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Carbachol stimulation of gastric acid secretion and its effects on the parietal cell

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1 The acid secretagogue effect of gastrin is mainly mediated by the release of enterochromaffin-like (ECL) cell histamine, but the mechanism of muscarinic stimulation of acid secretion remains unclear. The results of studying aminopyrine uptake in isolated parietal cells, and histamine release in isolated ECL cells suggest that muscarinic agents may act both directly on the parietal cell and indirectly via histamine release from ECL cells.

2 We examined parietal and ECL cell responses to the muscarinic agent carbamylcholine (carbachol) in conscious rats and in rat isolated vascularly perfused stomachs.

3 Intravenous carbachol stimulated acid secretion in conscious gastric fistula rats and increased H^+K^+ ATPase mRNA abundance, indicating activation of parietal cells. In these experiments there was no increase in portal venous histamine, or in oxyntic mucosal histidine decarboxylase (HDC) enzyme activity and HDC mRNA abundance.

4 In rat isolated stomachs stimulated with carbachol in the dose range 10 nM – 1 mM only the 1 μM concentration increased venous histamine significantly.

5 We concluded that the muscarinic agent carbachol stimulates acid secretion and H^+K^+ ATPase mRNA *in vivo* by a direct effect on the parietal cell, that does not depend on the release of ECL cell histamine.

Keywords: ECL cell; parietal cell; gastrin; histamine; muscarinic; H^+K^+-ATPase; histidine decarboxylase

Introduction

The stimulation of gastric acid secretion by histamine has been recognized since 1920, but for many years the physiological significance of this effect was in question (Popielski, 1920). The doubts were resolved when Black and colleagues discovered histamine H_2 receptor antagonists which they showed to block acid secretion stimulated by cholinoceptor secretagogues and gastrin (Black *et al*., 1972; Grossman & Konturek, 1974). The action of gastrin in releasing histamine has been the subject of intense debate. However, it is now generally agreed that gastrin acts mainly by releasing histamine from the enterochromaffin-like (ECL) cells in the oxyntic mucosa. The mechanism of action of cholinoceptor agonists on gastric acid secretion is less clear. In particular it is not certain whether, like gastrin, cholinoceptor agents act predominantly via histamine release from ECL cells. Previous studies have shown that in isolated canine parietal cells, carbamylcholine (carbachol) potently stimulates aminopyrine uptake (Soll, 1980) and induces expression of genes involved in acid secretion (Campbell & Yamada, 1989). Studies of receptor gene expression and ligand binding indicate that muscarinic M_3 receptors are present on parietal cells (Kajimura *et al*., 1992), and there is also evidence that carbachol acts on the M_3 receptor to increase acid secretion and intracellular calcium concentrations in the parietal cell (Wilkes *et al*., 1991).

Together these observations support the idea of a direct muscarinic stimulation of the parietal cells. However, set against this, are studies on oxyntic mucosal cell preparations enriched in ECL cells which show that carbachol releases histamine (Prinz *et al*., 1993). Moreover carbachol stimulated both basal and gastrin-driven release of histamine from isolated ECL cells, in a dose-dependent manner, probably by activation of an M_1 type receptor (Sandor *et al*., 1996). In addition, unilateral vagotomy produces a decreased argyrophil cell count on the denervated side of the rat stomach (Håkanson *et al*., 1984), and acetylcholine administered to frog isolated gastric mucosa gives a transient increase in histamine concentration in the serosal buffer (Ekblad, 1980).

The evidence available therefore suggests that muscarinic stimulation might act both directly on the parietal cell to stimulate acid secretion, and indirectly by releasing histamine from ECL cells. The relative importance of these two modes of action *in vivo* is not clear. In the present study we have sought to resolve this question by use of a range of approaches to determine if carbachol stimulates ECL-cell activity in intact, conscious rats when given in doses that potently stimulate the parietal cell. Furthermore, the effect of carbachol on histamine release from rat isolated, vascularly perfused stomachs was studied.

