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Time-course of deactivation of rat stomach ECL cells following cholecystokinin$_B$/gastrin receptor blockade

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1 The so-called enterochromaffin-like (ECL) cells constitute 65–75% of the endocrine cells in the acid-producing part of the rat stomach. They produce and secrete histamine and pancreastatin, a chromogranin A (CGA)-derived peptide, in response to gastrin. Cholecystokinin $(CCK)_B$/gastrin receptor blockade is known to suppress their activity.

2 We have examined the time course of the deactivation of the ECL cells following treatment with the selective CCK_B receptor antagonists RP73870 and YM022. The drugs were given by continuous subcutaneous infusion for a time span of 1 h to 3 weeks and the serum gastrin concentration and various ECL cell parameters were measured (oxyntic mucosal histidine decarboxylase (HDC) activity, histamine and pancreastatin concentrations, HDC mRNA and CGA mRNA levels, and circulating pancreastatin concentration).

3 The two antagonists caused a prompt and dramatic decline in the oxyntic mucosal HDC activity and HDC mRNA level. The HDC activity started to decline after 1–2 h, was reduced by 60–70% after 6 h and was maximally suppressed (80–90%) after 24–48 h. The HDC mRNA level was reduced after 12 h and was at about 20% of the pretreatment level after 2–4 days of infusion. The ECL cell histamine concentration was lowered by about 50% after 7–10 days.

4 RP73870 and YM022 lowered the serum pancreastatin concentration and the oxyntic mucosal CGA mRNA level. The serum pancreastatin concentration was reduced by 40% after 6 h and the reduction was maximal after 2–3 days. A decline in the oxyntic mucosal CGA mRNA level was noted after 12 h with a maximal reduction after 2–4 days of infusion. The ECL cell pancreastatin concentration was reduced by 30–40% after 3 weeks.

5 The infusion of RP73870 and YM022 induced hypergastrinaemia. The serum gastrin concentration started to rise after 2–4 h, there was a 2 fold increase after 6 h and maximal increase (3–4 fold) after 2–3 days of treatment.

6 In conclusion, CCK_B/gastrin receptor blockade promptly deactivates the ECL cells. Deactivation, manifested in a greatly reduced HDC activity, was apparent after 1–2 h of the infusion. The serum pancreastatin concentration and the oxyntic mucosal HDC mRNA and CGA mRNA levels were greatly reduced after 1–2 days. The ECL cell concentrations of histamine and pancreastatin declined quite slowly by comparison.

Keywords: ECL cells; gastrin; CCK_B/gastrin receptors; CCK_B/gastrin receptor antagonist; histidine decarboxylase (HDC); histamine; pancreastatin

Introduction

The enterochromaffin-like (ECL) cells constitute 65–75% of the endocrine cells in the oxyntic mucosa of the rat stomach (Håkanson *et al*., 1992; 1994). They respond to gastrin stimulation by the release of histamine and pancreastatin, a chromogranin A (CGA)-derived peptide, and by the accelerated synthesis of the histamine-forming enzyme histidine decarboxylase (HDC, EC 4.1.1.22) (Håkanson *et al*., 1992; 1993; 1994; 1995; Prinz *et al*., 1993; Chen *et al*., 1994). In fact, the HDC and CGA mRNA abundance in the ECL cells is regulated by circulating gastrin (Dimaline & Sandvik, 1991; Dimaline *et al*., 1993; Chen *et al*., 1994; Sandvik *et al*., 1994; Höcker *et al*., 1996). The ECL cells represent a major source of circulating pancreastatin-like peptides (Håkanson *et al*., 1995; Kimura *et al*., 1997; Norlén *et al*., 1997). The functional significance of pancreastatin in the ECL cells is not clear although CGA and CGA-derived peptides are thought to play a role in the formation and stabilization of secretory granules in neuroendocrine cells (Winkler *et al*., 1986; Winkler & Fischer-Colbrie, 1992). ECL cell histamine is known to play an important role in the control of gastric acid secretion (Black & Shankley, 1987; Waldum *et al*., 1991; Andersson *et al*., 1996a).

The ECL cells possess cholecystokinin $(CCK)_B$/gastrin receptors (Chiba *et al*., 1991; Roche *et al*., 1991a; b; Asahara *et al*., 1994), displaying high affinity binding for both sulphated and nonsulphated CCK-8 and for gastrin. By use of selective CCK_B/gastrin receptor antagonists, it has been shown that the gastrin-evoked histamine release and HDC activation of the ECL cells are mediated by CCK_B/gastrin receptors (Kawabata *et al*., 1991; Sandvik & Waldum, 1991; Prinz *et al*., 1994; Ding *et al*., 1995; Ding & Håkanson, 1996a). This conclusion has received substantial experimental support. We have shown that sustained CCK_B/gastrin receptor blockade for 7 days, with the potent and selective receptor antagonists RP73870 (Pendley *et al*., 1995) and YM022 (Nishida *et al*., 1994; 1995), will deactivate the ECL cells as reflected in the decrease in oxyntic mucosal HDC activity and serum pancreastatin concentration (Ding *et al*., 1997). The present study was designed to study how quickly the ECL cells become deactivated in response to sustained CCK_B/gastrin receptor blockade with RP73870 and YM022.

Methods

Animals

Male Sprague-Dawley rats, weighing 200–220 g at the start of the experiments, were kept in plastic Macrolon cages (4–6 animals in each cage) with free access to standard rat food

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pellets (ALAB, Stockholm, Sweden) and water throughout the study.

