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The effect of α_2-adrenoceptor agonists on the acid secretory responses of rat isolated gastric mucosa to electrical field stimulation

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1 The effects of clonidine, UK-14,304, noradrenaline, *para*-aminoclonidine and phenylephrine were examined on the acid secretory response of the rat isolated gastric mucosa preparation to electrical field stimulation.

2 Clonidine, UK-14,304, noradrenaline and *para*-aminoclonidine but not phenylephrine (10 μM) reduced the response of the gastric mucosa stimulated at 2.5 Hz; gastric mucosae stimulated at higher frequencies were insensitive to the action of these α_2-adrenoceptor agonists.

3 The inhibitory effect of the selective α_2-adrenoceptor agonist UK-14,304 was antagonized by idazoxan but not by prazosin.

4 These findings indicate that clonidine and other α_2-adrenoceptor agents inhibit the acid secretory response of the rat gastric mucosa to electrical field stimulation by an action at α_2-adrenoceptors, which are probably located on cholinergic nerve terminals.

Introduction

Clonidine inhibits gastric acid secretion in rats (Hoefke & Kobinger, 1966) and this action has been attributed to its α_2-adrenoceptor agonist activity. Recent work has extended these observations to other α_2-adrenoceptor agonists including UK-14,304, *para*-aminoclonidine and WHR1370A (Dettmar & Smeaton, 1985) and it has been suggested that α_2-adrenoceptors may be involved in the regulation of gastric acid secretion in the rat.

Whole animal experiments have indicated that clonidine may possess both a central and peripheral site of action (Pascaud & Roger, 1975; Jennewein, 1977), although the effect on acid secretion may be an increase or decrease in secretion, depending upon the experimental conditions adopted for the study (Del Tacca *et al.*, 1982). In the anaesthetized rat, clonidine was observed to inhibit gastric acid secretion induced by electrical stimulation of the vagus and this action has been ascribed to an inhibition of acetylcholine release at vagal nerve endings (Jennewein, 1977; Cheng *et al.*, 1981).

There have been few reports of experiments with clonidine using *in vitro* stomach preparations, although Del Tacca *et al.* (1982) found that in the guinea-pig isolated fundus preparation, clonidine appeared to increase acid secretion. We have investigated the action of clonidine and several α-adrenoceptor agents on the acid secretory responses elicited by electrical field stimulation of the rat isolated gastric mucosa preparation; field stimulation is believed to stimulate acid secretion through the mediation of postganglionic cholinergic nerves within the mucosa which are of vagal origin (Baird & Main, 1978). Preliminary results of this work have been presented previously (Dettmar & Lord, 1985).

Methods

Gastric mucosa preparations were set up and maintained in Krebs solution according to the method described by Main & Pearce (1978) using male Sprague Dawley rats of 150–180 g (field stimulation experiments) or 100–130 g (secretagogue experiments). For the dissection of the mucosa, the rat was anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and the stomach exteriorized. The muscle tissue overlying the non-antral region was removed by

blistering with modified Krebs solution using a fine needle to inject the solution beneath the serosa. The serosa was cut along the greater curvature and the muscle was separated from the mucosa. A 1 cm^2 section of mucosa was mounted, mucosal surface inwards, on a polypropylene cup and transferred to an organ bath maintained at 37°C; the tissue was superfused with a mucosal solution (unbuffered modified Krebs solution) at a flow rate of 1 ml min^{-1}. The pH of the superfusate flowing from the outlet of the perfusion cup was monitored continuously. Acid secretory responses were elicited by administration of secretagogues or by electrical field stimulation (5 V, 2.5 Hz, 0.5 ms for 15 min) when platinum electrodes placed above and below the mucosa were used. The preparation was equilibrated for 90 min after which a drug was added to the organ bath and field stimulation or secretagogue was applied 30 min later; control preparations were stimulated in the absence of drug. Where antagonists were used they were administered 15 min before the agonists. The acid secretory responses were measured in terms of μmol H^+ cm^{-2} h^{-1} and corrected for basal output which was taken as that just before the start of the response; the basal output was subtracted from the peak output in order to exclude the non-stimulated portion of acid secretion from the response of the preparation.

