The role of vagal adrenergic activity in the mechanism of gastric acid secretion after pylorus-ligation in the rat

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Abstract—The role of the vagus nerve and adrenoceptor stimulation in acid secretion after pylorus-ligation in the rat has been examined. All drugs were administered intraperitoneally. Atropine (5 mg kg^{-1}) depressed the H$^+$ output (111 μmol $\pm$ 33·8 vs 412·5 μmol $\pm$ 62·2, mean $\pm$ s.e.m., n = 10, $P < 0.001$); cimetidine (40 mg kg^{-1}) did not enhance this action, while vagotomy was more effective than atropine (32·7 μmol $\pm$ 4·9, mean $\pm$ s.e.m., n = 10, $P < 0.05$). Atropine (10 mg kg^{-1}) produced a similar depression to the 5 mg kg^{-1} dose. Cimetidine (100 mg kg^{-1}) depressed the H$^+$ output (248·5 μmol $\pm$ 46·8, mean $\pm$ s.e.m., n = 10, $P < 0.05$). Propranolol (5–20 mg kg^{-1}) had no significant effect on the H$^+$ output but dose-dependent inhibition was produced by phenoxybenzamine or phentolamine; an inhibition similar to that achieved by vagotomy was seen with the 20 mg kg^{-1} dose. Both these drugs (5 or 10 mg kg^{-1}) had no significant effect on the H$^+$ output when given with atropine (5 mg kg^{-1}) but the H$^+$ output was significantly lower than that produced by either drug at the same dose given alone. Atropine (5 mg kg^{-1}) with phenoxybenzamine or phentolamine (20 mg kg^{-1}) produced H$^+$ output not significantly different from that with vagotomy or either α-adrenoceptor given alone at 20 mg kg^{-1}, but the result was significantly ($P < 0.05$) lower than the H$^+$ output with atropine (5 mg kg^{-1}) alone. The results suggest that pylorus-ligation in the rat produces vagal α-adrenoceptor delivery to the stomach causing cholinergic and non-cholinergic stimulation to acid secretion.

Pylorus-ligation is a known stimulus for acid secretion in the rat and although the mechanism is uncertain it probably involves a vagovagal reflex (Brodie 1966; Brodie & Knapp 1966). It has been reported (Shay et al 1949; Donald & Code 1952) that vagotomy completely abolishes the acid response to pylorus ligation. However, Håkanson et al (1980) found a reduced, rather than abolished, acid response in vagotomized rats subjected to pylorus-ligation 2–8 weeks after the denervation. Cho et al (1978) reported that the α-adrenoceptor blocking drug phentolamine (5–20 mg kg^{-1}) depresses acid secretion in the pylorus-ligated rat. However, those investigators did not establish the influence of vagal activity on α-adrenoceptor stimulation in the mechanism of gastric acid secretion in this rat model. The present study was undertaken to further examine the role of the vagus nerve and adrenoceptor impulses in the mechanism of acid stimulation by pylorus-ligation in the rat.

Materials and methods

Animals. Groups of ten Sprague-Dawley rats of either sex, between 200–250 g, were housed in cages with wide mesh wire bottoms to prevent coprophagy. They had water only for 24 h before study and after surgery, they were fasted until killed.

Source and preparation of drugs. Propranolol hydrochloride BP (1 mg ampoules; Inderal, ICI, Cheshire, UK) was used in doses of 5, 10, 15 and 20 mg kg^{-1}. Phenoxybenzamine hydrochloride (100 mg ampoules; Dibenyline, SKF, Hertfordshire, UK) and phentolamine mesylate (50 mg ampoules; Rogitine, CIBA, Horsham, UK) were diluted with double distilled water to solutions of 1, 2, 3 and 4 mg mL^{-1} for the 5, 10, 15 and 20 mg kg^{-1} doses, respectively. Atropine sulphate powder (Sigma, St. Louis, Mo., USA) was dissolved in double distilled water to solutions of 1, 2 and 3 mg mL^{-1}. Cimetidine (200 mg ampoules,

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SKF, Hertfordshire, UK) was diluted with double distilled water to an 8 mg mL^{-1} solution for the 40 mg kg^{-1} dose and a 20 mg mL^{-1} solution for the 100 mg kg^{-1} dose. Control animals were given saline.