RP73870, dissolved in 0.9% saline (1 mg in 7.5 μl), and YM022, dissolved in polyethylene glycol (PEG) 300 (1 mg in 8.5 μl), were given to rats for various periods of time, from 1 h to 3 weeks, by subcutaneous infusion via osmotic minipumps (ALZET 2001, Alza Corporation, Palo Alto, CA, U.S.A.), implanted in the neck under Brietal anaesthesia (50 mg kg^{-1}, intraperitoneally). Vehicle (PEG 300) was given to control rats for 6 h, 7 days or 3 weeks. The dose used for both RP73870 and YM022 was 1 μmol kg^{-1} h^{-1} which has been found to be maximally effective in deactivating the ECL cells (Ding *et al*., 1997). The food intake was followed during the first 24 h of the infusion and the rats were weighed daily during the first week and then once a week to verify a normal food intake.

The rats were killed by exsanguination via the abdominal aorta under chloral hydrate anaesthesia (300 mg kg^{-1}, intraperitoneally). Serum was collected and stored at -20°C until determination of gastrin and pancreastatin. The stomachs were removed, opened along the major curvature and rinsed with ice-cold 0.9% saline. Each stomach was flattened on a glass plate with the mucosa upwards and the oxyntic mucosa was scraped off with a scalpel and stored at -80°C until analysed for HDC activity, for histamine and pancreastatin concentrations and for HDC mRNA and CGA mRNA levels.

Determination of gastrin and pancreastatin

Serum gastrin was measured by radioimmunoassay (Stadil & Rehfeld, 1973). Rat gastrin-17 (Research Plus, Bayonne, NJ, U.S.A.) was used as standard. The serum gastrin concentration was expressed as pmol equivalents l^{-1}.

Serum pancreastatin was measured by radioimmunoassay (Håkanson *et al*., 1995). Rat pancreastatin (CGA 264-314, Peninsula Europe, St. Helens, Merseyside, U.K.) was used as standard. The concentration of pancreastatin-like peptides in the serum was expressed as nmol equivalents l^{-1}.

Pancreastatin in the oxyntic mucosa was extracted by boiling in redistilled water for 10 min followed by centrifugation at 4000 *g* for 20 min. The supernatant was lyophilized and redissolved in 1 ml of 0.05 M sodium phosphate buffer (pH 7.4) containing 0.2% sodium azide, 0.25% bovine serum albumin, 0.25% ethylenediaminetetracetic acid, 500 iu ml^{-1} aprotinin (Trasylol; Bayer, Leverkusen, Germany) for determination of pancreastatin by radioimmunoassay as described above. The pancreastatin concentration in the oxyntic mucosa was expressed as pmol equivalents of synthetic rat pancreastatin g^{-1} wet weight.

Determination of histamine and HDC

The oxyntic mucosa was homogenized in ice-cold 0.01 M sodium phosphate buffer, pH 7.4, to a concentration of a 100 mg wet weight ml^{-1}. Aliquots (80 μl) of the oxyntic mucosal homogenates were incubated with L-[1-^{14}C]-histidine (sp.act. 50 mCi $mmol^{-1}$), 0.5 mM L-histidine, and 0.01 mM pyridoxal-5-phosphate in a total volume of 160 μl at 37°C for 1 h as described previously (Larsson *et al*., 1986). The HDC activity was expressed as pmol $^{14}CO_2$ mg^{-1} h^{-1}. The histamine concentration in the oxyntic mucosa was measured as described by Rönnberg & Håkanson (1984) and expressed as μg g^{-1} (wet weight).

Determination of HDC and CGA mRNA

Total RNA was extracted from the oxyntic mucosa by a modified Chomczynski and Sacchi method (Monstein *et al*., 1995). The integrity of the isolated total RNA was analysed electrophoretically on a denaturing 1% agarose-formaldehyde gel, followed by staining in ethidium bromide and transfer onto a positively charged nylon membrane (Boehringer Mannheim, Mannheim, Germany). The gels were loaded with 5 μg RNA in each lane. Specific complementary RNA (cRNA) probes for HDC and CGA were generated by *in vitro* transcription of DNA templates (Andersson *et al*., 1996b) in the presence of digoxigenin (Dig)-UTP (DIG RNA Labelling Kit, Boehringer Mannheim). Prehybridization and hybridization with the specific RNA probes were carried out as described elsewhere. The membranes were washed with 2 × SSC and 1% SDS for 20 min at 65°C, for 20 min at 70°C and with 0.1 × SSC and 1% SDS for 20 min at 75°C (1 × SSC = 150 mM trisodium citrate, 15 mM sodium chloride; SDS = sodium dodecyl sulphate). After the membranes had been washed, chemiluminescence was developed according to the Boehringer Mannheim manual. The signal was visualized by exposure for 2–5 min to Amersham MP X-ray film, by use of a DuPont intensifying screen. For internal controls the membranes were recycled in 0.5% SDS, 0.1 × SSC at 100°C for 1 h and rehybridized with a Dig-labelled-18S rRNA probe (Ambion, Austin, TX, U.S.A.). The individual bands were quantified with the image analysis system UVP SW 5000 (Ultraviolet Products Ltd., Cambridge, U.K.). The area under the curve (AUC) was calculated for CGA (one band) and for HDC (two bands) and divided by the AUC for the corresponding 18S rRNA band. Controls (rats killed at zero time) were set at 100. Results are expressed as % of control.

