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ECL Cell Histamine Mobilization Studied by Gastric Submucosal Microdialysis in Awake Rats: Methodological Considerations

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Abstract: The ECL cells are endocrine/paracrine cells in the acid-producing part of the stomach. They secrete histamine in response to circulating gastrin. Gastric submucosal microdialysis has been used to study ECL-cell histamine mobilization in awake rats. In the present study we assess the usefulness and limitations of the technique. Microdialysis probes were implanted in the gastric submucosa. Histological analysis of the stomach wall around the probe revealed a moderate, local inflammatory reaction 1–2 days after implantation; the inflammation persisted for at least 10 days. Experiments were conducted 3 days after the implantation. The “true” submucosal histamine concentration was determined by perfusing at different rates (the zero flow method) or with different concentrations of histamine at a constant rate (the no-net-flux method): in fasted rats it was calculated to be 87 ± 5 (means \pm S.E.M.) nmol/l and 76 ± 9 nmol/l, respectively. The corresponding histamine concentrations in fed rats were 93 ± 5 and 102 ± 8 nmol/l, respectively. With a perfusion rate of 74 μ l/hr the recovery of submucosal histamine was 49%, at 34 μ l/hr the recovery increased to 83%. At a perfusion rate below 20 μ l/hr the microdialysate histamine concentration was close to the actual concentration in the submucosa. The ECL-cell histamine mobilization was independent of the concentrations of Ca^{2+} in the perfusion medium (0–3.4 mmol/l Ca^{2+}). In one experiment, histamine mobilization in response to gastrin (10 nmol/kg/hr subcutaneously) was monitored in rats pretreated with prednisolone (60 mg/kg) or indomethacin (15 mg/kg). The two antiinflammatory agents failed to affect the concentration of histamine in the microdialysate either before or during the gastrin challenge, which was in accord with the observation that the inflammatory reaction was modest and that inflammatory cells were relatively few around the probe and in the wall of the probe. In another experiment, rats were given aminoguanidine (10 mg/kg) or metoprine (10 mg/kg) 4 hr before the start of gastrin infusion (5 nmol/kg/hr intravenously). Metoprine (inhibitor of histamine N-methyl transferase) did not affect the microdialysate histamine concentration, while aminoguanidine (inhibitor of diamine oxidase) raised both basal and gastrin-stimulated histamine concentrations. We conclude that microdialysis can be used to monitor changes in the concentration of histamine in the submucosa of the stomach, and that the inflammatory reaction to the probe is moderate and does not affect the submucosal histamine mobilization.

The ECL cells are endocrine/paracrine cells located in the basal half of the oxyntic gland area in the mammalian stomach. They are rich in histamine and in the histamine-forming enzyme histidine decarboxylase (Håkanson *et al.* 1971, 1986 & 1994; Kubota *et al.* 1984; Chen *et al.* 1999). Gastrin is a major stimulus, eliciting prompt mobilization of ECL-cell histamine (Kahlson *et al.* 1964; Sandvik *et al.* 1987; Kitano *et al.* 2000), which in turn causes the parietal cells to secrete HCl (Sandvik *et al.* 1987; Waldum *et al.* 1991; Andersson *et al.* 1996). The ECL cells are considered the primary source of histamine in the submucosa and mucosal interstitium since in the rat they harbour about 80% of mucosal histamine (Sundler & Håkanson 1991; Andersson *et al.* 1992; Nissinen & Panula 1993). The remaining 10–20% histamine is stored in mast cells (Håkanson *et al.* 1986; Andersson *et al.* 1992).

Since 1966 when so-called “dialysis sacs” were first im-

planted in subcutaneous tissue (Bito *et al.* 1966), the technique of microdialysis has developed into a sophisticated tool for monitoring the concentration of low molecular-weight substances in interstitial fluid (Benveniste 1989; Benveniste & Huttemeier 1990; Ungerstedt 1991; Justice 1993). Today a microdialysis probe consists of a thin tube of semipermeable dialysis membrane, which is perfused with a physiological salt solution. Any molecule can diffuse passively over the membrane as long as it is smaller in size than the membrane pores (and does not bind to the membrane). As a consequence, the technique can be used both to monitor the concentration of solutes in the extracellular fluid and to introduce low molecular-weight compounds locally into a tissue (by perfusing the microdialysis probe with a solution containing the molecule of interest at a higher concentration than in the surrounding tissue).

Recently a protocol was developed, based on previous reports on microdialysis in the brain, to adapt the microdialysis technique to the study of histamine mobilization from gastric ECL cells in the conscious rat (Kitano *et al.* 2000; Norlén *et al.* 2000a, b & 2001; Konagaya *et al.* 2001). For this purpose the microdialysis probe was implanted into the

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gastric submucosa. Gastric submucosal microdialysis offers advantages compared to other methods to monitor gastric histamine mobilization in that it allows the continuous sampling of submucosal histamine in fully conscious animals. However, there are potential limitations associated with the technique. Although the histamine concentration in the microdialysate reflects the submucosal histamine concentration, it does not reveal the "true" concentration. Once histamine is released, it is rapidly eliminated by histamine-degrading enzymes (histamine N-methyltransferase and diamine oxidase) (Maslinski & Fogel 1991). Although histamine in the microdialysate is protected from degrading enzymes, it may be degraded before reaching the probe, resulting in misleadingly low values. Further, inflammatory mediators have been shown to suppress ECL-cell histamine mobilization (Lindström *et al.* 1997; Lindström & Håkanson 2001; Norlén *et al.* 2001), suggesting that an inflammatory response due to the implantation of the probe may affect the microdialysate histamine concentration.

