Br. J. Pharmacol. (1989), **96**, 557–562

Histamine release in the isolated vascularly perfused stomach of the rat: regulation by autoreceptors

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1 In the isolated vascularly-perfused stomach of the rat, gastrin 1-17 (520 pmol l^{-1}) increased acid output from basal values of 13.7 ± 2.7 to 92.5 ± 11.4 μmol h^{-1} and venous histamine output from 10.1 ± 2.3 to 54.7 ± 7.9 nmol h^{-1} (mean ± s.e.mean).

2 The H_1 receptor agonist 2-methylhistamine (10 μmol l^{-1}) increased acid output to 21.6 ± 2.9 μmol h^{-1} ($P < 0.05$) and reduced basal histamine output to 4.0 ± 0.8 nmol h^{-1} ($P < 0.05$). Gastrin-stimulated acid secretion and vascular histamine output was not significantly affected by 2-methylhistamine (10 μmol l^{-1}).

3 The H_2 receptor agonist, impromidine, dose-dependently increased basal acid secretion, reaching a maximal value of 145.5 ± 11.7 μmol h^{-1} with impromidine (10 μmol l^{-1}), and maximal gastrin-stimulated acid secretion to 167.4 ± 15.1 μmol h^{-1} with impromidine (10 μmol l^{-1}). Impromidine dose-dependently inhibited basal and gastrin-stimulated vascular histamine output.

4 The H_3 receptor agonist **R**-a-methylhistamine, (1 and 10 μmol l^{-1}) minimally increased basal acid secretion. **R**-a-methylhistamine (10 μmol l^{-1}) did not significantly affect maximal gastrin-stimulated acid secretion. Basal and gastrin-stimulated vascular histamine outputs decreased to 4.0 ± 0.8 ($P < 0.05$) and 24.7 ± 4.7 nmol h^{-1} ($P = 0.05$) with **R**-a-methylhistamine (10 μmol l^{-1}).

5 The H_2 receptor antagonist ranitidine (2 μmol l^{-1}) did not inhibit basal acid secretion, but acid outputs with gastrin and all histamine agonists were reduced. Ranitidine did not affect histamine release in the basal state, with gastrin or with any histamine agonist tested.

6 We conclude that gastric histamine release in the rat is regulated via a histamine H_2 receptor sensitive to the histamine agonists tested, but not to ranitidine. It is unlikely that the inhibition of histamine release is secondary to increased gastric acidity.

Introduction

Histamine is recognized as an important intercellular messenger. It is released from histamine-storing cells in several tissues and regulates cellular function via histamine receptors on the effector cell surface. Previous research has led to the discovery of three specific subclasses of histamine receptors designed H_1, H_2 and H_3 receptors (Black *et al.*, 1972; Arrang *et al.*, 1983). The distribution of H_1 and H_2 receptors is very complex (Chand & Eyre, 1975) while so far, H_3 receptors have only been documented in histaminergic neurones.

Histamine is an important mediator substance in the physiological regulation of gastric function. Stimulation of H_1 receptors in the gastric vascular bed induces vasodilatation (Main & Whittle, 1976). The H_2 receptors on the parietal cell mediate the potent acid secretagogue effect of histamine (Black *et al.*, 1972; Soli & Berglindh, 1987), and administration of H_2 receptor antagonists inhibits the acid response to all natural stimuli (Grossman & Konturek, 1974). Until now there have been no studies concerning the presence of H_3 receptors in the gastric mucosa.

The central role of histamine in the regulation of gastric secretion has led to great interest in the mechanism regulating the liberation of this mediator from its mucosal stores. Our studies with the isolated, vascularly perfused stomach of the rat have shown that gastrin induces an immediate and dose-dependent histamine release preceding acid secretion (Sandvik *et al.*, 1987), and furthermore that somatostatin (Sandvik & Waldum, 1988a) and prostaglandin analogues (Sandvik & Waldum, 1988b) inhibit this release. Another interesting aspect of the regulation of gastric histamine release is the possible existence of autoregulatory mechanisms. Previous

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studies have suggested that in the rat, histamine can inhibit its own synthesis and release by a H_2-receptor-mediated feedback mechanism (Håkanson *et al.*, 1977; 1978; Maslinski & Sewing, 1977). While extensive studies have shown that H_3 receptor stimulation inhibits neuronal histamine synthesis and release (Arrang *et al.*, 1987), there are no studies on the role of H_3 receptors in the regulation of gastric histamine release.

