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# α-Fluoromethylhistidine depletes histamine from secreting but not from non-secreting rat stomach ECL cells

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**Abstract**

Histamine in the oxyntic mucosa of the rat stomach occurs in mast cells (10%) and ECL cells (90%). Unlike the mast cells, the ECL cells operate under the control of gastrin. α-Fluoromethylhistidine, an irreversible inhibitor of the histamine-forming enzyme, histidine decarboxylase depletes ECL-cell but not mast-cell histamine. This report shows that the effectiveness by which histidine decarboxylase inhibition depletes ECL-cell histamine depends on the rate of histamine secretion. Rats received α-fluoromethylhistidine by continuous subcutaneous infusion for 24 h. Maximally effective doses ($\geq 3$ mg/kg/h) inhibited histidine decarboxylase and reduced oxyntic mucosal histamine in fed rats by 80–90%. In fasted rats, the reduction was 50%. α-Fluoromethylhistidine greatly reduced the number of histamine-immunoreactive ECL cells (immunocytochemistry) and of secretory vesicles in the ECL cells (electron microscopy) in fed but not in fasted rats. The half-life of oxyntic mucosal histamine (determined upon histidine decarboxylase inhibition) was 2.6 h in fed rats and 19.4 h in fasted rats. The amount of histamine secreted in response to gastrin (monitored by gastric submucosal microdialysis) was greatly reduced by α-fluoromethylhistidine in fed rats but not in fasted rats. ECL cells were isolated from rat stomach by elutriation (80% purity). Their histamine content was determined after culture, with or without α-fluoromethylhistidine, in the presence of varying concentrations of gastrin. In a medium containing 10 nM gastrin, ECL cells responded to a maximally effective concentration of α-fluoromethylhistidine (0.1 nM) with 80% reduction in histamine content. In the absence of gastrin, ECL cells responded to α-fluoromethylhistidine with 45% reduction of histamine; the releasable histamine pool was unaffected. In conclusion, the combination of histidine decarboxylase inhibition and a high rate of histamine secretion will promptly exhaust the ECL-cell histamine pool, while histidine decarboxylase inhibition and a low secretion rate will affect the histamine pool much less. 

*Keywords:* α-Fluoromethylhistidine; Gastrin; Histamine; Histidine decarboxylase; Microdialysis in vivo; ECL cell; Secretory vesicle; Stomach

## 1. Introduction

Oxyntic mucosal histamine in the rat occurs in mast cells (10%) and ECL cells (90%) (Håkanson et al., 1986, 1994). The ECL cells respond to gastrin by production and secretion of histamine and chromogranin A-derived peptides, such as pancreastatin (Chen et al., 1994, 1996a; Håkanson et al., 1995; Kimura et al., 1997; Lindström et al., 1997; Norlén et al., 1997; Kitano et al., 2000). Histamine mobilized from the ECL cells mediates gastrin-stimulated acid secretion (Håkanson and Sundler, 1991; Waldum et al., 1991; Andersson et al., 1996a). Mast cells on the other hand, do not respond to gastrin (Soll et al., 1988) and are not believed to be involved in the regulation of acid secretion (Andersson et al., 1996a).

Histamine in the ECL cells is stored in secretory vesicles (Zhao et al., 1999a) and mobilized by exocytosis. The formation of histamine, catalyzed by the cytosolic enzyme histidine decarboxylase, can be blocked by the irreversible histidine decarboxylase inhibitor α-fluoromethylhistidine (Kollonitsch et al., 1978; Kollonitsch, 1982; Watanabe et

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al., 1990). Treatment with α-fluoromethylhistidine causes a prompt loss of ECL-cell histamine, while mast cell histamine seems to be resistant (Andersson et al., 1992, 1996b). The present report originated from a pilot study, showing that while α-fluoromethylhistidine depleted histamine from ECL cells in fed rats it failed to do so in fasted rats (see also Koyama et al., 1987). The effects of α-fluoromethylhistidine-induced histidine decarboxylase inhibition were studied with respect to (1) the histamine storage compartment of ECL cells (chemistry, immunocytochemistry and electron microscopy) and (2) the ability of ECL cells to mobilize histamine in response to gastrin (in vivo gastric submucosal microdialysis, isolated ECL cells). The results demonstrate that the rate and degree of α-fluoromethylhistidine-induced depletion of ECL-cell histamine depend upon the secretory activity of the ECL cells, thus explaining the different effects of α-fluoromethylhistidine on fasted versus fed rats.

## 2. Materials and methods

### 2.1. Drugs

α-Fluoromethylhistidine was obtained from Sigma (St. Louis, MO, USA) and dissolved in 0.9% NaCl. Human $Leu^{15}$-gastrin-17 was obtained from Research Plus (Bayonne, NJ, USA) and dissolved in 0.9% NaCl, containing 1% bovine serum albumin (Boehringer Mannheim, Mannheim, Germany). Matrigel® was from Collaborative Biomedical Products (Bedford, MA, USA). Dulbecco's modified Eagle's medium (DMEM)-Ham's F12 was from Sigma.