The aim of the present study was to assess the usefulness and limitations of the technique of gastric submucosal microdialysis for the study of ECL-cell histamine mobilization in conscious rats: 1) by determining the actual concentration of histamine in the submucosa of the acid-producing part of the stomach, 2) by exploring to what extent local inflammation, caused by the implantation of the microdialysis probe, will affect the histamine concentration in the microdialysate and 3) by assessing the importance of histamine-degrading enzymes.

Materials and Methods

Chemicals. Human Leu¹⁵-gastrin-17 was obtained from Research Plus (South Plainfield, NJ, USA) and dissolved in 0.9% saline. Histamine dihydrochloride, aminoguanidine hemisulfate and indomethacin were obtained from Sigma (St. Louis, MO, USA). Histamine dihydrochloride and aminoguanidine hemisulfate were dissolved in 0.9% saline. Indomethacin was dissolved in 5% NaHCO₃. Metoprine was a kind gift from Professor T. Watanabe (Tohoku University, Japan). It was dissolved in 1% lactic acid in 0.9% saline. Prednisolone sodium succinate (Precortalon[®]) was obtained from Organon (Oss, Holland). It was dissolved in sterile water at the time of injection. Krebs-Ringer solution (NaCl 0.12 mol/l, CaCl₂ 0, 1.2 or 3.4 mmol/l, KCl 4.72 mmol/l, KH₂PO₄ 1.19 mmol/l, MgSO₄ 1.19 mmol/l, NaHCO₃ 0.025 mol/l and HEPES 0.01 mol/l) and saline (0.9% NaCl) were prepared for perfusion via the microdialysis probes.

Implantation of the microdialysis probe. One hundred and twenty male Sprague-Dawley rats, weighing 250–300 g at the start of the experiment, were used. The rats were kept at a 12 hr light and 12 hr dark cycle in plastic cages (2–3 rats in each cage) with free access to standard rat food pellets (Lactamin, Vadstena, Sweden) and tap water. Surgery was performed under chloral hydrate anaesthesia (300 mg kg⁻¹, intraperitoneally) using clean but not sterile instruments. No antibiotics were used. A flexible microdialysis probe (MAB 3.8.10, AgnTho's AB, length 10 mm, outer diameter 0.57 mm, 35 kDa cut-off) was used. The abdomen was opened by a midline incision. The serosa of the dorsal aspect of the acid-producing part of the stomach was tangentially punctured by a needle (22G) and a tunnel (approximately 15 mm long) was made in the submucosal layer from the greater towards the lesser curvature. The

membrane of the microdialysis probe was inserted into the tunnel and kept in place with sutures. The inlet and outlet tubes were passed through the abdominal opening and tunneled under the skin to a point at the nape of the neck where they were secured with sutures. Rats to be infused intravenously with gastrin were fitted with a polyethylene catheter in the right jugular vein at the same surgical session. The rats were freely fed or fasted before start of sampling (as specified). When the rats were to be fasted, they were kept in individual fasting cages with wire-mesh bottoms for 48 hr with free access to water. If not otherwise stated the rats were subjected to microdialysis 3 days after the operation and each rat and each probe were subjected to one experiment only. After completion of the experiments the rats were killed by exsanguination from the abdominal aorta following an overdose of chloral hydrate intraperitoneally. Each stomach was dissected out and the position of the probe was determined by visual inspection and histological examination. The studies were approved by the local Animal Welfare Committee, Lund/Malmö.

Histology. Specimens of the stomach wall (5×5 mm) were collected from the area surrounding the microdialysis probe. They were fixed by immersion in 4% formaldehyde and embedded in paraffin after dehydration in ethanol. Sections (10 µm) were cut perpendicularly to the probe and stained with haematoxylin and eosin. The distance between the probe and the base of the oxyntic glands was routinely determined. Also the mucosal thickness and the distance between the muscle layer and the base of the glands were measured. The sections were examined for signs of inflammation by assessing: 1) the number of inflammatory cells invading the wall of the probe, 2) the oedema (by measuring the distance between the mucosa and the muscle layer (probe diameter subtracted)), and 3) the degree of fibrosis (by measuring the thickness of the fibrotic layer at its thickest point around the probe).

Experimental setup: Sampling of microdialysate. The rats were kept in Bollman-type restraining cages throughout the experiments. Each animal had been familiarized with the restraining cages for two weeks prior to the experiments. The inlet tube was connected to a microinfusion pump (Model 361, Sage Instrument, ATI Orion, Boston, USA) and the outlet was allowed to drain into 300 µl polyethylene vials. Perfusion of the microdialysis probes with saline (74 µl/hr if not otherwise stated) started at 7 a.m. and continued for 2 hr before sampling in order to obtain stable baseline values (equilibration period). Basal samples were collected for 2 hr before start of stimulation (if not otherwise stated). Gastrin-stimulated samples were collected for 3–4 hr. The effect of perfusion with different concentrations of Ca²⁺, the effect of inhibition of histamine-degrading enzymes and the effect of antiinflammatory agents on the microdialysate histamine concentration were assessed before and during intravenous (5 nmol/kg/hr, 1 ml/hr) or subcutaneous (10 nmol/kg/hr, 1 ml/hr) infusion of gastrin. Gastrin was dissolved in 0.9% saline and given by continuous infusion through the intravenous catheter or through a needle inserted in the neck (the needle was put in place immediately after collection of the two first basal samples). The microdialysis samples were stored at –20° until analysis of histamine by radioimmunoassay (see below).

Determination of equilibration period. Microdialysate was sampled every 30 min. after the start of perfusion. Sampling continued for 3 hr.

Calculation of submucosal histamine concentration: The zero flow method. After 2 hr of equilibration (perfusion rate 10 µl/hr) sampling of microdialysate started. The microdialysis probes were perfused with saline at increasing rates (10, 15, 20, 34, 74 and 150 µl/hr). Fifteen µl samples of microdialysate were collected at each perfusion rate. Each time the perfusion rate was increased sampling was preceded by an equilibration period corresponding to 10–15 µl. The experiment lasted for no more than 8 hr. The animals were

then returned to their cages with free access to food and water. On the following day the experiment was repeated with the rats in the fed state.