We carried out the present study to examine the effects of histamine H_1, H_2 and H_3 agonists on baseline and gastrin-stimulated histamine release in the isolated vascularly perfused stomach of the rat. In this acid-secreting, rat isolated stomach preparation the vascular perfusate is not recirculated, ensuring optimal control of the stimulants applied. Furthermore, the perfusate contains no histamine-storing cells (basophil leukocytes, thrombocytes), and the venous effluent is collected from the portal vein with minimal degradation of histamine. This ensures maximal sensitivity and reproducibility of the assay, with the functional integrity of the stomach preserved.

Methods

The rat isolated vascularly perfused stomachs were prepared as previously described (Kleveland *et al.*, 1986). Briefly, fasted rats weighing 240–260 g (mean 250 g) were anaesthetized with urethane and the stomachs isolated and transferred to an organ bath filled with Krebs and Ringer buffer. The vascular bed was perfused through an arterial catheter with a Krebs and Ringer buffer with an ionized calcium concentration of 1.12 mmol l^{-1}, dextran T70 40 mg ml^{-1}, glucose 5 mmol l^{-1}, pyruvate 5 mmol l^{-1} and 10% (vol/vol) washed ovine erythrocytes. The vascular perfusate was gassed with O_2 (96%) and CO_2 (4%) using a membrane oxygenator, and the stomachs perfused at a rate of 2 ml min^{-1}. The vascular effluent was not recirculated. The gastric lumen was perfused with distilled water (pH 7.0) gassed with 100% O_2. All perfusates and the organ bath were kept at 37°C.

The compounds, 2-methylhistamine (H_1 agonist), impromidine (H_2 agonist) and R-a-methylhistamine (H_3 agonist) were administered from the start of the vascular perfusion. After an initial 'washout' period of 20 min, isobutyl methylxanthine (IBMX) (50 μmol l^{-1}) was added to the vascular perfusate. Baseline acid secretion and histamine release rates were measured with IBMX only. In the stimulation studies, gastrin 1-17 (Gl-17) 520 pmol l^{-1} was added to the vascular perfusion medium after another 20 min and continued for 60 min. One stomach preparation was used as a single experiment, and each drug combination tested on five stomachs.

Sampling and analysis

The vascular and luminal effluents were collected in 10-min fractions. Acid output was determined by titrating each fraction to pH 7.0 with NaOH 1 mmol l^{-1} (Radiometer, Copenhagen, Denmark). The vascular effluent was collected and centrifugated at +4°C, and the supernatant kept frozen at −20°C until analysis for histamine.

The histamine analyses were performed by use of a commercial histamine radioimmunoassay kit (Immunotech, Marseille, France). The kits include tubes coated with monoclonal antibody against acylated histamine, acylating agent and ^{125}I acylated histamine as tracer. The sensitivity is 0.1 nmol l^{-1} histamine, intra-assay variance 6.1%. 2-Methylhistamine, impromidine and R-a-methylhistamine showed histamine crossreactivities of 0.005, 0.005 and 0.002, respectively. Gastrin, ranitidine and IBMX in the concentrations used, did not crossreact with histamine in the RIA.

Calculations

The results are expressed as total acid output and total venous histamine output during the 40–100 min perfusion period. Histamine-like immunoreactivity from the histamine agonists added to the perfusate was subtracted from the measured histamine concentrations when calculating histamine outputs. Differences in acid and histamine outputs were evaluated for significance by the Wilcoxon rank sum test for unpaired samples.

Drugs and solutions

The following drugs were used: isobutyl methylxanthine (Sigma), 2-methylhistamine (Smith, Kline & French), impromidine (Smith, Kline & French), ranitidine (Glaxo), R-a-methylhistamine (J-M Arrang, Paris), gastrin 1–17 (Professor E. Wünch, Munich). All drugs were dissolved in the perfusion buffer, except isobutyl methylxanthine which was first dissolved in 96% ethanol and subsequently diluted in normal saline and the perfusion buffer (final dilution 1 : 860).

Results

Acid secretion

Gastrin 1–17 (520 pmol l^{-1}) significantly ($P < 0.01$) increased acid output from basal (IBMX only) 13.7 ± 2.7 μmol h^{-1} to 92.5 ± 11.4 μmol h^{-1} (mean ± s.e.mean).