Chemicals

RP73870 (([N - (methoxy - 3 - phenyl) - N-(N-methyl-N-phenylcarbamoylmethyl) - carbamoy l- methyl]-3-ureido)-3-phenyl)-2-ethylsulphonate-(RS) was provided by Dr C. Guyon (Rhône-Poulenc Rorer, Vitry-Sur-Seine, France). YM022 ((R)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1H-1, 4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea) was provided by Dr K. Miyata (Yamanouchi Pharmaceutical, Ibaraki, Japan).

Statistical analysis

Results are expressed as means ± s.e.mean. Untreated rats (at zero time) were compared to drug-treated rats. Statistical significance was determined by analysis of variance (ANOVA) followed by Fisher's least significant difference test or Scheffé's *F* test. $P<0.05$ was considered significant.

Results

Time course of changes in body weight, oxyntic mucosal HDC activity, HDC mRNA level and histamine concentration

Infusion of RP73870, YM022 or vehicle did not affect the body weight (data not shown) but RP73870 and YM022 promptly reduced the oxyntic mucosal HDC activity. The HDC activity was reduced by about 30–40% ($P<0.05$) 1–2 h after the start of the infusion of RP73870 or YM022. After 6 h of infusion the HDC activity was 30–35% of the pretreatment level ($P<0.01$). Maximal inhibition (80–90%) was observed after 1–2 days of infusion (Figure 1). The oxyntic mucosal HDC mRNA concentration started to decline after 12 h ($P<0.05$) and was lowered maximally (about 80% reduction) after 2–4 days of infusion of RP73870 or YM022 (Figure 2). In contrast to the rapid decline in HDC activity and HDC mRNA level, the histamine concentration in the oxyntic mucosa was reduced much more slowly. The histamine concentration did not begin to decline until after 4 days of treatment with RP73870 and YM022. The ECL cell histamine concentration was lowered by about 50% after 7–10 days ($P<0.05$) (Figure 3).

Time course of reduction in serum pancreastatin concentration and in oxyntic mucosal CGA mRNA and pancreastatin concentrations

The serum pancreastatin concentration was reduced by about 40% ($P<0.05$) after 6 h of infusion with RP73870 and

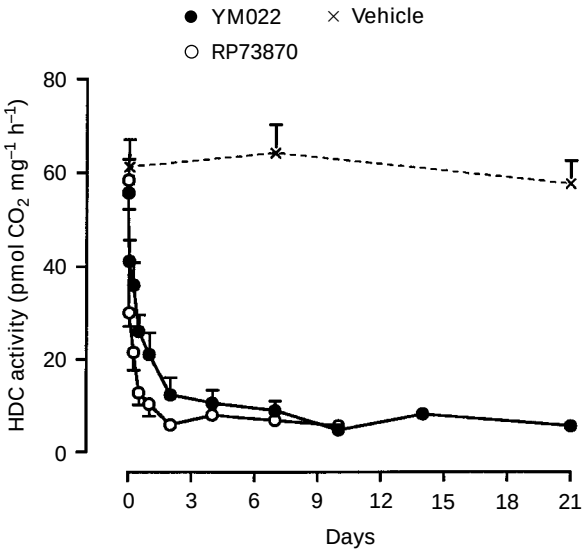

Figure 1 Time-course of the decrease in the rat oxyntic mucosal histidine decarboxylase (HDC) activity in response to CCK$_B$/gastrin receptor blockade. RP73870, YM022 (1 μmol kg^{-1} h^{-1}) and vehicle (PEG 300) were given by continuous subcutaneous infusion for different time periods. Means$\pm$s.e.mean (vertical lines) are shown (five to eight rats in each group).

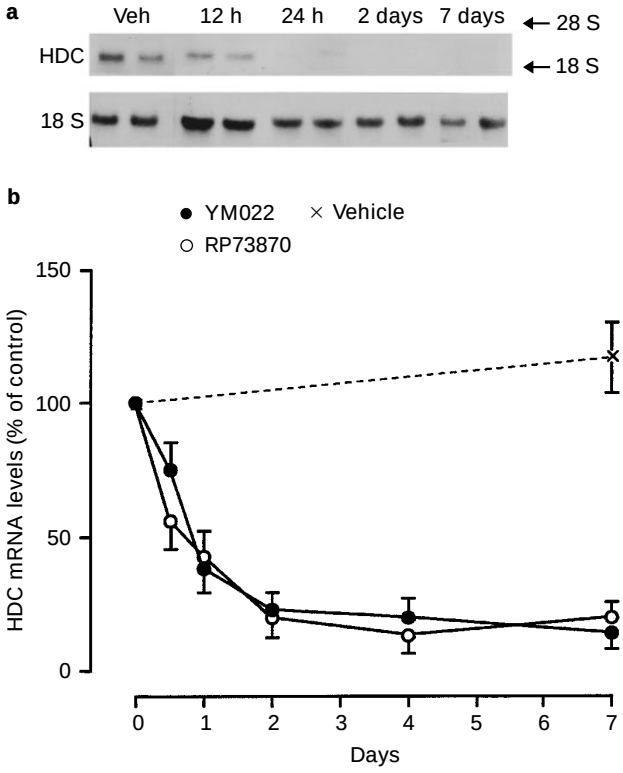

Figure 2 (a) Representative Northern blots: 5 μg of total oxyntic mucosal RNA from rats treated with RP73870 or YM022 for different time periods or with vehicle (PEG 300) for 7 days. The membranes were hybridized with specific probes for histidine decarboxylase (HDC) mRNA and after recycling for 18 S ribosomal RNA; 28 S and 18 S indicate the positions of the ribosomal RNAs. Only one of the two HDC mRNA bands can be seen in the photograph. Veh-vehicle. (b) Time-dependent effect of CCK$_B$/gastrin receptor blockade on the expression of HDC mRNA (signal densities for HDC mRNA divided by those for 18 S rRNA). The values in control rats (time zero) were set at 100. RP73870, YM022 (1 μmol kg^{-1} h^{-1}) and vehicle (PEG 300) were given by continuous subcutaneous infusion. Means$\pm$s.e.mean (vertical lines) are shown (five or six rats in each group).