Methods

Handling of animals

The animal experiments were approved by the Animal Welfare Committee of the University Hospital of Trondheim. The rats were housed in wire-mesh cages at 24°C and constant humidity with a 12 : 12 h light-dark cycle, and fed with a commercial rat diet and tap water *ad libitum*. Before all surgical procedures, the rats were anaesthetized with 0.2 ml 100 g^{-1} body weight of a combination of (per ml) 2.5 mg fluanison, 0.05 mg fentanyl, and 1.25 mg midazolam (Flecknell & Mitchell, 1984).

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Drug infusions in conscious rats

For drug infusions, a polyethylene catheter was inserted into the left internal jugular vein and tunnelled to the tail as previously described (Haarstad & Petersen, 1989; Sandvik *et al*., 1994), allowing continuous infusions to be given to conscious, minimally restrained animals. One group of animals also underwent splenectomy and had a catheter inserted from the central splenic vein into the portal vein, and tunnelled to the tail together with the jugular vein catheter.

Acid secretory studies in conscious gastric fistula rats

Two groups of rats were fitted with chronic gastric fistulas by use of standard surgical techniques. One week later, a jugular vein catheter was inserted. Animals were fasted with free access to tap water for 24 h after the operation and the catheter was kept open by continuous infusion of normal saline 1 ml h^{-1}. Before the experiments, the stomach was rinsed with normal saline through the fistula and gastric juice was collected by continuous passive drainage. After an initial 30 min washout period basal secretions were collected for 30 min. The animals then received 30 nmol kg^{-1} h^{-1} carbachol in normal saline or saline alone (controls), 4 rats in each group. This carbachol dose was chosen because pilot studies showed that it potently stimulated gastric acid secretion in fistula rats. The concentration of carbachol was adjusted so that the rats received 2 ml h^{-1} of intravenous fluid, compensating for the loss through the fistula. The gastric juice was collected from the fistulas every 30 min, the volume measured, and titrated to pH 7.0 with 0.1 M NaOH.

Carbachol and gastrin infusions with portal venous sampling

Animals were allowed a four-day recovery period after surgery. The jugular and the portal venous lines were kept open by continuous infusion with 1 ml h^{-1} of normal saline; or saline with 0.2% (w/v) bovine serum albumin (BSA) to the rats which were to receive gastrin infusion. After a 24 h fast with free access to tap water the experiments were started by sampling 0.2 ml portal blood in a heparin-treated syringe to prepare plasma for baseline histamine measurements. Animals then received continuous infusions (1 ml h^{-1}) of carbachol (30 nmol kg^{-1} h^{-1} in normal saline) or gastrin 1-17 (10 pmol kg^{-1} h^{-1} in normal saline with BSA). A previous study from our laboratories showed that this gastrin dose induced a rapid elevation of serum gastrin to values above the normal range for fed rats (Sandvik *et al*., 1994). Portal venous blood was sampled before and at 5, 10, 15, 30 and 45 min after the start of the drug infusion.

Carbachol infusion and tissue harvesting for histidine decarboxylase activity and mRNA abundance

Animals ($n = 4-6$) received intravenously either saline (controls) or carbachol 30 nmol kg^{-1} h^{-1} for 15 min, 30 min, 1 h, 2 h or 6 h. After the stimulation period the rats were immediately given anaesthetic intravenously and killed by decapitation. Trunk blood was collected for gastrin measurement, the stomach rapidly excised and a wedge-shaped full thickness sample for RNA extraction taken from the major curvature of the corpus near the forestomach. The remaining corpus mucosa was scraped for the histidine decarboxylase (HDC) enzyme assay. Tissue was homogenized for RNA extraction and HDC enzyme assay as previously described (Sandvik *et al*., 1994), and both homogenates were immediately frozen and kept at $-80°C$.