The Krebs solution contained (mM): NaCl 119, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, glucose 12 and $NaHCO_3$ 25; the solution was bubbled with 95% O_2 and 5% CO_2. The modified Krebs solution used to superfuse the mucosa had a similar composition except that the KH_2PO_4 and $NaHCO_3$ were omitted and replaced with equimolar NaCl; this solution was bubbled with 100% O_2 and had a pH of 6.2.

Drugs

The drugs used were acetylcholine chloride (Sigma), clonidine hydrochloride (Bonapace), histamine acid phosphate (BDH), idazoxan hydrochloride (Reckitt and Colman), noradrenaline bitartrate (Koch Light), pentagastrin (ICI), *para*-aminoclonidine hydrochloride (Research Biochemicals Inc.), phenylephrine hydrochloride (Koch-Light), prazosin hydrochloride (Pfizer), propranolol hydrochloride (Sigma) and UK-14,304 (5-bromo-6-[2-imidazolin-2-yl amino]-quinoxaline tartrate, Pfizer).

Statistics

Results are expressed as mean ± s.e.mean (n) where n is the number of observations. Statistical analysis of the unpaired data was performed using Student's t test and Dunnett's t test for multiple comparisons. P levels of <0.05 were considered significant.

Results

The responses of rat gastric mucosa to electrical stimulation and exogenous acetylcholine

Control preparations were found to secrete acid in response to electrical stimulation using the parameters of 5 V, 2.5 Hz and 0.5 ms for 15 min, with 2.5 Hz representing the lowest frequency at which measurable responses could be elicited (Table 1). Secretory responses which were obtained at 5 Hz and 10 Hz were less sensitive to the inhibitory actions of the α_2-adrenoceptor agonists being studied; as shown in Table 1 UK-14,304 (10 μM) reduced significantly ($P<0.01$) the responses of the gastric mucosa stimulated at 2.5 Hz, whereas at 5 Hz and 10 Hz, although the responses following UK-14,304 (10 μM) were less than the control values, they were not significantly different.

Acid secretory responses were observed in preparations to which acetylcholine (1 μM) had been administered (Table 1); these responses were not significantly different from responses obtained from preparations which had been exposed to UK-14,304 (10 μM). The responses of the mucosa to field stimulation were blocked by tetrodotoxin (0.6 μM) and by atropine (0.1 μM), but they were unaffected by cimetidine at a concentration as high as 1 mM.

The effect of selected α-adrenoceptor agonists on the acid secretory responses of rat gastric mucosa to electrical stimulation

Noradrenaline (0.1–10 μM), UK-14,304 (0.5–10 μM) and *para*-aminoclonidine (0.025–0.5 μM) reduced the acid secretory responses of the mucosa, subjected to field stimulation, (5 V, 2.5 Hz, 0.5 ms for 15 min) in a concentration-related manner (Figure 1); at the highest concentrations the responses were abolished. In the presence of clonidine (5–50 μM) the acid secretory responses were only partially inhibited. None of the compounds had any effect upon the basal acid output from the mucosa over the concentration-range studied; noradrenaline was observed to increase acid secretion at concentrations in excess of 10 μM. Phenylephrine (10 μM) had no effect upon the secretory responses of the mucosa subjected to field stimulation. The inhibition observed in the presence of noradrenaline was not affected by prior administration of propranolol (2 μM).