### 2.2. Animals

Male or female (as specified) Sprague–Dawley rats (200–250 g) were kept at a 12-h light and 12-h dark cycle in plastic cages (four to six in each cage) with free access to standard rat food pellets (Lactamin, Vadstena, Sweden) and tap water. During fasting, the rats were housed in individual cages with wire mesh bottoms for 24–48 h. Rats that were to receive gastrin by intravenous infusion received a catheter in the right jugular vein under chloral hydrate anaesthesia (300 mg/kg, intraperitoneally). Rats were kept in Bollman-type restraining cages when they received intravenous infusion or were subjected to gastric mucosal microdialysis (see below). α-Fluoromethylhistidine was administered via osmotic minipumps (Alzet 2001 D, Alza, Palo Alto, CA, USA), subcutaneously in the neck under methohexitone sodium (Brietal®) anaesthesia (1.5 ml/kg intraperitoneally, 30 mg/ml, Eli Lilly, Indianapolis, IN, USA). Before implantation, the minipumps were activated in 0.9% NaCl at 37°C for 4 h. The rats were killed by exsanguination from the abdominal aorta under chloral hydrate anaesthesia (as above). The stomach was opened along the major curvature, washed in ice-cold 0.9% saline, and placed on an ice-cooled glass surface with the mucosa upwards. The oxyntic mucosa was taken for histology (see below) and/or scraped off with a scalpel and stored at −80°C for determination of histidine decarboxylase activity and histamine concentration. The studies were approved by the local Animal Welfare Committee, Lund.

### 2.3. Dose–response and time–response experiments in intact rats

Forty-two female rats received α-fluoromethylhistidine at doses ranging from 0.03 to 30 mg/kg/h. They were killed 24 h after start of infusion. In a follow-up study, the time course of oxyntic mucosal histamine depletion was investigated by giving α-fluoromethylhistidine (3 mg/kg/h) for 1.5 to 24 h to freely fed rats and fasted rats (118 male rats in all). Sham-operated rats received a midline incision in the neck (the skin was immediately closed with sutures) under methohexitone anaesthesia (as above), and were killed after 1.5 to 24 h. Fasted rats were deprived of food for 24 h before start of the α-fluoromethylhistidine infusion. Fasting continued for as long as the infusion lasted (up to 24 h).

### 2.4. Histology and structural anatomy

Six fasted rats and six freely fed rats received α-fluoromethylhistidine (10 mg/kg/h) via osmotic minipumps (deposited under the skin of the neck) for 24 h, while six fasted rats and 6 freely fed rats were sham operated (as above). Tissue specimens were taken from the ventral aspect of the acid-producing part of the stomach for immunocytochemistry of histamine and for electron microscopy of ECL cells (see below).

### 2.5. Mobilization of ECL-cell histamine studied by in *vivo* microdialysis

One series of experiments was conducted using rats subjected to gastric submucosal microdialysis (Kitano et al., 2000). A flexible microdialysis probe (MAB3.8.10, AgnTho, Stockholm, length 10 mm, outer diameter 0.57 mm, 35 kDa cut-off) was implanted in the submucosal layer of the oxyntic mucosa as previously described (Kitano et al., 2000). 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. Each rat and each probe were used once only. Rats that were to be fasted were deprived of food starting 1 day after surgery. α-Fluoromethylhistidine infusion (3 mg/kg/h) was initiated on day 2 after surgery by implantation of osmotic minipumps (as above) and sampling of microdialysate started on day 3. The inlet tube was connected to a microinfusion pump

(Model 361, Sage instrument, ATI Orion, Boston, MA, USA) and the outlet was allowed to drain into 300 μl polyethylene vials. All rats subjected to submucosal microdialysis were conscious during the experiment since anaesthesia is known to suppress gastrin-induced histamine mobilization from the ECL cells (Norlén et al., 2000). Twenty-four male rats were fitted with a gastric submucosal microdialysis probe: 12 rats were fed and 12 were fasted (48 h) at the time of the experiment. Six fed and six fasted rats received α-fluoromethylhistidine by continuous subcutaneous infusion for 24 h before termination of the experiments, while six fed and six fasted rats did not receive α-fluoromethylhistidine. Perfusion of the microdialysis probes with degassed saline (1.2 μl/min) started at 7 a.m. After 2 h of stabilization, basal samples were collected for 2 h. ECL-cell histamine secretion was stimulated by infusion of gastrin-17 (5 nmol/kg/h, 1 ml/h for 4 h) via the jugular vein. Microdialysate samples were collected every 20 min during the first h after start of gastrin infusion and then hourly.

### 2.6. Isolation and primary culture of ECL cells: histamine content and secretion

ECL cells were purified as described in detail previously (Lindström et al., 1997). Briefly, cells were harvested from the oxyntic mucosa of 4 rats at a time. Repeated counter-flow elutriation resulted in an average yield of 2 million cells. The purity of the ECL cell preparation was assessed by immunocytochemistry, using a guinea-pig anti-rat histidine decarboxylase antiserum (1:750), and found to be at least 80%. The cells were cultured in 96-well plates pre-coated with Matrigel® (diluted 1:10 with DMEM-Ham's F12) (20,000 cells per well) in a humid atmosphere with 5% $CO_2$/95% air at 37°C for 48 h until the start of the experiments. The culture medium consisted of DMEM-Ham's F12 (1:1) supplemented with 2% fetal calf serum, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 250 ng/ml amphotericin B, 10 μg/ml insulin, 5.5 μg/ml transferrin, 5 ng/ml selenium, 1.25 μg/ml bovine serum albumin, 10 nM hydrocortisone, 15 mM HEPES and 10 μM pyridoxal-5-phosphate. α-Fluoromethylhistidine (1, 10 or 100 μM) and/or gastrin-17 (0.1 or 10 nM) were added to the medium at the start of culture. The cells were exposed to α-fluoromethylhistidine and/or gastrin-17 for 48 h. At that stage, the cells were either collected for measurement of histamine content or kept for studies of histamine secretion. The ECL-cell histamine content was determined by radioimmunoassay (see below) after extraction in hot redistilled water for $2 \times 5$ min. For study of histamine secretion, the culture medium was replaced with fresh serum-free, gastrin-free culture medium containing the desired concentration of α-fluoromethylhistidine. After equilibration for 1–2 h, the medium was again aspirated and replaced with secretion medium (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 10 HEPES at pH 7.0. Basal and stimulated (10 nM gastrin) secretion was measured (30 min incubation). The incubation was interrupted by centrifuging the plates at $220 \times g$ for 1 min. The supernatants were collected and stored at −20°C until measurement of histamine.