Isolated vascularly perfused stomachs of the rat

Totally isolated, vascularly perfused stomachs of the rat were made as previously described (Short *et al*., 1984; Kleveland *et al*., 1986). The study included six groups of isolated stomachs (4–6 stomachs in each group) all of which received carbachol in the arterial perfusate at 40–45, 60–65 and 80–85 min (perfusion time) in concentrations of either 10 nM, 100 nM, 1 μM, 10 μM, 100 μM or 1 mM. Each isolated stomach preparation received carbachol in one concentration only, and the perfusate was not recirculated ensuring that the capillary carcachol concentration was equal to the arterial concentration. The venous effluent was collected in the 1 min period immediately preceding carbachol, and during the third 1 min period after carbachol infusion was started. This mode of administration has previously been shown to induce an instantaneous, rapidly reversible histamine release when gastrin is given (Sandvik & Waldum, 1990). The venous effluent was frozen and kept at $-20°C$ until analysis for histamine.

Measurement of histidine decarboxylase activity

The procedure described by Beaven *et al*. (1978) with some modifications (Larsson *et al*., 1986) was used. An 80 μl aliquot of the oxyntic mucosal homogenate was incubated with [1-^{14}C]-L-histidine (24 nCi, 0.48 nmol), 0.5 mM L-histidine and 10 mM pyridoxal-5-phosphate in a total reaction volume of 160 μl at 37°C for 60 min. The reaction was stopped by adding 80 μl 2 M perchloric acid followed by incubation at 37°C for 30 min. The expelled $^{14}CO_2$ was trapped in 50 μl Protosol and counted in scintillation fluid.

Histidine decarboxylase and H^+K^+ ATPase mRNA quantification

Total RNA was isolated by ultracentrifugation of the homogenates on a caesium chloride cushion, precipitated with ethanol, electrophoresed on a formaldehyde-agarose gel and blotted onto Boehringer Mannheim nylon membranes by use of methods described previously (Dimaline *et al*., 1993). Plasmids encoding fragments of HDC, H^+K^+ ATPase, and GAPDH cDNA (Dimaline & Sandvik, 1991; Sandvik *et al*., 1994; Dimaline *et al*., 1997) were linearized, complementary RNA probes labelled with ^{32}P transcribed *in vitro* according to standard protocols, and purified on NucTrap columns (Stratagene). Membranes were prehybridized for 4 h at 65°C, then hybridized in the same solution containing RNA probe (2×10^6 c.p.m. ml^{-1}) for a further 18 h as previously described (Dimaline & Struthers, 1996). Following hybridization, membranes were washed in high-stringency salt conditions, exposed to a storage phosphor screen for 18 h and the screen scanned on a PhosphorImager 425 (Molecular Dynamics, Sevenoaks, U.K.). Images were quantified by use of ImageQuant software (Molecular Dynamics). The membranes were hybridized first with the HDC riboprobe, followed by H^+K^+ ATPase, and finally GAPDH; signals were removed between hybridizations with boiling 0.1% SDS. Estimations of mRNA size were made with reference to the positions of 18S and 28S ribosomal RNAs. The riboprobes generated hybridized to the membranes revealing specific bands of the predicted size for all mRNAs studied (Figure 1).

