J. Pharm. Pharmacol. 1987, 39: 562–564
Communicated November 27, 1986

Inhibition of basal and insulin-stimulated gastric acid secretion by phenoxybenzamine or phentolamine

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The present study in the rat was undertaken to investigate the possible involvement of α- and β-adrenoceptors in mediating gastric acid secretion in response to insulin-hypoglycaemia. Phenoxybenzamine, 15 mg kg^{-1}, depressed the basal acid secretion to a level similar to that associated with vagotomy; phentolamine, 15 mg kg^{-1}, had a similar effect. Insulin (1 unit kg^{-1}) significantly stimulated gastric acid secretion. Vagotomy or phenoxybenzamine or phentolamine, 15 mg kg^{-1} prevented this action. The β-adrenoceptor blocking drug, propranolol, 5–15 mg kg^{-1}, had no significant effect on the basal acid secretion or its stimulation by insulin. The similarity in action between vagotomy and large doses of phenoxybenzamine and phentolamine suggests that, in the rat, vagal α-adrenoceptor stimulation is involved in the mechanisms of basal and insulin-stimulated gastric acid secretion.

Insulin-hypoglycaemia activates the vagal nucleus in the medulla oblongata and if vagal innervation of the parietal cell mass is intact, this results in stimulation of gastric acid secretion. The insulin test used to detect the presence of intact nerve fibres after vagal operations for peptic ulcer is based on this (Hollander 1946).

Co-administration of glucose with the insulin (by injection) abolishes the acid secretory response (Hedenbro 1980) suggesting that the initial hypoglycaemia is a factor for stimulating acid secretion. Hypoglycaemia is stressful and as such can activate the hypothalamus producing central adrenergic discharge (Leonard et al 1964) in addition to stimulating gastric acid secretion (Peters & Richardson 1983). In experimental animals, insulin-hypoglycaemia activates the hypothalamus whilst inducing a vagally mediated stimulus to gastric acid secretion (Porter et al 1953; French et al 1953). These findings all tend to indicate a hypothalamic and vagal overactivity as the principal effector in mediating acid stimulation by insulin-hypoglycaemia.

In man, a significant correlation exists between adrenaline and serum gastrin levels during hypoglycaemia, and adrenaline given by infusion can induce gastrin secretion (Brandsborg et al 1975). Hayes et al (1972) observed that serum gastrin was elevated in patients with phaeochromocytoma and that administration of the α-adrenoceptor blocking drug, phenoxybenzamine, and removal of the tumour reduced the fasting plasma gastrin level to normal. Propranolol, a β-adrenoceptor blocking drug, has been shown to reduce the gastric secretory response to insulin-hypoglycaemia (Hodge et al 1972; Read et al 1972).

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These observations suggest that catecholamines may be involved in the stimulation of gastric acid secretion effected by insulin-hypoglycaemia. The possible involvement of α- and β-adrenoceptors in the effect of insulin-hypoglycaemia on gastric acid secretion has therefore been examined.

Methods

Animals. Groups of ten rats of either sex (200–250 g), fasted for 24 h but allowed free access to water, were housed in cages with a wire mesh base to prevent coprophagy.

Source and preparation of drugs. The β-adrenoceptor blocking drug, propranolol hydrochloride BP (1 mg ampoules; Inderal, ICI, Cheshire, UK), was used in doses of 5, 10 and 15 mg kg^{-1}. The α-adrenoceptor blocking drugs, phenoxybenzamine hydrochloride (100 mg ampoules; Dibenyline, SKF, Herts, UK) and phentolamine mesylate (50 mg ampoules; Rogitine, Ciba, Horsham, UK), were diluted with double distilled water to prepare solutions of 1, 2 and 3 mg mL^{-1} for the 5, 10 and 15 mg kg^{-1} doses, respectively. Atropine sulphate (Sigma, St Louis, MO, USA) was dissolved in double distilled water to prepare a 1 mg mL^{-1} solution. Cimetidine (200 mg ampoules; SKF, Herts, UK) was used with double distilled water to prepare an 8 mg mL^{-1} solution. Soluble insulin injection 80 units mL^{-1} BP (Wellcome, Crewe, UK) was used to prepare 40 mL of 1 unit mL^{-1} strength by adding 0·5 mL insulin from the original vial to 39·5 mL double distilled water. All drugs were freshly prepared each day and were administered intraperitoneally into the left iliac fossa using a 25 G needle.