### 2.7. Determination of histidine decarboxylase activity and histamine concentration

Oxyntic mucosa was homogenised in ice-cold 0.1 M sodium phosphate buffer, pH 7.4, to a concentration of 100 mg/ml. Aliquots (80 μl) of the oxyntic mucosal homogenates were incubated with L-[1-$^{14}$C]histidine (specific activity 50 mCi/mmol), 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). Histidine decarboxylase activity was expressed as pmol $^{14}CO_2$/mg/h. The histamine concentration in oxyntic mucosa, microdialysate and isolated ECL cells was measured by radioimmunoassay using a commercially available kit (Immunotech, Paris, France). The histamine concentration was expressed as μmol per g wet weight, nmol per liter or pmol per well.

### 2.8. Light microscopy

Tissue samples from the oxyntic mucosa ($2 \times 4$ mm) were fixed by immersion in 4% (w/v) 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (Sigma) in 0.1 M sodium phosphate buffer, pH 7.4, for 8–12 h at 4°C (Panula et al., 1988). After rinsing in 10% sucrose-enriched buffer overnight, the specimens were placed on wooden blocks and frozen in CRYOMATRIX embedding medium (Shandon Scientific, Cheswick, England) on dry ice. Frozen sections were cut perpendicular to the mucosal surface in a cryostat (Bright and Huntington, Cambridge, UK) at 10 μm thickness. The sections were thawed onto gelatin-coated glass slides and incubated at 4°C overnight (in a moist chamber) with an antiserum against histamine (code 8431, Eurodiagnostica, Malmö, Sweden) at a dilution of 1:1000 in phosphate-buffered saline (PBS), containing 0.1% Triton X-100 (PBS-T) and 1% bovine serum albumin. The sections were rinsed with PBS twice (10 min each time) and incubated for 2 h at room temperature with fluorescein isothiocyanate (FITC)-conjugated porcine anti-rabbit immunoglobulin G (code F250, Dako, Glostrup, Denmark), diluted 1:40 in PBS-T. After washing twice with PBS-T (10 min each time), the sections were mounted in a mixture of PBS and glycerol (1:3), and examined in an Olympus microscope (BX50, Japan). Photomicrographs were taken with black and white Kodak Tri-X film (ASA 400). Control studies included liquid-phase preabsorption overnight with the antigen (1 μg/ml) and the routine omission of primary antiserum and substitution with non-immune rabbit serum.

### 2.9. Electron microscopy

Minute tissue specimens ($< 1$ mm$^3$) from the oxyntic mucosa were immersed in a mixture of glutaraldehyde (1%) and formaldehyde (3%) in 0.075 M sodium phosphate buffer, pH 7.2, for 6 h. The specimens were post-fixed for 1 h in 1% osmium tetroxide, dehydrated in graded acetone solutions and embedded in Epon 812. Ultrathin sections (60–80 nm) were cut on an LKB MK III Ultrotome, routinely contrasted with uranyl acetate and lead citrate and examined in a transmission electron microscope (Philips CM10) (Zhao et al., 1999a). The ECL cells were identified by their characteristic ultrastructure (Zhao et al., 1999a). ECL cell profiles with nuclei were photographed for planimetric analysis as described by Weibel (1969) and Weibel and Bolender (1973). The granules/vesicles in the ECL cells were classified into granules, secretory vesicles and microvesicles (Zhao et al., 1999a). The granules were defined as cytoplasmic membrane-enclosed organelles, displaying an electron-dense core and a thin electron-lucent halo between the membrane and the dense core, the diameter of the dense core representing at least 50% of the diameter of the entire organelle (50–250 nm in diameter). Vesicles were defined as membrane-enclosed electron-lucent organelles without a dense core or possessing a small, often eccentrically located dense core, the diameter of the dense core being less than 50% of the diameter of the organelle. Vesicles belong to either of two populations: (1) secretory vesicles with a diameter of 125–500 nm (with a dense core, revealed by serial sectioning if not immediately apparent) and (2) clear, electron-lucent microvesicles (without dense core) with a diameter of 25–125 nm (Zhao et al., 1999a). In the present study, we measured the number and volume density of the secretory vesicles.

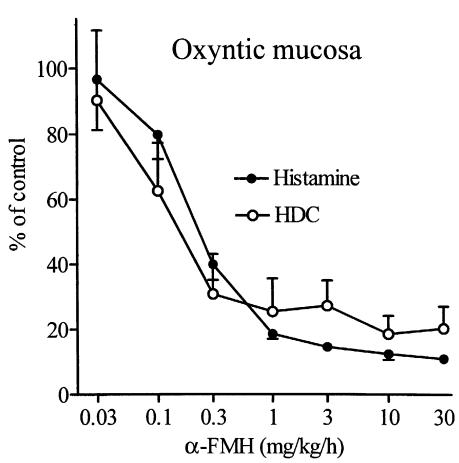


Fig. 1. Effects of increasing doses of α-fluoromethylhistidine (α-FMH) on oxyntic mucosal histidine decarboxylase (HDC) activity and histamine concentration (expressed as per cent of the values in untreated rats). Fed rats received 24 h infusion of α-fluoromethylhistidine from osmotic minipumps implanted under the skin of the neck. Means ± S.E.M. $n = 4$–8.