Drugs were prepared daily and injected intraperitoneally into the left iliac fossa. Atropine and cimetidine or atropine and phenoxybenzamine or phentolamine when used together were given seriatim.

Surgery. Animals were anaesthetized with diethyl ether (BP) and surgery was performed through a 2 cm upper midline laparotomy. The gastrohepatic ligament was divided to expose the abdominal oesophagus then the anterior and posterior vagal trunks were divided just below the oesophageal hiatus. The pyloric sphincter was ligated using a 5/0 silk tie, avoiding the blood supply to stomach or duodenum.

Experimental design. The experimental groups are listed in Tables 1 and 2. Vagotomy or a sham operation (exposing and identifying both vagal trunks) with pylorus-ligation was done. The abdomen was closed and animals were injected with saline or drugs. Six hours later rats were killed by ether overdose, the stomachs removed, gently shaken to mix gastric contents and opened along the greater curvature. The contents were collected and the mucosa rinsed with 2 mL double distilled water to wash any remaining gastric secretion. The H$^+$ output for each animal was determined by titration to pH 7·0 with 0·1 M NaOH using an automatic titrator (Radiometer, Copenhagen) and expressed as μmol/6 h, then the mean H$^+$ output was calculated for each study group.

To minimize day-to-day variation in response to treatment, the study was conducted over several days and on each of which

Table 1. Effect of atropine, cimetidine, α- or β-adrenoceptor blocking agents or vagotomy on H$^+$ output of the pylorus-ligation rat (n = 10).

Experimental group	H$^+$ output in μmol after 6 h (mean $\pm$ s.e.m.)
Saline 5 mL kg^{-1}	412·5 $\pm$ 62·2
Atropine 5 mg kg^{-1}	111 $\pm$ 33·8***
Cimetidine 40 mg kg^{-1}	467·5 $\pm$ 82·8
Atropine 5 mg kg^{-1} + Cimetidine 40 mg kg^{-1}	107·5 $\pm$ 26·5***
Cimetidine 100 mg kg^{-1}	248·5 $\pm$ 46·8*
Propranolol 5 mg kg^{-1}	443·2 $\pm$ 52·7
Propranolol 10 mg kg^{-1}	405·7 $\pm$ 59·5
Propranolol 15 mg kg^{-1}	452·5 $\pm$ 56·8
Propranolol 20 mg kg^{-1}	461·5 $\pm$ 68·6
Phenoxybenzamine 5 mg kg^{-1}	372·4 $\pm$ 51·5
Phenoxybenzamine 10 mg kg^{-1}	254 $\pm$ 32·1*
Phenoxybenzamine 15 mg kg^{-1}	95·7 $\pm$ 16·7***
Phenoxybenzamine 20 mg kg^{-1}	28·6 $\pm$ 6·1***
Phentolamine 5 mg kg^{-1}	381·5 $\pm$ 48·9
Phentolamine 10 mg kg^{-1}	247·4 $\pm$ 38·6*
Phentolamine 15 mg kg^{-1}	82·6 $\pm$ 21·5***
Phentolamine 20 mg kg^{-1}	34·5 $\pm$ 5·4***
Vagotomy + saline 5 mL kg^{-1}	32·7 $\pm$ 4·9***

* $P < 0.05$, *** $P < 0.001$ Mann–Whitney test comparing control group with treatment group.

Table 2. Effect of atropine alone or with alpha-adrenoceptor blockade and vagotomy on the H$^+$ output of the pylorus-ligation rat (n = 10).