The effect of idazoxan and prazosin on the inhibitory action of UK-14,304

Acid secretory responses to electrical stimulation were substantially reduced in preparations exposed to UK-14,304 (1 μM). However, responses obtained in the

presence of idazoxan (10 μM) and UK-14,304 (1 μM) were not significantly different from control, untreated preparations (Table 2). In addition, acid secretory responses could be restored to preparations exposed to UK-14,304 and continuous electrical stimulation by the administration of idazoxan. Prazosin (10 μM) however did not affect the inhibitory action of UK-14,304 (1 μM). It was observed that neither idazoxan nor prazosin affected basal acid secretion, and neither antagonist had any effect upon the magnitude of the acid secretory responses of the mucosa to field stimulation when administered alone.

The effect of UK-14,304 on the acid secretory responses of rat gastric mucosa to histamine and pentagastrin

The acid secretory responses to histamine (90 μM) and pentagastrin (64 nM) were unaffected by prior administration of UK-14,304 as shown in Table 3.

Table 1 Effect of UK-14,304 on acid secretory responses of rat gastric mucosa to field stimulation and exogenous acetylcholine (ACh)

Treatment	Frequency (Hz)	*Acid secretory response* (μmol H^+ cm^{-2} h^{-1})
Field stimulation		
Control	2.5	1.13 ± 0.17 (10)
UK-14,304 (10 μM)	2.5	0.06 ± 0.05 (4)**
Control	5	1.64 ± 0.52 (6)
UK-14,304 (10 μM)	5	0.95 ± 0.17 (6)
Control	10	1.52 ± 0.45 (6)
UK-14,304 (10 μM)	10	1.22 ± 0.19 (6)
ACh (1 μM)		
Control		1.92 ± 0.33 (6)
UK-14,304 (10 μM)		2.15 ± 0.36 (6)

Values are means ± s.e. (*n*). **$P<0.01$ versus control.

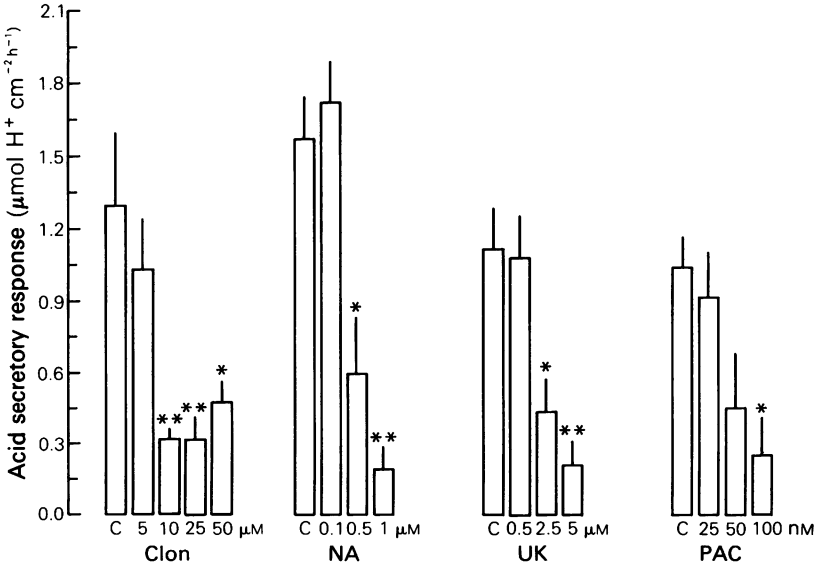

Figure 1 The effect of clonidine (Clon), noradrenaline (NA), UK-14,304 (UK) and *para*-aminoclonidine (PAC) on the acid secretory response to field stimulation. Rat gastric mucosa preparations were either untreated (C) or exposed to drug before electrical stimulation. Each column is the mean value with vertical lines showing s.e.mean. Ordinate scale: acid secretory response in μmol H^+ cm^{-2} h^{-1}. *$P<0.05$; **$P<0.01$.

