J. Pharm. Pharmacol. 1988, 40: 448–450
Communicated September 18, 1987

The role of adrenoceptors in the mechanism of reserpine-induced stimulation of gastric acid secretion in the rat

AWS S. SALIM, *University Department of Surgery, Royal Infirmary, Glasgow G4 0SF, UK*

Abstract—The role of α- and β-adrenoceptors in the mechanism of reserpine-induced stimulation of gastric acid secretion in the rat has been examined. After 6 h reserpine (0·1 mg kg^{-1} i.p.) significantly stimulated acid secretion relative to control values (176 ± 4 vs 60 ± 3 μmol, mean ± s.e.m., n = 10, $P < 0.001$). Neither coeliac ganglionectomy nor propranolol (5-15 mg kg^{-1}) influenced this action. Vagotomy prevented acid stimulation by reserpine and was associated with H^+ output similar to that of vagotomy controls (13 ± 1 vs 14 ± 1 μmol, mean ± s.e.m., n = 10). Dose-dependent inhibition of the reserpine-induced acid secretion was produced by phenoxybenzamine or phentolamine; an inhibition similar to that achieved by vagotomy was noted with the 15 mg kg^{-1} dose (13 ± 1 and 15 ± 1 μmol, respectively, vs 176 ± 4 μmol, mean ± s.e.m., n = 10, $P < 0.001$). The similarity in action between vagotomy and large doses of phenoxybenzamine or phentolamine suggests that, in the rat, vagal α-adrenoceptor stimulation is directly involved in the mechanism of reserpine-induced stimulation of gastric acid secretion.

In man, a significant correlation exists for adrenaline and serum gastrin levels during hypoglycaemia, while 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 either the α-adrenoceptor blocking drug, phenoxybenzamine, or removal of the tumour, turned the fasting plasma gastrin level to normal. Pylorus ligation is a known stimulus for acid secretion in the rat and the mechanism involves a vagovagal reflex (Brodie 1966). In this model, the α-adrenoceptor blocking drug, phentolamine (5–20 mg kg^{-1}), depresses acid secretion (Cho et al 1978). Propranolol, a β-adrenoceptor blocking drug, has been shown to reduce the gastric secretory response to insulin-hypoglycaemia in man (Hodge et al 1972; Read et al 1972). In the rat, the basal and insulin-stimulated acid secretions were not influenced by propranolol, but were inhibited by phenoxybenzamine or phentolamine, suggesting that vagal α-adrenoceptor stimulation is involved in the mechanisms of basal and insulin-stimulated acid secretion in the rat (Salim 1987). These observations suggest that catecholamines are involved in stimulation of gastric acid secretion.

The stimulation of gastric acid by intravenous reserpine exceeds the response to such potent stimulants as insulin, histamine and betazole (Kirsner & Ford 1957). Reserpine has been shown (Kim & Shore 1963; Emås & Fyrö 1965) to produce this stimulation in animals via a vagal action on the stomach causing release of acid secretagogues. However, reserpine activates the hypothalamus, causing central adrenergic discharge with stimulation of peripheral sympathetic pathways and its actions are mediated by noradrenaline or 5-hydroxytryptamine (5-HT) (Page 1958). The possible involvement of α- and β-adrenoceptors in the effect of reserpine on gastric acid secretion in the rat has, therefore, been examined.

Materials and 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 wide mesh wire bottoms to prevent coprophagy. After surgery, rats were fasted until killed.

Correspondence to: A. S. Salim, Department of Surgery, Royal Infirmary, Perth PH1 1NX, UK.

Source and preparation of drugs. A 0·1 mg mL^{-1} solution of reserpine was prepared by dissolving 8 mg powder (Sigma, St. Louis, Mo., USA) in 0·1 mL glacial acetic acid (BDH Chemicals, Poole, UK) and the volume made up to 80 mL with double distilled water. Phenoxybenzamine hydrochloride (Dibenyline, SKF, Herts UK) and phentolamine mesylate (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. Propranolol hydrochloride BP (Inderal, ICI, Cheshire, UK) was used in doses of 5, 10 and 15 mg kg^{-1}. Saline was given to control animals. Drugs were freshly prepared each day and were injected intraperitoneally into the left iliac fossa, using a 25 G needle.