The H_1 agonist 2-methylhistamine (10 μmol l^{-1}) increased basal acid output to 21.6 ± 2.9 μmol h^{-1} ($P < 0.05$). Impromidine in the range from

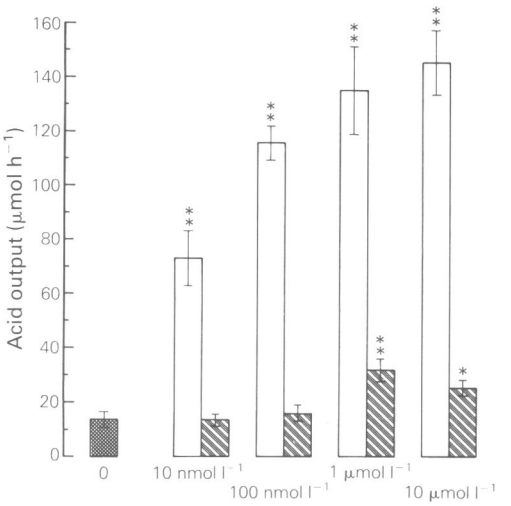

Figure 1 Acid output from rat isolated stomachs, under basal conditions and in response to increasing concentrations of histamine receptor agonist. Open columns impromidine (H_2 agonist), closed columns-**R**-a-methylhistamine (H_3 agonist). Significance of differences from baseline are indicated by * ($P < 0.05$) or ** ($P < 0.01$). Results expressed as mean of five experiments for baseline and each drug concentration; s.e.mean shown by vertical bars.

$10\,nmol\,l^{-1}$ to $10\,\mu mol\,l^{-1}$ dose-dependently increased basal acid output, reaching $145.5 \pm 11.7\,\mu mol\,h^{-1}$ with $10\,\mu mol\,l^{-1}$ (Figure 1). **R**-a-methylhistamine stimulated basal acid secretion, reaching a maximum value of $32.0 \pm 4.2\,\mu mol\,h^{-1}$ with $1\,\mu mol\,l^{-1}$ (Figure 1).

G1–17-stimulated acid output was not significantly changed after $10\,\mu mol\,l^{-1}$ of 2-methylhistamine or **R**-a-methylhistamine (115.0 ± 11.8 and $92.4 \pm 17.4\,\mu mol\,h^{-1}$, respectively). Impromidine increased G1–17-stimulated acid secretion, reaching a maximum value of $167.4 \pm 15.1\,\mu mol\,l^{-1}$ with $10\,\mu mol\,l^{-1}$ concentration (Figure 2).

Ranitidine ($2\,\mu mol\,l^{-1}$) did not affect basal acid output ($8.1 \pm 2.8\,\mu mol\,h^{-1}$). Gastrin-stimulated acid secretion was reduced to $18.7 \pm 6.3\,\mu mol\,h^{-1}$ with this concentration of ranitidine ($P < 0.01$). Ranitidine reduced the acid output stimulated by 2-methylhistamine ($10\,\mu mol\,l^{-1}$) to 14.9 ± 0.5 ($P = 0.05$), and with impromidine ($100\,nmol\,l^{-1}$) to 28.9 ± 0.9 ($P < 0.01$), and with **R**-a-methylhistamine ($10\,\mu mol\,l^{-1}$) to 10.8 ± 1.8 ($P < 0.05$) $\mu mol\,h^{-1}$.

Histamine release

G1–17 induced a prompt release of histamine to the venous effluent, the output increasing from basal 10.1 ± 2.3 to $54.7 \pm 7.9\,nmol\,h^{-1}$ ($P < 0.01$).

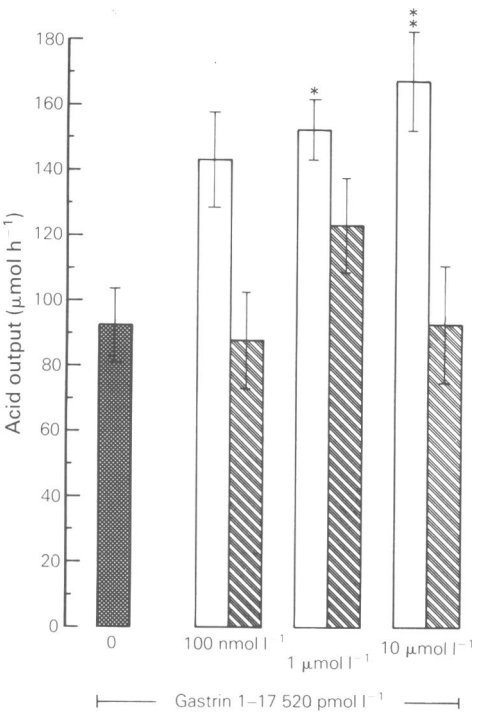

Figure 2 Acid output from rat isolated stomachs, with gastrin 1–17 ($520\,pmol\,l^{-1}$) alone, or in combination with increasing concentrations of histamine receptor agonist. Open columns, impromidine (H_2 agonist); closed columns, **R**-a-methylhistamine (H_3 agonist). Significance of differences from gastrin alone are indicated by *($P < 0.05$) or **($P < 0.01$). Results expressed as mean of five experiments for baseline and each drug concentration, vertical bars show s.e.mean.