Experimental group	H$^+$ output in μmol after 6 h (mean ± s.e.m.)
Saline 5 mL kg^{-1}	459·7 ± 53·2
Atropine 5 mg kg^{-1}	103·4 ± 28·5***
Atropine 10 mg kg^{-1}	114·1 ± 34·7***
Atropine 5 mg kg^{-1} + phenoxybenz. 5 mg kg^{-1}	119·5 ± 27·3***
Atropine 5 mg kg^{-1} + phenoxybenz. 10 mg kg^{-1}	106·6 ± 26·8***
Atropine 5 mg kg^{-1} + phenoxybenz. 15 mg kg^{-1}	89·5 ± 17·4***
Atropine 5 mg kg^{-1} + phenoxybenz. 20 mg kg^{-1}	30·4 ± 2·8***
Atropine 5 mg kg^{-1} + phentolamine 5 mg kg^{-1}	109·3 ± 37·2***
Atropine 5 mg kg^{-1} + phentolamine 10 mg kg^{-1}	101·5 ± 31·3***
Atropine 5 mg kg^{-1} + phentolamine 15 mg kg^{-1}	86·5 ± 21·2***
Atropine 5 mg kg^{-1} + phentolamine 20 mg kg^{-1}	32·1 ± 4·2***
Vagotomy + saline 5 mL kg^{-1}	28·5 ± 3·1***

*** $P < 0.001$ Mann–Whitney test comparing control group with treatment group.

animals were allocated to the control and all of the treatment groups within the experiment.

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

Results

The results are presented in Table 1 and 2.

Atropine (5 mg kg^{-1}) depressed the H$^+$ output of the pylorus-ligation rat and addition of cimetidine (40 mg kg^{-1}) did not enhance this action. Vagotomy was more effective than atropine in depressing the H$^+$ output (Table 1). Cimetidine, 40 mg kg^{-1} had no significant influence on the H$^+$ output relative to control values but the 100 mg kg^{-1} dose depressed this output (Table 1). Propranolol, 5–20 mg kg^{-1}, had no significant effect on the H$^+$ output but a dose-dependent inhibition was produced by phenoxybenzamine or phentolamine with an inhibition similar to that achieved by vagotomy being observed with the 20 mg kg^{-1} doses (Table 1).

In experiments designed to investigate the effect of atropine in doses higher than 5 mg kg^{-1} on the H$^+$ output, 10 mg kg^{-1} depressed the output to levels similar to those achieved by the 5 mg kg^{-1} dose (Table 2) while 15 mg kg^{-1} proved toxic to the animals.

Phenoxybenzamine or phentolamine, 5–10 mg kg^{-1} had no significant effect on the H$^+$ output caused by atropine, 5 mg kg^{-1}, but the H$^+$ output was significantly lower than that produced by the phenoxybenzamine or phentolamine given alone.

Atropine, 5 mg kg^{-1}, with 20 mg kg^{-1} of phenoxybenzamine or phentolamine produced H$^+$ output not significantly different from that attained with vagotomy or from the α-adrenoceptor blocking drugs given alone at 20 mg kg^{-1} (Table 2), but significantly ($P < 0.05$) lower than the H$^+$ output after atropine (5 mg kg^{-1}) alone.

Discussion

The results confirm previous reports that ligation of the pylorus is a powerful stimulus to acid secretion in the rat (Brodie 1966; Brodie & Knapp 1966; Håkanson et al 1980) and suggest that a cholinergic component is involved (Table 1). Failure of cimetidine to inhibit secretion at a dose normally regarded as antisecretory (40 mg kg^{-1}: Rainsford 1978) and its success at a

higher dose (100 mg kg^{-1}) suggests that histamine is not the primary stimulus for acid secretion in this model. However, blockade of H$_2$-receptors may still render the parietal cells less sensitive to stimulation by other secretagogues in accordance with the potentiation-interaction theory (Soll 1977, 1978; Soll & Walsh 1979).

The magnitude of acid depression by 5 mg kg^{-1} of atropine was similar to that afforded by the 10 mg kg^{-1} dose (Table 2) while 15 mg kg^{-1} was toxic. Vagotomy was significantly more effective in depressing H$^+$ output than atropine given alone or with cimetidine (Tables 1, 2) indicating that mediation of acid secretion in this model is primarily dependent on the vagus but that the mechanism has both cholinergic and non-cholinergic components. Whether this additional vagal component is a vagal gastrin drive (Emås 1964; Emås & Fyrö 1965; Hansky et al 1972) or some other vagally released acid secretion mediator is not known. The present results agree with those of Brodie (1966) and Brodie & Knapp (1966), who suggested that the vagus nerve mediates stimulation of acid secretion in the pylorus-ligated rat. The study also confirms the findings of Håkanson et al (1980) that atropine, but not the H$_2$-receptor antagonist metiamide, lowers the acid response to pylorus-ligation in the rat.