YM022. The maximum reduction (about 70%) was seen after 2–3 days of treatment (Figure 4). The oxyntic mucosal CGA mRNA level was lowered after 12 h ($P<0.05$), and maximal reduction (about 80%) was seen after 2–4 days (Figure 5). The oxyntic mucosal pancreastatin concentration was moderately reduced (30–40%, $P<0.05$) only after 3 weeks of infusion of YM022 (Figure 6).

Time course of hypergastrinaemia

Infusion of RP73870 or YM022 induced hypergastrinaemia. The serum gastrin concentration started to rise after 2–4 h and was increased almost 2 fold after 6 h of infusion ($P<0.05$). It was elevated 3–4 fold after 2–3 days of infusion (Figure 7).

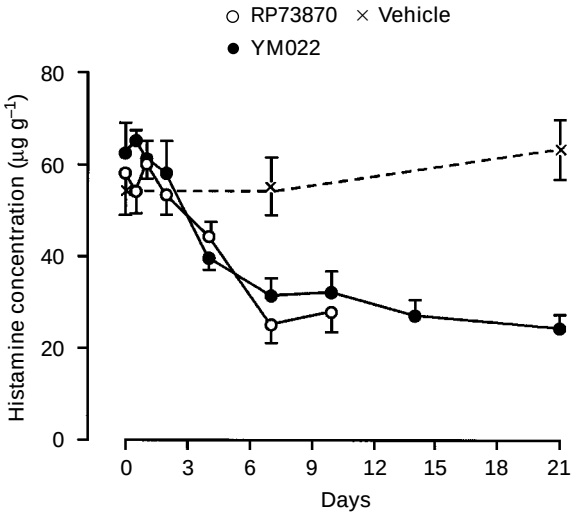

Figure 3 Time-course of the changes in the rat oxyntic mucosal histamine concentration in response to CCK$_B$/gastrin receptor blockade. RP73870, YM022 (1 μmol kg^{-1} h^{-1}) and vehicle (PEG 300) were given by continuous subcutaneous infusion for different time periods. Means$\pm$s.e.mean (vertical lines) are shown (five or six rats in each group).

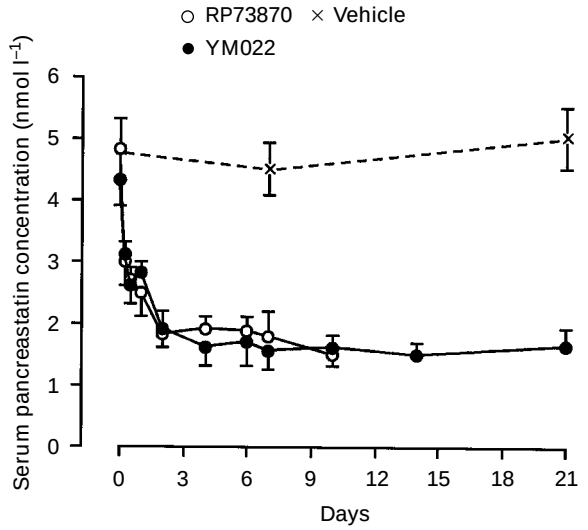

Figure 4 Time-course of the decline in serum pancreastatin concentration in response to CCK$_B$/gastrin receptor blockade. RP73870, YM022 (1 μmol kg^{-1} h^{-1}) and vehicle (PEG 300) were given by continuous subcutaneous infusion for different time periods. Means$\pm$s.e.mean (vertical lines) are shown (five or six rats in each group).

