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ECL-cell histamine mobilization in conscious rats: effects of locally applied regulatory peptides, candidate neurotransmitters and inflammatory mediators

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1 The ECL cells control gastric acid secretion by mobilizing histamine in response to circulating gastrin. In addition, the ECL cells are thought to operate under nervous control and to be influenced by local inflammatory processes.

2 The purpose of the present study was to monitor histamine mobilization from ECL cells in conscious rats in response to locally applied regulatory peptides, candidate neurotransmitters and inflammatory mediators.

3 Microdialysis probes were implanted in the submucosa of the acid-producing part of the rat stomach. Three days later, the agents to be tested were administered *via* the microdialysis probe and their effects on basal (48 h fast) and stimulated (intravenous infusion of gastrin-17, 3 nmol kg^{-1} h^{-1}) mobilization of ECL-cell histamine was monitored by continuous measurement of histamine in the perfusate (radioimmunoassay).

4 Locally administered gastrin-17 and sulfated cholecystokinin-8 mobilized histamine as did pituitary adenylate cyclase-activating peptide-27, vasoactive intestinal peptide, peptide YY, met-enkephalin, endothelin and noradrenaline, adrenaline and isoprenaline.

5 While gastrin, sulfated-cholecystokinin-8, met-enkephalin and isoprenaline induced a sustained elevation of the submucosal histamine concentration, endothelin, peptide YY, pituitary adenylate cyclase activating peptide, vasoactive intestinal peptide, noradrenaline and adrenaline induced a transient elevation.

6 Calcitonin gene-related peptide, galanin, somatostatin and the prostanoid misoprostol inhibited gastrin-stimulated histamine mobilization.

7 The gut hormones neurotensin and secretin and the neuropeptides gastrin-releasing peptide, neuropeptide Y and substance P failed to affect ECL-cell histamine mobilization, while motilin and neuromedin U-25 had weak stimulatory effects. Also acetylcholine, carbachol, serotonin and the amino acid neurotransmitters aspartate, γ-aminobutyric acid, glutamate and glycine were inactive or weakly active as was bradykinin.

8 In summary, a range of circulating hormones, local hormones, catecholamines, neuropeptides and inflammatory mediators participate in controlling the activity of rat stomach ECL cells *in situ*. *British Journal of Pharmacology* (2001) **134,** 1767–1777

Keywords: ECL cells; microdialysis; histamine; VIP; PACAP; galanin; somatostatin; prostaglandins

Abbreviations: CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; GABA, γ-aminobutyric acid; GRP, gastrin-releasing peptide; NMU, neuromedin U; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating peptide; PYY, peptide YY; SP, substance P; VIP, vasoactive intestinal peptide

Introduction

ECL cells in the oxyntic mucosa control gastric acid secretion (Håkanson & Sundler, 1991; Waldum *et al*., 1991; Andersson *et al*., 1996). They respond to gastrin with the release of histamine, flooding adjacent parietal cells. While the existence of a gastrin-ECL cell-parietal cell axis seems widely accepted today (Lindström *et al*., 2001), there is no consensus as to how the nervous system controls the ECL cells and the parietal cells. Most nerve fibres in the oxyntic mucosa form part of the enteric nervous system, which operates under vagal and sympathetic control. Candidate neurotransmitters in nerve fibres in the gastric mucosa include not only acetylcholine and noradrenaline (Schultzberg *et al*., 1980; Furness *et al*., 1983) but also neuropeptides such as calcitonin gene-related peptide (CGRP), enkephalins, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), neuropeptide Y (NPY), peptide YY (PYY, a constituent of both enteric nerves and endocrine/paracrine cells), neurotensin, neuromedin U (NMU), motilin, galanin, gastrin-releasing peptide (GRP), somatostatin and substance P (SP) (the latter two peptides occur in both enteric nerves and endocrine/paracrine cells) (Schultzberg *et al*., 1980; Sundler *et al*., 1982; 1992; Ekblad *et al*., 1985a, b; 1991; Sternini *et al*., 1987; Furness *et al*., 1989; Böttcher *et al*., 1993; Hannibal *et al*., 1998). Also, serotonin (a constituent of both nerves and endocrine cells) and amino acids such as aspartate, glycine, glutamate and γ-aminobutyric acid (GABA), known to act as neurotransmitters in the brain,

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may function as neurotransmitters in the enteric nervous system as well. Futhermore, inflammatory mediators, such as prostaglandins, seem to control the functional activity of the ECL cells (Sandvik & Waldum, 1988a; Lindström & Håkanson, 1998).

The purpose of the present study was to explore how ECL cells in intact, conscious rats respond to various locally applied neurotransmitter candidates and inflammatory mediators. Although isolated rat stomach ECL cells are known to be stimulated by gastrin, PACAP, VIP and adrenaline and inhibited by somatostatin, galanin and prostaglandins (Prinz *et al*., 1993; 1994a,b; Lawton *et al*., 1995; Sandor *et al*., 1996; Lindström *et al*., 1997; Lindström & Håkanson, 1998; 2001; Zeng *et al*., 1998; 1999), only gastrin has been studied *in vivo* (Kitano *et al*., 2000; Konagaya *et al*., 2001). The paucity of *in vivo* information reflects methodological shortcomings and the facts that (1) histamine is rapidly degraded once released from the ECL cells, (2) circulating histamine derives not only from ECL cells but also from mast cells and basophils and (3) systemically administered neurotransmitters have interfering extra-gastric effects. Recently it was shown that histamine mobilization from ECL cells *in situ* can be monitored in conscious rats by the use of gastric submucosal microdialysis (Kitano *et al*., 2000; Norlén *et al*., 2000). In this study, agents to be tested were administered locally in the gastric submucosa *via* a microdialysis probe and their effects on basal and gastrin-stimulated ECL-cell histamine mobilization were monitored.