Surgery. Animals were anaesthetized with Diethyl Ether (BP). All procedures were performed through a 5 cm transverse abdominal incision situated midway between the xyphoid process and the pubic symphysis.

Vagotomy. The gastrohepatic ligament was divided to expose the abdominal oesophagus, then the anterior and posterior vagal trunks were divided just below the oesophageal hiatus.

Gastric diversion. The procedure is described in detail elsewhere (Salim 1985). The blood supply was kept intact, and the distal end was ligated. The diversion system tube was placed just distal to the pyloric sphincter then the duodenum was tied over it.

Coeliac ganglionectomy. The coeliac ganglion was removed piecemeal and stored in 4% buffered neutral formalin for histological confirmation. The ventral and lateral sides of the aorta from the coeliac to below the superior mesenteric arteries were cleared of areolar tissue using a gauze pledget.

Secretory studies. Controlled studies were undertaken to determine the effect of vagotomy, phenoxybenzamine and phentolamine, propranolol, and coeliac ganglionectomy on the basal gastric acid secretion over 6 h in the gastric diversion rat. Gastric diversion with vagotomy or a sham operation (exposing and identifying both vagal trunks) and coeliac ganglionectomy or a sham operation (retracting the viscera and identifying the anatomical site of the ganglion behind the peritoneum) were done. The abdomen was closed and animals were injected with saline 1 mL kg^{-1}, phenoxybenzamine, phentolamine or propranolol, 5, 10 or 15 mg kg^{-1} (Table 1). In the experimental groups, Table 2, the procedure was similar. Animals were allowed to recover from anaesthesia and were then injected with saline (1 mL kg^{-1}) or reserpine (0·1 mg kg^{-1}).

Six hours later animals were killed by ether overdose, the stomach with its diversion system removed, then opened along the greater curvature and the bag contents collected. The bag and gastric mucosa were each rinsed with 1 mL double distilled water to remove any remaining H^+. The H^+ output was determined by titration to pH 7·0 with 0·1 M NaOH and expressed as the mean μmol/6 h ± s.e.m. for each study group.

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

Intraperitoneal administration of the vehicle for reserpine in a dose of 1 mL kg^{-1} had no significant effect on the H^+ output of the rat stomach.

Statistical analysis. Results are expressed as mean ± s.e.m. The statistical significance ($P < 0.05$) of observed differences between groups was determined using the Mann-Whitney U test for non-parametric data.

Results

Results are presented in Tables 1 and 2.

Table 1. Effect of vagotomy, α- or β-adrenoceptor blocking drugs or coeliac ganglionectomy on the basal gastric acid secretion in the gastric diversion rat.

Experimental group	n	μmol H^+ output/6 h (mean ± s.e.m.)
Saline 1 mL kg^{-1}	10	63 ± 2·5
Vagotomy saline 1 mL kg^{-1}	10	11 ± 0·9
Phenoxybenzamine 5 mg kg^{-1}	10	46 ± 2·1
Phenoxybenzamine 10 mg kg^{-1}	10	24·8 ± 1·5
Phenoxybenzamine 15 mg kg^{-1}	10	12·4 ± 0·5
Phentolamine 5 mg kg^{-1}	10	42 ± 2·9
Phentolamine 10 mg kg^{-1}	10	25·1 ± 1
Phentolamine 15 mg kg^{-1}	10	11·8 ± 0·6
Propranolol 5 mg kg^{-1}	10	60·8 ± 3·1
Propranolol 10 mg kg^{-1}	10	62·4 ± 2·8
Propranolol 15 mg kg^{-1}	10	60·5 ± 2·5
Coeliac ganglionectomy saline 1 mL kg^{-1}	10	61·2 ± 3·2

Table 2. Effect of vagotomy, α- or β-adrenoceptor blocking drugs or coelic ganglionectomy on the reserpine-induced gastric acid secretion in the gastric diversion rat.