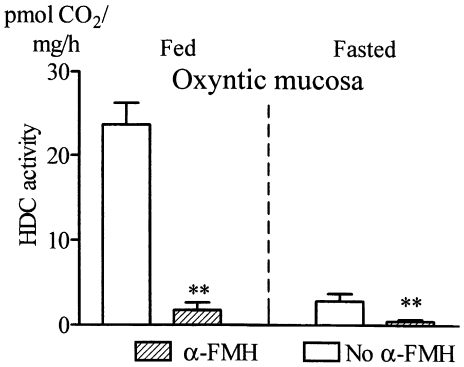


Fig. 2. Effect of α-fluoromethylhistidine (α-FMH) (3 mg/kg/h, 24 h infusion) or no α-fluoromethylhistidine on oxyntic mucosal histidine decarboxylase (HDC) activity in fed and fasted rats. Means ± S.E.M. $n = 6$. Differences between untreated and treated rats were assessed by the Student t-test. $^{**}p < 0.01$.

### 2.10. Statistics

The results are given as means ± S.E.M. Differences were statistically analysed by the Student *t*-test or by one-way Analysis of Variance (ANOVA) followed by the Bonferroni multiple comparison test. The half-life of the histamine pool was determined from plots of oxyntic

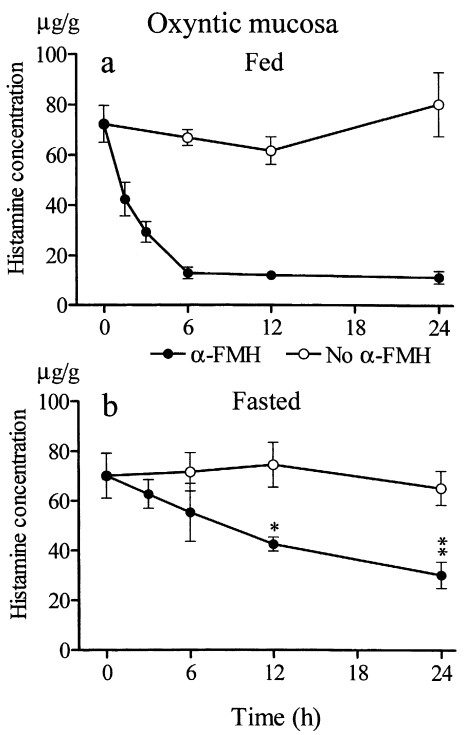


Fig. 3. Time-course of α-fluoromethylhistidine (α-FMH)-induced depletion of ECL-cell histamine in fed (a) and fasted rats (b). α-Fluoromethylhistidine (3 mg/kg/h) was given by infusion for 1.5 to 24 h from osmotic minipumps implanted under the skin of the neck. The effect of α-fluoromethylhistidine in fed rats was highly significant ($p < 0.001$, not shown) within 3 h. In fasted rats, the effect of α-fluoromethylhistidine was significant after 12 h ($p < 0.05$, $^{*}$) and 24 h ($p < 0.01$, $^{**}$). Means ± S.E.M. $n = 5$–7. Statistical differences were analysed by one-way ANOVA followed by the Bonferroni multiple comparison test.

mucosal histamine concentration versus time. The 95% confidence interval (CI) of the half-life was determined from where a horizontal line at half the $t_0$ control histamine value crossed the upper and lower 95% CI of the α-fluoromethylhistidine time–response curve.

## 3. Results

### 3.1. Effects of α-fluoromethylhistidine in intact rats

#### 3.1.1. Dose–response relationship

A wide range of doses of α-fluoromethylhistidine was given by continuous subcutaneous infusion for 24 h. Dose–response curves illustrating the α-fluoromethylhistidine-induced inhibition of histidine decarboxylase and the consequent depletion of histamine from the oxyntic mucosa of fed rats are shown in Fig. 1. $ED_{50}$ was 0.15 mg/kg/h for inhibition of histidine decarboxylase activity and 0.25 mg/kg/h for depletion of histamine. Maximum or near-maximum inhibition of histidine decarboxylase activity (80–90%) was achieved at a dose of about 1 mg/kg/h while maximum depletion of histamine (90%) was achieved at a dose of about 3 mg/kg/h (Fig. 1).

#### 3.1.2. Time-course of effects

A maximally effective dose of α-fluoromethylhistidine (3 mg/kg/h) reduced the histidine decarboxylase activity