Methods

Chemicals

Human Leu^{15}-gastrin-17 and sulphated cholecystokinin-8 (CCK-8s) were purchased from Research Plus (Bayonne, NJ, U.S.A.). Rat α-CGRP was from Sigma, St. Louis (MO, U.S.A.). All other peptides were from Peninsula Europe (Merseyside, St. Helens, U.K.) bradykinin, met-enkephalin, human endothelin-1, rat galanin, GRP, motilin, bovine neurotensin, rat NMU-25, NPY, PACAP-27, PYY, porcine secretin, somatostatin-14, SP and VIP. The prostaglandin E_1 agonist misoprostol was a kind gift from Searle (Skokie, IL, U.S.A.). All amino acids, amines and choline esters were from Sigma. All agents were dissolved in 0.9% saline and tested with respect to their interference with the radioimmunoassay of histamine. Only gastrin (at high concentrations) was found to interfere, precluding the use of concentrations greater than 0.1 mmol l^{-1} for local infusion.

Animals

Sprague-Dawley rats (250–300 g) were kept at a 12-h light/12-h dark cycle in plastic cages (4–6 animals in each cage) with free access to standard rat food pellets (Lactamin, Vadstena, Sweden) and tap water. Rats to be fasted were housed in individual cages with wire mesh bottoms for 48 h. During perfusion of the microdialysis probes, they were kept in Bollman-type restraining cages. Starting 1 week prior to the experiments all rats had been familiarized with the Bollman cages by daily training for 1–2 h. The studies were approved by the local Animal Welfare Committee, Lund.

Implantation of the microdialysis probe and sampling of microdialysate

A flexible microdialysis probe (MAB3.8.10, AgnTho's AB, Stockholm, length 10 mm, outer diameter 0.57 mm, 35 kDa cut-off) was used (Kitano *et al*., 2000). Surgery was performed under chloral hydrate anaesthesia (300 mg kg^{-1} intraperitoneally). The abdomen was opened by a midline incision. The serosa of the ventral aspect of the acid-producing part of the stomach was tangentially punctured by a needle (22 G) and a tunnel (15–20 mm) was made in the submucosal layer from the greater to the lesser curvature. The probe was inserted into the tunnel and kept in place with sutures. The inlet and outlet tubes were passed through the abdominal opening and tunnelled under the skin to a point at the nape of the neck. Rats to be infused intravenuously with gastrin were fitted with a catheter in the right jugular vein. This operation was done at the same time as the implantation of the probe. All rats were fasted for 48 h before start of the microdialysis.

Microdialysis was performed 3 days after the implantation of the probe. All rats remained conscious throughout the experiment since anaesthesia has been shown to inhibit mobilization of histamine from the ECL cells (Norlén *et al*., 2000). The inlet tube of the microdialysis probe was connected to a microinfusion pump (Model 361, Sage instrument, ATI Orion, Boston, MA, U.S.A.) and the outlet tube was allowed to drain into 300 μl polyethylene vials. Perfusion of the microdialysis probes with 0.9% saline (1.2 μl min^{-1}) started at 7 a.m. After a 2-h equilibration period, collection of microdialysate commenced. The time taken for the solution to be transported from membrane to the outlet of the probe was determined (3 min) as was the time taken for the solution to be transported from the inlet to the outlet of the probe (6 min). Each rat and each probe was used once only. After completion of the experiment, each rat was killed by exsanguination under chloral hydrate anaesthesia and the position of the probe in the submucosa was verified by dissection of the stomach. When the agents were to be applied locally they were dissolved in 0.9% saline and perfused through the microdialysis probe. All peptides and misoprostol were screened at a concentration of 0.1 mmol l^{-1} while amino acids, amines and choline esters were applied at a concentration of 10 mmol l^{-1} (if not otherwise stated) in at least three independent experiments. Stimulatory effects were assessed in fasted but otherwise untreated rats, while inhibitory effects were assessed during intravenous gastrin infusion (3 nmol kg^{-1} h^{-1}) in fasted rats. All agents were screened for stimulatory effects (see Table 1). All agents that failed to raise the microdialysate histamine concentration when applied to fasted rats were screened for inhibitory effects. Concentration-response curves were constructed for all agents that were found to stimulate or inhibit ECL-cell histamine mobilization.

Stimulatory effects

Microdialysate samples for determination of basal histamine secretion in fasted rats were collected during 2 h. At this time point (time zero) perfusion with saline was exchanged for perfusion with the agent to be tested (in saline). Samples were collected every 20 min during the first hour of stimulation

Table 1 Stimulatory (+) or suppressive (−) effects of various agents on histamine mobilization from ECL cells *in situ*

Substance	*Effect*	*Concentration* (mmol l^{-1})	*Integrated response* (%)	*Peak response* (%)	*Number of rats*
Peptides					
CCK-8s	+	0.1*	+220±35	+340±59	7 (32)
Gastrin	+	0.1*	+280±21	+460±170	5 (25)
Galanin	−	0.1*	−62±12		4 (25)
GRP	0	0.1			(6)
Met-enkephalin	+	3*	+380±120	+610±230	6 (23)
Motilin	0	0.1			(4)
Neurotensin	0	0.1			(5)
NMU-25	0	0.1			(6)
PACAP-27	+	0.1*	+200±31	+520±170	5 (23)
VIP	+	0.1*	+150±11	+510±150	6 (21)
PYY	+	0.1*	+280±46	+1200±140	4 (17)
NPY	0	0.1			(6)
Secretin	0	0.1			(4)
Somatostatin	−	1*	−78±9		4 (32)
SP	0	0.1			(6)
CGRP	−	0.1	−61±7		4 (21)
Endothelin	+	0.03*	+1700±180	+6700±1000	7 (24)
Amino acids and amines					
Acetylocholine	0	10			(7)
Carbachol	0	1			(17)
Adrenaline	+	10*	+440±100	+2400±800	4 (21)
Noradrenaline	+	10*	+300±92	+890±310	4 (20)
Isoprenaline	+	100*	+410±28	+450±59	5 (26)
Aspartate	0	10			(7)
GABA	0	10			(6)
Glutamate	0	10			(7)
Glycine	0	10			(7)
Serotonin	0	10			(6)
Inflammatory mediators					
Bradykinin	0	0.1			(3)
Misoprostol	−	0.3*	−77±8		5 (25)

Any effect smaller than the minimum response required to be regarded as a stimulator or inhibitor is given as 0. To be accepted as a stimulator or an inhibitor, an agent (at the screening concentration) should (at least) induce a 100% increase (+100%) over the basal histamine mobilization or a 50% reduction (−50%) in gastrin-stimulated histamine mobilization. Stimulatory effects are presented as the rise in microdialysate histamine concentration over basal levels during the 3 h stimulation period (integrated response) and as peak response. Inhibitory effects are presented as the integrated per cent inhibition of gastrin-stimulated rise in histamine mobilization (3 h). Concentration-response curves were constructed for all agents that qualified as stimulators or inhibitors. The near-maximally effective concentration (*) is given for all stimulators and inhibitors while the screening concentration is given for those agents that had no or little stimulatory or inhibitory effect. The integrated and peak response at a near-maximally effective concentration is shown for each stimulator or inhibitor. Mean values±s.e.mean, number of animals is given for the near-maximally effective concentration, total number of animal for each agent is presented in brackets.

and then every 60 min during the subsequent 2 h. To be accepted as a stimulator an agent should (at least) induce a 2 fold increase (integrated response) over the basal histamine mobilization. Concentration-response studies were carried out for all agents that were accepted as stimulators at the screening concentration.