Table 2 Effects of idazoxan and prazosin on the inhibitory action of UK-14,304 on rat gastric mucosa

Treatment	*Acid secretory response* (μmol H^+ cm^{-2} h^{-1})
Control	1.02 ± 0.12 (18)
UK-14,304 (1 μM)	0.15 ± 0.06 (6)**
UK-14,304 (1 μM) + idazoxan (10 μM)	0.86 ± 0.20 (6)
UK-14,304 (1 μM) + prazosin (10 μM)	0.27 ± 0.13 (6)**
Idazoxan (10 μM)	0.78 ± 0.24 (6)

Values are means ± s.e. (*n*). **$P < 0.01$ versus control.

Table 3 The effect of UK-14,304 on the acid secretory responses of rat gastric mucosa to histamine and pentagastrin

Treatment	*Acid secretory response* (μmol H^+ cm^{-2} h^{-1})
Histamine (90 μM)	0.87 ± 0.18 (6)
Histamine (90 μM) + UK-14,304 (10μM)	0.95 ± 0.13 (6)
Pentagastrin (64 nM)	0.84 ± 0.17 (6)
Pentagastrin (64 nM) + UK-14,304 (10 μM)	0.74 ± 0.15 (6)

Values are means ± s.e. (*n*).

Discussion

The acid secretory responses of the rat isolated gastric mucosa to electrical field stimulation were first described by Baird & Main (1978). The characteristics of the response led them to conclude that the effect was mediated by postganglionic cholinergic nerves of vagal origin; basically similar results were obtained with mouse stomach following electrical stimulation (Angus & Black, 1978; 1982). We obtained acid secretory responses which resembled closely those observed by Baird & Main (1978) in magnitude and duration, although the initial response to stimulation was invariably the largest of any responses obtained in one preparation and for this reason the first response only of any preparation was used in the studies described here.

In our studies, clonidine inhibited the acid secretory responses of the gastric mucosa to electrical stimulation, confirming in the isolated preparation of the stomach the observations of others who stimulated the vagus nerve and found an inhibition of gastric acid secretion by clonidine in the anaesthetized rat (Jennewein, 1977; Cheng *et al.*, 1981). The incomplete nature of the inhibitory effect of clonidine is suggestive of a partial agonist action as reported by Medgett *et al.* (1978). The inhibition of the acid secretory responses of the isolated gastric mucosa by α_2-adrenoceptor agonists, as studied in the experiments with the selective α_2-adrenoceptor agonist UK-14,304 (Cambridge, 1981), appeared to be dependent upon the frequency of stimulation since responses elicited at higher frequencies (5 Hz and 10 Hz) were relatively insensitive to the action of the agonist. The increased effectiveness of UK-14,304 at lower frequencies has also been reported for its inhibitory action on stomach contractile responses elicited by electrical field stimulation (Dettmar *et al.*, 1985). In the guinea-pig ileum preparation, Paton & Vizi (1969) observed that noradrenaline inhibited acetylcholine release only at low frequencies of stimulation.

In the rat isolated gastric mucosa preparation we were unable to detect any stimulatory effect on acid secretion following administration of clonidine, an action which was attributed to histamine-like properties in the guinea-pig isolated stomach preparation (Del Tacca *et al.*, 1982). We also found that cimetidine failed to block the effects of field stimulation as originally described by Baird & Main (1978), although in the mouse whole stomach preparation, metiamide at high concentration (1 mM) virtually abolished the secretory responses to field stimulation (Angus & Black, 1982). Bunce *et al.* (1976) have reported that the secretagogue action of cholinomimetics was insensitive to metiamide in the immature rat stomach preparation. Thus it appears there is a lack of involvement of histamine in the secretagogue action of acetylcholine in isolated stomach preparations of the rat in contrast to those from the mouse. The increases in acid secretion which we observed at high concentrations of noradrenaline have been reported by Canfield *et al.* (1981) to be due to an action at β-adrenoceptors.

