THE EFFECT OF SYMPATHOMIMETIC DRUGS AND THEIR ANTAGONISTS ON GASTRIC ACID SECRETION IN THE RAT

by

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"Such is the field of stomach physiology:
scattered pieces of knowledge and a
mass of hypotheses and theories ...."

Heinz and Öbrink
(1954)

ABSTRACT

The effects of sympathomimetic agonists and their antagonists have been studied in the rat, using both conscious animals and an isolated stomach preparation. Sympathomimetics have been reported to inhibit gastric acid secretion *in vivo* but it is not known if their inhibitory effect is due to a direct action on the gastric mucosa.

Pentagastrin-stimulated acid secretion was inhibited by isoprenaline in the Heidenhain pouch rat. This inhibition was antagonised by propranolol, practolol (a β_1-selective adrenoceptor antagonist) and butoxamine (β_2-selective). The acid response to pentagastrin was unaffected by practolol and butoxamine but was potentiated by propranolol. This confirms and extends other findings in the conscious rat and dog.

In the rat isolated stomach, β-adrenoceptor agonists (isoprenaline, salmefamol and salbutamol) and noradrenaline stimulated acid secretion in a concentration dependent manner. This stimulating effect was inhibited by the non-selective β-adrenoceptor antagonists propranolol, timolol and pindolol. Practolol and butoxamine antagonised the effect of noradrenaline but had no effect on isoprenaline or salmefamol stimulated acid secretion. Metiamide and atropine, at concentrations which inhibit respectively, histamine- and bethanechol-stimulated secretion in this preparation, had no effect on the action of isoprenaline or salmefamol.

The α-adrenoceptor agonists phenylephrine and methoxamine had no effect on acid secretion. Pentagastrin- and bethanechol-stimulated secretion were also unaffected by phenylephrine, suggesting that α-adrenoceptors are not directly involved in the regulation of acid secretion in the rat isolated stomach.

These results suggest that the rat isolated stomach contains β-adrenoceptors which, when activated, lead to acid secretion by a mechanism independent of cholinergic or histamine H_2-receptors. This further suggests that the inhibitory effect of β-adrenoceptor agonists *in vivo* must be an indirect action mediated by some other agent capable of inhibiting the direct effect of β-adrenoceptor agonists in the stomach.

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CONTENTS

		Page No.
Abstract		3
Acknowledgements		5
Contents		6
Chapter 1	Introduction: The Control of Acid Secretion	14
1.1	Histamine	15
1.2	Gastrin	16
1.3	Acetylcholine	17
1.4	Other possible physiological regulatory agents	19
1.5	Interdependence of the actions of secretagogues	20
1.6	Intracellular mediators of secretagogue action	23
Chapter 2	Introduction: The Action of Sympathomimetic Drugs and their Antagonists on Gastric Acid Secretion	28
2.1	Introduction	29
2.2	Alpha and Beta receptors	31
2.3	The gastric circulation	33
2.4	Sympathetic innervation of the gastric mucosa	35
2.5	The effect of naturally occurring catecholamines on gastric acid secretion	38
2.5.1	Experiments using conscious animals	38
(a)	Studies in the dog	38
(b)	Studies in man	39
(c)	Summary of studies in conscious animals	40
2.5.2	Experiments using anaesthetised animals	41
(a)	Studies in the dog and cat	41
(b)	Studies in the rat	43

		Page No.
2.5.3	Studies involving measurement of acid secretion and gastric mucosal blood flow	43
(a)	Noradrenaline	43
(b)	Adrenaline	44
2.5.4	Summary of studies using adrenaline and noradrenaline	45
2.6	The effect of β-adrenoceptor agonists on gastric acid secretion	46
2.6.1	Pentagastrin-stimulated acid secretion	46
(a)	Conscious dog	46
(b)	Conscious rat	48
2.6.2	Histamine-stimulated acid secretion	49
(a)	Conscious dog	49
(b)	Conscious rat	50
2.6.3	Studies in man	50
2.6.4	Summary	50
2.7	The effects of β-adrenoceptor antagonists alone on gastric acid secretion in conscious animals	51
(a)	Effects on pentagastrin-induced and basal secretion	51
(b)	Effects on histamine-stimulated acid secretion	53
2.8	The effects of β-adrenoceptor agonists and antagonists on gastric acid secretion in anaesthetised animals	54
(a)	Studies in the rat	54
(b)	Studies in the dog and cat	55
(c)	Summary	56
2.9	Aims of the project	56

		Page No.
Chapter 3	Methods	58
3.1	Rat isolated stomach	59
3.1.1	Introduction	59
3.1.2	Choice of immature rat	63
3.1.3	Isolated stomach or isolated mucosa?	63
3.1.4	Dietary pretreatment	65
3.1.5	Method for sacrifice of rat	66
3.1.6	Dissection and setting up of the stomach	66
3.1.7	Composition of salines	70
3.1.8	Gassing of the bathing solutions	70
3.1.9	Temperature	71
3.1.10	Experimental protocol	71
3.1.11	Measurement of acid secretion	72
(a)	pH	72
(b)	Titration	72
(c)	Use of 20mM NaOH	72
(d)	Determination of pump rate	73
(e)	Calculation of acid content	73
3.1.12	pH measurement or titration for the measurement of acid secretion?	75
(a)	Continuous flow pH recording	75
(b)	Titration	76
(c)	Non-HCl secretion	76
3.1.13	Expression of results: Secretory ratio	79
3.1.14	Drugs	80
3.1.15	Statistical analysis	81
3.2	Heidenhain pouch rat	81
3.2.1	Introduction	81

		Page No.
3.2.2	The rat as the experimental species for gastric acid secretion studies	83
3.2.3	Use of a conscious animal in preference to an anaesthetised animal	84
3.2.4	Operative technique	85
3.2.5	Post-operative feeding and care	87
3.2.6	Experimental protocol	88
3.2.7	The use of the perfused pouch method for collecting acid secretion	92
3.2.8	Expression of results	94
3.2.9	Drugs	94
3.2.10	Statistical analysis	95
Chapter 4	Results: Rat Isolated Stomach	96
4.1	The effect of salmefamol on gastric secretagogue induced acid secretion	97
4.1.1	The effect of salmefamol on pentagastrin-induced acid secretion	97
4.1.2	The effect of salmefamol on bethanechol-induced acid secretion	98
4.2	The effect of β-adrenoceptor agonists on gastric acid secretion	101
4.2.1	Examples of single responses to β-adrenoceptor agonists	101
4.2.2	Log concentration-response curves	105
4.3	The effect of adrenoceptor antagonists on β-adrenoceptor agonist stimulated acid secretion	108
4.3.1	The effect of non-selective β-adrenoceptor antagonists	109
(a)	Isoprenaline-stimulated acid secretion	109
(b)	Salmefamol-stimulated acid secretion	116

		Page No.
4.3.2	The effect of β_2-selective adrenoceptor antagonists	120
(a)	Isoprenaline-stimulated acid secretion	120
(b)	Salmefamol-stimulated acid secretion	124
4.3.3	The effect of a β_1-selective adrenoceptor antagonist	124
(a)	Isoprenaline-stimulated acid secretion	124
(b)	Salmefamol-stimulated acid secretion	127
4.4	The effect of β-adrenoceptor antagonists on basal acid secretion	127
4.5	The effect of the naturally occurring catecholamine, noradrenaline, on gastric acid secretion	128
4.6	The effect of β-adrenoceptor antagonists on noradrenaline-stimulated acid secretion	133
(a)	Non-selective β-adrenoceptor antagonists	133
(b)	A β_1-selective adrenoceptor antagonist	133
(c)	A β_2-selective antagonist	137
4.7	Summary	137
4.8	Other antagonists of gastric acid secretion	139
(a)	Atropine	139
(b)	Metiamide	139
4.9	The effect of metiamide and atropine on basal acid secretion	141
4.10	Possible non-specific effects of propranolol	142
(a)	The effects of propranolol on pentagastrin- and bethanechol-induced acid secretion	142
(b)	The effect of (+) propranolol on isoprenaline-stimulated acid secretion	144
4.11	The effect of α-adrenoceptor agonists on gastric acid secretion	146

		Page No.
4.12	The effect of an α-adrenoceptor agonist on the response to pentagastrin, histamine and bethanechol	146
4.13	The effect of α-adrenoceptor antagonists on sympathomimetic-stimulated acid secretion	150
(a)	Isoprenaline-stimulated acid secretion	150
(b)	Noradrenaline-stimulated secretion	150
Chapter 5	Results: Heidenhain Pouch Rat	153
5.1	Introduction	154
5.2	Spontaneous secretion in the Heidenhain pouch rat	154
5.3	Pentagastrin-stimulated acid secretion	155
(a)	Preliminary studies	155
(b)	The effect of pentagastrin on acid secretion in the Heidenhain pouch rat	156
5.4	Expression of results in Heidenhain pouch rat experiments	161
5.5	Choice of doses of β-adrenoceptor antagonists	162
5.6	The effects of isoprenaline alone and in the presence of β-adrenoceptor antagonists on pentagastrin-stimulated acid secretion	166
5.6.1	Isoprenaline	166
5.6.2	Propranolol + isoprenaline	166
5.6.3	Practolol + isoprenaline	166
5.6.4	Butoxamine + isoprenaline	169
5.6.5	An example of the effects of isoprenaline alone and in the presence of β-adrenoceptor antagonists in one rat	169
5.7	The effect of β-adrenoceptor agonists on pentagastrin-stimulated acid secretion in the Heidenhain pouch rat	172
5.7.1	Propranolol	172
5.7.2	Practolol	172
5.7.3	Butoxamine	177

		Page No.
5.7.4	(+) propranolol	175
5.7.5	The effects of β-adrenoceptor antagonists on the response to pentagastrin in single Heidenhain pouch rats	175
5.8	Summary	180
Chapter 6	Discussion	181
6.1	Studies in the rat isolated stomach	182
6.1.1	Action of β-adrenoceptor agonists	182
6.1.2	Possible mechanisms of action of sympathomimetics on gastric acid secretion in vitro	183
(a)	Release of endogenous histamine	183
(b)	Release of endogenous gastrin	185
(c)	Release of endogenous acetylcholine	187
(d)	Action of β-adrenoceptor antagonists	189
6.1.3	Evidence for β-adrenergic activation of a gastric mucosal adenylate cyclase/cAMP system	193
6.1.4	α-adrenoceptor effects on acid secretion	195
6.2	Studies in the Heidenhain pouch rat	197
6.2.1	The effect of isoprenaline	197
6.2.2	Mechanism of action of isoprenaline	198
(a)	Inhibition of histamine release or production	199
(b)	β-agonists and gastrin release	200
(c)	Effects on gastric mucosal blood flow	201
(d)	Hyperglycaemic effects of β-agonists	202
(e)	Central nervous effects of isoprenaline	204
(f)	Release of endogenous inhibitory agents by isoprenaline	204
(i)	5-hydroxytryptomine	205
(ii)	Somatostatin	208
(g)	Conclusion	209

		Page No.
6.2.3	Mechanism of action of the potentiation of pentagastrin-stimulated secretion by propranolol	209
6.2.4	General conclusion	213
6.3	Further experiments	216
Appendix I	Drug doses used in references quoted in Chapter 2	219
Appendix II	Statistical analysis of maximum responses shown on Figure 13	227
References		232
Index of Tables and Figures		275

CHAPTER 1

INTRODUCTION: THE CONTROL OF ACID SECRETION

The regulation of gastric acid secretion is one of the most complex physiological processes in the body. The control of acid secretion is mediated by chemical messengers which arrive at the target, the parietal cell, by one of three modes: neurocrine, paracrine or endocrine. Neurocrine transmission spans the very short distance of the neuroeffector junction and neurocrine effects can directly activate the parietal cell to secrete acid by release of acetylcholine from postganglionic fibres. Paracrine transmission occurs by diffusion of the chemical messenger through the intercellular space from the cell of origin to its target. The concept of paracrine secretion and its role in acid secretion remains hypothetical, but there is little doubt that locally secreted histamine has a major effect on parietal cell function. Endocrine transmission occurs via the circulation. Gastrin is the only hormone known to stimulate the parietal cell under physiological conditions. Thus, the three major secretagogues which are generally regarded as being of physiological importance are histamine, gastrin and acetylcholine. These secretagogues have been shown to be effective in both *in vitro* and *in vivo* studies in many species, including man.

1.1 Histamine

Histamine is present in large quantities in mammalian and human gastric mucosa (Troidl, 1975). It appeared in the upper gastrointestinal tract at the same point in the phylogenetic tree that the ability to secrete acid was acquired (Reite, 1972). Early studies on histamine showed it to be a potent stimulant of acid secretion (Keeton *et al.*,1920; Popielski, 1920) and subsequent studies have shown that histamine acts at H_2 receptors in the gastric mucosa (Black *et al.*, 1972). There is, however, some disagreement

concerning the cellular localisation of histamine. In man, rabbit, cat, dog, pig and hamster, Hakanson and co-workers (1969, 1970) reported that gastric mucosal histamine was stored only in mast cells. Gastric histaminocytes, although morphologically indistinguishable from mast cells seem to be physiologically distinct from perivascular mast cells. Perivascular mast cell histamine but not gastric mucosal histamine is released by compound 48/80 and by antigen-antibody reactions (Lorenz et al.,1969). In the rat and the mouse the majority of the histamine in the gastric mucosa is found at the base of the gastric glands (Thunberg, 1967; Kim and Glick 1968; Hakanson 1970). Hakanson (1970) reported that in the rat histamine is present in enterochromaffin-like cells although Pentilla and Hinoven (1969) claimed that the histamine was stored in basally located parietal cells.

1.2 Gastrin

Gastrin is the peptide hormone most clearly identified as an important stimulant of gastric acid secretion. The principal source of gastrin is the mucosa of the pyloric gland area. Gastrin is apparently synthesised by the G-cells which are most abundant in the mid-portion of the pyloric glands,and can be identified by immuno-histochemical techniques as well as by their typical appearance in electron microscopy. (Solcia et al., 1975). G-cells show cytoplasmic granules, in which the hormone already formed is stored and then released upon stimulation. The two main biologically active forms of gastrin in the pyloric gland mucosa are little gastrin and big gastrin (Walsh 1977). The potency of G-17 is six to eight times that of G-34 and it seems that G-17 is the principal biologically active form of gastrin in the circulation. Both G-17

and G-34 exist in sulphated and non-sulphated forms but sulphation does not affect acid stimulating activity. All mechanisms of gastrin release appear to operate through chemicals which act directly on G-cells, such as peptides and amino acids in contact with the gastric mucosa. The specific amino acids that are the most potent gastrin releasers are tryptophan and phenylalanine and these amino acids are the most potent stimulants of acid secretion (Byrne et al.,1977). There is evidence however that certain amino acids have acid stimulating effects independent of circulating gastrin levels.

Gastrin release is suppressed by acidification of the antral mucosa, an apparent example of a negative feedback relationship between gastrin release and gastric acid secretion. Conversely hypochlorhydria or achlorhydria have been associated with elevation of the fasting gastrin level and increased capacity to release gastrin in response to stimuli such as food.

1.3 Acetylcholine

Vagal release of gastrin is mediated by acetylcholine, the third major secretagogue, which is released by the postsynaptic nerve endings of the parasympathetic nervous system in the gastric mucosa. The nerves that innervate the gastric glands are post-ganglionic fibres that originate in ganglia located in submucosal and muscular layers of the stomach. The neuronal path can be activated both by vagal efferent fibres and by local reflex arcs within the wall of the stomach (Grossman 1967, 1978). Thus the cholinergic intramural or vago-vagal reflexes both release acetylcholine which either directly stimulates the parietal cells, or stimulates the G-cells to release gastrin. The effects mediated

by the vagal efferents and local reflex arcs are inhibited by muscarinic cholinergic antagonists such as atropine. The view that the postganglionic neuronal pathway utilises cholinergic transmitters is supported by reports that exogenous cholinomimetic drugs produce the same maximal response in laboratory animals as vagal stimulation, gastrin or histamine. In man, however, there is a smaller response to cholinomimetics (Roland et al., 1975). These observations in man may reflect cholinergic inhibitory pathways or non-cholinergic vagal efferent fibres.

Vagal release of gastrin in man also appears to be less important than in other species (e.g. dogs and rats). In the dog vagal excitation by sham feeding or insulin causes release of gastrin that is abolished by vagotomy and blocked by atropine (Tepperman et al., 1972; Csendes et al., 1972). Also in the dog, topical application of acetylcholine to the antral mucosa stimulates gastrin release (Robertson et al., 1950) but systemic administration of cholinomimetics is only weakly effective (Sjodin and Nelson 1973). In man, the existence of a cholinergic mechanism for gastrin release has not yet been established, either by showing that cholinomimetics release gastrin or by showing that atropine blocks some forms of gastrin release. In fact atropine, in man, enhances rather than inhibits gastrin release in response to feeding and insulin, even when intragastric pH is kept at a constant level. The serum gastrin response to insulin hypoglycaemia in humans is maintained after vagotomy (Christensen et al., 1976), and there is evidence that this form of gastrin release is mediated by circulating adrenaline (Christensen and Stadil 1976). The question remains as to whether the vagal release of gastrin in man is non-cholinergic or cholinergic, but atropine resistant.

1.4 Other Possible Physiological Regulatory Agents

There are numerous endogenous agents which have been found, under experimental conditions, to inhibit acid secretion in various species. Those that are present within the gastric mucosa may act as paracrine messengers whereas other agents which are released into the blood stream from other areas of the gastrointestinal tract such as the duodenum would act as endocrine messengers. Potential inhibitors that have been found in gastric mucosa are somatostatin (Konturek, 1976; Pearse et al., 1977), the prostaglandins (Robert, 1976; Main and Whittle1976), glucagon (Pearse et al, 1977, Konturek et al.,1975), dopamine (Valenzuela, 1976), and 5HT (Jaffe et al., 1977).

Vasoactive intestinal peptide (VIP) has been localised to nerve terminals in the gastric mucosa and has been proposed as a potential inhibitory neurotransmitter with neurocrine actions (Pearse et al.,1977).

The presence of fat in the duodenum has been shown to inhibit acid secretion in man. This fat-induced inhibition of gastric secretion is usually regarded as an endocrine mechanism since this can be elicited in vagally-denervated canine pouches. There are several likely candidates for this function. Somatostatin (Konturek, 1976), 5HT (Jaffe et al.,1977) and GIP (Pedersen et al.,1972) have all been proposed as mediators of fat induced inhibition of acid secretion. Neurotensin has also been shown to be released by oleic acid from the duodenum in man (Kihl et al., 1980). CCK has been shown to be released by fat in the duodenum, but this hormone cannot be solely responsible for inhibition by fat, since fat in the duodenum inhibits the Heidenhain pouch response to histamine, an effect which cannot be reproduced by CCK (Konturek, 1979).

Several of these peptides present in the gut also inhibit gastrin release. Somatostatin, which is present in the pyloric mucosa has been shown to be a potent inhibitor of gastrin release (Vat n et al., 1977; Philip et al., 1977). It is thought that it may mediate gastrin release by a local action on gastrin cells. Gastrin secreting (G) cells and somatostatin secreting (D) cells are located in antral mucosa at variable distances from each other in different species (Alumets et al., 1977; Helstaedler et al., 1977; Larsson et al., 1979). In rat and man somatostatin secreting cells extend dendritic like cytoplasmic processes that are in close apposition with gastrin secreting cells (Larsson et al., 1979). Saffouri and co workers (1980) have hypothesised that endogenous gastric somatostatin secretion exerts a continuous restraint on basal gastric secretion and that stimulation of gastrin secretion by cholinergic agents is mediated, in part, by inhibition of somatostatin.

In conclusion, the observation that the secretion of acid increases after food ingestion and is virtually abolished during the interdigestive period forms a basis for the theory that gastric secretion depends on the interplay of many stimulatory influences which arise within the central nervous system, the stomach and the intestines, and that inhibitory and stimulatory processes occur and interact continuously.

1.5 Interdependence of the Actions of Secretagogues

The relationship between histamine and the other two secretagogues has been of interest to gastroenterologists for many years and the interaction of these secretagogues is still

a controversial topic. For many years the main obstacle to investigation of this problem was the lack of a specific antagonist of the effect of histamine on gastric secretion. The discovery that this effect was mediated via a second type of histamine receptor, the H_2 receptor, and the development of specific H_2 antagonists such as cimetidine (Brimblecombe et al.,1975) gave investigators a tool with which to study the role of histamine in gastric secretion. No specific gastrin antagonist has been developed,which also hinders the study of secretagogue interdependence.

H_2 antagonists can block histamine stimulated gastric secretion in all species studied including man (Brimblecombe and Parsons, 1977). However, the secretory response to gastrin is also effectively blocked by H_2 antagonists, as are all other secretory stimulants in vivo, such as stable choline esters, 2-deoxyglucose and food. Since cimetidine is a specific antagonist at H_2 receptors the role of histamine in gastric secretion is clearly important.

Two major theories have been proposed to explain the role of histamine in secretagogue interactions. Code (1965), among others, proposed the "common mediator hypothesis" based on that initially put foreward by McIntosh (1938). This claims that secretagogues, other than histamine itself, do not act directly on the parietal cell but via the release of endogenous histamine. Observations such as those on the release of histamine into the gastric juice during vagal stimulation (McIntosh, 1938) and histamine turnover studies (Kahlson et al, 1964) culminated in this hypothesis. This theory does not, however, explain why metiamide is unable to inhibit acetylcholine induced acid

secretion *in vitro* (Bunce et al, 1976), although the responses to histamine and gastrin are inhibited. Rangachari (1979), using the isolated primate gastric mucosa, has confirmed that the secretory responses to histamine and pentagastrin are inhibited by metiamide but that those to acetylcholine are resistant. This would suggest that there are H_2 and acetylcholine receptors on the parietal cell, but that gastrin acts indirectly via endogenous histamine.

In an attempt to demonstrate separate receptors for the three major secretagogues on the parietal cell the isolated parietal cell and isolated gastric gland preparations were developed (Soll, 1978, Berglindh 1979). Gastric acid secretion cannot be measured directly in these preparations as for *in vitro* and *in vivo* preparations, however the rate of oxygen consumption and the accumulation of the weak base aminopyrine by the cells were considered to reflect parietal cell function. While these preparations have an advantage over *in vivo* and *in vitro* studies in that drugs have direct access to receptors, the interpretation of results must be made with two reservations. Firstly, the isolated parietal cell is far removed from its normal regulatory physiological environment. Secondly, the separation procedures required may alter or destroy receptors. The validity of using aminopyrine uptake as a direct measure of acid secretion has also been questioned since the parietal cell may become saturated with aminopyrine at a point where maximum secretory capacity has not been reached. However, isolated parietal cell studies have been helpful in the investigation of secretagogue interaction.

Recently Soll (1978), using isolated canine parietal cells has demonstrated a response to gastrin that is refractory to inhibition by H_2 receptor antagonists, and Parsons et al, (1980) have provided evidence for a small but significant part of the secretory response to gastrin which is independent of an involvement of histamine. Grossman and Konturek (1974) proposed a second theory known as the "interacting receptor hypothesis" which suggests that histamine, gastrin and cholinergic agents each act directly on specific receptors at the parietal cell itself. Soll (1978) has demonstrated that distinct receptors for histamine, acetylcholine and gastrin exist on the isolated canine parietal cells and that potentiating interactions occur between histamine and acetylcholine, histamine and gastrin, but not between acetylcholine and gastrin. He has modified Grossman's hypothesis and suggests that the constant threshold background of histamine in vivo serves to potentiate the effect of the other two secretagogues. The apparent non-specificity of H_2 blockers in vivo is explained by the abolition of the potentiating effects of histamine on gastrin or acetylcholine.

Although there is, in balance, more favourable evidence for separate receptors for histamine and acetylcholine and probably gastrin the controversy still remains unresolved.

1.6 Intracellular Mediators of Secretagogue Action

There is strong evidence that cyclic 3'5' adenosine monophosphate (cAMP) is an intracellular mediator of gastric acid secretion in amphibian gastric mucosa. Nakajima and co workers (1971) found that pentagastrin and histamine were stimulators of adenylate cyclase. Exogenously applied cAMP was found to stimulate H^+ and Cl^-

transport (Harris and Alonso 1965). Furthermore phosphodiesterase inhibitors such as methylxanthines produced an increase in the mucosal content of cAMP which preceded the secretory response. (Harries et al., 1969).

In the mammal there is conflicting evidence as to the involvement of cAMP in the regulation of acid secretion. Studies in vivo have shown that, in the rat, stimulation by pentagastrin, histamine or cholinergic agents is accompanied by an increase in mucosal cAMP content (Ruoff and Sewing 1973, 1974; Domschke et al., 1972). In the dog, in vivo, there have been reports that none of the major secretagogues stimulate gastric mucosal cAMP (Mao et al., (1973; Tague et al., 1977). However, Bieck et al.,(1973) have found that histamine did increase the cAMP content of canine gastric mucosa. The interpretation of in vivo studies on cAMP levels has recently been questioned by Ruoff (1977) who reported a rapid postmortem rise in gastric mucosal cAMP in the rat. This was blocked by propranolol. Adrenalectomy prevented the post mortem rise in cAMP, therefore it seems likely that, in vivo, circulating adrenaline can cause a rise in gastric mucosal cAMP, and for this reason interpretation of in vivo results must be made with this fact in mind.

Therefore, in vitro studies would seem preferable for investigating intracellular mediators of acid secretion. Stimulation of gastric secretion by db cAMP has been reported in the rat isolated stomach (Bunce et al.,1976), and the rabbit isolated mucosa (Fromm, Schwartz and Quijano, 1975). Phosphodiesterase inhibitors also stimulate acid secretion in the rabbit isolated mucosa (Fromm et al.,1975) and in the guinea pig isolated stomach (Holton & Spencer, 1975). Furthermore, in the guinea pig a mucosal adenylate cyclase

has been shown to be stimulated by histamine (Perrier and Laster, 1970). This was confirmed by Canfield et al. (1977) who found that histamine stimulated both acid secretion and cAMP formation in the guinea pig isolated stomach.

Broken cell preparations have also been used to study cAMP and gastric acid secretion. Histamine has been shown to increase adenylate cyclase activity in guinea pig broken cell preparations (Perrier and Laster, 1970) and dog cell preparations (Dozois et al., 1977), although this result was not obtained by Thompson's group (1977 a) in the dog. Thompson and co workers (1977 b) have also reported that histamine did not activate adenylate cyclase from the rat gastric mucosa broken cell preparation although other workers such as McNeill and Verma (1974) showed histamine activation of rat broken cell adenyl cyclase.

Many workers have failed to show that secretagogues other than histamine affect adenylate cyclase activity in mammalian gastric mucosa in vitro. However, recently there has been a report of a gastrin sensitive adenyl cyclase in rat by Lewin and his associates (1976, 1977) although this enzyme preparation is not affected by histamine. The failure of other secretagogues to stimulate cAMP in vitro may be interpreted in several ways. The gastrin and acetylcholine receptors may be damaged by the homogenisation and centrifugation, however it seems unlikely that histamine receptors would escape unaffected. Furthermore gastric plasma membranes prepared from rat fundic mucosa bind ^{3}H-labelled gastrin (Lewin et al, 1977) which shows that gastrin receptors are still present but not necessarily viable. Secondly, gastrin and acetylcholine may act via a different intracellular mediator such as calcium ions.

Alteration of calcium fluxes across the plasma membrane or alteration in the membrane content of cellular calcium may play a role in the responses to carbachol and gastrin, although the role of calcium in the control of gastric secretion is not fully understood. In some reports removal of calcium has no effect on the acid response to gastrin (Bunce, Honey and Parsons, 1979) while in others the response is potentiated (Main and Pearce, 1977) or diminished (Black and Welch, 1977). Bunce et al.,(1979) have shown that the acid response to acetylcholine was dependant on the extracellular calcium concentration in the rat isolated stomach although this contrasts with the results of Main and Pearce (1977 b) using methacholine in the rat gastric mucosa preparation. Further support of the view that cholinergic stimulation does not utilise cyclic nucleotides as intracellular mediators comes from the observation that bethanechol did not stimulate either cAMP or cGMP in the guinea pig isolated stomach (Canfield et al., 1979).

The use of the isolated parietal cell preparation has been of help in studying the problem of intracellular mediators of acid secretion. In the canine isolated parietal cell preparation histamine but not carbachol or gastrin stimulated cAMP production (Soll and Wollin, 1977). In cell separation experiments this effect of histamine was localised to the parietal cell itself. The dose-response relation for histamine stimulation of cAMP production correlated closely with histamine stimulation of the parietal cell acid secretory mechanisms (as indicated by stimulation of both oxygen uptake and amidopyrine accumulation.) Phosphodiesterase inhibitors such as IBMX produced similar potentiation of histamine stimulation of amidopyrine uptake and histamine stimulation of cAMP production. In contrast,

Ray, Jacobson and Thompson (1977) reported that histamine, pentagastrin and carbachol do not alter cAMP levels in isolated rat parietal cells.

In summary, it seems possible that cAMP is the intracellular mediator for histamine stimulated acid secretion, although some reports do not substantiate this. There is no solid candidate yet for acetylcholine or gastrin stimulated secretion, however calcium may be involved in the pathway of acetylcholine stimulated secretion.

CHAPTER 2

INTRODUCTION: THE ACTION OF SYMPATHOMIMETIC DRUGS AND THEIR ANTAGONISTS ON GASTRIC ACID SECRETION

2.1 Introduction

The effects of catecholamines on gastric secretion have been studied since extracts of the adrenal medulla were first used at the beginning of this century. However, the actions of sympathomimetic drugs on gastric acid secretion is still a matter of some controversy. Similarly, although it is generally agreed that section of the sympathetic nerves to the stomach increases acid output and that electrical stimulation of these nerves reduces it, the possible physiological role of the sympathetic nervous system in gastric secretion is not fully understood.

The literature on the action of sympathomimetic drugs on acid secretion contains many contradictions. In 1951 Code summarised the situation in the following way: "The overwhelming impression derived from a review of the literature dealing with the effect of epinephrine on gastric secretion is that it does not have an important action on the secretory process. In some tests it increased gastric secretion, in others it decreased secretion and in still others it had no effect. A systematic search has not been made of its effect on the excitability of the mucosa to other stimuli but such changes as have been observed when it has been given with other stimuli have been small and variable." By 1965 it was established that the effect of catecholamines on gastric secretion was rather larger than had been originally supposed but there was still considerable confusion as to what effect they had. Jacobson (1965) writes "Catecholamines have been among the most widely studied vasoactive substances. The influence of epinephrine and norepinephrine on gastric secretion has received much attention. The actions of these agents on both secretion and blood flow to the stomach are still a matter of some considerable controversy." However, to date, the predominant view is that sympathomimetics have an anti-secretory action *in vivo* (Holton, 1973).

The widely contradictory reports on catecholamine actions on gastric secretion may be accounted for by differences of species, drug dosage, route of administration, and different experimental procedures, such as the use of anaesthesia or vagotomised animals. The situation is further complicated by the effects of these drugs on the cardiovascular system and the interdependence of secretion and blood flow to the mucosa.

The more recent availability of both synthetic agonists and antagonists showing a selective profile of activity at adrenoceptors has allowed some clarification of this situation and the available information may be conveniently considered under three headings:

1. The effects of naturally occuring catecholamines on gastric acid secretion.
2. The effects of β-adrenoceptor agonists on gastric acid secretion.
3. The effects of β-adrenoceptor antagonists alone on gastric acid secretion.

It now seems likely that the mechanism of action of the inhibition of acid secretion by the naturally occuring catecholamines may be secondary to the reduction of gastric mucosal blood flow which they cause (Sanders, 1976). However, in recent years many studies have been carried out on the effects of β-adrenoceptor agonists on acid secretion in vivo. There have been reports of a stimulation of acid, an inhibition or no effect at all. If these drugs are inhibitory, as the main body of evidence suggests, their mechanism of action cannot be explained by an effect on blood flow since β-adrenoceptor agonists have a vasodilator action. These topics will be discussed below.

However, it will be useful to first consider some general characteristics of gastric mucosal blood flow, sympathetic innervation of the gastric mucosa, and the classification of adrenoceptors into α and β types.

2.2 Alpha and Beta Receptors

The framework for our present understanding of adrenergic responses is based on the dual receptor theory of Ahlquist (1948) which was conceived to explain the quantitative differences in response to catecholamines. The classification of adrenoceptors into α and β types is now well accepted and highly selective agonists and antagonists of α and β receptors are known. The large body of evidence allowing pharmacological classification of adrenoceptors has created an illusion of their morphological reality, however although the nature and localisation of α and β-adrenoceptors are still unknown, a strong implication of functionally and morphologically distinct, well defined, static membrane structures has been inherent in many studies. In the past few years a more dynamic image of receptors with molecular plasticity has emerged. Kunos (1976) suggested that the balance of the two forms of receptor may be important in the regulation of tissue responsiveness to various stimuli under different physiological and pathological conditions.

The existence of distinct β-adrenoceptor receptor subtypes was first postulated by Lands and co workers (1967). The classification of β_1 (cardiac), and β_2 (bronchial and vascular) receptors was based on the rank order of potency of a series of drugs eliciting pharmacological responses.

There is now evidence that there are also α-adrenergic receptor subtypes. The term α_1 and α_2 had originally been proposed as synonyms for the post and presynaptic α-receptors (Langer, 1974). Presynaptic α-receptors occur at all noradrenergic terminals where they have been sought. It soon became clear that the presynaptic α-receptors differed from the classical postsynaptic receptor in their sensitivity to drugs. More recently there has been evidence for the existence of similar receptors to the α_2 type other than on noradrenergic neurones, for instance in cholinergic neurones where α_2-receptors mediate inhibition of acetylcholine release (Drew, 1978). This has made it necessary to use the prefixes of α_1 and α_2 solely for receptors with different sensitivities to drugs, regardless of location or function (Starke and Langer, 1979).

With the distinction between α and β-receptors, it became clear that noradrenaline acts mainly on α-receptors, isoprenaline mainly on β receptors and that adrenaline acts on both receptors. β_1-adrenergic receptors have approximately the same affinity for adrenaline and noradrenaline, whereas β_2-receptors have a greater affinity for adrenaline than noradrenaline (Minneman and Molinoff, 1980).

These classifications have, however, been developed for receptors in other systems than that in this study, and it may be that hypothetical receptors in the gastric mucosa will not necessarily fall into any of the established categories.

2.3 The Gastric Circulation

When interpreting the effect of a drug on gastric acid secretion *in vivo* it is necessary to take account of any influence it may have on blood flow within the gastric mucosa. Although there is evidence that gastric secretion can be supported by an anaerobic mechanism (McQuarrie & Vennes, 1969) any long-lasting energy consuming process depends to a large extent on an adequate supply of substrates and maintenance of a suitable environment which can only occur during sufficient capillary blood flow. It is well established that gastric mucosal blood flow and gastric secretion are linked (Swan and Jacobson, 1967; Bynum and Jacobson, 1971). An increase in gastric secretion is always accompanied by an increase in mucosal blood flow. However, blood flow does not itself *cause* gastric secretion (Cowley and Code, 1969) although it can increase secretion if this has been limited by an inadequate blood supply.

Blood vessels in parallel with the mucosal capillaries have been recognised for some years. In 1951 Barlow and co workers described submucosal arteriovenous anastomoses in the human stomach, and on the basis of glass microsphere injection studies Walder (1952) concluded that these shunts play a major role in the control of gastric mucosal blood flow. Normally, shunt resistance is higher than mucosal arteriolar resistance and most of the blood flows through the mucosa. In the presence of agents or stimuli which constrict the arterioles mucosal arteriolar resistance increases and more blood passes through the shunts. However, the existence of shunts has been questioned by investigators using radioactive labelled microspheres. When Delaney and Grim (1964) injected microspheres into the left gastric artery in the dog little or none of the microspheres were recovered in

the venous effluent. In similar studies Shoemaker and Powers (1966) and Archibald et al., (1974) did not recover any spheres in the venous outflow. Guth and Rosenberg (1972) using a visual, image-splitting technique in the rat showed completely separate arterial and venous plexi without any shunts. They concluded that the submucosal arterioles are the major resistance vessels in the gastric microcirculatory bed. Constriction of these vessels would result in an increased resistance and a decrease in mucosal blood flow, while dilatation of these vessels decreases resistance and increases mucosal blood flow. Guth and Rosenberg (1972) suggested these vessels were under both nervous and humoral control. The parasympathetic nerves subserve vasodilation while the sympathetic nerves subserve vasoconstriction. There are various humoral agents to which the gastric vasculature is sensitive. Of these, catecholamines and histamine are thought to be important with catecholamines constricting the vessels and histamine causing vasodilatation and changes in vascular permeability.

Two further factors which are thought to alter gastric mucosal blood flow are changes in capillary resistance and variations in portal venous pressure. Jansson and co workers (1966, 1970) have shown that, in the cat, there are both precapillary and postcapillary resistance vessels and that catecholamines constrict both postcapillary and precapillary vessels. Since the gastric circulation drains into the splenic vein any change in portal venous pressure will alter blood flow to the stomach. There is evidence for the existence of both vasodilator and vasoconstrictor actions of catecholamines in the hepatic circulation (Goodman and Gillman, 1970; Ross and Kirassch, 1969) and that catecholamines increase splenic venous pressure (Jacobson, 1963; Holton, 1973).

Therefore, there is a close association between the gastric mucosal circulation and parietal cell function. The mucosal circulation must also be considered in conjunction with the sympathetic innervation of the mucosa since this is thought to play a major part in the control of mucosal blood flow.

2.4 Sympathetic Innervation of the Gastric Mucosa

The sympathetic innervation of the stomach derives from the sixth to tenth spinal nerves (Loeweneck, 1974). Sympathetic preganglionic efferent fibres destined for the stomach form the greater splanchnic nerves which pass through the diaphragm to enter the abdomen and terminate in the coeliac ganglia from whence the postganglionic fibres reach the stomach. Synapses between pre- and post-ganglionic fibres also occur beyond the coeliac ganglion on the gastric arterial walls. Most of these fibres accompany the gastric arteries and mix with vagal fibres in the gastric wall.

It is generally agreed that sympathetic nerve section increases the output of gastric acid (Shafer and Kittle, 1951; Oberhelman et al.,1951; Brucknër et al.,1971; Kasuya et al.,1977). Several groups of early workers, however, produced contrary results (Thomsen, 1924; Heslop, 1938). Forrest and Code (1954) concluded that "the sympathetic nerves which reach the stomach have no direct effect on the secretory mechanism involved in the production of acid and pepsin by the gastric mucosa." Brucknër (1974) however showed that sympathetic nerve section increased acid output in both the cat and Shay rat and concluded that the

sympathetic nervous system exerts "a depressing and moderating influence on gastric secretion." Furthermore he suggested that the splanchnic nerves exert a vasoconstrictor effect on the gastric vasculature which decreases acid secretion. Electrical stimulation of the splanchnic nerves reduces gastric activity. This has been shown for gastric muscle activity (Reed and Sanders, 1971), gastric acid secretion (Reed et al.,1971) and gastric mucosal blood flow (Reed and Sanders, 1971; Thompson and Vane, 1953). Gastric oxygen consumption is also inhibited (Grund et al, 1975). There has also been evidence for a possible involvement of the sympathetic nervous system in regulating serum gastrin levels. Matsuo and co workers (1974) showed that splanchnectomised dogs have larger serum gastrin responses to feeding as well as increased acid and pepsin responses. Blair and co workers (1975) have shown that electrical stimulation of the sympathetic nerve in the rat reduces the serum gastrin response to food in antral pouches and also inhibits the simultaneously occuring increase in acid secretion and mucosal blood flow. Mucosal blood flow is consistently reduced by sympathetic nerve stimulation (Reed et al.,1971; Grund et al, 1975). Stimulation of the splanchnic nerves in anaesthetised cats only reduced the acid, pepsin and mucosal blood flow responses to vagal stimulation if the normal rise in arterial blood pressure was prevented by inclusion of a pressure resevoir in the circulation. This suggests that the rise in systemic arterial pressure counteracts the splanchnic induced reduction of secretion, suggesting a vasoconstrictor action of sympathetic nerves, but not the agent responsible.

The mechanism of action of the sympathetic nerves to the stomach does not seem to involve circulating adrenaline since the degree of inhibition of acid secretion was not affected by adrenalectomy. (Reed, Sanders and Thorpe, 1971). However, the inhibition was reversed by guanethidine. This suggests that the inhibitory pathway is dependent on noradrenaline release from sympathetic nerve terminals in the gastric mucosa. The range of stimulants against which the sympathetic nerves exert an inhibition also suggests that the inhibition is directed at points after the inhibition of parietal, peptic and G cell receptor mechanisms.

Grund and co workers (1975) measured gastric oxygen consumption during sympathetic nerve stimulation in an attempt to test for a direct antisecretory effect of nerve stimulation and found that oxygen consumption decreased in parallel with acid secretion and that there was no change in oxygen extraction. If the sympathetic nerves inhibit gastric secretion by a vasoconstrictor mechanism the parietal cells may attempt to extract more oxygen from the circulation to enable continued secretion. This was not the case, which would suggest an antisecretory action on the parietal cell. Sanders (1976), however, suggested that the parietal cell may be extracting maximal oxygen already and that no further extraction was possible. Clarification of this point must await further evidence. Further support for a vasoconstrictor effect of the sympathetic nerves comes from microanatomical evidence. The small arterioles at the base of the cat gastric mucosa have been reported to be sympathetically innervated (Furness, 1971). Direct observation of the small arterioles which make up the submucosal plexus of the rat gastric mucosa showed that they were reduced in diameter

on sympathetic nerve stimulation (Guth and Smith, 1974) and these blood vessels are known to be sympathetically innervated (Norberg, 1967).

In summary, it seems that the sympathetic nerves supplying the stomach can inhibit gastric mucosal function. The mechanism may depend on a vasoconstrictor action of the sympathetic nerves.

2.5 The Effect of Naturally Occuring Catecholamines on Gastric Acid Secretion

Doses of sympathomimetic agonists and antagonists used in the references which are quoted are shown in Appendix I.

2.5.1 Experiments using Conscious Animals

(a) Studies in the Dog

The first direct evidence of an inhibitory effect of catecholamines on gastric acid secretion was published by Hess and Gundlach (1920). They found a decrease in the amount of acid secreted from Pavlov pouch dogs in response to feeding following intramuscular injection of an extract of adrenal glands. Such extracts were unpurified and contained both noradrenaline and adrenaline. Therefore, it was not possible to compare the effects of these two catecholamines in many experiments performed during this period.

The first quantitative study on the inhibitory effect of individual catecholamines was carried out by Forrest and Code (1954) who showed that both adrenaline and noradrenaline produced a dose-related decrease in histamine-induced gastric acid secretion in dogs with Heidenhain pouches. Adrenaline was the more potent of the two compounds. Harries (1956) also showed that noradrenaline

caused a dose related inhibition of histamine-stimulated secretion in gastric fistula dogs. Pradhan and Wingate (1962) found that both catecholamines decreased acid secretion from dogs prepared with Necheles fundic pouches in response to feeding and bethanechol as well as histamine. Many other studies with catecholamines in the conscious dog have confirmed their inhibitory effect in both gastric fistulae and Heidenhain pouch animals (Nicoloff et al., 1964; Holton et al.,1969; Greshiskin, 1970; Jacobson, 1966, 1967, 1970).

(b) Studies in Man

The earliest convincing study on the action of catecholamines on gastric secretion in man was carried out by Moll and Flint (1928) who showed that catecholamines inhibited acid secretion stimulated by a test meal in hyperchlorhydric patients. Bernstein (1952) showed that acid secretion from the resting human stomach was inhibited by adrenaline, whilst Leonsis and Waddel (1958) showed that noradrenaline inhibited acid secretion in response to histamine, hypoglycaemia or food. More recently Christensen and Stadil (1976) have investigated the effect of much lower doses of catecholamines in man, in an attempt to make a more physiological study. They used two doses of both adrenaline and noradrenaline (5 and 50 ng $kg^{-1}min^{-1}$). The low dose of adrenaline and both doses of noradrenaline were considered to be physiologic as Vendsalu (1960) showed that the basal secretion rate of adrenaline in man was 8 ng $kg^{-1}min^{-1}$, and noradrenaline was 18 ng $kg^{-1}min^{-1}$.

The study of Christensen and Stadil showed that the higher dose of adrenaline had no effect on acid secretion, but the low dose stimulated acid secretion. They also measured gastrin release, and these findings will be discussed in Chapter six. Noradrenaline in the low dose caused no change in acid secretion but the higher dose inhibited acid secretion. Prior administration of pindolol, a β-adrenoceptor antagonist completely abolished the effect of both catecholamines on acid secretion. The observation that adrenaline at a low dose stimulated resting secretion in man is interesting since Holton (1973) also reported that occasionally small doses of adrenaline, ($0.2\mu g\ kg^{-1}min^{-1}$) in conscious dogs with Heidenhain pouches, enhanced histamine stimulated secretion.

(c) Summary of Studies in Conscious Animals

The main conclusions to be drawn from studies on conscious animals is that naturally occuring catecholamines in doses of the order of $1\mu g\ kg^{-1}min^{-1}$ i.v. inhibited acid secretion produced by feeding, histamine and cholinergic stimulation in both innervated stomachs and vagally denervated pouches. Higher doses of catecholamines given intramuscularly or subcutaneously have also been shown to have an inhibitory effect. Much smaller doses of adrenaline may stimulate acid secretion. The majority of experiments on conscious animals have been carried out in the dog and in man. Few studies on the effects of adrenaline and noradrenaline have been carried out using the conscious rat, although

Fjalland (1972) reported an inhibitory effect of these catecholamines in the conscious fistula rat. Other studies in the rat have been carried out on anaesthetised preparations and these will be discussed below (2.5.2 (b)).

2.5.2 Experiments Using Anaesthetised Animals

Several studies have been carried out in acute experiments on anaesthetised animals, for either convenience of method or to facilitate a more detailed investigation of the effects of catecholamines on the gastric vasculature. The results of such studies have shown far less agreement than those in conscious animals where there seems to be a clear cut inhibition of acid secretion. Interpretation of results in acute experiments must take into account the effects on acid secretion of laparotomy, manipulation of the stomach and anaesthesia (see Chapter 3). Studies on pylorus ligated rats have been included under this heading since they undergo laparotomy at the start of the experiment, although they are allowed to recover from anaesthesia following ligation.

(a) Studies in the Dog and Cat

It is generally agreed that noradrenaline decreases histamine stimulated acid secretion and total blood flow in the dog. (Cumming et al.,1961; Cumming et al.,1963; Nicoloff et al., 1964). This effect could be prevented by administration of the α-adrenoceptor antagonist phenoxy benzamine (Haigh and Steadman, 1968). In contrast Parrot and Verliac (1941) reported that adrenaline enhanced acid secretion in the dog. Enhancement of histamine

induced acid secretion by adrenaline was also observed in the anaesthetised dog by Holton and Jacoby (1966). Cumming and co workers (1961, 1963) observed that adrenaline always increased total blood flow to the stomach but had a variable effect on acid secretion, either increasing or decreasing it. A decrease in both total blood flow and acid secretion in the presence of adrenaline was recorded by Peter et al (1962). Thompson and Vane (1953) showed that the effect of adrenaline on total stomach blood flow in the cat seemed to be dependent on the dose used, small doses increasing and large doses decreasing the flow. They suggested that low doses stimulated β-adrenergic vasodilator receptors and high doses stimulated vasoconstrictor α-receptors. However, this hypothesis cannot explain the results of Haigh and co workers (1967) who reported that adrenaline increased total gastric blood flow but decreased acid secretion in anaesthetised dogs stimulated with pentagastrin, suggesting perhaps a direct inhibitory effect on acid secretion. Peters and Womack (1958) obtained similar results with adrenaline during histamine stimulated acid secretion. Thus, these various conflicting results of the effects of adrenaline in the anaesthetised animal were associated with uncertainty regarding the relationship between acid secretion and blood flow to the stomach.

The study of Peters and Womack (1958) also showed that adrenaline had dilated the arteriovenous shunts, which suggested that a more precise relationship might be found between acid secretion and mucosal rather than total blood flow. This led to studies on mucosal blood flow rather than whole stomach blood flow, both in anaesthetised and conscious animals, and will be discussed in 2.5.3.

(b) Studies in the Rat

As the rat is the species used in this work it is considered under a separate heading. The first study of the effects of catecholamines in the rat was performed by Bass and Patterson (1967) using the pylorus ligation technique. They found that both noradrenaline and adrenaline inhibited basal secretion. Yamaguchi and co workers (1977), also using pylorus ligated rats found that noradrenaline inhibited basal secretion, however, neither α nor β-adrenoceptors antagonists prevented this inhibition. Tani and co workers (1980) using the Ghosh and Shild perfused rat stomach preparation showed that noradrenaline inhibited tetragastrin, carbamylcholine and histamine stimulated acid secretion. Adrenaline on the other hand, at a lower dose than that used by Bass and Patterson, enhanced acid secretion stimulated by tetragastrin. Thus, results in the rat are very similar to those reported in the dog and cat.

2.5.3 Studies Involving Measurement of Acid Secretion and Gastric Mucosal Blood Flow

(a) Noradrenaline

Noradrenaline has been shown to decrease mucosal blood flow both in the anaesthetised and conscious dog (Delaney and Grim, 1965; Cowley and Code 1969, 1970; Jacobson, 1970). It is still not certain whether its effect in secretion is secondary to decreased blood flow or a direct action on the secretory mechanism. Since a primary reduction of secretion would decrease tissue metabolism it would be expected to be accompanied by decreased mucosal blood flow. Jacobson (1970), working with conscious dogs

calculated the ratio, R, of aminopyrine clearance to secretory volume and showed that it decreased with noradrenaline. Since aminopyrine clearance is a measure of blood flow a fall in R implies a greater effect on blood flow than on secretion and Jacobson concluded that noradrenaline exerted its primary effect on the gastric vasculature.

Reed and co workers (1971) studied aminopyrine clearance and acid secretion in anaesthetised cats (with vagus and splanchnic nerves sectioned) and found that splanchnic nerve stimulation decreased both aminopyrine clearance and acid secretion induced by pentagastrin or stimulation of the peripheral end of the vagus. These workers provided evidence that the effects were not due to stimulation of the adrenal medulla and concluded that they were due to noradrenaline from sympathetic nerve endings. They also pointed out that it is more significant to relate mucosal blood flow to acid secretion rather than to the volume of secretion and recent work on mucosal blood flow has used this ratio in preference to Jacobson's ratio. Curwain and Holton (1972) have shown that 'noradrenaline decreased acid secretion and mucosal blood flow' confirming the earlier reports of Jacobson.

(b) Adrenaline

The effect of adrenaline on mucosal blood flow seems to be less clear cut, for instance Cowley and Code (1969) found an increase in amenophenazone clearance in conscious dogs, whereas Jacobson and co workers (1966) found a decrease in amenophenazone clearance (mucosal blood flow) in the conscious dog using adrenaline.

Thus measurement of mucosal blood flow have suggested that noradrenaline acts primarily at the gastric vasculature to reduce blood flow, whereas the results with adrenaline do not lead to a clear conclusion. This may result from the ability of adrenaline to stimulate both α and β- adrenoceptors.

2.5.4 Summary of Studies Using Adrenaline and Noradrenaline

The results described so far have shown that noradrenaline, a predominantly α-adrenoceptor agonist decreases acid secretion in both anaesthetised and conscious animals. This is possibly secondary to vasoconstriction which reduces gastric mucosal blood flow. The results obtained with adrenaline have been conflicting. Adrenaline stimulates both α and β-adrenoceptors, at high doses stimulating predominantly α-adrenoceptors and low doses predominantly β-adrenoceptors. Therefore analysis of its action at specific receptors is complicated. There are large differences between the effects of adrenaline in conscious and anaesthetised animals and furthermore there has been no clear evidence that adrenaline reduces gastric mucosal blood flow. The effect of catecholamines at α-receptors may be at the vascular level, however, no studies in the isolated stomach have been carried out to investigate the question of a direct action on the mucosa. The question of the site of action of catecholamines which stimulate β-adrenoceptors led to the use of specific β-adrenoceptor agonists such as isoprenaline, and later to the use of specific receptor agonists and antagonists for the two β-adrenoceptor sub-types; β_1 and β_2. This avoided the possibility of stimulating both α and β-receptors when using adrenaline.

2.6 The Effect of β-Adrenoceptor Agonists on Gastric Acid Secretion

β-adrenoceptor agonists have been shown to inhibit secretagogue stimulated gastric acid secretion in the conscious dog and rat. These are described under headings in relation to stimulus used to induce acid secretion; pentagastrin and histamine. Observations on other forms of stimulation of acid secretion are less extensive and are included under headings where appropriate to the work discussed.

2.6.1 Pentagastrin-Stimulated Acid Secretion

(a) Conscious Dog

In the Heidenhain pouch dog isoprenaline inhibited gastric secretion with no change in gastric mucosal blood flow (Curwain and Holton, 1972). Magee (1976) also reported that isoprenaline reduced pentagastrin-stimulated secretion in the dog and that the β-adrenoceptor antagonists propranolol reversed this inhibition. Daly and Stables (1977) using lower doses of isoprenaline than Curwain and Holton (1972) also found an inhibition of pentagastrin-stimulated secretion in the Heidenhain pouch dog.

Studies in the dog were extended to investigate the effect of specific β_2-adrenoceptor agonists on acid secretion by several groups of workers, in order to further characterise the receptor sub-types involved. Curwain, Holton and Spencer (1972) showed that both salbutamol and terbutaline inhibited acid secretion in a dose dependent manner. This effect was abolished by propranolol. The ratio of blood flow to acid

secretion increased markedly in each case indicating that the inhibition by β_2-adrenoceptor agonists was not due to a reduction in mucosal blood flow. The inhibitory effect of salbutamol in the conscious dog was confirmed by Curwain, Fielding and Russel (1974) who found that it inhibited acid secretion in response to feeding.

Daly, Long and Stables (1978) took these studies in the Heidenhain pouch dog one step further by investigating the interactions of the selective antagonist practolol (β_1), H 35/25 (β_2) as well as the non-selective β-antagonist propranolol on the inhibitory effects of salbutamol and isoprenaline. They found that the gastric antisecretory action of salbutamol was significantly blocked by propranolol and H 35/25 but not by practolol. The (+) isomer of propranolol, which has no action at β-adrenoceptors, had no effect on this inhibition. The inhibitory effect of isoprenaline was significantly blocked by propranolol and practolol but not by H 35/25 or (+) propranolol. This suggests that both β_1 and β_2 sub-types can mediate inhibitory effects on acid secretion.

Gottrup and Ornsholt (1978) have reported that salmefamol (a β_2-agonist) inhibited pentagastrin stimulated acid secretion in gastric fistula dogs in a dose dependent manner. This inhibition was prevented by propranolol, but not by practolol. Gottrup, Ornsholt & Andersen (1980) also studied the effect of salmefamol in vagotomised gastric fistula dogs and found that vagotomy did not alter the inhibitory effects found in the earlier study (1978).

McCloy and co workers (1979) have also reported that salbutamol inhibited both pentagastrin and feeding stimulated secretion in the dog. Propranolol abolished the effect of salbutamol with both stimuli.

Summary

In the conscious dog, isoprenaline and two β_2 adrenoceptor agonists inhibit pentagastrin stimulated acid secretion by a mechanism that doesnot seem to involve blood flow. (Archibald et al 1974; Curwain, Holton and Spencer; 1972, Curwain and Holton, 1972). Furthermore it has been shown by Daly and co workers (1978) that both β_1 and β_2 adrenoceptors can mediate this inhibition.

(b) Conscious Rat

In the conscious rat isoprenaline was first shown to inhibit acid secretion by Misher and colleagues (1969). Isoprenaline markedly reduced basal acid output in the fistula rat for three hours after drug administration. Fjalland (1972) also showed isoprenaline to exhibit antisecretory action on basal secretion and this inhibition was reversed by propranolol but not by phenoxybenzamine Lundell and Svensson (1974) studied the effects of isoprenaline on pentagastrin stimulated acid secretion in the Heidenhain pouch rat. They found that isoprenaline inhibited the response and that this effect was blocked by propranolol. They also reported that the dose of isoprenaline used had no effect on blood pressure. Lundell, Svensson and Nillson (1976) found that basal acid output and feeding stimulated secretion were also inhibited by isoprenaline.

Summary

Isoprenaline inhibits basal, pentagastrin, and food-stimulated secretion in the rat. There are no data on blood flow available, but no evidence to suggest that it is involved in these responses. These findings in the rat are similar to those found in the dog.

2.6.2 Histamine Stimulated Acid Secretion

(a) Conscious Dog

The effect of β-adrenoceptor agonists on histamine stimulated secretion is more complex. Harries (1957), using dogs with gastric fistulae reported that isoprenaline inhibited acid secretion. Jacobson and co workers (1967) found that isoprenaline increased mucosal blood flow without affecting histamine-stimulated secretion. Holton (1973) reported that histamine-induced acid secretion was enhanced by small doses of isoprenaline in Heidenhain pouch dogs. This was in contrast to her findings of an inhibitory action of these doses of isoprenaline against pentagastrin or food-stimulated acid secretion. Gottrup and Ornsholt (1979) studied the effect of salmefamol in the gastric fistula dog and showed it to be a less potent inhibitor of histamine than pentagastrin. McCloy and co workers (1979) have also reported that histamine stimulated secretion in the dog was less easily inhibited by salbutamol than was pentagastrin stimulated secretion. This is in agreement with the report of Curwain and Holton (1972) which showed that isoprenaline had a greater inhibitory effect on acid secretion induced by pentagastrin and by feeding than that induced by histamine.