Agents that were found to stimulate ECL-cell histamine mobilization less than 2 fold at the screening concentration, were usually applied at a 10 times higher concentration. Concentration-response studies were conducted if the agent induced more than 2 fold increase in histamine mobilization at the higher concentration.

Inhibitory effects

After 2 h of sampling from fasted rats for determination of the basal histamine concentration in the gastric submucosa, ECL-cell histamine mobilization was induced by continuous intravenous infusion of gastrin-17 (3 nmol kg^{-1} h^{-1}, 1 ml h^{-1}). Samples were collected every 20 min during the first hour and then every hour. After 2 h of gastrin infusion, perfusion of the microdialysis probe with saline was exchanged for perfusion with saline containing the agent to be studied. Intravenous gastrin infusion continued throughout the experiment. Blood was drawn from the tail vein for analysis of serum gastrin. The basal serum gastrin concentration was determined (radioimmunoassay) during the equilibration period preceding the first sampling of microdialysate, while the serum gastrin concentration during gastrin infusion was determined after the last microdialysate fraction had been collected. To be accepted as an inhibitor an agent should (at least) induce a 50% decrease in gastrin-stimulated histamine mobilization during the 3 h period. Concentration response studies were performed for all agents that qualified as inhibitors.

Analysis of microdialysate and serum samples

Histamine in the microdialysates was measured by radioimmunoassay using a commercially available kit (Immuno-

tech, Marseille, France). The histamine concentration was expressed as nmoles per litre. The serum gastrin concentration was determined by radioimmunoassay as previously described (Stadil & Rehfeld, 1973), using antiserum no. 2604 (a kind gift from Dr J.F. Rehfeld, Copenhagen, Denmark), and expressed as picomole equivalents of rat gastrin-17 per litre.

Statistical analysis

Data are presented as means ± s.e.mean. Stimulatory effects are presented as the rise in microdialysate histamine concentration over basal levels during the 3 h stimulation period (integrated response) or as peak response. Inhibitory effects are presented as the integrated per cent inhibition of the gastrin-stimulated rise in histamine mobilization during 3 h; the rise in histamine was calculated by subtracting the basal microdialysate histamine concentration from the microdialysate histamine concentration during the second hour of gastrin infusion. Concentration-response curves were constructed using a GraphPad PRISM program (version 3.00, GraphPad Software, San Diego, CA, U.S.A.). EC_{50} and IC_{50} values (i.e. the concentrations that induced half maximal effect) were not calculated because they are affected by a variety of factors beside the kinetics of ligand-receptor interaction (rate of diffusion over the microdialysis membrane, penetration to target, degradation in tissue) and consequently the values are not meaningful.

Results

The basal microdialysate histamine concentration was 27 ± 2 pmol l^{-1} ($n = 30$). Intravenous infusion of gastrin (3 nmol kg^{-1} h^{-1}) resulted in a 30 fold elevation of serum gastrin compared to untreated fasted rats (from 22 ± 3 to 620 ± 67 pmol l^{-1}, $n = 8$) and raised the microdialysate histamine concentration 3 fold (from 30 ± 3 to 88 ± 5, $n = 15$).

Gastrin and CCK

Both gastrin-17 and CCK-8s are known to stimulate ECL-cell histamine mobilization. In the present study, the peptides were applied by local perfusion and found to induce a strong histamine response. At a near-maximally effective concentration of gastrin or CCK (0.1 mmol l^{-1} in the microdialysate), the histamine concentration in the microdialysate was four times higher than basal (Figure 1A–D).

Choline esters

Neither acetylcholine (10 mmol l^{-1} in the microdialysate) (Table 1) nor carbachol (1 mmol l^{-1}) (Figure 2A) raised the microdialysate histamine concentration and neither acetylcholine nor carbachol inhibited the gastrin-induced histamine mobilization (not shown in Figure 2). Attempts to give higher concentrations of carbachol than 1 mmol l^{-1} were discontinued because of systemic effects (salivation and pupil constriction).

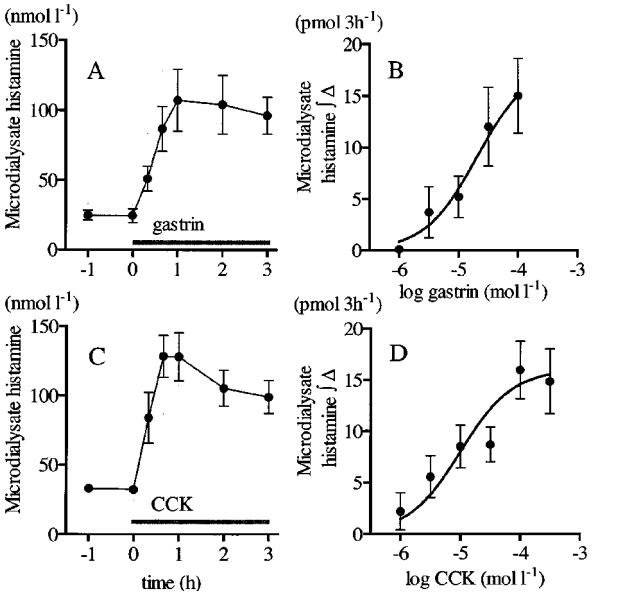

Figure 1 Time course (A,C) and concentration-response (B,D) curves for gastrin-17 (A,B) and CCK-8S (C,D). Gastrin and CCK were administered *via* the microdialysis probe. The concentration administered in (A) and (C) was 0.1 mmol l^{-1}. Stimulation started at time zero and lasted for 3 h (as indicated by horizontal line). The integrated response (B,D) was calculated in each experiment. Mean ± s.e.mean, $n = 4–7$.