Propranolol (5–20 mg kg^{-1}) had no significant influence on the H$^+$ output (Table 1) demonstrating that β-adrenoceptors are not involved in the mechanism of stimulation of this output by pylorus-ligation. Phentolamine (5–20 mg kg^{-1}) has been previously reported (Cho et al 1978) to suppress acid secretion in the model used and in the present study dose-dependent inhibition of the H$^+$ output associated with pylorus-ligation was provided by phenoxybenzamine or phentolamine, an inhibition similar to that achieved by vagotomy being observed at 20 mg kg^{-1} (Table 1). This similarity in action suggests that the vagus nerve has α-adrenoceptors directly involved in the mechanism of stimulation of gastric acid secretion by pylorus ligation.

To determine the association between α-adrenoceptor and cholinergic stimulation in the mechanism of acid secretion in the model used and to explore the possibility that the vagus delivers α-adrenoceptor impulses to the stomach which, in turn, initiate cholinergic and non-cholinergic stimulation of the parietal cells, it was necessary to examine the combined effect of α-adrenoceptor and cholinergic blockade on the H$^+$ output of the pylorus-ligated rat (Table 2). Phenoxybenzamine or phentolamine, 5–10 mg kg^{-1} had no significant effect on the H$^+$ output.

These results show that the effect of cholinergic and α-adrenoceptor blockade on the H$^+$ output are not synergistic or additive. When the anticholinergic drug was given with α-adrenoceptor blocking agents, the resulting amount of acid depression was similar to the extent of H$^+$ output suppression achieved by either antagonist acting alone (Table 2). Therefore, cholinergic and α-adrenoceptor antagonists block the stimulus to acid secretion at different points of the cycle. The initial secretory impulse appears to be delivered to the stomach by vagal α-adrenoceptor stimulation which, in turn, triggers the cholinergic and non-cholinergic impulses directly acting on the parietal cells.

Adrenergic nerve fibres in the main cervical trunk and gastric branches of the vagus nerve of various species have been demonstrated, e.g. rats, guinea-pigs, rabbits, cats and dogs (Muryobayashi et al 1968; Nielsen et al 1969; Tansy et al 1971). These fibres have also been observed at subdiaphragmatic levels of the vagus nerve in man (Lundberg et al 1976). Using the histochemical fluorescence method in cats and dogs, Muryobayashi et al (1968) reported that noradrenaline is the chemical transmitter in vagal adrenergic fibres. Current theory of acid secretion shows that the parietal cell may be responsive to three agents (acetylcholine, histamine, gastrin) which although capable of independent stimulation, may each interact to potentiate

the other (Soll 1977, 1978; Soll & Walsh 1979). It, thus, appears from these observations and the results of the present study that pylorus-ligation in the rat affects vagal α-adrenoceptor delivery to the stomach causing cholinergic and non-cholinergic stimulation of acid secretion.

The digestive system contains at least 90% of total body 5-HT (Erspamer 1954) and this agent has been shown to liberate histamine (Feldberg & Smith 1953) and acetylcholine (Erspamer 1954). These findings suggest that 5-HT might be implicated in the mechanism of gastric acid secretion. In animals, vagal adrenergic activity releases gastrointestinal 5-HT (Hohenleitner et al 1971; Tansy et al 1971; Ahlman et al 1976), therefore, additional studies are needed to determine whether in the pylorus-ligated rat the stimulation of acid secretion by vagal alpha-activity is mediated by 5-HT.

In conclusion, the results of the present study suggest that pylorus ligation in the rat produces vagal α-adrenoceptor delivery to the stomach causing cholinergic and non-cholinergic stimulation to acid secretion.

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