Gottrup, Ornsholt and Andersen (1980) also looked at the effect of salmefamol in vagotomised fistula dogs where they found that it had little or no effect. Thus vagotomy appears to enhance the difference in potency of β-agonist inhibition of pentagastrin- and histamine-stimulated acid secretion.

(b) Conscious Rat

In the rat isoprenaline inhibited histamine-stimulated acid secretion to a lesser extent than had previously been shown for pentagastrin stimulated secretion (Lundell, Svensson and Nillson, 1976). This appears to be the only published account of the effects of β-agonists on histamine stimulation in the conscious rat.

2.6.3 Studies in Man

There have been few reports on the effect of β-adrenoceptor agonists in man. Fielding and co workers (1975) showed that salbutamol inhibited basal and pentagastrin-induced acid secretion in man, however, there has also been one report of isoprenaline either stimulating or having no effect on basal secretion in duodenal ulcer patients (Abasov and Iof, 1978).

2.6.4 Summary

Evidence from studies in the dog and rat indicates that β-adrenoceptor agonists inhibit acid secretion in response to pentagastrin, feeding and cholinergic agents but that histamine-stimulated acid secretion is more resistant to inhibition by these agents. This does not seem to involve a reduction in the

ratio of gastric mucosal blood flow to acid secretion in contrast with findings with noradrenaline. Both β_1 and β_2 receptor sub-types may be involved.

2.7 The Effects of β-Adrenoceptor Antagonists Alone on Gastric Acid Secretion in Conscious Animals

(a) Effects on Pentagastrin-Induced and Basal Secretion

These studies have been carried out mostly in the dog and man, with a few reports of studies in the conscious rat. The first report of a potentiation of pentagastrin induced acid secretion by propranolol was by Evans and Lin (1970) who found that it increased both the acid and mucosal blood flow response to pentagastrin in the Heidenhain pouch and gastric fistula dog. This suggested that one of the mechanisms of propranolol potentiation was by increasing blood flow, however, the ratio of aminopyrine in the gastric juice to that in the blood decreased, suggesting that propranolol might also have a direct effect on the acid secretory mechanism. They further reported that propranolol augmented secretion by the Heidenhain pouch to a greater extent than that of the gastric fistula preparation (Lin and Evans, 1973). Lin, Evans and Spray (1973) found similar results using the β_2 adrenoceptor antagonist butoxamine.

Curwain, Holton and Spencer (1973) have also reported an enhancement of pentagastrin induced acid secretion by propranolol in the Heidenhain pouch dog. This was accompanied by a parallel increase in gastric mucosal blood flow. The ratio of MBF/acid secretion remained constant or decreased. The potentiating effect of propranolol on pentagastrin induced acid secretion has been confirmed by McCloy et al.,(1979)in the gastric fistula dog

and Gottrup, Ornsholt & Andersen (1979) in the vagotomised gastric fistula dog. The β_1-antagonist practolol had no effect on pentagastrin stimulated acid secretion in the dog (Curwain et al., 1974; Gottrup, Ornsholt & Andersen, 1979).

In the Heidenhain pouch rat Lundell and Svensson (1974) reported a potentiation of pentagastrin-stimulated secretion by propranolol.

The effects of β-adrenoceptor antagonists in man are variable and in some respects contradictory. In healthy subjects an i.v. injection of propranolol significantly suppressed basal acid secretion (Geumi et al., 1969). Konturek and Oleksy (1969) showed that, in man, the maximal response to pentagastrin was unaffected by propranolol although they did note a slight tendency of propranolol to shift the peak of the dose response curve to the left. Propranolol alone slightly increased basal acid secretion. Kronborg (1975) reported that propranolol had no effect on spontaneous acid secretion in man, while the maximum pentagastrin induced acid secretion was slightly reduced. Christensen and co workers (1976) also found no change in basal acid secretion in the presence of propranolol. More recently Schoon and co workers (1980) showed that both propranolol and metroprolol did not alter the acid response to submaximal pentagastrin stimulation in man.

Summary

It seems that, at least in the dog and rat, propranolol augments pentagastrin stimulated secretion but has no effect on basal acid secretion. Butoxamine (β_2) but not practolol (β_1)

has also been reported to enhance pentagastrin induced acid secretion. The situation in man seems to be open to question.

(b) Effects on Histamine-Stimulated Acid Secretion

The effects of propranolol on histamine-induced acid secretion has been less extensively studied. Curwain and co-workers (1974) reported that in the Heidenhain pouch dog propranolol had no effect on a submaximal dose of histamine. However, Bruckner and Mullerabe (1972) reported that in the gastric fistula cat propranolol potentiated the acid response to histamine. They also showed an increase in spontaneous acid output with propranolol. Geumi and co workers (1972) report an antisecretory action of propranolol on histamine induced acid secretion in man. More recently Gottrup, Andersen & Jorgensen (1980) have studied the effects of β_1 and β_2-adrenoceptor antagonists on basal and histamine stimulated acid secretion in non-vagotomised and vagotomised gastric fistula dogs. Neither propranolol nor practolol had any significant effect on basal or histamine stimulated acid secretion in either preparation.

Summary

As with the β-adrenoceptor agonist studies there is greater disagreement on the effect of β-antagonists on histamine induced acid secretion than pentagastrin induced secretion.

2.8 The Effects of β-Adrenoceptor Agonists and Antagonists on Gastric Acid Secretion in Anaesthetised Animals

There have been fewer reports of studies on anaesthetised animals and of these the majority have been performed on the pylorus-ligated rat.

(a) Studies in the Rat

Isoprenaline reduced the volume of secretion in the pylorus-ligated rat and this effect was prevented by propranolol (Yamaguchi, 1977). Also in the pylorus-ligated rat the adrenoceptor antagonists dichloro-isoprenaline and pronethalol decreased basal secretory volume (Bass and Patterson, 1966). Okabe, Suziki and Tagaki (1970) measured both secretory volume and acid output in pylorus-ligated rats and found that propranolol and alprenolol reduced both basal acid output and secretory volume. This inhibitory effect of propranolol in pylorus-ligated rats was also shown by Danhof and Geumi (1972). Debnath and co workers (1974) however,reported a significant increase of acid output in the pylorus ligated rat with a similar dose of propranolol, although a much higher dose significantly decreased resting secretion. Goel and co workers (1977) reported that pindolol, a non-selective β-adrenoceptor antagonist and practolol a β_1 antagonist stimulated acid secretion in small doses but at higher doses significantly inhibited it.

Using the Ghosh and Schild rat preparation, Tani and co workers (1979, 1980) showed that isoprenaline stimulated acid secretion in a dose dependent manner in the resting stomach and

that this effect was blocked by propranolol. Isoprenaline also enhanced the acid response to submaximal tetragastrin, histamine and carbamylcholine. The effect of isoprenaline alone was increased by theophylline but not by metiamide. Theophylline alone had no effect on the responses to secretagogue stimulated secretion.

(b) Studies in the Dog and Cat

In the cat close arterial injection of isoprenaline produced an increase in mucosal blood flow with little or no change in acid secretion in the resting stomach (Reed and Smy, 1968; Reed and Sanders, 1971; Reed, Smy, Venables and Harries, 1973). However, isoprenaline did produce an increase in acid secretion and mucosal blood flow in response to a small dose of gastrin (Reed and Smy, 1968), but with little increase in acid secretion when given during the peak responses to large doses of gastrin. (Reed et al, 1973). However when isoprenaline was given by an intravenous route there was an increase in both acid secretion and mucosal blood flow using maximal doses of gastrin. Clearly there is a difference in the effect of isoprenaline in the anaesthetised cat compared with the conscious rat and dog. However, isoprenaline has been shown to inhibit pentagastrin induced acid secretion in the conscious cat with little or no change in mucosal blood flow (Holland: personal communication), suggesting that the difference may be due to anaesthesia rather than species.

Okabe and co workers (1977) have shown that, in the anaesthetised dog, propranolol produced a potentiation of tetragastrin induced acid secretion but had no influence on the

secretion induced by methacholine. Increasing the dose of propranolol, however, produced a slight reduction in the tetragastrin induced secretion and a significant reduction of methacholine induced secretion. Histamine induced gastric secretion was unaffected by either dose of propranolol.

(c) Summary

Reports on the effect of β-adrenoceptor agonists and antagonists have been conflicting when studied in anaesthetised animals and show a wide variation. This may be due to differences of species, doses, routes of administration and experimental preparation. The absence of more information on the effects in anaesthetised animals does not allow any definite conclusions to be made, whereas in the conscious animal it seems that, at least when using pentagastrin as a secretory stimulant, β-agonists exert an inhibitory effect, and some β-antagonists a potentiating effect on acid secretion.

2.9 Aims of the Project

It is apparent from this literature review of the effects of sympathomimetic drugs and their antagonists on gastric acid secretion that many studies have been carried out in the intact animal and there are contradictory reports on the effects of β-adrenoceptor agonists. The interpretation of experiments using intact animals is complicated since sympathomimetic drugs have such a widespread activity in the body. Actions on the central nervous and cardiovascular systems may alter acid output indirectly. Laparotomy may reduce gastrointestinal blood flow due to sympathomimetic vasoconstriction and may alter the effects seen

with β-adrenoceptor agonists compared with experiments in conscious animals of the same species. The study of the actions of β-adrenoceptor antagonists is further complicated by their potentiating effect on pentagastrin stimulated secretion in the conscious rat and dog. Intact animal studies also do not localise the site of the adrenoceptors concerned in the acid secretory response to sympathomimetic drugs.

To date there have been no reports on the effects of catecholamines on gastric secretion *in vitro* in mammals.

Because of the problems of interpreting results *in vivo*, and of comparing different species, a study was undertaken to compare results from conscious Heidenhain pouch rats with those from an isolated stomach preparation in the rat. If sympathomimetic agonists and antagonists act directly upon the stomach their effects should be demonstra ble *in vitro*. An extensive study of the effects of β-adrenoceptor agonists and antagonists on gastric acid secretion *in vitro* was carried out. The actions of established inhibitors of acid secretion on these responses were also studied, and the effects of noradrenaline and an α-adrenoceptor agonist were investigated.

CHAPTER 3

METHODS

3.1 RAT ISOLATED STOMACH

3.1.1 Introduction

The isolated stomach and isolated gastric mucosa, where the muscle wall has been removed, provide experimental advantages for the study of gastric function since complicating factors such as variation in blood flow, or nervous control can either be reduced or abolished. The amphibian isolated mucosal preparation has been used extensively and is well documented. (For review Forte and Solberg, 1973) more recently mammalian isolated stomachs and mucosae have been used successfully to study gastric function, in particular gastric acid secretion.

Rat isolated stomach preparations secrete acid spontaneously and respond to various secretagogues. Several groups of workers have previously stimulated H^+ secretion from the isolated whole rat stomach or gastric mucosa (Patterson and Stetton, 1949; Cummins and Vaughan, 1965; Dickstein and Sulman, 1965; Wan et al., 1974; Brennan et al., 1975; Bunce and Parsons, 1976; Hearn and Main, 1975), although quantitative data have only been provided by Wan et al., (1974), Bunce and Parsons (1976) and Hearn and Main (1975). Resting secretion in this preparation (3.63 ± 0.2μmol H^+ cm^{-2} h^{-1}) is slightly higher than that reported by Bunce and Parsons (1976) which was 2.5μmol H^+ h^{-1}. Sernka and Harris (1972) obtained a resting secretion of 1.6 - 2.1μequiv cm^{-2} h^{-1} in the rat isolated mucosa. Other workers have obtained basal secretory rates of 30μequiv h^{-1} (Brennan et al., 1975) and up to 15μ equiv cm^{-2} 30 min^{-1} (Wan et al., 1974). Some values found by other

workers, of basal and maximal acid secretion in isolated mucosa and stomach preparations are shown in Table 1. In the present study the spontaneous acid secretion was stable and showed little variation over each hour during which time agonists were studied. This is shown in Figure 1.

While *in vitro* preparations are ideal for the study of direct effects of drugs on gastric acid secretion there are limitations which must be balanced against the more obvious advantages of the isolated preparation. By its very nature the isolated system will not permit the kinds of total integrative analysis which are possible with intact systems; isolation requires interruption of important nerves, particularly the vagus, loss of blood supply which carried oxygen and glucose and removes bicarbonate and in isolated mucosal preparations removal of the muscle coats virtually eliminates sub-epithelial neural plexi.

Interpretation of results comparing different species both *in vivo* and *in vitro* may be problematic since some secretagogues such as pentagastrin may be of different potency from species to species. However, in the present study the aim was to compare the effects of sympathomimetic drugs and their antagonists *in vivo* and *in vitro* using the same species. In spite of these various problems the convenience, simplicity and carefully controlled conditions provided by isolated preparations substantiate their role in experimental studies of gastric secretion.

In conclusion, the rat isolated stomach has been chosen as a model to study the effects of sympathomimetic agonists and antagonists on gastric secretion.

TABLE 1

A COMPARISON OF BASAL AND STIMULATED ACID SECRETION IN THE ISOLATED STOMACHS AND MUCOSAE OF DIFFERENT SPECIES

uequiv/cm^2/h (except where stated)

SPECIES	ISOLATED MUCOSA	BASAL ACID	MAXIMAL ACID
Rat	Sernka & Harris, 1972	1.6 - 2.1	-
Rat	Hearn & Main, 1975	-	Up to 10 eq
Piglet	Forte, Forte & Machen, 1975	0 - 1	Up to 15
Guinea Pig	Sjostrand, Ryberg & Olbe, 1977	1.81	5.1
Kitten	Tepperman, Schofield & Tepperman, 1975	0.29 $\pm$ 0.03	3.11 $\pm$ 0.62
Dog	Kuo & Shanbour, 1978	0 - 0.58	4.24 $\pm$ 0.35
Primate	Rangachari, 1979	0.15	5.1
Rabbit	Fromm, Schwartz & Quizano, 1975	2.4 $\pm$ 0.2	Up to 5
	ISOLATED STOMACH		
Guinea Pig	Holton & Spencer, 1975	2 - 4	Up to 13
Rat	Wan, Assem & Schild, 1974	Up to 12	Up to 23
Rat	Sernka & Harris, 1972	2.7	-
Rat	Brennan, Arbakov, Stefankiewicz & Groves, 1975	30 - 34µeq/hr	-
Rat Immature	Bunce & Parsons, 1976	2.5 mol x 10^{-8} H^+ h^{-1}	Up to 5.8µmol H^+ h^{-1}

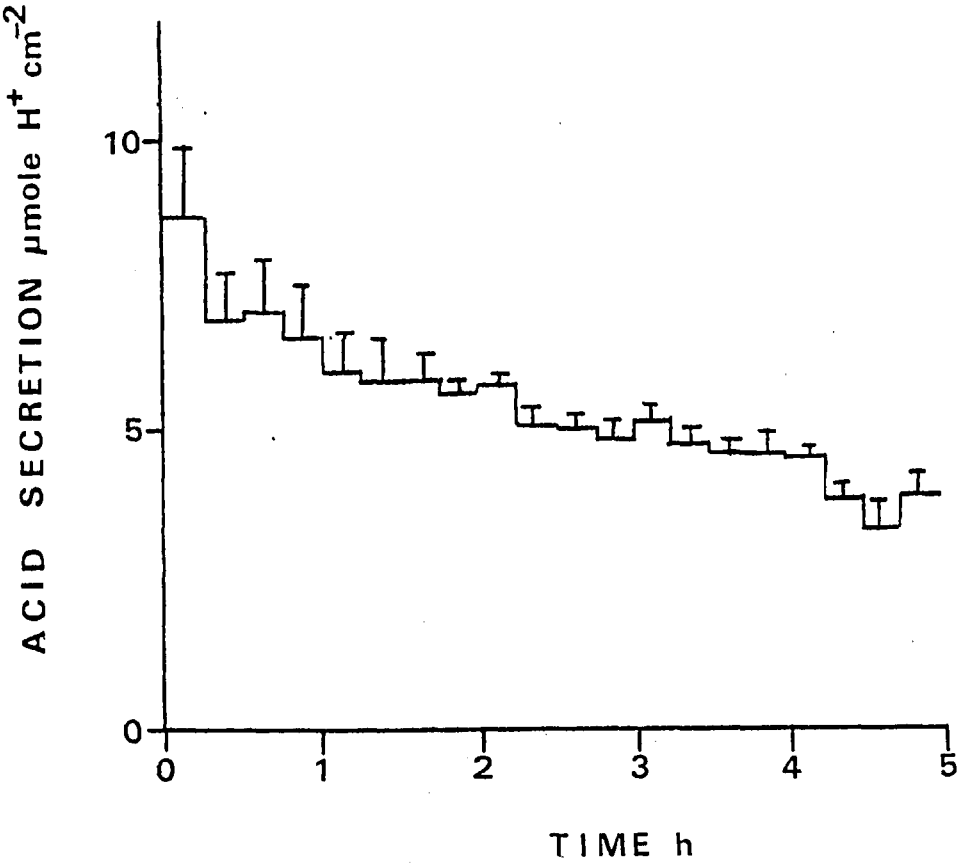


Figure 1 Mean resting secretion of 6 stomachs for 5 hours

Bars = s.e.m.

3.1.2 Choice of the Immature Rat

Stomachs from immature rats were used to study acid secretion *in vitro*. Adequate oxygenation is a basic problem in all isolated preparations therefore a very thin preparation such as the young rat stomach is an advantage since it offers a short diffusion path for oxygen. The approximate thickness of the rat stomach was determined experimentally over an age range of 19 to 22 days and the results are shown in Table 2. Between the ages of 19 and 22 days the thickness of the stomach only increased from 0.032 cm to 0.038 cm and this was the age range at which they were used. This factor was considered to be of prime importance since the rate at which oxygen can be supplied is thought to limit the rate of acid secretion in isolated stomachs.

The adult rat stomachs used by Wan, Assem and Schild (1973) were 1mm thick and isolated stomachs from other species can be several mm's thick. This will reduce the diffusion of oxygen into the mucosa which is required to support acid secretion. Therefore by using the thinnest preparation described to date the possibility of inadequate oxygenation is minimised.

3.1.3 Isolated Stomach or Isolated Mucosa ?

There are several disadvantages associated with removal of the muscle layer either by blunt dissection (Sernka and Hogben, 1969) or by a blistering technique (Forte, Forte and Machen, 1975; Hearn and Main, 1975). The most obvious drawback to this procedure is the possibility of damage to the epithelial integrity when the muscle coat is separated from the mucosa. Stretching of the mucosa may occur as the muscle layer is dissected from the mucosa, and this itself may stimulate acid secretion which makes interpretation of

TABLE 2

Age (days)	n	Combined Wet Weight (g)	Average Weight of Stomach (g)	Thickness (mm)
19	5	0.180	0.036	0.032
20	6	0.246	0.041	0.036
21	5	0.199	0.040	0.035
22	6	0.258	0.043	0.038

Stomachs which had been used for acid secretory studies were cut from the mucosal tubes to which they were tied during the experiment (see Figure 2, 3.1.6) around the circumference at the end of the tube, blotted and weighed in groups of 5 or 6. The area of the mucosa was calculated from the area of the tube (1.13 cm^2). Assuming a density of 1g/cc the thickness (x) was calculated using the formula:

$$\text{Mass} = \text{Volume } (x \times 1.13\ cm^2) \times \text{density } (1)$$

results difficult. The main argument for the isolated mucosa preparation has been that it is a thinner preparation, however, the muscle coat only contributes about 20% to the thickness of the stomach (Sernka and Hogben, 1969). These workers have also shown that in the rat and guinea pig the serosal coat can be ignored as a substantial diffusion barrier and that it represents only a passive barrier to ion movement. On the whole, the isolated gastric mucosal preparation is a less physiological preparation than the isolated stomach and direct comparison of *in vivo* and *in vitro* results are less valid.

Since the adult rat isolated mucosa is thicker than the immature rat stomach the advantages gained from stripping the muscle coat away from the mucosa in the immature rat would be negligible, and it is extremely unlikely that the procedure would be feasible because of the delicacy of the thin stomach. Furthermore, Holton and Spencer (1975) found that in the guinea pig stomach (0.05 cm thick) removal of the muscle layer caused no change in observed results compared with the whole stomach. Therefore the immature rat isolated stomach was considered to be the best choice for this study.

3.1.4 Dietary Pre-Treatment

Male rats, age 19 days, together with a lactating female were obtained from dealers. They remained in good health and suffered no obvious ill effect from travelling. The rats were used within two days of arrival and during this time began to wean themselves. This had no obvious effect on the results obtained.

Rats were not starved for a number of reasons. Immature rats deprived of food either prior to or after weaning would not develop normally, and would be less healthy than fed animals. Bunce and Parsons (1976) showed that stomachs from fed animals were more responsive to secretagogues than those from fasted animals and similar observations have also been made by Kittahara, Fox and Hogben (1969) and Brennan et al. (1975).

3.1.5 Method for Sacrifice of Rat

Rats were killed by cervical dislocation. Other methods that have been used for the rat include ether anaesthesia (Sernka and Hogben, 1969) and injection of sodium pentobarbitone (Bunce and Parsons, 1976). However, ether is stressful to the animal and may cause hormonal changes which could affect gastric secretion (Cutting et al., 1937; Skyring, 1961) in the intact animal. Since it is only a matter of seconds between cervical dislocation and removal of the stomach, the advantage of maintaining the blood supply, as in the setting up of an isolated whole stomach, is not applicable here.

3.1.6 Dissection and Setting Up of the Stomach

The stomach was rapidly removed with the minimum of contact with the instruments of dissection and cut along the lesser curvature. The mucosa was washed with cold 0.9% saline. The secretory portion of each stomach was stretched tightly and tied over the end of a small plastic tube of end area 1.13cm^2 with the mucosa facing upwards into the lumen of the tube.

A diagrammatic representation of the apparatus is shown in Figure 2. The secretory portion of the stomach was considered to be the main fundic region excluding the rumen and,where the size of the stomach allowed,this portion only was used. In some of the smaller stomachs some rumen had to be included since it was impossible to stretch the fundic portion without damaging it, to tie over the end of the plastic tube.

Each stomach preparation was set up at 34°C in separate baths containing 30ml of buffered saline (PH 7.4) and bubbled with 95% O_2 5% CO_2. The temperature was maintained by suspension of the serosal bath in a large water bath, water being circulated at a constant 34°C by a Churchill thermoregulator. The mucosal surface was bathed with 5ml of unbuffered saline and bubbled with 100% O_2. The composition of the salines are shown in Table 3 and discussed in 3.1.7.

The levels of the solutions on either side of the preparation were approximately the same to minimise any external pressure. However, a slight difference in the levels showed that the stomachs had ruptured or the ties become loose, since any hole in the stomach would result in a levelling of the serosal and mucosal bathing fluids. Bubbling was maintained at a brisk rate to ensure adequate oxygenation and to mix solutions on addition of drugs. However, care was taken not to apply too vigorous 'bubbling' since damage to the tissue might ensue.

The apparatus was thoroughly cleaned daily using distilled water since it has been shown that thorough cleaning of the organ baths is essential for a reproducible secretory response to secretagogues (Spencer, 1974).

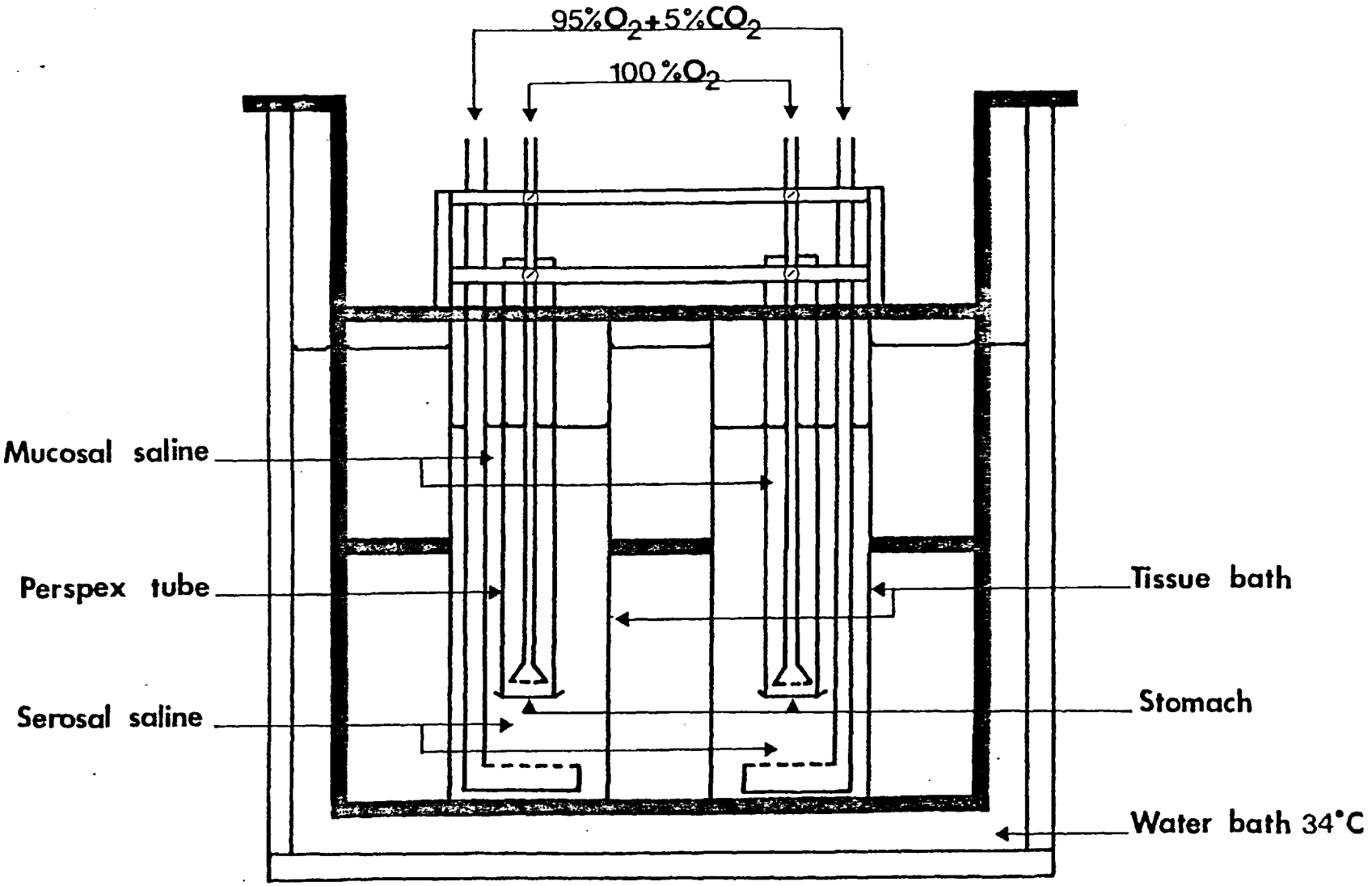


Figure 2 Diagrammatic representation of experimental apparatus used in studies on the rat isolated stomach.

TABLE 3

COMPOSITION OF SALINES

Sernka & Hogben, 1969

	Serosal Saline	Mucosal Saline
	µeq/litre	µeq/litre
Na^+	136.0	136.0
K^+	5.0	5.0
Mg^{2+}	2.4	2.4
Ca^{2+}	7.2	7.2
Cl^-	124.6	150.6
HCO_3^-	26.0	-
Glucose	16.7mM	16.7mM

3.1.7 Composition of Salines

Table 3 shows the composition of mucosal and serosal salines. These were made up according to Sernka and Hogben (1969) for the rat, and used in their studies on active ion transport by the isolated gastric mucosa of the rat. These salines have also been used in studies on the isolated gastric mucosa of the rat. These salines have also been used in studies on the isolated guinea pig stomach (Holton and Spencer, 1975) and were found to be an appropriate composition for successful secretagogue stimulation and maintenance of resting secretion in the rat isolated stomach (Spencer: personal communication).

3.1.8 Gassing of the Bathing Solutions

The gastric mucosa requires more carbon dioxide than is produced by tissue metabolism. Carbon dioxide is incorporated into HCO_3^- which is liberated into the serosal solution (in vitro) or blood (in vivo), in exchange for H^+ secreted into the gastric lumen (Davies, 1948; Teorell, 1951) It has been shown by Davies and Langmuir (1948) that in the absence of CO_2 gassing the rate of H^+ production does not exceed that of CO_2 produced by metabolism. On maximal stimulation by histamine ulceration was seen which they proposed was due to hydroxylgroup accumulation since ulceration was not apparent with adequate CO_2 supplies. For these reasons, in the isolated stomach 5% CO_2 was supplied in the gassing mixture to the serosal solution. The mucosal solution was unbuffered and gassed with 100% O_2 to minimalise buffering of the secreted acid. Inadequate gassing of the mucosal saline causes a reduction in acid secretion; this may or may not be reversible depending on the duration of O_2 starvation of the tissue.

3.1.9 Temperature

Holton and Spencer (1975) found that, for the guinea pig, reducing the temperature from 37°C to 34°C caused no appreciable change in acid production. This was also found to be true for the rat isolated stomach preparation. (Spencer: personal communication).

3.1.10 Experimental Protocol

Six stomach preparations were set up concurrently. Once in the serosal bath the oxygen and oxygen/carbon dioxide supplies were adjusted for optimum gassing, the temperature of the large bath checked to ensure a constant temperature, and the first 5ml of mucosal saline discarded and replaced with fresh mucosal saline. Measurements were commenced after 15 minutes, the mucosal saline removed every 15 minutes during the experiment for acid measurement. Mucosal salines for the six stomachs were removed using 5ml syringes with a short attachment of tubing, each syringe labelled from 1 to 6 to ensure that samples from each stomach were clearly marked.

All drugs were added to the serosal bathing fluid. After setting up the stomach the basal acid secretion was allowed to stabilise for one hour both under control conditions and in the presence of antagonists. At one hour basal secretion had reached a steady state as shown in Figure 1. Agonists were added to the serosal fluid at this point and left in contact with the serosal surface for one hour after which the serosal saline was removed and the tissues washed out twice. Return to prestimulated levels of secretion was more rapid after two washes than one. The serosal saline was replaced with or without antagonist as

appropriate and acid measurement continued for a further hour. Each tissue received only one dose of agonist for all experiments except the pilot experiments described in 4.1.1 and 4.1.2.

Drugs were prepared daily and administered in as small a volume as was practical. The volumes of drug added ranged from 10 µl (as in some commercially available vials) to 1ml (solutions made up in the laboratory). An equivalent amount of saline was removed before addition of the drug solution.

3.1.11 Measurement of Acid Secretion (See Figure 3)

The mucosal saline was removed every 15 minutes.

(a) PH

The pH of each 15 minute sample was measured with an Inteck polymer bodied pH electrode on a Beckman model 3500 digital pH meter.

(b) Titration

The sample was titrated by continuous addition of 20mM NaOH using a Watson Marlow Delta B pump (flow rate 6ml h^{-1}) which was switched off manually at pH7. The pH change was recorded on a Devices two channel recorder which gave a reading of time taken to reach pH7.

(c) Use of 20mM NaOH

The desired experimental protocol required that six samples be titrated in less than 15 minutes. Rapid addition of OH^- to the sample can minimise titration time, however, the glass pH electrode does not respond to a change in pH instantaneously as H^+ must come to equilibrium across the H^+ sensitive glass. Thus, if the pH change is very rapid the electrode output will lag behind

and inaccuracies will result. Another consideration is that dilute solutions of NaOH are prone to take up carbon dioxide from the atmosphere and may become appreciably titrated. Using the titration system, it has been shown that the estimated H^+ content of a sample of acidified mucosal saline was the same using 1, 10 or 20mM NaOH (Canfield, unpublished observation). Therefore 20mM NaOH was used for titration and on average six such titrations required 8-10 minutes. The 20mM NaOH was made up each day by dilution of a IN volumetric standard NaOH solution.

(d) Determination of Pump Rate

Regular determinations of pump flow rate were carried out to check that the flow rate remained constant. This was achieved by weighing a volume of NaOH pumped in a known time.

(e) Calculation of Acid Content

The volume of NaOH required to titrate the sample to pH7 was calculated from time taken to reach pH7 multiplied by pump rate. This can be converted to mole H^+ cm^{-2} h^{-1} by multiplying the time of the 15 minutes sample by four and dividing by the area of the preparation (1.13 cm^2). Absolute values of acid content have only been used to quantify resting secretion. Some values for the maximum responses to agonists are given in the results for comparative purposes. Results of agonist - antagonist studies have been expressed as the secretory ratio. This is discussed in 3.1.13.

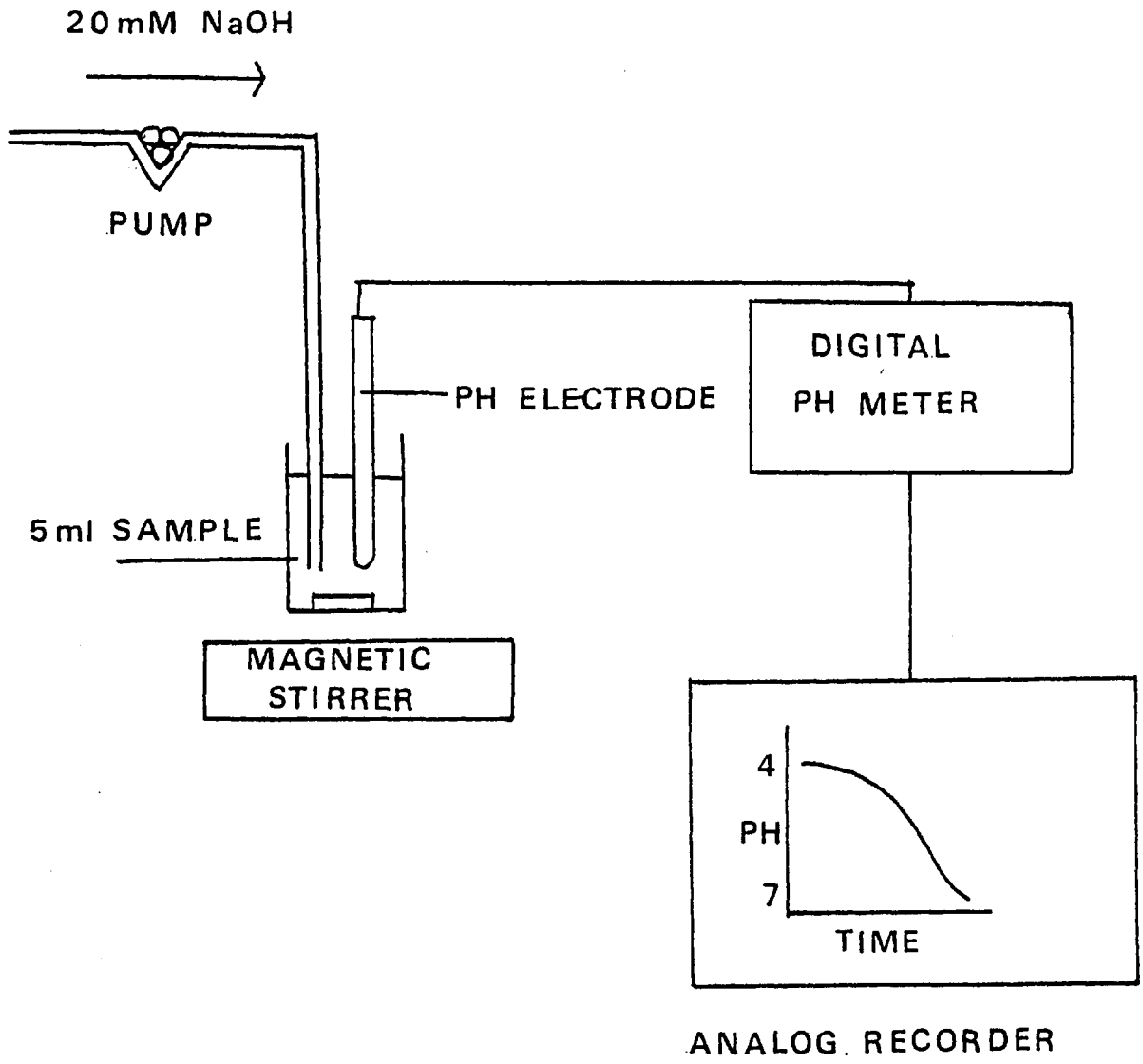


Figure 3 Diagrammatic representation of measurement of acid secretion

3.1.12 pH Measurement or Titration for the Measurement of Acid Secretion?

The glass pH electrode measures the H^+ activity in the sample, the activity coefficient being a function of ionic strength. As pH = -log a $\{H^+\}$ where a is the activity coefficient, passing the electrode output through an antilog-preamplifier will generate a signal which is a function of a $\{H^+\}$. This would save time taken to titrate all the samples. The major disadvantage of pH measurement is that it would not record any H^+ which were secreted and buffered, for instance by mucin, and would not give any indication of any buffering occurring.

However titration will include any buffer which may have been secreted in a partially titrated state, for instance, with associated H^+ which were not of parietal cell origin. The two methods both have advantages and disadvantages and it is best to consider which method suits one's own experimental design and limitation of experimental resources. Titration and pH measurement have both been used by other workers and are discussed further below.

(a) Continuous Flow pH Recording

This method has been used in the isolated perfused whole rat stomach by Bunce and Parsons (1976) and the isolated perfused whole mouse stomach by Angus and Black (1978). An "on line" recording of the pH of gastric secretion is obtained by the use of a flow pH electrode. This method has the advantage of providing a continuous recording of secretory events in the isolated stomach and a continous output of results. This method cannot, however,

record any H^+ which had been buffered as discussed above. The perfusing fluid is not bubbled with oxygen whilst in contact with the mucosa and a possible build up of carbon dioxide may occur. Continuous flow recording is expensive if six stomachs are set up concurrently,since each stomach requires a separate pH electrode, multiple electrode pH meter facility and antilog channel.

(b) Titration

This method has been used by Holton and Spencer (1975) and Hearn and Main (1975) and has several advantages. It gives a reading of total acid output and the titration curve will give an indication of any buffering occurring in the sample. The technology is simple and inexpensive, a fact to be taken into consideration when setting up six stomachs concurrently. The disadvantages of this method are related mainly to convenience. There is a delay before one can obtain the secretory rate data since each titration must be read off the recorder separately and the time resolution is in 15 minute periods. This is then converted into a graphical visualisation of secretory events in each stomach, so it takes longer to show that the tissue has reached peak secretion. However, for the purposes of this study, taking advantages and disadvantages of each method into account, it was decided that titration provided the most suitable method for measurement of acid secretion.

(c) Non-HCl Secretion

It must be noted that neither method can distinguish between HCl and H^+ from other sources. There are three possible sources of H^+ other than HCl secretion. These are lactate/pyruvate

from metabolism, mucin which acts as a gastric buffer and CO_2/ HCO_3^- from the serosal surface. In this preparation any CO_2 diffusing from the serosa is probably blown off by bubbling with O_2 in the mucosal saline. In the guinea pig stomach preparation Spencer (1974) found that sodium thiocyanate always abolished the stimulated secretion of HCl, whereas in the unstimulated stomach she found a portion of acid that was not inhibited by sodium thiocyanate. Sernka (1969) showed that a buffer secreted by the gastric mucosa had a pKa of about 6 and suggested that this was mucin. Unresponsive preparations of the rat isolated stomach often produced samples of about pH6. It is possible that the non HCl portion of resting acid secretion reported by Spencer was mucin and so only resting secretion would be significantly affected by mucin release in the guinea pig stomach.

In order to test whether the titration values from these preparations represent HCl and not non-HCl secretion titration samples from six stimulated stomachs have been compared to a titration obtained from 5ml oxygenated mucosal saline acidified with a small volume ($\simeq$ 20µl) of concentrated HCl to a similar pH as stimulated samples. The results are shown in Figure 4. Each filled circle represents the mean titration curve of six stomachs at five second intervals. The open circles represent the values obtained from titrating HCl/mucosal saline. The samples (stimulated + basal acid output) behaved like the mucosal saline to which only HCl has been added. Therefore any non-HCl secretion is not a significant factor in this preparation. Even if basal acid secretion contains some non-HCl component this remains constant over a period of hours as basal secretion remaims stable.

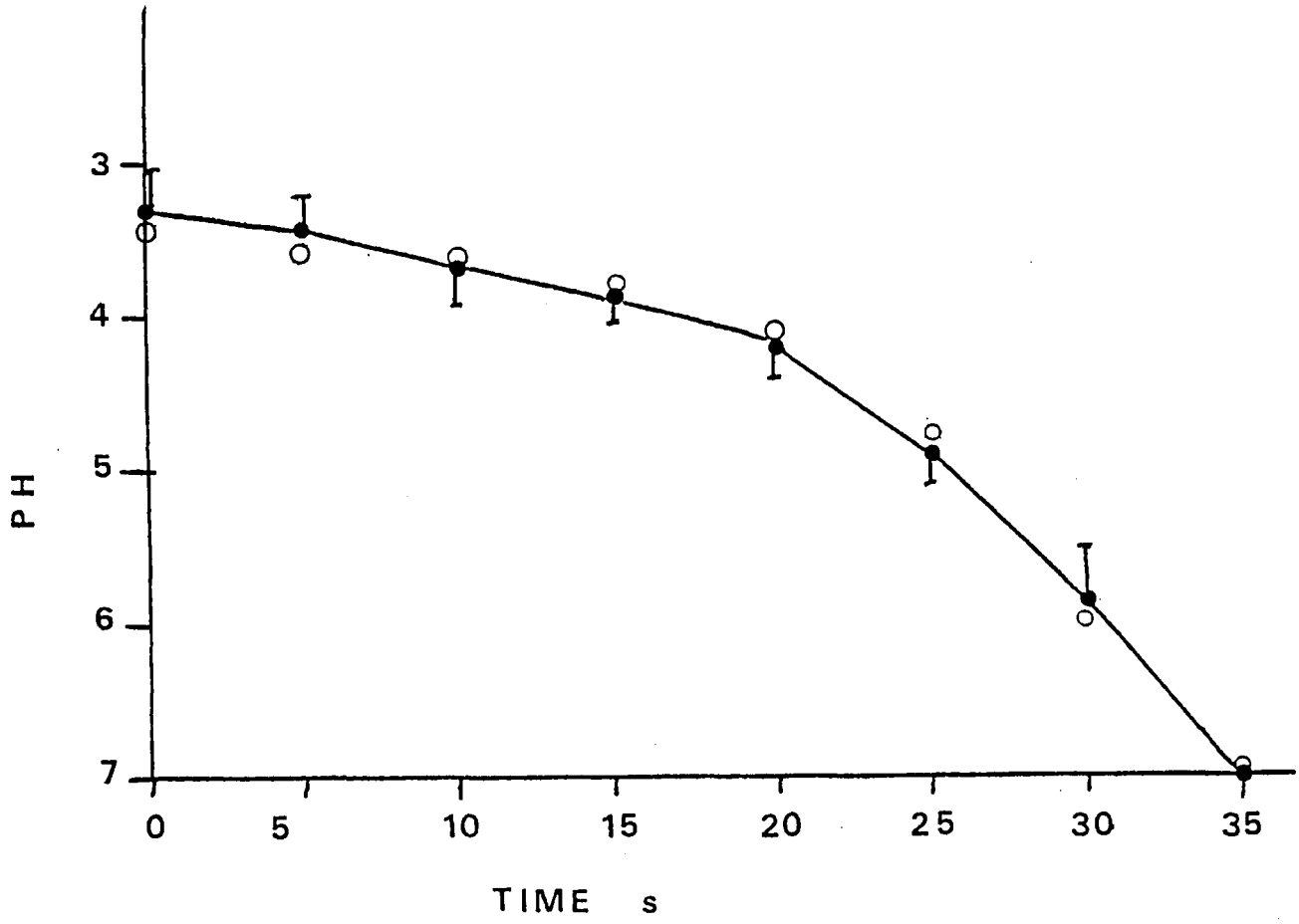


Figure 4 Mean titration curve of six secreting stomachs compared with a titration curve from mucosal saline + HCL (open circles). Each filled circle represents the mean of six stomachs. Bars = s.e.m.

3.1.13 Expression of Results: Secretory Ratio

The variation of the rate of basal secretion between different stomachs was some threefold. In general stomachs with a high basal secretion gave larger responses to a given concentration of secretagogue than those stomachs with low basal rates. This variation between stomachs could have been due to a number of factors such as exact age of animal, weight, stage of gastric development, adequate maternal nutrition or disturbances caused by shipment of litter. As the animals were not necessarily litter mates there was also the possibility of variation due to different genetic origins. In principal many of these variations could be studied by suitably designed experiments in which the results were subjected to analysis of variance. However circumstances required that animals were obtained from a commercial dealer so that many of the possible sources of variation were not under direct control. For this reason it was felt that such an experimental investigation would not be appropriate. A further complication was variability in stomach size between animals of nominally the same age. In smaller stomachs part of the area of perspex tube was covered by non secreting rumen, so it was not possible to determine the exact secretory area with any accuracy so expression of results as μmole H^+ cm^{-2} h^{-1} was not considered to be suitable.

For the reasons discussed it was decided to compare secretory responses by using the secretory ratio R.

$$R = \frac{\text{Average rate of secretion at plateau of response}}{\text{Average spontaneous rate of secretion}}$$

Thus a doubling of acid secretion represents a secretory ratio of 2. The use of this ratio assumes that the basal rate remains stable over the period of the experiment. This is shown in Figure 1. In some experiments acid secretion did not quite return to pre-stimulated levels. In these cases an average was taken of pre-stimulated and post-stimulated basal secretion.

3.1.14 Drugs

Atropine sulphate (BDH)

Adrenaline (MacCarthys)

Bethanechol chloride (Urecholine. Glenwood Labs Ltd.)

Butoxamine hydrochloride (Wellcome Research Labs)

Carbachol (MacCarthys)

Cimetidine (Smith Kline & French Labs Ltd)

Histamine acid phosphate (MacCarthys)

Hassle H35/25 (Uniscience Ltd)

Isoprenaline sulphate (MacCarthys)

Labetalol hydrochloride (Glaxo)

Metiamide (Smith Kline & French Labs Ltd)

Methoxamine hydrochloride (Wellcome Research Labs Ltd)

Noradrenaline (Levophed. Winthrop)

Pentagastrin (Peptavlon. ICI Ltd)

Propranolol hydrochloride (Inderal. ICI Ltd)

(+) Propranolol (ICI Ltd)

Practolol (ICI Ltd)

Phentolamine (Rogitine. CIBA)

Phenylephrine (Koch - Light)

Pindolol (Sandoz Ltd)

Pronethalol (ICI Ltd)

Salbutamol sulphate (Glaxo)

Salmefamol (Glaxo)

Thymoxamine hydrochloride (Warner - Lambert (UK) Ltd)

Timolol (Merck, Sharp & Dohme)

3.1.15 Statistical Analysis

Control secretory ratios (with agonists alone) were compared with test ratios (agonists after preincubation with an antagonist) using both non-parametric Mann-Whitney U test and student's t-test. Both tests yielded the same results throughout the work. The levels of significance given in this work are those derived from the Mann-Whitney test as this test does not require the assumptions about the normal distribution of the data which are required for the t-test.

3.2 HEIDENHAIN POUCH RAT

3.2.1 Introduction

Heidenhain described the first gastric pouch from the fundic gland area in 1879. It was made from the greater curvature, the vagal nerve fibres passing to this area from the lesser curvature were therefore transected, hence the term "denervated" pouch. Gregory (1962) pointed out that this may be a misnomer since along with the splenic and epiploic blood vessels sympathetic nerves reach the greater curvature. Since Heidenhain's description of his denervated pouch various adaptions of this procedure have been widely adopted in physiologic and pharmacological studies. The present study was carried out using a

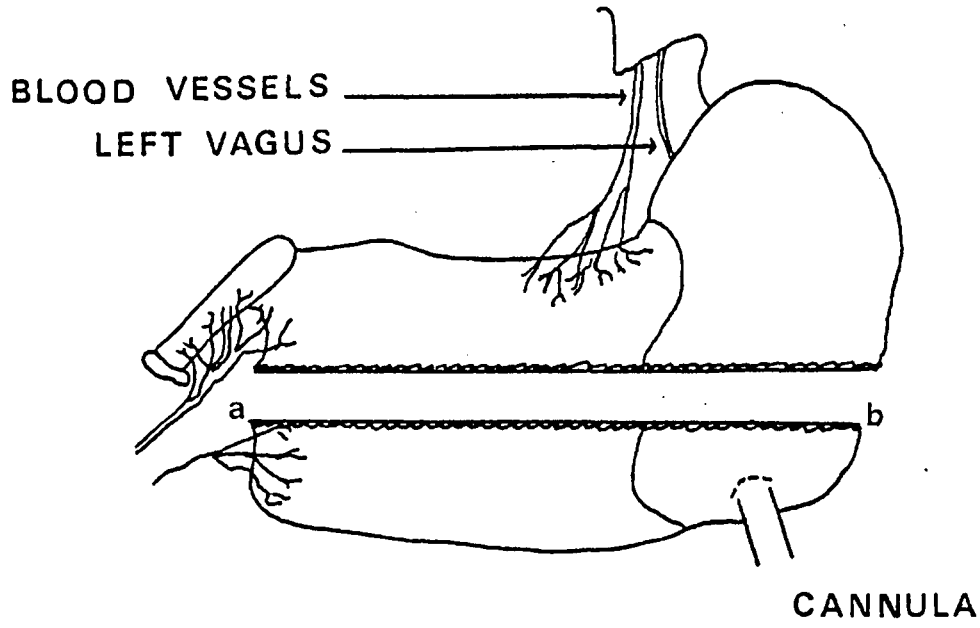


Figure 5 Diagrammatic representation of a Heidenhain pouch.
a - b = line along which cuttings are made.

method adapted from Alphin and Lin (1959) for the preparation of a chronic denervated pouch. A Heidenhain pouch consists of a small portion of the squamous or non-glandular portion of the stomach which houses the indwelling cannula and about 50% of the acid secreting portion of the stomach (Figure 5).

The Heidenhain pouch rat was used in preference to the fistula rat or Pavlov pouch rat for the following reasons. The stomach of the fistula rat can never be completely emptied of food, and this factor would make interpretation of results more difficult since any food in the stomach would itself cause acid secretion. The technique for the preparation of the Pavlov pouch rat is rather more complex and Pavlov pouch animals have a higher resting secretion so are more difficult to maintain in peak health. Since studies on gastric secretion in the Heidenhain pouch rat in respect of sympathomimetic drugs are well documented this preparation was considered to be the most appropriate in these studies.

3.2.2 The Rat as the Experimental Species for Gastric Acid Secretion Studies

Rats were used in this study to enable a direct comparison between the isolated stomach and the conscious whole animal in the same species. Rats of a uniform strain can be bred in large numbers and can be purchased inexpensively. They can be housed and maintained for little expense compared with the costs for cats and dogs. They can be kept in the laboratory and can therefore receive personal attention and care. They can be used for experiments within the confines of a small laboratory.

After training and with gentle handling they will remain quiet during the course of the experiments.

One objection to using the rat for acid secretory studies is its unique metabolism of histamine. Since histamine is by now well established in its involvement in acid secretion, this factor may be of importance. The rat is the only species in which diamine oxidase occurs in the gastric mucosa (Kobayaski and Ivy, 1959) and this enzyme is the major route of histamine metabolism in the rat. Histamine methyltransferase is found in the gastric mucosa of all species examined except the rat (See Bunce et al.,1979). In other species the methylating pathway predominates (Snyder, Axelrod & Bauer, 1964). Histidine decarboxylase is also present in the gastric mucosa of the rat and mouse in much greater quantities than those found in other species (Kahlson, Rosengren and Thunberg, 1964). Such factors obviously must be taken into account when comparing the rat to other species, however, the rat has been widely used as an experimental animal in the study of gastric secretion and material for comparison of gastric secretagogues and inhibitors is therefore available in the literature.

3.2.3 Use of a Conscious Animal in Preference to an Anaesthetised Animal

Considerable experience is required for the satisfactory preparation, maintenance and use of conscious pouched animals. An anaesthetised animal would have presented fewer diffulties. However, anaesthesia may exert an inhibitory influence on acid secretion and this appears to be related to the nervous control of gastric secretion (Cutting et al., 1937; Skyring et al., 1961).

Urethane and pentobarbitone inhibit insulin stimulated acid secretion. (Bralow, Komarov and Shay, 1961; Jagi and Unvas, 1949;). Anaesthesia followed by laparotomy and manipulation of the viscera results in radical change of the physiological condition of the stomach because of prolonged reflex sympathetic discharge. (Holton, 1973). The resulting depression in gastrointestinal movement lasts for several hours after afferent stimulation and may be abolished by local anaesthesia of the lumbar spinal cord (Markowitz and Campbell, 1927). Thus, acute experiments in anaesthetised animals, while they are more convenient, may not give a true indication of physiological responses.

3.2.4 Operative Technique

Wistar rats of both sexes weighing 200-350g were used. The rats were starved for 24 hours on a grid surface but allowed free access to water. The rat was anaesthetised using small animal Immobilon, 0.6-0.8 ml/kg i.p. Immobilon is a neuroleptic analgesic comprising Etorphine HCl BP (Vet) 0.074 mg/ml and methotrimeprazine BP (Vet) 18 mg/ml in a solution containing chlorocresol BP 0.1% w/v, sodium citrate BP, citric acid BP, Na metabisulphate BP, ascorbic acid BP and disodium edetate BP. A dose of 60 µg atropine sulphate was given simultaneously to alleviate any respiratory problems caused by bronchial secretions.

The hair was clipped from the upper abdomen over the stomach and this area was swabbed with chlorhexidine 0.5% in 70% methylated spirits (Hibitane). A mid line incision was made along the abdominal wall and the stomach gently exteriorised

using blunt toothed forceps and laid on a saline soaked gauze. A purse string suture was sewn into the squamous portion of the stomach using 5/0 silk and a cut made within the circle of stitches, wide enough for the insertion of a nylon cannula. (Length 20mm. Internal bore 2mm). The interior of the stomach was gently cleansed by insertion of a saline soaked cotton wool bud. The cannula was inserted and the purse string suture pulled tight. Topical phenylephrine (1:1000) was applied to the exterior of the stomach at this stage to discourage bleeding once the cuts were made.

A small cut was made in the squamous portion of the stomach in order to insert two haemostatic clamps dividing the acid secreting area, being careful not to injure blood vessels supplying the stomach and pouch. A clean cut with the scalpel was made on the pouch side of each clamp. The clamping and cutting of each side of the stomach was done separately. During the sewing of the pouch the clamps remained in position to reduce bleeding. The pouch and stomach were closed separately by continuous inverting sutures using 5/0 silk, inverting the last rosette of the mucosa into the inside. This was achieved by placing a stitch over the inversion.

The pouch and stomach were washed with sterile saline and replaced in the abdomen and the cannula brought through a small cut in the body wall to the left of the main incision. The cannula was sewn to the peritoneal surface of the body wall with 4/0 silk and the incision closed also using 4/0 silk, sewing the body wall and skin separately. The skin was again swabbed with Hibitane and a brass bite block screwed onto the plastic cannula. The Immobilon antidote Revivon was injected subcutaneously. Small animal Revivon contains Diprenorphine 0.272mg/ml

in a solution containing chlorocresol BP. An equivalent dose of Revivon to that of Immobilon was used. The animals recovered consciousness in approximately 10 minutes.

3.2.5 Post-Operative Feeding and Care

Food was witheld from the animals for 48 hours after construction of the pouch. Animals were allowed a choice of water or 0.9% sterile saline to help maintain bodily electrolyte balance. The rats were also given subcutaneous sterile saline if they appeared weak after recovery from the anaesthetic. Animals with pouches were kept singly in cages lined with paper. Sawdust was found to block the cannula entrance and thus prevent free drainage of secretion from the pouch.

Initial operations to construct Heidenhain pouches were often unsuccessful due to inexperience. These difficulties were eventually overcome and the animals survived up to six months. At first there was little spontaneous acid secretion from the pouches, but towards the end of the three or four month period an increase in spontaneous secretion caused acid burns around the cannula and subsequently lesions developed. These could be stitched with some success but eventually it become impossible to re-operate and close up the lesions. In some cases the cannula dropped out altogether and although cannulae could be reinserted into the rats and stitched to the body wall this procedure was not always successful. Occasionally rats with well established pouches developed alimentary tract obstructions, possibly due to decreased stomach size. It was considered that the useful life of a Heidenhain pouch rat depended mainly on the maintenance of good physical condition of the skin around the pouch. The

incidence of post operative problems decreased with experience in constructing the pouch, minimising the time under anaesthesia and blood loss.

The Heidenhain pouch rats started secreting acid 5-14 days after the operation and were never used until two weeks after the operation. Post-operative animals were kept in the laboratory which had a constant temperature of $21^{o} \pm 1^{o}C$. The pouches were regularly flushed with 0.9% saline using a 5ml syringe with a short length of 20 gauge polyethylene tubing, to remove mucus or blood clots. As soon as newly operated rats had recovered, and started secreting acid a longer bite block was screwed onto the cannula to increase the distance of acid secretion from the body wall. Rats were placed in Bollman restraining cages for a few hours each day to ensure that they became used to the experimental environment.

3.2.6 Experimental Protocol (Figures 6 and Plate 1)

Rats were starved for 24 hours prior to the experiment but allowed free access to water or 0.9% saline. The animals were set up in Bollman restraining cages as seen in Plate 1. Two animals were set up concurrently. The pouch was continuously perfused with 0.9% saline (6ml hr^{-1}) which was collected by gravity drainage and titrated to pH7 at 15 minute intervals as described for the rat isolated stomach. 100mM NaOH was used since the pouch secretions contained a high concentration of H^{+} and could not be titrated with the 15 minute intervals using 20mM NaOH. The inflow was inserted into the tip of the pouch to ensure complete washout of the acid.