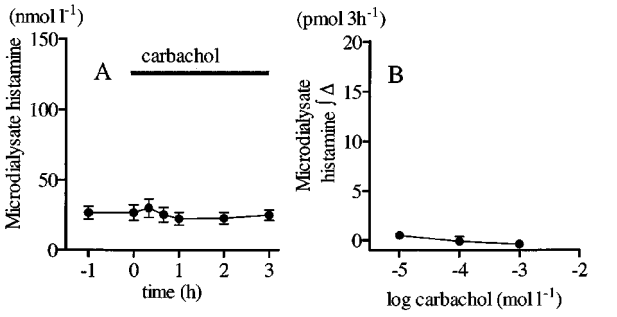

Figure 2 Time course (A) and concentration-response (B) curves for carbachol. The substance was administered *via* the microdialysis probe. The concentration administered in (A) was 1 mmol l^{-1}. Stimulation started at time zero and lasted for 3 h (as indicated by horizontal line). The integrated response (B) was calculated. Mean ± s.e.mean, $n = 4–6$.

Catecholamines

Noradrenaline and adrenaline mobilized histamine quite effectively. At a near-maximally effective concentration (10 mmol l^{-1}), the noradrenaline and adrenaline-stimulated histamine release displayed a peak response about 10 and 20 times higher than the basal level. The response was characteristically transient: it peaked after 20–40 min, its subsequent decline was apparent 1 h after start of infusion and the histamine concentration in the microdialysate was back to pre-stimulation levels after about 2 h despite continued infusion of the catecholamines. Administration of isoprenaline (100 mmol l^{-1}) resulted in a 5 fold increase in the microdialysate histamine level; with this agent the histamine concentration remained at a plateau throughout the stimulation period (Figure 3A–F).

Amino acids and serotonin

All amino acids were applied at a concentration of 10 mmol l^{-1} in the microdialysate. GABA had a weak stimulatory effect at the screening concentration (59±18% increase, integrated response, $n=3$). At a concentration of 100 mmol l^{-1} GABA increased the microdialysate histamine concentration by 58±14% ($n=3$). Thus, it did not qualify as a stimulator. Aspartate, glutamate, glycine, and serotonin were without stimulatory effect and did not reduce gastrin-evoked histamine mobilization (not shown).

Regulatory peptides

All peptides were applied at a concentration of 0.1 mmol l^{-1} (if not otherwise stated). Endothelin, met-enkephalin, PYY, PACAP and VIP had strong stimulatory, concentration-dependent effects on ECL-cell histamine mobilization (Table 1). Near-maximally effective concentrations of PACAP and VIP (0.1 mmol l^{-1}) resulted in a prompt 6 fold increase in the microdialysate histamine concentration within 20–40 min, followed by a decline to levels about twice higher than the basal histamine concentration after 3 h (Figure 4A–D). Within 40 min, endothelin (0.03 mmol l^{-1}) produced a peak in the microdialysate histamine concentration about 50 times higher than the basal level. The histamine concentration approached pre-stimulation levels after 3 h (Figure 5A,B). PYY (but not NPY) stimulated histamine mobilization more than 10 fold (peak response). The response was prompt but short-lasting and the microdialysate histamine concentration was back to basal within 3 h (Figure 5C,D). The stimulatory effect of met-enkephalin (4 fold increase of the microdialysate histamine concentration at a concentration of 1 mmol l^{-1}) persisted throughout the stimulation period (Figure 5E,F). NMU-25 and motilin had weak stimulatory effects at the screening concentration (the integrated microdialysate histamine concentration increased by 88±29%, $n=6$ and 47±18%, $n=4$, respectively). Secretin increased the microdialysate histamine by 37±23% ($n=4$). CGRP, galanin, GRP, neurotensin, NPY, somatostatin and SP were without effect on basal histamine secretion (not shown).

Somatostatin, galanin and CGRP inhibited the gastrin-evoked histamine response concentration-dependently (Figure 6A–F). The inhibitory effects of somatostatin, galanin and CGRP were 78±9 ($n=4$), 62±12 ($n=6$) and 61±7 ($n=4$) %, at a concentration of 1, 0.1 and 0.1 mmol l^{-1}, respectively (Table 1). GRP, neurotensin, NPY, PYY and SP were without effect (not shown). Neuropeptides that stimulated basal histamine secretion were not tested for inhibitory effects (with the exception of PYY that was tested for inhibitory effects despite its stimulatory action).

Inflammatory mediators

Bradykinin (0.1 mmol l^{-1}) had a weak stimulatory effect (42±6% increase, $n=3$) (integrated response). The prostaglandin E_1 analogue misoprostol (0.1 mmol l^{-1}) was without effect on basal histamine mobilization.