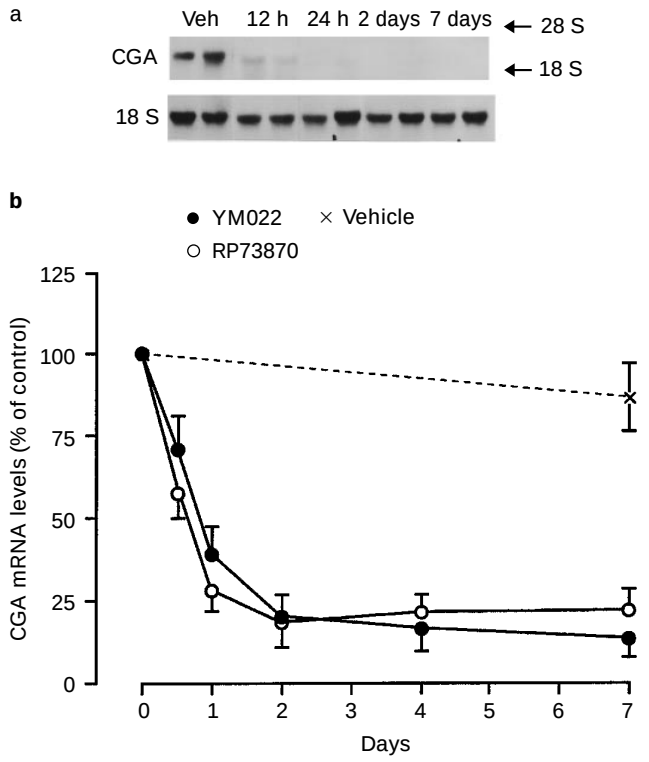

Figure 5 (a) Representative Northern blots: 5 μg of total oxyntic mucosal RNA from rats treated with RP73870 or YM022 for different time periods or with vehicle (PEG 300) for 7 days. The membranes were hybridized with specific probes for chromogranin A (CGA) mRNA and after recycling for 18 S ribosomal RNA; 28 S and 18 S indicate the positions of the ribosomal RNAs. Veh = vehicle. (b) Time-dependent effect of CCK_B/gastrin receptor blockade on the expression of CGA mRNA (signal densities for CGA mRNA divided by those for 18 S rRNA). The values in control rats (time zero) were set at 100. RP73870, YM022 (1 μmol kg^{-1} h^{-1}) and vehicle (PEG 300) were given by continuous subcutaneous infusion. Means ± s.e.mean (vertical lines) are shown (five or six rats in each group).

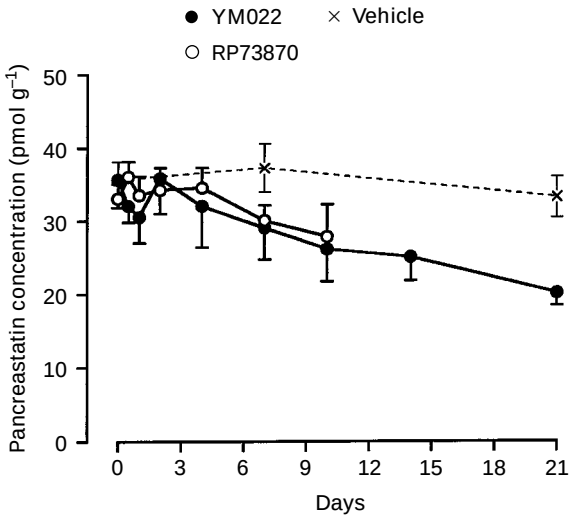

Figure 6 Oxyntic mucosal pancreastatin concentration at different times during the CCK_B/gastrin receptor blockade. RP73870, YM022 (1 μmol kg^{-1} h^{-1}) and vehicle (PEG 300) were given by continuous subcutaneous infusion. Means ± s.e.mean (vertical lines) are shown (five or six rats in each group).

Discussion

Gastrin activates the ECL cells by interacting with CCK_B/gastrin receptors. The development of specific and potent

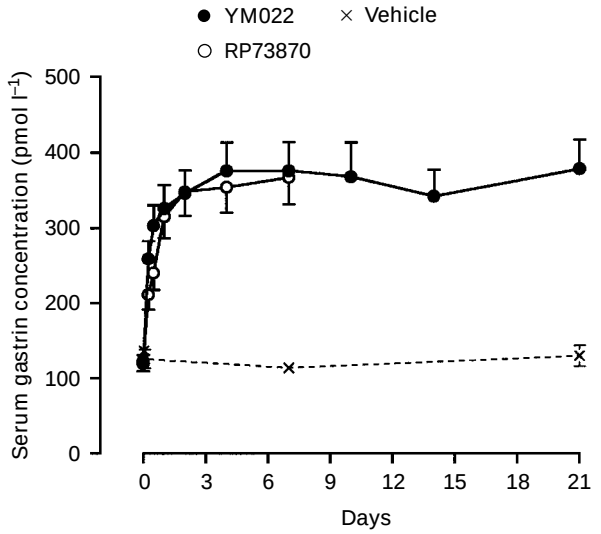

Figure 7 Time-course of the rise in serum gastrin concentration in response to CCK_B/gastrin receptor blockade. RP73870, YM022 (1 μmol kg^{-1} h^{-1}) and vehicle (PEG 300) were given by continuous subcutaneous infusion. Means ± s.e.mean (vertical lines) are shown (five or six rats in each group).

CCK_B/gastrin receptor antagonists has provided useful pharmacological tools for the study of how gastrin controls the ECL cells. YM022, a benzodiazepine derivative, and RP73870, an ureidoacetamide compound, have been shown to bind to CCK_B/gastrin receptors within the nanomolar concentration range and to antagonize gastrin-evoked gastric acid secretion in anaesthetized rats (Nishida *et al*., 1994; Pendley *et al*., 1995). Previously, we have evaluated a series of novel CCK_B/gastrin receptor antagonists and showed that both YM022 and RP73870 effectively antagonized the gastrin-evoked activation of the ECL cells (Ding & Håkanson, 1996a) and the gastrin-evoked acid secretion in chronic gastric fistula rats (Ding & Håkanson, 1996b). Sustained CCK_B/gastrin receptor blockade was found to deactivate the ECL cells, as indicated by the reduced oxyntic mucosal HDC activity, histamine concentration and HDC mRNA and CGA mRNA levels, and by the reduced serum pancreastatin concentration (Ding *et al*., 1997). The deactivation of the ECL cells by CCK_B/gastrin receptor blockade supports the view that they depend on circulating gastrin for maintained activity. The close gastrin control of the ECL cell HDC activity (Håkanson *et al*., 1992; 1994; Chen *et al*., 1994) suggest that by comparison the vagal input might be less important for HDC activity. It should be noted that although the ECL cells seem to be under vagal control (Håkanson *et al*., 1994), the cholinomimetics carbachol and bethanechol failed to stimulate histamine and pancreastatin secretion from isolated ECL cells (Lindström *et al*., 1997).