For calculation of the submucosal concentration of histamine a population approach of the method of Jacobson *et al.* (1985) was used. The microdialysate histamine concentration (C_{dial}) is a function of the perfusion rate (F), the submucosal histamine concentration (C_{hist}), and the product of the mass transfer coefficient and the active area of the probe (K), according to the formula:

$$C_{\text{dial}} = C_{\text{hist}} (1 - \exp(-K/F))$$

Non-linear regression analyses were performed for the observations obtained from the rats in fasting and fed state, respectively, using the NONMEM program, estimating interindividual variation in C_{hist} and K . Individual Bayesian estimates of C_{hist} and K were obtained from the program.

Calculation of submucosal histamine concentration: The no-net-flux method. The microdialysis probes were perfused with increasing concentrations of histamine. After 2 hr, i.e. at the end of the equilibration period, a basal 30 min. sample was collected. Subsequently, the saline perfusate was replaced by a solution of 75 nM histamine in saline. After equilibration for 15 min., microdialysate was collected for 30 min. This procedure was repeated for each concentration of histamine (75, 150 and 300 nM histamine). The animals were then returned to their cages with free access to food and water. The experiment was repeated on the following day with the rats in the fed state.

Subtracting the influx histamine concentration from the efflux histamine concentration yields the Δ histamine concentration. The microdialysate histamine concentration that resulted in a Δ histamine concentration = 0 (influx concentration equal to efflux concentration) was calculated for each rat. The slope of the regression analysis is a measure of the probe efficiency and can be assumed identical in all experiments using the same probe. Initially, a linear model with common slopes and individual intercepts was used (SAS proc GLM), then the common slope was used to transform all observations to the x-axis, using the formula: x-intercept = perfusate concentration - Δ /slope. The calculated x-intercepts were then used in a repeated measures analysis (SAS proc MIXED) estimating least squares means of the x-intercepts for fasting and freely fed rats, respectively, and the confidence interval of the difference between these two states.

Composition of the perfusion medium. The microdialysis probes were perfused with 0.9% saline or Krebs-Ringer solution with different concentrations of Ca^{2+} (0, 1.2 or 3.4 mmol/l Ca^{2+}). Basal samples were collected for 2 hr after which ECL-cell histamine secretion was stimulated by subcutaneous infusion of gastrin-17 (10 nmol/kg/hr) for 3 hr. Microdialysis samples were collected every 20 min during the first hour of stimulation and then every hour.

Inhibition of histamine-degrading enzymes. Seven rats were given a bolus dose of metoprine (10 mg/kg) (Prell *et al.* 1997) and 6 rats were given a bolus dose of aminoguanidine (10 mg/kg) (Kahlson *et al.* 1963) subcutaneously 2 hr before the experiment. Fourteen rats received either metoprine vehicle (1% lactic acid in 0.9% saline) or aminoguanidine vehicle (saline). Perfusion of the microdialysis probes with saline (74 $\mu\text{l/hr}$) started at 7 a.m. and continued for 2 hr before sampling. Basal samples were collected for 2 hr after which ECL-cell histamine secretion was stimulated by intravenous infusion of gastrin-17 for 4 hr (5 nmol/kg/hr) (Konagaya *et al.* 2001). Microdialysis samples were collected every 20 min. during the first hour of stimulation and then every hour.

Antiinflammatory agents. Six rats received prednisolone (60 mg/kg, intramuscularly) (Yamazaki *et al.* 1986) and 7 rats vehicle 72 hr before the sampling started. Another group of 5 rats received indomethacin (15 mg/kg, intraperitoneally) (Lorenzetti & Ferreira 1985;

Peskar *et al.* 1991) and 6 rats received the vehicle 2 hr before the experiment. At the time of the experiment, basal samples were collected hourly for 2 hr before the start of subcutaneous gastrin infusion for 3 hr. Microdialysis samples were collected every 20 min. during the first h of gastrin infusion and then every hr.

Determination of microdialysate histamine. Histamine in the microdialysate was determined by radioimmunoassay using a commercially available kit (Immunotech, Paris, France). The limit of detection, defined as the lowest concentration of histamine significantly different from zero with the probability of 95%, is 0.2 nmol per litre. The histamine concentration was expressed as nmoles per litre.

Statistical analysis. Data are presented as mean values \pm S.E.M.. Differences were analysed for statistical significance by one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test or by calculation of the area under the curve during gastrin stimulation followed by the Student's unpaired t-test. $P < 0.05$ was considered significant.

Results

Determination of equilibration period. The microdialysate histamine concentration was slightly higher in the beginning than at the end of the experiment (32 ± 4 nmol/l versus 24 ± 4 nmol/l) (fig. 1). The difference was not statistically significant ($P > 0.05$).

Determination of the submucosal histamine concentration: the zero flow method. Varying the perfusion rate, greatly affected the histamine concentration in the perfusate (fig. 2). The submucosal histamine concentration was calculated from the curve relating the microdialysate histamine concentration to the perfusion rate (see Materials and Methods) and found to be 87 ± 5 (means \pm S.E.M.) nmol/l in fasted rats and 93 ± 5 nmol/l in fed rats (no statistically significant difference). With a perfusion rate of 74 $\mu\text{l/hr}$ the recovery of submucosal histamine was 49%, with a perfusion rate of 34 $\mu\text{l/hr}$ it was 83%. From fig. 2 it seems that with a perfusion rate below 20 $\mu\text{l/hr}$, the microdialysate