Experimental group	n	μmol H^+ output/6 h (mean ± s.e.m.)
Saline 1 mL kg^{-1} saline 1 mL kg^{-1}	10	60 ± 3
Vagotomy saline 1 mL kg^{-1} saline 1 mL kg^{-1}	10	14 ± 1
Saline 1 mL kg^{-1} reserpine 0·1 mg kg^{-1}	10	176 ± 4
Vagotomy saline 1 mL kg^{-1} reserpine 0·1 mg kg^{-1}	10	13 ± 1
Phenoxybenzamine 5 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	82 ± 3
Phenoxybenzamine 10 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	37 ± 2
Phenoxybenzamine 15 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	13 ± 1
Phentolamine 5 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	90 ± 5
Phentolamine 10 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	44 ± 3
Phentolamine 15 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	15 ± 1
Propranolol 5 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	170 ± 5
Propranolol 10 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	172 ± 4
Propranolol 15 mg kg^{-1} reserpine 0·1 mg kg^{-1}	10	178 ± 4
Coeliac ganglionectomy saline 1 mL kg^{-1} reserpine 0·1 mg kg^{-1}	10	180 ± 5

Histology examination confirmed removal of the coeliac ganglion in all members of the ganglionectomy group.

Coeliac ganglionectomy or propranolol, 5–15 mg kg^{-1}, had no significant effect on the basal gastric acid secretion. Dose-dependent inhibition of this secretion was produced by phenoxybenzamine or phentolamine, an inhibition similar to that achieved by vagotomy was produced by the 15 mg kg^{-1} dose (12·4 ± 0·5 and 11·8 ± 0·6 μmol respectively, vs 11 ± 0·9 μmol).

Reserpine significantly ($P < 0.001$) stimulated gastric acid secretion relative to control values (176 ± 4 vs 60 ± 3 μmol). Coeliac ganglionectomy or propranolol 5–15 mg kg^{-1}, had no significant effect on this action. Vagotomy prevented stimulation of acid by reserpine and was associated with H^+ output similar to that of vagotomy controls (13 ± 1 vs 14 ± 1 μmol). Dose-dependent inhibiton of the reserpine-induced gastric acid secretion was produced by phenoxybenzamine or phentolamine, an inhibition similar to that achieved by vagotomy was noted with the 15 mg kg^{-1} dose (13 ± 1 and 15 ± 1 μmol, respectively, vs 176 ± 4 μmol, $P < 0.001$).

Discussion

Phenoxybenzamine and phentolamine are both α-adrenoceptor blocking drugs, the latter being the more specific (Nickerson 1970). Phenoxybenzamine has been reported to block 5-HT, muscarinic and histamine H_1-receptors, acetylcholinesterase and both the neuronal and extraneuronal uptake of catecholamines at similar concentrations and exposure times to those required for α-adrenoceptor blockade. Phentolamine, too, has been reported to block neuronal uptake (Foster 1968), although at a concentration (10^{-5} M) probably higher than those likely to be achieved in the present study. For those reasons, phenoxybenzamine and phentolamine were used in order to achieve a more accurate assessment of the involvement of α-adrenoceptors in the mechanism of reserpine-induced stimulation of gastric acid secretion in the rat.

While propranolol, 5–15 mg kg^{-1}, has no significant influence on the basal or insulin-stimulated gastric acid secretion (Salim 1987), phentolamine (5–20 mg kg^{-1}) has been reported (Cho et al 1978) to depress acid secretion in pylorus-ligated rats. Phenoxybenzamine and phentolamine (5–15 mg kg^{-1}) also inhibit basal and insulin-stimulated gastric acid secretion in rats without pylorus ligation (Salim 1987).

In the rat, the basal gastric acid secretion is mediated by vagal stimulation which releases the acid secretagogues acetylcholine, histamine and gastrin (Hedenbro 1980). These secretagogues are capable of independent stimulation of the parietal cell and may each interact to potentiate the other (Soll 1977, 1978). This study shows that the vagus mediates basal acid secretion in the rat (Table 1).