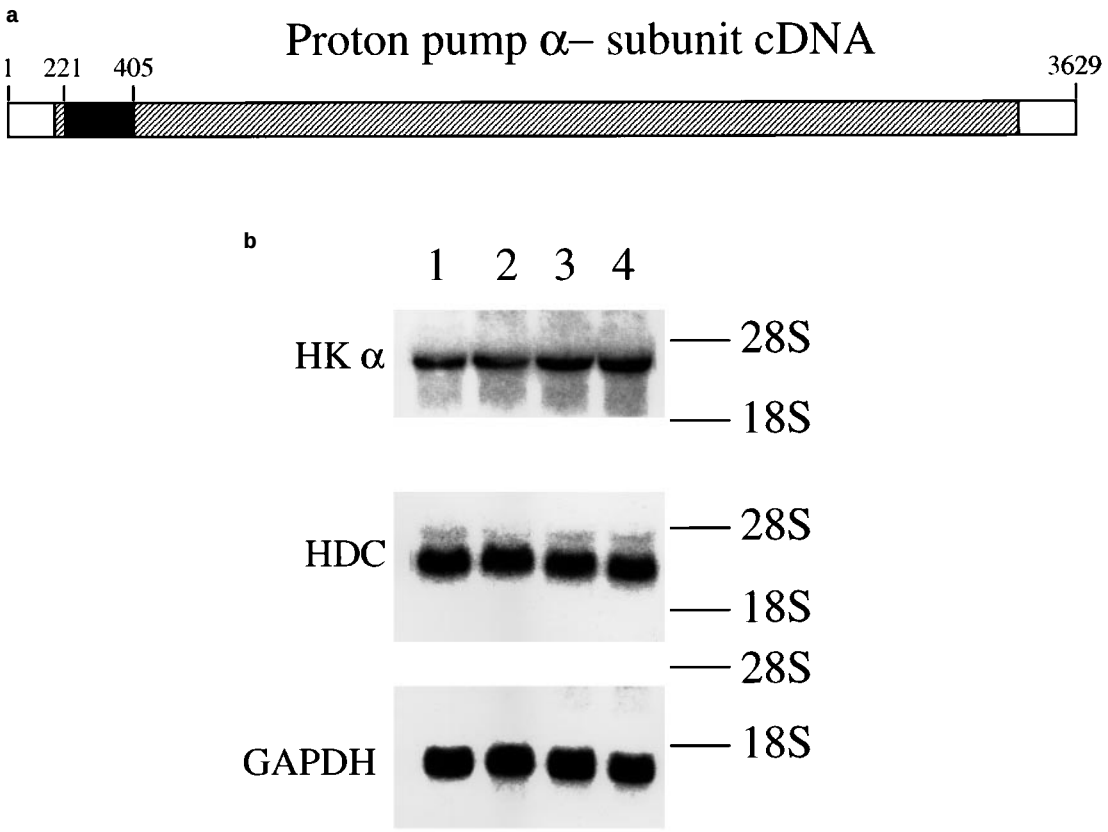

Figure 1 (a) Localization of the cloned fragment of the α-subunit of H^+K^+-ATPase cDNA used as riboprobe template (solid bar) within the protein coding region (stippled). (b) Representative Northern blot hybridization patterns with cRNA probes against H^+K^+-ATPase (HKα, ~4 kb), histidine decarboxylase (HDC, 2.5 kb (major) and 3.5 kb (minor)) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.4 kb). Samples of total RNA (20 μg) were analysed from the gastric corpus of rats receiving infusions of saline (lanes 1 and 2) or carbachol 30 nmol kg^{-1} h^{-1} (lanes 3 and 4). Note increase in H^+K^+-ATPase, but not HDC or GAPDH signal with carbachol infusion.

Gastrin radioimmunoassay

Gastrin in rat plasma was measured with a previously described method by use of a rabbit antiserum directed against the biologically active part of the gastrin molecule (Kleveland *et al*., 1985).

Materials

^{32}P labelled nucleotides were obtained from Amersham (Buckinghamshire, U.K.); [1-^{14}C]-L-histidine and Protosol from New England Nuclear (Boston, Mass). Nylon membranes for the Northern blots were obtained from Boehringer Mannheim (Mannheim, Germany). Unsulphated human heptadecapeptide gastrin and other chemicals were purchased from Sigma (St. Louis, MO). Male Wistar rats weighing 240–260 (mean 250) g were obtained from Møllegaard (Skensved, Denmark).

Statistics and calculations

The HDC enzyme activity and the different mRNA abundances are expressed as % of basal values. Messenger RNA abundances were normalized to GAPDH values. Differences in plasma gastrin, oxyntic mucosal HDC activity and mRNA abundances were evaluated by use of the Mann–Whitney U test for unpaired samples. Changes in venous histamine concentrations (portal venous blood or rat isolated stomach vascular effluent) were evaluated by means of the Wilcoxon matched pairs test. All numerical values are expressed as mean ± s.e.mean.