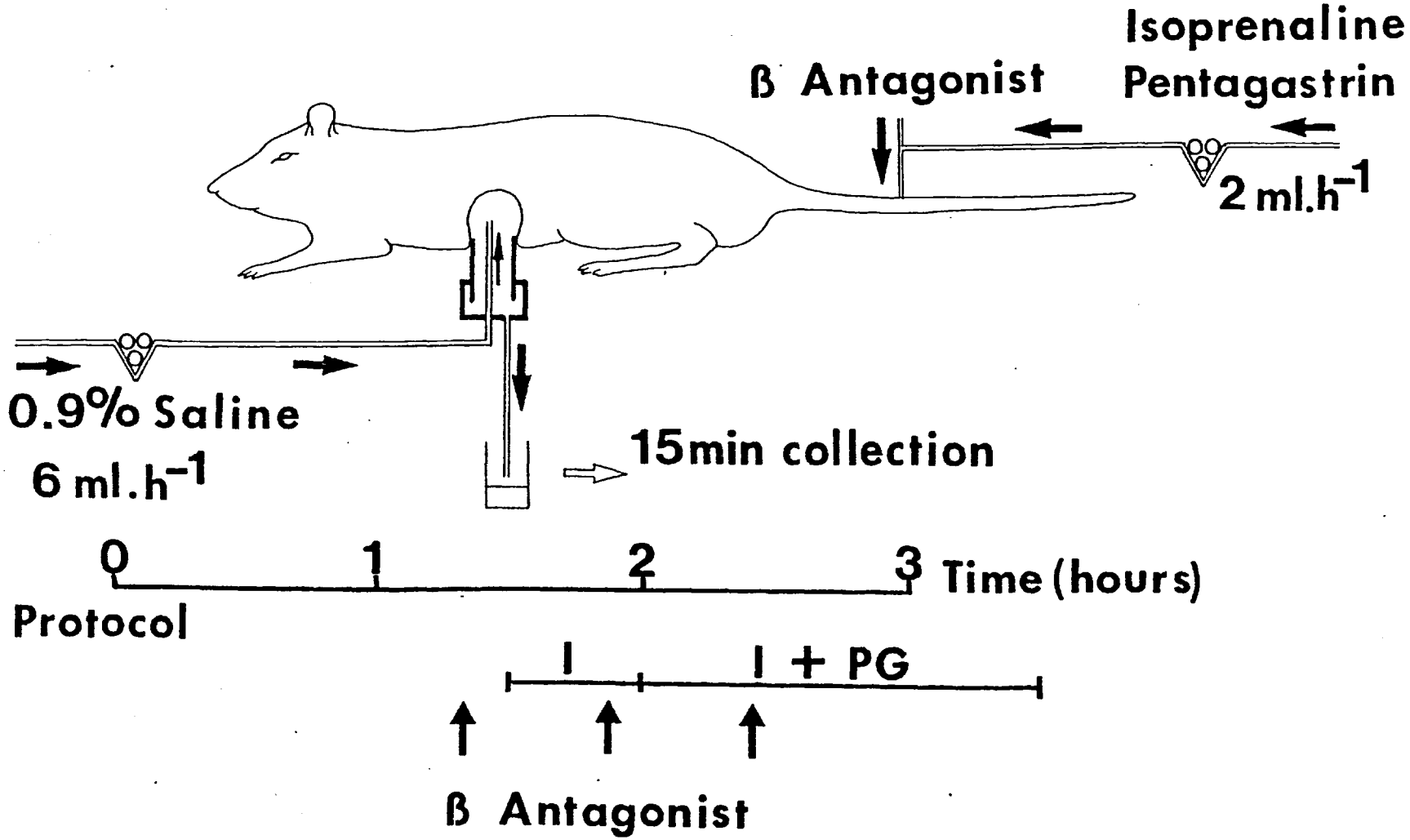
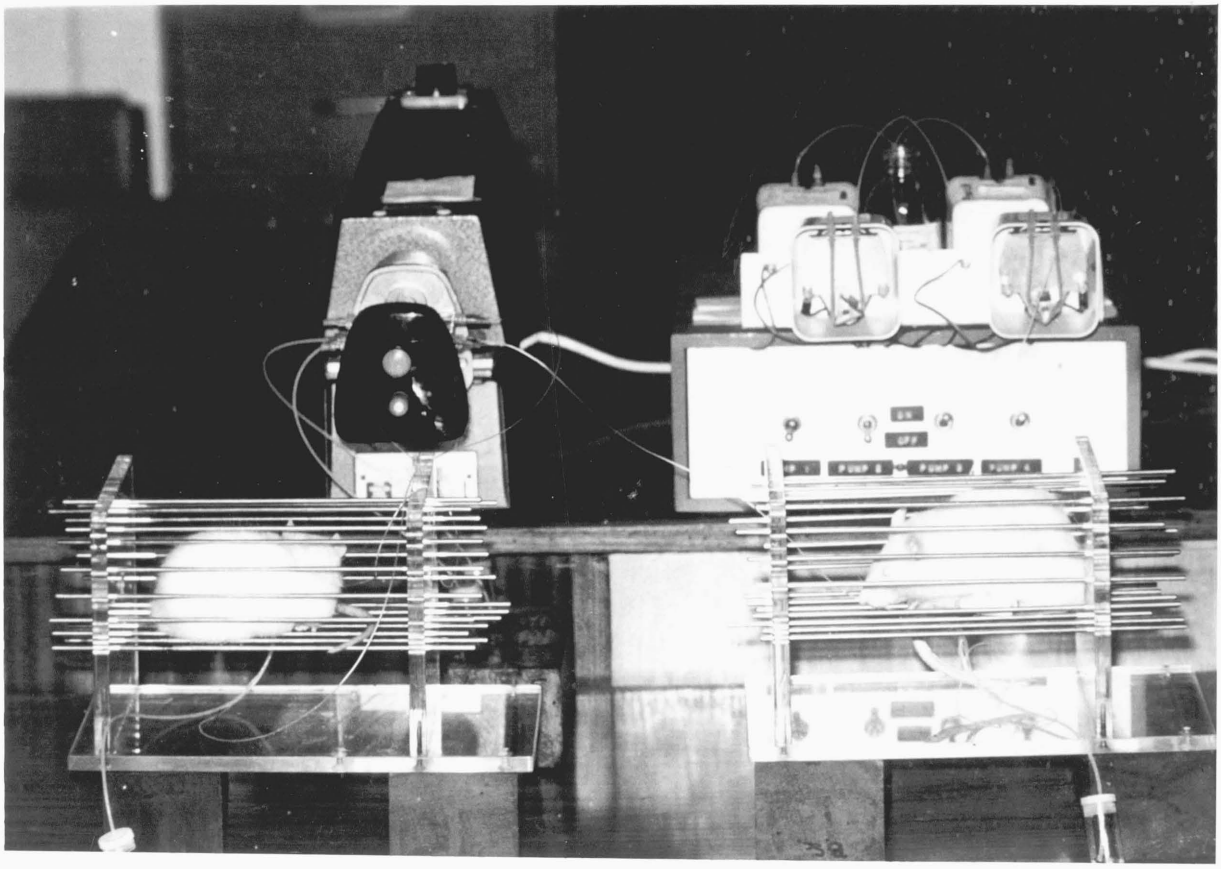


Figure 6 Experimental protocol for Heidenhain pouch rats.

Plate 1 Experimental apparatus used in Heidenhain pouch rat experiments showing Bollman restraining cages in the foreground and peristaltic pumps behind the cages.


ON
OFF

Pentagastrin and isoprenaline were administered by continuous intravenous infusion (2ml hr^{-1}) via a catheter, (Abocath T 20G), inserted into a tail vein prior to the experiment, under light halothane anaesthesia. It was secured in place using adhesive tape. Antagonists were administered by i.v. injection via the tail vein catheter.

Experiments followed one of four protocols.

(i) I.v. saline 1.5hr. I.v. pentagastrin 1.5hr.

(ii) I.v. saline 1.0hr. I.v. isoprenaline 0.5hr, i.v. isoprenaline and pentagastrin 1.5hr.

(iii) As (ii) but antagonist injected 10 minutes before infusion of isoprenaline and twice again at 30 minute intervals.

(iv) As (iii) but without isoprenaline infusion.

In the case of a rat becoming over anxious or agitated the experiment was abandoned since stress has been shown to cause changes in gastric mucosal function. Figure 7 shows this effect in one experiment. A period of stress at one hour inhibited acid secretion, this could not be fully reversed by pentagastrin even after the rat had calmed down, and the experiment was terminated.

3.2.7 The Use of the Perfused Pouch Method for Collecting Acid Secretion

The perfused pouch method has a number of advantages for gastric secretion studies. This method ensures that almost all, if not all the gastric juice secreted by the pouch is washed off the gastric mucosa, whereas using gravity drainage completeness of collection can never be ensured. The continuous washout of

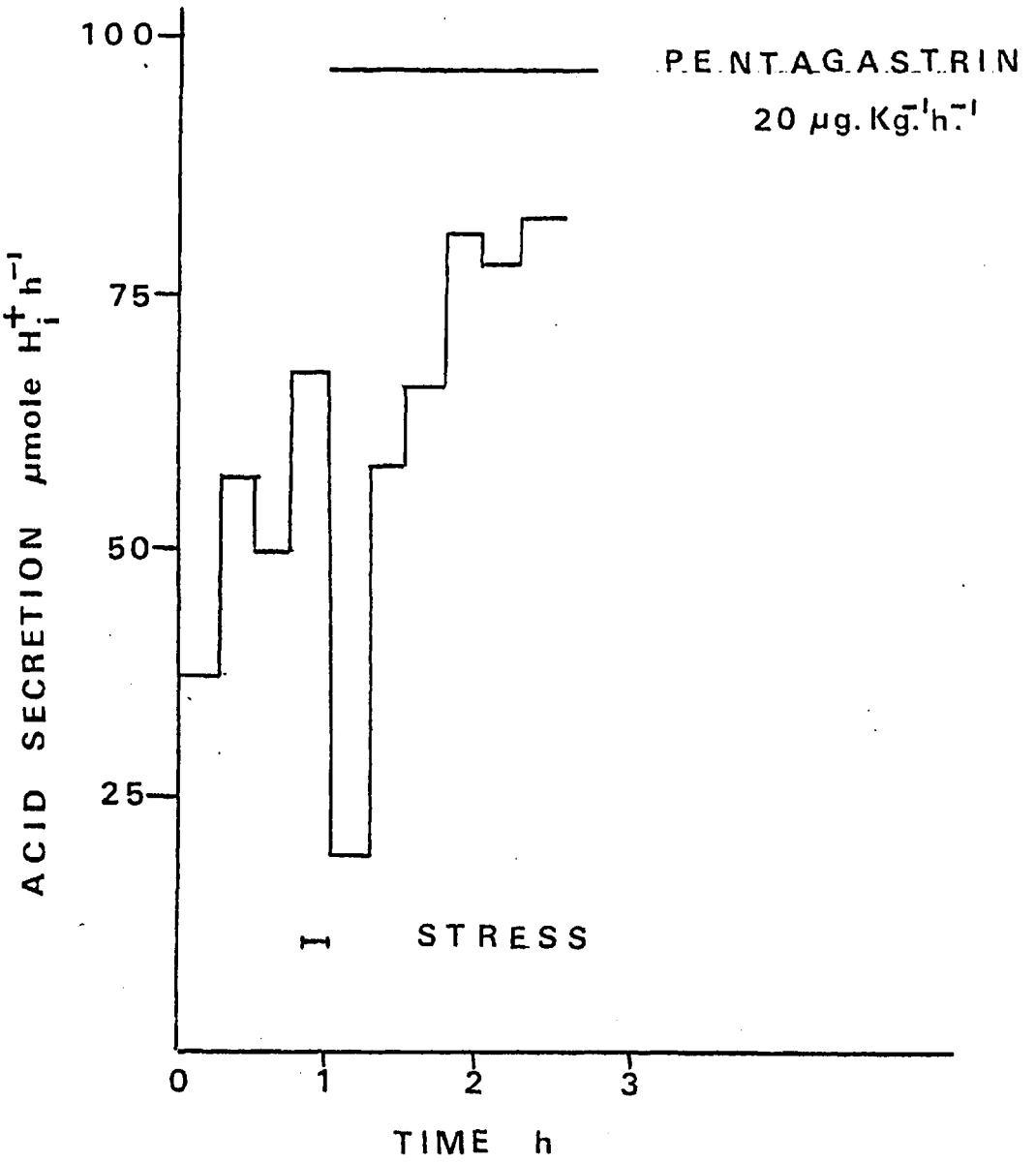


Figure 7 Effect of a period of stress during an experiment on one Heidenhain pouch rat.
The animal showed anxiety, agitated movement and rapid breathing.

the acid from the stomach greatly reduces the possibility of re-absorption of hydrogen ions which has been demonstrated through the gastric mucosa when acid solutions are instilled into the rat stomach (Lindner, 1964). Continuous gastric perfusion allows the time course of the response to an agonist or antagonist to be followed more accurately than gravity drainage since cessation of secretory events are immediately apparent. Using gravity drainage a reduction in secretion may not be immediately apparent if drainage is inadequate or the rat alters its position and causes pooling of the acid in the pouch.

3.2.8 Expression of Results

The secretory rate was expressed as the percentage change in response compared with pentagastrin alone in the same animal. This method was chosen since absolute rates of acid secretion vary with the size of individual pouches.

3.2.9 Drugs

Drugs were diluted in sterile saline (0.9%) and were made up freshly each day.

The following drugs were use:

Isoprenaline sulphate (MacCarthys)

Pentagastrin (Peptavlon, ICI)

Propranolol (Inderal, ICI)

(+) Propranolol (ICI)

Practolol (ICI)

Butoxamine (Burroughs Wellcome)

3.2.10 Statistics

The use of non-parametric statistical analysis (Mann-Whitney U test) compared to the student's t-test has been discussed in 3.1.15. The significant levels quoted here are derived from the Mann-Whitney U test.

CHAPTER 4

RESULTS: RAT ISOLATED STOMACH

4.1 THE EFFECT OF SALMEFAMOL ON GASTRIC SECRETAGOGUE INDUCED ACID SECRETION IN THE RAT ISOLATED STOMACH

Salmefamol, a β_2-adrenoceptor agonist has previously been shown to inhibit pentagastrin- and bethanechol-induced acid secretion in the Heidenhain pouch dog in this laboratory. (Canfield, Curwain, King and Price, 1978). Preliminary work in this study was intended to look for a comparable inhibitory effect of salmefamol *in vitro* using the rat isolated stomach preparation. For this reason the experimental protocol differed from that described for the main body of work on the rat isolated stomach (Chapter 3), which was chosen after these pilot studies as a suitable experimental design.

A response to a submaximal concentration of pentagastrin was followed by a second application of pentagastrin. Salmefamol (10^{-5}M) was added to the serosal saline after a plateau had been reached during this second response. In a second experiment a response to a submaximal dose of bethanechol was followed by a response to bethanechol in the presence of salmefamol (10^{-5}M). Results are expressed as secretory ratio and in μmole $H^+cm^{-2}h^{-1}$ to facilitate comparison with results in the Heidenhain pouch dog and work carried out by other laboratories.

4.1.1 The Effect of Salmefamol on Pentagastrin-Induced Acid Secretion

In contrast with results in the Heidenhain pouch dog, salmefamol did not inhibit pentagastrin-induced acid secretion. Results obtained in one rat isolated stomach are shown in Figure 8. Pentagastrin (5.4×10^{-7}M) produced acid responses

of 3.50 and 2.10μmole $H^+cm^{-2}h^{-1}$ (secretory ratios of 1.82 and 1.42). Tachyphylaxis to pentagastrin as well as other secretagogues has been shown by many workers in the isolated stomach or mucosa. Salmefamol ($10^{-5}M$) was added to the serosal saline during the maximum response to the second dose of pentagastrin. Clearly there is no evidence of an inhibition of the response, in fact salmefamol appeared to potentiate the response to pentagastrin with an acid response of 3.66μmole $H^+cm^{-2}h^{-1}$ (secretory ratio of 1.73).

The mean secretory ratio due to pentagastrin alone was 1.69 ± 0.14 and in the presence of salmefamol was 2.47 ± 0.37 (n = 3).

4.1.2 The Effect of Salmefamol on Bethanechol-Induced Acid Secretion

Salmefamol ($10^{-5}M$) was also ineffective in inhibiting bethanechol-induced acid secretion in the rat isolated stomach. The results obtained in one rat isolated stomach are shown in Figure 9. Bethanechol ($1.7 \times 10^{-6}M$) stimulated gastric acid secretion, with a response of 4.60μmole $H^+cm^{-2}h^{-1}$ in the absence of salmefamol and 6.30μmole $H^+cm^{-2}h^{-1}$ in the presence of salmefamol. (Secretory ratios of 2.9 and 3.7 respectively). When bethanechol was removed the rate of acid secretion did not fall to basal level in the presence of salmefamol, as might have been expected, but did so later in the experiment when both drugs were removed.

The mean secretory ratio in response to bethanechol alone was 2.81 ± 0.72 and in the presence of salmefamol was 3.58 ± 0.42 (n = 3).

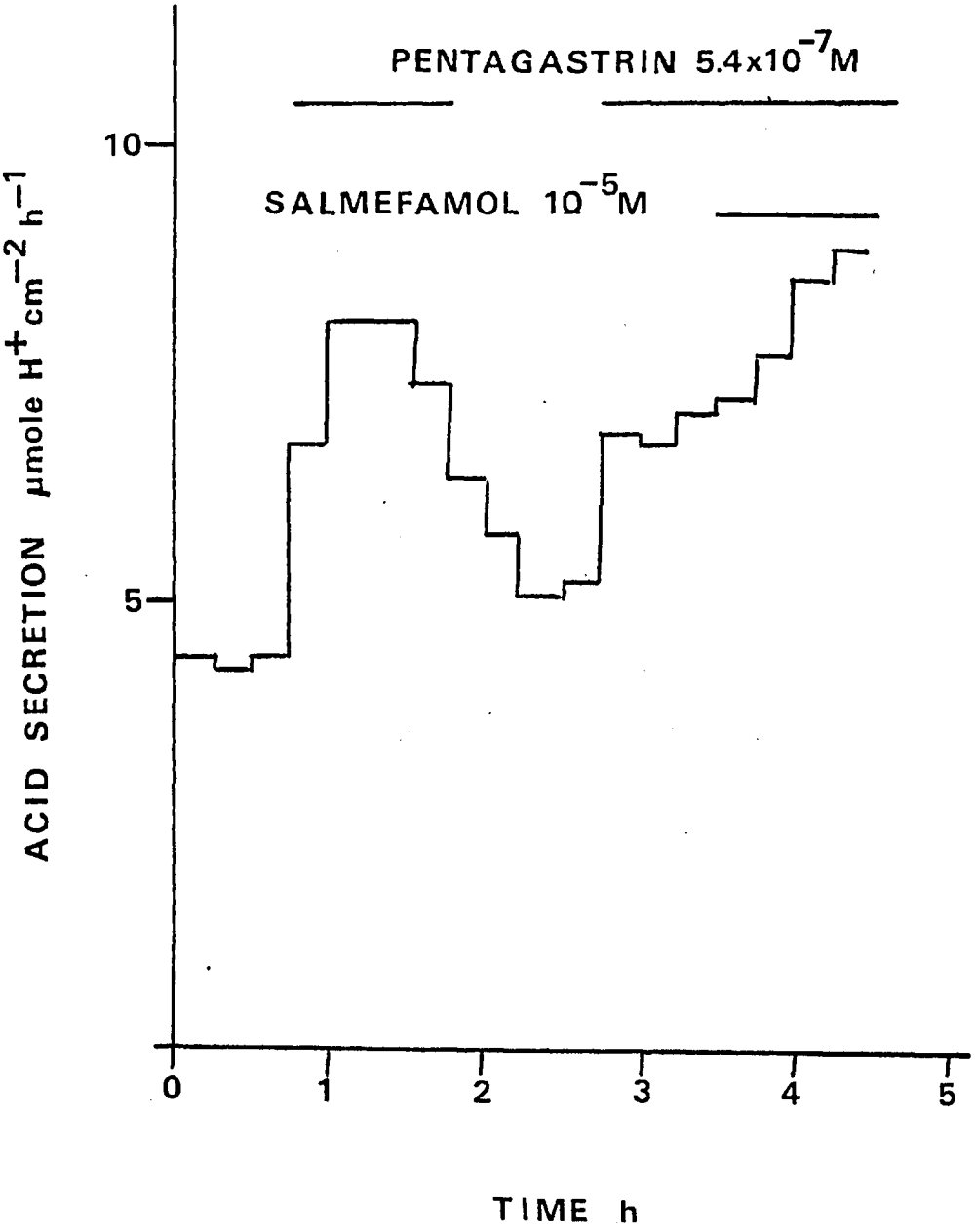


Figure 8 The effect of salmefamol on pentagastrin-induced acid secretion in one rat isolated stomach.

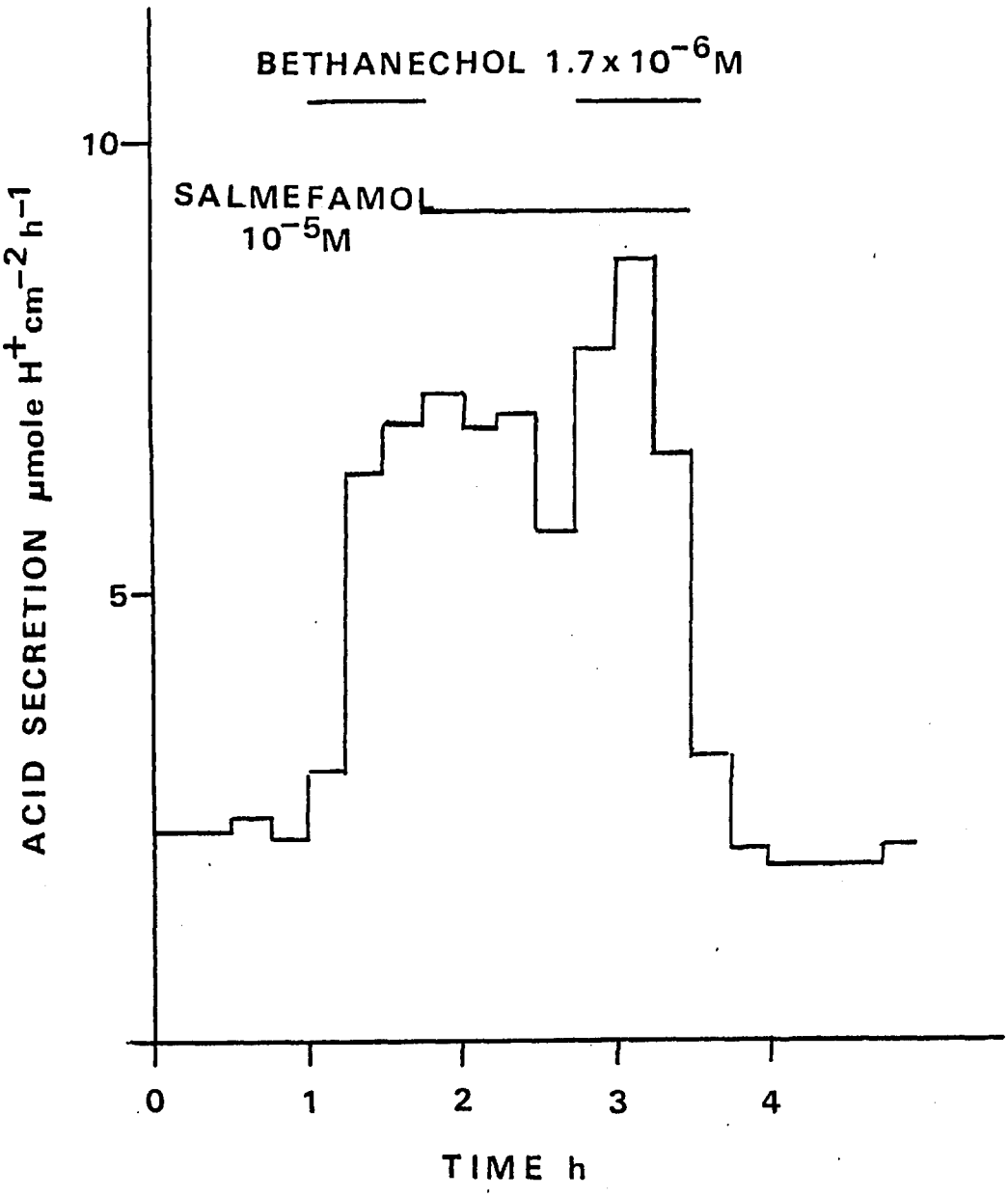


Figure 9 The effect of salmefamol on bethanechol-induced acid secretion in one rat isolated stomach.

Clearly, salmefamol (10^{-5}M) did not inhibit either pentagastrin- or bethanechol-induced acid secretion in the rat isolated stomach. The apparent potentiation of responses and the observation that the rate of secretion remained raised in the presence of salmefamol alone led to the study of the effects of β-adrenoceptor agonists alone on gastric acid secretion in this preparation.

4.2 THE EFFECT OF β-ADRENOCEPTOR AGONISTS ON GASTRIC ACID SECRETION

4.2.1 Examples of Single Responses of β-Adrenoceptor Agonists

Salmefamol, salbutamol and isoprenaline all stimulated acid secretion in the rat isolated stomach.

Figures 10, 11 and 12 show responses to the three agonists used, in three separate experiments.

Salmefamol (5×10^{-6}M) produced an increase in acid secretion of 4.8 μmole $H^+cm^{-2}h^{-1}$ above basal. This represents a secretory ratio of 2.4. The secretory rate returned to the unstimulated level within ninety minutes, which was considerably slower than the recovery time seen after stimulation with histamine, bethanechol or pentagastrin in this preparation, where the secretory rate returns to basal after approximately forty-five minutes (J.E. Spencer: personal communication). Construction of concentration response curves using β-adrenoceptor agonists (4.2.2) were carried out using one concentration in each stomach.

Salbutamol (5×10^{-6}M) produced an acid response of 3.50 μmole $H^+cm^{-2}h^{-1}$ above basal. This represents a secretory ratio of 1.61. This particular response was more transient than others in this study, however "fade" was occasionally observed, and for this reason the figure is included here.

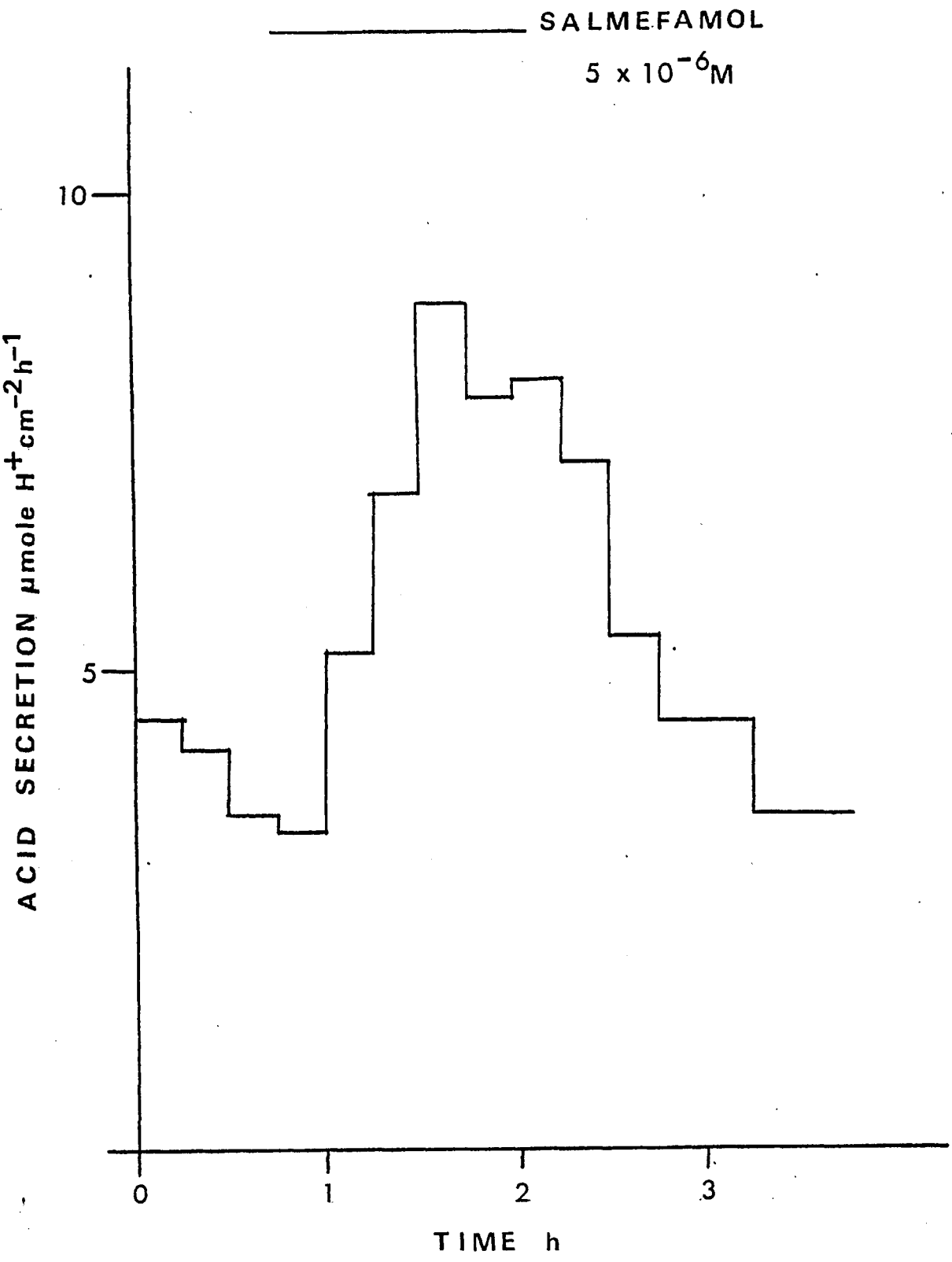


Figure 10 The effect of salmefamol on gastric acid secretion in one rat isolated stomach.

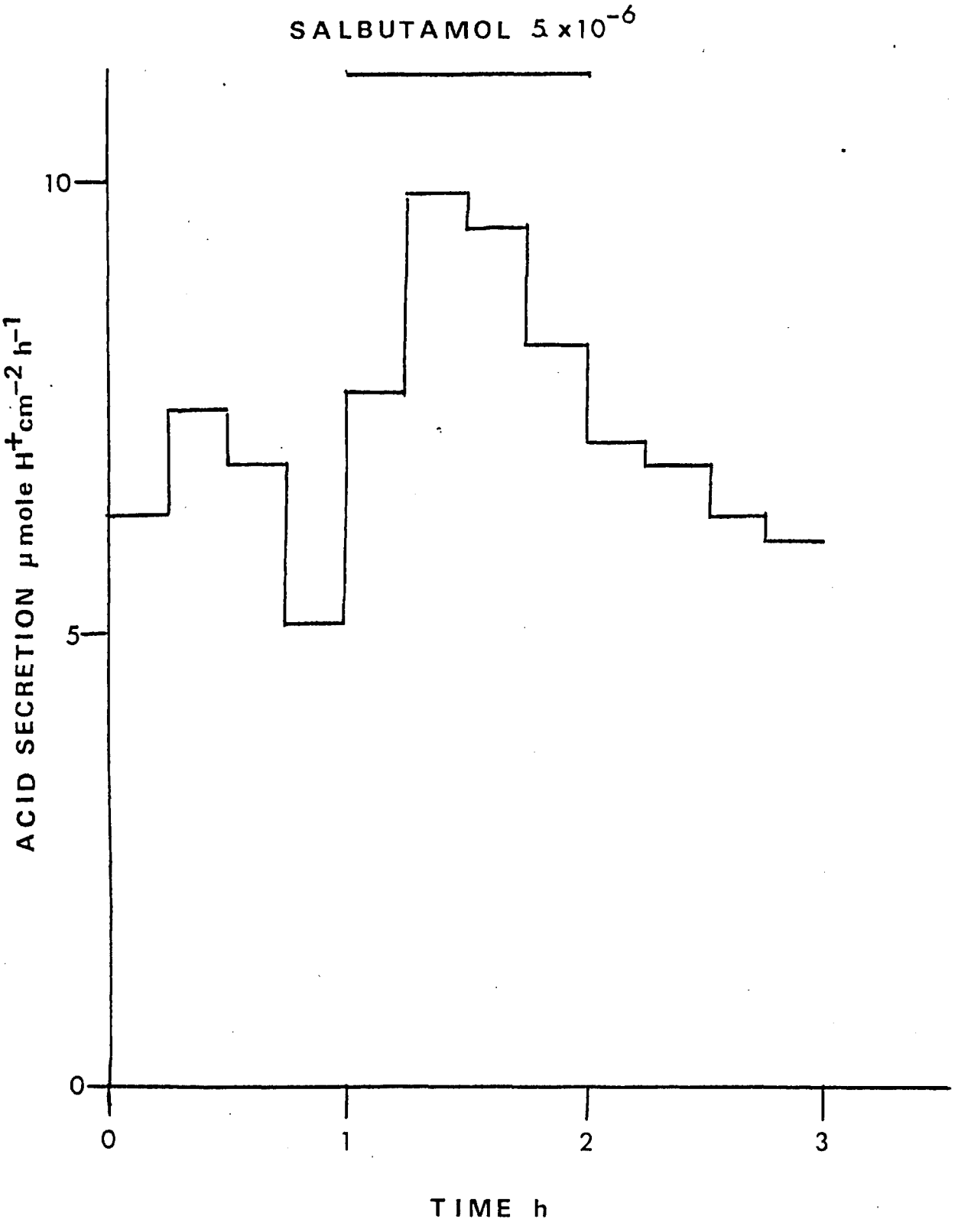


Figure 11 The effect of salbutamol on gastric acid secretion in one rat isolated stomach.

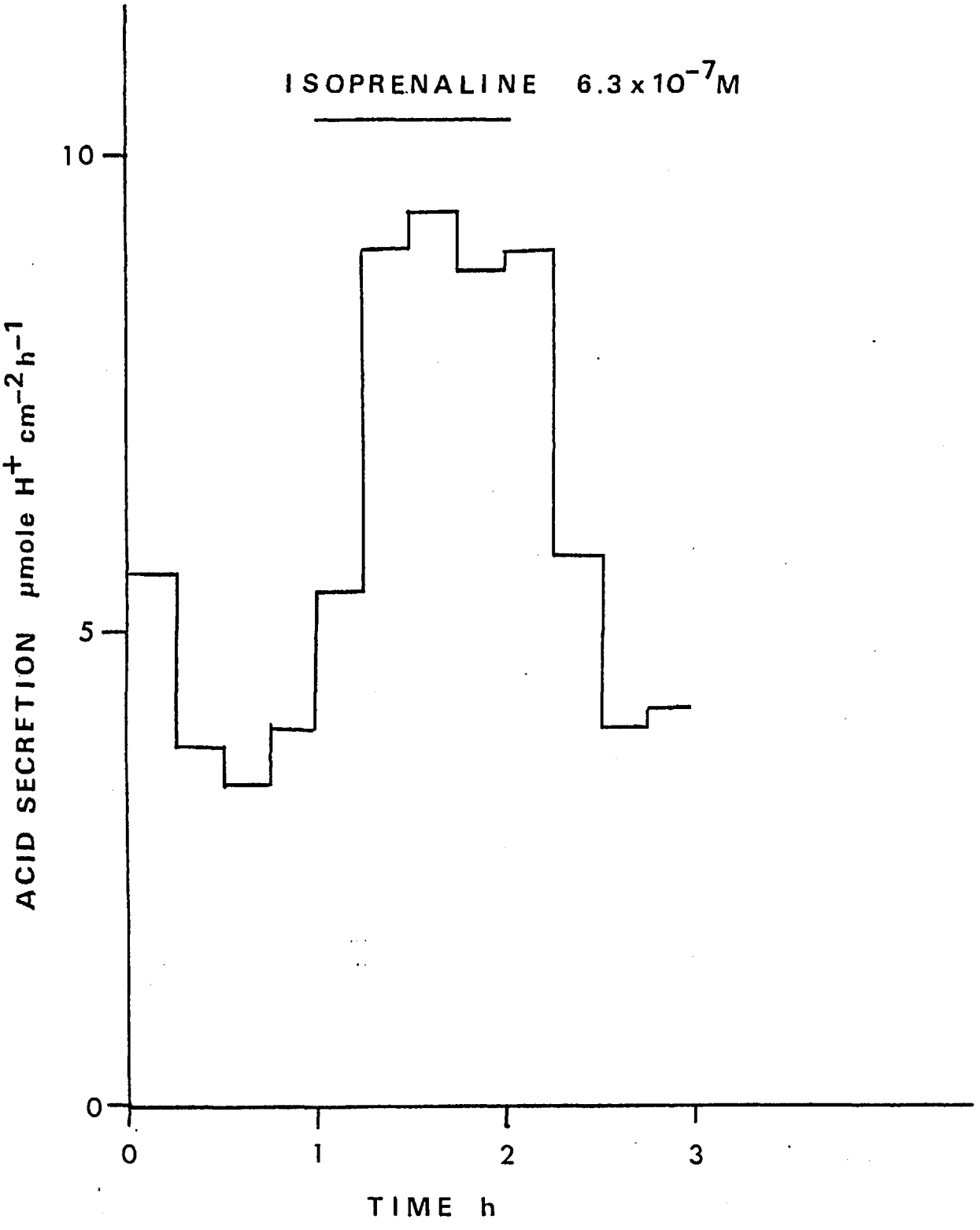


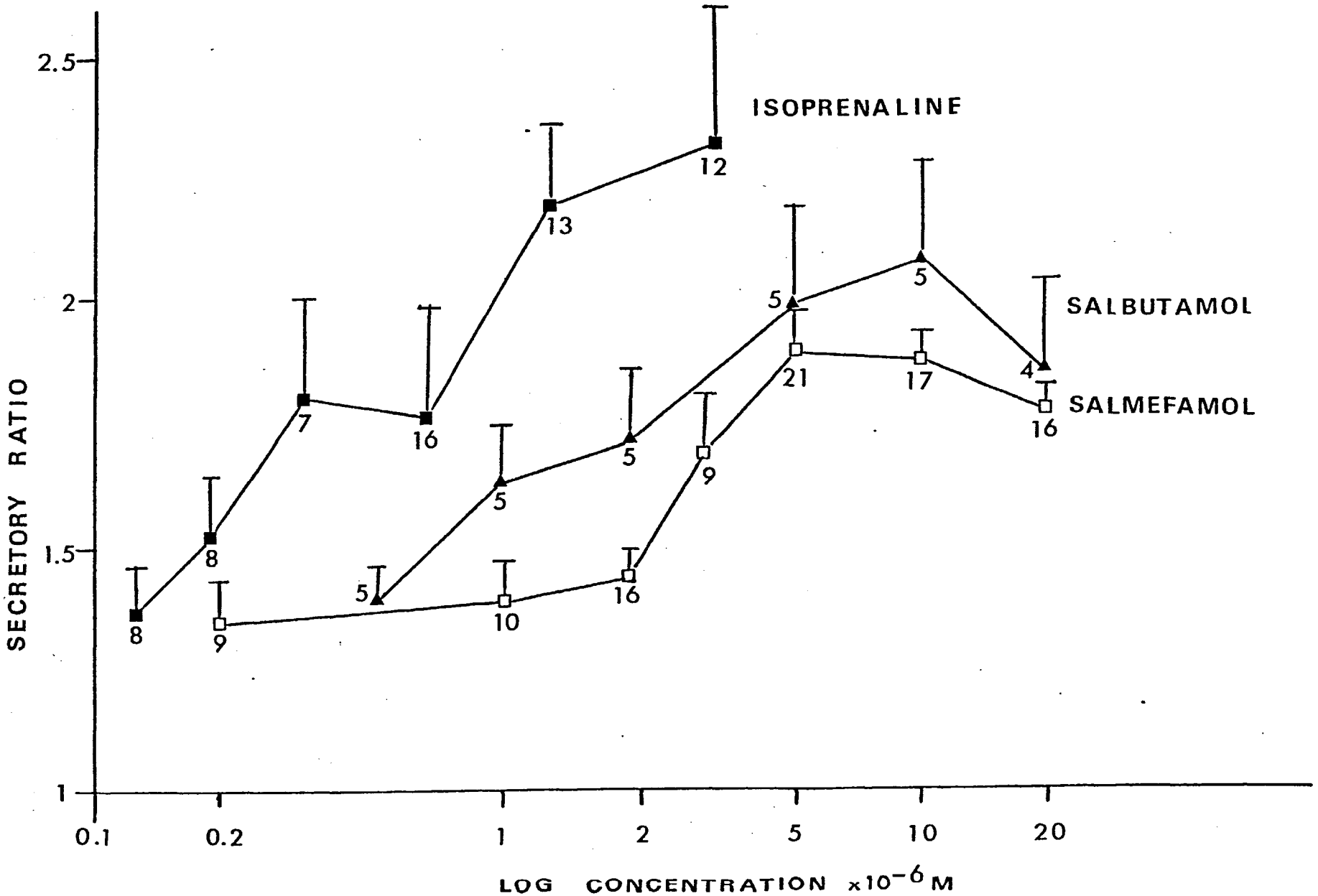
Figure 12 The effect of isoprenaline on gastric acid secretion in one rat isolated stomach.

Isoprenaline gave more consistent and more reproducible responses than those to salmefamol or salbutamol, which returned to baseline within forty-five minutes. In this example an acid response of 5.3 μmole $H^+cm^{-2}h^{-1}$ above basal was produced by 6 x 10^{-7}M isoprenaline. The recovery time after washout for isoprenaline was more typical of that of other gastric secretagogues in this preparation. This fact, coupled with the prompt nature of, and maintenance of the response led to the use of isoprenaline in the majority of studies on the effects of antagonists on β-adrenoceptor agonists.

4.2.2 Log Concentration-Response Curves

Figure 13 shows the log concentration-response curves for the β_2 adrenoceptor agonists salmefamol and salbutamol, and also the curve for isoprenaline, a non-selective β-adrenoceptor agonist. All three compounds were found to stimulate acid secretion in a concentration-dependent manner, in the range of 0.2 - 20 μM for salmefamol, 0.5 - 20 μM for salbutamol and 0.1 - 10 μM for isoprenaline. The maximum responses and slopes of the linear portions of the curve arecomparablefor each drug. The mean maximum secretory ratios with the S.E. of the means were: salmefamol 1.90 ± 0.09 (n = 21); salbutamol 2.10 ± 0.21 (n = 5); isoprenaline 2.33 ± 0.28 (n = 12). Absolute values for these maxima were 3.84 ± 0.28; 3.61 ± 0.28 and 5.45 ± 0.72 μmole $H^+ cm^{-2} h^{-1}$ respectively. Isoprenaline was the most potent agonist.

Figure 13 Drug concentration on log scale plotted against secretory ratio. Points are mean values with s.e. bars and number of observations are shown below each point. See also Appendix II.


2.5
2
1.5
1
SECRETORY RATIO
ISOPRENALINE
SALBUTAMOL
SALMEFAMOL
12
13
7
16
8
9
5
5
10
5
9
16
5
21
5
17
4
16
0.1
0.2
1
2
5
10
20
LOG CONCENTRATION x10⁻⁶ M

The maximum secretory ratios obtained and the slopes of the linear portion of the curves are similar to results obtained in this preparation with histamine, pentagastrin, bethanechol and theophylline (J.E. Spencer: personal communication).

4.3 THE EFFECT OF ADRENOCEPTOR ANTAGONISTS ON β-ADRENOCEPTOR AGONIST STIMULATED ACID SECRETION

In order to determine whether the stimulatory effect of β-adrenoceptor agonists on gastric acid secretion was really the result of β-receptor stimulation the effects of β-antagonists were studied on the acid response to a submaximal concentration of isoprenaline and salmefamol. Each stomach was exposed to only one concentration of the agonist and was randomly allocated to either the test group containing the antagonist or to the control group. Each series of experiments contained its own control group since the secretory responses were subject to variability, and control groups for each series of experiments are shown in the figures. However, no significant differences between mean value for the different control groups were apparent.

Results are expressed as secretory ratio (3.113) and shown as histograms, with control group (agonist alone) shown on the left of each pair of columns. The concentration of agonist or antagonist are shown in the legend, or on the figure. The concentration of β-adrenoceptor antagonists used are rather higher than those required to inhibit β-adrenoceptor agonists in other tissues, and the possible reason for this will be discussed in Chapter 6.

Concentrations of isoprenaline (1.25×10^{-6}M) and salmefamol (5×10^{-6}M) were used throughout all antagonist studies.

4.3.1 The Effect of Non-Selective β-Adrenoceptor Antagonists

(a) Isoprenaline-Stimulated Acid Secretion

Timolol, propranolol and pindolol all produced a significant inhibition of the acid response to isoprenaline (1.25×10^{-6}M).

Figures 14 and 15 show the effects of timolol (2×10^{-5}M) and propranolol (2×10^{-5}M) on the responses to isoprenaline, with reductions in the secretory ratio from 2.00 ± 0.22 ($n = 5$) to 1.26 ± 0.11 ($n = 6$) ($p < 0.01$) and from 1.98 ± 0.18 to 1.52 ± 0.19 ($n = 12$) ($p < 0.01$) respectively. These values represent a 74% and 47% reduction in the acid response.

Figure 16 shows a two-point concentration-response curve for isoprenaline alone and in the presence of propranolol. Propranolol (2×10^{-5}M) caused a parallel shift of the concentration-response curve to the right.

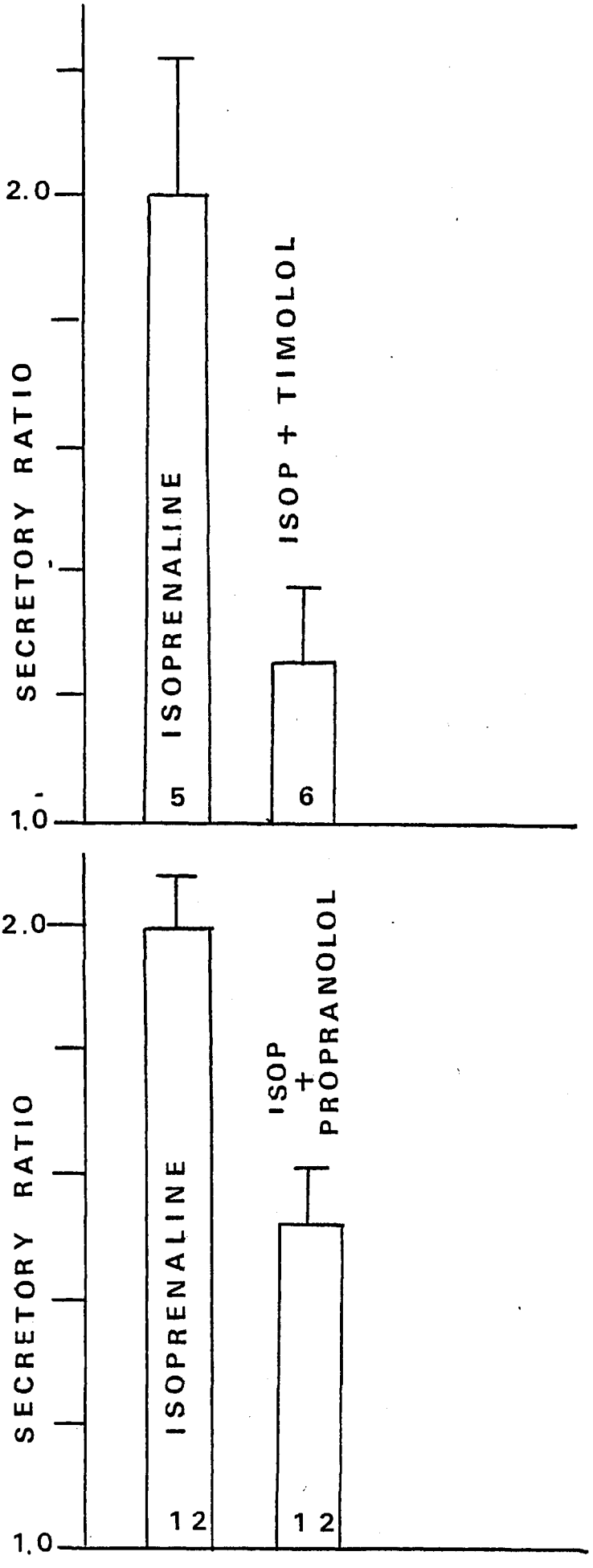
Figure 17 shows the effect of pindolol (2×10^{-5}M) on the acid response to isoprenaline, with a reduction of secretory ratio from 1.70 ± 0.08 to 1.55 ± 0.09 ($n = 6$) ($p < 0.05$). This represents a 21% reduction in response.

Figure 18 shows the effect of increasing the concentration of pindolol and timolol. Timolol (10^{-4}M) produced no further inhibition of the response with a reduction in secretory ratio from 1.73 ± 0.10 ($n = 13$) to 1.29 ± 0.39 ($n = 11$) ($p < 0.01$). This represents a 60% reduction in the response.

Pindolol (10^{-4}M), however, reduced the secretory ratio from 1.73 to 0.10 ($n = 13$) to 1.33 ± 0.04 ($n = 10$) ($p < 0.05$). This was a 55% reduction compared with a 21% reduction in response in the presence of pindolol (2×10^{-5}M).

Figure 14 The effect of timolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

Figure 15 The effect of propranolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.


SECRETORY RATIO
2.0
1.0
ISOPRENALINE
5
ISOP + TIMOLOL
6
SECRETORY RATIO
2.0
1.0
ISOPRENALINE
12
ISOP
+
PROPRANOLOL
12

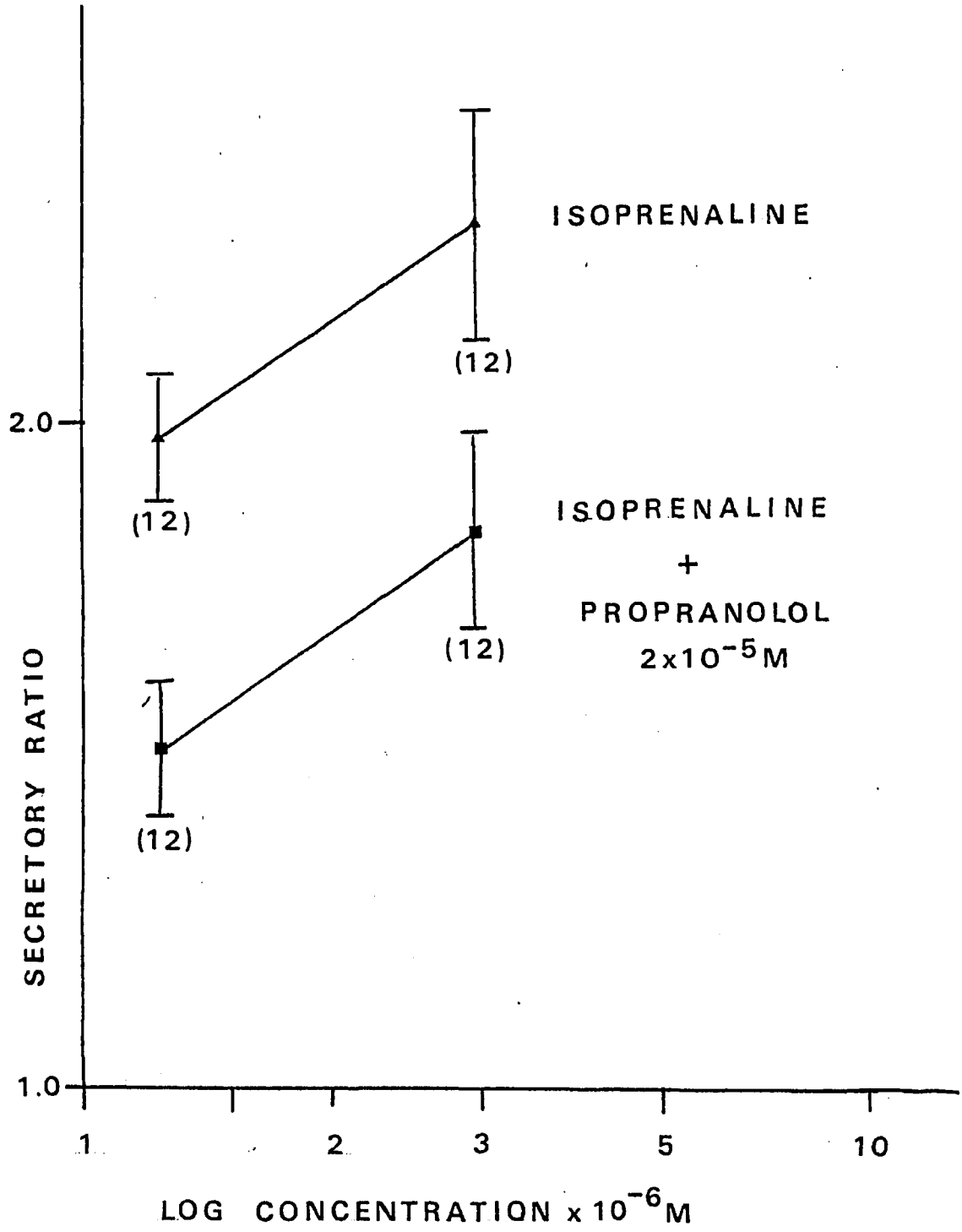
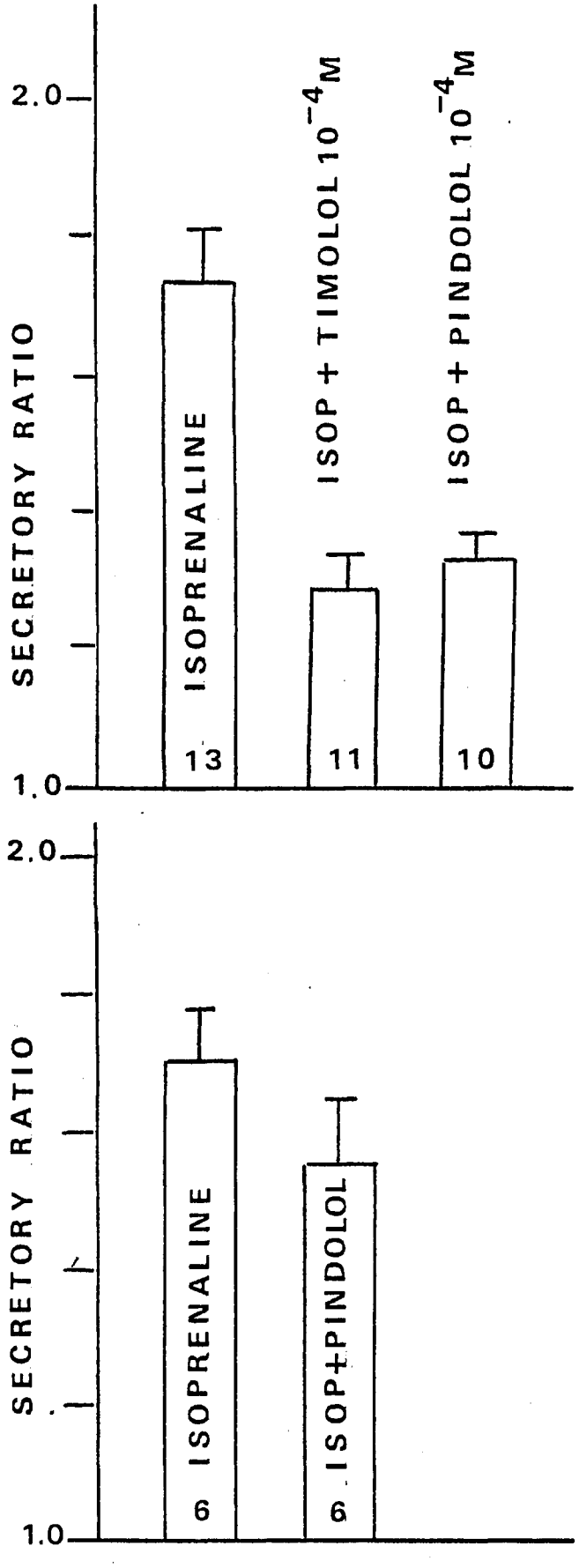


Figure 16 The effect of propranolol (2×10^{-5}M) on the response to isoprenaline compared with the response in the absence of antagonist. Points are mean values with s.e. bars and numbers of observations are in parentheses.

Figure 18 The effect of pindolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

Figure 17 The effect of pindolol (10^{-4}M) and timolol (10^{-4}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.