In this study we showed that HDC activity and the serum pancreastatin concentration declined promptly in response to CCK_B/gastrin receptor blockade. A decline in HDC activity was noted as early as 1–2 h after start of infusion and it was maximal (about 80–90% reduction) after 1–2 days. The decline in serum pancreastatin concentration probably reflects inhibition of pancreastatin secretion from the ECL cells, since they represent the major source of circulating pancreastatin in the rat (Håkanson *et al*., 1995; Kimura *et al*., 1997; Norlén *et al*., 1997). The serum gastrin concentration increased 2 fold after 6 h of infusion, reaching a maximum (3 fold increase) after 2–3 days of infusion. Nishida *et al*. (1995) described hypergastrinaemia after 13 weeks treatment with YM022, while the dipeptoid compound PD136450 failed to affect the serum gastrin concentration (Eissele *et al*., 1992), perhaps due to its agonistic rather than antagonistic activity (Schmassmann *et al*., 1994; Ding *et al*., 1995). The hypergastrinaemia that ensues upon CCK_B/gastrin receptor blockade may reflect the

inhibition of acid secretion and the consequent abolishment of the acid feed-back inhibition of gastrin release. In addition, the elevated serum gastrin concentration may reflect attenuation of CCK_B/gastrin receptor-mediated autofeed-back inhibition of gastrin release.

For a long time, the target cells for gastrin in the stomach have been debatable. The lack of demonstrable CCK_B/gastrin receptors on rat parietal cells (Song *et al*., 1996) and the fact that depletion of ECL cell-histamine completely abolishes gastrin-evoked acid secretion (Andersson *et al*., 1996a) seem to favour the view that the ECL cells rather than the parietal cells are the major targets for gastrin. If this is the case, the antagonist-evoked rise in serum gastrin concentration may reflect inhibition of histamine release from the ECL cells rather than a direct inhibitory effect of the antagonists on the parietal cells. The decrease in the pancreastatin concentration and the increase in the gastrin concentration in the circulation following CCK_B/gastrin receptor blockade occurred along the same time course with a rapid and quite dramatic change within 6 h, reaching a maximum after 2–3 days. Indeed, it has been shown that gastrin induces a parallel release of histamine and pancreastatin from isolated ECL cells and that YM022 effectively inhibits the gastrin-evoked release of both histamine and pancreastatin (Lindström *et al*., 1997).

CCK_B receptor blockade lowered the HDC mRNA and CGA mRNA expression in the ECL cells. The concentrations of HDC mRNA and CGA mRNA in the oxyntic mucosa were reduced after 12 h of treatment, with a maximal reduction after 2–4 days. These observations support the view that gastrin is important for the transcription of both the HDC and the CGA genes (Dimaline & Sandvik, 1991; Dimaline *et al*., 1993; Höcker *et al*., 1996).

The oxyntic mucosal histamine and pancreastatin concentrations decreased only slowly after CCK_B receptor blockade. The histamine concentration was unchanged until 4 days of treatment and reduced by about 50% after 7–10 days. In contrast, the inhibition of histamine synthesis was manifest already after a few hours of infusion. The concentration of pancreastatin in the oxyntic mucosa remained unaffected for at least two weeks, probably reflecting the simultaneous suppression of CGA synthesis and pancreastatin secretion. It seems that CCK_B/gastrin receptor blockade prevents both the formation and the release of secretory products from the ECL cells (resulting in a very gradual loss of secretory products in general). Further, the results suggest that the ability of the ECL cells to maintain the pancreastatin concentration is greater than its ability to maintain histamine.

CCK_B/gastrin receptors in the central nervous system are thought to be involved with anxiety and/or the control of feeding (Willis *et al*., 1986; Garlichi *et al*., 1990). However, the body weight of the rats was unaffected by the CCK_B/gastrin receptor blockade, suggesting that the food intake did not differ between untreated rats and rats receiving the drugs.

In conclusion, CCK_B/gastrin receptor blockade deactivates the ECL cells. Deactivation was apparent after only 1–2 h (reduced HDC activity) but required several weeks before it was fully manifested (lowered histamine and pancreastatin content).

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References

ANDERSSON, K., CABERO, J.L., MATTSSON, H. & HÅKANSON, R. (1996a). Gastric acid secretion after depletion of enterochromaffin-like cells histamine. *Scand. J. Gastroenterol*., **31,** 24–30.

ANDERSSON, K., LINDSTRÖM, E., CHEN, D., MONSTEIN, H.-J., BOKETOFT, Å. & HÅKANSON, R. (1996b). Depletion of enterochromaffin-like cells histamine increases histidine decarboxylase and chromogranin A mRNA levels in rat stomach by a gastrin-independent mechanism. *Scand. J. Gastroenterol*., **31,** 959–965.

ASAHARA, M., KINOSHITA, Y., NAKATA, H., MATSUSHIMA, Y., NARIBAYASHI, Y., NAKAMURA, A., MATSUI, H., CHIHARA, K., YAMAMOTO, J., ICHIKAWA, A. & CHIBA, T. (1994). Gastrin receptor genes are expressed in gastric parietal and enterochromaffine-like cells of Mastomys natalensis. *Dig. Dis. Sci*., **39,** 2149–2156.