Results

Acid secretion

Carbachol (30 nmol kg^{-1} h^{-1}) stimulated acid secretion approximately four fold from 20 ± 2.9 μmol 30 min^{-1} to 84.3 ± 20.8 μmol 30 min^{-1} ($P<0.05$) over 30 min, and rates of secretion remained significantly elevated for the next 120 min. No stimulation of acid secretion was seen in animals infused with saline (range 17.5 ± 2.5 to 30.9 ± 5.0 μmol 30 - min^{-1}), over a comparable period (Figure 2).

Histamine release to portal venous blood

Infusion of carbachol (30 nmol kg^{-1} h^{-1}), had no effect on portal venous histamine concentrations, which were 98.0 ± 9.8 nM before carbachol and 85.0 ± 11.9 nM immediately after, and which varied between 88.0 ± 12.3 and 94.2 ± 13.0 nM during the 45 min infusion (Figure 3). In marked contrast, in response to infusion of gastrin (10 pmol kg^{-1} h^{-1}), the histamine in plasma from portal blood was promptly increased from 128.3 ± 13.1 nM before infusion to 222.0 ± 17.4 nM within 5 min, and varied between 191.7 ± 11.7 and 146.7 ± 23.0 nM during the infusion period (Figure 3).

Oxyntic mucosal H^+K^+-ATPase mRNA abundance

In response to infusion of carbachol (30 nmol kg^{-1} h^{-1}) there was an increase in proton pump mRNA abundance of $140.6 \pm 11.8\%$ relative to basal ($P<0.05$), after 1 h (Figures 1 and 2). The H^+K^+-ATPase mRNA returned to basal levels after 6 h. GAPDH mRNA abundance was unaffected by carbachol infusion.

Oxyntic mucosal HDC enzyme activity and mRNA abundance

During carbachol infusion there were no significant changes in either HDC enzyme activity or HDC mRNA abundance. Enzyme activity varied from 81.6 ± 10.6 to $109.3 \pm 11.1\%$ of basal, and HDC mRNA abundance varied from 93.5 ± 8.2 to $106.2 \pm 8.7\%$ of basal throughout the carbachol infusion (Figure 4).

Histamine release from rat isolated stomachs

Over the range 10 nM to 1 mM carbachol failed to stimulate histamine release, except the 1 μM dose which elicited a modest rise from 88 ± 11 nM preinfusion to 119 ± 13 nM during carbachol ($P<0.01$).

Serum gastrin concentrations

In the groups of animals used to determine acid secretion, HDC enzyme activity and mRNA abundances, serum gastrin during carbachol infusion varied from 24.0 ± 3.3 to 18.8 ± 0.5 pM. In the rats with portal vein catheters, serum gastrin varied from 11.2 ± 1.0 to 13.6 ± 1.2 pM during infusion. Carbachol did not induce significant changes in serum gastrin in any group. In the rats that received gastrin infusions, serum gastrin increased from 12.0 ± 1.2 pM before the peptide, to 233.0 ± 14.0 pM immediately after. During the 45 min infusion period, serum gastrin varied from 238 ± 7.0 to 245.0 ± 15.1 pM.

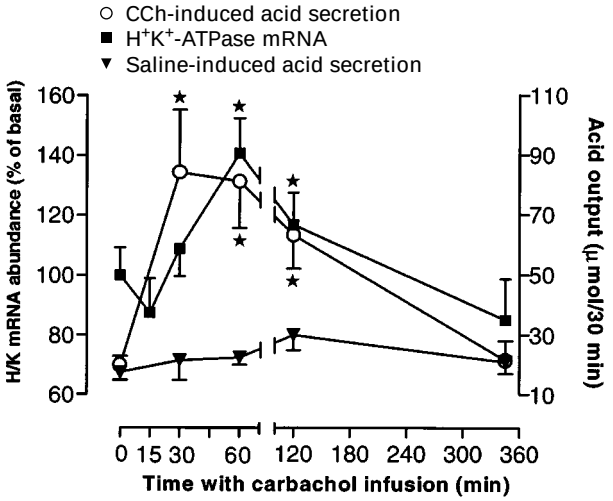

Figure 2 Acid secretion and gastric corpus mRNA abundances in rats infused with carbachol (CCH, 30 nmol kg^{-1} h^{-1}) or saline (controls). In one group ($n=4$) gastric acid secretions were collected for 15 min each side of the time points; in a second group ($n=4-6$) gastric corpus total RNA was harvested at the times indicated. Results are mean and vertical lines show s.e.mean. *$P<0.05$ relative to basal.