2.0
1.0
SECRETORY RATIO
ISOPRENALINE
13
ISOP + TIMOLOL 10^{-4}M
11
ISOP + PINDOLOL 10^{-4}M
10
2.0
1.0
SECRETORY RATIO
ISOPRENALINE
6
ISOP+PINDOLOL
6

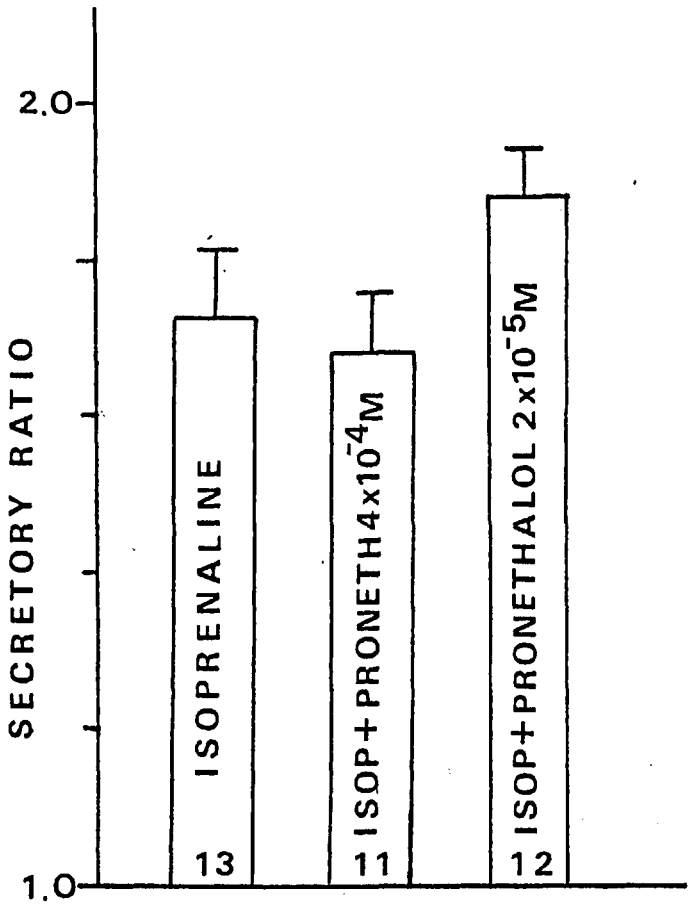


Figure 19 The effect of pronethalol (4×10^{-4}M and 2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

Pronethalol is structurally very similar to propranolol, however it was ineffective in inhibiting the secretory response to isoprenaline. The results are shown in Figure 19. The control ratio to isoprenaline in this series of experiments was 1.73 ± 0.02 ($n = 13$) and ratios were 1.89 ± 0.06 ($n = 12$) and 1.69 ± 0.07 ($n = 11$) in the presence of 2×10^{-5}M and 4×10^{-4}M pronethalol respectively.

In summary, propranolol, timolol and pindolol all produced a significant inhibition of the acid secretory response to isoprenaline. However, even at these high concentrations complete inhibition was not produced. The two point concentration-response curve for isoprenaline in the presence of propranolol is consistent with a competitive antagonism of the response. Further conclusions cannot be drawn on the basis of this observation since only one dose of propranolol was investigated. Pronethalol was unable to inhibit the isoprenaline response.

(b) Salmefamol-Stimulated Acid Secretion

Figure 20 shows the effects of propranolol and timolol on salmefamol induced acid secretion. Propranolol (2×10^{-5}M) produced a significant inhibition of the response to salmefamol (5×10^{-6}M) reducing the secretory ratio from 2.18 ± 0.12 to 1.35 ± 0.08 ($p < 0.01$). This represents a 70% inhibition of the response.

Timolol (2×10^{-5}M) reduced the secretory ratio from 2.12 ± 0.13 ($n = 6$) to 1.18 ± 0.08 ($n = 5$) ($p < 0.01$). This was an 84% inhibition of response.

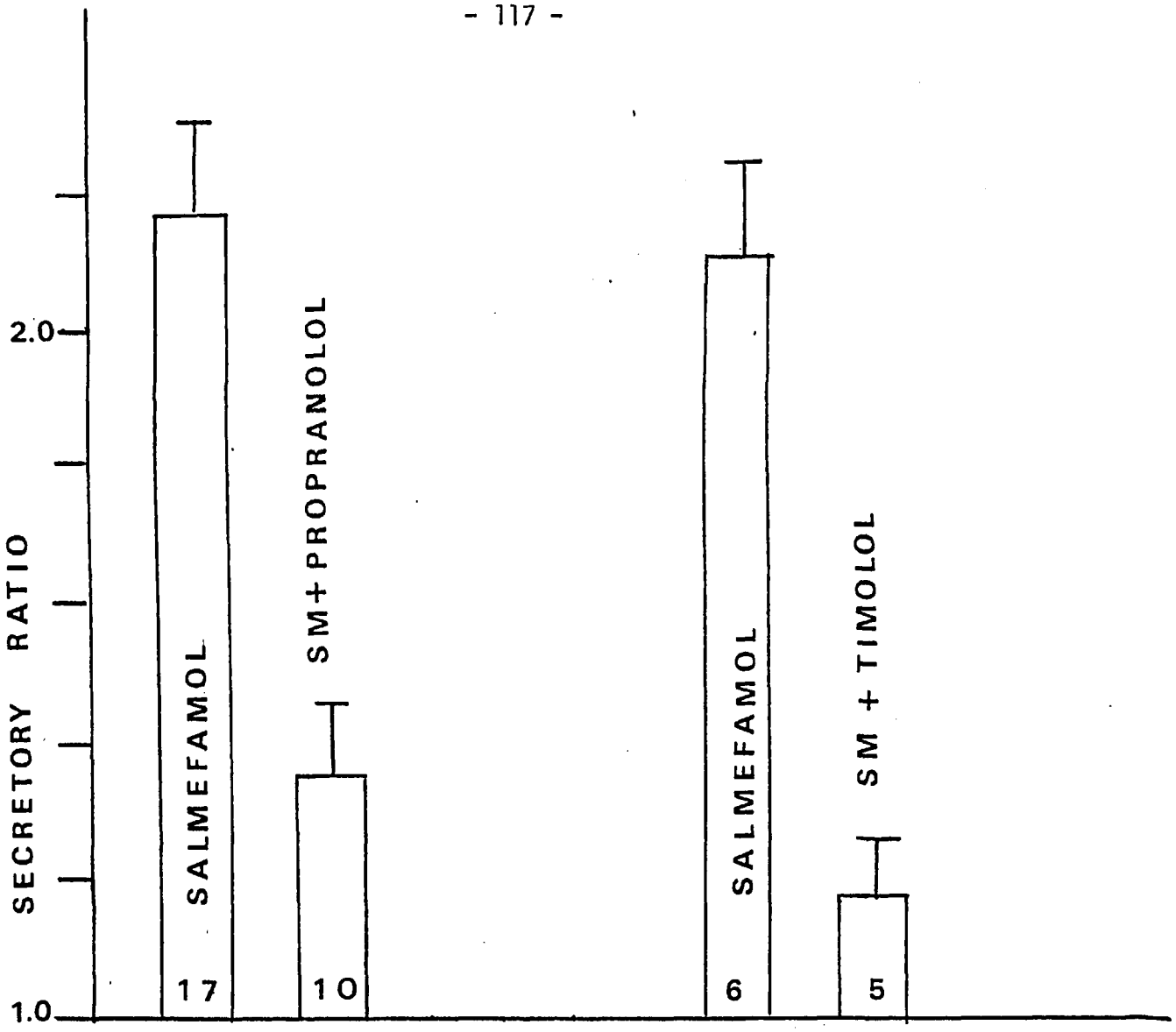


Figure 20 The effect of propranolol (2×10^{-5}M) and timolol (2×10^{-5}M) on the secretory ratio obtained with salmefamol (5×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

Figure 21 The effect of propranolol (2×10^{-5}M) on the response to salmefamol compared with the response in the absence of antagonist. Points are mean values with s.e. bars and numbers of observations are shown below each point. The concentration-response curve for salmefamol alone is taken from Figure 13, and values after the maximum response have been omitted.

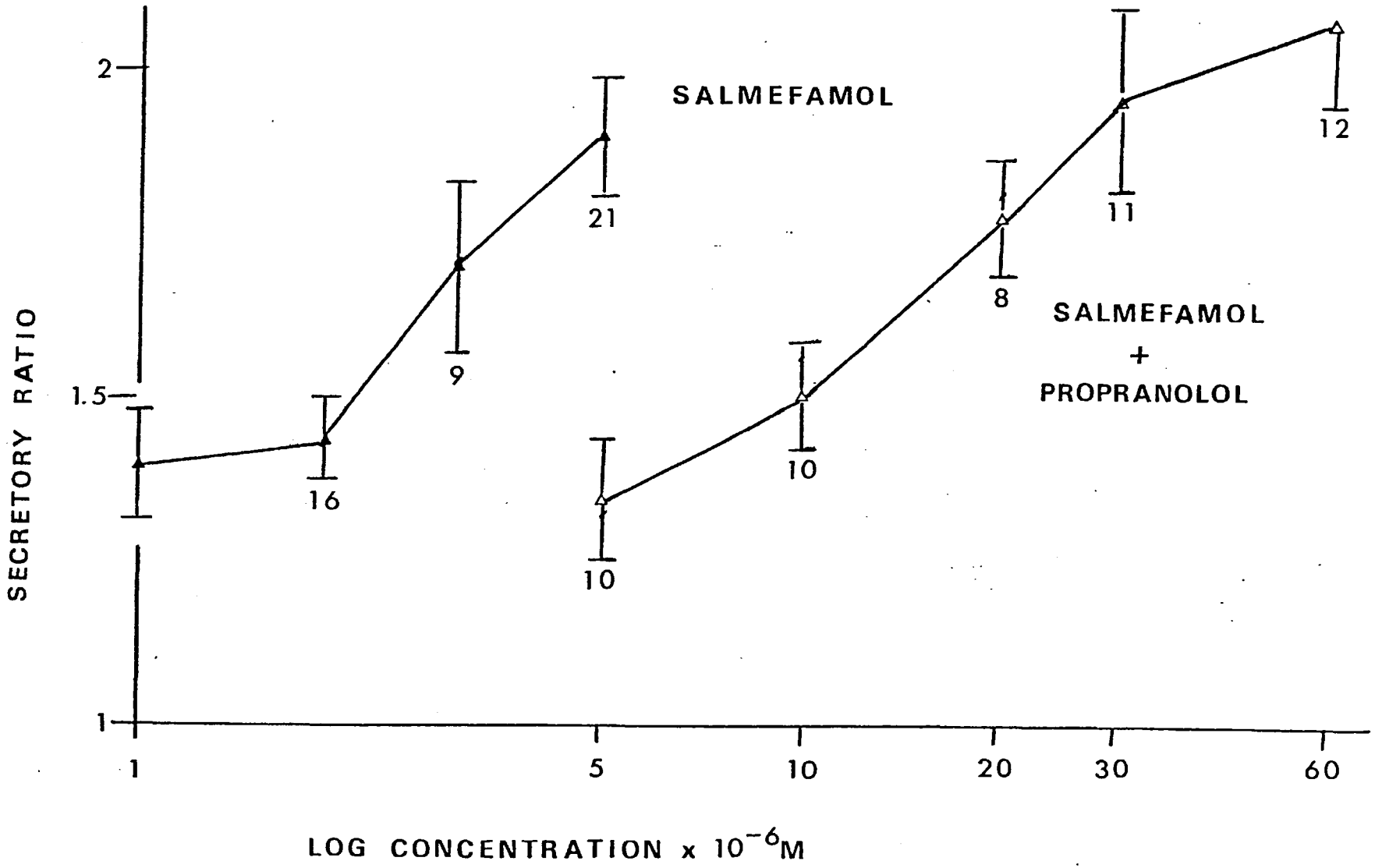

SECRETORY RATIO
2
1.5
1
SALMEFAMOL
SALMEFAMOL
+
PROPRANOLOL
21
16
9
10
10
8
11
12
1
5
10
20
30
60
LOG CONCENTRATION x 10^{-6}M

Figure 21 shows the log-concentration response curve for salmefamol in the absence and presence of propranolol (2×10^{-5}M). The maximum response to salmefamol in the presence of propranolol occurred at 6×10^{-5}M where the secretory ratio was 2.07 ± 0.12 (n = 12) (2.95 ± 0.28 μmole $cm^{-2}h^{-1}$). This can be compared to the maximum response to salmefamol alone of 1.90 ± 0.09 (n = 21) at 5×10^{-6}M).

These results are consistent with a competitive antagonism of the response as there is no apparent change of slope and the maximum response is not reduced in the presence of propranolol.

In summary, salmefamol was inhibited by propranolol and timolol in a similar manner to isoprenaline.

4.3.2 The Effect of β_2-Selective Adrenoceptor Antagonists

Since the β_2-selective agonists salmefamol and salbutamol stimulated acid secretion in a concentration-dependent manner, the effect of β_2-selective antagonists were investigated on both salmefamol and isoprenaline stimulated secretion in order to further elucidate the mechanisms involved in these responses.

(a) Isoprenaline-Stimulated Acid Secretion

Figure 22 shows that butoxamine (2×10^{-5}M and 10^{-4}M) had no effect on isoprenaline-induced acid secretion. The mean secretory ratio in the group receiving isoprenaline (1.25×10^{-6}M) alone was 2.28 ± 0.11 (n = 6), and 2.30 ± 0.25 (n = 5) and 2.41 ± 0.06 (n = 6) for butoxamine 2×10^{-5}M and 10^{-4}M respectively.

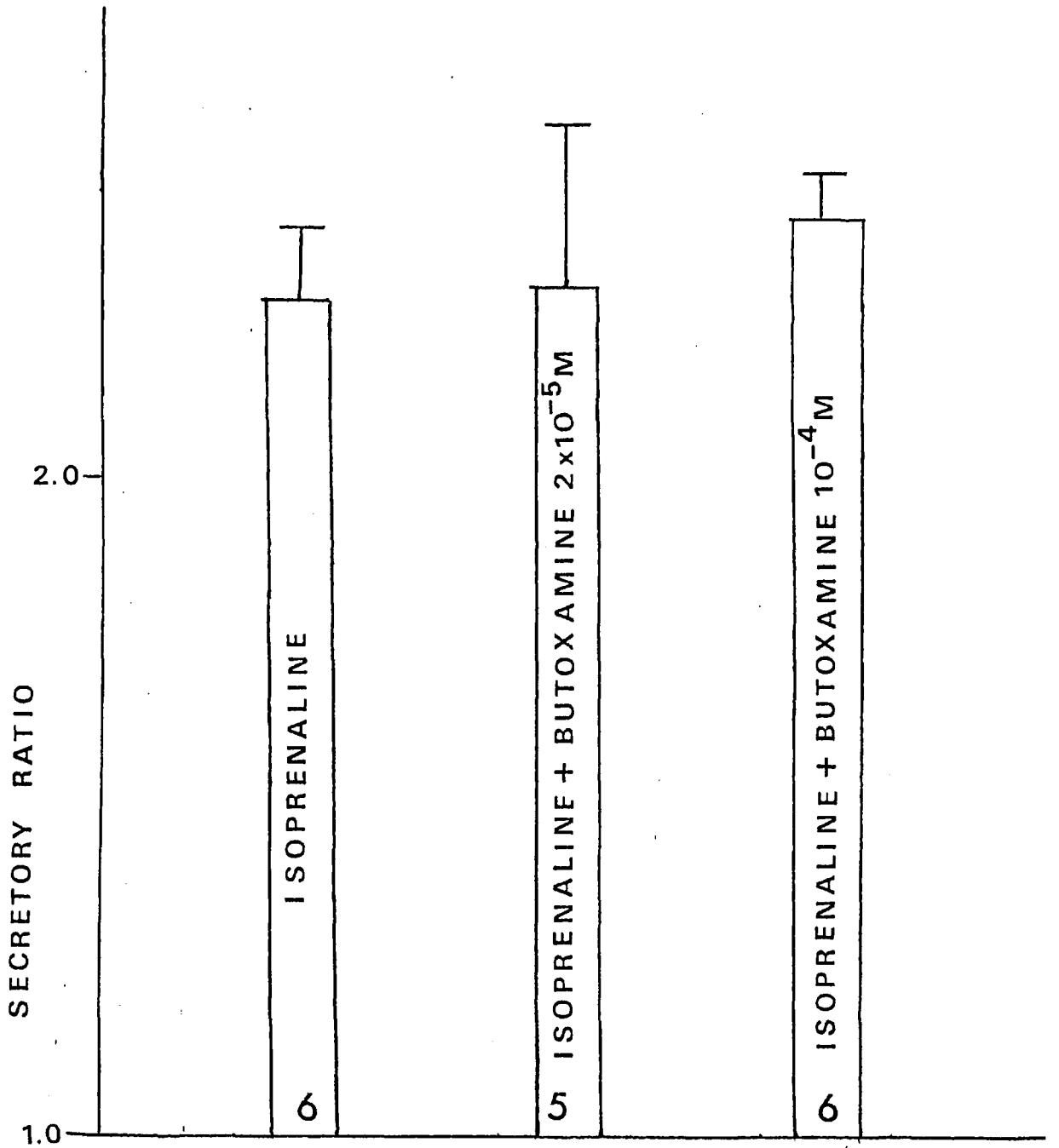


Figure 22 The effect of butoxamine (2×10^{-5}M and 10^{-4}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

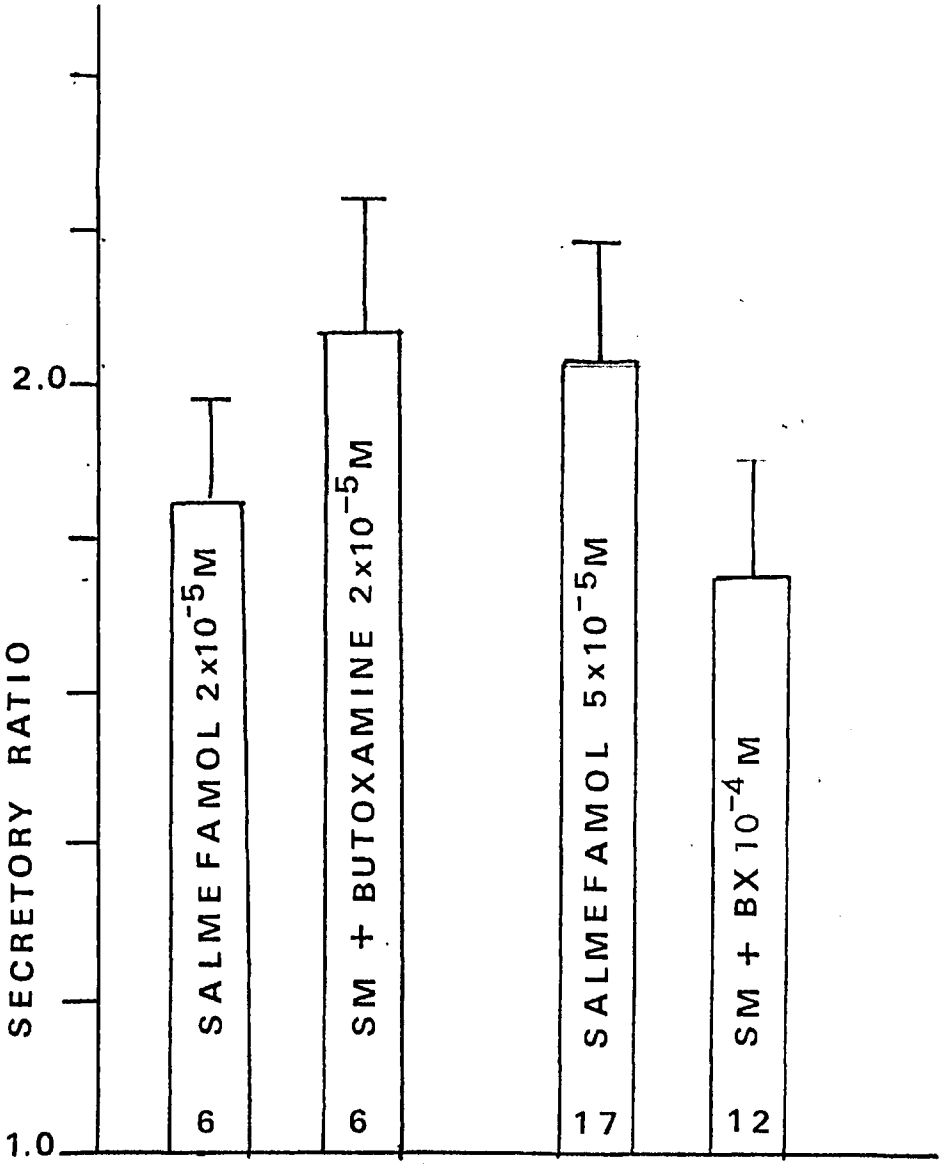


Figure 23 The effect of butoxamine (2×10^{-5}M and 10^{-4}M) on the secretory ratio obtained with salmefamol (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

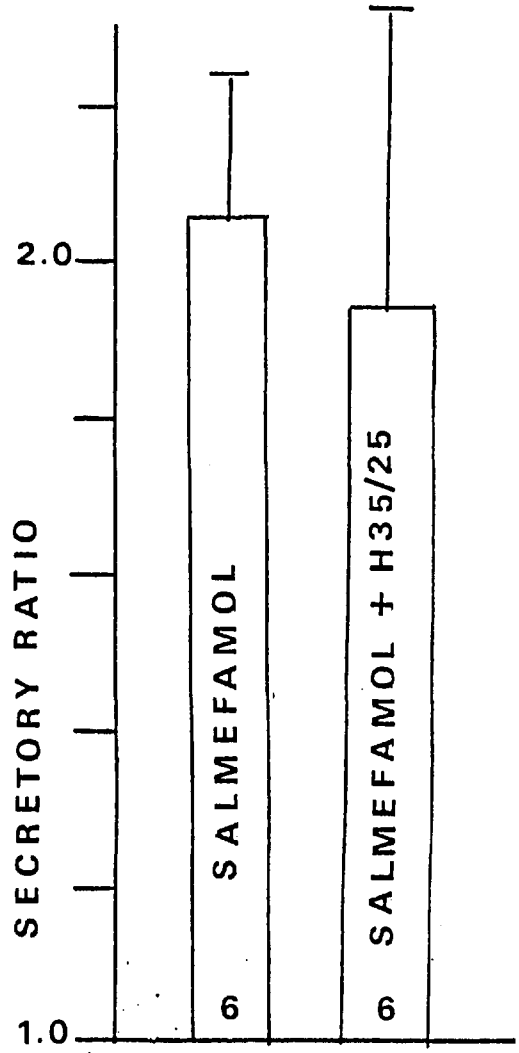


Figure 24 The effect of $H_{35/25}$ (10^{-4}M) on the secretory ratio obtained with salmefamol (5×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

(b) Salmefamol-Stimulated Acid Secretion

Butoxamine also failed to inhibit salmefamol stimulated secretion. The results are shown in Figure 23. The control secretory ratios with salmefamol (5 x 10^{-6}M) alone were 1.85 ± 0.14 (n = 6) and 2.02 ± 0.26 (n = 6) and ratios in the presence of butoxamine 2 x 10^{-5}M and 10^{-4}M were 2.10 ± 0.17 (n = 6) and 1.75 ± 0.15 (n = 5) respectively.

Figure 24 shows that the β_2-selective antagonist $H_{35/25}$ was also ineffective in inhibiting the acid response to salmefamol. The mean secretory ratio for salmefamol alone was 2.05 ± 0.19 (n = 6) and in the presence of $H_{35/25}$ (10^{-4}M) was 1.94 ± 0.390 (n = 6).

Thus, although β_2-selective agonists could stimulate acid secretion, β_2-selective antagonists did not reverse this effect at concentrations up to 10^{-4}M.

4.3.3 The Effect of a β_1-Selective Adrenoceptor Antagonist

(a) Isoprenaline-Stimulated Acid Secretion

Practolol had no effect on the acid secretory response to isoprenaline.

Figure 25 shows the effect of two concentrations of practolol (2 x 10^{-5}M and 10^{-4}M on the response to isoprenaline (1.25 x 10^{-6}M). At the lower concentration of the antagonist the control ratio was 1.98 ± 0.19 and test 2.37 ± 0.27 (n = 12 for both groups), whilst at the higher concentration the control ratio was 2.20 ± 0.12 and test 1.93 ± 0.12 (n = 6 in both groups).

The apparent increase in secretory ratio with the lower dose of practolol was not statistically significant.

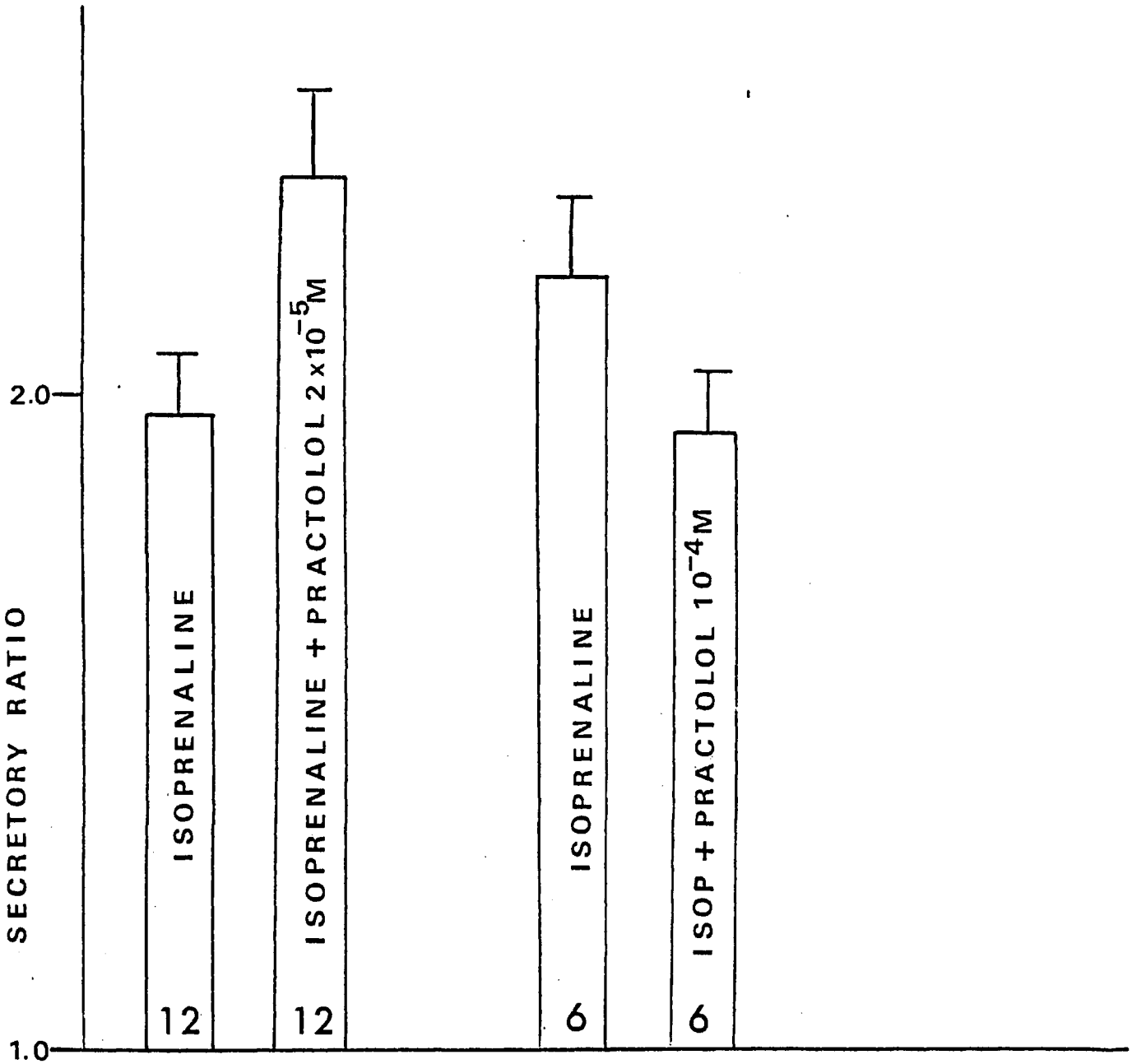


Figure 25 The effect of practolol (2 x 10^{-5}M and 10^{-4}M) on the secretory ratio obtained with isoprenaline (1.25 x 10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

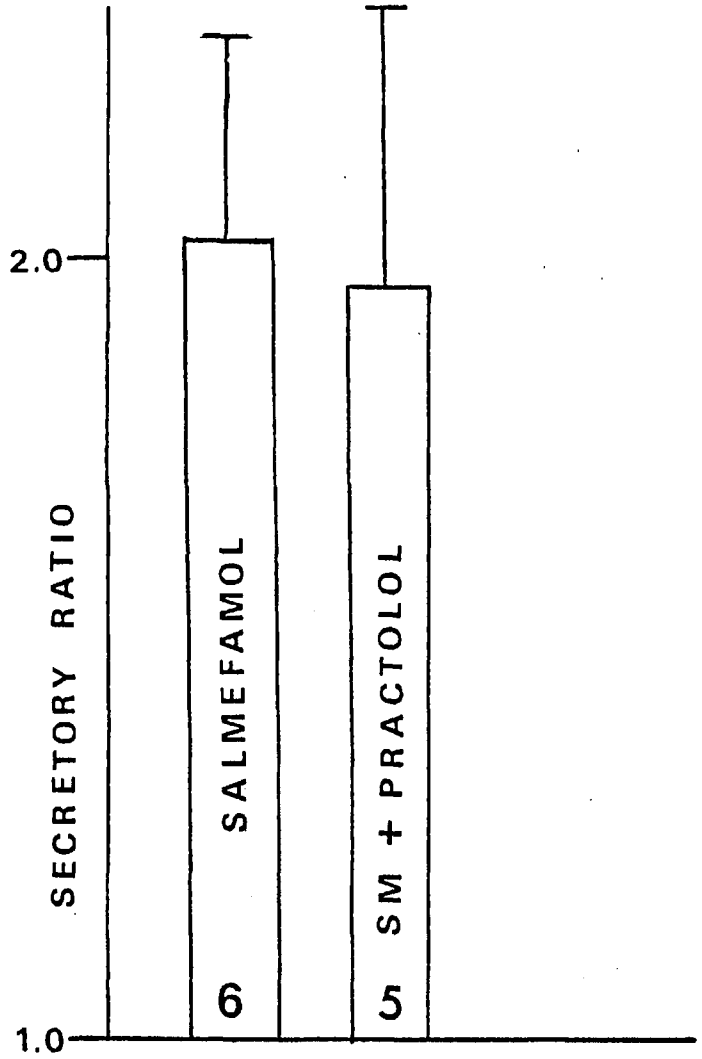


Figure 26 The effect of practolol (2×10^{-5}M) on the secretory ratio obtained with salmefamol (5×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

(b) Salmefamol-Stimulated Acid Secretion

Salmefamol was also unaffected by practolol. Figure 26 shows the mean secretory ratios for salmefamol (5×10^{-6}M) in the absence and presence of practolol (2×10^{-5}M); control ratio was 2.02 ± 0.26 (n = 6) and test ratio was 1.96 ± 0.41 (n = 5).

4.4 THE EFFECT OF β-ADRENOCEPTOR ANTAGONISTS ON BASAL ACID SECRETION

Although there were no specific experiments in this study to investigate the effects of antagonists on basal secretion over a prolonged period of time, an analysis of the effects of some of the antagonists was carried out comparing basal secretion in control experiments and basal secretion during pre-incubation with antagonist. The results are shown in Table 4, showing pooled mean basal secretions taken randomly from series of experiments using the appropriate antagonist.

Propranolol (2×10^{-5}M) slightly reduced basal secretion from 3.63 ± 0.21 (n = 24) to 3.06 ± 0.14μmole $H^+cm^{-2}h^{-1}$ (n = 12) ($p < 0.05$). This would tend to increase the secretory ratio in the agonist/antagonist studies and slightly mask inhibition. However, significant inhibition of the agonists was always observed with propranolol.

Practolol (2×10^{-5}M) slightly increased basal secretion from 3.63 ± 0.21 (n = 24) to 4.22 ± 0.17 (n = 12) μmole $H^+cm^{-2}h^{-1}$. This would tend to decrease the secretory ratio and apparently potentiate any inhibition. However practolol was always observed to be unable to inhibit β-agonist stimulated secretion.

Butoxamine (2×10^{-5}M), pindolol (10^{-4}M) and timolol (2×10^{-5}M) produced no significant change in basal acid output.

TABLE 4

THE EFFECT OF β-ADRENOCEPTOR ANTAGONISTS ON BASAL ACID SECRETION

Antagonist	Rate of Secretion (μmole $H^+cm^{-2}h^{-1}$)			
	mean	s.e.m.	n	p
Control	3.63	0.21	24	
Propranolol (2×10^{-5}M)	3.06	0.14	12	0.05
Practolol (2×10^{-5}M)	4.22	0.17	12	0.05
Butoxamine (2×10^{-5}M)	3.51	0.16	26	
Control	4.01	0.33	12	
Pindolol (10^{-4}M)	3.80	0.34	12	
Timolol (2×10^{-5}M)	3.40	0.21	12	

4.5 THE EFFECT OF THE NATURALLY OCCURRING CATECHOLAMINE NORADRENALINE ON GASTRIC ACID SECRETION

Concentration-Response Curve

Noradrenaline produced a concentration-dependent stimulation of acid output in the range of 5×10^{-7}M to 2×10^{-5}M with a mean maximum secretory ratio of 2.03 ± 0.13 (n = 6) (3.19 ± 0.38 μmole $H^+cm^{-2}h^{-1}$) (Figure 27).

Figure 28 shows the log concentration-response curves for all four sympathomimetics used, and shows that the maximum responses and slopes of the linear portion of the curves are similar for all

Figure 27 Noradrenaline concentration on log scale plotted against secretory ratio. Points are mean values with s.e. bars and numbers of observations are shown below each point.

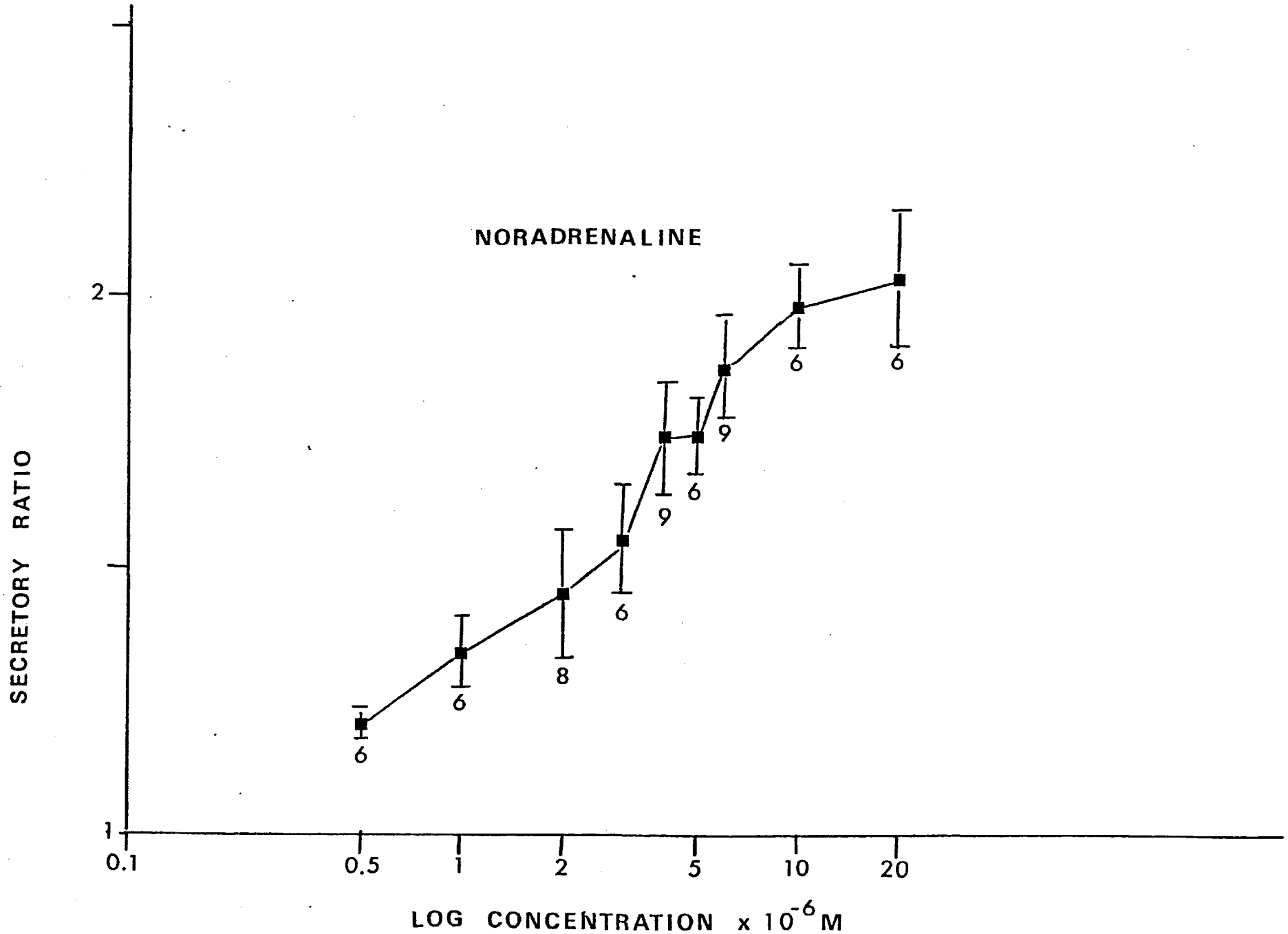
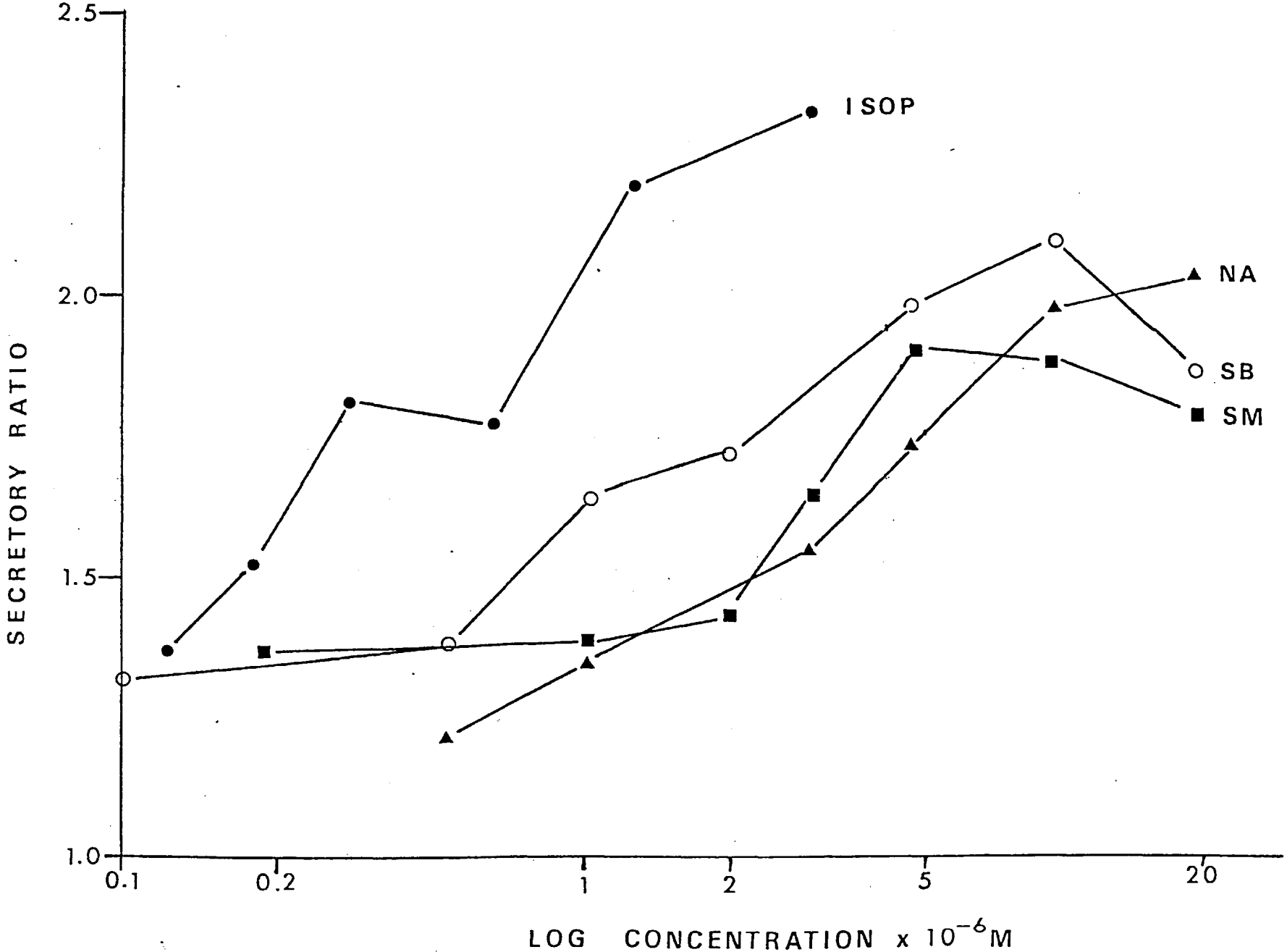

NORADRENALINE
SECRETORY RATIO
2
1
0.1
0.5
1
2
5
10
20
LOG CONCENTRATION x 10^{-6}M
6
6
8
6
9
6
9
6
6

Figure 28 β-agonist concentration on log scale plotted against secretory ratio. Points are mean values with s.e. bars and numbers of observations are shown below each point.


2.5
2.0
1.5
1.0
SECRETORY RATIO
ISOP
NA
SB
SM
0.1
0.2
1
2
5
20
LOG CONCENTRATION x 10^{-6}M

four drugs. Noradrenaline appears to be least potent but no uptake inhibitors were used in the bath and this may partly account for this observation.

4.6 THE EFFECT OF β-ADRENOCEPTOR ANTAGONISTS ON NORADRENALINE-STIMULATED ACID SECRETION

A concentration of 5×10^{-6}M noradrenaline was used throughout these studies.

(a) Non-Selective β-Adrenoceptor Antagonists

Timolol, pindolol and propranolol (2×10^{-5}M) all produced a significant inhibition of the acid response to noradrenaline (5×10^{6}M). Mean secretory ratios are shown in Figure 29.

The control response to noradrenaline produced a secretory ratio of 1.84 ± 0.07 ($n = 8$). Ratios in the presence of timolol, propranolol and pindolol (2×10^{-5}M) were 1.30 ± 0.03 ($n= 8$), 1.49 ± 0.09 ($n = 8$) and 1.37 ± 0.05 ($n = 7$) respectively ($p< 0.05$ for all drugs). These represent 64%, 42% and 56% inhibitions.

(b) A β_1-Selective Adrenoceptor Antagonist

Practolol (2×10^{-5}M) did not inhibit the acid response to noradrenaline. Increasing the concentration to 10^{-4}M did, however, produce a significant inhibition. The results are shown in Figure 30.

The control response to noradrenaline produced a secretory ratio of 2.13 ± 0.23 ($n = 6$) and test ratio in the presence of practolol (2×10^{-5}M) was 2.10 ± 0.19 ($n =6$) control and test ratios for noradrenaline alone and with practolol (10^{-4}M) were 1.74 ± 0.15 and 1.28 ± 0.07 ($n = 6$) ($p< 0.01$) respectively.

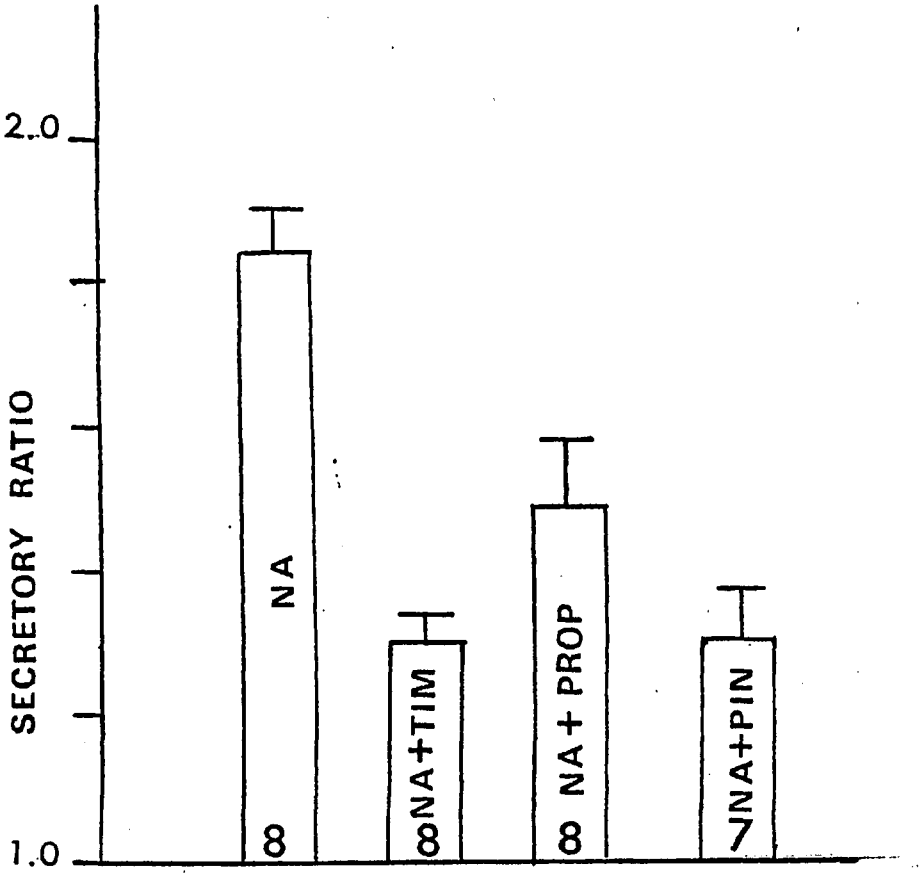


Figure 29 The effect of timolol (2×10^{-5}M), propranolol (2×10^{-5}M) and pindolol (2×10^{-5}M) on the secretory ratio obtained with noradrenaline (5×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

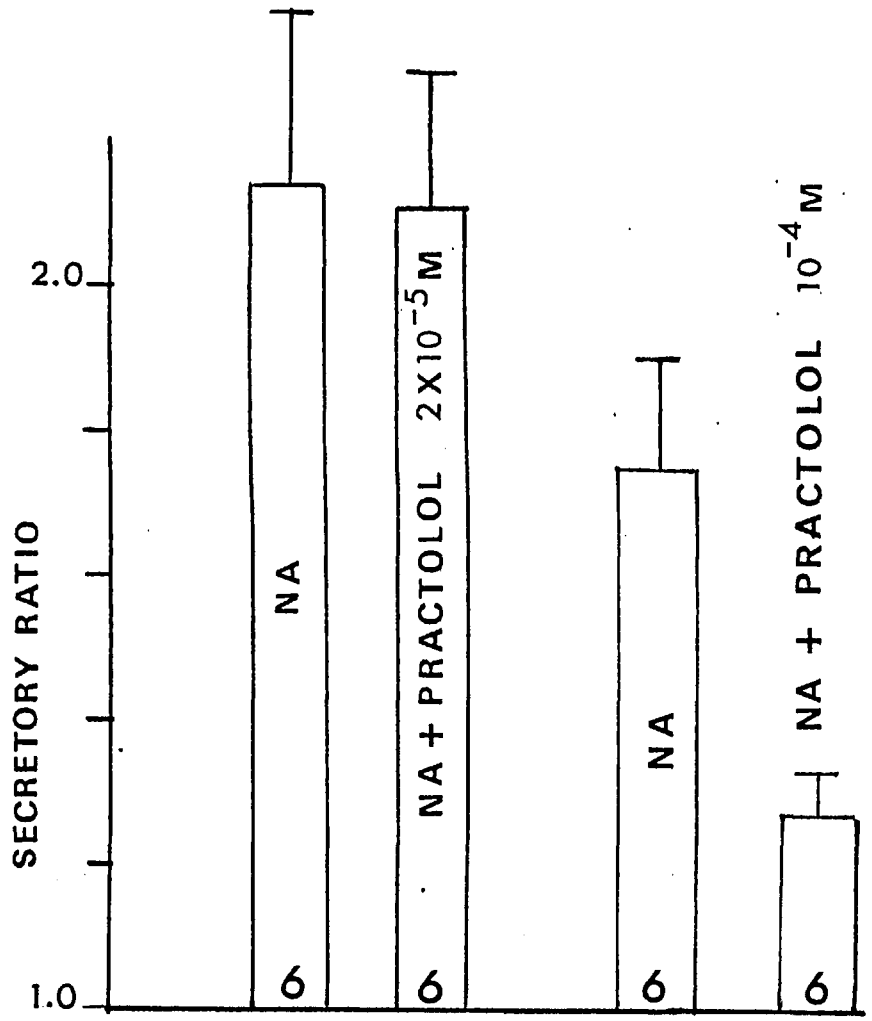


Figure 30 The effect of practolol (2 x 10^{-5}M and 10^{-4}M) on the secretory ratio obtained with noradrenaline (5 x 10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

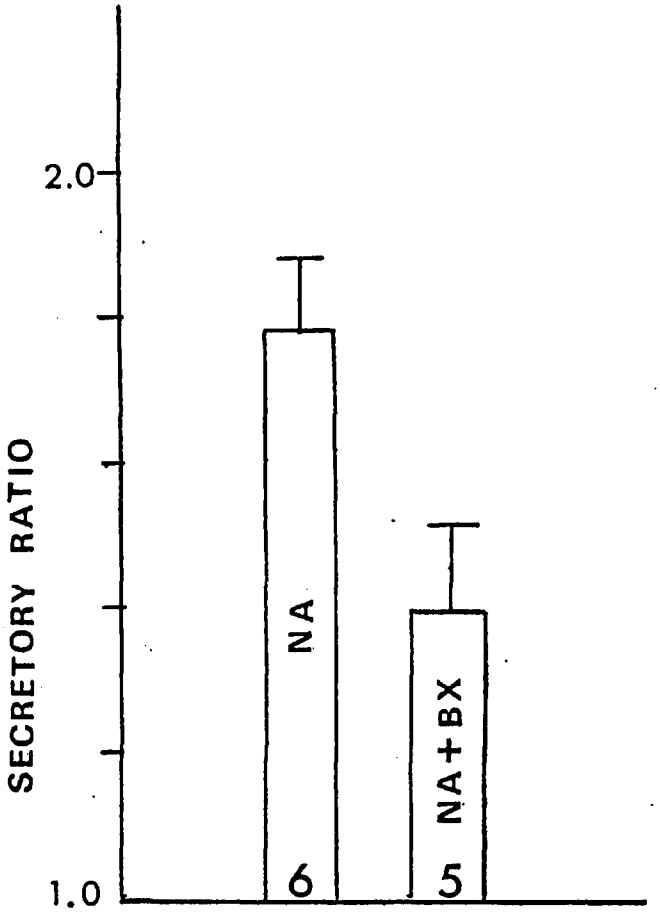


Figure 31 The effect of butoxamine (2 x 10^{-5}M) on the secretory ratio obtained with noradrenaline (5 x 10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

In this study, only the lower dose of practolol was used against salmefamol stimulated secretion and was shown to have no effect. It has subsequently been shown by workers in this laboratory that practolol (10^{-4}M) did not inhibit the secretory response to salmefamol (Canfield et al., 1981). Thus practolol (10^{-4}M) had no effect on salmefamol or isoprenaline stimulated secretion (4.3.3 (a)), whereas at the same concentration practolol produced a significant inhibition of noradrenaline stimulated secretion.

(c) A β_2-Selective Antagonist

In contrast with results obtained with isoprenaline and salmefamol, butoxamine (2×10^{-5}M) produced a significant inhibition of the response to noradrenaline (5×10^{-6}M). Results are shown in Figure 31. The control response to noradrenaline produced a secretory ratio of 1.73 ± 0.09 ($n = 6$) and the secretory ratio in the presence of butoxamine was 1.39 ± 0.11 ($n = 5$) ($p < 0.05$).

4.7 SUMMARY

Table 5 summarises the effects of various antagonists on the responses to three adrenoceptor agonists, isoprenaline, salmefamol and noradrenaline. Submaximal responses to all three drugs were inhibited by propranolol, timolol and pindolol. The response to isoprenaline was unaffected by pronethelol. The results with propranolol on salmefamol and isoprenaline were consistent with competitive antagonism. Both β_1- and β_2-selective antagonists inhibited the response to noradrenaline at concentrations which were ineffective against the other two agonists.

TABLE 5

SUMMARY OF AGONIST-ANTAGONIST STUDIES

+ = Significant inhibition
- = No effect
0 = No study carried out

	ISOPRENALINE 1.25×10^{-6}M	SALMEFAMOL 5×10^{-6}M	NORADRENALINE 5×10^{-6}M
Propranolol 2×10^{-5}M	+	+	+
Pindolol 2×10^{-5}M	+	0	+
Pindolol 10^{-4}M	+	0	0
Timolol 2×10^{-5}M	+	+	+
Timolol 10^{-4}M	+	0	0
Pronethalol 2×10^{-5}M	-	0	0
Pronethalol 4×10^{-4}M	-	0	0
Practolol 2×10^{-5}M	-	-	-
Practolol 10^{-4}M	-	- (Canfield et al., 1981)	±
Butoxamine 2×10^{-5}M	-	-	+
Butoxamine 10^{-4}M	-	-	0
H 35/25 10^{-4}M	0	-	0

4.8 OTHER ANTAGONISTS OF GASTRIC ACID SECRETION

β-adrenoceptor agonists may partly stimulate acid secretion in the rat isolated stomach by releasing one of the endogenous secretagogues. This possibility was tested by investigating the effects of atropine and metiamide using the same experimental design as the β-agonist studies.

Figure 32 shows the results.

(a) Atropine (10^{-5}M)

Atropine (10^{-5}M) caused a significant inhibition of the response to bethanechol (1.7×10^{-5}M) but had no effect on the sympathomimetic drugs. The mean control and test ratios with bethanechol were 1.89 ± 0.05 ($n = 5$) and 1.10 ± 0.05 ($n = 6$) ($p < 0.01$). Isoprenaline gave control and test values of 1.98 ± 0.09 ($n = 12$) and 1.95 ± 0.15 ($n = 11$).

Salmefamol gave control and test ratios of 2.02 ± 0.26 ($n = 6$) and 1.94 ± 0.4 ($n = 6$).

Noradrenaline gave control and test ratios of 1.98 ± 0.07 ($n = 8$) and 1.78 ± 0.07 ($n = 6$).

(b) Metiamide (10^{-4}M)

Bunce, Parsons and Rollings (1976) reported that metiamide inhibited the response to gastrin in the rat isolated stomach, and thus the use of this drug should have enabled the exclusion of the possibility that β-adrenoceptor agonists act by releasing either histamine or gastrin. Metiamide (10^{-4}M) inhibited the response to histamine (5.4×10^{-5}M) but was without effect on the β-adrenoceptor agonists responses or the response to pentagastrin (2.17×10^{-7}M).

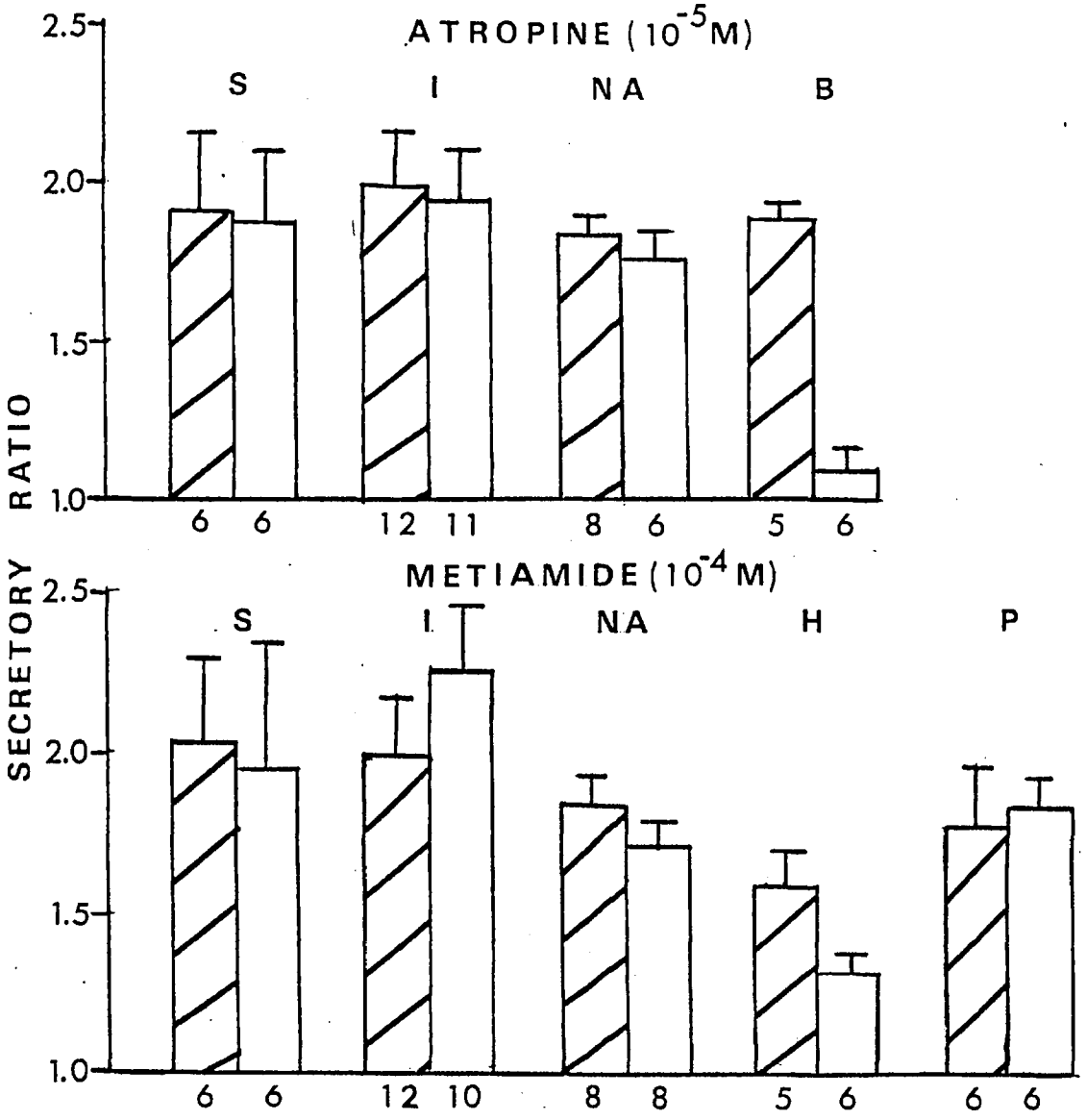


Figure 32 The effects of atropine and metiamide on the secretory ratios obtained with salmefamol (S, 5 x 10^{-6}M), isoprenaline (I, 1.25 x 10^{-6}M), noradrenaline (NA, 5 x 10^{-6}M), bethanechol (B, 1.7 x 10^{-5}M), histamine (H, 5.4 x 10^{-5}M) and pentagastrin (P, 2.2 x 10^{-7}M). Columns are mean values with s.e. bars. The shaded columns are control values in the absence of the antagonist. Numbers of observations are at the base of each column.