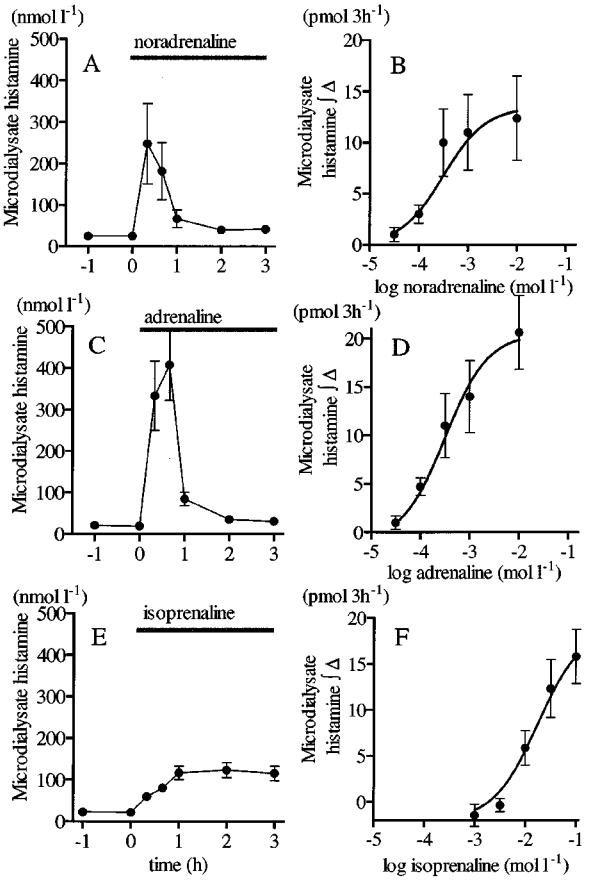

Figure 3 Time course (A,C,E) and concentration-response (B,D,F) curves for noradrenaline (A,B), adrenaline (C,D) and isoprenaline (E,F). The substances were administered *via* the microdialysis probe. The concentration administered in (A) and (C) was 10 mmol l^{-1} and in (E) 100 mmol l^{-1}. Stimulation started at time zero and lasted for 3 h (as indicated by horizontal line). The integrated response (B,D,F) was calculated. Mean±s.e.mean, $n=4-6$.

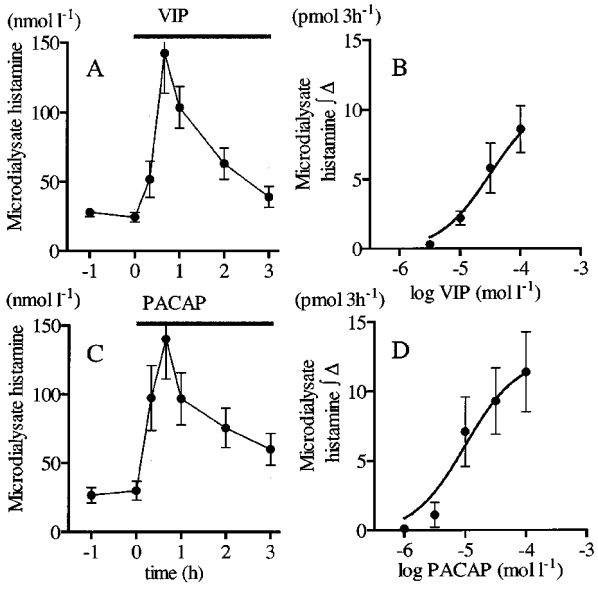

Figure 4 Time course (A,C) and concentration-response (B,D) curves for VIP (A,B) and PACAP-27 (C,D). VIP and PACAP were administered *via* the microdialysis probe. The concentration administered in (A) and (C) was 0.1 mmol l^{-1}. Stimulation started at time zero and lasted for 3 h (as indicated by horizontal line). The integrated response (B,D) was calculated. Mean±s.e.mean, $n=4-7$.

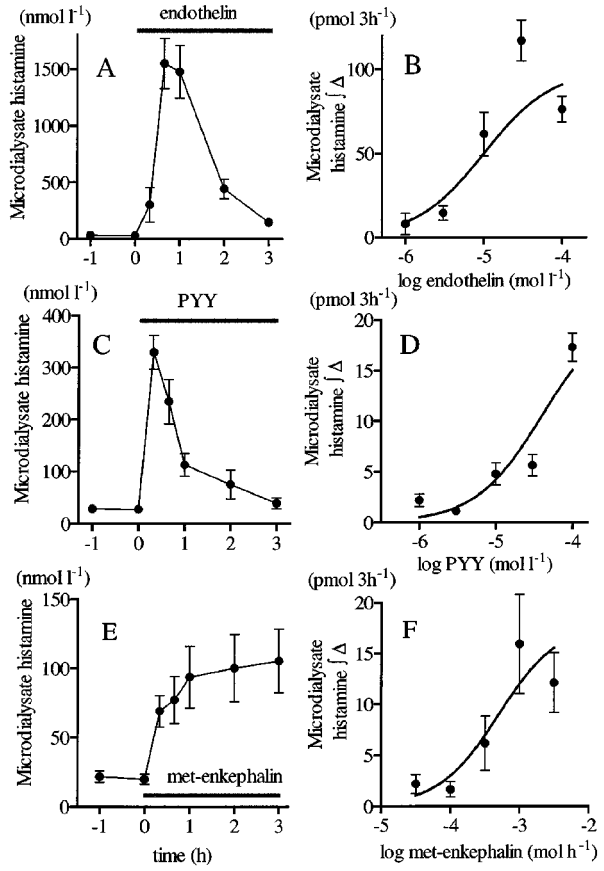

Figure 5 Time course (A,C,E) and concentration-response (B,D,F) curves for endothelin (A,B), PYY (C,D) and met-enkephalin (E,F). The substances were administered *via* the microdialysis probe. In (A), (C) and (E), endothelin, PYY and met-enkephalin were administered *via* the microdialysis probe at a concentration of 0.03, 0.1 and 3 mmol l^{-1}, respectively. Stimulation started at time zero and lasted for 3 h (as indicated by horizontal line). The integrated response (B,D,F) was calculated. Mean ± s.e.mean, $n = 4-7$.