The relative potencies of the α_2-adrenoceptor agonists (*para*-aminoclonidine > noradrenaline > UK-14,304 > clonidine) in reducing the acid secretory responses of the gastric mucosa to field stimulation is suggestive of an action at α_2-adrenoceptors. This is supported by the ineffectiveness of phenylephrine, an α_1-adrenoceptor agonist. The lack of any postjunctional effect against responses elicited by acetylcholine, which acts directly upon the parietal cells to stimulate acid release, and the lack of any effect against responses elicited by pentagastrin or histamine, would indicate that the α_2-adrenoceptors are located prejunctionally on the cholinergic fibres supplying the mucosa. Direct evidence that clonidine

reduced acetylcholine released by the vagus nerve in isolated stomach preparations has been obtained by Del Tacca *et al.* (1982) who assayed the release of transmitter; they also found that the inhibition of acetylcholine by clonidine was antagonized by the α_2-adrenoceptor antagonist, yohimbine. In the acid secretory experiments in rat gastric mucosa we found that the selective α_2-adrenoceptor antagonist, idazoxan (Chapleo *et al.*, 1981) antagonized the effects of UK-14,304, the selective α_2-adrenoceptor agonist, while the α_1-antagonist, prazosin, was ineffective, providing strong evidence of the nature of the α-adrenoceptors on the nerve terminals. Similar conclusions regarding the nature of the α-adrenoceptors on the cholinergic nerve terminals in the rat gastric fundus were reached by Verplanken *et al.* (1984) who examined electrically-induced contractions of longitudinal muscle strips. In a study of the relaxant effect of α_2-adrenoceptor agonists they found noradrenaline to be more potent than clonidine or UK-14,304 although in a departure from our findings they found evidence of a prejunctional action with phenylephrine.

The concentration of idazoxan required to antagonize the inhibitory effect of UK-14,304 on acid secretion resulting from field stimulation was substantially greater than those which are effective in other *in vitro* systems such as guinea-pig ileum and rat vas deferens preparations where concentrations in the range of 40 and 400 nM idazoxan have been used to displace the concentration-response curve for clonidine (Doxey *et al.*, 1983). In isolated stomach preparations of the rat, where electrical stimulation-induced contractions were measured, Dettmar *et al.* (1985) used a concentration of idazoxan of 1 μM to obtain displacement of the dose-effect curve of UK-14,304 and a similar concentration of yohimbine was used by Verplanken *et al.* (1984) for a similar purpose. It is characteristic of perfused isolated stomach preparations that higher antagonist concentrations are required to block the effects of secretagogues (Angus & Black, 1979; Bunce *et al.*, 1977). Angus & Black (1979) found that the pK_B for atropine when measured against bethanechol-induced acid secretion was significantly lower than in the perfused isolated stomach of the mouse when measured against bethanechol-induced smooth muscle contraction. In our experiments a relatively high concentration of 100 nM atropine was required to reduce the acid secretory response to nerve stimulation and Angus & Black (1982) made a similar observation in the electrically stimulated mouse stomach preparation. Angus & Black (1978) have suggested there is removal of atropine from the receptor compartment in the isolated stomach preparation of the mouse and loss to the perfusion solution which affects the equilibrium conditions and leads to anomalously high values for the pK_B of atropine. Presumably the same process occurs with the α_2-adrenoceptor antagonist employed in this study and similar consequences with regard to the concentrations required for antagonism.

We have thus demonstrated in an isolated stomach preparation that clonidine and other α_2-adrenoceptor agonists inhibit the acid secretory response to nerve stimulation, confirming the experiments of others who have used intact animal preparations and have suggested a peripheral component to the action of clonidine in addition to a central component (Jennewein, 1977; Cheng *et al.*, 1981). The effectiveness of these α_2-adrenoceptor agonists, which include noradrenaline, in reducing acid secretion by an action that is evidently mediated by α_2-adrenoceptors located on postganglionic cholinergic terminals of the vagus suggests that in gastric acid secretion adrenergic nerves may have an inhibitory role exerted upon enteric cholinergic neurones as proposed by Furness & Costa (1974) in relation to the control of gastrointestinal motility.

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