2-Methylhistamine ($10\,\mu mol\,l^{-1}$) reduced basal histamine output to $4.0 \pm 0.8\,nmol\,h^{-1}$ ($P < 0.05$). Impromidine dose-dependently inhibited basal histamine output, to a minimum value of $3.7 \pm 0.6\,nmol\,h^{-1}$ with $1\,\mu mol\,l^{-1}$ (Figure 3). **R**-a-methylhistamine depressed basal histamine output to $4.0 \pm 0.9\,nmol\,h^{-1}$ at $10\,\mu mol\,l^{-1}$ ($P = 0.05$) (Figure 3).

During the infusion of 2-methylhistamine ($10\,\mu mol\,l^{-1}$), gastrin-stimulated histamine release was reduced to $23.6 \pm 5.3\,nmol\,h^{-1}$ ($P = 0.05$). Impromidine induced a dose-dependent inhibition of stimulated histamine output, reaching a minimum value of $9.5 \pm 1.0\,nmol\,h^{-1}$ at $10\,\mu mol\,l^{-1}$ (Figure 4). With **R**-a-methylhistamine ($10\,\mu mol\,l^{-1}$), gastrin-stimulated histamine release was reduced to $24.7 \pm 4.7\,nmol\,h^{-1}$ ($P = 0.05$) (Figure 4).

Ranitidine ($2\,\mu mol\,l^{-1}$) did not significantly affect histamine release in the basal state or with gastrin, H_1, H_2 or H_3 agonist, the histamine outputs being

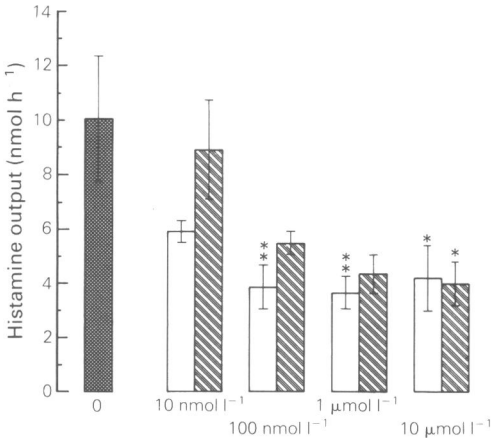

Figure 3 Venous histamine output from rat isolated stomachs, under basal conditions and in response to increasing concentrations of histamine receptor agonist. Open columns, impromidine (H_2 agonist), closed columns, R-a-methylhistamine (H_3 agonist). Significance of differences from baseline are indicated by * ($P < 0.05$) or ** ($P < 0.01$). Results expressed as mean of five experiments for baseline and each drug concentration, vertical bars show s.e.mean.

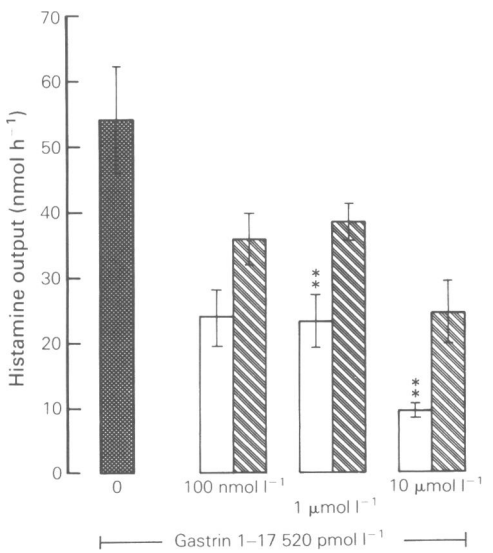

Figure 4 Venous histamine output from rat isolated stomachs, with gastrin 1–17 (520 pmol l^{-1}) alone, or in combination with increasing concentrations histamine receptor agonist. Open bars impromidine (H_2 agonist), closed bars – R-a-methylhistamine (H_3 agonist). Significance of differences from gastrin alone are indicated by ** ($P < 0.01$). Results expressed as mean of five experiments for each drug concentration; vertical bars show s.e.mean.

7.9 ± 1.3, 39.9 ± 5.5, 6.3 ± 0.9, 5.5 ± 1.0 and 4.5 ± 1.0 nmol h^{-1}, respectively.