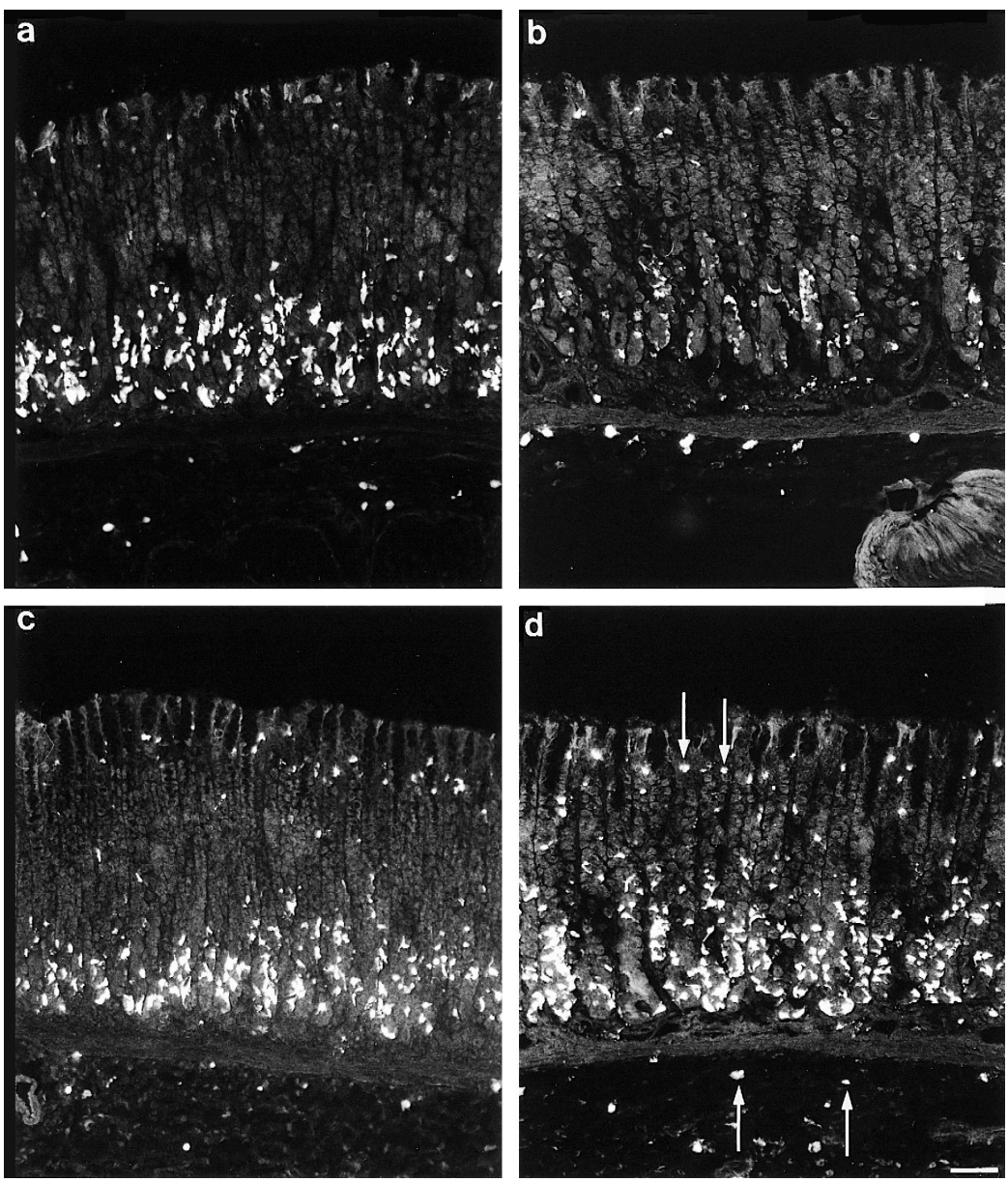


Fig. 4. Histamine immunofluorescence in ECL cells in the stomach of fed (a, b) and fasted (c, d) rats, treated with α-fluoromethylhistidine (b, d) or untreated (a, c). α-Fluoromethylhistidine (10 mg/kg/h) was given by infusion for 24 h from osmotic minipumps implanted under the skin of the neck. Note the near-complete loss of immunofluorescent ECL cells in b but not in d and the persisting presence of histamine-immunofluorescent mast cells (for example, see arrows) at the mucosal surface and in the submucosa. Bar = 100 μM.

in fed rats by 75% ($p < 0.001$) within 1.5 h (not shown). After 24 h the histidine decarboxylase activity was reduced from $24 \pm 2.6$ to $1.8 \pm 0.9$ pmol $CO_2$/mg/h (Fig. 2). The oxyntic mucosal histamine concentration was reduced by about 80% within 6 h, the calculated half-life being 2.6 h (95% CI = 1.1 to 3.9 h) (Fig. 3a).

Fasting for 48 h greatly reduced the oxyntic mucosal histidine decarboxylase activity (Fig. 2) (see also Håkanson et al., 1975) without affecting the histamine concentration (Fig. 3). Infusion of α-fluoromethylhistidine for 24 h reduced the histidine decarboxylase activity from $2.8 \pm 0.9$ pmol to $0.5 \pm 0.2$ pmol $CO_2$/mg/h (about 80%, $p < 0.01$) (Fig. 2). The histamine concentration in these rats was reduced slowly by α-fluoromethylhistidine, with a calculated half-life of 19.4 h (95% CI = 3.8 to > 24 h) (Fig. 3b).

### *3.1.3. Histamine immunostaining and ECL-cell ultrastructure*

In fed rats, treatment with α-fluoromethylhistidine (10 mg/kg/h) for 24 h reduced the immunofluorescence intensity of the ECL cells and their apparent number (Fig. 4a,b). Mast cells were unaffected (see also Andersson et al., 1992). In fasted rats treated with α-fluoromethylhistidine, histamine immunostaining revealed an unchanged immunofluorescence intensity and number of both ECL cells and mast cells (Fig. 4c,d).