BLACK, J.W. & SHANKLEY, N.P. (1987). How does gastrin act to stimulate oxyntic cell secretion? *Trends Pharmacol. Sci*., **8,** 486–490.

CHEN, D., MONSTEIN, H.-J., NYLANDER, A.-G., ZHAO, C.-M., SUNDLER, F. & HÅKANSON, R. (1994). Acute responses of rat stomach enterochromaffin-like cells to gastrin: Secretory activation and adaptation. *Gastroenterology*, **107,** 18–27.

CHIBA, T., KINOSHITA, Y., MORISHITA, T., NAKATA, H., NAKAMURA, A. & HOSODA, S. (1991). Receptors for gastrin on gastric carcinoid tumor membranes of Mastomys natalensis. *Biochem. Biophys. Res. Commun*., **177,** 739–744.

DIMALINE, R. & SANDVIK, A.K. (1991). Histidine decarboxylase gene expression in rat fundus is regulated by gastrin. *FEBS Lett*., **28,** 20–22.

DIMALINE, R., EVANS, D., FORSTER, E.R., SANDVIK, A.K. & DOCKRAY, G.J. (1993). Control of gastric corpus chromogranin A messenger RNA abundance in the rat. *Am. J. Physiol*., **264,** G583–588.

DING, X.-Q., CHEN, D. & HÅKANSON, R. (1995). Cholecystokinin-B receptor ligands of the dipeptoid series act as agonists on rat stomach histidine decarboxylase. *Gastroenterology*, **109,** 1181–1187.

DING, X.-Q. & HÅKANSON, R. (1996a). Evaluation of potency and specificity of a series of cholecystokinin-B/gastrin receptor antagonists. *Pharmacol. Toxicol*., **79,** 124–130.

DING, X.-Q. & HÅKANSON, R. (1996b). Effect of cholecystokinin-B/gastrin receptor blockade on gastric acid secretion in conscious rats. *Pharmacol. Toxicol*., **79,** 324–330.

DING, X.-Q., LINDSTRÖM, E. & HÅKANSON, R. (1997). Cholecystokinin-B/gastrin receptor blockade suppresses the activity of rat stomach ECL cells. *Pharmacol. Toxicol*., **81,** 19–25.

EISSELE, R., PATBERG, H., KOOP, H., KRACK, W., LORENZ, W., McKNIGHT, A.T. & ARNOLD, R. (1992). Effect of gastrin receptor blockade on endocrine cells in rats during achlorhydria. *Gastroenterology*, **103,** 1596–1601.

GARLICHI, J., KONTUREK, P.K., MAJKA, J., KWIECIEN, N. & KONTUREK, S.J. (1990). Cholecystokinin receptor and vagal nerves in the control of food intake in rat. *Am. J. Physiol*., **258,** E40–45.

HÅKANSON, R., CHEN, D. & SUNDLER, F. (1994). The ECL cells. In *Physiology of the Gastrointestinal Tract*. ed. Johnson, L.R. pp. 1171–1184. New York: Raven Press.

HÅKANSON, R., DING, X.-Q., NORLÉN, P. & CHEN, D. (1995). Circulating pancreastatin is a marker for the enterochromaffin-like cells of the rat stomach. *Gastroenterology*, **108,** 1445–1452.

HÅKANSON, R., TIELEMANS, Y., CHEN, D., ANDERSSON, K., MATTSSON, H. & SUNDLER, F. (1992). The biology and pathobiology of the ECL cells. *Yale J. Biol. Med*., **65,** 761–764.

HÅKANSON, R., TIELEMANS, Y., CHEN, D., ANDERSSON, K., MATTSSON, H. & SUNDLER, F. (1993). Time-dependent changes in enterochromaffin like cell kinetics in stomach of hypergastrinemic rats. *Gastroenterology*, **105,** 15–21.

HÖCKER, M., ZHANG, Z.-S., FENSTERMACHER, D.A., TÅGERUD, S., CHULAK, M., JOSEPH, D. & WANG, T.C. (1996). Rat histidine decarboxylase promoter is regulated by gastrin through a protein kinase C pathway. *Am. J. Physiol*., **270,** G619–633.

KAWABATA, S., KANAYAMA, S., SHINOMURA, Y., MIYAZAKI, Y., IMAMURA, I., MORIWAKI, K., WADA, H. & TARUI, S. (1991). Effect of CCK receptor antagonists, MK-329 and L-365,260 on CCK-induced acid secretion and HDC activity in the rat. *Regul. Pept*., **35,** 1–10.

KIMURA, K., CHEN, D., LINDSTRÖM, E., ZHAO, C.-M. & HÅKANSON, R. (1997). Evidence that rat stomach ECL cells represent the main source of circulating pancreastatin. *Regul. Pept*., **68,** 177–180.

LARSSON, H., CARLSSON, E., MATTSSON, H., LUNDELL, L., SUNDLER, F., SUNDELL, G., WALLMARK, B., WATANABE, T. & HÅKANSON, R. (1986). Plasma gastrin and gastric enterochromaffin like cell activation and proliferation. Studies with omeprazole and ranitidine in intact and antrectomized rats. *Gastroenterology*, **90,** 391 – 399.

LINDSTRÖM, E., BJÖRKQVIST, M., BOKETOFT, Å., CHEN, D., ZHAO, C.-M., KIMURA, K. & HÅKANSON, R. (1997). Neurohormonal regulation of histamine and pancreastatin secretion from isolated rat stomach ECL cells. *Regul. Pept*., (in press).