Discussion

Support for the idea that gastrin mainly acts to stimulate acid secretion via the release of ECL cell histamine comes from four lines of evidence: firstly, detailed pharmacological studies on the action of histamine H_2 receptor antagonists on acid secretion in the isolated perfused stomach of the mouse (Black & Shankley, 1987). Secondly, studies on the rat isolated perfused stomach which show that gastrin evokes an immediate, dose-dependent histamine release sufficient to explain the subsequent stimulation of acid secretion in the rat (Sandvik *et al.*, 1987), and this effect is mediated by cholecystokinin$_B$ (CCK_B) type receptor (Sandvik & Waldum, 1991); similarly gastrin increases venous histamine output from the canine stomach (Gerber & Payne, 1992). Thirdly, gastrin releases histamine from small-cell enriched fractions of gastric fundic mucosal cells from rat, rabbit and dog (Chuang *et al.*, 1992; Roche *et al.*, 1991a, b; Prinz *et al.*, 1993; Sandor *et*

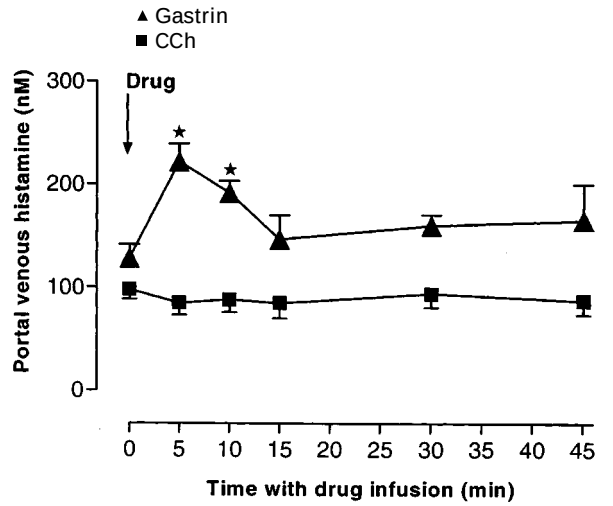

Figure 3 Histamine output to the portal venous blood during intravenous infusion with carbachol 30 nmol kg^{-1} h^{-1} (CCh) or gastrin 1–17 10 pmol kg^{-1} h^{-1}. Results are mean and vertical lines show s.e.mean, $n=5$. *$P<0.05$.

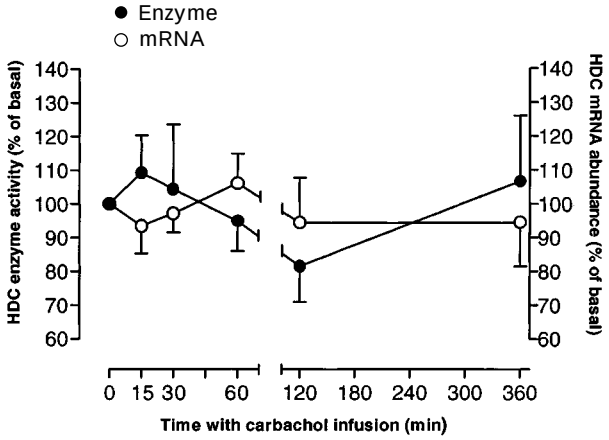

Figure 4 Histidine decarboxylase (HDC) enzyme activity (Enzyme) and mRNA abundance (mRNA) in rats stimulated with carbachol 30 nmol kg^{-1} h^{-1} from 0–6 h. Results are mean and vertical lines show s.e.mean, $n=4-6$.

al., 1996). Fourthly, it is now clear that a neuroendocrine cell, the enterochromaffin-like (ECL) cells, synthesizes, stores and releases histamine upon gastrin stimulation (Roche *et al*., 1991a, b; Prinz *et al*., 1993; Sandor *et al*., 1996), and is also a target for gastrin in stimulating ECL cell growth in animals and in man (Borch *et al*., 1985; Lönroth *et al*., 1990; Bordi *et al*., 1995).