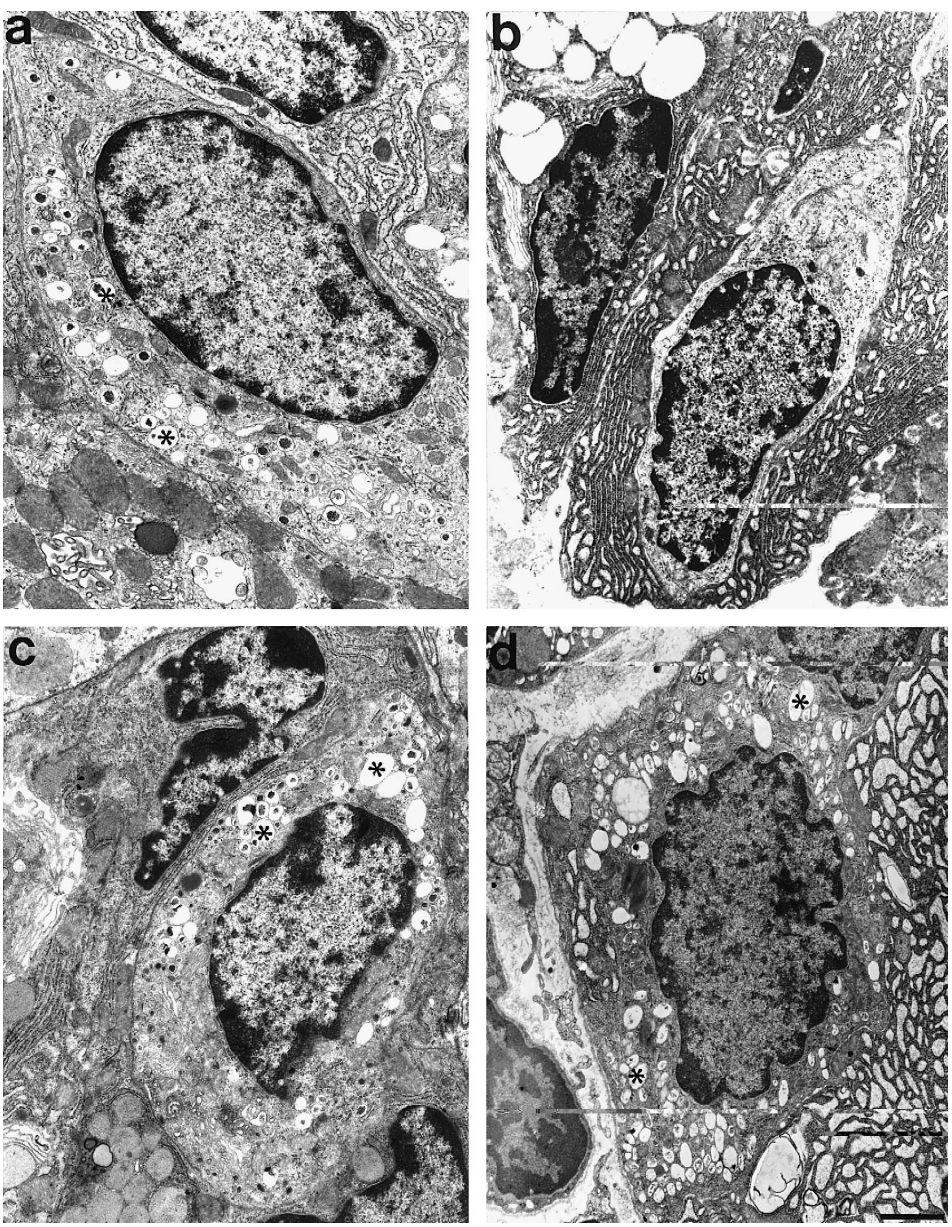


Fig. 5. Electron micrographs of ECL cells in the stomach of fed (a, b) and fasted (c, d) rats, treated with α-fluoromethylhistidine (b, d) or untreated (a, c). α-Fluoromethylhistidine (10 mg/kg/h) was given by infusion for 24 h from osmotic minipumps implanted under the skin of the neck. Examples of typical secretory vesicles are indicated by asterisks. Note the loss of secretory vesicles in (b) but not in (d). Bar = 200 nm.

The ultrastructure of most ECL cells in the fed rats was greatly affected by α-fluoromethylhistidine in that the number of secretory vesicles and the volume of the secretory vesicle compartment were reduced, only 25–30% remaining. Although the majority of the ECL cells were virtually devoid of secretory vesicles, about 10% retained a fair number. In contrast, the ECL cells of the fasted rats failed to respond overtly to α-fluoromethylhistidine (Figs. 5 and 6).

### 3.1.4. Histamine secretion studied by in vivo microdialysis

Intravenous infusion of gastrin to fed rats resulted in a two to three-fold increase in microdialysate histamine concentration within 20 min. Pretreatment with 3 mg/kg/h α-fluoromethylhistidine for 24 h lowered basal microdialysate histamine by about 70% and almost abolished the gastrin-evoked histamine secretion (Fig. 7a). In fasted rats, the basal microdialysate histamine concentration was about 60% of that in fed rats. Gastrin raised the microdialysate histamine concentration three-fold within 20–40 min (to the level seen in fed rats). Interestingly, α-fluoromethylhistidine had little effect on either basal or gastrin-induced histamine secretion in fasted rats, except that gastrin-stimulated histamine secretion was attenuated after 3–4 h of gastrin infusion. In fasted rats not receiving α-fluoromethylhistidine, gastrin-stimulated histamine secretion was maintained at a high level throughout the experiment (Fig. 6b).

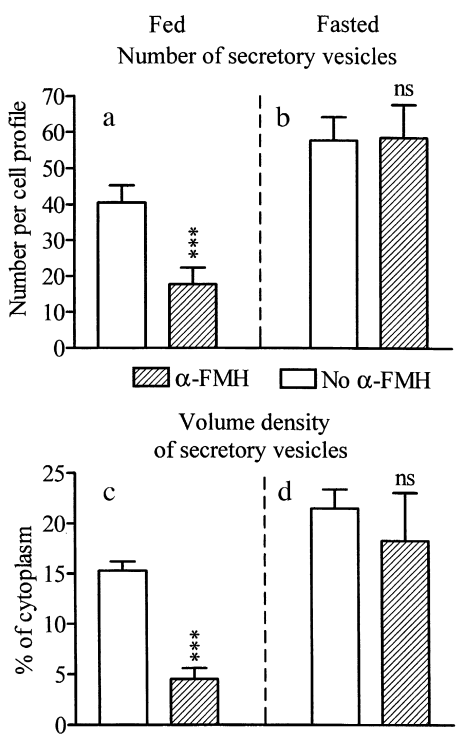


Fig. 6. Effect of α-fluoromethylhistidine (α-FMH) or no α-fluoromethylhistidine on the number and volume density of the secretory vesicles in ECL cells of fed (a, c) and fasted rats (b, d). Six rats in each group, 23–45 ECL cells from each group were examined. Means ± S.E.M. Differences between α-fluoromethylhistidine-treated rats and untreated rats were assessed by the Student *t*-test. *** $P < 0.001$, ns, not significant.