MONSTEIN, H.-J., NYLANDER, A.-G. & CHEN, D. (1995). RNA extraction from gastrointestinal tract and pancreas by a modified Chomczynski and Sacchi method. *Bio. Technol*., **19,** 340 – 343.

NISHIDA, A., MIYATA, K., TSUTSUMI, R., YUKI, H., AKUZAWA, S., KOBAYASHI, A., KAMATO, T., ITO, H., YAMANO, M., KATUYAMA, Y., SATOH, M., OHTA, M. & HONDA, K. (1994). Pharmacological profile of (R)-1-[2,3 dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl) urea (YM022), a new potent and selective gastrin/CCK-B receptor antagonist, *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther*., **269,** 725 – 731.

NISHIDA, A., KOBAYASHI, A., AKUZAWA, S., TAKINAMI, Y., SHISHIDO, T., KAMATO, T., ITO, H., YAMANO, M., YUKI, H., NAGAKURA, Y., HONDA, K. & MIYATA, K. (1995). Gastrin receptor antagonist YM022 prevents hypersecretion after long term acid suppression. *Am. J. Physiol*., **269,** G699 – 705.

NORLÉN, P., CURRY, W.J., CHEN, D., ZHAO, C.-M., JOHNSTON, C.F. & HÅKANSON, R. (1997). Expression of the chromogranin A-derived peptides pancreastatin and WE_{14} in rat stomach ECL cells. *Regul. Pept*., (in press).

PENDLEY, C.E., FITZPATRICK, L.R., CAPOLINO, A.-J., DAVIS, M.A., ESTERLINE, N.-J., JAKUBOWSKA, A., BERTRAND, P., GUYON, C., DUBROEUCQ, M.-C. & MARTIN, G.E. (1995). RP73870, a gastrin/CCK-B receptor antagonist with potent anti-ulcer activity in the rat. *J. Pharmacol. Exp. Ther*., **273,** 1015 – 1022.

PRINZ, C., KAJIMURA, M., SCOTT, D.-R., MERCIER, F., HELANDER, H.-F. & SACHS, G. (1993). Histamine release from rat enterochromaffin like cells. *Gastroenterology*, **105,** 449 – 461.

PRINZ, C., SCOTT, D.-R., HURWITZ, D., HELANDER, H.-F. & SACHS, G. (1994). Gastrin effects on isolated rat enterochromaffin like cells in primary culture. *Am. J. Physiol*., **267,** G663 – 672.

ROCHE, S., GUSDINAR, T., BALI, J.-P. & MAGOUS, R. (1991a). 'Gastrin' and 'CCK' receptors on histamine- and somatostatin-containing cells from rabbit fundic mucosa. I. Characterization by means of agonists. *Biochem. Pharmacol*., **42,** 765 – 770.

ROCHE, S., GUSDINAR, T., BALI, J.-P. & MAGOUS, R. (1991b). 'Gastrin' and 'CCK' receptors on histamine- and somatostatin-containing cells from rabbit fundic mucosa. II. Characterization by means of selective antagonists L-364,718 and L-365,260. *Biochem. Pharmacol*., **42,** 771 – 776.

RÖNNBERG, A.-L. & HÅKANSON, R. (1984). A simplified procedure for fluorometric determination of histamine in rat stomach. *Agents Actions*, **14,** 195 – 199.

SANDVIK, A.K., DIMALINE, R., MÅRVIK, R., BRENNA, E. & WALDUM, H.-L. (1994). Gastrin regulates histidine decarboxylase activity and mRNA abundance in rat oxyntic mucosa. *Am. J. Physiol*., **267,** G254 – G258.

SANDVIK, A.K. & WALDUM, H.L. (1991). CCK-B (gastrin) receptor regulates gastric histamine release and acid secretion. *Am. J. Physiol*., **260,** G925 – 928.

SCHMASSMANN, A., GARNER, A., FLOGERZI, B., HASAN, M.Y., SANNER, M., VARGA, L. & HALTER, F. (1994). Cholecystokinin type B receptor antagonist PD136,450 is a partial agonist in the stomach and a full agonist in the pancreas of the rat. *Gut*, **35,** 270 – 274.

SONG, M., WONG, H., OHNING, G. & WALSH, J.H. (1996). Immunohistochemical localization of the gastrin/CCK-B receptor in the rat stomach (abs). *Gastroenterology*, **110,** A1120.

STADIL, F. & REHFELD, J.-F. (1973). Determination of serum gastrin. An evaluation of the reliability of a radioimmunoassay. *Scand. J. Gastroenterol*., **8,** 101 – 112.

WALDUM, H.L., SANDVIK, A.-K., BRENNA, E. & PETERSEN, H. (1991). The gastrin-histamine sequence in the regulation of gastric acid secretion. *Gut*, **32,** 698 – 700.

WILLIS, G.L., HANSKY, J. & SMITH, G.C. (1986). Central and peripheral proglumide administration and cholecystokinin-induced satiety. *Regul. Pept*., **15,** 87 – 98.

WINKLER, H., APPS, D.-K. & FISCHER-COLBRIE, R. (1986). The molecular function of adrenal chromaffin granules: established facts and unresolved topics. *Neuroscience*, **18,** 261 – 290.

WINKLER, H. & FISCHER-COLBRIE, R. (1992). The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience*, **49,** 497 – 528.

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