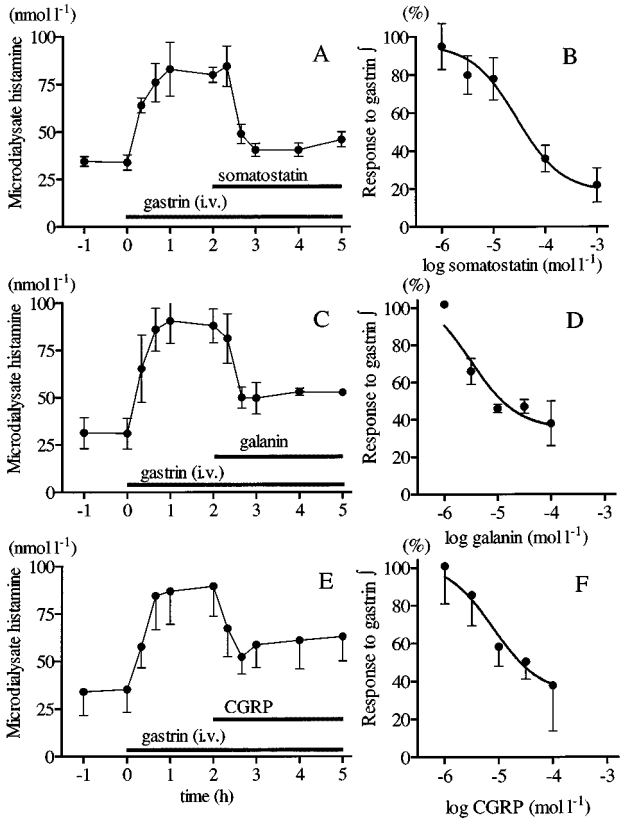

Figure 6 Time course (A,C,E) and concentration-response (B,D,F) curves for somatostatin (A,B), galanin (C,D) and CGRP (E,F). The rats received intravenous infusion of gastrin (3 nmol kg^{-1} h^{-1}), starting at time zero and lasting for 5 h (as indicated by horizontal line). In (A), (C) and (E), somatostatin, galanin and CGRP were administered *via* the microdialysis probe at a concentration of 1, 0.1 and 0.1 mmol l^{-1}, respectively, starting 2 h after gastrin (horizontal line). The integrated response (B,D,F) was calculated. Mean ± s.e.mean, $n = 4-10$.

Misoprostol inhibited gastrin-stimulated histamine mobilization concentration-dependently, the maximal inhibition being 77 ± 8% (0.3 mmol l^{-1}, $n = 4$, Figure 7A,B, Table 1).

Discussion

Vagal excitation and elevated levels of circulating gastrin are known to stimulate acid secretion, while the effects of sympathetic nerve stimulation are less clear-cut. Gastrin acts by causing the ECL cells to secrete histamine, which in turn stimulates the parietal cells to secrete HCl (Kahlson *et al*., 1964; Code, 1965; Black & Shankley, 1987; Håkanson & Sundler, 1991; Waldum *et al*., 1991; Andersson *et al*., 1996). The vagal input to the stomach is transmitted to special command neurons in the myenteric ganglia (Schemann, 1992). Thus, neurotransmitters in vagal nerve fibres are unlikely to activate either ECL cells or parietal cells directly. Instead, transmitters in vagally controlled enteric neurons, such as acetylcholine, CGRP, enkephalins, galanin, PACAP and VIP may act directly on the parietal cells to modulate acid secretion and/or indirectly *via* the G cells (gastrin secretion) or the ECL cells (histamine secretion). While ECL cells in primary culture do not respond to acetylcholine (Lindström *et al*., 1997; Lindström & Håkanson, 2001), they respond readily to gastrin and the neuropeptides PACAP and VIP (Sandor *et al*., 1996; Lindström *et al*., 1997; Zeng *et al*., 1999; Lindström & Håkanson, 2001). They also respond to adrenaline and noradrenaline, probably *via* β-adrenoceptor activation (Lawton *et al*., 1995; Lindström *et al*., 1997; Lindström & Håkanson, 2001). Stimulated ECL cells in primary culture are inhibited by somatostatin, by the neuropeptide galanin and by inflammatory mediators of the prostaglandin E_1 and E_2 type (Prinz *et al*., 1994b; Sandor *et al*., 1996; Lindström *et al*., 1997; Lindström & Håkanson, 1998; Zeng *et al*., 1998). How various neuropeptides and local hormones affect ECL-cell histamine mobilization *in vivo* is still largely unknown. One reason that this area remains unexplored is that administration of candidate neurotransmitters, local hormones and inflammatory mediators by the conventional routes is likely to cause systemic effects that will complicate the interpretation of the results.

The technique of microdialysis offers an advantage in that administration of agents *via* the microdialysis probe will result in high local concentrations with little risk of systemic effects. Moreover, since locally administered agents should affect acid secretion only locally, they are not likely to affect

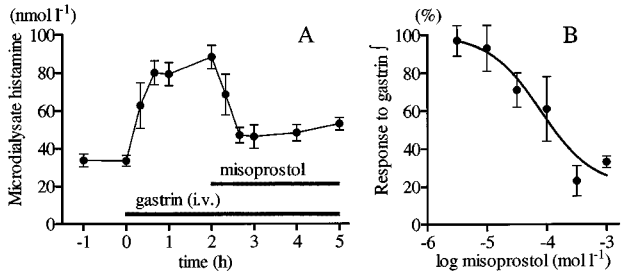

Figure 7 Time course (A) and concentration-response (B) curves for the prostaglandin E_1 congener misoprostol. The rats received intravenous infusion of gastrin-17 (3 nmol kg^{-1} h^{-1}), starting at time zero and lasting for 5 h (as indicated by horizontal line). In (A), misoprostol was administered *via* the microdialysis probe at a concentration of 0.3 mmol l^{-1}, starting 2 h after gastrin (horizontal line). The integrated response (B) was calculated. Mean ± s.e.mean, $n = 4-5$.

the intragastric pH. This is important since any change in the intragastric pH will affect the circulating concentrations of gastrin and as a consequence influence histamine mobilization from the ECL cells. A problem with the local administration of agents *via* the microdialysis probe is that the agent will create a concentration gradient in the tissue surrounding the probe and that the actual concentration that produces the response cannot be determined. Nonetheless, concentration-response experiments were conducted, not in an attempt to define the potency of the various agents but rather to make sure that maximally or near-maximally effective concentrations of the different agents were used.

We propose that to be characterized tentatively as a stimulator an agent should induce at least a 2 fold increase (integrated response) in the amount of histamine mobilized during 3 h. To be characterized as an inhibitor an agent should induce at least 50% decrease in the gastrin-stimulated histamine mobilization. The results are summarized in Table 1.

Stimulatory agents

Gastrin and CCK As expected both agents stimulated ECL-cell histamine mobilization. At near-maximal concentrations (0.1 mmol l^{-1}) they induced a sustained 4 fold increase in microdialysate histamine, similar to what has previously been reported after continuous intravenous administration of maximally effective doses of gastrin to fasted rats (Kitano *et al*., 2000; Konagaya *et al*., 2001). When gastrin is given intravenously, ECL cells respond with near-maximum histamine mobilization to a circulating gastrin concentration of approximately 1 nmol l^{-1} (Kitano *et al*., 2000; Konagaya *et al*., 2001). In the present study, a gastrin concentration of 0.1 mmol l^{-1} in the perfusate had a near-maximum effect, suggesting that the resulting local gastrin concentration was in the range of 1 nmol l^{-1}.