The mean control and test ratios for the action of metiamide on histamine were 1.58 ± 0.11 (n = 5) and 1.12 ± 0.05 (n = 6). With pentagastrin corresponding values were 1.66 ± 0.04 (n = 5) and 1.68 ± 0.05 (n = 6). Mean secretory ratios for isoprenaline (1.25×10^{-6}M) alone and in the presence of metiamide were 1.98 ± 0.09 (n = 12) and 2.25 ± 0.21 (n = 10).

Control and test ratios for salmefamol 5×10^{-6}M were 2.02 ± 0.26 (n = 6) and 1.94 ± 0.4 (n = 6), and for noradrenaline were 1.98 ± 0.07 (n = 8) and 1.69 ± 0.10 (n = 8)

Thus, these studies indicate that β-adrenoceptor agonists do not stimulate acid secretion by the release of acetylcholine or histamine. The finding that metiamide was unable to inhibit the response to pentagastrin was unexpected. From this study it cannot be concluded that β-adrenoceptor agonists do not release gastrin. However, further work in this laboratory has indicated that PGE_2 significantly inhibited pentagastrin but not the response to isoprenaline. This will be discussed in Chapter 6.

4.9 THE EFFECT OF METIAMIDE (10^{-4}M) AND ATROPINE (10^{-5}M) ON BASAL ACID SECRETION

TABLE 6

	μmole $H^+cm^{-2}h^{-1}$		
	mean	sem	n
Control	3.63	0.21	24
Metiamide (10^{-4}M)	4.06	0.47	11
Atropine (10^{-5}M)	3.32	0.28	12

Table 6 shows the effect of metiamide and atropine on basal secretion, using the method described in 4.3.4. Neither drug had any significant effect upon basal secretion.

4.10 POSSIBLE NON-SPECIFIC EFFECTS OF PROPRANOLOL

The responses to all three sympathomimetics studied were antagonised by propranolol. This drug is known to have a membrane stabilising effect in other systems (Goodman & Gilman, 1981). This raised the possibility that propranolol was exerting a non-specific antisecretory action. If this was the case, propranolol, at the concentration used in this study, would be expected to inhibit other secretagogues such as bethanechol and pentagastrin. This possibility was investigated by testing the effects of propranolol (2×10^{-5}M) on pentagastrin (5×10^{-7}M) and bethanechol (1.7×10^{-5}M) stimulated acid secretion. Both concentrations of secretagogues have been shown to be submaximal in this preparation (J.E. Spencer: personal communication). A further test for non-specific effects of propranolol was carried out using the isomer (+) propranolol which does not have β-antagonist action but does have similar membrane stabilising effects to the active isomer.

The results of these two studies are discussed below.

(a) The Effects of Propranolol (2×10^{-5}M) on Pentagastrin and Bethanechol Induced Acid Secretion

Propranolol had no significant effect on the response to either secretagogue. Results are shown in Figure 33.

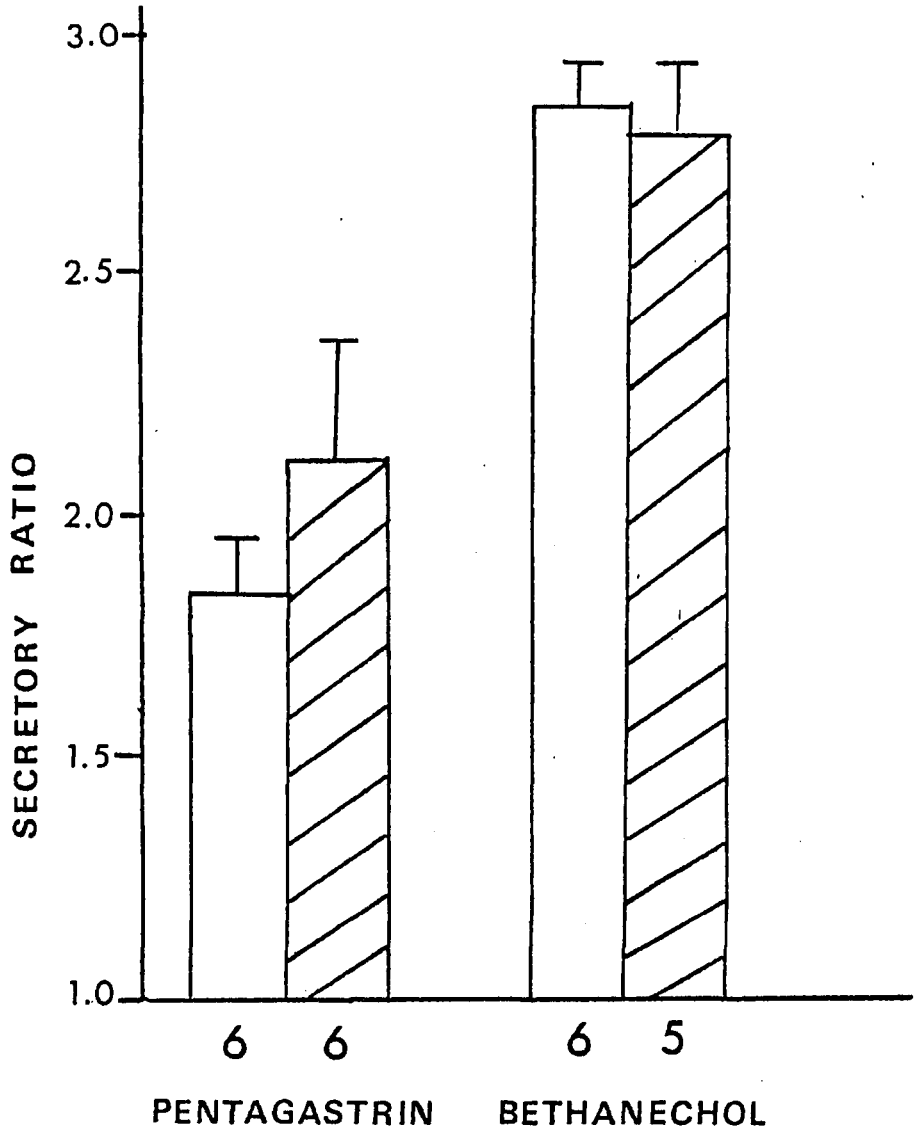


Figure 33 The effects of propranolol (2 x 10^{-5}M) on the responses to pentagastrin (5 x 10^{-7}M) and bethanechol (1.7 x 10^{-5}M) are shown by the hatched columns, control values by the open columns. The columns are mean values with s.e. bars and the number of observations are shown at the base of each column.

The control and test ratios for pentagastrin were 1.83 ± 0.1 (n = 6) (3.36 ± 0.27μmole $H^+cm^{-2}h^{-1}$) and 2.11 ± 0.20 (n = 6) (3.71 ± 0.3μmole $H^+cm^{-2}h^{-1}$), and with bethanechol 2.84 ± 0.08 (n = 6) (4.89 ± 0.74μmole $H^+cm^{-2}h^{-1}$) and 2.82 ± 0.17 (n = 5) (4.67 ± 0.74μmole $H^+cm^{-2}h^{-1}$).

The response to bethanechol was unusually large in this series of experiments. In the experience of workers in this laboratory this stimulant shows larger variations between groups of animals than any other of the secretagogues which have been used in this preparation.

(b) The Effect of (+) Propranolol on Isoprenaline Stimulated Acid Secretion

Results are shown in Figure 34. At 2 x 10^{-5}M (+) propranolol did not inhibit isoprenaline (1.25 x 10^{-6}M) whereas the same concentration of (±) propranolol did produce a significant inhibition.

Mean secretory ratios were 2.00 ± 0.12 (n = 6), 1.49 ± 0.09 (n = 5) and 1.97 ± 0.17 (n = 6) for isoprenaline, isoprenaline and (±) propranolol and isoprenaline and (+) propranolol respectively.

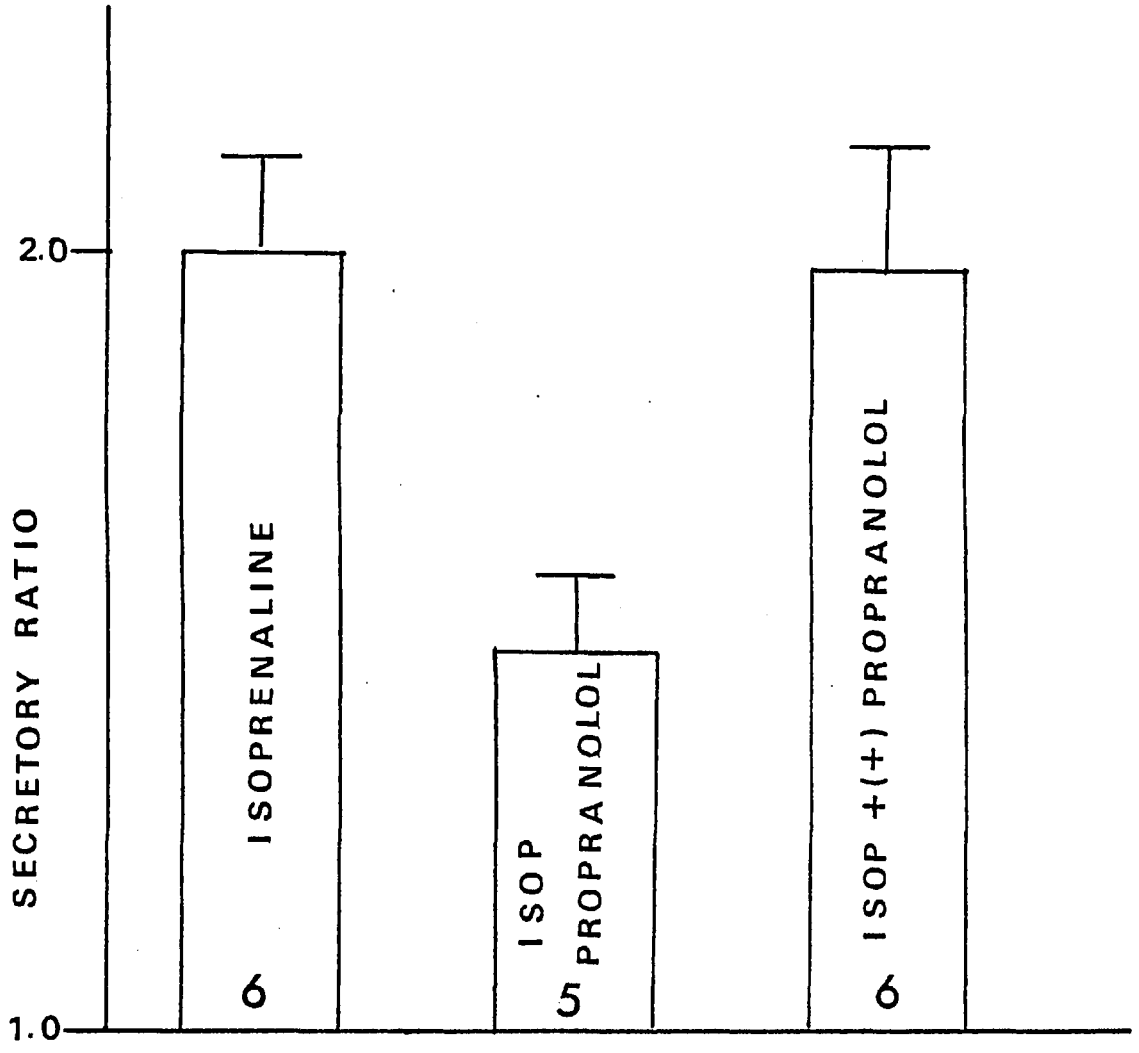


Figure 34 The effect of (±) propranolol (2×10^{-5}M) and (+) propranolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

4.11 THE EFFECT OF α-ADRENOCEPTOR AGONISTS ON GASTRIC ACID SECRETION

As α-adrenoceptor agonists have been reported to inhibit acid secretion *in vivo* their effects have been examined *in vitro* on both basal and secretagogue stimulated acid secretion.

Figure 35 shows the effects of phenylephrine (10^{-5}-10^{-3}M) and methoxamine (10^{-5}-10^{-4}M) on basal acid secretion. The concentration response curve for noradrenaline is included for comparison. Clearly α-adrenoceptor agonists have no significant effects upon acid secretion. The mean secretory ratios were 1.05 ± 0.04 (n = 6) (0.11 ± 0.09μmole $H^+cm^{-2}h^{-1}$) for methoxamine and 1.14 ± 0.08 (n = 6) (0.32 ± 0.21μmole $H^+cm^{-2}h^{-1}$) for phenylephrine both at 10^{-4}M.

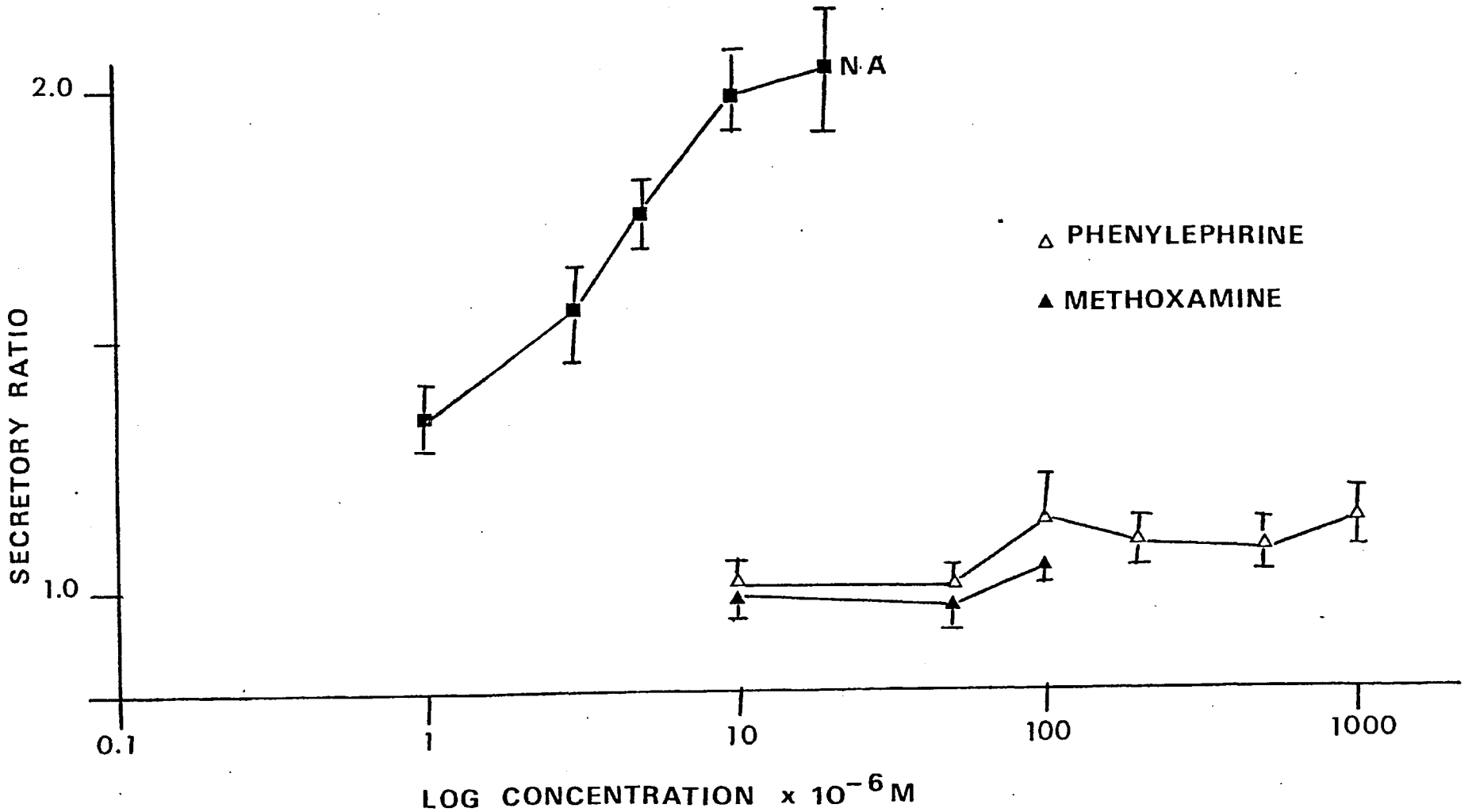
4.12 THE EFFECT OF AN α-ADRENOCEPTOR AGONIST ON THE RESPONSE TO PENTAGASTRIN, HISTAMINE AND BETHANECHOL

The action of phenylephrine (2×10^{-5}M) on the response to pentagastrin (2.17×10^{-7}M) histamine (5.4×10^{-5}M) and bethanechol (1.7×10^{-5}M) was investigated. Results are shown in Figure 36.

The mean control and test secretory ratios were: pentagastrin 1.90 ± 0.08 (n = 6) and 1.86 ± 0.13 (n = 5); histamine 2.11 ± 0.27 (n = 6) and 1.91 ± 0.1 (n = 6); bethanechol 1.92 ± 0.08 (n = 6) and 1.80 ± 0.07 (n = 6).

Thus, phenylephrine had no significant effect of any of the secretagogues tested.

Figure 35 Phenylephrine and methoxamine concentration on log scale plotted against secretory ratio. Points are mean values of at least five experiments.


2.0
1.0
SECRETORY RATIO
N·A
△ PHENYLEPHRINE
▲ METHOXAMINE
0.1
1
10
100
1000
LOG CONCENTRATION x 10⁻⁶ M

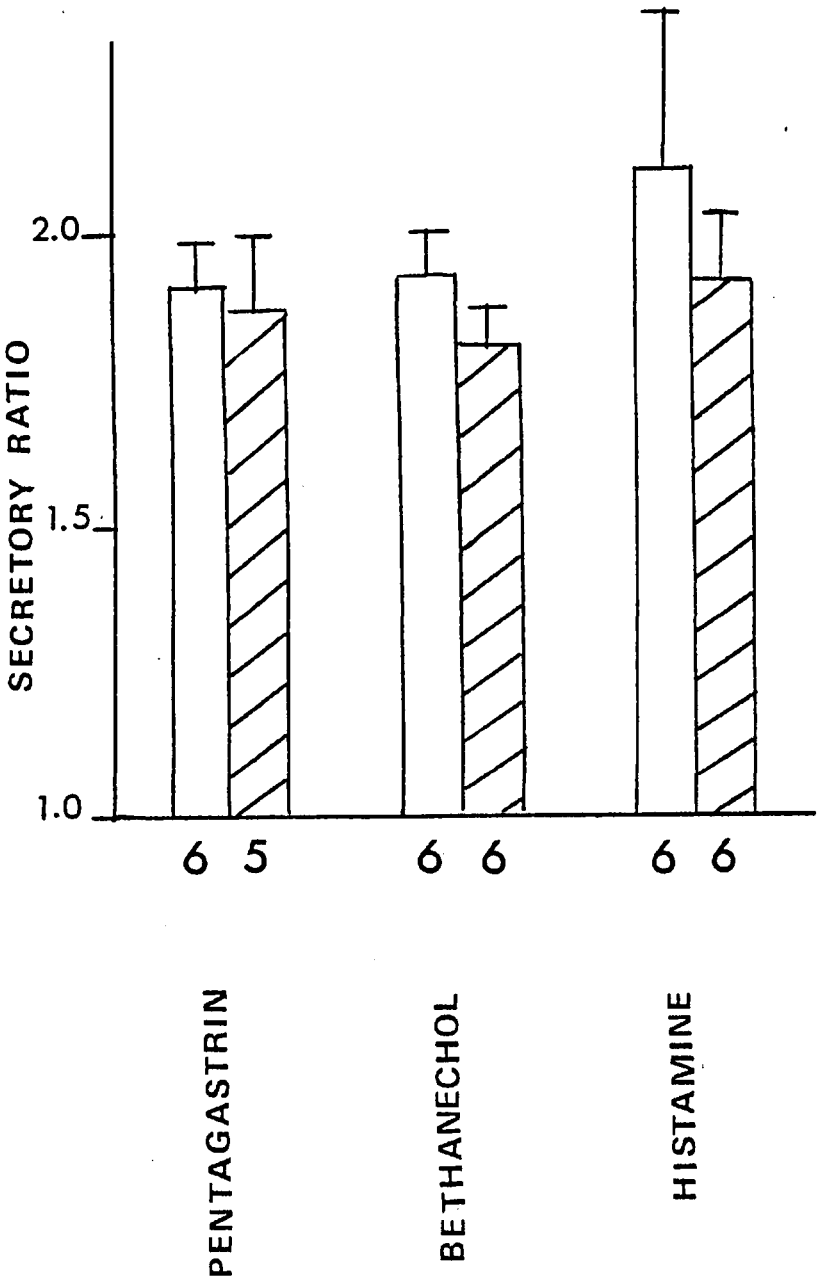


Figure 36 The effect of phenylephrine (2×10^{-5}M) on the responses to pentagastrin (2.17×10^{-7}M), bethanechol (1.7×10^{-5}M) and histamine (5.4×10^{-5}M) are shown by the hatched columns, control values by the open columns. The columns are mean values with s.e. bars, with the numbers of observations shown at the base of each column.

4.13 THE EFFECT OF α-ADRENOCEPTOR ANTAGONISTS ON SYMPATHOMIMETIC STIMULATED ACID SECRETION

(a) Isoprenaline-Stimulated Acid Secretion (1.25×10^{-6}M)

Figure 37 shows the secretory ratios for the responses to isoprenaline (1.25×10^{-6}M) in the absence and presence of phentolamine (2×10^{-5}M). Clearly phentolamine had no effect, with mean secretory ratios of 2.20 ± 0.12 (n = 6) and 1.96 ± 0.07 (n = 5).

(b) Noradrenaline-Stimulated Acid Secretion (5×10^{-6}M)

Phentolamine (10^{-4}M) and thymoxamine (10^{-4}M) also had no effect on noradrenaline stimulated acid secretion. The results are shown in Figure 38.

The control response to noradrenaline gave a mean secretory ratio of 1.73 ± 0.06 (n = 8). Ratios in the presence of phentolamine and thymoxamine were 1.69 ± 0.09 (n = 8) and 1.79 ± 0.14 (n = 7) respectively.

Summary

α-adrenoceptor agonists do not stimulate acid secretion in the rat isolated stomach. The ability of sympathomimetics to stimulate acid secretion does not seem to involve α-adrenoceptors since α-antagonists are unable to inhibit these responses, nor do α-agonists affect responses to histamine, pentagastrin or bethanechol.

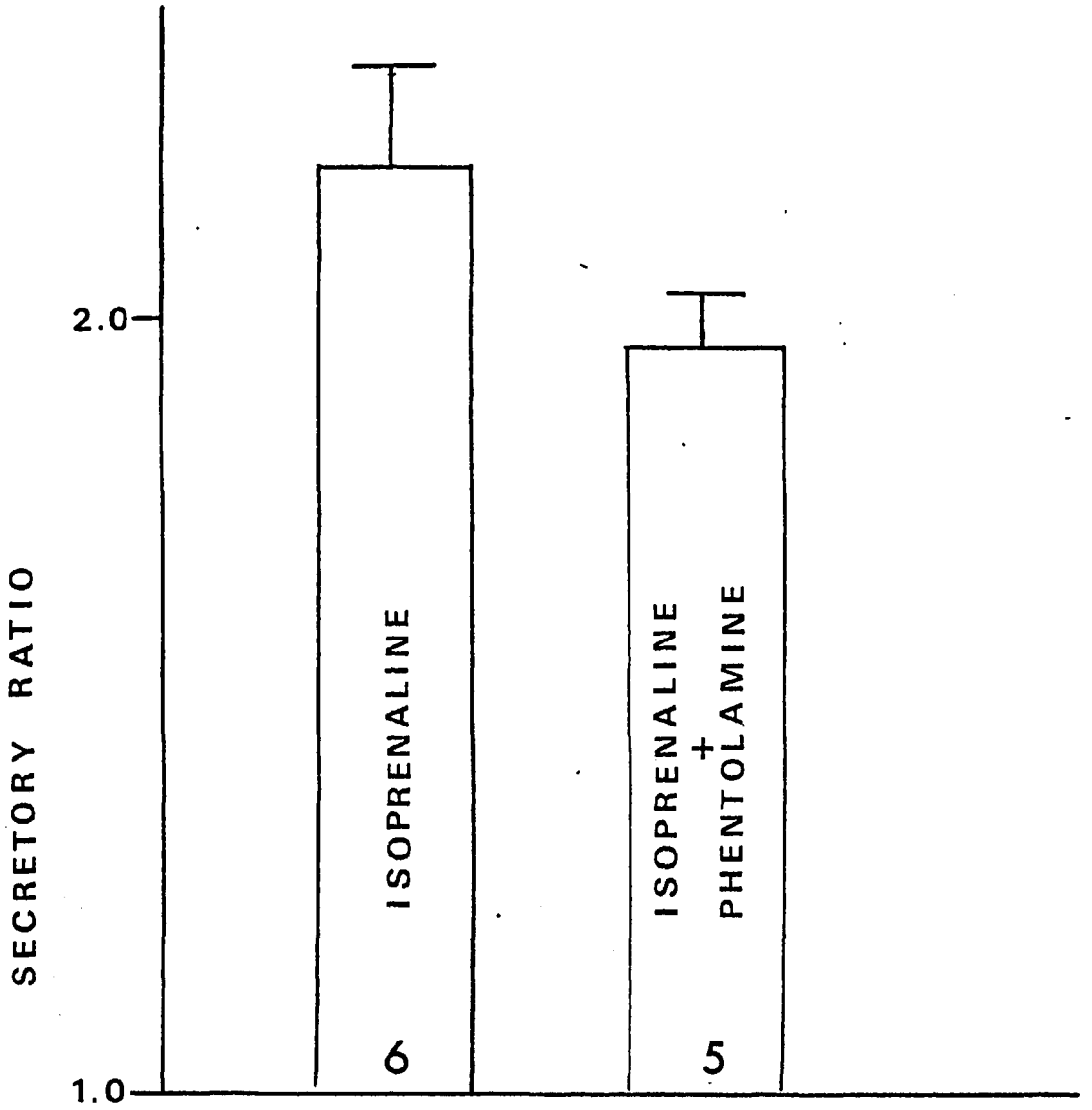


Figure 37 The effect of phentolamine (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

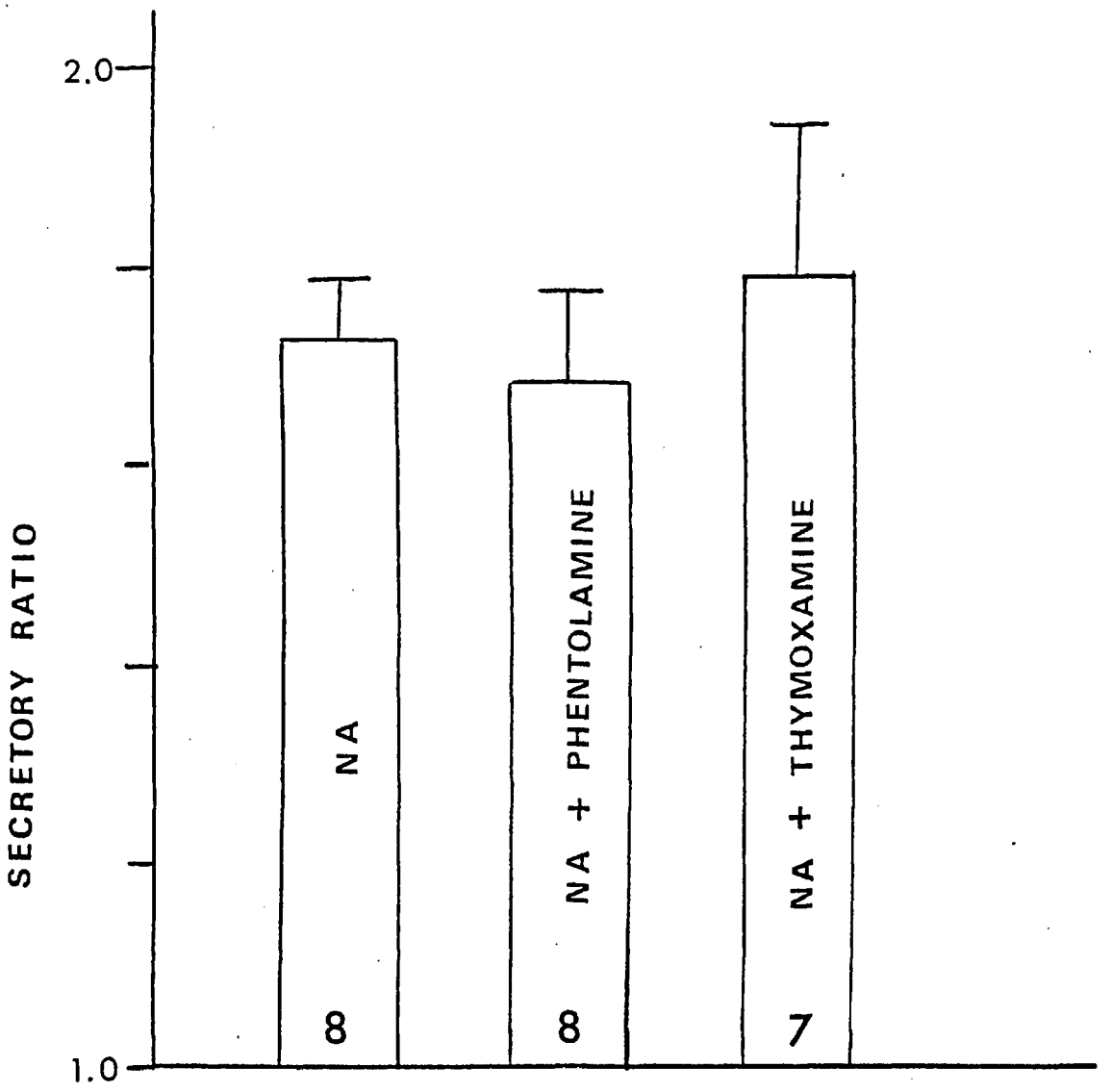


Figure 38 The effect of phentolamine (10^{-4}M) and thymoxamine (10^{-4}M) on the secretory ratio obtained with noradrenaline (5×10^{-6}M). Columns indicate mean values with s.e. bars. Number of observations are shown at the base of each column.

CHAPTER 5

RESULTS: HEIDENHAIN POUCH RAT

5.1 INTRODUCTION

The aim of this study in the Heidenhain pouch rat was to confirm that β-adrenoceptor agonists inhibit acid secretion in the conscious rat, a finding in contrast with the work described in Chapter 4 which showed that β-agonists stimulate acid secretion *in vitro*. The study was intended to confirm the finding of Lundell and Svensson (1974), that isoprenaline inhibits the response to pentagastrin in the Heidenhain pouch rat, and to extend their findings with β-adrenoceptor antagonists in an attempt to further study the mechanism of β-agonist inhibition of acid secretion.

5.2 SPONTANEOUS SECRETION IN THE HEIDENHAIN POUCH RAT

Immediately after surgery the pouches were flushed with sterile saline. This procedure was carried out once daily until pouch contents were shown to be acidic (litmus paper blue to red). Spontaneous secretion in newly operated rats was seen 5-14 days after pouch construction. Heidenhain pouch rats were not used for experiments until at least 14 days after their surgery.

Spontaneous secretion varied between different Heidenhain pouch rats, presumably due to variation in area of glandular mucosa contained in the pouches.

The mean rate of spontaneous secretion of all animals used in this study was 3.93 ± 0.64 μmole $H^{+}h^{-1}$ ($n = 10$). These measurements were taken within one month of pouch construction.

This value is similar to that of Svensson (1970) who reported a spontaneous secretion of 1.6 $\pm$ 0.22 µequiv/30 min (n = 10) in the Heidenhain pouch rat. Svensson also showed that basal secretion in vagally denervated pouch rats was some ten times lower than in Pavlov pouch rats, where innervation is intact.

Spontaneous secretion did tend to increase with length of time after pouch construction. This might suggest that there was partial reinnervation of the mucosa contained in the pouch. However, the increase in spontaneous secretion did not occur in all rats used in this study.

5.3 PENTAGASTRIN-STIMULATED ACID SECRETION

(a) Preliminary Studies

Dose-response curves were not constructed for each animal in this study, since McIsaac (1976) performed an extensive study in our laboratory on the effect of pentagastrin in the Heidenhain pouch rat. He showed that pentagastrin produced a dose-related response to pentagastrin in the range of 1 - 32 µg $kg^{-1}h^{-1}$. Lundell and Svensson (1974) used two doses of pentagastrin 0.25 µg/h and 16 µg/h (250g rat).

A short preliminary study was carried out in two rats in which dose-response curves were constructed in the range of 5 - 200 µg $kg^{-1}h^{-1}$. Pentagastrin was infused in steps of increasing doses. Each dose of pentagastrin was infused for 1.25 hr before giving the next dose. In each rat two experiments were performed giving the lower range and higher range of doses on separate days. Acid secretion in response to pentagastrin was taken as the mean of the last two fifteen minute periods at each level.

The results are shown in Figure 39. The maximum acid response for rat 1 was 191.1 μmole H^+h^{-1} (at 50 μg $kg^{-1}h^{-1}$) and for rat 2 was 84.3 μmole H^+h^{-1} (at 70 μg $kg^{-1}h^{-1}$).

Figure 40 shows the results plotted as a percentage of the maximum response.

A dose of 20 μg $kg^{-1}h^{-1}$ was chosen for this study for the following reasons.

1. 20 μg $kg^{-1}h^{-1}$ lies within the range of doses used by McIsaac (1976) in our laboratory.
2. This dose lies between the two doses used by Lundell and Svensson (1974) in their studies on the effect of isoprenaline on pentagastrin-stimulated acid secretion in the Heidenhain pouch rat.
3. In our preliminary study, 20 μg $kg^{-1}h^{-1}$ gave a good secretory response which was submaximal. Figure 40 shows that in rat 1, 20 μg $kg^{-1}h^{-1}$ produced 35% of the maximum response and in rat 2, produced 65% of the maximum response.

(b) <u>The Effect of Pentagastrin (20 μg $kg^{-1}h^{-1}$) on Acid Secretion in the Heidenhain Pouch Rat</u>

The mean response to pentagastrin in all animals used in this study was 62.3 $\pm$ 7.3 μ mole H^+h^{-1} (n = 10).

Table 7 shows the range of responses from which this mean was obtained.

Figure 39 Acid secretory responses to an intravenous infusion of pentagastrin in two Heidenhain pouch rats.

● Rat 1

o Rat 2

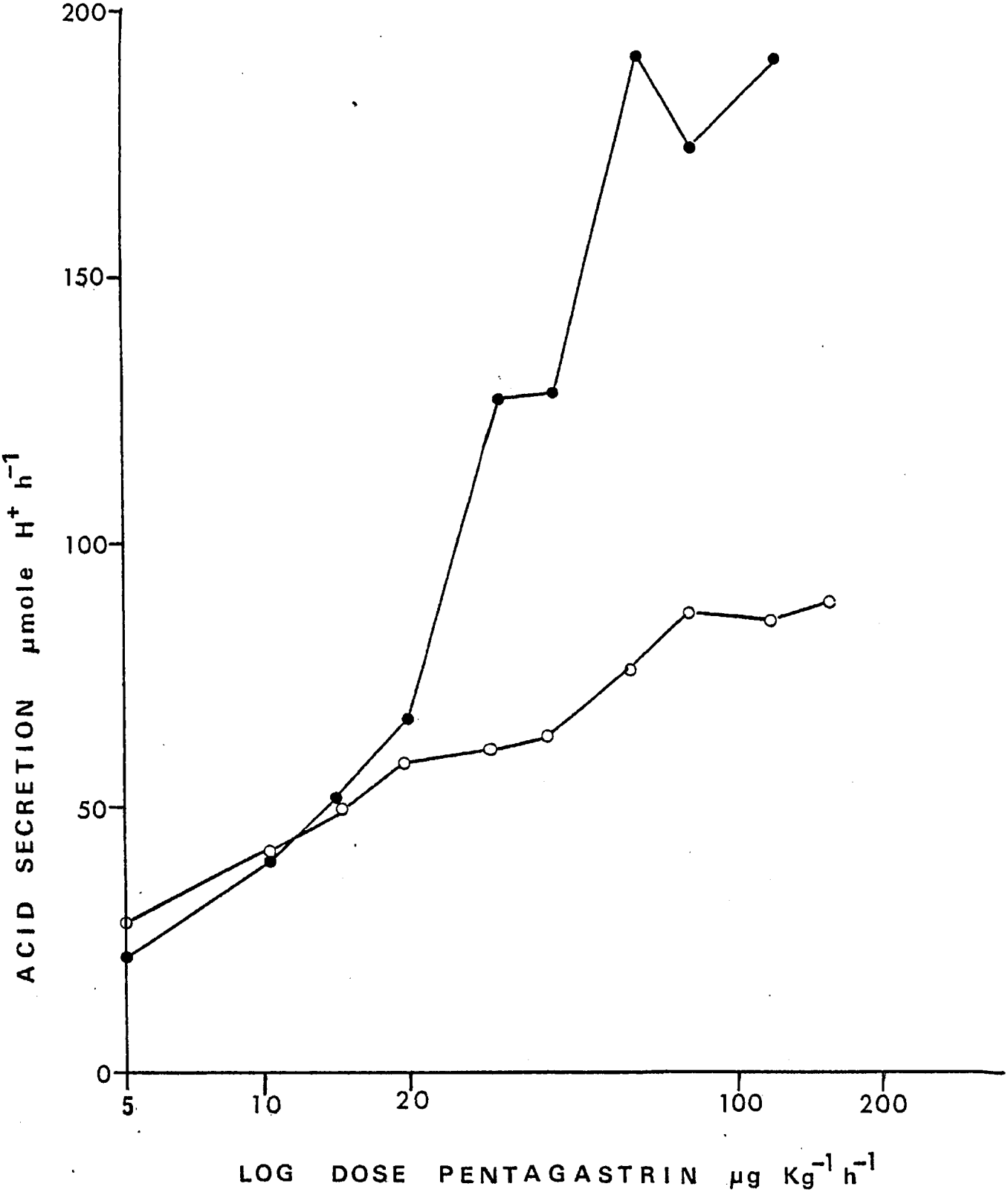

200
150
100
50
0
ACID SECRETION µmole H^+ h^{-1}
5
10
20
100
200
LOG DOSE PENTAGASTRIN µg Kg^{-1} h^{-1}

Figure 40 Acid secretory responses to an intravenous infusion of pentagastrin in two Heidenhain pouch rats. The acid responses are expressed as percentage of the maximum response.

- ● Rat 1
- o Rat 2

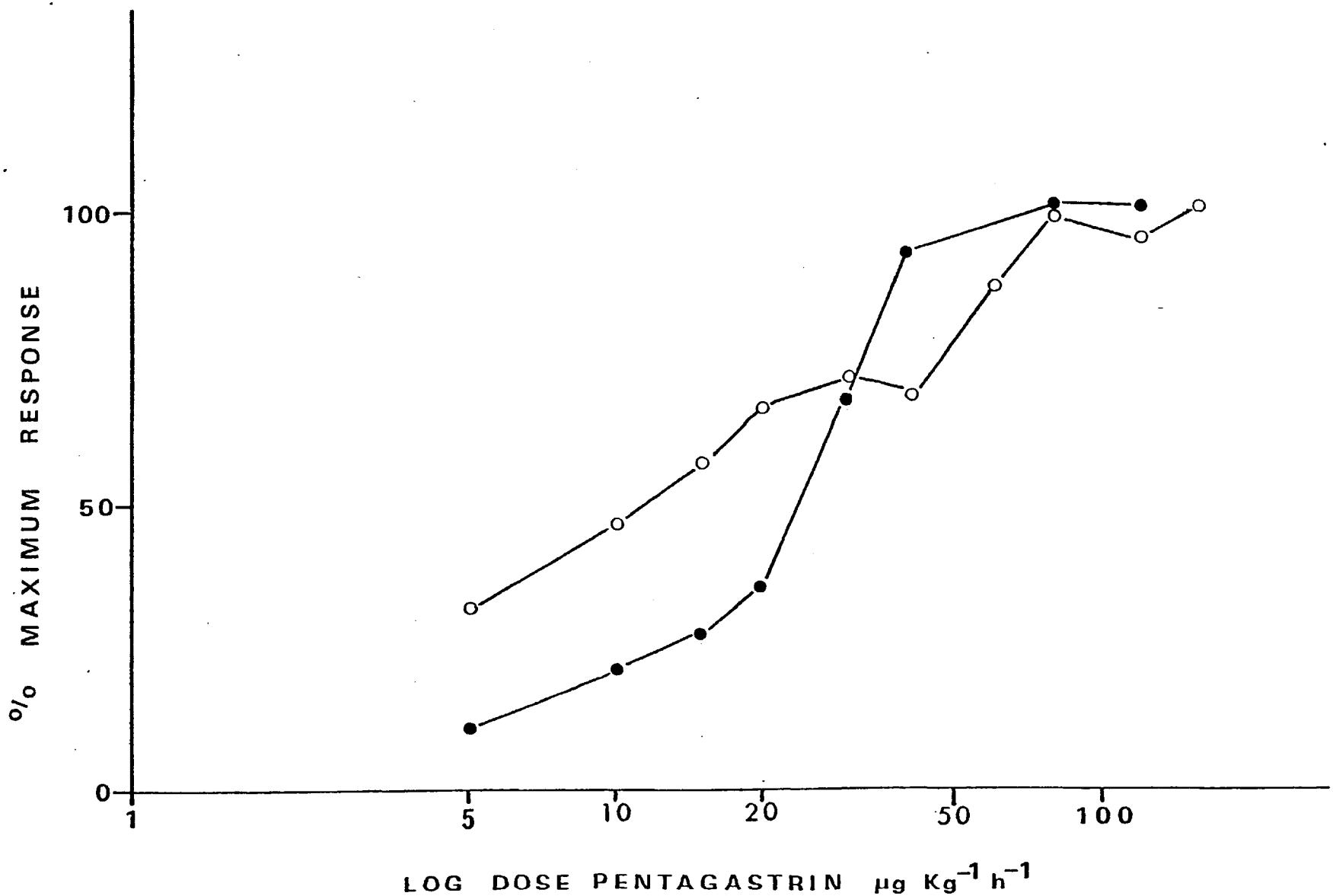

% MAXIMUM RESPONSE
100
50
0
1
5
10
20
50
100
LOG DOSE PENTAGASTRIN µg Kg⁻¹ h⁻¹

TABLE 7

SECRETORY RESPONSES OBTAINED IN RESPONSE TO AN INFUSION OF PENTAGASTRIN ($20 \mu g\ kg^{-1} h^{-1}$) IN EACH OF THE HEIDENHAIN POUCH RATS USED IN THIS STUDY

$\mu mole\ H^+ h^{-1}$	
101.00	56.3
66.6	59.0
23.6	89.1
62.3	79.4
42.9	42.9

5.4 EXPRESSION OF RESULTS IN HEIDENHAIN POUCH RAT EXPERIMENTS

From the values in Table 7 it can be seen that there is a wide variation in responses from different Heidenhain pouch rats. This is not unexpected since each Heidenhain pouch is not of identical size, although an attempt was made to construct pouches of approximately the same size. Although rats were of a similar weight the sizes of the stomachs varied from rat to rat, and in the case where a stomach was small, the pouch had to be constructed correspondingly smaller in order to leave the remaining stomach sufficiently large to maintain normal feeding.

In order to make the experimental results comparable, in all subsequent experiments the secretory rate was expressed as percentage change in response compared with pentagastrin alone in the same animal. Thus the response to pentagastrin (20 μg $kg^{-1}h^{-1}$) acted as the control experiment in each animal.

In some animals spontaneous secretion increased with time after construction of the pouch (5.2). In these animals the response to pentagastrin was also larger, and in these cases, a new control response to pentagastrin was obtained for the second series of experiments.

5.5 CHOICE OF DOSES OF ISOPRENALINE AND β-ADRENOCEPTOR ANTAGONISTS

5.5.1 Isoprenaline

Since the purpose of this study *in vivo* was to confirm the inhibitory effect of isoprenaline on gastric secretion in the conscious rat, a dose of 40μg $kg^{-1}h^{-1}$ was chosen, since this dose has previously been shown to inhibit pentagastrin-stimulated secretion in the Heidenhain pouch rat (Lundell and Svensson, 1974).

5.5.2 β-Adrenoceptor Antagonists

As a result of experiments with different β-adrenoceptor agonists and antagonists it has been suggested that β-adrenoceptors may be divided into sub-groups denoted β_1 or β_2 (Lands *et al.*, 1967). Myocardial muscle β-adrenoceptors which mediate an increase in heart rate (positive chronotropic) and an increase in force of contraction (positive inotropic) have been classified as β_1,

whereas β-receptors in the smooth muscle of the vascular bed (vasodilation), skeletal muscle (tremor) and bronchioles (bronchiolar dilation) have been classified as β_2.

Non-specific antagonists such as propranolol block both types of adrenoceptor. The criteria for β_1 and β_2-selectivity has been based on the relative potency of the antagonists blocking the action of β-agonists at the appropriate receptor site. Thus the β_1-selective antagonist, practolol at doses which have little effect on the bronchodilator and vasodepressor effects of isoprenaline, antagonises the positive chronotropic effect of isoprenaline in the anaesthetised dog. The β_2-selective antagonist butoxamine will block the vasodepressor and bronchodilator effects of isoprenaline to a greater extent than the positive chronotropic responses in the dog. (Daly, Flook and Levy, 1975). The selectivity of β_2-antagonists has been reported to be less clear cut than that of β_1-antagonists.

It is not known whether β_1- and β_2-adrenoceptor antagonists would show the same pattern of selectivity at the adrenoceptors involved in isoprenalines antisecretory action as at those described in the heart (β_1), vascular smooth muscle and bronchial tree (β_2). In the absence of this information the choice of doses of all β-antagonists have been based on reports of their antagonism of the action of isoprenaline on the heart, as a bronchodilator and as a vasodepressant in vivo.

(a) Propranolol

Propranolol ($1 mgkg^{-1}$) has been shown to reduce the positive chronotropic, vasodepressor and bronchodilator effect of

isoprenaline in the anaesthetised dog. (Daly, Flook and Levy, 1975). Doses of propranolol, in a similar range have been used by Curwain, Holton & Spencer (1972) (1mg kg^{-1}), Lundell and Svensson (1974) (2mg kg^{-1}) and Okabe and co workers (1977) (1,5, 10mg kg^{-1}) in their studies on the antisecretory effects of isoprenaline. Therefore a dose of 2mg kg^{-1} i.v. was chosen.

(b) Practolol

Daly and co workers (1975) have shown that practolol in doses of 0.3, 3, 30mg kg^{-1} reduced the positive chronotropic effect of isoprenaline in the anaesthetised dog. At doses up to 10mg kg^{-1} practolol had little or no effect on the bronchodilator effect of isoprenaline. At 30 mg kg^{-1} practolol reduced the vasodepressor response to isoprenaline. Parratt and Wadsworth (1970) also showed that practolol, (10mg kg^{-1}), markedly reduced the effects of isoprenaline on heart rate, myocardial blood flow and had no effect on isoprenaline induced vasodepression in the cat. Therefore a dose of 8mg kg^{-1} practolol was used in this study.

(c) Butoxamine

Levy (1966) showed that butoxamine at cumulative doses up to 9mg kg^{-1} produced no significant effect on heart rate in the dog. Following a total cumulative dose of 9 to 16mg kg^{-1} butoxamine the vasodepressor effect of isoprenaline was reversed. Daly and co workers (1975) reported that butoxamine, at doses up to and including 10 mg kg^{-1} had no effect on isoprenalines positive chronotropic responses in the dog.

At 10mg kg^{-1} butoxamine reduced the bronchodilator response to isoprenaline by 7-10 fold. A dose of 8mg kg^{-1} i.v. butoxamine was chosen for this study.

The doses of β-antagonists used in this study were therefore large to ensure adequate blockade but at the doses employed have been shown to retain their relative selectivity in studies in the cat and dog. There is little or no information available on the selective actions of practolol and butoxamine *in vivo* in the rat as most of the studies on these drugs have been carried out in the cat and dog, however the assumption has been made, that in a similar dose range as employed in the cat and dog the β-adrenoceptor agonists used here will have similar selectivity.

5.6 THE EFFECT OF ISOPRENALINE ALONE AND IN THE PRESENCE OF β-ADRENOCEPTOR ANTAGONISTS ON PENTAGASTRIN STIMULATED ACID SECRETION

The results are shown in Figure 41.

5.6.1 Isoprenaline

Isoprenaline (40 μg $kg^{-1}h^{-1}$) caused a marked reduction in the response to pentagastrin with a mean value of 37.5 ± 8.4% ($n = 4$ $p < 0.01$) of that with pentagastrin alone.

The mode of action of isoprenaline on pentagastrin-induced secretion may involve a β-receptor if the effect was blocked by a β-antagonist, therefore the effects of a non-selective, β_1-selective and β_2-selective antagonists were investigated.

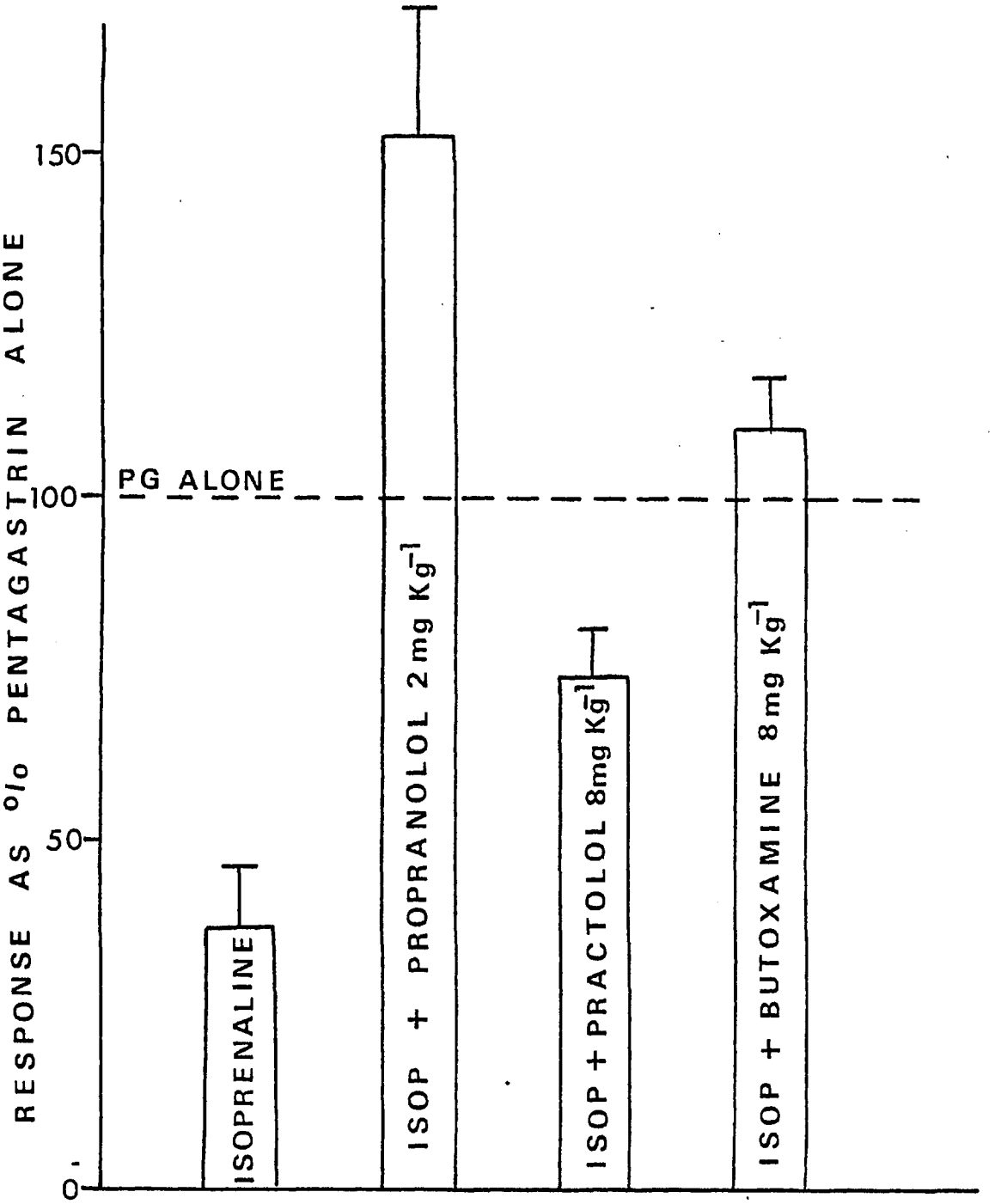
5.6.2 Propranolol + Isoprenaline

Propranolol (2mg kg^{-1}) reversed this inhibition, the response being 152.5 ± 18.2% ($n = 4$) of that of pentagastrin alone. The increased response was not statistically significant ($0.05 > p < 0.1$).

5.6.3 Practolol + Isoprenaline

Practolol (8mg kg^{-1}) antagonised the isoprenaline inhibition ($p < 0.01$) but the mean response (74.0 ± 7.0% $n = 4$) was still less than that with pentagastrin alone ($p < 0.05$).

Figure 41 The effect of isoprenaline infusion ($40\mu gkg^{-1}h^{-1}$) on the response to pentagastrin infusion ($20\mu gkg^{-1}h^{-1}$) alone and in the presence of intravenous injections of propranolol, practolol or butoxamine. Doses of β-antagonists are shown in the columns which show means with s.e. bars. n = 4 in each case. The ordinate is a percentage of the response to the same dose of pentagastrin alone in each animal.


RESPONSE AS % PENTAGASTRIN ALONE
150
100
50
0
PG ALONE
ISOPRENALINE
ISOP + PROPRANOLOL 2 mg Kg⁻¹
ISOP + PRACTOLOL 8mg Kg⁻¹
ISOP + BUTOXAMINE 8 mg Kg⁻¹

5.6.4 Butoxamine + Isoprenaline

Butoxamine (8mg kg^{-1}) also prevented the inhibitory effect of isoprenaline, the response being not significantly different from that to pentagastrin alone (110.3 $\pm$ 7.6% n = 4).

5.6.5 An Example of the Effects of Isoprenaline Alone and in the Presence of β-Adrenoceptor Antagonists in One Rat

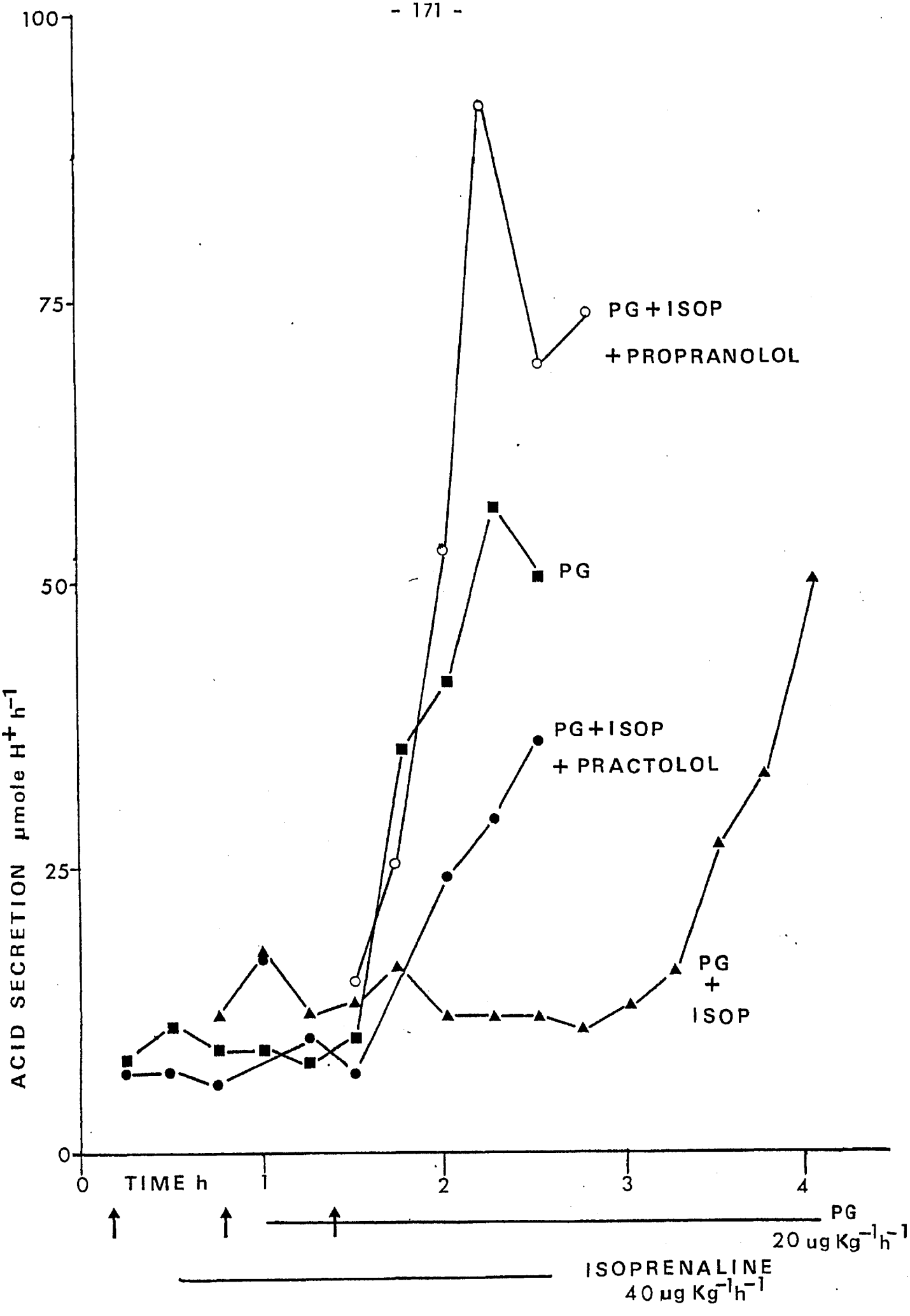
Figure 42 shows the results of four separate experiments in one rat. Spontaneous secretion in this rat was 7.1μmole H^+h^{-1}. An infusion of pentagastrin (20μg $kg^{-1}h^{-1}$) alone produced a response of 57.5μmole H^+h^{-1} at 1.25 hr after beginning the infusion. Isoprenaline, in this experiment produced an almost complete inhibition of the response to pentagastrin with a secretory rate of 12.9μmole H^+h^{-1} 1.5 hr after the start of its infusion. In this particular experiment the pentagastrin infusion was continued after completing the isoprenaline infusion. The figure shows that the inhibition by isoprenaline is rapidly reversed with an acid secretory rate of 50.2μmole H^+h^{-1} 1.5 hr later.