Secretory studies. Saline, 5 mL kg^{-1}, was administered intraperitoneally into two groups and each of phenoxybenzamine 5, 10 and 15 mg kg^{-1} and propranolol 5, 10 and 15 mg kg^{-1} were similarly given to an experimental group (Table 1). After 15 min animals were anaesthetized with intraperitoneal pentobarbitone 25 mg kg^{-1} and submitted to tracheostomy and intubated orally with a 6 FG tube (Infants Feeding Tube 400/220, Portex Ltd, Hythe, UK). Following intubation, one of the groups pretreated with saline was vagotomized whilst in all other groups a sham operation was performed (exposing and identifying both vagal trunks). Gastric fasting secretion was recovered by slowly instilling 1 mL double distilled water and recovering all gastric contents. Thereafter, gastric secretion was collected for 1 h.

then insulin (1 unit kg^{-1}) was administered intraperitoneally and gastric secretion collected for another hour.

Using a similar experimental design conducted in a new group of rats, the study then evaluated the effect of vagotomy, intraperitoneal atropine (5 mg kg^{-1}), cimetidine (40 mg kg^{-1}) and phentolamine (5, 10 and 15 mg kg^{-1}) on the basal and insulin (1 unit kg^{-1})-stimulated gastric acid secretion (Table 1).

To minimize day-to-day variation in response to treatment, animals were allocated to the control and all of the treatment groups within the experiment on each experimental day.

The H^+ output was determined by titration to pH 7·0 with 0·1 M NaOH using an automatic titrator (Radiometer, Copenhagen) and expressed as the mean μmol h^{-1} ± s.e.m. for each study group.

The Wilcoxon test was used to determine whether results within a particular group were significantly different and the Mann-Whitney U test was used to establish the statistical significance ($P < 0·05$) of observed differences between groups.

Results

Results are shown in Table 1. Administration of insulin significantly stimulated the basal acid output (38 μmol h^{-1} ± 3·5 vs 12·1 μmol h^{-1} ± 0·7, $P < 0·001$), an effect prevented by both phenoxybenzamine and vagotomy. Phenoxybenzamine given in a dose of 15 mg kg^{-1} depressed the basal acid output to a level similar to that associated with vagotomy (3·7 μmol h^{-1} ± 0·5 vs 3 μmol h^{-1} ± 0·3) and insulin had no influence on this action (3·8 μmol h^{-1} ± 0·5 vs 3·7 μmol h^{-1} ± 0·5). Propranolol treatment had no influence on the basal acid output or its stimulation by insulin.

The basal output was significantly depressed by both atropine and cimetidine (7·6 μmol h^{-1} ± 0·3 and 6·9 μmol h^{-1} ± 0·5, respectively, vs 12·9 μmol h^{-1} ± 0·8, $P < 0·01$). Similarly, both agents significantly prevented stimulation of acid secretion by insulin (16·1 μmol h^{-1} ± 0·4 and 18·6 μmol h^{-1} ± 0·7, respectively, vs 37·1 μmol h^{-1} ± 1·7, $P < 0·001$). However, vagotomy was significantly more effective than atropine or cimetidine in depressing the basal acid output ($P < 0·01$) and in preventing insulin stimulation of acid secretion ($P < 0·001$). Administration of phentolamine in a dose of 5 mg kg^{-1} had no significant effect on the basal acid secretion (10·1 μmol h^{-1} ± 0·3 vs 12·9 μmol h^{-1} ± 0·8), but prevented its stimulation by insulin (10·1 μmol h^{-1} ± 0·3 vs 12·5 μmol h^{-1} ± 0·4). Larger doses of phentolamine significantly depressed basal acid secretion and insulin had no effect on this action. With 10 mg kg^{-1} phentolamine this depression in acid output was similar to that produced by each of atropine and cimetidine, however at a dose of 15 mg kg^{-1}, the antisecretory effect was similar to that achieved with vagotomy (3·2 μmol h^{-1} ± 0·5 vs 2·9 μmol h^{-1} ± 0·4).

Table 1. Effect of α- and β-adrenoceptor blockade on gastric acid output induced by insulin-hypoglycaemia in the rat (n = 10).