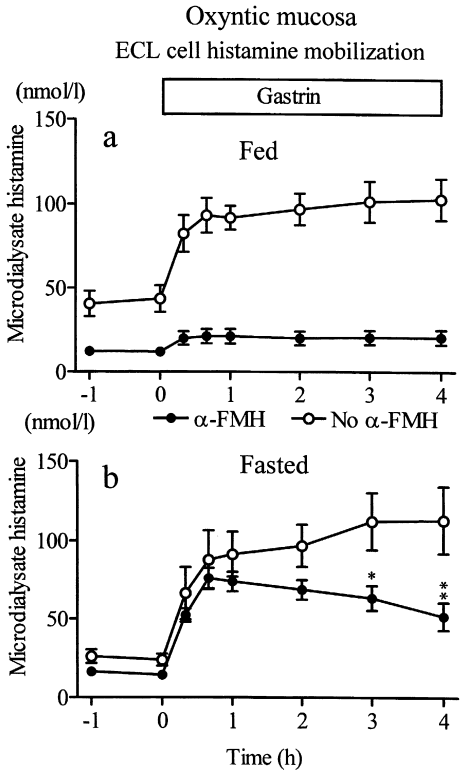


Fig. 7. Effect of α-fluoromethylhistidine (α-FMH) on gastrin-induced mobilization of ECL-cell histamine from the stomach of fed (a) or fasted rats (b). α-Fluoromethylhistidine (3 mg/kg/h) was given by infusion for 24 h from osmotic minipumps. Gastrin-17 (5 nmol/kg/h) was given by continuous intravenous infusion for the last 4 h of the 24-h infusion period. Histamine mobilization was monitored continuously by gastric submucosal microdialysis for the final 6 h before the end of the study. Note that the histamine-mobilizing effect of gastrin was abolished by α-fluoromethylhistidine in fed rats but not in fasted rats. In fed rats, the effect of α-fluoromethylhistidine was highly significant (ampared to control) at all time points ($p < 0.001$, not shown). In fasted rats, the effect of α-fluoromethylhistidine was significant 3 h ($p < 0.05$, *) and 4 h ($p < 0.01$, **) after the start of gastrin infusion. Means ± S.E.M. $n = 6$. One-way ANOVA followed by the Bonferroni multiple comparison test was used to analyse statistical differences.

## 3.2. Effect of α-fluoromethylhistidine on isolated ECL cells

### 3.2.1. Histamine content

Incubation of isolated ECL cells with varying concentrations of α-fluoromethylhistidine for 48 h reduced the histamine content concentration-dependently (Fig. 8a, see also Lindström et al., 2000). The α-fluoromethylhistidine-induced reduction in ECL-cell histamine seemed to correspond to the concentration of gastrin in the medium. The higher the gastrin concentration the more effective the α-fluoromethylhistidine-evoked ECL-cell histamine depletion. The maximally effective concentration of α-fluoromethylhistidine was about 0.1 mM with 10 nM gastrin in

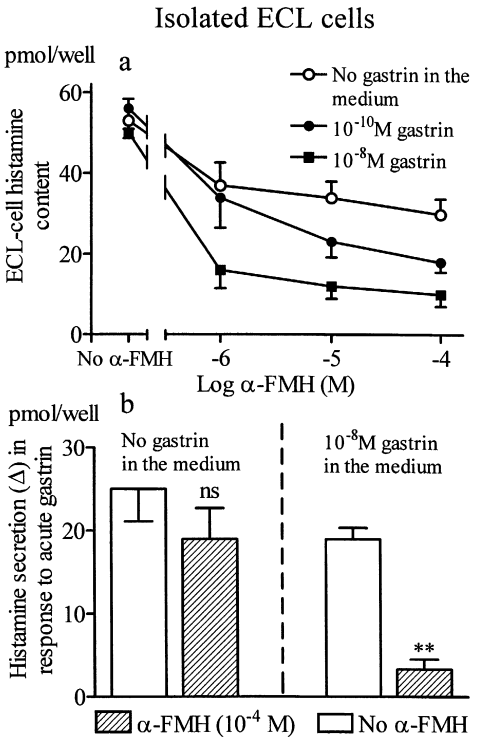


Fig. 8. Effect of α-fluoromethylhistidine (α-FMH) on histamine content and secretion from isolated ECL cells. Histamine depletion results from increasing the concentration of α-fluoromethylhistidine (a), but the depletion is favoured by the presence of gastrin in the medium. The effect of α-fluoromethylhistidine pretreatment on the ability of the ECL cells to secrete histamine in response to gastrin depends on whether or not they have been exposed to gastrin during culture (b). Means ± S.E.M. $n = 6$. The Student t-test was used to assess the statistical significance of the differences between α-fluoromethylhistidine-treated and untreated cells. $^{**}p < 0.01$, ns, not significant.

the medium; under these circumstances the histamine content was reduced by 80%. In the absence of gastrin, α-fluoromethylhistidine reduced the histamine content by only about 40% (Fig. 8a).

