}$ or $0.2\ \mu g\ kg^{-1}\ min^{-1}$ for 30 minutes).

Calculation of results

In each experiment the values for acid secretion and aniline clearance for three successive gastric juice samples (10 or 15 min periods) immediately before giving the test drug were used as control observations. After giving the drug one sample was omitted and the next three samples were used for test observations. The ratio, R_a, of gastric mucosal blood flow (ml/min aniline clearance) to acid secretory rate (μM/min) was calculated for each sample from these data. The observations of acid secretory rate and of R_a from each set of experiments at one dose level of a single drug with each secretagogue were treated separately. The difference between the means of the test and control observations was expressed as a percentage of the mean control. Each set of observations was subjected to an analysis of variance. Variance between experiments was extracted and the ratio of the variance between test and control observations to residual variance was calculated. The probability that this variance ratio was fortuitous was obtained from Table V in Fisher & Yates (1967).

Results

Enhancement of acid secretion by propranolol and phenylephrine

The effects of propranolol (0.1, 0.4 mg/kg) on pentagastrin-induced secretion and mucosal blood flow are illustrated in Figure 1. It can be seen that secretion was enhanced, gastric mucosal blood flow increased concomitantly with secretion and the ratio (R_a) of mucosal blood flow to acid

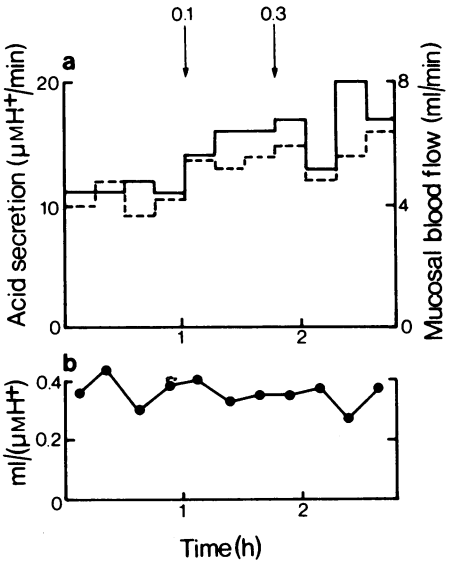

Fig. 1 The effect of intravenous propranolol on submaximal gastric acid secretion and mucosal blood flow in response to pentagastrin. (a) Acid secretion (solid line) and mucosal blood flow (broken line). Propranolol (0.1, 0.3 mg/kg i.v.) was given as indicated. Both secretion and mucosal blood flow increased in response to propranolol. (b) The ratio R_a of blood flow to acid secretory rate calculated from the results in (a). R_a did not vary significantly during the experiment.

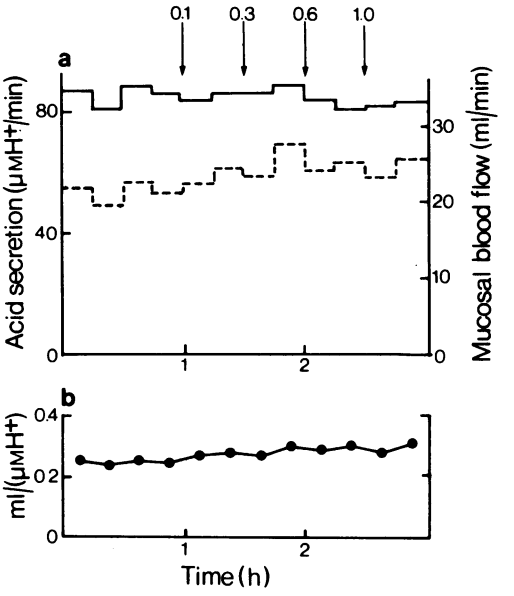

Fig. 2 The effect of intravenous propranolol on submaximal gastric secretion and mucosal blood flow in response to histamine. (a) Acid secretion (solid line) and mucosal blood flow (broken line). Propranolol (0.1, 0.3, 0.6 and 1.0 mg/kg i.v.) was given as indicated. Neither acid secretion nor mucosal blood flow was significantly affected. (b) The ratio R_a of blood flow to acid secretory rate calculated from the results in (a).

secretory rate remained constant. These effects of propranolol on pentagastrin-induced secretion were dose-related and were confirmed in every experiment.