PACAP and VIP PACAP and VIP are well known constituents of enteric neurons (Ekblad *et al*., 1991; Sundler *et al*., 1992; Hannibal *et al*., 1998) and have been shown to inhibit acid secretion in anaesthetized rats and in the isolated perfused rat stomach (Schorr *et al*., 1974; Viller *et al*., 1976; Makhlouf *et al*., 1978; Mungan *et al*., 1992; 1995; Li *et al*., 2000). It is notable therefore that histamine secretion from isolated ECL cells in primary culture is stimulated by both PACAP and VIP (Sandor *et al*., 1996; Lindström *et al*., 1997; Zeng *et al*., 1999; Lindström & Håkanson, 2001). One way to reconcile the inhibitory effect of PACAP and VIP on acid secretion with their stimulating effect on ECL-cell histamine secretion, is to suggest that the two neuropeptides mobilize also somatostatin (from D cells in the oxyntic mucosa) (Chiba *et al*., 1980a, b; 1985; Saffouri *et al*., 1984; Schubert, 1991), which in turn inhibits both parietal cells and ECL cells (Chew, 1983; Sandvik & Waldum, 1988b; Prinz *et al*., 1994b). Indeed, it was recently reported that PACAP stimulates acid secretion in anaesthetized rats when somatostatin was eliminated by immunoneutralization (Zeng *et al*., 1999). The present findings favour the view that PACAP and VIP stimulate ECL-cell histamine mobilization in conscious rats.

Other regulatory peptides Met-enkephalin, which has been demonstrated in nerve cell bodies and fibres of the enteric nervous system (Ekblad *et al*., 1985a; 1991), increased the microdialysate histamine concentration 3–4 fold. This is surprising in that enkephalins did not stimulate secretion from isolated ECL cells in primary culture (Lindström *et al*., 1997). Possibly, the effects seen *in vivo* are exerted on non-ECL cells such as D cells: indeed met-enkephalin has been shown to inhibit somatostatin secretion from gastric D cells (Chiba *et al*., 1980b). PYY occurs in a special neuronal system in the rat stomach (Böttcher *et al*., 1993). In this study, PYY had a powerful albeit transient stimulatory effect on ECL-cell histamine mobilization which contrasts with earlier reports that PYY inhibits rather than stimulates acid secretion (Guo *et al*., 1987; Eissele *et al*., 1990). NPY was without effect. Endothelin is a potent vasoconstrictor peptide (Yanagisawa *et al*., 1988) and is produced by both vascular endothelial and mucosal epithelial cells in many sites in the gastrointestinal tract (Takahashi *et al*., 1990). This regulatory peptide induced a transient 50 fold elevation in the microdialysate histamine concentration (peak response). Since endothelin does not stimulate isolated ECL cells (Lindström *et al*., unpublished observation) it seems that the effects observed are indirect. Endothelin has been shown to cause gastric ulceration after systemic (Wallace *et al*., 1989) and submucosal injection (Watanabe *et al*., 2000) and is thought to be involved in ischaemia-reperfusion injury of the gastric mucosa (Michida *et al*., 1994). In the present study, mucosal haemorrhagic lesions were noted after exposure to near-maximally effective concentrations of endothelin. It is therefore tempting to suggest that the powerful histamine-mobilizing effect of endothelin is secondary to vasoconstriction and to the consequent ischaemic tissue damage.

Adrenergic agents Adrenergic agonists have previously been shown to stimulate secretion of histamine from isolated ECL cells in a manner suggesting the involvement of β_2 type-receptors (Prinz *et al*., 1993; Lawton *et al*., 1995; Sandor *et al*., 1996; Lindström *et al*., 1997; Lindström & Håkanson, 2001). In the present study, noradrenaline and adrenaline raised the microdialysate histamine concentration greatly (10 and 20 fold, respectively, peak response), but transiently. Isoprenaline, on the other hand, induced a 5 fold increase in microdialysate histamine that persisted throughout the treatment period. It thus seems that noradrenaline/adrenaline

and isoprenaline stimulate the ECL cells *via* different pathways. Presumably α-adrenergic stimulation contributes to the noradrenaline/adrenaline-induced release of histamine from the ECL cells whereas isoprenaline act *via* β-receptors. Since isolated ECL cells seem to be equipped mainly with β-receptors, generating a moderate response upon activation (Lindström *et al*., 1997; Lindström & Håkanson, 2001), the spectacular but transient effects of noradrenaline and adrenaline are likely to be at least partly indirect. Inspection of the oxyntic mucosa after exposure to near-maximally effective concentrations of noradrenaline and adrenaline revealed local haemorrhagic lesions, and it cannot be excluded that the transient stimulatory effects of noradrenaline and adrenaline are secondary to tissue destruction. The mechanisms behind the mucosal bleedings, the release of histamine in response to adrenaline and noradrenaline, and the nature of the receptors involved will be the subject of another report (Bernsand *et al*., in preparation).

Regulatory peptides and candidate neurotransmitters with weak stimulatory effects NMU-25 almost qualified as a stimulator of ECL cell histamine mobilization (90% increase, integrated response) while secretin exerted weaker stimulatory effects (35% increase). This is in line with the previously demonstrated weak stimulatory effect of NMU-25 and secretin on isolated ECL cells (Lindström *et al*., 1997). Motilin increased the microdialysate histamine concentration slightly (45% increase), an effect that cannot be reproduced in primary ECL-cell cultures (Lindström *et al*., 1997). Bradykinin, which is without effect on isolated ECL cells (Lindström *et al*., 1997), exerted a weak histamine-mobilizing effect (40% increase). Surprisingly, GABA was found to raise the microdialysate hisamine concentration by 60% (integrated response). Previously, GABA has been shown to inhibit somatostatin release (Koop & Arnold, 1986; Weigert *et al*., 1998), while having no effect on isolated ECL cells (Lindström *et al*., 1997). Hence, the weak stimulatory effect of GABA on ECL-cell histamine mobilization is likely to be indirect.