### *3.2.2. Histamine secretion*

Without gastrin in the culture medium, α-fluoromethylhistidine pretreatment for 48 h failed to affect the histamine secretion induced by an acute gastrin stimulus. With 10 nM gastrin in the culture medium throughout the 48 h incubation with α-fluoromethylhistidine, the greatly reduced histamine content was associated with a failure to respond with histamine secretion to an acute gastrin challenge (Fig. 8b).

## 4. Discussion

Previously, we reported that continuous subcutaneous infusion of α-fluoromethylhistidine for 24 h depleted histamine from the ECL cells of mouse, rat and hamster (Andersson et al., 1992, 1996b). More than a decade ago, Koyama et al. (1987) found that gastric histamine was reduced by α-fluoromethylhistidine in fed but not in fasted animals. Indeed, in this study α-fluoromethylhistidine-evoked ECL-cell histamine depletion was prompt in fed rats but slow in fasted rats. The calculated half-life of oxyntic mucosal histamine was 2.6 h in fed rats and 19.4 h in fasted rats. We suggest that the rate by which histidine decarboxylase inhibition depletes histamine from the ECL cells reflects the rate of histamine secretion (see also Koyama et al., 1987). A high rate of histamine secretion (for instance in response to high circulating concentrations of gastrin) will rapidly exhaust the histamine store if the synthesis of new histamine is prevented. In the fasted state, the rate of ECL-cell histamine secretion is low, and consequently, histamine depletion following histidine decarboxylase inhibition will be slow.

The results of histamine immunocytochemistry agreed with the chemical findings. In fed rats, infusion of a maximally effective dose of α-fluoromethylhistidine for 24 h rendered the ECL cells virtually non-immunoreactive (see also Andersson et al., 1992), while in fasted rats α-fluoromethylhistidine seemed to have little effect on their immunofluorescence intensity. Electron microscopy revealed that the α-fluoromethylhistidine-induced exhaustion of ECL-cell histamine in fed rats was associated with a greatly reduced number of secretory vesicles in the cytoplasm (Andersson et al., 1992, 1996b), which is in line with the view that these organelles represent the subcellular storage site of ECL-cell histamine (Zhao et al., 1999a,b). The secretory vesicles are thought to arise from small dense, Golgi-derived granules, that are being transformed gradually into secretory vesicles by the accumulation of histamine from the cytosol (Andersson et al., 1992, 1996b; Chen et al., 1996b; Zhao et al., 1999a,b). When histamine is not available (as is the case after α-fluoromethylhistidine-induced inhibition of histidine decarboxylase), the granules cannot metamorphose into secretory vesicles. In the ECL cells of fasted rats, α-fluoromethylhistidine failed to eliminate histamine and secretory vesicles, in support of the view that these organelles store histamine.

Microdialysis experiments revealed that α-fluoromethylhistidine pretreatment impaired the ability of gastrin to mobilize gastric histamine in fed rats. In fasted rats that had not been treated with α-fluoromethylhistidine, the amounts of histamine that were mobilized remained high throughout a 4 h gastrin challenge. After 24 h of α-fluoromethylhistidine treatment, the peak response to gastrin was similar to that seen in rats not treated with α-fluoromethylhistidine, but there was a gradual decline with time in the amount of histamine secreted. Conceivably, this decline in gastrin-induced histamine mobilization reflects the gradual exhaustion of the releasable histamine pool in a situation when de novo synthesis of histamine is prevented. In rats not treated with α-fluoromethylhistidine, a gastrin challenge will result not only in histamine secretion but also in accelerated histamine formation (to replenish the histamine

stores) (Chen et al., 1994). This explains the ability of the ECL cells to maintain high histamine secretion over long periods of time in response to prolonged gastrin stimulation. From the microdialysis studies it also appears that although the total amount of histamine in the ECL cells of fasted rats is halved by α-fluoromethylhistidine, the releasable histamine pool remains intact.

Isolated ECL cells responded to α-fluoromethylhistidine in much the same way as did ECL cells in vivo. As expected, histidine decarboxylase inhibition effectively depleted histamine from isolated ECL cells when they were cultured for 48 h in a medium containing α-fluoromethylhistidine and gastrin. When cultured in a medium without gastrin, the ECL cells responded poorly to α-fluoromethylhistidine (50–60% histamine remaining after 48 h); under these circumstances the amount of histamine secreted in response to an acute gastrin stimulus was unaffected. Thus, the effectiveness of α-fluoromethylhistidine as a histamine-depleting agent in isolated ECL cells was directly correlated to the degree of the gastrin stimulus. It remains to be explained why the ECL-cell histamine content is lowered by α-fluoromethylhistidine in the absence of gastrin. It is unlikely that this loss of histamine reflects the effects of gastrin contaminating the culture medium since the concentration of gastrin in foetal calf serum (and in the culture medium) was too low to be measurable (data not shown). Instead, the gastrin-independent loss of histamine might reflect a constitutive, continuous release of small amounts of histamine. The pancreastatin content of the ECL cells and the secretion of this peptide from the ECL cells were not affected by α-fluoromethylhistidine (unpublished observations) (see also Lindström et al., 2000; Kitano et al., 2000), indicating that histamine depletion does not impair the ability of the ECL cells to secrete other products.

In conclusion, the depletion of ECL-cell histamine following α-fluoromethylhistidine-induced inhibition of histidine decarboxylase depends on the rate of histamine secretion: a high secretion rate (for instance in response to gastrin) will rapidly exhaust ECL-cell histamine, while a low secretion rate will result in a maintained or slowly diminishing histamine content.

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