	μmol H^+ output (mean ± s.e.m.)	
Experimental groups	Pre-insulin hour	Post-insulin hour
Saline 5 mL kg^{-1} i.p.	12·1 ± 0·7	38 ± 3·5
Vagotomy	3 ± 0·3	2·8 ± 0·5
Phenoxybenzamine 5 mg kg^{-1} i.p.	9·6 ± 0·9	15·7 ± 0·4
Phenoxybenzamine 10 mg kg^{-1} i.p.	5·8 ± 0·3	5·4 ± 0·6
Phenoxybenzamine 15 mg kg^{-1} i.p.	3·7 ± 0·5	3·8 ± 0·5
Propranolol 5 mg kg^{-1} i.p.	12·5 ± 0·5	37·4 ± 3·3
Propranolol 10 mg kg^{-1} i.p.	11·9 ± 0·6	36·8 ± 4
Propranolol 15 mg kg^{-1} i.p.	13 ± 0·3	37·2 ± 2·9
Saline 5 mL kg^{-1} i.p.	12·9 ± 0·8	37·1 ± 1·7
Vagotomy	2·9 ± 0·4	3·1 ± 0·3
Atropine 5 mg kg^{-1} i.p.	7·6 ± 0·3	16·1 ± 0·4
Cimetidine 40 mg kg^{-1} i.p.	6·9 ± 0·5	18·6 ± 0·7
Phentolamine 5 mg kg^{-1} i.p.	10·1 ± 0·3	12·5 ± 0·4
Phentolamine 10 mg kg^{-1} i.p.	6·1 ± 0·2	6 ± 0·5
Phentolamine 15 mg kg^{-1} i.p.	3·2 ± 0·5	2·8 ± 0·6

Discussion

Phenoxybenzamine and phentolamine are α-adrenoceptor blocking agents, the latter being the more specific (Nickerson 1970). Phentolamine (5–20 mg kg^{-1}) has been previously reported (Cho et al 1978) to depress basal acid secretion in pylorus-ligated rats. The present study confirms these results (Table 1) and shows that at 10 mg kg^{-1} both phentolamine and phenoxybenzamine produce similar inhibition of acid secretion to that observed with atropine or cimetidine. However, at 15 mg kg^{-1} they are similar to vagotomy in their antisecretory potency suggesting that, in the rat, the vagus nerve has α-adrenoceptors directly involved in the mechanism of basal acid secretion.

In the rat, insulin-hypoglycaemia causes vagal excitation which stimulates acid secretion by liberating acetylcholine, histamine and gastrin (Hedenbro 1980). This study shows that the vagus nerve mediates insulin stimulation of acid secretion (Table 1) and that this vagal action involves more than cholinergic and histaminic components. Vagotomy was more effective ($P < 0·001$) than atropine or cimetidine in blocking insulin stimulation of acid secretion. Furthermore, whilst each of atropine and cimetidine significantly ($P < 0·001$) prevented insulin stimulation of acid secretion relative to control values, their insulin-associated acid output was still significantly higher than pre-insulin values (Table 1). It thus appears that whilst atropine and cimetidine block their relative receptors, they depress parietal cell responses to additional stimulants in accordance with the potentiation-interactions concept of secretagogues (Soll & Walsh 1979).

At 10 mg kg^{-1} phenoxybenzamine or phentolamine depressed basal acid secretion to levels similar to those produced by atropine or cimetidine, but insulin did not influence this depression (Table 1). Since acetylcholine, histamine and gastrin mediate insulin stimulation of acid secretion (Hedenbro 1980) and blockade of cholinergic or histaminic receptors still allows insulin to increase acid secretion (Table 1), it appears that 10

mg kg^{-1} of the α-adrenoceptor antagonists blocked receptors to all three secretagogues or blocked the primary stimulus responsible for their liberation. There is no evidence that these antagonists have any blocking action on gastrin receptors. Furthermore, at 15 mg kg^{-1} they depresssed the basal acid secretion to a level similar to that achieved by vagotomy, and insulin did not influence this action (Table 1). These results suggest that, in the rat, the vagus nerve has α-adrenoceptors directly involved in the mechanism of basal acid secretion and its stimulation by insulin and that these receptors mediate secretagogue liberation.

Noradrenergic fibres have been demonstrated in the cervical and gastric branches of the vagus nerve in man and animals including the rat (Muryobayashi et al 1968; Lundberg et al 1976). In animals, vagal adrenergic activity releases gastrointestinal 5-hydroxytryptamine (5-HT) (Hohenleitner et al 1971; Tansy et al 1971; Ahlman et al 1976) and evidence has recently been presented in the rat that 5-HT liberates gastric acid secretagogues (acetylcholine, gastrin, histamine) by a paracrine action (Salim 1985). This 5-HT action is at odds with the knowledge that it inhibits gastric acid secretion in the rat isolated stomach (Canfield & Spencer 1983). However, available evidence shows that acid-induced 5-HT release causes diminution of gastric blood flow and depression of acid secretion (Peskin & Miller 1962). Therefore, 5-HT may be a key factor in the physiological control of acid secretion where its vagally induced release enhances acid secretion by a paracrine action whereas its acid-induced release inhibits acid secretion by a humoral effect depressing gastric blood flow.

In conclusion, the findings of this study suggest that in the rat, vagal α-adrenoceptor stimulation is involved in the mechanisms of basal and insulin-stimulated gastric acid secretion.

The skilful technical assistance of Mr D. McMillan and the secretarial work of Miss L. Cook are gratefully acknowledged.

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