In doses of 0.1 mg/kg, 0.4 mg/kg and 2 mg/kg, propranolol had no effect on histamine-induced secretion or gastric mucosal blood flow (Fig. 2), although after 1 mg/kg propranolol, histamine-induced secretion was slightly increased in 2 out of 5 experiments and the mean increase of 12% was just significant ($P < 0.05$). In these experiments mean R_a fell by 18% (Table 1). We conclude that in the dose range 0.1 mg/kg to 2 mg/kg propranolol has no effect on histamine-induced secretion. The effect of propranolol on acid secretion was expressed quantitatively as described in the methods section and the results of all the experiments are summarized in Figure 3.

Table 1 The effects of the drugs on R_a (the ratio of mucosal blood flow to acid secretory rate) in the dog

Secretagogue		*Pentagastrin*			*Histamine*		
Drug	*Dose*	*% Change*	n	P	*% Change*	n	P
Propranolol	0.4	−10	12	>0.05	−1	17	>0.05
	1.0	−4	12	>0.05	−18	16	<0.05
Phenylephrine	1.0	−18	18	<0.01	−14	12	<0.05
Isoprenaline	0.2	+175	12	<0.01	+12	24	>0.05
Phentolamine	2.0	+7	12	<0.01	−30	24	<0.01

Doses of propranolol and phentolamine are expressed as mg kg^{-1} i.v. Doses of phenylephrine and isoprenaline are expressed as μg kg^{-1} min^{-1} for 20 and 30 min respectively. The mean percentage change was calculated from experiments on 2 to 5 dogs as described in the methods section. *n* is the number of observations. *P* is the probability that the change is fortuitous.