Propranolol completely abolished the inhibitory effect of isoprenaline, and augmented the response to pentagastrin, with a maximum response of 92.1 μmole H^+h^{-1} at 1.25 hr after beginning the pentagastrin infusion.

At 1.5 hr after isoprenaline infusion the response to pentagastrin was 37.1μmole H^+h^{-1} in the presence of practolol. Thus, in this experiment practolol did not completely reverse the inhibitory effect of isoprenaline.

Figure 42 Effects on acid secretion produced by intravenous infusion of pentagastrin ($20\mu gkg^{-1}h^{-1}$) alone, (■) and in the presence of isoprenaline ($40\mu gkg^{-1}h^{-1}$), (▲) isoprenaline + practolol (8mgkg i.v.) (●), and isoprenaline + propranolol (○) ($2mgkg^{-1}$ i.v.) in one Heidenhain pouch rat. Arrows denote injection of β-antagonist. Experimental protocol is shown in 3.2.6.

Readings were not taken during the first 0.5 hr of the experiment.


100
75
50
25
0
ACID SECRETION µmole $H^+ h^{-1}$
PG + ISOP
+ PROPRANOLOL
PG
PG + ISOP
+ PRACTOLOL
PG
+
ISOP
0
TIME h
1
2
3
4
PG
20 ug $Kg^{-1}h^{-1}$
ISOPRENALINE
40 µg $Kg^{-1}h^{-1}$

5.7 THE EFFECT OF β-ADRENOCEPTOR AGONISTS ON PENTAGASTRIN-STIMULATED ACID SECRETION IN THE HEIDENHAIN POUCH RAT

The aim of this part of the study was to investigate the effects of β-adrenoceptor antagonists alone on pentagastrin-stimulated acid secretion. Propranolol has been shown to potentiate the response to pentagastrin in the dog and rat (Curwain, Holton and Spencer, 1973; Lundell and Svensson, 1974). The effects of selective β_1 and β_2 adrenoceptor antagonists have not previously been studied on the conscious rat.

The results are shown in Figure 43.

5.7.1 Propranolol

Propranolol (2mg kg^{-1}) produced a significant ($p<0.01$) increase in the response to pentagastrin, being 165.8 $\pm$ 9.7% ($n = 4$) of the response to pentagastrin alone.

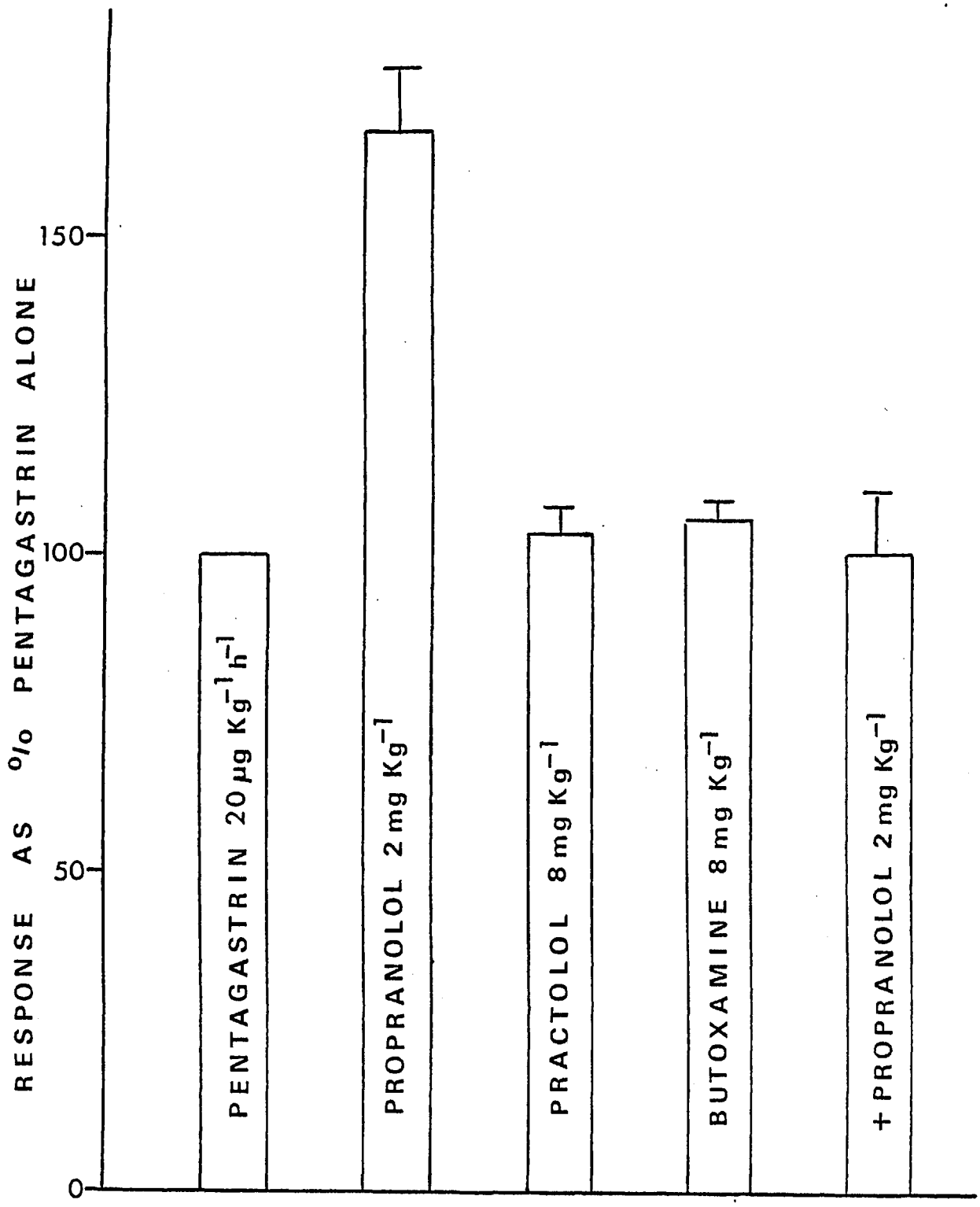
5.7.2 Practolol

There was no significant difference in the response to pentagastrin alone or in the presence of practolol (8mg kg^{-1}) with a response in the presence of practolol of 104.0 $\pm$ 4.2% ($n = 4$) of the response to pentagastrin alone.

5.7.3 Butoxamine

Butoxamine (8mg kg^{-1}) also did not produce any significant change in the response to pentagastrin. The response in the presence of butoxamine was 106.0 $\pm$ 5.5% ($n = 4$) of the response with pentagastrin alone.

Figure 43 The effects of intravenous injection of propranolol, practolol, butoxamine and (+) propranolol on the response to an infusion of pentagastrin. Doses are shown in the columns which show means with s.e. bars. n = 4 in each case. The ordinate is a percentage of the response to the same dose of pentagastrin alone in each animal.


RESPONSE AS % PENTAGASTRIN ALONE
150
100
50
0
PENTAGASTRIN 20 µg Kg⁻¹ h⁻¹
PROPRANOLOL 2 mg Kg⁻¹
PRACTOLOL 8 mg Kg⁻¹
BUTOXAMINE 8 mg Kg⁻¹
+ PROPRANOLOL 2 mg Kg⁻¹

5.7.4 (+) Propranolol

As in the experiments in vitro there remained the possibility that propranolol was acting via a non-specific membrane stabilising effect. Therefore the inactive (+) isomer of propranolol was investigated, and was found to have no effect on the response to pentagastrin producing a response of 95.2 ± 9.7% of that of pentagastrin alone.

5.7.5 The Effects of β-Adrenoceptor Antagonists on the Response to Pentagastrin in Single Heidenhain Pouch Rats

Figure 44 shows the effect of four separate experiments in one rat. Spontaneous secretion in this rat was high. The mean spontaneous secretion from three experiments was 20.4 ± 3.76 μmole H^+h^{-1}.

Pentagastrin stimulation produced a response of 89.1 μmole H^+h^{-1}. Pentagastrin in the presence of butoxamine produced a similar response being 80.5 μmole H^+h^{-1}. In this animal pentagastrin in the presence of practolol produced a response of 103.1 μmole H^+h^{-1}. In the presence of propranolol the response to pentagastrin was augmented giving an acid secretory rate of 173.9 μmole H^+h^{-1}.

Figure 45 shows the effect of (+) propranolol on the response to pentagastrin in one rat. Spontaneous secretion in this rat was also high, with a value of 16.1 μmole H^+h^{-1} at the start of the experiment using (+) propranolol. Although it seemed that, at the start of the experiment using pentagastrin alone, there was some evidence of a stimulation of acid secretion, this returned to baseline before the start of the pentagastrin infusion and for this reason the experiment was continued.

Figure 44 Effects on acid secretion produced by an intravenous infusion of pentagastrin (20μg $kg^{-1}h^{-1}$) alone (□) and in the presence of practolol (8mg kg^{-1}) (○) propranolol (2mg kg^{-1}) (■), and butoxamine (8mg kg^{-1}) (●) in one Heidenhain pouch rat. Arrows denote injection of β-antagonist. Readings were not taken for the first 0.5hr of the experiment.

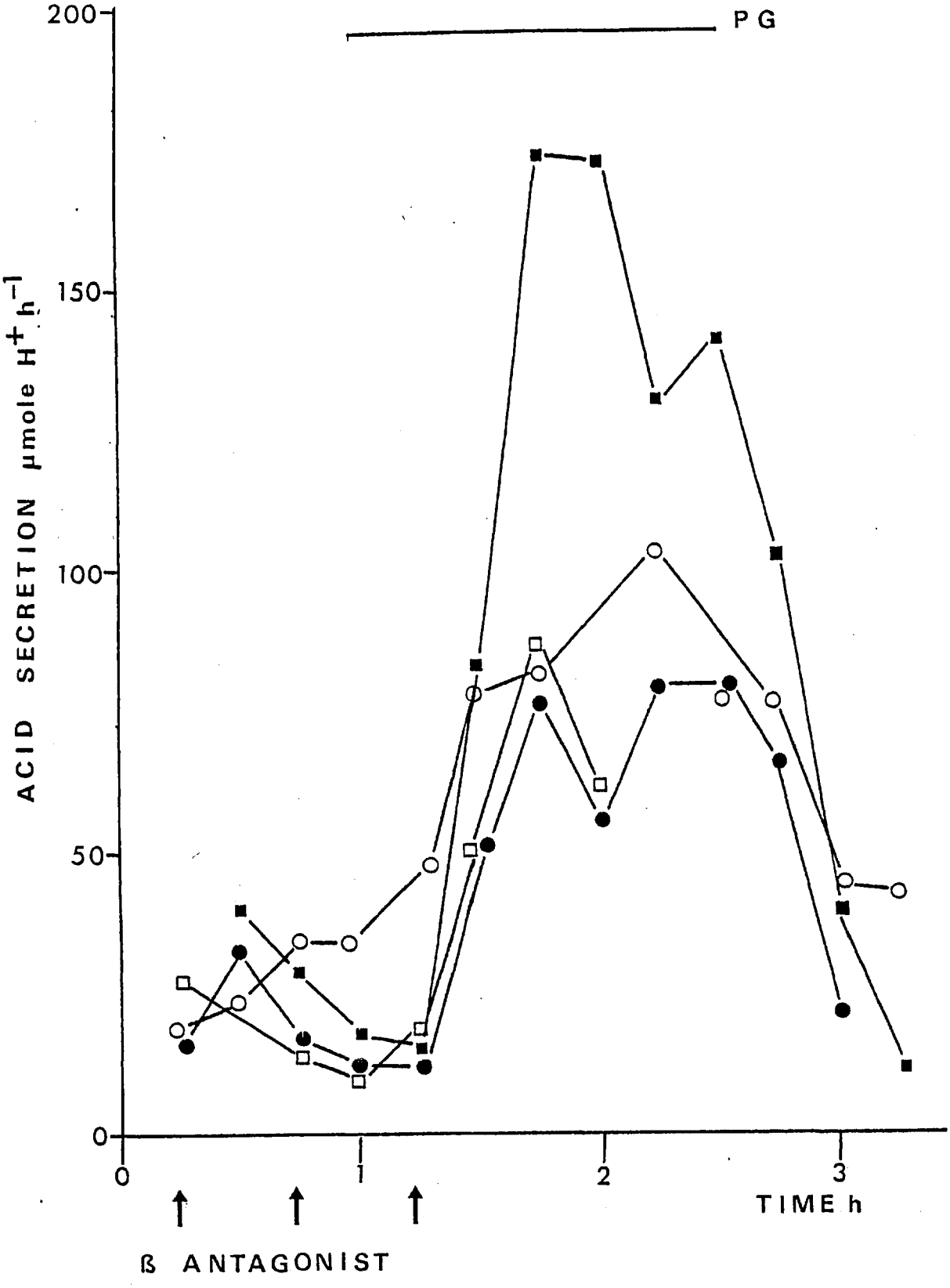
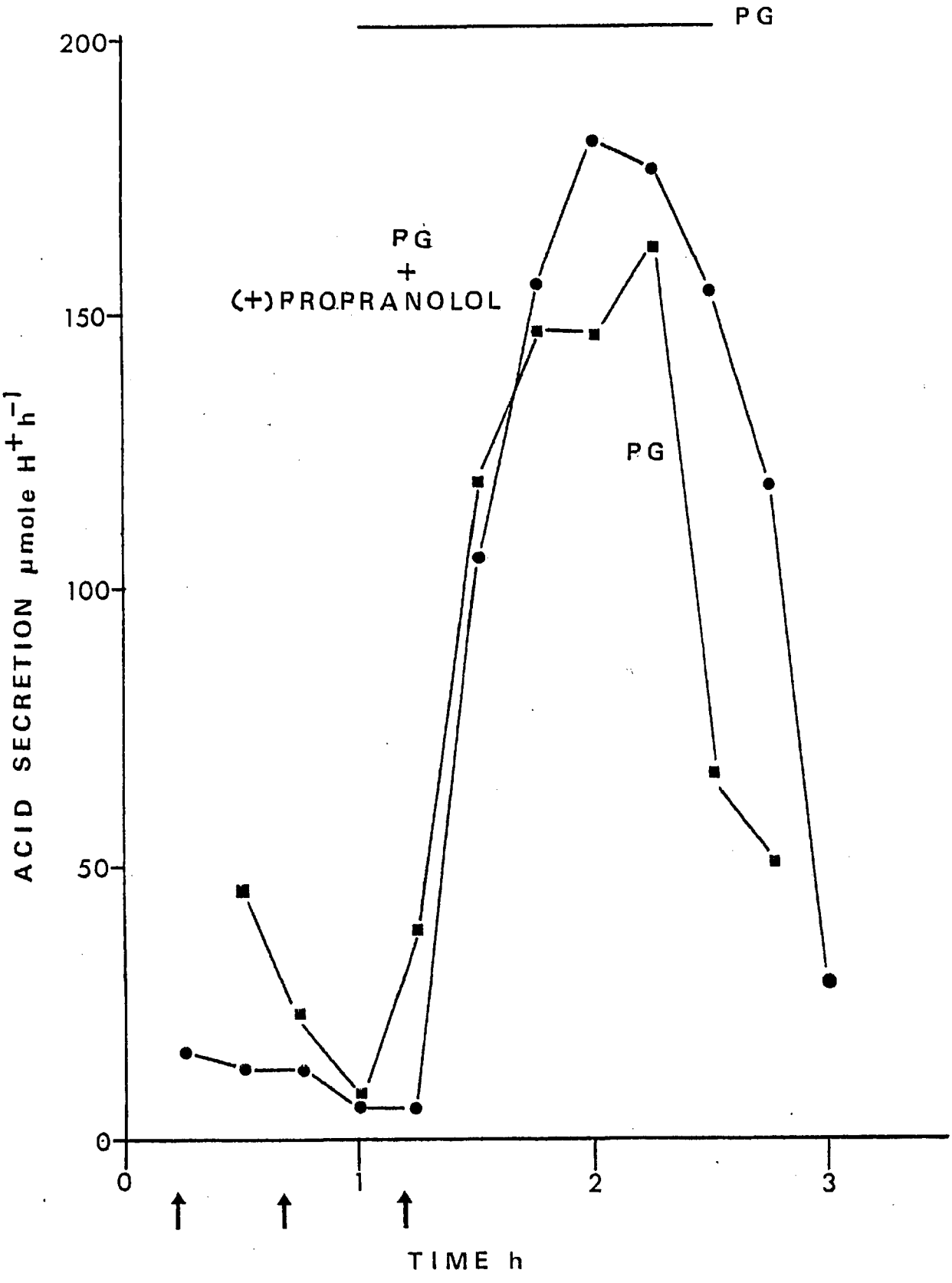

200
150
100
50
0
PG
ACID SECRETION µmole H+ h-1
0
1
2
3
TIME h
ß ANTAGONIST

Figure 45 Effects on acid secretion produced by an intravenous infusion of pentagastrin (20μg $kg^{-1}h^{-1}$) alone (■) and in the presence of (+) propranolol (2mg kg^{-1}) (●) in one Heidenhain pouch rat. Arrows denote injection of (+) propranolol. Readings were not taken for the first 0.5 hr of the experiment.


PG
200
150
100
50
0
PG
+
(+) PROPRANOLOL
PG
ACID SECRETION µmole $H^+ h^{-1}$
0
1
2
3
TIME h

Responses to pentagastrin alone and in the presence of (+) propranolol were similar giving acid secretory rates of 163.2 and 182.5 μmole $H^{+}h^{-1}$ respectively.

5.8 SUMMARY

Isoprenaline infusion inhibited pentagastrin-stimulated acid secretion and this effect was completely blocked by propranolol and butoxamine. Practolol partially reversed the inhibition of pentagastrin-stimulated acid secretion.

Propranolol was shown to potentiate the response to pentagastrin in the Heidenhain pouch rat, an effect which was not seen with either practolol or butoxamine, nor with the inactive isomer of propranolol.

CHAPTER 6

DISCUSSION

6.1 STUDIES IN THE RAT ISOLATED STOMACH

6.1.1 Action of β-Adrenoceptor Agonists

Isoprenaline, salmefamol, salbutamol and noradrenaline all produced a concentration dependent increase in the rate of acid secretion in the immature rat isolated stomach preparation. Adrenaline has also been shown to produce a similar effect in this preparation, giving a similar maximum acid secretory response (Canfield, Hughes, Price and Spencer, 1981). Thus, two naturally occurring catecholamines and three synthetic β-adrenoceptor agonists have been shown to stimulate acid secretion in the rat isolated stomach. The maximum responses obtained were similar to those seen with other secretagogues in this preparation.

The stimulatory effect of β-agonists found *in vitro* is in contrast with their effect *in vivo* where they have been shown to inhibit acid secretion in the conscious animal. To my knowledge, this is the first study performed on the effect of catecholamines *in vitro* on mammalian gastric acid secretion. However, there have been two studies in the amphibian isolated gastric mucosa, where isoprenaline and adrenaline were reported to have no effect on acid secretion by the bullfrog isolated gastric mucosa at concentrations up to 10^{-4}M (Thorpe, Frusco, Bass and Hug, 1971; Flemström, 1978). Flemström (1978) also reported a significant inhibition of basal secretion by isoprenaline at 10^{-3}M, and Thorpe and co workers (1971) showed that this concentration of isoprenaline inhibited histamine-stimulated secretion in the bullfrog isolated gastric mucosa.

In the rat isolated stomach preparation a concentration of 10^{-3}M isoprenaline was shown to cause a significant stimulation of acid secretion (Canfield and Price, 1981). Thus, the rat stomach behaves differently from the amphibian stomach in respect of adrenoceptor agonists.

6.1.2 Possible Mechanisms of Action of Sympathomimetics on Gastric Acid Secretion In Vitro

The stimulatory effect of β-adrenoceptor agonists may be brought about by the release of an endogenous gastric secretagogue. However, as shown in the results metiamide and atropine did not inhibit adrenoceptor agonist responses. The conclusions that can be drawn from this, and other work in our laboratory using prostaglandin E_2 will be discussed under the following headings:

(a) Release of endogenous histamine.

(b) Release of endogenous gastrin.

(c) Release of endogenous acetylcholine.

(d) The effect of β-adrenoceptor antagonists.

(a) Release of Endogenous Histamine

If adrenoceptor agonists stimulate acid secretion via the release of endogenous histamine, the response to these drugs would be inhibited by an H_2 antagonist.

Metiamide has been shown to inhibit histamine stimulated acid secretion in the rat isolated whole stomach in a concentration range of 3×10^{-6}M to 3×10^{-5}M (Bunce and Parsons, 1976).

Similar results were obtained in the kitten isolated gastric mucosa (Tepperman, Schofield and Tepperman, 1975) and the isolated primate gastric mucosa (Rangachari, 1979), although higher concentrations of metiamide were used.

In this study metiamide (10^{-4}M) produced a significant inhibition of a submaximal concentration of histamine but had no effect on isoprenaline, salmefamol or noradrenaline stimulated secretion. From this result it is concluded that these drugs are not acting via release of histamine.

This result was not unexpected since there is evidence from other tissues that isoprenaline inhibits histamine release and formation. Assem (1974) stated that "in virtually every variety of preparation studied to date, some inhibition of antigen-induced histamine release by sympathomimetic amines has been found". For instance, Assem and Feigenbaun found that isoprenaline decreased histamine formation by human leucocytes and Assem & Schild (1969, 1971) showed that histamine release was inhibited by isoprenaline in sensitised lung tissue.

However, the histamine containing cells in the rat gastric mucosa have been shown to have biochemical and morphological differences from mast cells, as shown by Soll, Lewin and Beaven (1981) in their studies on rat isolated histamine containing cells. Rat gastric histamine containing cells did not possess receptors for IgE or release histamine on exposure to compound 48/80, in contrast to rat peritoneal mast cells. To date, the physiological stimulus for histamine release from these gastric cells is unknown and there is no evidence to suggest it may involve catecholamines.

From our present knowledge, it seems likely that β-adrenoceptor agonists do not release histamine from the rat gastric mucosa, since the acid response to these drugs is resistant to H_2 receptor antagonism.

(b) Release of Endogenous Gastrin

There are a number of reports showing that catecholamines increase serum gastrin concentrations in vivo and these will be discussed in more detail in section 6.2.2 (b). Such a mechanism would be a possible explanation for the observed stimulatory effect of catecholamines in vitro. Testing this possibility is more difficult as there is no specific gastrin antagonist available. Metiamide has been shown to inhibit the response to gastrin in the rat isolated whole stomach at concentrations of 10^{-5}M and 3×10^{-5}M (Bunce, Parsons and Rollings, 1976) and it was anticipated that this H_2 antagonist could be used to test the possibility of adrenoceptor agonists releasing gastrin in the rat isolated stomach. At 10^{-4}M metiamide was unable to inhibit the response to a submaximal dose of pentagastrin in this preparation, a concentration which also had no effect on adrenoceptor agonist stimulated secretion. Parsons, Price and Sykes (1980) have shown that,in the rat isolated whole stomach and the rat fundic mucosa,there was a small portion of the gastrin response that was refractory to H_2 antagonism, an observation in agreement with Soll (1978) who demonstrated a response to gastrin in canine isolated parietal cells which was refractory to inhibition by H_2 receptor antagonists.

Main and Pearce (1978) also found that metiamide only partly abolished pentagastrin-stimulated secretion in the rat isolated gastric mucosa. More recently Ackerman (1981) has provided evidence that, in rats, the acid secretory response to pentagastrin occurs before the histamine-mediated system is fully functional. He showed that the responsiveness to impromidine developed between 19 and 22 days *in vivo* in the rat, and that at the age when animals were unresponsive to impromidine, the response to pentagastrin was not inhibited by cimetidine. Therefore age differences may account for the variations in reports of pentagastrin susceptibility to H_2 antagonism.

Therefore it was not possible to use an H_2 antagonist to investigate the possibility of a release of gastrin by isoprenaline.

Prostaglandin E_2 has been shown to inhibit pentagastrin-stimulated secretion in the rat isolated gastric mucosa (Hearn and Main, 1975). This led to the investigation of the effects of prostaglandin E_2 on the responses to pentagastrin, isoprenaline and adrenaline by other workers in our laboratory. Prostaglandin E_2 produced a significant inhibition of the acid response to pentagastrin but was ineffective in inhibiting the responses to isoprenaline and adrenaline (Canfield *et al.*, 1981). In contrast to the effect of prostaglandin E_2 on pentagastrin-stimulated secretion in the rat isolated stomach and mucosa, Boughton-Smith and Whittle (1981) reported that prostaglandin E_2 had inconsistent actions on pentagastrin-stimulated secretion in the rat isolated whole stomach. It is possible that this difference may be accounted for by the absence of the antrum in the isolated stomach and mucosa preparation.

In these experiments, and those of other workers, the assumption has been made that pentagastrin (or synthetic human gastrin) is acting like endogenous rat gastrin. It would be preferable to use rat gastrin, but the lack of commercially available rat gastrin precluded this.

However, with that assumption, it is concluded that β-adrenoceptor agonists do not act via the release of gastrin since a potent inhibitor of pentagastrin-stimulated secretion in this preparation was unable to inhibit β-agonist stimulated secretion. Studies on rat gastrin cells under tissue culture conditions have shown that isoprenaline, noradrenaline and adrenaline could stimulate gastrin release from these cells (DeSchryver-Kecskemeti and Greider, 1978). The release of gastrin by noradrenaline was inhibited by prostaglandin E_2 (100ng). Therefore if catecholamines were releasing gastrin in the rat isolated stomach, prostaglandin E_2 may have been expected to inhibit this effect. This was not the case. It is not known whether there are sufficient G cells in this preparation to cause significant gastrin release and to investigate this gastrin levels in the serosal and mucosal saline could be measured.

(c) Release of Endogenous Acetylcholine

Atropine (10^{-5}M) has been shown to inhibit the acid secretory response to acetylcholine in the isolated whole stomach of the rat (Bunce, Marsh and Parsons, 1977). The effects of atropine on endogenous acetylcholine has been studied by electrical field stimulation of the rat isolated mucosa and mouse isolated whole stomach. (Baird and Main, 1978; Angus and Black, 1978 (a) and (b)).

In both preparations (10^{-7}M) atropine substantially inhibited the effect of field stimulation. However, in the mouse isolated stomach field stimulation could also be inhibited by metiamide, at a dose which had no effect on bethanechol stimulated secretion, suggesting an involvement of histamine in the effect of endogenous acetylcholine. This is also seen in the rat whole stomach but the effect is small (C.A. Price: unpublished observation). Therefore by studying the effect of a high concentration of atropine (10^{-5}M) on β-agonist stimulated secretion some inhibition should have been seen if β-agonists were releasing endogenous acetylcholine. However, although atropine (10^{-5}M) markedly inhibited bethanechol-stimulated secretion, this concentration had no effect on any of the β-adrenoceptor agonists tested. This indicates that release of endogenous acetylcholine is not involved in these responses.

CONCLUSION

From the results using antagonists of the three major secretagogues which were unable to inhibit adrenoceptor agonist-stimulated secretion it seems possible that the stimulatory effect of these drugs is a direct effect on the parietal cell. The rats used in this study were immature animals and the sympathetic innervation of the gastrointestinal tract is incomplete at this time (Yoshida, Taniyama and Tanaka, 1979). The whole stomach wall preparation also contains intact neural plexi. Both of these considerations may explain the results obtained with adrenoceptor agonists. However, Canfield and co workers (1981)

have shown that, in the adult rat isolated mucosa, adrenaline stimulated acid secretion in a concentration dependent manner. Isoprenaline also stimulates this preparation. (C.A. Price: unpublished observation). Therefore in a preparation with fully developed sympathetic innervation and the neural plexi disrupted by dissection, catecholamines still stimulated acid secretion, an effect which, from results in the immature rat, would seem to be a direct effect on the parietal cell.

Both β_1-selective (noradrenaline) and β_2-selective (salmefamol, salbutamol) agonists stimulated acid output suggesting that both types of receptors may be involved. This may partly explain the lower potency of these selective agonists compared with isoprenaline. The lack of an inhibitory effect of phentolomine suggests that α-adrenoceptors are not involved in these responses.

(d) Action of β-Adrenoceptor Antagonists

If the stimulatory effect of adrenoceptor agonists is mediated via a β-adrenoceptor then their action should be inhibited by β-adrenoceptor antagonists.

Since both β_1-selective and β_2-selective agonists stimulated acid secretion in this preparation the effects of some non-selective β-antagonists were first investigated.

Propranolol, timolol and pindolol all produced a significant inhibition of the responses to isoprenaline, salmefamol and noradrenaline. The results with propranolol on salmefamol and isoprenaline were consistent with competitive inhibition. The (+) isomer of propranolol did not inhibit β-agonist-stimulated

secretion at a concentration at which the (±) isomer was active, indicating that propranolol was not exerting a non-specific membrane stabilising effect. This was further supported by the fact that (±) propranolol at this concentration had no effect on acid secretion stimulated by bethanechol or pentagastrin.

Neither β_1- nor β_2-selective antagonists inhibited the responses to isoprenaline or salmefamol. However, at the same concentrations used in the above studies these selective antagonists produced a significant inhibition of noradrenaline-stimulated secretion. This was not only true for practolol and butoxamine, as shown in this study, but also for atenolol (β_1) and ICI 118551 (β_2) as shown by Canfield and co workers (1981). However, although atenolol had no effect on isoprenaline, salmefamol and adrenaline-stimulated secretion, ICI 118551 was effective in inhibiting the responses to these agonists. Noradrenaline was consistently more succeptible to inhibition than other agonists used in this study. Noradrenaline is considered to act predominantly at α-adrenoceptors (Alquist, 1948), however phentolamine had no effect on noradrenaline-stimulated acid secretion, indicating that in the rat isolated stomach noradrenaline is acting at β-adrenoceptors.

With both the selective and non-selective antagonists the concentration that produced significant inhibition is greater than might be expected from the pA_2 values reported for the antagonists. β-adrenoceptors have been classified in terms of relative potencies of a series of agonists and determination of the potencies of competitive selective antagonists. In vitro determinations of pA_2 values for β-antagonists in a variety of tissues have been carried out by Levy and Apperley (1978).

They quote a pA_2 value for propranolol and practolol, at β_1 adrenoceptors in guinea pig, rabbit and chick left atria, of 8.3 - 8.8 and 6.5 - 6.9 respectively. These values are equivalent to concentrations of 5×10^{-9} - 1.6×10^{-9}M and 3.2×10^{-7} - 1.3×10^{-7}M for propranolol and practolol respectively. Thus the concentrations used in this study (2×10^{-5}M) are higher than would be predicted. Bunce, Marsh and Parsons (1977) also found that, in the rat isolated whole stomach relatively high concentrations of atropine were required to inhibit acetylcholine-stimulated secretion. More recently Black and his colleagues (Angus and Black, 1979; Angus, Black and Stone, 1980) have drawn attention to the anomalous pA_2 values obtained with atropine and metiamide for inhibition of acid-secretion in the isolated mouse whole stomach preparation, compared with other tissues such as heart or uterus for metiamide, and guinea pig ileum for atropine. They pointed 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 meant that local concentrations of antagonist in the region of the basement membrane could not be in equilibrium. Where the antagonist passes the parietal cell membrane and appears in the effluent then the concentration of antagonist in the region of the receptors will be lower than that obtained if equilibrium was achieved. However their addition of metiamide to the lumen perfusate, to remove the concentration gradient down which metiamide would pass from serosal to mucosal saline, did not alter the anomalous pA_2 value obtained with metiamide on the serosal side only. Thus it is possible that the factor

responsible for the anomalous pA_2 values obtained was primarily the act of secretion.

Although in these studies the stomachs were not perfused, obviously the problems of active secretion remain the same, and removal of mucosal saline every fifteen minutes would also hamper the equilibration of receptor with antagonist. This would presumably also be true for agonists. Therefore comparison of pA_2 values obtained in other tissues with those obtained in acid-secretion experiments in vitro may not be valid. The problems discussed above would also mean that one cannot interpret results with selective antagonists with certainty since the selectivity of an antagonist is not absolute, but relative to the concentration of antagonist used. For instance, practolol (β_1) has been shown to have a pA_2 value of 4.6 - 5.1 at β_2-andrenoceptors in frog ventricle and guinea pig trachea (Levy and Apperley, 1978). This would be equivalent to concentrations of 2.5×10^{-5} - 8×10^{-6}M. Therefore although the β_2-selective antagonist ICI 118551 inhibited noradrenaline, adrenaline salmefamol and isoprenaline at 10^{-5}M, at this concentration it does not act selectively, although the above discussion of anomalous pA_2 values will still be a complicating factor.

These considerations do not, however, explain the results with noradrenaline where butoxamine and practolol inhibited the response at concentrations which were ineffective against the other agonists. The present experiments can provide no explanation for this, and the whole question of type of receptor involved will require experiments using isolated parietal cells which would be free of the discrepancies discussed regarding isolated stomach preparations.

It is clear however from these studies *in vitro* using β-adrenoceptor agonists and antagonists that the stimulatory effect of β-agonists on acid secretion appears to be mediated by a β-receptor, and not by release of an endogenous secretagogue. Both β_1 and β_2 subtypes may be involved in these responses. This conclusion is supported by recent findings concerning adrenoceptor stimulation of cyclic AMP in rat isolated parietal cells.

6.1.3 Evidence for β-Adrenergic Activation of a Gastric Mucosal Adenylate Cyclase/cAMP System

In recent years it has been well established that the adenylate cyclase/cAMP system plays an important role in the regulation of gastric acid secretion (for references see Chapter 1), particularly histamine-stimulated secretion.

In most tissues occupation of β-adrenoceptors by β-agonists leads to an increase in adenylate cyclase activity (Coyle and Snyder, 1980; Minnerman and Molinoff, 1980).

An increase in gastric mucosal cAMP has been reported from *in vivo* studies in the rat after β-adrenergic stimulation, an effect which was antagonised by propranolol (Ruoff, 1977). Thompson and co workers (1977) using rat gastric mucosal particulate preparations showed that isoprenaline and adrenaline stimulated adenyl cyclase activity. Studies of cAMP in whole gastric mucosa are complicated by the heterogeneity of the gastric mucosa, in which the acid secreting parietal cells account for only part of the total cell population. Since adenyl cyclase has a universal cellular distribution more recent

studies have used isolated parietal cells to test the responsiveness of parietal cell adenyl cyclase to secretagogues. Gunther, Wagner and Ruoff (1980) have shown that adrenaline and hexoprenaline caused a concentration dependent rise in adenylate cyclase activity in rat isolated parietal cells. This stimulatory action could be blocked by propranolol but not by phenoxybenzamine.

Thompson's group have extended their earlier studies to investigate the effects of catecholamines on enriched rat parietal cell adenylate cyclase (Thompson, Chang and Rosenfeld, 1981). They have shown that isoprenaline (100μM), l-noradrenaline (100μM) and adrenaline (100μM) all activated adenylate cyclase. GPP (NH)p was required for the full effect but each agonist had a significant effect in its absence. The EC_{50} for isoprenaline was 0.5μM. This can be compared to a concentration of 1μM of isoprenaline which produced approximately 50% of the maximum response in the rat isolated stomach in the work described in this thesis. Propranolol (100μM) fully inhibited the adrenaline activation of adenyl cyclase while phentolamine had no effect. The effective concentrations for adenyl cyclase activation were near those observed for other tissues (Ross et al., 1977). Further studies in isolated purified rat parietal cells have shown a β_2-adrenergic receptor as judged by adenyl cyclase criteria (W.J. Thompson to S.P. Canfield: personal communication).

This evidence in rat isolated parietal cells strongly supports the conclusion from this study that β-adrenergic receptors may be present on the parietal cell, although in the isolated parietal cell system, this has not, as yet, been linked to secretory function. The question remains whether, in vivo

these effects are mediated via circulating catecholamines or noradrenaline from adrenergic innervation of parietal cells, or endocrine cells in the gastric mucosa. The majority of studies on adrenergic innervation of the stomach have concentrated on the gastric musculature. It has been shown that there is a rich contribution of adrenergic nerve fibres to the intramural nerve plexuses (Norberg, 1967) and to the arteries of the gut wall (Furness (1971)). Costa and Gabella (1971), using fluorescent histochemical techniques found that adrenergic innervation in and around gland cells, close to epithelial cells in the rabbit and guinea pig, and fluorescent fibres could be traced as far as the inner surface of the stomach. Kataoka and co workers (1979) found catecholamine containing endocrine cells in the cat fundic mucosa. However there have been no similar studies in the rat, which would resolve the question of origin of catecholamines in the gastric mucosa.

6.1.4 α-Adrenoceptor Effects on Acid Secretion

There seems to be general agreement that, α-agonists inhibit acid secretion *in vivo*. (For references see Chapter 2). This conclusion has been drawn from studies using noradrenaline which acts predominantly at α-receptors. The inhibitory effect of noradrenaline has been considered to result simply from the reduction in mucosal blood flow that it produces.

The results with α-adrenoceptor agonists in this study provide support for an indirect effect on acid secretion *in vivo* since methoxamine and phenylephrine in concentrations as high as 10^{-3}M were without effect *in vitro*. Phenylephrine had no

effect on bethanechol-, pentagastrin-, or histamine-stimulated secretion. Therefore it is likely that *in vivo* noradrenaline stimulates α-adrenoceptors on the gastric vasculature which mediate vasoconstriction, causing a reduction in gastric mucosal blood flow. The lack of an inhibitory effect of phentolamine on noradrenaline stimulated acid secretion also supports the idea that α-adrenoceptors are not involved in catecholamine-stimulated secretion.

These studies are in agreement with those of Thompson and co workers (1981) who found that, in rat isolated parietal cells, methoxamine did not stimulate adenyl cyclase activity.

An earlier report by Ruoff (1977) showed that adrenaline, which acts on both α and β-receptors stimulated both cAMP and cGMP in rat gastric mucosa. Blockade of β-adrenoceptors suppressed the adrenaline effect on cAMP and increased its effect on cGMP. The opposite was found on α-adrenoceptor blockade. Phenylephrine enhanced the concentration of cGMP in rats pretreated with propranolol at low concentrations and at high concentrations raised cGMP levels significantly. He suggested a reciprocal inhibitory interaction between α and β-receptors since β-receptor activation stimulated cAMP and α-receptor activation stimulated cGMP. In view of the complete lack of effect of α-agonists on acid secretion in the rat isolated stomach it seems unlikely that the increase in cyclic GMP seen by Ruoff was directly linked to acid secretory function. Furthermore Ruoff's study was performed in the intact gastric mucosa, therefore changes in cGMP could not be localised to parietal cells. It is concluded that α-adrenoceptors are not involved in the control of acid secretion *in vitro*.

6.2 STUDIES IN THE HEIDENHAIN POUCH RAT

6.2.1 The Effect of Isoprenaline

The results from this study confirm and extend the findings of Lundell and Svensson (1974) in this preparation. Isoprenaline infusion resulted in a marked inhibition of pentagastrin-stimulated secretion, and this effect was completely blocked by propranolol. Similar findings on the effect of isoprenaline have been reported in the conscious dog. (Magee, 1976; Daly and Stables, 1977; Curwain and Holton, 1972).

β_2-selective adrenoceptor agonists such as salbutamol and salmefamol also inhibit pentagastrin-stimulated acid secretion in the conscious dog (Curwain, Holton and Spencer, 1972; Daly, Long and Stables, 1978; Gottrup and Ornsholt, 1978). In these studies propranolol was again effective in reversing the inhibitory effects.

In addition to previous findings in the conscious rat, this study has shown that both β_1- and β_2-selective antagonists reversed the inhibitory effect of isoprenaline, with butoxamine completely abolishing and practolol partially reversing it. This suggests an involvement of both β-receptor subtypes, a finding similar to that of Daly and co-workers (1978) in dogs with a Heidenhain pouch. However in the study of Daly and co-workers the β_2-selective antagonist $H_{35/25}$ had no effect on the antisecretory action of isoprenaline, in contrast to its blockade of the anti-secretory action of salbutamol. This suggested that the anti-secretory effect of isoprenaline in the dog was mediated by β_1-receptors only. Thus there seems to be a difference in the action of isoprenaline in the dog and rat.

However, in both species activation of both β_1- and β_2-adrenoceptors can produce an inhibition of pentagastrin-induced acid secretion. As there have been no studies on the effects of β_1- and β_2- selective antagonists on the antisecretory action of β-agonists on histamine or bethanechol stimulated secretion in the rat no further comparisons can be made.

6.2.2 Mechanism of Action of Isoprenaline

Since β-agonists have been shown to stimulate acid secretion in vitro, the inhibition seen in vivo must be an indirect effect on the gastric mucosa. Alternatively, β-agonists may be inhibitory at low doses and stimulatory at higher doses. Although it is difficult to compare doses in vivo and in vitro, the fact that isoprenaline (10^{-7}M) produced a stimulation of acid secretion in the rat isolated stomach suggests that this is not the case.

β-agonists have been reported to inhibit pentagastrin- and feeding-stimulated acid secretion in the rat and dog and bethanechol-stimulated secretion in the dog, in conscious animal experiments. (Lundell and Svensson, 1974; Canfield, Curwain, King and Price, 1978). This suggests a site of action beyond the secretagogue receptors. This inhibitory effect must also be able to inhibit the direct stimulant action of β-agonists on the gastric mucosa.

The experiments in vivo in this study do not themselves provide any information about the inhibitory mechanism involved in the action of isoprenaline, other than suggesting an involvement of both β_1- and β_2-receptors. It was not the purpose of the work described in this thesis to undertake an experimental investigation of this aspect of the action of β-agonists on acid

secretion. However, there are a number of possible ways that the inhibition could be produced. Their mechanism of action may be an indirect effect on the stomach via vascular or neural pathways, an inhibition of release of an endogenous secretagogue, or a stimulation of the release of an inhibitory agent from the stomach or another site.

Evidence for and against these possibilities will be discussed below.

(a) Inhibition of Histamine Release or Production

As discussed in 6.1.2 (a), isoprenaline can inhibit the release of histamine in many tissues. In the Heidenhain pouch rat isoprenaline has been shown to inhibit the activation of histidine decarboxylase (HDC) caused by pentagastrin, at a dose which also inhibited the acid secretion response to pentagastrin (Lundell and Svensson, 1974). This effect was completely blocked by propranolol. In the rat isolated stomach the mechanism of action of isoprenaline did not involve histamine, however this does not preclude an effect *in vivo* where co-operative effects of histamine in acid secretion may be more important.

Histamine is clearly involved in pentagastrin-stimulated secretion, as shown by the ability of H_2-antagonists to inhibit pentagastrin *in vivo*. However it is by no means certain that histamine is the final common mediator of pentagastrin-stimulated acid secretion. Lundell and Svensson (1974) suggested that from the evidence in their study, isoprenaline inhibited acid secretion by restraining histamine mobilisation. There has been no evidence of a direct stimulation of histamine release by pentagastrin in the rat. Furthermore there is evidence of a dissociation between

HDC activation and acid secretion in the rat *in vivo* by secretin (Caren *et al.*, 1969) and *in vitro* from time-course studies (C.C. Sykes: personal communication). Therefore the action of pentagastrin on HDC may not be directly related to acid output, and thus isoprenaline's effect on pentagastrin activated HDC may be unrelated to its antisecretory effect. If isoprenaline was acting by restraining histamine mobilisation, it would be unlikely to inhibit histamine stimulated acid secretion in the Heidenhain pouch rat, an effect shown by Lundell, Svensson & Nillson (1976). Furthermore exogenous histamine failed to prevent the inhibitory effect of isoprenaline in the conscious rat (Lundell *et al.*, 1976), which also suggests that isoprenaline's mode of action cannot be entirely explained by an effect on histamine mobilisation or release.

(b) β-agonists and Gastrin Release

There is no evidence to suggest that β-agonists inhibit gastrin release *in vivo*. In fact catecholamines have been shown to raise serum gastrin levels in man, dog and rat (Stadil and Rehfeld, 1973; Hayes *et al.*, 1972; Hsu and Cooper 1977).

Hsu and Cooper (1977) found that, in the rat, isoprenaline and adrenaline increased serum gastrin levels and that propranolol completely inhibited the hypergastrinaemic response to isoprenaline. Acid secretion was not measured in this study, therefore it cannot be concluded whether this was a direct or an indirect effect, resulting from an elevated antral pH during inhibition of acid secretion.

Therefore if isoprenaline is also causing gastrin release inthe rat, the indirect mechanism whereby acid secretion is inhibited must be such as to counteract the effect of raised serum gastrin levels.

(c) Effects on Gastric Mucosal Blood Flow

β_2-receptors normally mediate vasodilation by relaxation of vascular smooth muscle. It seems unlikely that isoprenaline's inhibitory effect could be a consequence of a reduction of mucosal blood flow. One possibility has been raised by the recent reports of presynaptic β-adrenoceptors in noradrenergic nerve endings (Langer and Dubocovich,1978). Activation of these receptors by β-agonists leads to an increase in transmitter release from these nerve endings. If these presynaptic receptors existed on noradrenergic nerve endings in the gastric vasculature then isoprenaline would increase sympathetic tone to these vessels and hence inhibition of acid secretion would result.

There is however strong evidence from studies in the dog and cat that this is not the case.

Curwain and Holton (1972) showed that the ratio of mucosal blood flow to acid secretion was increased during isoprenaline inhibition in Heidenhain pouch dogs. Archibald, Moody and Simons (1974) have shown that isoprenaline actually increased gastric mucosal blood flow in the anaesthetised dog. Zinner, Kerr and Reynolds (1975) report that isoprenaline, at a dose which other workers have shown to inhibit acid secretion, caused vasodilation of both right and left gastric circulations in the anaesthetised dog.

Isoprenaline also inhibited pentagastrin stimulated acid secretion in the conscious fistula cat with no change in gastric mucosal blood flow (Holland 1980). Blood flow studies have not been performed in the rat during experiments on isoprenaline's anti-secretory effect, either in this study or the work of others. Lundell and Svensson (1974) reported that a dose of isoprenaline which inhibited acid secretion had no effect on arterial blood pressure in the Heidenhain pouch rat.

From the evidence discussed it is concluded that isoprenaline does not decrease gastric mucosal blood flow and that its action on acid secretion is not the result of vascular changes.

(d) Hyperglycaemic Effects of β-Agonists

β-agonists have been shown to produce hyperglycaemia in man, dog, cat and rat (Himms Hagen,1967; Ellis, 1967). In the cat this effect of isoprenaline is β_2-mediated (Loakpradit and Lockwood,1977). Since hyperglycaemia *per se* can inhibit acid secretion the possibility of isoprenaline working by this mechanism must be considered.

Whilst adrenaline is a potent hyperglycaemic agent in all species studied, there are variations in the effects of isoprenaline from species to species. Thus, while isoprenaline has strong hyperglycaemic activity in the dog, in the rat it is weak. Fleming and Kenny (1964) showed that the hyperglycaemic effect of isoprenaline in the rat was dependent on the state of nourishment. In the normal, fed rat isoprenaline was relatively inactive and in fasted rats isoprenaline produced a modest hyperglycaemia only.

The rats in the study in this thesis were starved, as is normal for studies on gastric secretion, thus a small hyperglycaemic response might be expected.

Potter and Ellis (1975) showed that, in the rat the hyperglycaemic effect of isoprenaline was limited by its stimulating effect on insulin release, and suggested that this factor explained the relative inactivity of this catecholamine in the rat. If isoprenaline stimulates insulin release then a stimulation of acid secretion would be expected which would counteract any hyperglycaemic inhibitory effect on acid secretion. However in the Heidenhain pouch rat isoprenaline markedly inhibited pentagastrin stimulated acid secretion.

Unfortunately there have been no experiments performed in the rat to investigate the effects of an infusion of glucose on acid secretion and blood glucose levels simultaneously. Therefore it is not known whether the modest hyperglycaemia after isoprenaline infusion reported by Potter and Ellis (1975) in the fasted rat would be sufficient in itself to inhibit acid secretion. An investigation of this kind during isoprenaline infusion would further clarify the problem. McCloy and co workers (1979) showed that, in the dog, an infusion of salbutamol produced a 33% rise in glucose and a 63% inhibition of acid, whereas an infusion of dextrose which raised blood glucose to half this level only inhibited acid output by 19%. Thus, in the dog, hyperglycaemia may account for only some of the inhibitory effect of β-agonists.

The possibility that isoprenaline inhibits acid secretion via a hyperglycaemic effect in the rat cannot be excluded, although it seems less likely that the entire effect could be due to hyperglycaemia since isoprenaline has also been reported to stimulate insulin release in the rat.

(e) Central Nervous Effects of Isoprenaline

Although isoprenaline is considered not to cross the blood brain barrier in significant amounts, an infusion of isoprenaline caused restlessness in dogs (Holton 1973) and in the rats used in this study. Isoprenaline has also been reported to cause apprehension and anxiety in man. Therefore there is a possibility of stimulation of the sympathetic nerves to the stomach and secretion of adrenaline from the adrenal medulla. The reflex effects of sympathetic nerves can be excluded in conscious dogs since inhibition of acid secretion occurs after pharmacologically effective doses of guanethidine and hexamethonium (Curwain et al., 1971).

The precise areas in the central nervous system which control vagal activity, and gastric secretion have not been identified, although stimulation of the hypothalamus has been shown to produce an alteration in gastric acid secretion. (Rideley and Brooks 1965; Misher and Brooks 1966; Osumi et al., 1977). Since inhibitory pathways in the central nervous system, controlling acid secretion are poorly understood, it is not possible to discuss this question further, but merely to point out that central nervous effects may be contributing towards the inhibitory effect of isoprenaline in vivo.

(f) Release of Endogenous Inhibitory Agents by Isoprenaline

The mechanism by which isoprenaline inhibits acid secretion in vivo must be capable of nullifying the effects of increased gastrin and insulin release which it also causes in vivo and of inhibiting the direct stimulatory effect on acid secretion shown in vitro.

Isoprenaline has been shown to release both 5-hydroxy-tryptamine (5-HT) and somatostatin from the gastrointestinal tract, and both to these agents are potent inhibitors of acid secretion, with inhibitory profiles similar to that of isoprenaline, which is less effective in inhibiting histamine than other secretagogue stimulated secretion.

(i) 5-Hydroxytryptomine (5-HT)

Adrenaline, noradrenaline, isoprenaline and sympathetic nerve stimulation all release 5-HT from the duodenal mucosa. (Pettersson, 1979). The effect of adrenaline was blocked by propranolol indicating that this was a β-adrenergic effect. The gastrointestinal tract is the most important mammalian source of 5-HT (Erspamer, 1954; Thompson, 1971). Within the GI tract 5-HT is localised within the myenteric plexus (Robinson and Gershon, 1971) and within enterochromaffin cells (Solcia and Sampietro, 1967). The inhibitory effect of 5-HT on acid secretion *in vivo* is now well documented (For review see Jaffe 1979). Thus 5-HT and its precursor 5-hydroxytryptophan, inhibit basal-, cholinergic-, histamine- and pentagastrin-stimulated acid secretion in the dog. (Black, Fisher and Smith, 1958; Haverback, Bogdanski and Hogben, 1958). Histamine-stimulated secretion seemed more resistant to inhibition. Thus Black *et al.*, (1958) reported that bilateral cervical vagotomy abolished the inhibitory effect of 5-HT on histamine-induced secretion in the anaesthetised dog, and Haverback and co workers (1958) reported that histamine was not inhibited by a dose of 5-HT which was effective against urecholine stimulated secretion in the conscious dog. Thus 5-HT and isoprenaline have both been reported

to be less effective against histamine-stimulated secretion *in vivo*.

Canfield and Spencer (1981) have studied the effects of 5-HT in the rat isolated stomach preparation used in this work. They showed that 5-HT was capable of inhibiting the direct stimulatory effect of isoprenaline. It also inhibited the response to pentagastrin, but unlike the *in vivo* studies described above, 5-HT was unable to inhibit bethanechol-induced secretion *in vitro*, although it was effective against histamine. Therefore, their *in vitro* study shows that 5-HT does fulfill one important criteria as a mediator of *in vivo* adrenergic inhibition, namely that it can block isoprenaline's direct stimulatory effect. However the *in vitro* studies do not fully explain the *in vivo* inhibitory profile shown for 5-HT and this problem requires further elucidation.

A local or paracrine role for 5-HT in the gut has been suggested since several reports have shown that considerable amounts of 5-HT are inactivated during hepatic transit (Thompson, 1971) or rapidly taken up into platelets (Born and Gilson, 1959). There appears to be no published information regarding a β-adrenergic 5-HT release from the stomach similar to that found for the duodenum. It seems unlikely that 5-HT is released from the stomach since *in vitro* isoprenaline stimulates acid secretion, although it is possible that *in vitro*, sufficient 5-HT is not released to overcome the stimulatory effect of isoprenaline. If 5-HT, released from the duodenum, is to explain the inhibitory mechanism of isoprenaline then sufficient of it must be present in the systemic circulation to produce an inhibitory effect.

There have been several reports that suggest this could happen despite its rapid inactivation in the liver. A rise in circulating 5-HT has been reported after a meal (Kellum and Jaffe, 1976). 5-HT is released into the portal circulation in measurable quantities during duodenal acidification. Jaffe, Kopen and Lazan (1977) have shown that, in the dog, duodenal acidification results in significant inhibition of acid output and produces an elevation of peripheral venous blood levels of 5-HT. Infusion of exogenous 5-HT to comparable blood levels resulted in a similar inhibition of acid secretion. They also found that significant amounts of immunoreactive 5-HT escaped hepatic inactivation and that during release of endogenous 5-HT, 92% of portal venous 5-HT was bound to platelets. Jaffe and co workers therefore suggested that endogenously released 5-HT, escaping inactivation in the liver and lung due to binding to platelets could play a physiological role in the intestinal control of acid secretion.

Further support for an effect of circulating 5-HT comes from acid secretion studies where the inhibition seen in vivo by 5-HT is long lasting. Thus, in the dog, an infusion of 5-HT for 30 minutes produced an inhibition of histamine-stimulated secretion in vagally intact animals for 1½ - 2 hours (Black 1958). This may be explained if circulating 5-HT was bound to platelets.

Therefore, as discussed above, there is some evidence to suggest that the release of 5-HT by β-agonists, and the inhibition of acid secretion by these agents may be linked, and that isoprenaline may act in vivo by this mechanism.

(ii) Somatostatin

Isoprenaline and adrenaline stimulate gastric somatostatin release in the isolated perfused rat stomach (Koop et al., 1980) A role for β-adrenoceptors has also been shown in the isolated canine pancreas where isoprenaline-stimulated somatostatin release was abolished by propranolol (Samols and Weir, 1979).

Somatostatin is present in D cells throughout the gastrointestinal mucosa and in intestinal nerve fibres. It has been shown to have a wide range of inhibitory actions in the gastrointestinal tract including inhibition of gastric acid and pepsin secretion, the release of gastrin, secretin, CCK-PZ, GIP, VIP and motilin. Immunoreactive somatostatin is also released under basal conditions into the blood draining the stomach and pancreas, and gastric or duodenal acidification results in a marked rise in plasma somatostatin (Schusdiazarra et al., 1978). Thus somatostatin may be a modulator of many inhibitory mechanisms in the gastrointestinal tract, acting either as a paracrine or hormonal agent.

Somatostatin has been shown to be a potent inhibitor of pentagastrin stimulated secretion in vivo in the cat and dog (Gomez-Pan et al., 1975; Barros D'Sa et al., 1975) and feeding- and cholinergic-stimulated secretion in the dog (Konturek et al., 1976). Histamine-stimulated secretion has been reported to be more resistant to somatostatin inhibition in the dog (Thomas 1980; Konturek et al., 1976; Hummelt et al., 1977).

Thus, somatostatin, like 5-HT, has a similar inhibitory profile to isoprenaline in vivo, and thus is a candidate for a possible inhibitory agent released by β-agonists in vivo, mediating their antisecretory effect. It is not known whether somatostatin

inhibits isoprenaline stimulated secretion *in vitro*, and further experiments are needed to clarify this question.