Coeliac ganglionectomy, to interrupt autonomic sympathetic delivery to the stomach (Leonard et al 1964), had no significant influence on this secretion (Table 1) suggesting that the sympathetic delivery to the rat stomach via the coeliac plexus is not involved in the mechanism of basal gastric acid secretion. Propranolol in doses of 5–15 mg kg^{-1} had no significant effect on this secretion (Table 1). Dose-dependent inhibition of the basal acid secretion of the rat stomach was afforded by phenoxybenzamine or phentolamine, an inhibition similar to that achieved by vagotomy was noted with the 15 mg kg^{-1} doses (Table 1). The similarity in action between vagotomy and large doses of phenoxybenzamine or phentolamine suggests that, in the rat, the vagus nerve has α-adrenoceptors directly involved in the mechanism of basal gastric acid secretion.

Reserpine has been shown (Kim & Shore 1963; Emås & Fyrö 1965) to produce a vagal action on the stomach of rats and cats, causing secretagogue release and stimulation of acid secretion.

The present study demonstrates that vagotomy protects the rat stomach against the reserpine-induced stimulation of acid secretion (Table 2). Neither coeliac ganglionectomy nor propranolol (5–15 mg kg^{-1}) had significant influence on the basal or reserpine-induced gastric acid secretion (Tables 1, 2). The results, therefore, suggest that β-adrenoceptors and the autonomic sympathetic system, are not involved in the mechanism of reserpine-induced stimulation of acid secretion.

The similarity in action between the α-adrenoceptor blocking drugs and vagotomy (Table 2) suggests that the vagus nerve has α-adrenoceptors directly involved in the mechanism of reserpine-induced stimulation of acid secretion. The small standard errors which represent coefficient of variance values between 2·2 and 7·7% of this study are much less than the range of 9–90% obtained by Kim & Shore (1963) in similarly conceived studies in the conscious rat. This reduction in apparent variance may be due to the differences in experimental technique.

Noradrenergic fibres have been demonstrated in the cervical and gastric branches of the vagus nerve of humans and animals including the rat (Muryobayashi et al 1968; Lundberg et al 1976). In animals, vagal adrenergic activity releases gastrointestinal 5-HT (Hohenleitner et al 1971; Tansy et al 1971; Ahlman et al 1976) and in the rat 5-HT liberates gastric acid secretagogues (acetylcholine, gastrin, histamine) by a paracrine action (Salim 1985). Since reserpine releases gastrointestinal 5-HT (Page 1958) and acid secretagogues (Kim & Shore 1963; Emås & Fyrö 1965), it appears that, in the rat, reserpine causes vagal α-adrenoceptor stimulation that releases 5-HT which liberates the secretagogues that stimulate acid secretion. This is at odds with the knowledge that 5-HT inhibits gastric acid secretion in the rat isolated stomach (Canfield & Spencer 1983). However, acid-induced 5-HT release causes depression of gastric blood flow and acid secretion (Peskin & Miller 1962). Therefore, 5-HT may be of central importance in the control of acid secretion where its vagally induced release stimulates 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 results of the present study suggest that in the rat, vagal α-adrenoceptor stimulation is directly involved in the mechanism of basal and reserpine-induced stimulation of gastric acid secretion.

The skilful technical assistance of Mr Donald McMillan and secretarial work of Miss Lesley Cook are gratefully acknowledged.

References

Ahlman, H., Dahlström, A., Kewenter, J., Lundberg, J. (1976) Vagal influence on serotonin concentration in enterochromaffin cells in the cat. Acta Physiol. Scand. 97: 362–368

Brandsborg, O., Brandsborg, M., Christensen, N. J. (1975) Plasma adrenaline and serum gastrin: studies in insulin-induced hypoglycemia and after adrenaline infusions. Gastroenterology 68: 455–460

Brodie, D. A. (1966) The mechanism of gastric hyperacidity produced by pylorus ligation in the rat. Am. J. Dig. Dis. 11: 231–241

Canfield, S. P., Spencer, J. E. (1983) The inhibitory effect of 5-hydroxytryptamine on gastric acid secretion in the rat isolated stomach. Br. J. Pharmacol. 78: 123–129