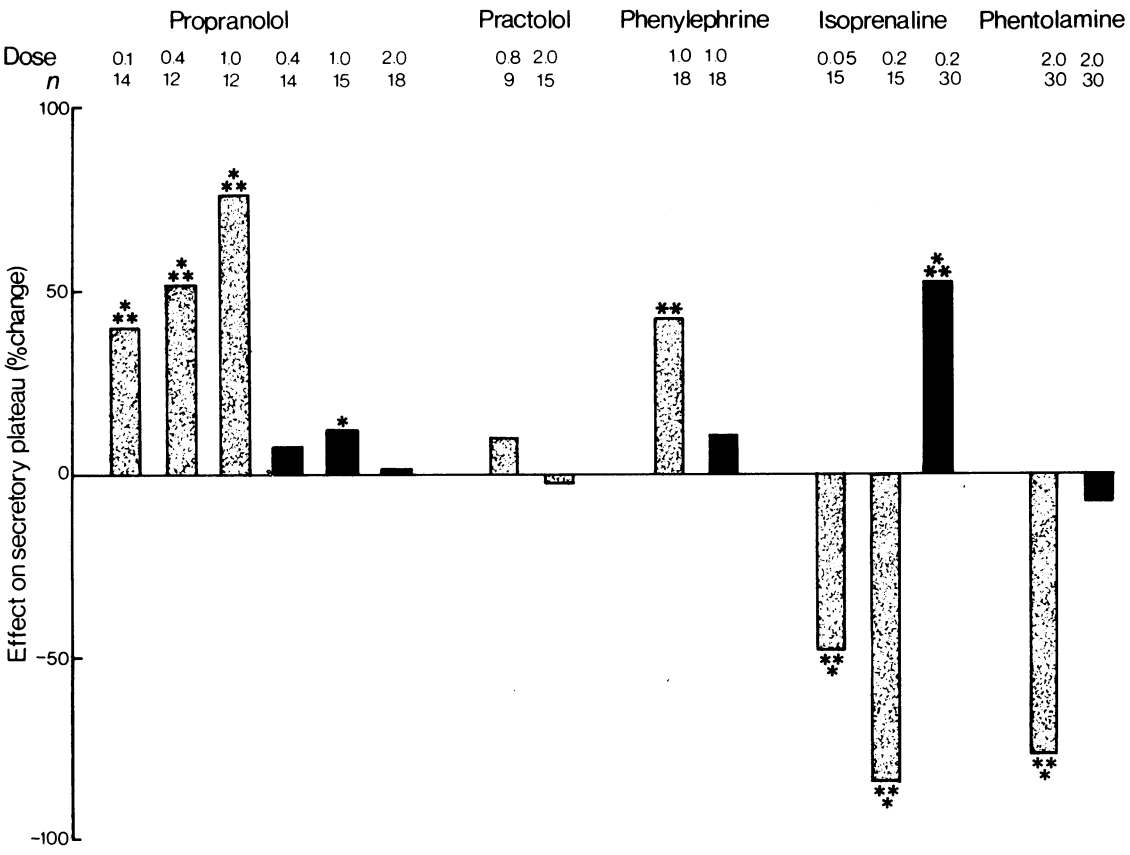

Fig. 3 Effects of adrenoceptor drugs on submaximal gastric secretion induced by histamine (solid columns) and pentagastrin (stippled columns). The columns are the differences between the means of test and control observations expressed as a percentage of the mean control secretory rate (see methods section). Three, two, one or no asterisks above a column denote that the probability that the change is fortuitous is less than 0.001, 0.01, 0.05, or greater than 0.05 respectively (for calculation see methods section). Note that propranolol and phenylephrine increased and isoprenaline and phentolamine decreased pentagastrin-induced secretion. Isoprenaline increased histamine-induced secretion but the other drugs had no significant effect. Doses of propranolol, practolol, and phentolamine are expressed as mg kg^{-1} i.v. Doses of phenylephrine and isoprenaline are expressed as $\mu g\ kg^{-1}\ min^{-1}$ i.v. for 20 and 30 min respectively. Each column is based on experiments from 3 to 5 dogs. *n* is the number of observations.

As shown in Fig. 3 phenylephrine also increased pentagastrin-induced secretion but not histamine-induced secretion. Gastric mucosal blood flow increased but R_a fell (Table 1). Practolol had a negligible effect. Neither propranolol nor phenylephrine caused secretion in the fasting unstimulated animal.

Inhibition of acid secretion by phentolamine and isoprenaline

These results are also summarized in Figure 3. Phentolamine (2 mg/kg) markedly decreased pentagastrin-induced secretion. The effects of isoprenaline which have been reported previously are also shown in Figure 3. Isoprenaline markedly decreased pentagastrin-induced secretion but increased histamine-induced secretion. Isoprenaline increased R_a during pentagastrin-induced secretion. Phentolamine increased R_a during pentagastrin-induced secretion but decreased R_a during histamine-induced secretion (Table 1).

Discussion

The aim of these experiments was to investigate the correlation between the actions of adreno-

ceptor agonists and antagonists on pentagastrin-induced gastric secretion in dogs with their known actions on histamine-forming capacity. Table 2 shows that drugs which increase histamine formation (propranolol and phenylephrine) also increase the response to pentagastrin whereas drugs which decrease histamine formation (isoprenaline and phentolamine) decrease the response to pentagastrin. Histamine-induced secretion is either unaffected or affected in the opposite direction. Before we can accept the hypothesis that these drugs have a primary action on the secretory mechanism it is necessary to analyse their effects on mucosal blood flow. The relevance of the changes in gastric mucosal blood flow is that whereas primary changes in secretion are accompanied by commensurate changes in mucosal blood flow so that R_a remains constant, a primary decrease in blood flow with decreased R_a would be expected to decrease secretion when blood flow became a limiting factor. A primary increase in blood flow does not *cause* secretion (Cowley & Code, 1970) but may increase it in circumstances where blood flow is limiting the action of a secretagogue (Holton, 1973). According to these criteria it appears that the actions of the drugs on pentagastrin-induced secretion are independent of their effects on gastric mucosal blood flow. As shown in Tables 1 and 2 the drugs which increased pentagastrin-induced secretion either had no effect or decreased R_a whereas the drugs which decreased secretion increased R_a. Isoprenaline was the only drug which significantly affected histamine-induced secretion and both secretion and R_a were increased. In this instance, therefore, the increased secretion may have been secondary to increased mucosal blood flow.