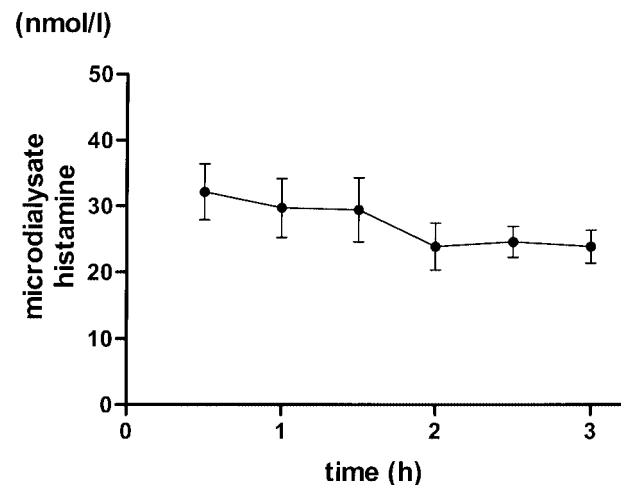


Fig. 1. Determination of equilibration period. Microdialysate histamine concentration in probes perfused with saline for 3 hr (means \pm S.E.M., 7 rats).

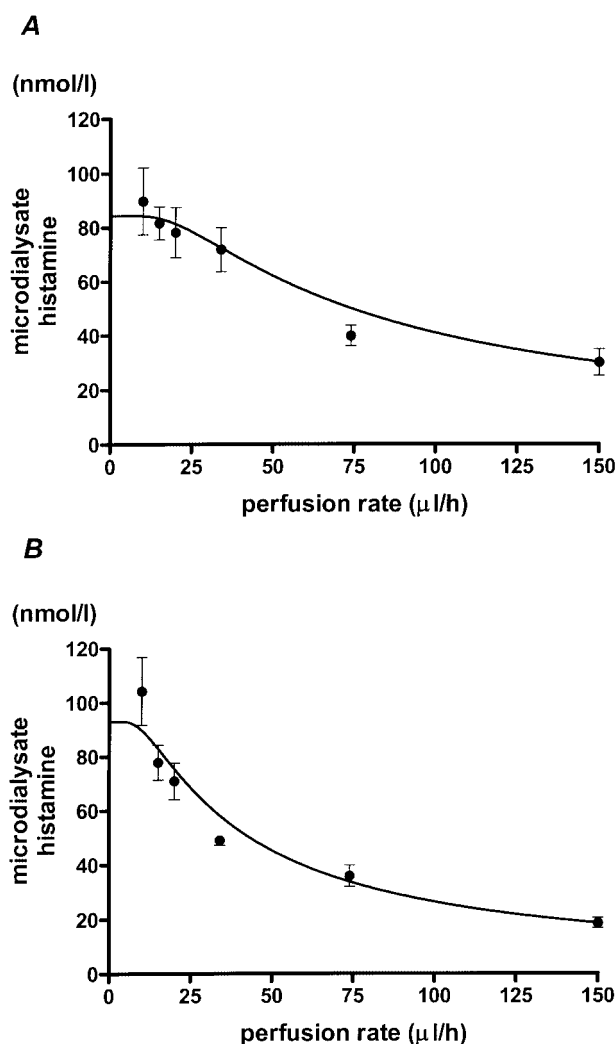


Fig. 2. The microdialysate histamine concentration as a function of the perfusion rate in fasted rats (A) and fed rats (B). The y-intercepts represent the "true" submucosal histamine concentrations. The submucosal histamine concentration in fasted rats was 87 ± 5 nmol/l and in fed rats 93 ± 5 nmol/l. Means \pm S.E.M. (8–12 rats in each group).

histamine concentration is quite close to the "true" submucosal histamine concentration.

Determination of the submucosal histamine concentration: the no-net-flux method. The Δ histamine concentration (efflux histamine – influx histamine) was plotted against the histamine concentration in the perfusate (fig. 3). The calculated (see Materials and Methods) submucosal histamine concentration was 76 ± 9 nmol/l in the fasted rats and 102 ± 8 nmol/l in the fed rats ($P < 0.05$).

Composition of perfusion medium. Perfusion with various physiological salt solutions, 0.9% saline or Krebs-Ringer variants (0, 1.2 and 3.4 mmol/l Ca^{2+}) failed to affect either basal or gastrin-induced histamine mobilization (fig. 4).