The main finding of the present study was that the muscarinic agonist carbachol potently stimulated gastric acid secretion and proton pump gene expression in conscious, minimally restrained rats. But, unlike gastrin, carbachol had no effect on ECL cell function. The oxyntic mucosal H^+K^+-ATPase mRNA abundance increased with acid secretion and was significantly higher than basal after 1 h with carbachol, lagging slightly behind the increase in acid secretion. The time-scale and the relative increase of H^+K^+-ATPase mRNA abundance is comparable to that observed after histamine infusion in rats anaesthetized with pentobarbitone (Tari *et al*., 1994). Similar increases in H^+K^+-ATPase mRNA abundance have also been described in purified preparations of canine parietal cells in response to histamine and carbachol (Campbell & Yamada, 1989).

In marked contrast, carbachol failed to influence a range of indicators of ECL cell activity. Thus carbachol had no effect on histamine release to portal blood, HDC enzyme activity or HDC mRNA abundance. This contrasts sharply with findings in previous studies from our laboratories and others, which showed that intravenous infusions of gastrin, that elicit serum gastrin concentrations achieved in the present study, produced significant, time- and dose-dependent increases in HDC enzyme activity and HDC mRNA abundance (Sandvik *et al*., 1994; Chen *et al*., 1994). Those effects could not be reproduced with carbachol. In the present study we also examined and compared the effects of intravenous infusion of carbachol and gastrin on the release of histamine to portal venous blood. While gastrin elicited significant increases in portal venous histamine concentration, carbachol had no effect. Taken together, the present data strongly suggest that the acid secretagogue effect of carbachol is not mediated by ECL cell histamine release *in vivo*.

The isolated, vascularly perfused stomach of the rat is a very sensitive model for the study of gastrin-induced histamine release; even in the low picomolar range gastrin induces a significant increase in venous histamine output (Sandvik & Waldum, 1990). Moreover, this preparation excludes the possibility that the extrinsic innervation influences ECL cell responses. However, carbachol did not induce dose-dependent changes in histamine release over the range 10 nM – 1 mM. The increase in histamine output observed with 1 μM carbachol may indicate that there is a slight muscarinic stimulation of the ECL cell in the rat isolated stomach preparation, in line with the findings from studies on isolated, enriched ECL cells (Prinz *et al*., 1993; Sandor *et al*., 1996). However, the lack of a dose-response relationship between carbachol and the venous histamine output is in striking contrast to that found with gastrin, again suggesting that the acid secretagogue effect of carbachol is not dependent on the stimulation of histamine release.

On the other hand, a previous study has shown that electrical vagal stimulation of isolated vascularly perfused stomachs of the rat increases acid secretion and induces a modest histamine release which is inhibited by atropine (Sandvik *et al*., 1988). However, the venous histamine output measured in this study was much lower than that induced by a gastrin dose eliciting a similar acid output, suggesting that it is likely to have only a minor role. Recent studies on enriched ECL cell preparations demonstrated that carbachol (1 nM to 100 mM) released histamine (Prinz *et al*., 1993; Sandor *et al*., 1996), but again, the amount of histamine released was modest compared to that found after stimulation with gastrin (1 nM). Thus, those studies also indicate that there is a dominating, direct acid secretagogue effect of muscarinic agents on the parietal cell. Several studies show that histamine H_2 receptor antagonists attenuate the acid secretagogue effect of cholinoceptor agents. This effect may be explained by removal of a potentiating interaction between histamine and cholinoceptic agents on the parietal cell (Grossman & Konturek, 1974).

In conclusion, the findings of the present study strongly suggest that the acid secretagogue effect of the muscarinic agent carbachol and therefore endogenous muscarinic activity, is mainly exerted directly on the parietal cell in the rat, and cast doubt on the physiological significance of acid secretion of muscarinic receptors on ECL cells.

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