Discussion

The present study clearly shows that the H_2-agonist impromidine inhibits both baseline and gastrin-stimulated histamine release from the isolated vascularly perfused stomach of the rat. The ECL cell stores most of the histamine present in rat gastric mucosa (Håkanson & Sundler, 1987). The histamine measured in the venous effluent of this stomach preparation therefore most probably originates from those cells. This indicates that autoregulatory histamine H_2 receptors are present on the ECL cell surface. This is in accordance with previous studies (Håkanson *et al.*, 1977; 1978; Maslinski & Sewing, 1977) showing H_2 receptor-mediated inhibition of histamine synthesis (histidine decarboxylase activity) and of histamine mobilization from rat gastric mucosa after pentagastrin infusion.

An additional finding of the present study is that impromidine suppressed histamine output even at concentrations not giving maximal acid secretion. This suggests that the H_2 receptor-mediated suppression of histamine release could be induced by physiological concentrations of free histamine within the gastric mucosa. However, the H_2 antagonist ranitidine in a concentration completely inhibiting gastrin-stimulated acid secretion did not affect venous histamine output. This may suggest that the histamine receptor on the ECL cell is different from the H_2 receptor on the parietal cells, interacting with the H_2 agonist impromidine but not with the H_2 antagonist ranitidine.

The inhibition of histamine release by impromidine could also be secondary to increased acidity of the gastric lumen. Previously we have shown that somatostatin inhibits gastric histamine release (Sandvik & Waldum, 1988a) and the observed inhibition of histamine release could be due to paracrine release of somatostatin induced by acidification. However, the present results make this explanation unlikely since impromidine in increasing concentrations from 100 nmol l^{-1} to 10 μmol l^{-1} given together with gastrin only slightly increased acid output (or lowered gastric pH), and still progressively suppressed histamine release. Furthermore, ranitidine potently inhibited impromidine-induced acid secretion without significantly affecting vascular histamine output. We conclude that the described effect of impromidine on histamine liberation is not due to stimulation of acid secretion.

Histamine H_1 and H_3 receptor agonists as administered in the present study have marginal effects on acid secretion. Furthermore, at high concentrations, these agents suppressed basal vascular histamine

release. We were not able to find effects of H_3 receptor stimulation comparable to the potent suppression of histamine release described in central and peripheral histaminergic neurones (Arrang *et al.*, 1987). This would indicate that the ECL cells do not possess H_3 receptors, and it is unlikely that a significant fraction of the venous histamine comes from gastric histaminergic neurones. The effects of 2-methylhistamine and **R**-a-methylhistamine on acid secretion and histamine release could be explained by the relative unspecificity of the agents used. The relative H_2 agonist potencies of 2-methylhistamine and **R**-a-methylhistamine vs. histamine are 0.04 and 0.01, respectively (Schwartz *et al.*, 1986; Arrang *et al.*, 1987). Parietal cells probably possess H_2 receptors only, but 2-methylhistamine and **R**-a-methylhistamine in the concentrations tested slightly stimulated acid secretion. This suggests that the H_1 and H_3 receptor agonists in the concentrations used show some affinity for H_2 receptors, and this is further supported by the finding that ranitidine inhibited the acid secretagogue effect of those substances. Likewise, the inhibition of histamine release induced by these agents could be a H_2-mediated effect. IBMX sensitizes the rat isolated stomach to the major gastric secretagogues like gastrin, histamine and muscarinic agents, inducing a leftward shift of the dose-response curve. Furthermore, IBMX stimulates gastrin release while the release of histamine and somatostatin is unaffected (Sandvik *et al.*, 1988), and since endogenous gastrin is drained via the venous effluent there are probably no significant indirect effects of IBMX on the parietal and ECL cells in this model. Previous studies from our laboratory have shown that the histamine release from the isolated vascularly perfused stomach of the rat can be stimulated by gastrin (Sandvik *et al.*, 1987), and inhibited by somatostatin (Sandvik & Waldum, 1988a) and misoprostol, a methylated prostaglandin E_1 analogue (Sandvik & Waldum, 1988b). Together with the present results, it is evident that several of the most important substances regulating gastric acid secretion also modulate ECL cell histamine release. This makes endogenous histamine release a probable mechanism of at least some of the effects of these substances on gastric acid secretion in the rat.

This work was supported by the Norwegian Research Council for Science and the Humanities, and the Norwegian Cancer Society. The financial support of the Cancer Fund at the Regional and University Hospital, Trondheim, is also acknowledged. We thank Britt Søgnen and Tore Skei for skilful technical assistance.

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(Received June 8, 1988
Revised September 28, 1988
Accepted October 11, 1988)