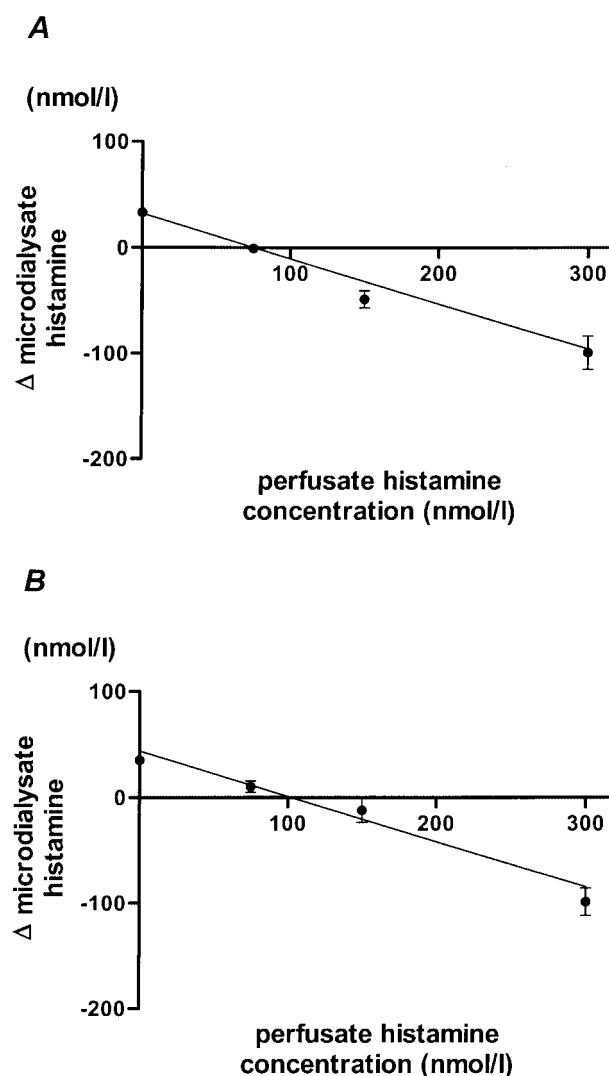


Fig. 3. The Δ histamine concentration (efflux histamine – influx histamine) plotted against the histamine concentration in the perfusate in fasted rats (A) and fed rats (B). The x-intercepts represent the "true" submucosal histamine concentrations. The submucosal histamine concentration was 76 ± 9 nmol/l in the fasted rats and 102 ± 8 nmol/l in the fed rats. Means \pm S.E.M. (6 rats in each group). The submucosal histamine concentration in the fed rats was significantly higher than in the fasted rats ($P < 0.05$).

Inhibition of histamine degrading enzymes. In rats treated with aminoguanidine, the microdialysate histamine concentration was higher than in vehicle-treated control rats (fig. 5A) when differences were analysed for statistical significance by one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test but not when the Student's unpaired t-test was used to assess the difference between area under the curve during gastrin stimulation. The increase in microdialysate histamine in rats treated with metoprine was not statistically significant (fig. 5B).

Histology. The mucosal thickness was $590 \mu\text{m} \pm 20$ ($n=20$). The distance between the probe and the base of the oxyntic mucosa ranged from 50 to 700 μm , with a mean value of

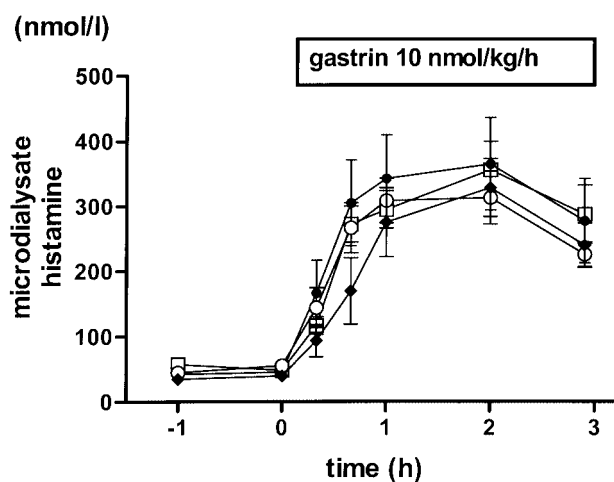


Fig. 4. The Ca^{2+} concentration in the medium had no effect on either basal or gastrin-stimulated ECL-cell histamine mobilization. The microdialysis probes were perfused with 0.9% saline (\blacklozenge) or Krebs-Ringer solutions with different concentrations of Ca^{2+} (0 mmol/l Ca^{2+} ; \circ , 1.2 mmol/l Ca^{2+} ; \blacksquare , and 3.4 mmol/l Ca^{2+} ; \bullet). Gastrin-17 was given by subcutaneous infusion for 3 hr as indicated. The microdialysate histamine concentration and not the submucosal concentration are given. Means \pm S.E.M. (5–8 rats in each group).

220 $\mu\text{m} \pm 30$, $n = 20$. Small bleedings around the probe were noted in 1/3 of the specimens and there was an inflammatory response to the microdialysis probe in the submucosa of all rats examined (fig 6). Expressed as number of inflammatory cells invading the probe, there was a peak 3–4 days after implantation of the probe (138 cells per probe profile), the cell number declined somewhat around day 5–10 (87 cells) (table 1). Oedema increased the distance between the mucosa and the muscle layer already 1–2 days after implantation of the probe, the distance remained increased throughout the study (table 1). Fibrosis was seen around the probe already a few days after the implantation; it was more prominent after 5–10 days.

Antiinflammatory agents. There was no difference in the submucosal histamine concentration between vehicle-treated rats and those treated with either prednisolone (fig. 7A) or indomethacin (fig. 7B).

Discussion

The oxyntic mucosa of the rat stomach is rich in histamine-producing ECL cells. Histamine, however, occurs not only in ECL cells but also in mast cells, although the latter are few in the rat stomach (Håkanson *et al.* 1986; Andersson *et al.* 1992 & 1996). ECL cells are mainly located in the basal half of the glands (Håkanson *et al.* 1986 & 1994), whereas mast cells occur at the mucosal surface and in the submucosa (Håkanson *et al.* 1967). Recently, it was shown that ECL-cell histamine mobilization can be monitored by means of gastric submucosal microdialysis (Kitano *et al.* 2000; Norlén *et al.* 2000a, b & 2001; Konagaya *et al.* 2001). The major