(g) Conclusion

It is unlikely that the mechanism of action of isoprenaline is to interfere directly with histamine, gastrin or acetylcholine interactions in the stomach itself as no inhibitory effect of isoprenaline is demonstrable *in vitro*. From the evidence discussed, an action on gastric mucosal blood flow is excluded. The possibility of a contribution of central nervous effects of isoprenaline is acknowledged.

Two possible mechanisms of action are suggested by reports in the literature. Firstly isoprenaline may be acting by a hyperglycaemic effect. Alternatively, or additionally, isoprenaline may act through the release of one or more inhibitory agents such as somatostatin or 5-HT. This would explain its wide spectrum of antisecretory activity *in vivo*.

6.2.3 Mechanism of Action of the Potentiation of Pentagastrin-Stimulated Secretion by Propranolol

Propranolol alone potentiated the acid response to pentagastrin in the Heidenhain pouch rat. This is in agreement with the work of Lundell and Svensson (1974) in the Heidenhain pouch rat and Curwain, Holton and Spencer (1972) and Lin and Evans (1973) in the Heidenhain pouch dog. This potentiation was not seen with either practolol or butoxamine, nor with the inactive isomer of propranolol which has the same membrane stabilising action as ($\pm$) propranolol but no β-blocking activity. Thus, in the rat, the potentiating effect must be either specific for the

active isomer of propranolol or alternatively, requires simultaneous blockade of both β_1- and β_2-adrenoceptors. The lack of ability of butoxamine to potentiate pentagastrin-stimulated secretion is in contrast to the report of this effect by Lin, Evans and Spray (1973) in the dog.

It has been suggested that this potentiating action may be related to the effects of propranolol upon histamine metabolism in the stomach (Curwain, Holton and Spencer 1973). This would be consistent with the lack of effect of propranolol in potentiating histamine-stimulated secretion in the dog. (Curwain et al., 1974; Gottrup et al., 1980). However one might have expected a similar finding in the isolated stomach but this study has shown that propranolol is without effect on pentagastrin-stimulated acid output.

Gottrup, Anderson and Ornsholt (1979) suggested that there is a vagal tonus on acid secretion, counterbalanced by tonic adrenergic activity. This balance would be disturbed in Heidenhain pouch animals. The unopposed adrenergic activity in the absence of the vagus should therefore result in a decreased acid response to gastrin. This effect has been demonstrated in the Heidenhain pouch rat (Svensson 1970) and the vagotomised gastric fistula dog (Gottrup et al., 1980). Thus β-blockade with propranolol would, according to this theory augment pentagastrin-stimulated secretion. In the vagotomised gastric fistula dog propranolol returned pentagastrin-stimulated acid output to prevagotomy level. This led Gottrup and co workers to suggest that the parietal cells were subject to sympathetic tone, and that propranolol returned the balance after vagotomy. This argument is persuasive, however, in the rat this cannot be the case, for the following reason.

The study on the rat isolated stomach has shown a β-adrenergic mediated stimulation of acid secretion by the sympathetic neurotransmitter noradrenaline, which excludes the possibility of sympathetic tonic inhibitory effects directly on the parietal cell.

However it is possible that an endogenous inhibitory agent, such as somatostatin or 5-HT acts *in vivo* to restrain or modulate acid secretion. Somatostatin has been shown to be released under basal conditions from the pancreas and stomach (Schusdiazzara *et al.*, 1979). As discussed earlier there is strong evidence for a role of β-adrenoceptors in the control of somatostatin release in the dog and rat. If somatostatin is released by a β-adrenergic mechanism then either circulating catecholamines or sympathetic neurotransmitter release could control somatostatin release and thus play a role in modulating acid secretion *in vivo*. Removal of this restraint by β-blockade would result in a fall in somatostatin release and an enhanced response to pentagastrin. Further evidence for this comes from chemically sympathectomised dogs which have an attenuated response of antral, fundic and pancreatic somatostatin release following the ingestion of a meal (Schusdiazzara & Unger, 1981). As somatostatin also inhibits cholinergically stimulated acid secretion, propranolol might be expected to potentiate bethanechol-induced secretion. Further experiments are needed to test this.

Since both acetylcholine and vagal stimulation results in a reduction in basal somatostatin release in the perfused rat stomach (McIntosh and Pederson, 1981), the theory of Gottrup and co workers (1979), that a balance exists between vagal and

adrenergic tonus on the parietal cell could be modified to a balance between vagal and adrenergic tonus on somatostatin release. Thus, in vagally denervated animals the unopposed adrenergic activity would result in an increase in basal somatostatin release and thus a decreased acid response to gastrin. Blockade of β-receptors would then redress this balance and return the response to gastrin to prevagotomised levels. This theory will also hold true for the Heidenhain pouch rat where propranolol potentiated the acid response to pentagastrin, if the somatostatin is predominantly released from the stomach.

Similarly this hypothesis may be applied to 5-hydroxytryptamine, with tonic release of inhibitor responsible for a tonic inhibition of acid secretion. Therefore it is possible that the potentiation of pentagastrin stimulated secretion by propranolol, shown in this study and those of other workers, is a consequence of a β-adrenergic blockade of the release of an inhibitory agent which acts as a modulator of gastric acid secretion. Since butoxamine and practolol did not show this potentiating effect it is possible that blockade of both adrenoceptors is required. Propranolol is the only β-antagonist that has been shown to have this potentiating effect and further experiments are required to determine if this effect is specific to propranolol or can be exhibited by other antagonists such as timolol or pindolol. This hypothesis would also explain the relatively weak effect of β-agonists on histamine induced acid secretion, and would predict that propranolol would potentiate bethanechol-induced acid secretion.

6.2.4 General Conclusion

From the work in this study on the rat isolated stomach, and the resports of β-adrenergic stimulation of isolated parietal cells, it is concluded that there are β-adrenoceptors present on rat parietal cells which mediate acid secretion via a cyclic AMP dependent mechanism. Therefore the inhibition of acid secretion demonstrated in vivo in the rat must be an indirect effect. The lack of effect of α-agonists in vitro supports the conclusion that α-agonists produce their inhibitiory effect in vivo by a reduction in gastric mucosal blood flow.

Both β-agonists and the naturally occurring catecholamines stimulated acid secretion. Thus if β-receptors are involved in the physiological control of acid secretion they may be stimulated by catecholamines from two sources; noradrenaline from sympathetic nerve endings or adrenaline from the adrenal medulla. During stress it would be advantageous if acid secretion could be switched off. Thus a large increase in circulating catecholamines could achieve this, if it stimulated the release of an endogenous antisecretory agent such as 5-HT or somatostatin. This would not only inhibit acid secretion due to vagal stimulation and gastrin, but also overcome β-adrenergic stimulated acid secretion during "fight or flight" conditions. As discussed earlier somatostatin or 5-HT release may be controlled by a balance between adrenergic and cholinergic influence. Under conditions of stress the adrenergic tonus would predominate, and thus cause the required inhibition of acid secretion.

The question remains as to the function of the β-receptors on rat parietal cells. The evidence suggests that parietal cells of rats have adenyl-cyclase coupled β-receptors which, when activated by β-adrenoceptor agonists, including noradrenaline,

stimulated acid secretion. This suggests that there is a possible role for the sympathetic nervous system in the regulation of acid secretion. The role of the parasympathetic nervous system in the control of acid secretion has long been established as it is easy to achieve complete vagotomy due to the focal nature of parasympathetic innervation. However, the sympathetic nervous system is diffuse and it is doubtful whether it is ever possible to achieve complete sympathectomy of the gastric mucosa, thus the possible role of the sympathetic nervous system in acid secretion has never been completely clarified. Obviously intravenous infusions of catecholamines can never mimic any local neurally mediated sympathetic effects which may be present. However, there now seems sufficient evidence to prompt a re-examination of the possible influence of the sympathetic nervous system in the regulation of gastric acid secretion.

In conclusion, a dual mechanism of β-adrenoceptor agonists on gastric secretion in the rat is proposed, with a direct stimulatory effect on the stomach masked by an indirect inhibitory effect *in vivo*. This might explain the conflicting results of β-adrenoceptor agonist studies on gastric acid secretion in the literature since the effects observed would depend on the balance between inhibitory and stimulatory actions, which might vary with species, anaesthesia, choice of drug and route of administration. A proposed model of this duel effect is shown in Figure 46, and further experiments which may help to prove or disprove this are discussed below.

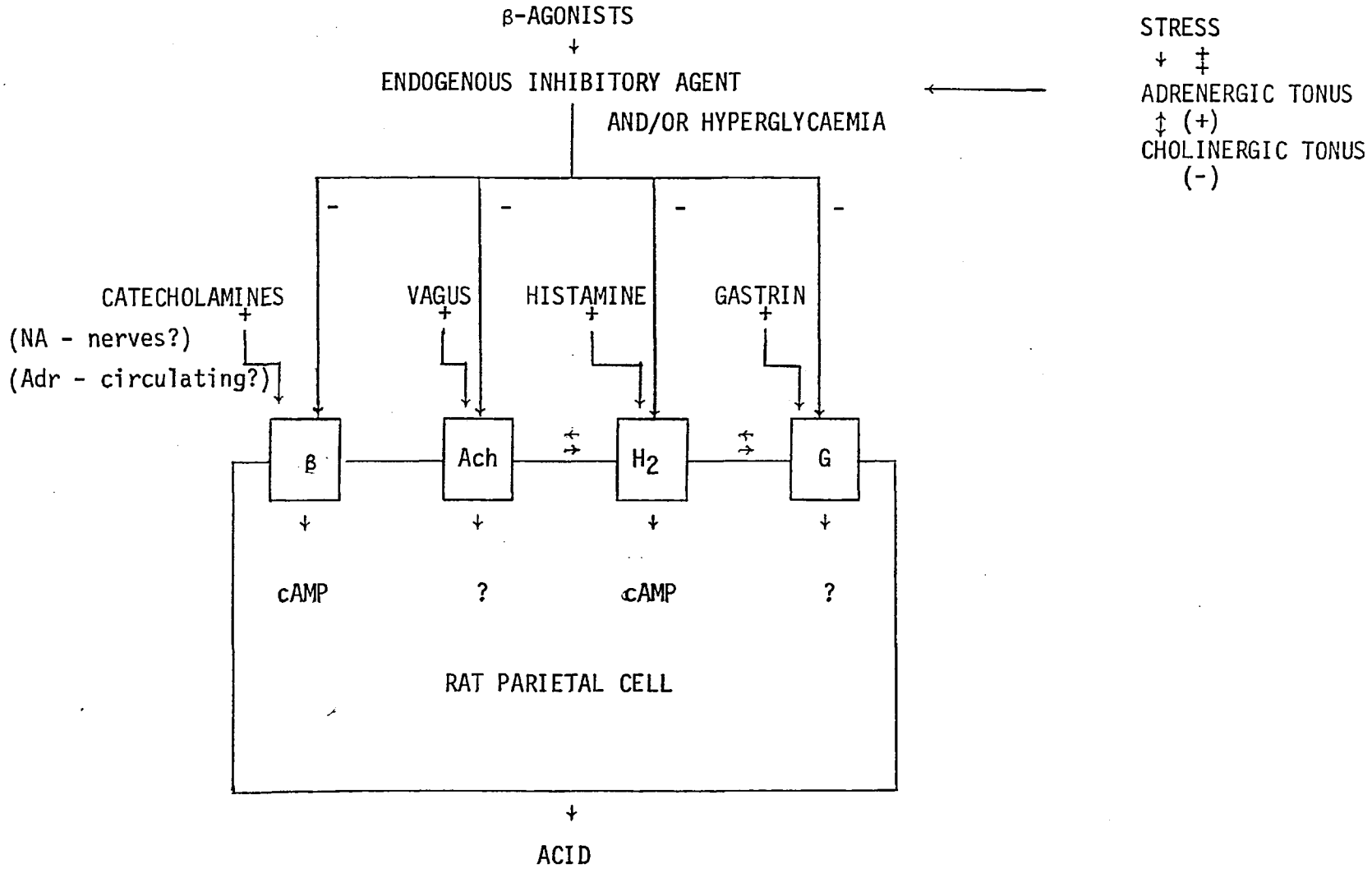


Figure 46: Proposed model for dual effects of β-agonists on gastric acid secretion in the rat (NA = noradrenaline, Adr = adrenaline)

6.3 FURTHER EXPERIMENTS

Answers to the following questions would provide evidence for or against the suggestions put forward in the discussion of this study.

6.3.1 Mechanism of Action of Isoprenaline In Vivo

(a) Hyperglycaemia

(i) Do blood glucose levels rise during an infusion of a dose of isoprenaline which inhibits pentagastrin-induced acid secretion?

(ii) Does an infusion of glucose which produces a similar blood glucose level to that seen during isoprenaline infusion, produce an equivalent antisecretory effect?

(iii) If glucose infusion inhibits pentagastrin-stimulated secretion, can this effect be blocked by β-antagonists?

(b) 5-HT

(i) Does a 5-HT antagonist block the antisecretory effect of isoprenaline? This would be expected if isoprenaline is producing its effect by releasing 5-HT.

(c) Somatostatin

(i) Does blood somatostatin-like immunoreactivity rise during isoprenaline infusion?

(ii) If this effect is seen, can it be reduced by β-adrenoceptor antagonists?

(iii) Does an infusion of somatostatin which produces similar blood levels as seen during isoprenaline infusion, produce a similar antisecretory effect?

6.3.2 Potentiation of Pentagastrin-Stimulated Secretion by Propranolol

(i) Is this effect specific to propranolol, or do other non-selective β-antagonists such as pindolol or timolol produce a potentiation of pentagastrin-stimulated acid secretion?

(ii) Does a 5-HT antagonist mimic this effect? This would be expected if propranolol's potentiating action is a consequence of β-adrenergic blockade of 5-HT release.

(iii) Does circulating somatostatin-like immunoreactivity fall during propranolol infusion?

(iv) Are somatostatin levels in the blood lower during propranolol potentiation of pentagastrin than during pentagastrin alone?

(v) Does propranolol potentiate bethanechol-induced gastric acid secretion?

6.3.3 Stimulation of Acid Secretion In Vivo

(i) Is it possible to unmask the direct stimulatory effect of β-agonists *in vivo*?

Examination of the literature shows that there are a few reports of stimulation by catecholamines *in vivo* in other species. Thus Christensen and Stadil (1976) showed that, in man, a low "physiological" dose of adrenaline stimulated acid secretion, whereas a higher dose had no effect. At both doses adrenaline stimulated gastrin release. Geumei and co workers (1969) have

also shown that a β-agonist nylidrin stimulated spontaneous acid secretion in man.* In the dog a significant enhancement of histamine-stimulated secretion by low doses of adrenaline has been demonstrated, both in the conscious and anaesthetised animal. (Holton, 1973, Holton and Jacoby, 1966).

If the stimulatory effect of β-agonists in the rat is neurally mediated then it may not be possible to show a stimulatory effect in vivo as β-agonists cannot be introduced locally into the gastric mucosa, and it is possible that any infusion of β-agonist will always produce an inhibitory effect.

* and nylidrin has been shown to inhibit gastric secretion in the conscious dog (Daly, Long and Stables, 1978)

APPENDIX I: DRUG DOSES USED IN REFERENCES QUOTED IN CHAPTER 2

(2.5 - 2.9)

KEY

A - Adrenaline
NA - Noradrenaline
I - Isoprenaline
DcI - Dichloroisoprenaline
Sb - Salbutomol
Sm - Salmefamol
Adr. gl. ext. - Adrenal gland extract

AUTHORS	DATE	DRUG	DOSE
Abasov and Iof (Man)	1978	I	No dose quoted
Bass and Patterson (Rat)	1967	NA	0.1 & 0.4mg kg^{-1} i.v.
		A	0.2 & 0.4mg kg^{-1} i.v.
		DCi	15 & 50ng kg^{-1} i.v.
		Pronethalol	16 & 65ng kg^{-1} i.v.
Bernstein (Man)	1952	A	0.6 - 1µg i.m.
Bruckner & Muller-Rabe (Cat)	1959	Propranolol	90mg kg s.c.
Christensen & Stadil (Man)	1976	A	5 & 50ng kg^{-1} min^{-1} i.v.
		NA	5 & 50ng kg^{-1} min^{-1} i.v.
Christensen et al. (Man)	1976	Propranolol	5µg kg^{-1} min^{-1} i.v.
Cowley and Code (Dog)	1969	A	1µg kg^{-1} min^{-1} i.v.
		NA	0.5µg kg^{-1} min^{-1} i.v.
	1970	NA	0.5µg kg^{-1} min^{-1} i.v.

AUTHORS	DATE	DRUG	DOSE
Cumming et al. (Dog)	1961	NA	1-8μg kg^{-1} min^{-1} i.v.
	1963	NA	1-8μg kg^{-1} min^{-1} i.v.
Curwain, Fielding & Russel (Dog)	1974	Sb	0.2μg kg^{-1} min^{-1} i.v.
Curwain & Holton (Dog)	1972	NA	0.05 - 2.0μg kg^{-1} min^{-1} i.v.
		I	0.02 - 20μg kg^{-1} min^{-1} i.v.
Curwain et al. (Dog)	1974	Practolol	2ng kg^{-1} i.v.
		Propranolol	0.1 & 1mg kg^{-1} i.v.
Curwain, Holton & Spencer (Dog)	1972	Sb	0.1 - 0.5μg kg^{-1} min^{-1} i.v.
		Terbutaline	0.1 - 0.5μg kg^{-1} min^{-1} i.v.
		Propranolol	1mg kg^{-1} i.v.
Curwain, Holton & Spencer (Dog)	1973	Propranolol	0.1 - 1mg kg^{-1} i.v.
Danhof & Geumei (Rat)	1972	Propranolol	1mg kg^{-1} i.v.
Daly & Stables (Dog)	1977	I	1 & 3 ng kg^{-1} min^{-1} i.v.

AUTHORS	DATE	DRUG	DOSE
Daly, Long and Stables (Dog)	1978	I	3 - 10ng $kg^{-1}min^{-1}$ i.v.
		Sb	100 & 300ng $kg^{-1}min^{-1}$ i.v.
		Propranolol	0.1mg kg^{-1} i.v.
		H 35/25	3mg kg^{-1} i.v.
		Practolol	1.0mg kg^{-1} i.v.
Debnath et al. (Rat)	1974	Propranolol	1mg & 50mg kg^{-1} i.p.
Delaney & Grim (Dog)	1965	NA	2µg kg^{-1} min^{-1} i.v.
Evans & Lin (Dog)	1970	Propranolol	0.5mg kg^{-1} i.v.
Fielding et al. (Man)	1975	Sb	0.2µg $kg^{-1}min^{-1}$ i.v.
Fjalland (Rat)	1972	I	0.01mg kg^{-1} s.c.
		Propranolol	5mg kg^{-1} s.c.
		Phenoxybenzamine	2mg kg^{-1} s.c.
Forrest and Code (Dog)	1954	A	20 - 40µg $kg^{-1}hr^{-1}$ i.v.
		NA	10 - 40µg kg^{-1} hr^{-1} i.v.

AUTHORS	DATE	DRUG	DOSE
Gottrup, Anderson & Jorgenson (Dog)	1980	Propranolol Practolol	0.5mg kg^{-1} i.v. 1.0mg kg^{-1} i.v.
Gottrup & Ornsholt (Dog)	1978	Sm	0.05 - 0.8µg $kg^{-1}min^{-1}$ i.v.
Gottrup & Ornsholt (Dog)	1979	Sm	0.05 - 0.8µg $kg^{-1}min^{-1}$ i.v.
Gottrup, Ornsholt & Anderson (Dog)	1979	Propranolol Practolol	0.5mg kg^{-1} i.v. 1.0mg kg^{-1} i.v.
Gottrup, Ornsholt & Anderson (Dog)	1980	Sm	0.2 - 0.8µg $kg^{-1}min^{-1}$ i.v.
Geumi et al. (Man)	1969	Propranolol	10mg i.v.
Geumi et al. (Man)	1972	Propranolol	10mg i.v.
Greshishkin (Dog)	1970	A	1µg $kg^{-1}min^{-1}$ i.v.
Haigh, Moffit & Steedman (Dog)	1967	A	1µg $kg^{-1}min^{-1}$ i.v.

AUTHORS	DATE	DRUG	DOSE
Haigh & Steedman (Dog)	1968	A	1.0µg $kg^{-1}min^{-1}$ i.v.
		NA	1.0µg $kg^{-1}min^{-1}$ i.v.
		Phenoxybenzamine	2.5 or 5.0mg kg^{-1} i.v. or i.a.
Harries (Dog)	1956	NA	1.2µg $kg^{-1}min^{-1}$ i.v.
Harries (Dog)	1957	I	0.75 - 2.75µg $kg^{-1}min^{-1}$ i.v.
Hess & Gundlach (Dog)	1920	Adr. gl. extract	1mg i.m.
Holland (Cat)	1980	I	32µg $kg^{-1}hr^{-1}$ i.v.
Holton (Dog)	1973	A	0.2µg $kg^{-1}min^{-1}$ i.v.
		I	0.1 - 0.5µg $kg^{-1}min^{-1}$ i.v.
Holton et al. (Dog)	1969	A	1µg $kg^{-1}min^{-1}$ i.v.
		NA	1µg $kg^{-1}min^{-1}$ i.v.
Holton & Jacoby	1966	A	1 - 4µg $kg^{-1}min^{-1}$ i.v.
Jacobson (Dog)	1970	NA	0.1 & 1.0µg $kg^{-1}min^{-1}$ i.v.

AUTHORS	DATE	DRUG	DOSE
Jacobson et al. (Dog)	1966	A	0.25 - 2.5μg $kg^{-1}min^{-1}$ i.v.
		I	1.2 & 2.4μg $kg^{-1}min^{-1}$ i.v.
Jacobson et al. (Dog)	1967	I	1 & 2μg $kg^{-1}min^{-1}$ i.v.
Konturek & Oleksy (Man)	1969	Propranolol	30, 60, 90μg kg^{-1} i.v.
Kronburg (Man)	1975	Propranolol	10mg
Leonsins & Waddel (Man)	1958	NA	0.5μg $kg^{-1}min^{-1}$
Lin & Evans (Dog)	1973	Propranolol	0.5mg kg^{-1} i.v.
Lin, Evans & Spray (Dog)	1973	Butoxamine	50μg kg^{-1} i.v.
Lundell & Svensson (Rat)	1974	I	20 & 40μg $kg^{-1}hr^{-1}$ i.v.
		Propranolol	2mg kg^{-1} i.v.
Magee (Dog)	1976	I	0.125 - 0.5μg min^{-1}
		Propranolol	25mg dog^{-1}

AUTHORS	DATE	DRUG	DOSE
MCloy et al. (Dog)	1979	Sb	0.05 - 0.2µg $kg^{-1}min^{-1}$ i.v.
		Propranolol	0.1mg kg^{-1} i.v.
Mishet et al. (Rat)	1969	I	0.125, 0.25 & 0.5mg kg^{-1} i.p.
Moll & Flint (Man)	1928	Adr. gl. extract	1mg s.c.
Nicolloff et al. (Dog)	1964	A	1µg $kg^{-1}min^{-1}$ i.v.
		NA	1µg $kg^{-1}min^{-1}$ i.v.
Okabe et al. (Dog)	1977	Propranolol	0.5, 1, 5 or 10mg kg^{-1} i.v.
Okabe et al. (Rat)	1970	Propranolol	20 & 50mg kg^{-1} s.c.
		Alprenolol	40mg kg^{-1} s.c.
Peter et al. (Dog)	1962	A	No dose given (Abstract)
Peters & Womack (Dog)	1958	A	2.5µg $kg^{-1}min^{-1}$ i.v.
Pradhan & Wingate (Dog)	1962	A	75µg kg^{-1} s.c.
		NA	75µg kg^{-1} s.c.

AUTHORS	DATE	DRUG	DOSE
Reed & Sanders (Cat)	1971	I	5µg min^{-1} c.a.
Reed & Smy (Cat)	1968	I	2 - 4µg $kg^{-1}min^{-1}$ i.v.
Reed et al. (Cat)	1973	I	5µg min^{-1} c.a.
Schoon et al. (Man)	1980	Propranolol	0.1mg kg^{-1} i.v.
		Metroprolol	0.2mg kg^{-1} i.v.
Tani & Muto (Rat)	1979	I	100µg kg^{-1} hr i.v.
Tani et al. (Rat)	1980	I	100µg kg^{-1} i.v.
		NA	25 - 100µg kg^{-1} i.v.
		A	12.5 - 50µg kg^{-1} i.v.
Thompson and Vane (Cat)	1953	A	10µg min^{-1} i.v.
Yamaguchi (Rat)	1977	NA	0.25 - 4.0mg kg^{-1} s.c.
		Phentolamine	4.0mg kg^{-1} s.c.
		Propranolol	4mg kg^{-1} s.c.
		I	0.016 - 4mg kg^{-1} s.c.

APPENDIX II: STATISTICAL ANALYSIS OF MAXIMUM RESPONSES SHOWN ON FIGURE 13

Figure 13 shows the log concentration response curves for isoprenaline, salmefamol and salbutamol. The maximum responses were similar for each drug. Mann-Whitney U tests have been performed on the values obtained at the maximum concentration for each drug, and this is shown below. From the statistical analysis it can be seen that there was no significant difference between the maximum responses obtained for salmefamol and salbutamol ($z = 0.94$, $n = 21$ and $n = 5$ respectively) or those obtained for salbutamol and isoprenaline ($U = 66.5$, $n = 5$ and $n = 12$ respectively). The maximum responses to salmefamol and isoprenaline were significantly different ($z = 2.51$, $p < 0.01$, $n = 21$ and $n = 12$ respectively).

The concentration-response curves to the three adrenoceptor agonists were not determined at the same time, and these experiments were designed to investigate whether β-agonist stimulation of acid secretion was concentration-dependent, rather than to analyse maximum responses. Although the rat isolated stomach has been found to be a good preparation for the study of gastric acid secretion, between preparation variability was found, as discussed in 3.1.13. Thus in the studies using antagonists, each series of experiments had its own control group. In order to directly compare maximum responses experiments could be performed by randomising β-agonists between groups of rats. However, for the purposes of this thesis the concentration-response curves show that β-agonists produce a concentration-dependent stimulation of acid secretion, with maximum responses which are similar, although the largest maximum response was significantly different from the smallest maximum response.

Mann-Whitney U tests were performed according to Siegel (1956) (Non-parametric statistics for the behavioural sciences, McGraw-Hill, Japan).

The following formulae were used

$$U = n_1 n_2 + \frac{n_1(n_1 + 1)}{2} - R_1$$

$$U = n_1 n_2 + \frac{n_2(n_2 + 1)}{2} - R_2$$

R_1 or R_2=sum of ranks assigned to group whose sample size is n_1 or n_2.

Large samples ($n_2 > 20$)

$$z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{(n_1)(n_2)(n_1 + n_2 + 1)}{12}}}$$

1. Salmefamol/Isoprenaline

Salmefamol		Isoprenaline	
Secretory Ratio	Rank No.	Secretory Ratio	Rank No.
2.05	20	2.56	29
1.48	3	3.19	33
3.04	31	2.32	23
2.50	28	1.81	12
2.39	25	1.70	8
1.55	4	2.10	21
1.74	10	2.40	26
1.71	9	3.07	32
1.62	6	2.33	24
2.00	19	1.93	17
1.88	14.5	1.92	16
2.13	22	2.68	30
1.64	7		271 Sum
1.76	11		
1.88	14.5		
1.85	13		
1.37	1		
2.44	27		
1.94	18		
1.47	2		
1.56	5		
	290 Sum		

$$U^1 = 21.12 + \frac{21(22)}{2} - 290 = 193$$

$$U = 21.12 + 12(13) - 271 = 59$$

$$z = \frac{59 - \frac{252}{2}}{\sqrt{\frac{252(34)}{12}}} = -2.51 \quad \text{sig} < .01$$

2. Salmefamol/Salbutamol

Salmefamol n = 21		Salbutamol n = 5	
Secretory Ratio	Rank No.	Secretory Ratio	Rank No.
2.05	18	1.70	9
1.48	3	2.25	20
3.04	26	1.50	4
2.50	23	2.52	24
2.39	21	2.53	25
1.55	5		82 Sum
1.74	11		
1.71	10		
1.62	7		
2.00	17		
1.88	14.5		
2.13	19		
1.64	8		
1.76	12		
1.88	14.5		
1.85	13		
1.37	1		
2.44	22		
1.94	16		
1.47	2		
1.56	6		
	269 Sum		

$$U^1 = 21.5 + \frac{21.22}{2} - 269 = 67$$

$$U = 21.5 + \frac{5.6}{2} - 82$$

$$= 38$$

$$z = 38 - \frac{5.21}{2} = -0.94 \text{ n/s}$$

$$\sqrt{\frac{5.21(27)}{12}}$$

3. Salbutamol/Isoprenaline

Salbutamol (n = 5)		Isoprenaline (n = 12)	
Secretory Ratio	Rank No.	Secretory Ratio	Rank No.
1.70	2.5	2.56	14
2.25	8	3.19	17
1.50	1	2.32	9
2.52	12	1.81	4
2.53	13	1.70	2.5
	36.5 Sum	2.10	7
		2.40	11
		3.07	16
		2.33	10
		1.93	6
		1.92	5
		2.68	15
			116.5 Sum

$$U^1 = 5.12 + \frac{5(6)}{2} - 36.5 = 83.5$$

$$U = 5.12 + \frac{12(13)}{2} - 116.5 = 66.5 \quad n/s$$

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INDEX OF TABLES AND FIGURES

TABLE 1 A comparison of basal and stimulated acid secretion in the isolated stomachs and mucosae of different species.

TABLE 2 Measurement of thickness of isolated stomachs.

TABLE 3 Composition of salines.

TABLE 4 The effect of β-adrenoceptor antagonists on basal acid secretion.

TABLE 5 Summary of agonist-antagonist studies.

TABLE 6 The effect of metiamide (10^{-4}M) and atropine (10^{-5}M) on basal acid secretion.

TABLE 7 Secretory responses obtained in response to an infusion of pentagastrin (20μg $kg^{-1}h^{-1}$) in each of the Heidenhain pouch rats used in this study.

FIGURE 1 Mean resting secretion of 6 stomachs for 5 hours.

FIGURE 2 Diagrammatic representation of experimental apparatus used in studies on the rat isolated stomach.

FIGURE 3 Diagrammatic representation of measurement of acid secretion.

FIGURE 4 Mean titration curve of six secreting stomachs compared with titration curve from mucosal saline + HCl.

FIGURE 5 Diagrammatic representation of a Heidenhain pouch.

FIGURE 6 Experimental protocol for Heidenhain pouch rats.

FIGURE 7 Effect of a period of stress during an experiment on one Heidenhain pouch rat.

FIGURE 8 The effect of salmefamol on pentagastrin-induced acid secretion in one rat isolated stomach.

FIGURE 9 The effect of salmefamol on bethanechol-induced acid secretion in one rat isolated stomach.

FIGURE 10 The effect of salmefamol on gastric acid secretion in one rat isolated stomach.

FIGURE 11 The effect of salbutamol on gastric acid secretion in one rat isolated stomach.

FIGURE 12 The effect of isoprenaline on gastric acid secretion in one rat isolated stomach.

FIGURE 13 Drug concentration on log scale plotted against secretory ratio.

FIGURE 14 The effect of timolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 15 The effect of propranolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 16 The effect of propranolol (2×10^{-5}M) on the response to isoprenaline compared with the response in the absence of antagonist.

FIGURE 17 The effect of pindolol (10^{-4}M) and timolol (10^{-4}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 18 The effect of pindolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 19 The effect of pronethalol (4×10^{-4}M and 2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 20 The effect of propranolol (2×10^{-5}M) and timolol (2×10^{-5}M) on the secretory ratio obtained with salmefamol (5×10^{-6}M).

FIGURE 21 The effect of propranolol (2×10^{-5}M) on the response to salmefamol compared with the response in the absence of antagonist.

FIGURE 22 The effect of butoxamine (2×10^{-5}M and 10^{-4}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 23 The effect of butoxamine (2×10^{-5}M and 10^{-4}M) on the secretory ratio obtained with salmefamol (1.25×10^{-6}M).

FIGURE 24 The effect of $H_{35/25}$(10^{-4}M) on the secretory ratio obtained with salmefamol (5×10^{-6}M).

FIGURE 25 The effect of practolol (2×10^{-5}M and 10^{-4}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 26 The effect of practolol (2×10^{-5}M) on the secretory ratio obtained with salmefamol (5×10^{-6}M).

FIGURE 27 Noradrenaline concentration on log scale plotted against secretory ratio.

FIGURE 28 β-agonist concentration on log scale plotted against secretory ratio.

FIGURE 29 The effect of timolol (2×10^{-5}M), propranolol (2×10^{-5}M) and pindolol (2×10^{-5}M) on the secretory ratio obtained with noradrenaline (5×10^{-6}M).

FIGURE 30 The effect of practolol (2×10^{-5}M and 10^{-4}M) on the secretory ratio obtained with noradrenaline (5×10^{-6}M).

FIGURE 31 The effect of butoxamine (2×10^{-5}M) on the secretory ratio obtained with noradrenaline (5×10^{-6}M).

FIGURE 32 The effects of atropine and metiamide on the secretory ratios obtained with salmefamol (5×10^{-6}M), isoprenaline (1.25×10^{-6}M), noradrenaline (5×10^{-6}M), bethanechol (1.7×10^{-5}M), histamine (5.4×10^{-5}M) and pentagastrin (2.2×10^{-7}M).

FIGURE 33 The effects of propranolol (2×10^{-5}M) on the responses to pentagastrin (5×10^{-7}M) and bethanechol (1.7×10^{-5}M).

FIGURE 34 The effect of (±) propranolol (2×10^{-5}M) and (+) propranolol (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 35 Phenylephrine and methoxamine concentration on log scale plotted against secretory ratio.

FIGURE 36 The effect of phenylephrine (2×10^{-5}M) on the responses to pentagastrin (2.17×10^{-7}M), bethanechol (1.7×10^{-5}M) and histamine (5.4×10^{-5}M).

FIGURE 37 The effect of phentolamine (2×10^{-5}M) on the secretory ratio obtained with isoprenaline (1.25×10^{-6}M).

FIGURE 38 The effect of phentolamine (10^{-4}M) and thymoxamine (10^{-4}M) on the secretory ratio obtained with noradrenaline (5×10^{-6}M).

FIGURE 39 Acid secretory responses to an intravenous infusion of pentagastrin in two Heidenhain pouch rats.

FIGURE 40 Acid secretory responses to an intravenous infusion of pentagastrin in two Heidenhain pouch rats.

FIGURE 41 The effect of isoprenaline infusion ($40\mu gkg^{-1}h^{-1}$) on the response to pentagastrin infusion ($20\mu gkg^{-1}h^{-1}$) alone and in the presence of intravenous injections of propranolol, practolol or butoxamine.

FIGURE 42 Effects of acid secretion produced by intravenous infusion of pentagastrin ($20\mu gkg^{-1}h^{-1}$) alone, and in the presence of of isoprenaline ($40\mu gkg^{-1}h^{-1}$), isoprenaline + practolol (8mgkg i.v.) and isoprenaline + propranolol ($2mgkg^{-1}$ i.v.) in one Heidenhain pouch rat.

FIGURE 43 The effects of intravenous injection of propranolol, practolol, butoxamine and (+) propranolol on the response to an infusion of pentagastrin.

FIGURE 44 Effects on acid secretion produced by an intravenous infusion of pentagastrin ($20_{\mu}g\ kg^{-1}h^{-1}$) alone and in the presence of practolol ($8mg_{\mu}kg^{-1}$) propranolol ($2mg_{\mu}kg^{-1}$), and butoxamine ($8mg\mu kg^{-1}$) in one Heidenhain pouch rat.

FIGURE 45 Effects on acid secretion produced by an intravenous infusion of pentagastrin (20 g $kg^{-1}h^{-1}$ alone and in the presence of (+) propranolol (2mg kg^{-1}) in one Heidenhain pouch rat.

FIGURE 46 Proposed model for dual effects of β-agonists on gastric acid secretion in the rat .

PLATE 1 Experimental apparatus used in Heidenhain pouch rat experiments showing Bollman restraining cages in the foreground and peristaltic pumps behind the cages.

Salmefamol: inhibitor or stimulant of gastric acid secretion?

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β_2-Adrenoceptor agonists have been shown to inhibit pentagastrin-induced gastric acid secretion in both dog (Curwain, Holton & Spencer, 1972; Daly & Stables, 1977) and rat (Lundell & Svensson, 1974). We have investigated the action of a drug of this type, salmefamol, on gastric acid secretion and mucosal blood flow (MBF) in 4 conscious dogs with well-established Heidenhain pouches and also in a rat isolated stomach preparation. The results showed the expected inhibition of secretion in the dog but there was a stimulation of acid output by salmefamol in the *in vitro* preparation.

Submaximal doses of either pentagastrin (2μg kg^{-1} h^{-1}) or bethanechol chloride (1μg kg^{-1} min^{-1}) were infused i.v. in saline (1 ml/min) in the dogs until a plateau of acid secretion was obtained. MBF was estimated by radioactive aniline clearance (Curwain & Holton, 1973). Salmefamol (0.2 or 0.8 μg kg^{-1} min^{-1} i.v. for 30 min) was tested against each secretagogue in each of the 4 dogs. The control rates of acid secretion and MBF were taken as the mean values for the 4 successive 15 min periods immediately before giving salmefamol. The means of the 2 successive 15 min samples giving the lowest rate of acid secretion were used as test values. Secretory rate was profoundly decreased (between 33 and 98%, mean 76%) in each of the 8 experiments. Changes in MBF were variable but in each experiment the ratio of blood flow to secretion increased.

The isolated stomachs from rats (35–50 g) were set up as described for the guinea pig by Holton & Spencer (1976) and were stimulated with either bethanechol chloride (1.7×10^{-5}M) or pentagastrin (2×10^{-5}M). The acid output was measured over four 15 min periods. The drug was then washed out and the tissues incubated for 1 h with salmefamol (10^{-5}M) before a second dose of the same stimulant was added in the presence of salmefamol. The results are expressed as the percentage increase above the spontaneous secretory rate in the absence of drugs.

The mean increase in acid output due to the pentagastrin alone was 185 ± 4.7% and 299 ± 15.6% in the presence of pentagastrin and salmefamol (s.e., $n = 3$). The values for bethanechol were 276 ± 88% and 356 ± 72% ($n = 3$) respectively. The results do not show any evidence of inhibition of the secretory response. Paired t tests indicated that the increase in the response in the presence of salmefamol was significant both for bethanechol ($P<0.05$) and pentagastrin ($P<0.02$). Salmefamol alone (10^{-5}M) increased the rate of acid secretion by 231 ± 19% ($n = 6$).

The concentration dependence of this salmefamol-stimulated acid secretion and the action upon it of various established inhibitors of acid output is being investigated.

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Comparative assay of histamine H_2-receptor antagonists using the isolated mouse stomach

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There is still insufficient evidence about the nature of the interaction between H_2-receptor antagonists and histamine on gastric acid secretion to argue that the inhibition of physiologically-induced acid secretion is due to blockade of histamine receptors. Bunce & Parsons (1976), using isolated rat stomachs, found a pA_2 value for metiamide equivalent to that measured in atrial and uterine tissues but the slope of the Schild plot of 0.73 was significantly different from unity. Apparently the necessary conditions for simple competitive inhibition were not met. To assess the significance of this finding we have assayed the activity of burimamide, metiamide and cimetidine on the isolated, lumen-perfused whole mouse stomach preparation (Angus & Black, 1978).

Each preparation was equilibrated with a single

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Mucosal cell turnover in the upper gut of the mouse and its modification by carbenoxolone

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In 1970 Lipkin claimed that carbenoxolone reduced the rate of mucosal cell proliferation in the acid secreting part of the mouse stomach (fundus) and increased the time these cells took to migrate to the point of exfoliation. Neither effect was apparent in duodenum or colon. This work has now been repeated and extended.

Mice received radioactive [^{3}H]-thymidine i.v. and the radioactivity remaining in the fundic, antral and duodenal mucosae was measured during the six days following this injection. A minimum of 22 mice were used to derive each value.

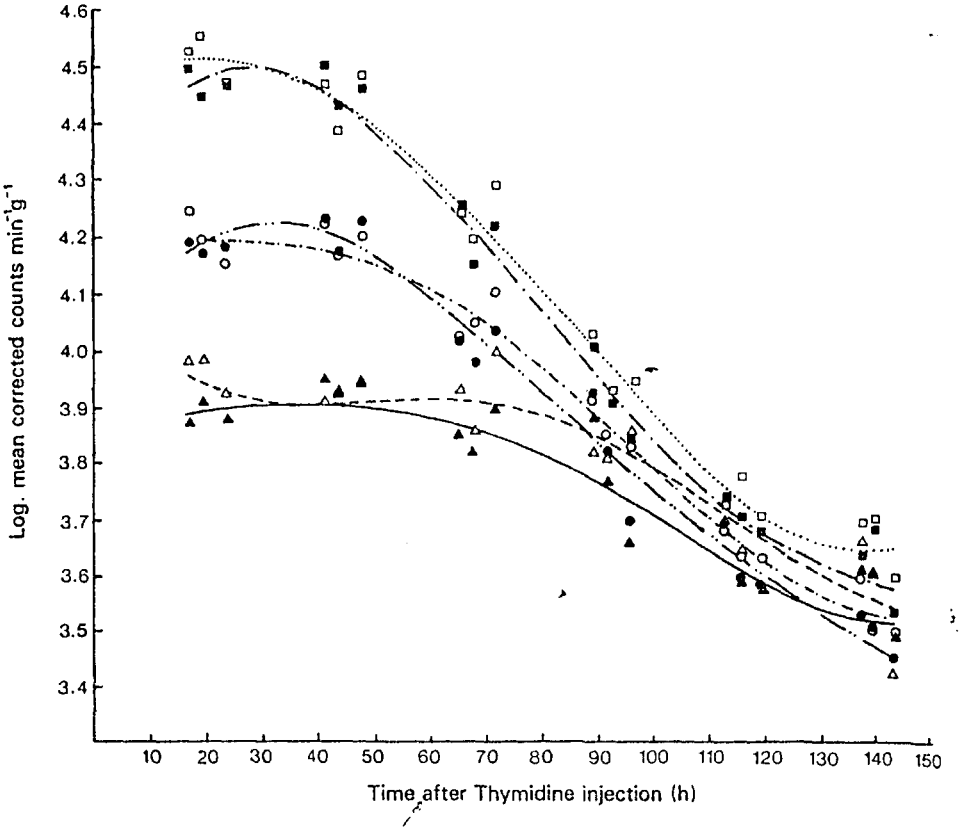


Figure 1 Mouse mucosal cell lifespan. Cells were taken from the (■) duodenal, (●) antral and (▲) fundic mucosae before (filled symbols) and after (open symbols) carbenoxolone.

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β-Adrenoceptor agonist stimulation of acid secretion in rat isolated stomach

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β-Adrenoceptor agonists inhibit pentagastrin-induced acid secretion from gastric pouches in conscious dog (Curwain, Holton and Spencer, 1972; Daly & Stables, 1977) and rat (Lundell and Svensson, 1974). Salmefamol, a β_2-adrenoceptor agonist inhibits bethanechol-induced acid secretion (Canfield, Curwain, King & Price, 1978) whilst in high doses the related drug, salbutamol will inhibit histamine-stimulated secretion in the dog (Daly, Long & Stables, 1978). We have recently shown that salmefamol does not inhibit acid secretion stimulated by pentagastrin, histamine or bethanechol in the rat isolated stomach (Canfield *et al.*, 1978), but appears to act as a stimulant of secretion. The present results extend this finding to include salbutamol and isoprenaline and the action of various antagonists on this stimulation by β-adrenoceptor agonists of acid secretion in the isolated stomach.

The rat stomach preparations were set up as described for the guinea pig by Holton & Spencer, 1976. All drugs were applied to the serosal bathing fluid. When required the tissues were pre-incubated with an inhibitor for one hour before addition of the β-adrenoceptor agonist for a further 60–90 minutes. Each tissue was exposed to only one concentration of salmefamol, salbutamol or isoprenaline, and the acid output was measured over fifteen-min periods. The change in rate of secretion is expressed as the ratio of the rate at the plateau of response to the average rate in the two control periods in the same tissue immediately before exposure to the agonist. The average control secretion in one series was 3.39 ± 0.190 μmol H^+ $cm^{-2}h^{-1}$ (s.e. mean, $n = 36$).

Both salmefamol (0.2–20 μM) and isoprenaline (0.06–6 μM) caused a concentration-dependent increase in the rate of acid secretion. The mean maximum observed value for the secretory ratio with salmefamol was 1.90 ± 0.09 ($n = 21$) and with isoprenaline 2.33 ± 0.28 ($n = 12$). Although the full concentration response curve remains to be investigated, salbutamol (0.1–20 μM) also caused an increase in acid secretion.

The responses to isoprenaline (1.25 μM) or salmefamol (5 μM) were not reduced by atropine (10 μM), metiamide (100 μM) or practolol (20 μM). However, propranolol (20 μM) did cause a significant decrease in the secretory ratio for both salmefamol and isoprenaline (*t*-test $P < 0.01$ in each case; $n = 12$ and 10 respectively) and caused a shift in the salmefamol concentration–response curve to the right with no change in slope of the linear region.

These results confirm that β-adrenoceptor agonists stimulate acid secretion by the rat isolated stomach. This action appears not to involve either histamine or cholinergic receptors and may be mediated by β_2 receptors. The reasons for the difference in response between the isolated preparation and the intact animal remain to be investigated.

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Isoprenaline and gastric acid secretion in the rat

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We have previously shown (Canfield, Curwain & Price, 1978) that isoprenaline stimulates acid secretion by the rat isolated stomach preparation and that this response was antagonized by (±)propranolol (2×10^{-5} M) but not by either atropine (10^{-5} M) or metiamide (10^{-4} M).

This contrasts with the findings of Lundell & Svensson (1974) that isoprenaline alone had no effect on acid secretion by conscious Heidenhain pouch rats but did inhibit the secretory response to pentagastrin. We have set out to confirm the work of Lundell and Svensson and compare the receptors involved both in *vivo* and *in vitro* by the use of a number of β-adrenoceptor antagonists.

The isolated stomach was set up as described previously (Canfield *et al.* 1978), drugs were added to the serosal bathing fluid and tissues were pre-incubated with antagonists for 1 hr where appropriate before addition of isoprenaline. The rate of acid secretion is expressed as the ratio of the stimulated to the spontaneous rate in each stomach.

Heidenhain pouch rats were set up in Bollman-type restraining cages, food having been withheld for 24 hr. Isoprenaline and pentagastrin were administered by continuous intravenous infusion (2 ml. hr^{-1}) and antagonists by I.V. injection, both via a tail vein catheter inserted under light halothane anaesthesia prior to setting up the animal. The pouch was continuously perfused with 0·9% saline (6 ml. hr^{-1}) which was collected by gravity drainage and titrated to pH 7·0 at 15 min intervals. The secretory rate is expressed as the percentage change in response compared with pentagastrin alone in the same animal.

The response of the isolated stomach to isoprenaline ($1{\cdot}25 \times 10^{-6}$ M) was significantly reduced ($P < 0{\cdot}05$ in each case) by (±)propranolol (2×10^{-5} M), timolol (2×10^{-5} M) and pindolol (10^{-4} M). No significant inhibition was seen with (+)-propranolol (2×10^{-5} M), pronethalol, practolol or butoxamine (all up to 10^{-4} M).

In the Heidenhain pouch rats, isoprenaline (40 μg kg^{-1} hr^{-1}) infusion begun 30 min before pentagastrin (20 μg kg^{-1} hr^{-1}) reduced the response to 37·5 ± 8·4% (S.E. of the mean, $n = 4$) of that due to pentagastrin alone. This inhibitory effect of isoprenaline was antagonized by propranolol (2 mg kg^{-1}), practolol (8 mg kg^{-1}) and butoxamine (8 mg kg^{-1}). The respective mean responses with S.E. of the mean ($n = 4$ in each case) were; 152 ± 18·2%, 74·0 ± 7·0% and 110 ± 7·6%.

These results confirm the different actions of isoprenaline on acid secretion in the rat *in vivo* and *in vitro* and suggest that different receptors may be involved in the two types of response.

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Protection against aspirin-induced gastric lesions in the rat by the H_2-receptor antagonists ranitidine and cimetidine

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Conflicting evidence has been reported for the ability of cimetidine to reduce the formation of aspirin-induced gastric lesions in rats dosed orally with exogenous acid. Carmichael, Nelson & Russell (1978) concluded that cimetidine was ineffective, whereas Guth, Aures & Paulsen (1979) found cimetidine to be effective in this type of test. In view of this situation it is now appropriate to report the effects of the newer H_2-receptor antagonist ranitidine on aspirin-induced lesions, in the absence or presence of exogenous acid, in the rat.

In control experiments groups of at least 10 female rats (Hooded Lister, 100–120 g: received 300 mg/kg aspirin (suspended in 0.5% w/v carboxymethylcellulose, 5 ml/kg) orally. The rats were sacrificed after 5 h, the stomachs removed, and the petechial lesions that occurred in the glandular region of the gastric mucosa were counted. Other groups of rats received either oral ranitidine (0.3, 1, 3 or 10 mg/kg) or cimetidine (3, 10 or 30 mg/kg) 15 min prior to aspirin administration. Both ranitidine and cimetidine produced a dose-related inhibition of lesion formation, the respective oral ED_{50} values (95% confidence limits) being 0.78 (0.56–1.05) and 3.93 (2.67–5.47) mg/kg, showing ranitidine to be five times more potent than cimetidine (seven times more potent on a molar basis) in this respect.

A second series of experiments was carried out to determine the effect of ranitidine on aspirin-induced lesion formation in the presence of exogenous acid. In these experiments both ranitidine (3, 30 or 300 mg/kg) and aspirin were administered orally in 160 mM HCl, and further doses of 160 mM HCl (5 ml/kg) were given at 1, 2, 3 and 4 h after aspirin. The rats were killed after 5 hours. Under these conditions ranitidine was still effective, but the ED_{50} value of 23.22 (14.07–37.97) mg/kg p.o. was higher than that obtained in the absence of exogenous acid.

In the final series of experiments the effect of parenterally administered ranitidine (0.3 and 3 mg/kg s.c.) on aspirin-induced lesion formation was investigated. The ED_{50} values for ranitidine against aspirin-induced lesion formation both in the absence and presence of exogenous acid were respectively 1.06 (0.68–1.69) and 1.65 (0.82–6.21) mg/kg s.c. These experiments show that the effect of ranitidine on aspirin-induced lesions formed in the presence of exogenous acid can be influenced by its route of administration.

Thus it can be concluded that the H_2-receptor antagonists ranitidine and cimetidine protect against the formation of aspirin-induced lesions. Furthermore, the effectiveness of ranitidine in the presence of exogenous acid shows that this protection is at least in part through an H_2-receptor mediated mechanism other than the inhibition of gastric acid secretion.

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Noradrenaline and gastric acid secretion by the rat isolated stomach

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Noradrenaline reduces both gastric mucosal blood flow and acid secretion in the intact animal in a number of species by an action at α-adrenoceptors (Holton, 1973). The reduction in acid output may be secondary to the reduced blood flow or be a direct effect on the mucosal cells which have been reported to receive a sympathetic nerve supply in the rat (Costa & Gabella, 1971). We have investigated the effect of noradrenaline on acid secretion by the rat isolated stomach where complications due to cardiovascular effects are avoided.

The isolated stomach was set up as described previously for guinea-pigs (Holton & Spencer, 1976). Drugs were added to the serosal bathing fluid and

Where appropriate, tissues were pre-incubated with antagonists for 1 h prior to addition of noradrenaline. The responses are expressed as the mean secretory ratio (R) with standard error and number of observations. R was calculated as the ratio of stimulated to spontaneous secretion in each stomach.

Noradrenaline stimulated acid secretion over the range 5×10^{-7} M to 2×10^{-5} M. Neither methoxamine or phenylephrine (up to 10^{-4} M) stimulated acid output and the response to noradrenaline (5×10^{-6} M, $R = 1.83 \pm 0.072$, $n = 8$) was not significantly inhibited by phentolamine or thymoxamine (up to 10^{-4} M). Respective values were $R = 1.69 \pm 0.093$, ($n = 8$); $R = 1.79 \pm 0.135$ ($n = 7$). Propranolol (2×10^{-5} M) did cause a significant inhibition of the response ($R = 1.49 \pm 0.086$, $n = 8$; $P < 0.01$).

The noradrenaline response does not appear to involve histaminic receptors or cholinoceptors as it was not inhibited by either metiamide (10^{-4} M, $R = 1.69 \pm 0.103$, $n = 6$) or atropine (10^{-5} M, $R = 1.78 \pm 0.069$, $n = 8$).

Catecholamines increase serum gastrin levels in intact rats by acting on β-adrenoceptors (Hsu & Cooper, 1977) and noradrenaline may act *in vitro* by releasing stored gastrin. The mean maximum value of R obtained with pentagastrin was 1.81 ± 0.061 ($n = 11$) and with noradrenaline $R = 2.03 \pm 0.127$ ($n = 6$); the difference between them not being significant. Straight lines were fitted to the central part of the dose response curves for pentagastrin and noradrenaline giving regression coefficients of 0.529 and 0.700 respectively. Analysis showed these not to be significantly different. Metiamide has been reported to inhibit gastrin stimulated acid secretion in the rat isolated stomach (Bunce, Parsons & Rollings, 1976) whereas we have shown it to be without effect on the noradrenaline response. We tested this apparent distinction by observing the action of cimetidine (1.3×10^{-4} M) on both pentagastrin (2.17×10^{-7} M) and histamine (5.4×10^{-5} M) in the same stomachs. With pentagastrin alone the value of $R = 1.71 \pm 0.082$ ($n = 5$) and with cimetidine $R = 1.68 \pm 0.098$ ($n = 6$). Control histamine $R = 1.58 \pm 0.106$ ($n = 5$), and with cimetidine $R = 1.12 \pm 0.050$ ($n = 6$). Thus, there was a significant inhibition of histamine but not pentagastrin in our preparation.

In conclusion, noradrenaline behaves similarly to isoprenaline (Canfield & Price, 1979) in stimulating acid secretion by an action at β-adrenoceptors in the rat isolated stomach. This does not appear to involve either histamine or cholinergic receptors but we cannot exclude the possibility that it results from the release of stored gastrin.

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Modification of radioreceptor assay for 1,25-dihydroxyvitamin D using gut cytosolic receptor from adult male or immature Japanese quail

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Recently a sensitive and highly specific radioreceptor assay for 1,25-dihydroxyvitamin D using cytosolic receptor-chromatin prepared from duodenal mucosae of egglaying Japanese quail, has been reported (Abel de la Cruz *et al.*, 1980). Unlike previous chicken radioreceptor assays for this hormone (Brumbaugh *et al.*, 1974), the quail are not fed with a rachitogenic diet prior to harvesting the mucosae.

We now report that gut mucosae from either adult male ($\simeq$6 months) or immature mixed sex (4 weeks) Japanese quail can also be used as a basis for this assay. Like the egglaying hens, these birds were fed normal Purina game bird Layena and Startena diets prior to killing. Specific ($\simeq$90%) and nonspecific binding were determined for the receptors. Receptors from both groups of birds were saturable with 1,25-dihy-

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A COMPARISON OF THE EFFECTS OF SYMPATHOMIMETIC AGENTS ON GASTRIC ACID SECRETION BY THE RAT STOMACH *IN VIVO* AND *IN VITRO*

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(*Received 14 July 1980*)

SUMMARY

1. The action of isoprenaline on gastric acid secretion in rats with Heidenhain pouches has been compared with its action in a rat isolated stomach preparation.

2. Isoprenaline (40 μg kg^{-1} h^{-1}) inhibited the acid secretion in response to pentagastrin (20 μg kg^{-1} h^{-1}) in conscious rats with Heidenhain pouches.

3. This inhibition could be abolished by propranolol (2 mg kg^{-1}) and butoxamine (8 mg kg^{-1}) and partially reversed by practolol (8 mg kg^{-1}).

4. Propranolol (2 mg kg^{-1}) significantly increased the response to pentagastrin (20 μg kg^{-1} h^{-1}) but butoxamine and practolol (both at 8 mg kg^{-1}) and the inactive isomer (+)-propranolol (2 mg kg^{-1}) were without any effect on the pentagastrin response in the rats with pouches.

5. In the rat isolated stomach preparation isoprenaline stimulated acid secretion over the range 10^{-7} M–10^{-3} M whereas phenylephrine and methoxamine were without effect.

6. Propranolol (2×10^{-5} M) inhibited this stimulatory effect of isoprenaline *in vitro* but (+)-propranolol (2×10^{-5} M), practolol and butoxamine (both at 10^{-4} M) had no effect on the response.

7. Propranolol (2×10^{-5} M) did not have any effect on the response of the isolated stomach to pentagastrin (5×10^{-7} M) or bethanechol ($1{\cdot}7 \times 10^{-5}$ M).

8. Phenylephrine (2×10^{-5} M) did not affect the *in vitro* responses to pentagastrin ($2{\cdot}17 \times 10^{-7}$ M), bethanechol ($1{\cdot}7 \times 10^{-5}$ M) or histamine ($5{\cdot}4 \times 10^{-5}$ M).

9. It is concluded that isoprenaline has a direct stimulatory effect and an indirect inhibitory effect on gastric acid secretion in the rat. Both effects involve stimulation of β-adrenoceptors. The relative predominance of one or other of these two opposing effects may help to explain the contradictory results in the literature regarding the actions of β-adrenoceptor agonists on gastric acid secretion.