Cho, C. H., Dai, S., Ogle, C. W. (1978) Some observations on the gastric effects of phentolamine in rats. Eur. J. Pharmacol. 48: 51–55

Emås, S., Fyrö, B. (1965) Vagal release of gastrin in cats following reserpine. Acta Physiol. Scand. 63: 358–369

Foster, R. W. (1968) A correlation between inhibition of the uptake of ^3H from (±)-^3H-noradrenaline and potentiation of the responses to (−)-noradrenaline in the guinea pig isolated trachea. Br. J. Pharmacol. Chemother. 33: 357–367

Hayes, J. R., Adrill, J., Kennedy, T. L., Shanks, R. G. (1972) Stimulation of gastrin release by catecholamines. Lancet i: 819–821

Hedenbro, J. (1980) Stimulation of gastric acid secretion. Vagus-gastrin-histamine interactions in the rat. Bull. 20, Lund Univ., Sweden

Hodge, A. J., Masarei, J. R., Catchpole, B. N. (1972) The role of the sympathetic nervous system in hypoglycaemia-stimulated gastric secretion. Gut 13: 341–345

Hohenleitner, F. J., Tansy, M. F., Golder, R. H. (1971) Effect of vagal stimulation on duodenal serotonin in the guinea pig. J. Pharm. Sci. 60: 471–472

Kim, K. S., Shore, P. A. (1963) Mechanism of action of reserpine and insulin on gastric amines and gastric acid secretion, and the effect of monoamine oxidase inhibition. J. Pharmacol. Exp. Ther. 141: 321–325

Kirsner, J. B., Ford, H. (1957) The effect of reserpine upon basal gastric secretion in man. A.M.A. Arch. Int. Med. 99: 390–400

Leonard, A. S., Long, D., French, L. A., Peter, E. T., Wangensteen, O. H. (1964) Pendular pattern in gastric secretion and blood flow following hypothalamic stimulation-origin of stress ulcer. Surgery 56: 109–120

Lundberg, J., Ahlman, H., Dahlström, A., Kewenter, J. (1976) Catecholamine-containing nerve fibres in the human abdominal vagus. Gastroenterology 70: 472–474

Muryobayashi, T., Mori, J., Fujiwara, M., Shimamoto, K. (1968) Fluorescence histochemical demonstration of adrenergic nerve fibres in the vagus nerve of cats and dogs. Jap. J. Pharmacol. 18: 285–293

Nickerson, M. (1970) Drugs inhibiting adrenergic nerves and structures innervated by them. In: Goodman, L. S., Gilman, A. G. (eds) The Pharmacological Basis of Therapeutics. Macmillan, London, Ch. 26, pp 550–570

Page, I. H. (1958) Serotonin (5-hydroxytryptamine); the last four years. Physiol. Rev. 38: 277–335

Peskin, G. W., Miller, L. D. (1962) The role of serotonin as an antral-inhibitory substance. Surg. Forum. 13: 245–247

Read, R. C., Thompson, B. W., Hall, W. H. (1972) Conversion of Hollander tests in man from positive to negative. Arch. Surg. 104: 573–578

Salim, A. S. (1985) The adrenergic hypothalamovagal pathway and acute gastric mucosal injury. PhD Thesis. University of Glasgow, UK

Salim, A. S. (1987) Inhibition of basal and insulin-stimulated gastric acid secretion by phenoxybenzamine or phentolamine. J. Pharm. Pharmacol. 39: 562–564

Soll, A. H. (1977) Secretagogue stimulation of O_2 consumption and ^{14}C-aminopyrine uptake by enriched canine parietal cells. Gastroenterology 72: 1166 (Abst)

Soll, A. H., (1978) The actions of secretagogues on oxygen uptake by isolated mammalian parietal cells. J. Clin. Invest. 61: 370–380

Tansy, M. F., Rothman, G., Bartlett, J., Farber, P., Hohenleitner, F. J. (1971) Vagal adrenergic degranulation of enterochromaffin cell system in guinea pig duodenum. J. Pharm. Sci. 60: 81–84