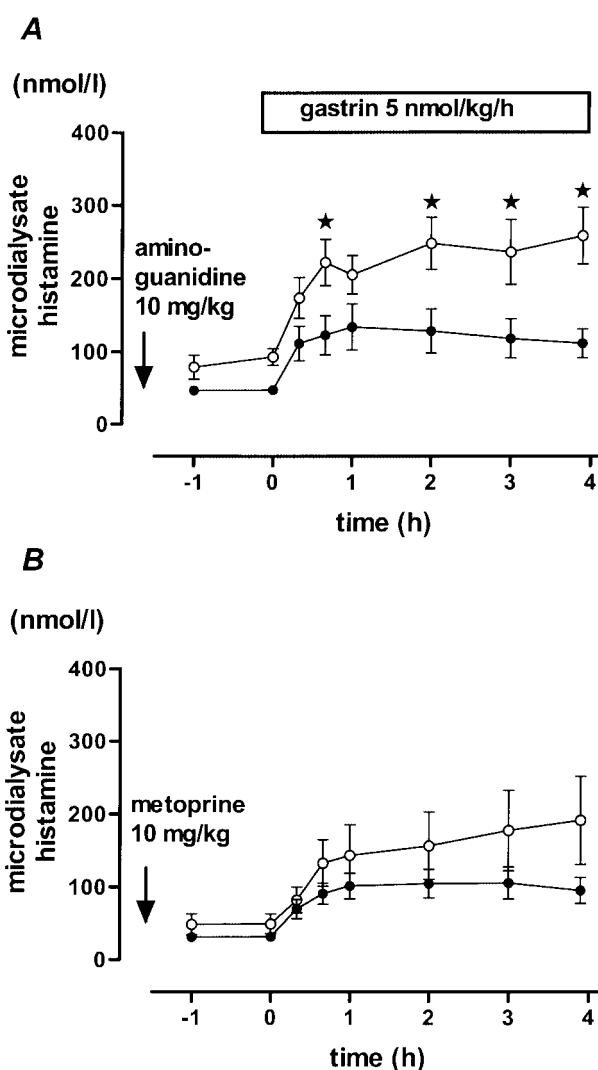


Fig. 5. Effect of aminoguanidine (inhibitor of diamine oxidase) (A) and metoprine (inhibitor of histamine N-methyl transferase) (B) for basal and gastrin-stimulated ECL-cell histamine mobilization. Gastrin-17 was given by intravenous infusion for 4 hr as indicated. A) Aminoguanidine (\circ) or vehicle (\bullet) were given by subcutaneous injection 2 hr before sampling started. B) Metoprine (\circ) or vehicle (\bullet) were given by subcutaneous injection 2 hr before sampling started. Means \pm S.E.M. (6–7 rats in each group). The microdialysate histamine concentration was higher ($P < 0.05$) in rats treated with aminoguanidine than in vehicle-treated rats as indicated by asterisk (*) when using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test but not when comparing the area under the curve during gastrin stimulation (Student's *t*-test) ($P > 0.05$).

advantage of this method lies in the fact that it makes it possible to measure ECL-cell histamine secretion in response to physiological stimuli in fully conscious animals.

However, the technique has potential limitations. The histamine concentration in the microdialysate is proportional, rather than identical to, the "true" submucosal concentration. Accurate estimation of the "true" histamine concentration is complicated by the fact that once released, histamine is being continuously degraded by extracellular

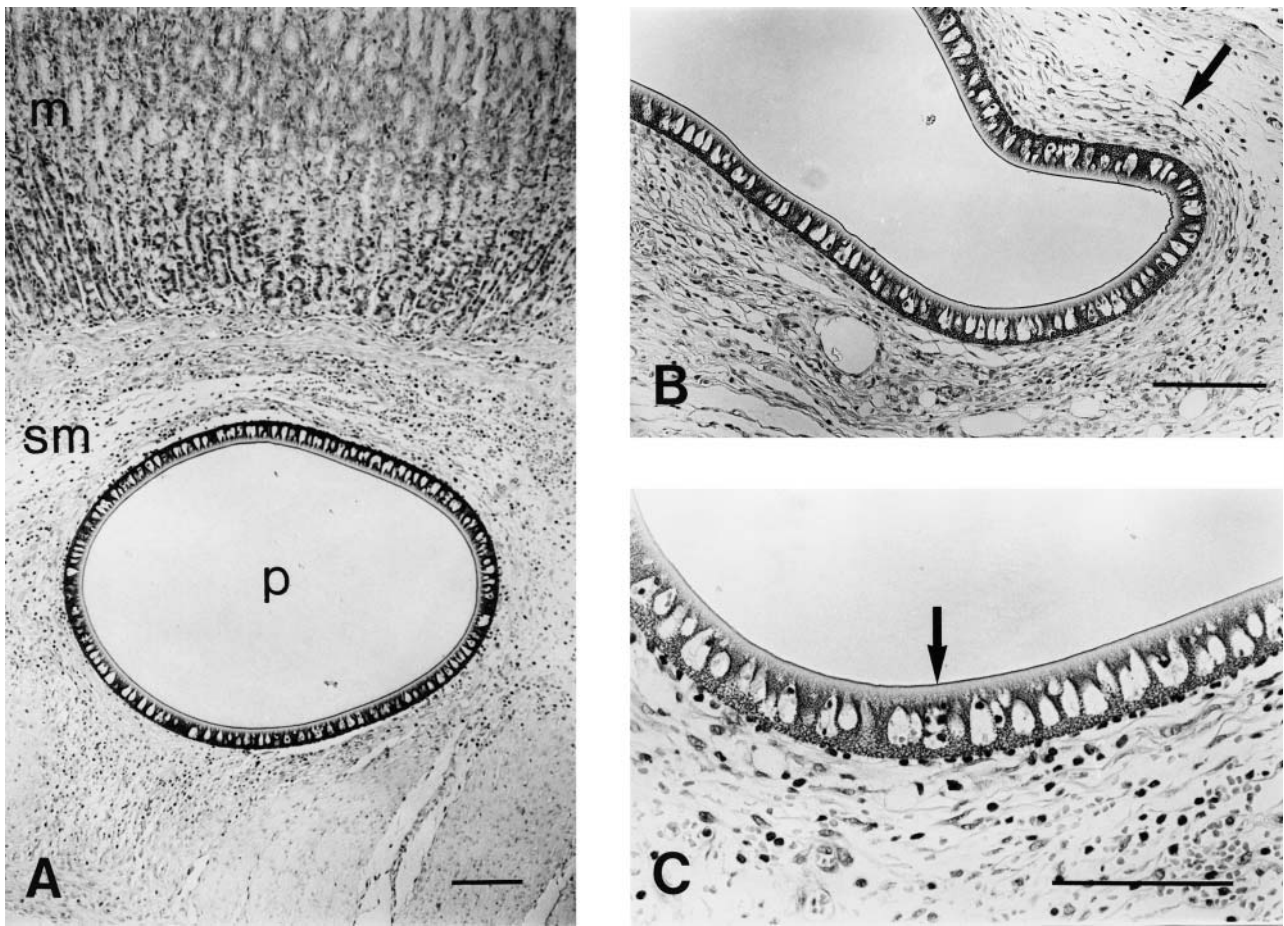


Fig. 6. Transverse sections of the gastric wall (stained with haematoxylin and eosin) showing: A) mucosa (m), and microdialysis probe (p) in the submucosal compartment (sm), B) fibrosis around the probe (for example, see arrow) and C) inflammatory cells invading the probe (for example, see arrow). Bar=100 μm .