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INTRODUCTION

There have been many reports on the effects of sympathomimetic drugs and their antagonists on gastric acid secretion (for reviews see: Holton, 1973; Sanders, 1976). There appears to be general agreement that α-adrenoceptor agonists inhibit acid output. The mechanism is uncertain but may be secondary to the accompanying reduction of gastric mucosal blood flow. There are contradictory reports in the literature on the action of β-adrenoceptor agonists, some indicating stimulation of acid secretion whilst others show an inhibitory effect (for additional references see Debnath, Gode, Das & Sanyal, 1974).

The interpretation of the results from experiments using intact animals is complicated because sympathomimetic drugs have such a wide spread of activity in the body. Actions on the central nervous and cardio-vascular systems may alter acid output indirectly. Laparotomy may reduce gasto-intestinal blood flow due to sympathetic vasoconstriction and alter the effects seen with β-adrenoceptor agonists compared with those seen in similar experiments with conscious animals of the same species that have a gastric pouch (Holton, 1973). The use of the β-adrenoceptor antagonist propranolol is a further complication because this drug potentiates the secretory response to pentagastrin in rat and dog (Lundell & Svensson, 1974; Curwain, Holton, McIsaac & Spencer, 1974). Studies on intact animals also do not localize the site of the adrenoceptors concerned in the acid secretory response to sympathomimetic drugs (Daly, Long & Stables, 1978).

Because of these problems we undertook a study to compare the results from conscious animals with a Heidenhain pouch with those from an isolated stomach preparation from the same species. If sympathomimetic agonists and antagonists act directly upon the stomach their effects should be demonstrable *in vitro*. Some of these results have been communicated to the British Pharmacological and Physiological Societies (Canfield, Curwain & Price, 1978; Canfield & Price, 1980).

METHODS

Heidenhain pouch experiments

Pouches were prepared in Wistar rats (250–300 g) of both sexes following the technique of Alphin & Lin (1959). A post-operative recovery period of 3–4 weeks was allowed during which time the pouches were regularly flushed with saline and the animals familiarized with the Bollman-type restraining cages used in the experiments. Animals were fed on a normal laboratory diet and had a choice of water or 0·9% saline to drink. Food was witheld for 24 h before the experiments. Isoprenaline and pentagastrin were administered by continuous I.V. infusion (2 ml hr^{-1}) and antagonists by I.V. injection, both via a catheter (Abocath T 20G) inserted in a tail vein under light halothane anaesthesia before beginning the experiment. The pouch was continuously pefused with 0·9% saline (6 ml h^{-1}) which was collected by gravity drainage and titrated to pH 7·0 with 100 mM-sodium hydroxide at 15 min intervals. The secretory rate is expressed as the percentage change in response compared with pentagastrin alone in the same animal, as the absolute rates vary with the size of individual pouches. Thus the response to pentagastrin (20 μg kg^{-1} h^{-1}) acted as the control experiment in each animal. The dose used gave about 50% of maximum response. Each animal received only a single exposure to pentagastrin on any one day following one of the four protocols below:

(i) I.V. saline 1·5 h, I.V. pentagastrin 1·5 h.

(ii) I.V. saline 1 h, I.V. isoprenaline 0·5 h, I.V. isoprenaline + pentagastrin 1·5 h.

(iii) As (ii) but β-adrenoceptor antagonist injected 10 min before infusion of isoprenaline and twice more at 30 min intervals.

(iv) As (iii) but without isoprenaline infusion.

Isolated stomach preparations

Stomachs were obtained from immature Wistar rats weighing 30–50 g which had been kept with a lactating female. Animals were killed by cervical dislocation, the stomach rapidly removed, cut along the lesser curvature, everted and washed with cold saline. The stomachs were set up in a water bath at 34 °C as described previously for the guinea-pig by Holton & Spencer (1976). All drugs were added to the buffered serosal saline (30 ml, pH 7·4) gassed with 95% oxygen/5% carbon dioxide. The unbuffered mucosal saline (5 ml) was gassed with 100% oxygen; it was replaced at 15 min intervals, its pH was measured, and titrated to pH 7 with 20 mM-sodium hydroxide. This preparation secretes acid spontaneously, the mean rate in one group of experiments being $3{\cdot}63 \pm 0{\cdot}208$ μmol H^+ cm^{-2} h^{-1} (S.E. of mean, $n = 24$). A variable proportion of the immature rat stomach preparation consists of non-secretory rumen and part of this was included within the area of the Perspex tube in some preparations where the rumen was relatively large. This reduced the area of secretory mucosa to less than 1·1 cm^2. As it was not possible to estimate accurately the size of this reduction the responses to drug addition have been expressed as the secretory ratio (R) where

$$R = \frac{\text{Average plateau response to drug}}{\text{Average spontaneous secretion}}.$$

The average spontaneous secretion was obtained from the rates for the two periods immediately before drug addition. Thus a value of $R = 1$ indicates no stimulation whilst $R = 2$ shows a doubling of the rate of secretion in the presence of the drug. Thus, the spontaneous secretion has been used as an index of the amount of secretory mucosa. Some absolute mean values of acid secretion (response − spontaneous, in μmol H^+ cm^{-2} h^{-1}) with standard errors have been included in the text to facilitate comparison of this preparation with other published work on isolated stomachs.

Control experiments showed that the spontaneous secretion did not change significantly over a 4 h period and that the antagonists were without effect on spontaneous secretion compared with control stomachs. Agonists were left in contact with the mucosa for 1 h and, where appropriate, tissues were pre-incubated with antagonist for 1 h before addition of the agonist.

Drugs

Drugs given to the rats with a pouch were diluted in sterile saline (0·9%) and all drugs were freshly made up for each experiment. The following drugs were used: isoprenaline sulphate (Macarthys); pentagastrin (Peptavlon, ICI); propranolol (Inderal, ICI); (+)-propranolol, practolol (ICI); butoxamine, methoxiamine (Burroughs Wellcome); phenylephrine (Koch-Light).

Statistics

Responses in the presence of an antagonist were compared with control values using both the Student's t test and the Mann–Witney U test. Both tests were in agreement for all the comparisons. The significance levels quoted here were obtained from the Mann–Witney test as this does not require assumptions to be made regarding the distribution of the data. All tests were carried out on the secretory ratio values.

RESULTS

Heidenhain pouch experiments

The mean rate of spontaneous acid secretion from all the animals in this study was $3{\cdot}93 \pm 0{\cdot}64$ μmol H^+ cm^{-2} h^{-1} (S.E. of mean, $n = 10$) and the mean response to pentagastrin stimulation (20 μg kg^{-1} h^{-1}) was $62{\cdot}5 \pm 7{\cdot}8$ (S.E. of mean, $n = 10$). Results for the various drug treatments are shown in Figs. 1 and 2, plotted as a percentage of the response to pentagastrin alone in the same animals. Isoprenaline (40 μg kg^{-1} h^{-1}) caused a marked reduction in the response to pentagastrin to a mean value of $37{\cdot}5 \pm 8{\cdot}4$% (S.E. of mean, $n = 4$, $P < 0{\cdot}01$) of that with pentagastrin alone.

This inhibition was reversed by propranolol (2 mg kg^{-1}), the response being $152{\cdot}5 \pm 18{\cdot}2\%$ (S.E. of mean, $n = 4$). The increased response was not statistically significant ($0{\cdot}05 > P < 0{\cdot}1$). Butoxamine (8 mg kg^{-1}) also prevented the inhibitory effect of isoprenaline, the response being not significantly different from that to pentagastrin alone ($110{\cdot}3 \pm 7{\cdot}6\%$: S.E. of mean, $n = 4$). Practolol (8 mg kg^{-1}) antagonized the isoprenaline inhibition ($P < 0{\cdot}01$) but the mean response ($74{\cdot}0 \pm 7{\cdot}0\%$: S.E. of mean, $n = 4$) was still less than that with pentagastrin alone ($P < 0{\cdot}05$).

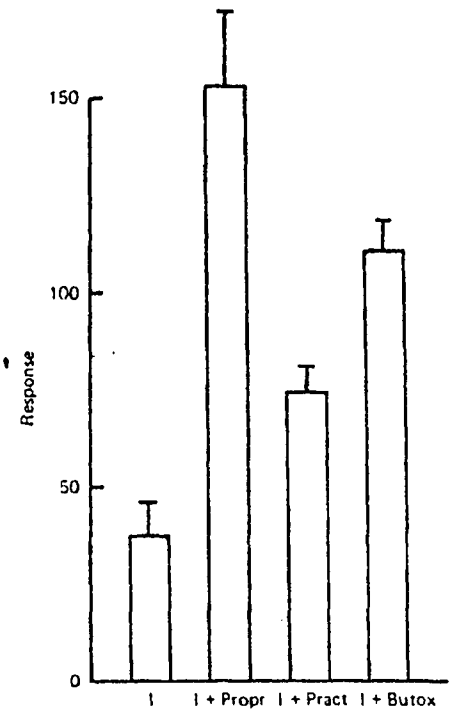


Fig. 1. Rat with a Heidenhain pouch. The effect of isoprenaline (I) infusion (40 μg kg^{-1} h^{-1}) on the response to pentagastrin infusion (20 μg kg^{-1} h^{-1}) alone and in the presence of I.V. injection of propranolol (Propr. 2 mg kg^{-1}), practolol (Pract. 8 mg kg^{-1}) or butoxamine (Butox. 8 mg kg^{-1}). Columns show means with S.E. bars. $n = 4$ in each case. The ordinate is the response as a percentage of the response to the same dose of pentagastrin alone in each animal.

Fig. 2 shows the effect of the β-adrenoceptor antagonists on the response to pentagastrin alone (20 μg kg^{-1} h^{-1}). Propranolol causes a significant increase in response ($165{\cdot}8 \pm 9{\cdot}7\%$: S.E. of mean, $n = 4$, $P < 0.01$) but neither practolol ($104{\cdot}0 \pm 4{\cdot}2\%$: S.E. of mean, $n = 4$) nor butoxamine ($106{\cdot}0 \pm 5{\cdot}5\%$: S.E. of mean, $n = 4$) caused any change. The inactive isomer (+)-propranolol also did not alter the response ($95{\cdot}2 \pm 9{\cdot}7\%$: S.E. of mean, $n = 4$).

As there appears to be no disagreement about the effect of α-adrenoceptor agonists on acid secretion *in vivo* these drugs were not studied in the animals with a pouch.

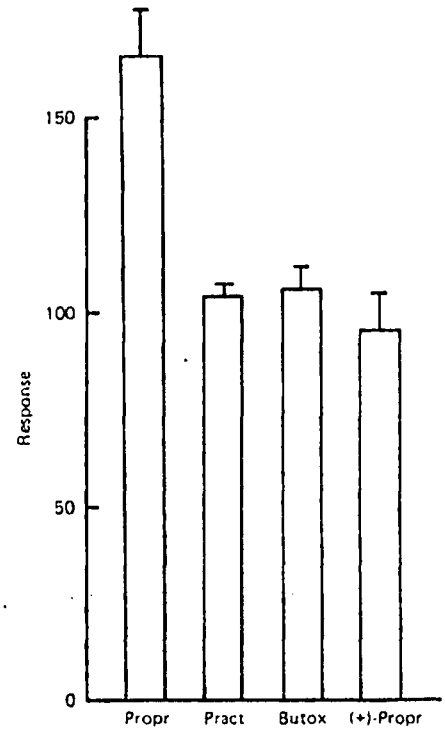


Fig. 2. Rat with a Heidenhain pouch. The effects of I.V. injection of propranolol (Propr, 2 mg kg^{-1}), practolol (Pract, 8 mg kg^{-1}), butoxamine (Butox, 8 mg kg^{-1}) or (+)-propranolol (+)-Propr, 2 mg kg^{-1}) on the response to an infusion of pentagastrin (20 μg kg^{-1} h^{-1}). Columns show means with S.E. bars. $n = 4$ in each case. The ordinate is the response as a percentage of the response to the same dose of pentagastrin alone in each animal.

Isolated stomach preparations

Log concentration–response curves for the β-adrenoceptor agonist isoprenaline and the α-adrenoceptor agonists phenylephrine and methoxamine are shown in Fig. 3. Isoprenaline caused a concentration-dependent increase in the secretory ratio (R) over the range 10^{-7} M–10^{-5} M up to an observed maximum of $R = 2{\cdot}33 \pm 0{\cdot}28$ (S.E. of mean, $n = 12$) at 3×10^{-6} M ($5{\cdot}45 \pm 0{\cdot}72$ μmol H^+ cm^{-2} h^{-1}). The α-adrenoceptor agonists had no significant effects upon acid secretion. The mean values of R were, for methoxamine $1{\cdot}05 \pm 0{\cdot}04$ (S.E. of mean, $n = 6$) ($0{\cdot}11 \pm 0{\cdot}09$ μmol H^+ cm^{-2} h^{-1}) and phenylephrine $1{\cdot}14 \pm 0{\cdot}08$ (S.E. of mean, $n = 6$) ($0{\cdot}32 \pm 0{\cdot}21$ μmol H^+ cm^{-2} h^{-1}), both drugs at a concentration of 10^{-4} M.

The remaining figures show experiments of a similar type in which each stomach received only a single exposure to the agonist and was randomly allocated to either the control or test group containing the antagonist. The chosen agonist concentrations gave about 60–70 % of maximum response in each case.

The effects of β-adrenoceptor antagonists on the response (R) to isoprenaline ($1{\cdot}25 \times 10^{-6}$ M) are shown in Fig. 4. Propranolol (2×10^{-5} M) reduced R from a control value of $2{\cdot}18 \pm 0{\cdot}10$ (S.E. of mean, $n = 22$) ($3{\cdot}9 \pm 0{\cdot}39$ μmol H^+ cm^{-2} h^{-1}) to $1{\cdot}52 \pm 0{\cdot}19$

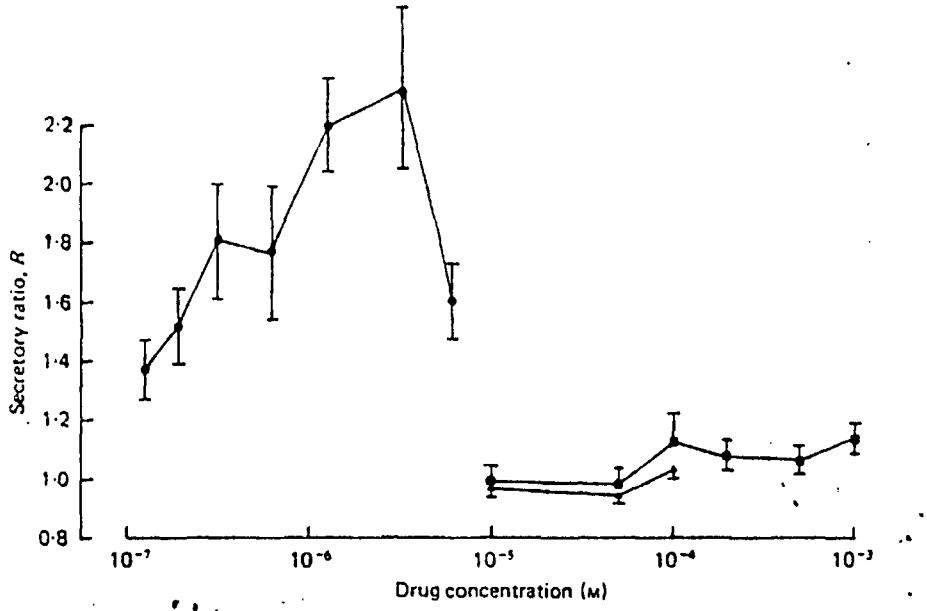


Fig. 3. Rat isolated stomach. Log drug concentration–response curves for isoprenaline (●——●), methoxamine (▲——▲) and phenylephrine (■——■). The points are mean values with S.E. bars, n at least 6 in each case.

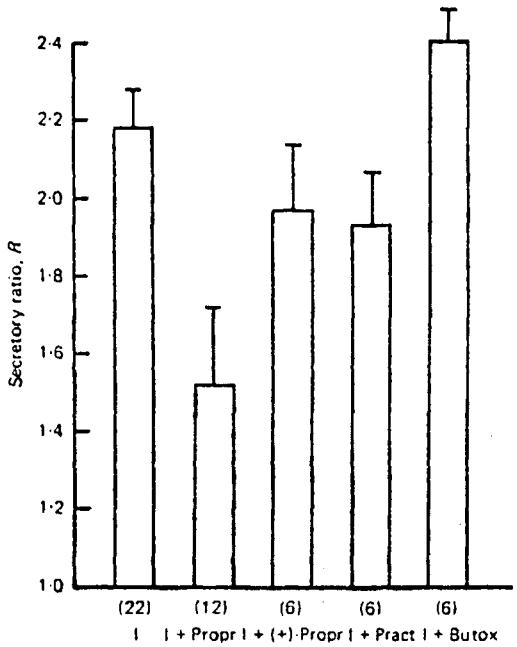


Fig. 4. Rat isolated stomach. The columns show the mean secretory ratio (R) with S.E. bars for isoprenaline (I) alone ($1{\cdot}25 \times 10^{-6}$ M) and in the presence of propranolol (Propr), (+)-propranolol ((+)-Propr) (both at 2×10^{-5} M), practolol (Pract) or butoxamine (Butox) (both at 10^{-4} M). Number of observations shown in brackets at the base of each column.

(S.E. of mean, $n = 12$, $P < 0{\cdot}01$) ($1{\cdot}75 \pm 0{\cdot}23$ μmol H^+ cm^{-2} h^{-1}). The inactive isomer (+)-propranolol, which has similar membrane stabilizing effects to the active isomer, showed no inhibitory action: $R = 1{\cdot}97 \pm 0{\cdot}17$ (S.E. of mean, $n = 6$) ($3{\cdot}63 \pm 0{\cdot}43$ μmol H^+ cm^{-2} h^{-1}). Neither practolol (10^{-4} M), with $R = 1{\cdot}93 \pm 0{\cdot}14$ (S.E. of mean, $n = 6$) ($3{\cdot}14 \pm 0{\cdot}25$ μmol H^+ cm^{-2} h^{-1}), nor butoxamine (10^{-4} M), with $R = 2{\cdot}41 \pm 0{\cdot}08$ (S.E. of mean, $n = 6$) ($4{\cdot}97 \pm 0{\cdot}35$ μmol H^+ cm^{-2} h^{-1}) caused any significant inhibition of the response to isoprenaline.

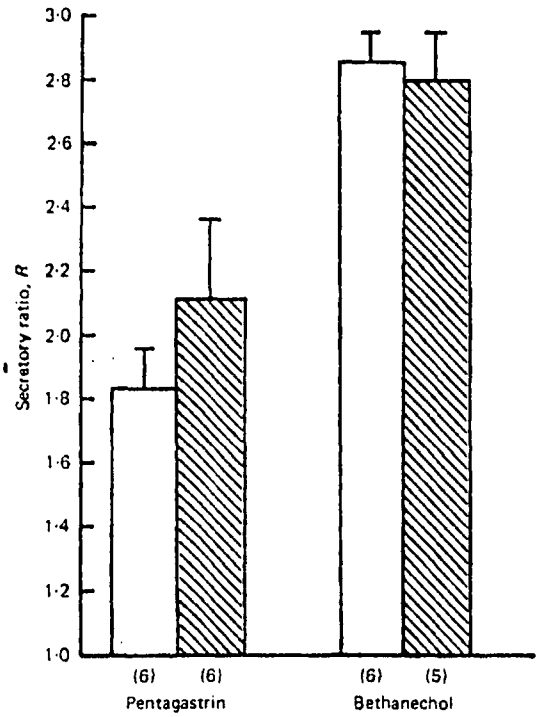


Fig. 5. Rat isolated stomach. The effects of propranolol (2×10^{-5} M) on the responses to pentagastrin (5×10^{-7} M) and bethanechol ($1{\cdot}7 \times 10^{-5}$ M) are shown by the hatched columns, control values by the open columns. The columns are mean values with S.E. bars, with the number of observations in brackets at the base of each column.

Fig. 5 shows the effect of propranolol (2×10^{-5} M) on the response (R) to pentagastrin (5×10^{-7} M) and bethanechol ($1{\cdot}7 \times 10^{-5}$ M). The control and test values for R with pentagastrin were $1{\cdot}83 \pm 0{\cdot}10$ (S.E. of mean, $n = 6$) ($3{\cdot}36 \pm 0{\cdot}27$ μmol H^+ cm^{-2} h^{-1}) and $2{\cdot}11 \pm 0{\cdot}20$ (S.E. of mean, $n = 6$) ($3{\cdot}71 \pm 0{\cdot}3$ μmol H^+ cm^{-2} h^{-1}) and with bethanechol, $2{\cdot}84 \pm 0{\cdot}08$ (S.E. of mean, $n = 6$) ($4{\cdot}89 \pm 0{\cdot}74$ μmol H^+ cm^{-2} h^{-1}) and $2{\cdot}82 \pm 0{\cdot}17$ (S.E. of mean, $n = 5$) ($4{\cdot}67 \pm 0{\cdot}74$ μmol H^+ cm^{-2} h^{-1}). Propranolol had no significant effect on the response to either secretagogue. The response to bethanechol was unusually large in this series of experiments and our experience has been that this stimulant shows larger variations between groups of animals than any of the other secretagogues which we have used.

The action of phenylephrine (2×10^{-5} M) on the response to pentagastrin ($2{\cdot}17 \times 10^{-7}$ M), histamine ($5{\cdot}4 \times 10^{-5}$ M) and bethanechol ($1{\cdot}7 \times 10^{-5}$ M) was investigated. The results are shown in Fig. 6. The mean control and test secretory ratios

together with the S.E. of the means were: pentagastrin $1{\cdot}90 \pm 0{\cdot}08$ ($n = 6$) and $1{\cdot}86 \pm 0{\cdot}13$ ($n = 5$) ($1{\cdot}76 \pm 0{\cdot}39$ and $2{\cdot}36 \pm 0{\cdot}51$ μmol H^+ cm^{-2} h^{-1}); histamine $2{\cdot}11 \pm 0{\cdot}27$ ($n = 6$) and $1{\cdot}91 \pm 0{\cdot}12$ ($n = 6$) ($2{\cdot}89 \pm 0{\cdot}54$ and $2{\cdot}33 \pm 0{\cdot}40$ μmol H^+ cm^{-2} h^{-1}); bethanechol $1{\cdot}92 \pm 0{\cdot}08$ ($n = 6$) and $1{\cdot}80 \pm 0{\cdot}07$ ($n = 6$) ($2{\cdot}53 \pm 0{\cdot}17$ and $2{\cdot}15 \pm 0{\cdot}36$ μmol H^+ cm^{-2} h^{-1}).

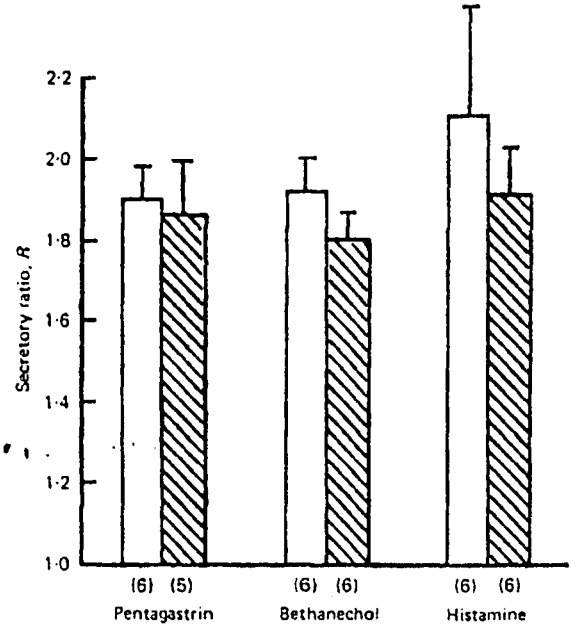


Fig. 6. Rat isolated stomach. The effects of phenylephrine (2×10^{-5} M) on the responses to pentagastrin ($2{\cdot}17 \times 10^{-7}$ M), bethanechol ($1{\cdot}7 \times 10^{-5}$ M) and histamine ($5{\cdot}4 \times 10^{-5}$ M) are shown by the hatched columns, control values by the open columns. The columns are mean values with S.E. bars, with the number of observations in brackets at the base of each column.

High concentrations of isoprenaline

Isoprenaline and adrenaline have been reported not to affect acid secretion by bullfrog isolated mucosa at concentrations up to 10^{-4} M (Thorpe, Frusco, Bass & Hug, 1971; Flemström, 1978), but isoprenaline was reported to inhibit the response to histamine at a concentration of 10^{-3} M (Thorpe *et al.* 1971). Application of 10^{-3} M-isoprenaline to the rat stomach preparation caused a significant ($P = 0{\cdot}05$) increase in the secretory ratio with a mean value of R of $1{\cdot}51 \pm 0{\cdot}06$ (S.E. of mean, $n = 6$) ($2{\cdot}24 \pm 0{\cdot}28$ μmol H^+ cm^{-2} h^{-1}) compared with a mean control value of $0{\cdot}953 \pm 0{\cdot}12$ (S.E. of mean, $n = 4$) ($0{\cdot}13 \pm 0{\cdot}34$ μmol H^+ cm^{-2} h^{-1}). Thus, the rat stomach behaves differently from the amphibian *in vitro* in respect of adrenoceptor agonists.

DISCUSSION

Heidenhain pouch experiments

Our results confirm and extend the findings of Lundell & Svensson in this preparation (1974). Isoprenaline infusion resulted in a marked inhibition of pentagastrin-stimulated acid secretio : and this effect was completely blocked by

propranolol. Additionally, the isoprenaline inhibition was reversed by the β_2-selective antagonist butoxamine and partially reversed by practolol (β_1-selective). These findings suggest an involvement of both β-receptor subtypes, a finding similar to that of Daly *et al.* (1978) in dogs with a Heidenhain pouch.

We have also confirmed the observation that propranolol alone potentiates the response to pentagastrin (Lundell & Svensson, 1974; Curwain *et al.* 1974). This potentiation was not seen with either practolol or butoxamine, nor with the inactive isomer of propranolol, which has the same membrane stabilizing action as the active isomer but no β-blocking activity. Thus, the potentiation effect must either be specific for the active isomer of propranolol or alternatively requires simultaneous blockade of both β_1- and β_2-receptors. It has been suggested that this potentiating action may be related to the actions of propranolol on histamine metabolism in the stomach (Curwain, Holton & Spencer, 1973), histamine-stimulated acid secretion being unaffected by propranolol. If this hypothesis were correct, one might expect a similar finding in the isolated stomach, but our results show propranolol to be without effect on either pentagastrin-or bethanechol-stimulated acid output. Thus, the mode of action of propranolol in potentiating the response to pentagastrin is uncertain, but it remains as a complicating factor when it is used in experiments to study the effects of sympathomimetic agents on acid secretion *in vivo*.

Gastric mucosal blood flow was not measured in these experiments. However, there is no evidence to suggest that the inhibitory action of isoprenaline is the result of a reduction in blood flow to the stomach. Lundell & Svensson (1974) reported that the dose of isoprenaline used here (40 μg kg^{-1} h^{-1}) does not alter the blood pressure in the rat whilst the ratio of mucosal blood flow to acid secretion was increased during isoprenaline inhibition in dogs with a pouch (Curwain & Holton, 1972).

Isolated stomach preparations

Isoprenaline clearly stimulated gastric acid secretion by the isolated stomach over a concentration range of 10^{-7} to 10^{-3} M. The maximum secretory ratio obtained and the slope of the central part of the curve are similar to results obtained in this preparation with histamine, pentagastrin, bethanechol and theophylline (J. E. Spencer, personal communication). The response was antagonized by propranolol at a concentration which had no effect on the response to etiher pentagastrin or bethanechol. The inactive isomer (+)-propranolol had no effect, and nor did practolol or butoxamine significantly inhibit the response. This may simply indicate that neither of these selective β-adrenoceptor antagonists was able to reach the active site *in vitro*. Alternatively the β-receptor involved may not fit easily into the β_1 and β_2 subdivision established from work on smooth muscle systems, so that a non-selective β-adrenoceptor antagonist might be effective whilst those showing subtype selectivity would not. The concentration of propranolol used is high compared with its accepted pA_2 value and this problem is discussed in more detail in the following paper (Canfield, Hughes, Price & Spencer, 1981). However, the results with the (+) isomer and the lack of effect on other secretagogues suggest that the propranolol was not having any non-specific antisecretory action.

The α-adrenoceptor agonists appear not to influence either basal acid secretion or the response to pentagastrin, histamine or bethanechol. This is perhaps surprising

in view of the report by Ruoff (1977) that α-agonists and α-antagonists can respectively alter the cyclic GMP and cyclic AMP content of rat gastric mucosa *in vivo*. However, Ruoff did not measure acid secretion in his experiments and his techniques did not localize the nucleotide changes to particular cell types. Thus, if the nucleotide changes were related to effects on pepsin or mucus secretion then they would not be apparent in our experiments where only acid secretion was measured.

Comparison of in vitro *and* in vivo *results*

Our results indicate opposing effects of isoprenaline *in vivo* and *in vitro*. The inhibition *in vivo* cannot result from a direct action on the gastric mucosa. It affects histamine-, pentagastrin-, cholinergic-and feeding-stimulated acid secretion (Lundell & Svensson, 1974; Canfield, Curwain, King & Price, 1978), suggesting a possible site of action beyond the secretagogue receptors. Our experiments *in vivo* provide no information about the inhibitory mechanism involved in the action of isoprenaline. It may act by causing the release of some inhibitory substance from elsewhere in the gastro-intestinal tract. For example, adrenaline has been reported to release serotonin from the duodenal mucosa by a β-adrenergic effect (Pettersson, 1979). It seems unlikely that its action is to interfere directly with histamine, gastrin and acetylcholine interactions in the stomach itself as no inhibitory effect of isoprenaline is demonstrable *in vitro*. Thus isoprenaline may act *in vivo* both to stimulate acid secretion as it does *in vitro* and also to inhibit it by two separate mechanisms, with the inhibitory effect predominating in the conscious dog and rat. If this dual effect is also found in other species it might explain the conflicting results of β-adrenoceptor agonist studies on gastric acid secretion. The effects observed would depend upon the balance between inhibitory and stimulatory actions, which might vary with species, anaesthesia, choice of drug and route of administration.

Our present experiments provide no information on the the possible mechanism of acid stimulation by isoprenaline *in vitro*. In other tissues β-adrenoceptor stimulation is associated with an increase in tissue cyclic AMP and this has been implicated as the intracellular mediator for histamine-stimulated acid secretion by many investigators. Thus, isoprenaline may act at β-adrenoceptors on the parietal cell to stimulate acid secretion via a cyclic-AMP-dependent mechanism. Alternatively it may act indirectly via a mechanism involving either cholinergic, histamine or gastrin receptors. We have investigated the action of sympathomimetic compounds in the *in vitro* preparation in more detail and these results are presented in the following paper (Canfield *et al.* 1981).

This work was supported by a grant from the Wellcome Trust. We would like to thank ICI Ltd and Burroughs Wellcome for gifts of drugs.

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THE ACTION OF β-ADRENOCEPTOR AGONISTS ON ACID SECRETION BY THE RAT ISOLATED STOMACH

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SUMMARY

1. The action of β-adrenoceptor agonists on acid secretion by an immature rat isolated stomach preparation has been studied.

2. Isoprenaline, salbutamol, salmefamol, adrenaline and noradrenaline all stimulated acid output over a concentration range of 2×10^{-7} M–10^{-5} M.

3. These responses were antagonized by propranolol (2×10^{-5} M), pindolol and timolol (10^{-6} M).

4. The antagonism of isoprenaline and salmefamol by propranolol was consistent with competitive inhibition.

5. Selective β-adrenoceptor antagonists (practolol, atenolol, butoxamine and ICI 118 551) caused significant inhibition of noradrenaline-stimulated secretion but not of that due to the other agonists.

6. An adult rat isolated mucosa preparation responded to adrenaline in a similar manner to the immature stomach preparation.

7. Acid secretion stimulated by β-adrenoceptor agonists was not antagonized by atropine (10^{-5} M), metiamide (10^{-4} M) or prostaglandin E_2 (10^{-5} M). The concentrations of these three antagonists caused marked inhibition of the responses to submaximal concentrations of bethanechol, histamine and pentagastrin respectively.

8. The results are discussed in relation to the possible mechanisms of action of β-adrenoceptor stimulation of acid secretion: it is concluded that the response is probably mediated by β-receptors on the parietal cell.

INTRODUCTION

In the preceding paper (Canfield & Price, 1981) we have shown that isoprenaline stimulates acid secretion by the immature rat isolated stomach preparation, in contrast to the inhibitory effect of this and similar compounds *in vivo*. In this paper we present the results of a more extensive investigation of the effects of β-adrenoceptor agonists on acid secretion *in vitro* and the action of both adrenoceptor antagonists

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and other established inhibitors of acid secretion on these responses. The results are discussed in relation to the possible mechanisms of β-adrenoceptor-stimulated acid secretion in the isolated stomach. Some of this work has been briefly communicated to the Physiological and Pharmacological Societies (Canfield, Curwain, King & Price, 1978; Canfield, Curwain & Price, 1978; Canfield & Price, 1980; Canfield, Price & Spencer, 1980).

METHODS

Isolated stomach preparations

Isolated stomach preparations were set up as detailed in the preceding paper (Canfield & Price, 1981). All drugs were added to the serosal bathing fluid and the unbuffered mucosal solution was replaced at 15 min intervals for estimation of acid secretion by titration. The response to drug addition in each stomach is expressed as the secretory ratio (R), where

$$R = \frac{\text{Average plateau response to drug}}{\text{Average spontaneous secretion}},$$

as described in the preceding paper. Some absolute values (response − spontaneous secretion, μmol H^+ cm^{-2} h^{-1}) are included in brackets in the text to facilitate comparison of the data with other published work using isolated stomachs.

Adult mucosa

Stomachs were removed from Wistar rats weighing 250–350 g and pinned out, mucosal surface down, on a moist cork board. Mucosal saline was injected between the mucosal and muscle layers to separate them and the muscle layer then dissected away. The isolated mucosal preparation was then treated in the same manner as the immature stomach.

Drugs

The following drugs were used: histamine acid phosphate, isoprenaline sulphate (MacCarthys); salbutamol, salmefamol (Glaxo Research); adrenaline (Antigen); noradrenaline (Winthrop); bethanechol chloride (Glenwood Laboratories); pentagastrin, propranolol, practolol, atenolol, ICI 118 551 (ICI Ltd); petholamine (Ciba); butoxamine (Burroughs Wellcome); pindolol (Sandoz); timolol (Merck, Sharp & Dohme); atropine sulphate (BDH Ltd); metiamide (S.K. & F. Ltd); and prostaglandin E_2 (Upjohn).

Statistics

Responses in the presence of an antagonist were compared with control stomachs using the Mann–Witney U test as in the preceding paper. Tests were performed on the secretory ratios.

RESULTS

Concentration–response curves

Fig. 1*A* shows the log concentration–response curves for the β_2-selective agonists salmefamol and salbutamol and also the isoprenaline curve from the preceding paper for comparison (Canfield & Price, 1981), whilst Fig. 1*B* shows the results with the two naturally occurring catecholamines. The maximum responses and slopes of the relationships are similar for all five drugs. The mean maximum secretory ratios with their S.E. of the means were: salmefamol $1{\cdot}90 \pm 0{\cdot}09$ ($n = 21$) ($3{\cdot}84 \pm 0{\cdot}28$ μmol H^+ cm^{-2} h^{-1}), salbutamol $2{\cdot}10 \pm 0{\cdot}21$ ($n = 5$) ($3{\cdot}61 \pm 0{\cdot}28$ μmol H^+ cm^{-2} h^{-1}), adrenaline $2{\cdot}22 \pm 0{\cdot}08$ ($n = 7$) ($3{\cdot}02 \pm 0{\cdot}44$ μmol H^+ cm^{-2} h^{-1}) and noradrenaline $2{\cdot}03 \pm 0{\cdot}13$ ($n = 6$) ($3{\cdot}19 \pm 0{\cdot}38$ μmol H^+ cm^{-2} h^{-1}). Isoprenaline was the most potent agonist and noradrenaline the least, but no uptake inhibitors were used and this may partly account for the noradrenaline results. Neither β_2-selective agonist was as potent as isoprenaline or adrenaline.

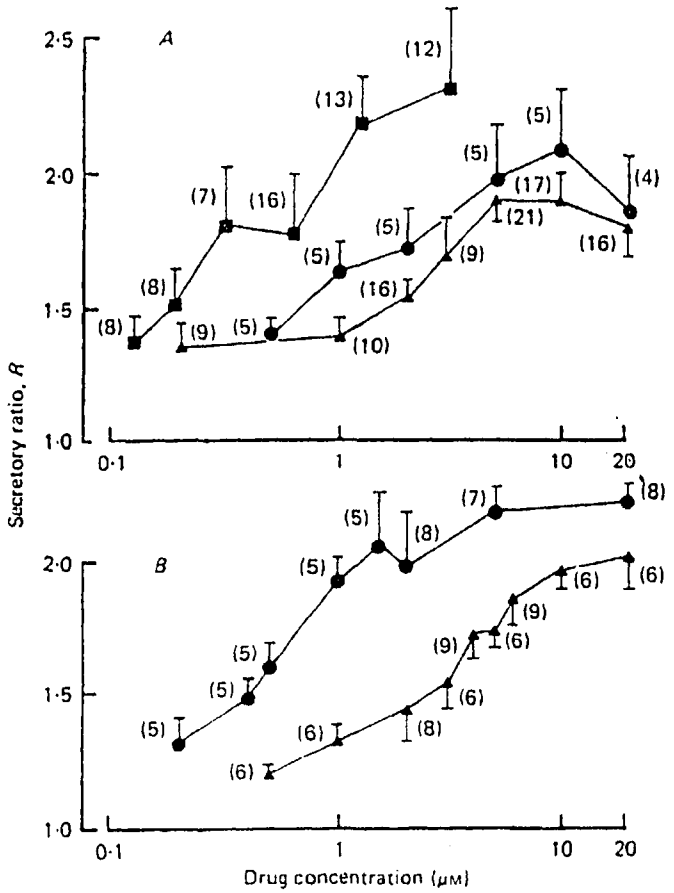


Fig. 1. Drug concentration on log scale plotted against secretory ratio. Points are mean values with S.E. bars, and numbers of observations are in parentheses. *A*, Isoprenaline (■), salmefamol (▲) and salbutamol (●). *B*, adrenaline (●) and noradrenaline (▲).

Adrenoceptor antagonists

Fig. 2 summarizes the effects of various antagonists on the responses to four of the adrenoceptor agonists. Each stomach was exposed to only one concentration of the agonists and was randomly allocated to either the test group containing the antagonist or to the control group. Not all possible agonist/antagonist pairs were studied and no antagonist studies were undertaken with salbutamol. The non-selective β-adrenoceptor antagonists caused inhibition of the response to all four agonists. The effects of the selective antagonists are less clear, with noradrenaline appearing to be consistently more susceptible to inhibition than the other agonists. For noradrenaline the control value of R was $1{\cdot}84 \pm 0{\cdot}07$ (S.E. of mean) ($3{\cdot}04 \pm 0{\cdot}32$ μmol H^+ cm^{-2} h^{-1}). In the presence of antagonists mean values of R ($\pm$S.E. of means) were: propranolol $1{\cdot}49 \pm 0{\cdot}09$ ($n = 8$) ($1{\cdot}82 \pm 0{\cdot}40$ μmol H^+ cm^{-2} h^{-1}), $2{\cdot}5 \times 10^{-5}$ M-butoxamine $1{\cdot}42 \pm 0{\cdot}05$ ($n = 13$) ($1{\cdot}41 \pm 0{\cdot}09$ μmol H^+ cm^{-2} h^{-1}) 10^{-4} M-practolol $1{\cdot}27 \pm 0{\cdot}07$ ($n = 6$) ($0{\cdot}68 \pm 0{\cdot}19$ μmole H^+ cm^{-2} h^{-1}) and 10^{-4} M-phentolamine $1{\cdot}69 \pm 0{\cdot}09$ ($n = 8$) ($2{\cdot}43 \pm 0{\cdot}34$ μmol H^+ cm^{-2} h^{-1}). This inhibitory effect of butoxamine and practolol may be compared with their lack of effect against isoprenaline described in the

previous paper (Canfield & Price, 1981). This is dealt with in more detail in the Discussion.

Fig. 3 shows the log concentration–response curve for salmefamol and a two-point curve for isoprenaline in the presence of propranolol (2×10^{-5} M). The maximum response to salmefamol in the presence of propranolol occurred at 6×10^{-5} M where

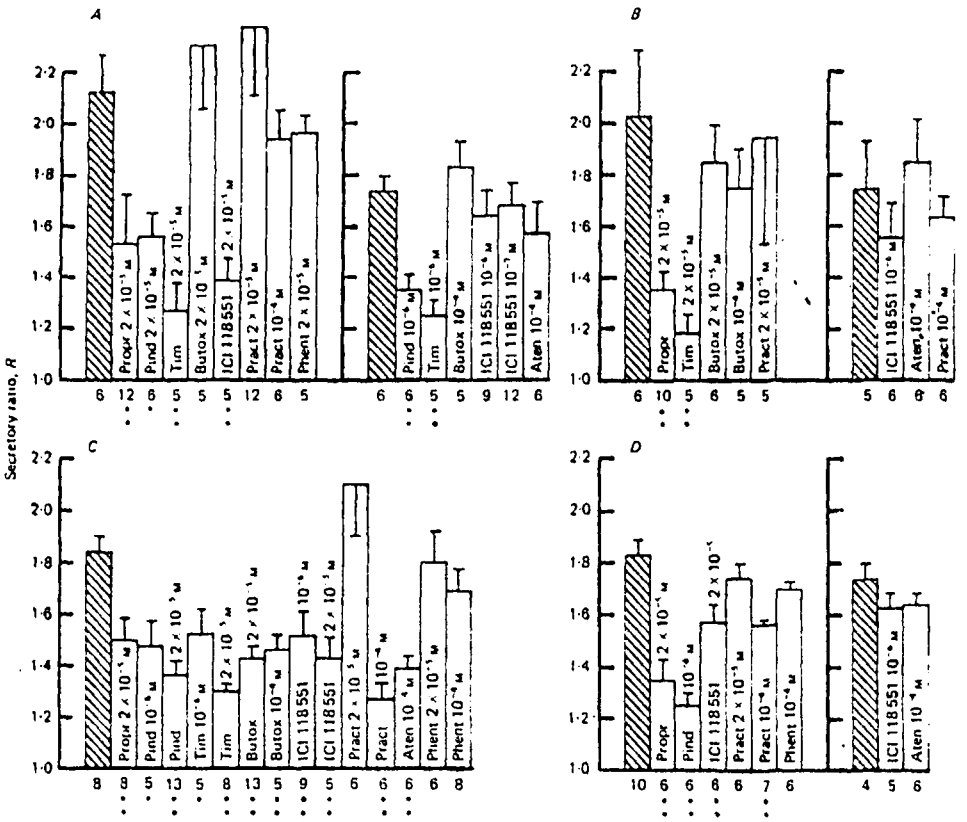


Fig. 2. The effect of various adrenoceptor antagonists on the secretory ratio obtained with four β-adrenoceptor agonists at a single submaximal concentration. Columns indicate mean values with S.E. bars, number of observations being shown at the base of each column. Statistically significant differences are shown by * = $P < 0.05$; ** = $P < 0.01$. Where agonist/antagonist interactions were studied in more than one series of experiments, the control values appropriate to each series are shown by the shaded columns. *A*, isoprenaline (1.25×10^{-6} M); *B*, salmefamol (5×10^{-6} M); *C*, noradrenaline (5×10^{-6} M); *D*, adrenaline (2×10^{-6} M). Aten, atenolol; Butox, butoxamine; Phent, phentolamine; Pind, pindolol; Pract, practolol; Propr, propranolol; Tim, timolol.

$R = 2.07 \pm 0.12$ (S.E. of mean, $n = 12$) (2.95 ± 0.28 μmol H^+ cm^{-2} h^{-1}). These results are consistent with a competitive antagonism of the response as there is no apparent change of slope and the maximum response to salmefamol is not reduced in the presence of propranolol. The α-adrenoceptor antagonist phentolamine at concentrations up to 10^{-4} M had no effect on the response to the β-adrenoceptor agonists.

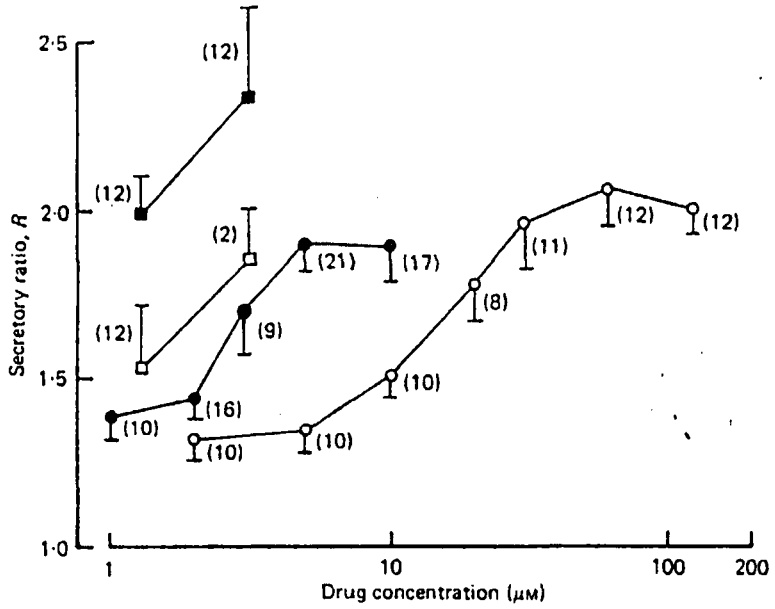


Fig. 3. The effect of propranolol (2×10^{-5} M, open symbols) on the response to isoprenaline (□) and salmefamol (○) compared with the response in the absence of antagonist (closed symbols). Points are mean values with S.E. bars. and numbers of observations are in parentheses.

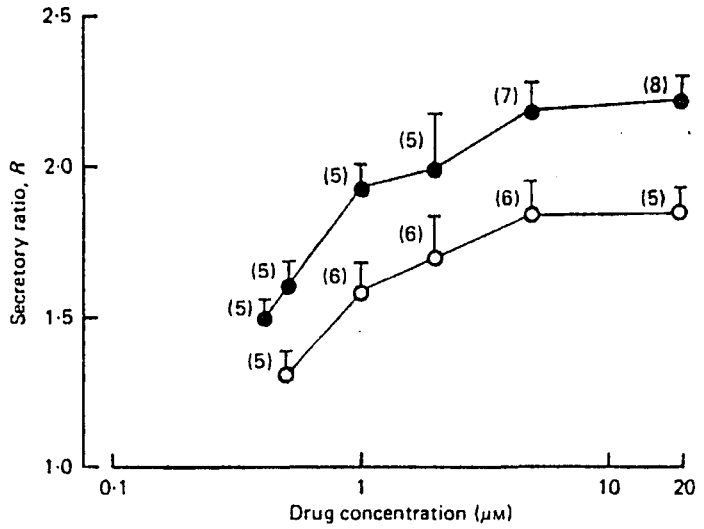


Fig. 4. Log adrenaline concentration plotted against secretory ratio for the immature stomach (●) and the adult mucosa preparation (○). Points are mean values with S.E. bars. and numbers of observations are in parentheses.

Adult mucosa

The rats used in this study were immature animals (age 15–20 days) and the sympathetic innervation of the gastro-intestinal tract is incomplete at this time (Yoshida, Taniyama & Tanaka, 1979). The whole stomach wall preparation also contains intact neural plexi. Both of these considerations might explain the results described above. We therefore undertook a few experiments with an isolated mucosa preparation from adult rats where the sympathetic innervation would be fully developed and the neural plexi substantially disrupted. The effects of adrenaline on the adult isolated mucosa are shown in Fig. 4 together with the original adrenaline curve from the immature rat preparations. The maximum R observed with adrenaline stimulation in the adult mucosa was $1{\cdot}85 \pm 0{\cdot}12$ (S.E. of mean, $n = 6$) ($1{\cdot}87 \pm 0{\cdot}50$ μmol H^+ cm^{-2} h^{-1}). Clearly adrenaline stimulated acid secretion in the adult preparation. Isoprenaline also stimulates this preparation (C. A. Price, personal communication). No further experiments were carried out with the adult mucosa as this preparation is 50% thicker than the whole stomach wall from the immature rat, as judged from the blotted weights.

Other antagonists of acid secretion

β-Adrenoceptor agonists may stimulate acid secretion in our experiments by releasing one of the endogenous secretagogues. In the intact rat, adrenaline raises serum gastrin, for example (Hsu & Cooper, 1977). We have investigated this possibility by testing the effects of atropine, metiamide and prostaglandin E_2 using the same experimental design as for Fig. 2. The results are shown in Fig. 5.

Atropine (10^{-5} M) caused a significant inhibition of the response to bethanechol ($1{\cdot}7 \times 10^{-5}$ M) but had no effect on the sympathomimetic drugs. The mean control and test ratios (±S.E. of means) with bethanechol were $1{\cdot}89 \pm 0{\cdot}05$ ($n = 5$) ($4{\cdot}07 \pm 0{\cdot}96$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}10 \pm 0{\cdot}05$ ($n = 6$) ($0{\cdot}48 \pm 0{\cdot}25$ μmol H^+ cm^{-2} h^{-1}) and with noradrenaline $1{\cdot}83 \pm 0{\cdot}07$ ($n = 8$) ($3{\cdot}03 \pm 0{\cdot}32$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}69 \pm 0{\cdot}10$ ($n = 8$) ($3{\cdot}42 \pm 0{\cdot}30$ μmol H^+ cm^{-2} h^{-1}). Bunce, Parsons & Rollings (1976) reported that metiamide inhibited the response to gastrin in the rat isolated stomach and we anticipated that the use of this H_2 antagonist would enable us to exclude the possibility of β-adrenoceptor agonists releasing either histamine or gastrin. Metiamide (10^{-4} M) inhibited the response to histamine ($5{\cdot}4 \times 10^{-5}$ M) but was without effect on the β-adrenoceptor agonist responses or the response to pentagastrin ($2{\cdot}17 \times 10^{-7}$ M). The mean control and test ratios (±S.E. of means) for the action of metiamide on histamine were $1{\cdot}58 \pm 0{\cdot}11$ ($n = 5$) ($1{\cdot}56 \pm 0{\cdot}24$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}12 \pm 0{\cdot}05$ ($n = 6$) ($0{\cdot}22 \pm 0{\cdot}08$ μmol H^+ cm^{-2} h^{-1}). With pentagastrin corresponding values were $1{\cdot}66 \pm 0{\cdot}04$ ($n = 5$) ($2{\cdot}58 \pm 0{\cdot}16$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}68 \pm 0{\cdot}05$ ($n = 6$) ($1{\cdot}97 \pm 0{\cdot}36$ μmol H^+ cm^{-2} h^{-1}). Cimetidine ($1{\cdot}3 \times 10^{-4}$ M) was also without inhibitory action on pentagastrin-stimulated secretion (unpublished observation).

As a result of this finding we extended the study to include prostaglandin E_2, which significantly inhibited pentagastrin but not the responses to isoprenaline or adrenaline. The mean control and test ratios (±S.E. of means) for the action of prostaglandin E_2 on pentagastrin were $1{\cdot}66 \pm 0{\cdot}04$ ($n = 5$) ($2{\cdot}50 \pm 0{\cdot}68$ μmol H^+ cm^{-2} h^{-1}) and $1{\cdot}36 \pm 0{\cdot}05$ ($n = 6$) ($1{\cdot}42 \pm 0{\cdot}40$ μmol H^+ cm^{-2} h^{-1}). Corresponding values for isopren-

aline were 2·02±0·18 ($n = 5$) (3·33±0·78 μmol H^+ cm^{-2} h^{-1}) and 1·88±0·08 ($n = 5$) (2·5±0·57 μmol H^+ cm^{-2} h^{-1}). It is clear from these results that acid secretion stimulated by β-adrenoceptor agonists is unaffected by any of these three potent inhibitors of other established secretagogues.

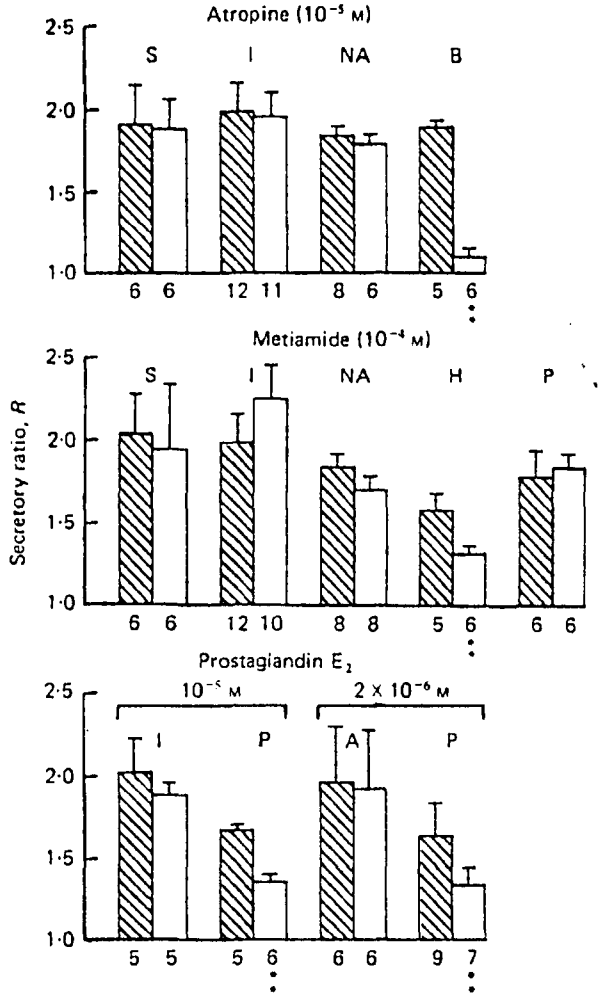


Fig. 5. The effects of atropine, metiamide and prostaglandin E_2 on the secretory ratios obtained with salmefamol (S, 5×10^{-6} M), isoprenaline (I, $1{\cdot}25 \times 10^{-6}$ M), noradrenaline (NA, 5×10^{-6} M), adrenaline (A, 2×10^{-6} M), bethanechol (B, $1{\cdot}7 \times 10^{-5}$ M), histamine (H, $5{\cdot}4 \times 10^{-5}$ M) and pentagastrin (P, $2{\cdot}2 \times 10^{-7}$ M). Columns are mean values with S.E. bars (the shaded columns being control values in the absence of antagonist), with numbers of observations at the base of each column.

DISCUSSION

The two naturally occurring catecholamines and the three synthetic β-adrenoceptor agonists all caused concentration-dependent increases in the rate of acid secretion by the immature rat stomach preparation. Both β_1-selective (noradrenaline) and β_2-selective (salmefamol, salbutamol) agonists stimulated acid output, suggesting that

both subtypes of receptors may be involved. This may partly explain the lower potency of these selective agonists compared with isoprenaline and adrenaline. The lack of inhibitory effect of phentolamine suggests that α-adrenoceptors are not involved in these responses. The response of the adult isolated mucosa to adrenaline and isoprenaline stimulation indicates that our results cannot be completely explained in terms of either the presence of intact neural plexi or immaturity of the preparation.

β-Adrenoceptor antagonists

The three non-selective antagonists (propranolol, pindolol and timolol) all significantly inhibited the response to β-adrenoceptor stimulation. The results with propranolol and salmefamol on isoprenaline (Fig. 3) were consistent with this being due to competitive inhibition.

The results with the selective β-adrenoceptor antagonists are more difficult to interpret. In both selective and non-selective cases, the concentration of antagonist required to produce significant inhibition is greater than might be expected from their respective pA_2 values. Recently Black and his colleagues (Angus & Black, 1979; Angus, Black & Stone, 1980) have drawn attention to the anomalous pA_2 values obtained with atropine and metiamide for inhibition of acid secretion in an isolated stomach preparation. They suggest that because of the act of secretion the antagonist concentration at the parietal cell never achieves equilibrium with the bathing medium and as a result anomalous values of pA_2 are obtained. This would presumably apply to all antagonist studies involving acid secretion *in vitro*, so that pA_2 values for drugs obtained on other tissues are of dubious value in the stomach. This would also mean that one cannot interpret the results obtained using selective antagonists with any certainty as the selectivity is not absolute but relative to the concentration of antagonist used. Thus, the β-selective antagonist ICI 118 551 at 10^{-5} M inhibited all four agonists but at this concentration is not likely to act selectively.

These considerations do not, however, explain the results with noradrenaline, where both the β_1- and β_2-selective antagonists inhibited the response at concentrations which were ineffective against the other agonists. The present experiments can provide no explanation for this for the reasons discussed above, and the whole question of the receptor type involved in these responses will probably require experiments using isolated parietal cell preparations where the problems discussed by Black *et al.* should no longer complicate the interpretation of the results.

Mechanism of action

The stimulatory effect of β-adrenoceptor agonists on acid secretion may be brought about by the release of some other secretagogue in the stomach. However, the lack of any inhibitory action of atropine, metiamide or prostaglandin e_2 on the adrenoceptor agonist responses indicates that they are not acting via release of the three established secretagogues: acetylcholine, histamine or gastrin. The simplest explanation of our findings is that there are β-adrenoceptors on the parietal cell which, when stimulated, bring about acid secretion. This explanation is supported by the recent finding that adrenaline over the same concentration range as used in this work caused a concentration-dependent increase in the cyclic AMP content of rat isolated parietal

cells (Günther, Wagner & Ruoff, 1980). In summary, our results confirm the findings of the preceding paper (Canfield & Price, 1981) that β-adrenoceptor agonists stimulate acid secretion *in vitro* by a β-receptor mechanism.

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