The previous work on the effect of these drugs on gastric secretion presents a rather confused picture which may be clarified by considering them in the light of the hypothesis that pentagastrin acts by liberating histamine from the gastric mucosa (Kahlson *et al.*, 1973). An extension of this hypothesis is that α-adrenoceptor agonists and β_2-adrenoceptor antagonists increase the rate of pentagastrin-induced histamine liberation but β-adrenoceptor agonists and α-adrenoceptor antagonists decrease histamine liberation in the gastric mucosa. These drugs would not affect the direct action of histamine. Whether the drugs would affect secretion in response to vagal stimulation depends on the possible role of histamine liberation in this type of secretion.

A considerable volume of work on the effects of α- and β-adrenoceptor agonists and antagonists on gastric secretion in various species has been described (see Holton, 1973, for earlier references). Many of the experiments have been carried out with large doses of the drugs which may be expected to affect gastric mucosal blood flow and to have other actions, e.g. propranolol has a local anaesthetic action (Bowman, Rand & West, 1968) and isoprenaline blocks β-receptors and stimulates α-receptors (Butterworth, 1963). We are concerned with the specific effects of small doses of the drugs which we suggest can be related to their effects on histamine formation. Small doses (0.03-0.5 mg/kg) of propranolol have been reported to increase submaximal pentagastrin-induced gastric secretion in man (Konturek & Olesky, 1969), conscious dogs (Evans & Lin, 1970; Lin, Evans & Spray, 1973) and spontaneous secretion in pylorus-ligated rats (Rossoff & Goldman, 1968; Debnath, Govinda Das, Gode & Sanyal, personal communication). Histamine-induced secretion has been reported as unaffected in anaesthetized dogs (Haigh & Steedman, 1968) or decreased in man (Geumei, Issa, El-Gendi & Abd-El-Samie, 1972) and pigeons (Geumei, Issa & Abd-El-Samie, 1972). In pylorus-ligated rats larger

Table 2 Comparison of the effects of drugs on histamine-forming capacity (HFC) with the effects on gastric acid secretion observed in our experiments

Drug	*Effect on HFC**	*Effect on gastric acid secretion*	
		Pentagastrin	*Histamine*
Propranolol	↑	↑	0
Phenylephrine	↑	↑	0
Practolol	0**	0	
Isoprenaline	↓	↓	↑
Phentolamine	↓	↓	0

* From Assem & Feigenbaum (1972).
** Assem & Feigenbaum, personal communication.

doses of propranolol decreased spontaneous secretion (Okabe, Saziki & Takagi, 1970; Debnath *et al.*, personal communication; Danhof & Geumei, 1972). Large doses of pronethalol and dichloroisoprenaline also decreased spontaneous secretion in pylorus-ligated rats (Bass & Patterson, 1967).

Phenylephrine has been studied by Misher, Pendleton & Staples (1969) who found that in small doses (0.25 mg/kg) it potentiated spontaneous secretion in rats with gastric fistulae. Larger doses (0.5 mg/kg and 1.0 mg/kg) caused a potentiation followed by a fall in secretion.

Phentolamine decreased secretion in response to food (i.e. gastrin-induced) in conscious dogs (Pradhan & Wingate, 1966) and spontaneous secretion in pylorus-ligated rats (Bass & Patterson, 1967). Phenoxybenzamine decreased pentagastrin-induced secretion in conscious dogs (Lin *et al.*, 1973) but increased secretion in pylorus-ligated rats. Small doses of isoprenaline (0.2 $\mu g\ kg^{-1}\ min^{-1}$) decreased secretion induced by pentagastrin or food and larger doses also decreased histamine-induced secretion (for references see Curwain & Holton, 1972a).

The experiments described in this study confirm and extend the previous findings with small doses of these drugs and demonstrate that their effects are not due to changes in gastric mucosal blood flow. The results are compatible with the hypothesis that the effects are due to modification of the amount of histamine liberated in response to pentagastrin.

We are grateful to the M.R.C. and the Wellcome Trust for support. R.L.McI. is a St Mary's Hospital Medical School postgraduate research scholar. Our thanks are due to I.C.I. Ltd for gifts of drugs.

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(Received August 16, 1973)