enzymes. Although histamine is protected from degrading enzymes inside the microdialysis membrane, histamine may be metabolized before reaching the inside of the probe. Moreover, implantation of the microdialysis probe is associated with tissue damage and a consequent local inflammatory response which may complicate histamine determination in two ways: 1) ECL cells are known to be inhibited by inflammatory mediators, such as prostaglandin E_2 and IL-1 β , both *in situ* and in primary culture (Lindström *et al.* 1997; Prinz *et al.* 1997; Lindström & Håkanson 2001; Norlén *et al.* 2001), and 2) inflammatory cells may represent an additional source of submucosal histamine as well as a source of histamine degrading enzymes. Hence, local inflammation *per se* might be expected to affect the way the ECL cells respond to stimuli and the concentration of histamine in the submucosa.

Also, the composition of the perfusion medium, in particular the Ca^{2+} concentration, may affect the way ECL cells respond to stimuli.

One purpose of the present study was to assess how closely the histamine concentration in the microdialysate corresponds to the actual concentration in the submucosa. Basal and gastrin-induced histamine mobilization was

monitored. After showing that the basal concentration of histamine was stable over time, an attempt was made to determine the "true" submucosal histamine concentration by 1) the no net-flux method (Lönnroth *et al.* 1987; Justice 1993) and 2) the zero flow method (Jacobson *et al.* 1985; Parsons & Justice 1992). By using these two methods, the submucosal histamine concentration in fasted rats was found to be 75–90 nmol/l. As expected, the concentration

Table 1.

Variations in histological parameters reflecting the inflammatory response after implantation of the microdialysis probe in the submucosal compartment of the rat stomach.

Days after implantation	Inflammatory cells ^{a)}	Mucosa-muscle distance (μm) ^{b)}	Fibrosis (μm) ^{c)}	Number of rats
1–2	92 \pm 21	566 \pm 34	91 \pm 28	4
3–4	138 \pm 48	483 \pm 105	86 \pm 21	5
5–10	87 \pm 23	525 \pm 38	145 \pm 25	8

Means \pm S.E.M. ^{a)}Number of cells in the probe profile. ^{b)}The distance between the mucosa and the muscle layer (probe diameter subtracted). The distance between mucosa and muscle in intact rats was 228 \pm 18 μm (n=20). ^{c)}The thickness of the fibrotic layer at its thickest point around the probe.

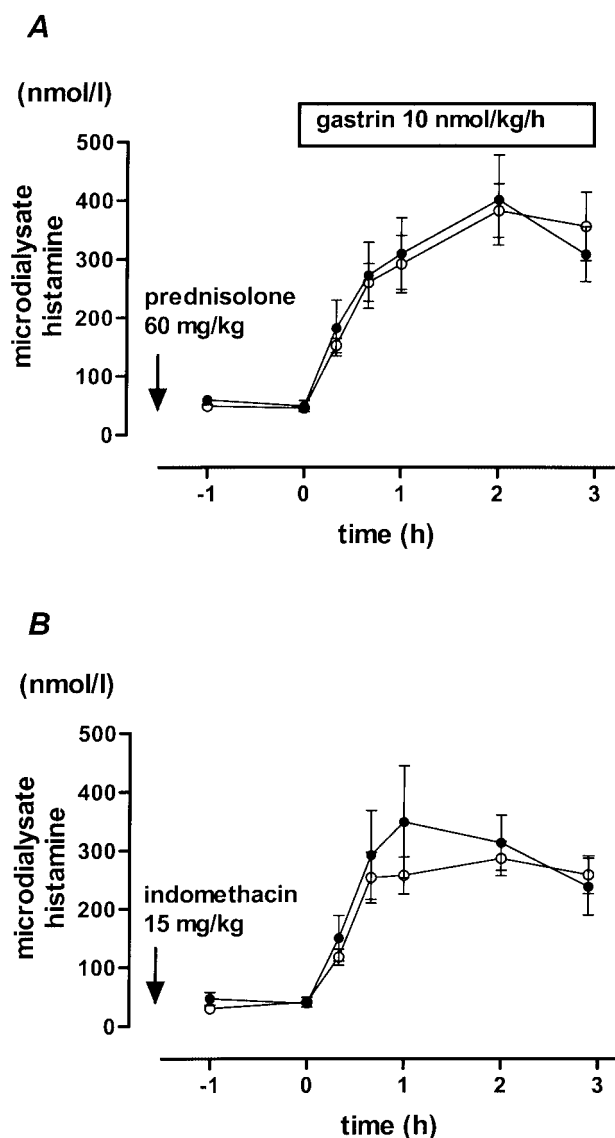


Fig. 7. Effect of prednisolone (A) and indomethacin (B) on basal and gastrin-stimulated ECL-cell histamine mobilization. Gastrin-17 was given by subcutaneous infusion for 3 hr as indicated. The values given are the microdialysate concentrations (49% recovery). A) Prednisolone (○) or vehicle (●) were given by intramuscular injection 72 hr before the sampling started. B) Indomethacin (○) or vehicle (●) were given by intraperitoneal injection 2 hr before the sampling was started. Means \pm S.E.M. (5–7 rats in each group).

in fed rats was somewhat higher, in the 90–100 nmol/l range. From the results it is possible to argue that at perfusion rates below 20 μ l/hr, the microdialysate histamine concentration is very close to the “true” submucosal histamine concentration.