Inhibitory agents

Somatostatin, galanin and CGRP In line with the results of previous *in vivo* and *in vitro* studies (Sandvik & Waldum, 1988b; Gerber & Payne, 1992; Kondo *et al*., 1993; Prinz *et al*., 1994b; Lindström *et al*., 1997) locally applied somatostatin concentration-dependently inhibited gastrin-evoked histamine mobilization in conscious rats. It is likely that this effect mimics that of endogenous somatostatin mobilized from paracrine D cells in the oxyntic mucosa (Alumets *et al*., 1979; Larsson *et al*., 1979).

Galanin is present in high concentrations in myenteric and submucous ganglia and nerve fibres in the rat stomach (Ekblad *et al*., 1985b; 1991; Melander *et al*., 1985). It inhibits both gastrin secretion (Madaus *et al*., 1988; Schepp *et al*., 1990) and acid secretion (Soldani *et al*., 1988; Rossowski & Coy, 1989). Soldani *et al*. (1988) found galanin to inhibit pentagastrin-evoked acid secretion but not histamine-evoked acid secretion (see also Kato *et al*., 1998). This implied a direct inhibitory action of galanin on the ECL cells, an effect which could subsequently be confirmed using isolated ECL cells in primary culture (Lindström *et al*., 1997; Zeng *et al*., 1998). The inhibitory efficacy of galanin (at maximum concentration) in the present study (60% reduction) was lower than that of somatostatin (80% reduction) (see also Lindström *et al*., 1997; Lindström & Håkanson, 2001).

CGRP, another neuropeptide present in enteric neurons of the rat stomach (Ekblad *et al*., 1985a; Sternini *et al*., 1987; Green & Dockray, 1988), was found to inhibit gastrin-induced histamine secretion. This is surprising since isolated ECL cells in primary culture were not inhibited by CGRP (Lindström *et al*., 1997; Lindström & Håkanson, 2001). Presumably, CGRP acts on non-ECL cells, e.g. to release somatostatin from D cells (Dunning & Taborsky, 1987; Zdon *et al*., 1988; Bunnett *et al*., 1990; Inui *et al*., 1991), thereby suppressing the activity of the ECL-cell *in situ*.

Prostaglandins Prostaglandins E_1 and E_2 are known to inhibit histamine secretion in the isolated perfused rat stomach (Sandvik & Waldum, 1988a) and from isolated ECL cells in primary culture (Lindström & Håkanson, 1998). In the present investigation, locally applied misoprostol was found to be a powerful inhibitor of gastrin-induced histamine mobilization (77% inhibition).

Agents without stimulating or inhibiting effects

Choline esters, such as acetylcholine and carbachol, have been claimed to mobilize histamine from rat and dog stomachs (Stubrin *et al*., 1965; Sandvik *et al*., 1988c; Gerber & Payne, 1992). On the other hand, there are reports demonstrating a lack of effect of choline esters on ECL-cell histamine mobilization (Rosengren & Svensson, 1969; Sewing, 1969; Koyama *et al*., 1987; Lindström *et al*., 1997; Sandvik *et al*., 1998; Lindström & Håkanson, 2001). In fact, in the present study, neither of the two choline esters stimulated histamine mobilization *in vivo*, nor did they inhibit gastrin-stimulated histamine release. Conceivably therefore, the acid-stimulating effect of choline esters (for review see Taché, 1988) is exerted on the parietal cells directly or on intramural ganglia that control the parietal cells.

GRP, neurotensin, NPY and SP were without stimulatory or inhibitory effects on histamine mobilization as were aspartate, glutamate, glycine and serotonin.

Concluding remarks

On the whole, our results are in line with what could be expected from previous studies of isolated ECL cells, namely that gastrin-17, CCK-8s, PACAP-27, VIP and adrenaline/noradrenaline stimulate and that galanin, somatostatin and certain prostaglandins inhibit ECL-cell histamine mobilization. Also the finding that choline esters failed to mobilize histamine from the ECL cells was to be anticipated from previous findings on isolated cells (Lindström *et al*., 1997; Andersson *et al*., 1999; Lindström & Håkanson, 2001). However, the demonstration of stimulatory effects of endothelin, met-enkephalin, PYY and CGRP were unexpected. Since these agents were without effect on isolated ECL cells (Lindström *et al*., 1997; Lindström & Håkanson, 2001) they probably affect the ECL cells indirectly *via* an effect on adjacent non-ECL cells.

Together our results suggest that a range of circulating hormones, local hormones and catecholamines, neuropeptides

and inflammatory mediators act in conjunction – directly and/or indirectly – to regulate ECL-cell histamine secretion *in vivo*. Quite unexpectedly we found ECL-cell histamine to be mobilized according to either of two distinct patterns. While gastrin-17, CCK-8s, met-enkephalin and isoprenaline induced a sustained response, endothelin, PYY, PACAP-27, VIP, adrenaline and noradrenaline gave rise to a strong, rather short-lasting response. Possible explanations for the transient histamine mobilization in response to the latter agents are being explored in a separate study, based on the following considerations: they may (1) exhaust the pool of releasable histamine in the ECL-cells; (2) release not only histamine (from the ECL cells) but also endogenous inhibitory agents (from ECL cells and non-ECL cells), such as somatostatin or prostaglandins, which (with some delay) will suppress the release of histamine; or (3) down-regulate the receptors responsible. In addition, some of these agents may affect microcirculation: agents that cause vasoconstriction (such as endothelin, adrenaline and noradrenaline) or vasodilation (such as CGRP, PACAP, VIP) may affect the local histamine concentration not by affecting the rate of histamine release but by influencing its wash-out from the submucosa. To the parietal cells it may be irrelevant whether the histamine concentration in the environment is elevated because of accelerated mobilization from the ECL cells or because of slow clearance from the mucosa. Still, for a better understanding of the *modus vivendi* of the ECL cells *in situ* this issue is critical.

According to the concept of the gastrin-ECL cell-parietal cell axis (Lindström *et al*., 2001), mobilization of ECL-cell histamine should be accompanied by gastric acid secretion. Hence, not only gastrin/CCK but also all other agents that stimulate secretion of ECL-cell histamine, i.e. adrenaline/noradrenaline, VIP/PACAP, endothelin, enkephalin and PYY, should be expected to activate the parietal cells. However, this simplistic view fails to consider the possibility that some of the histamine-mobilizing agents mentioned above may in addition cause the release of a parietal-cell inhibitor from adjacent non-ECL cells or they may cause vasoconstriction that will lead to impaired parietal-cell function.

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