In previous studies using gastric submucosal microdialysis 0.9% saline was used as perfusion medium (Kitano *et al.* 2000; Norlén *et al.* 2000a, b & 2001; Konagaya *et al.* 2001). Over the years, several other physiological salt solutions have been tested as perfusion media in microdialysis studies (Benveniste & Huttemeier 1990). In the present study we wanted to investigate to what extent ECL-cell histamine mo-

bilization depends on the perfusion medium and on the Ca^{2+} concentration of the medium. The results revealed no statistically significant difference in the microdialysate histamine concentration when the microdialysis probes were perfused with 0.9% saline or with Krebs-Ringer solutions with different concentrations of Ca^{2+} (0, 1.2 or 3.4 mmol/l Ca^{2+}). From previous studies, both *in vitro* and *in vivo*, exocytosis in ECL cells is known to depend upon Ca^{2+} entry (optimal extracellular Ca^{2+} concentration \sim 1–2 mM) (Sandvik *et al.* 1993; Zeng *et al.* 1996 & 1999; Lindström *et al.* 2001; Zanner *et al.* 2002). Since the absence or presence of Ca^{2+} in the perfusion medium did not seem to affect ECL-cell histamine mobilization we conclude that the Ca^{2+} concentration in the submucosa is sufficient to allow the ECL cells to secrete histamine, and that the perfusion medium has little impact on the local Ca^{2+} concentration.

Introduction of the microdialysis probe into the gastric submucosa is unavoidably associated with tissue damage and a reactive inflammatory response. There was minor local bleeding in 1/3 of the rats, oedema (increased thickness of submucosa), invasion of inflammatory cells into the wall of the probe, and fibrosis around the probe. Oedema and other inflammatory changes were revealed by histological examination already during the first few days after implantation of the probe. The edema persisted throughout the study. Invasion of inflammatory cells into the probe peaked during the first 3–4 days after the implantation of the microdialysis probe and declined subsequently. Fibrosis was slow and remained modest for the first couple of days, increasing thereafter. All things considered, day 3 or 4 after implantation of the probe would seem suitable for performing gastric submucosal microdialysis studies since at this point in time the edema was stable and the fibrosis was still inconspicuous.

Inflammatory mediators, such as prostaglandin E_2 and IL-1 β , released during the inflammatory response are powerful inhibitors of the stimulated synthesis and release of histamine from ECL cells (Prinz *et al.* 1997; Lindström & Håkanson 1998). The fact that pretreatment with anti-inflammatory agents (prednisolone and indomethacin) failed to affect the concentration of histamine in the microdialysate was in accord with the observation that inflammatory cells were quite few around the probe and in the wall of the probe. Although the results revealed local inflammation – albeit moderate – in response to the implantation of the probe, the inflammatory reaction did not impair the mobilization of ECL-cell histamine.

The average distance that histamine will have to diffuse from the base of the glands (the ECL cells occur basally in the glands) to the collecting probe in the submucosa was found to be 200 μ m. It must be realized that the submucosal histamine concentration is likely to be lower than the histamine concentration in the vicinity of the ECL cells. The distance between the ECL cells and the submucosa will cause gradual dilution of mobilized histamine and increase the likelihood of histamine being degraded before reaching the probe. In several species, e.g. in dogs and rabbits, N-

methylation seems to be the major route of inactivation (Brown *et al.* 1959; Code *et al.* 1976; Wollin 1987). However, in the rat stomach diamine oxidase seems to be more important for the metabolism of histamine than histamine N-methyl transferase (Schayer 1966). Pretreatment with metoprine (inhibitor of histamine N-methyl transferase) did not affect either basal or gastrin-stimulated histamine mobilization. Pretreatment with aminoguanidine (inhibitor of diamine oxidase) raised both basal and gastrin-stimulated histamine mobilization (~50%), supporting the view that diamine oxidase may be more important than N-methyl transferase for the degradation of ECL-cell histamine. The likelihood that histamine is being degraded during the passage from the mucosa to the probe rather illustrates the shortcomings of earlier attempts to measure mobilized gastric histamine in the venous outflow or in peripheral blood. Still, the technique of gastric submucosal microdialysis will not provide data on the "true" histamine concentration in the vicinity of either the ECL cells or the parietal cells.

In studies of the isolated, vascularly perfused rat stomach, Sandvik *et al.* (1987) showed that maximally effective doses of gastrin induced a vascular histamine concentration of about 500 nmol l⁻¹. Such a blood concentration produced a near-maximal stimulation of acid secretion (Kleveland *et al.* 1987). Based on these observations it was argued that the parietal cells are stimulated by blood-borne histamine mobilized by gastrin. Since the distance between the microdialysis probe and the ECL cell-rich region corresponds to that between the ECL cells and the parietal cells, it may be assumed that the histamine concentration in the parietal cell region is similar to that in the submucosa. In the present study, the "true" concentration of histamine in the submucosa (and probably around the parietal cells as well) following gastrin stimulation was around 800–900 nmol l⁻¹ (after correction for 49% recovery). This finding is consistent with the view that mobilized ECL-cell histamine reaches the parietal cells by diffusion but does not exclude the alternative pathway, namely that histamine reaches the parietal cells via the blood.

In conclusion, gastric submucosal microdialysis is a reliable method to study ECL-cell histamine mobilization. The technique offers the advantage that awake animals can be used, allowing "real-time" studies of "real life" situations. The potential problems of the technique are: 1) the inflammatory response to the insertion of the microdialysis probe may affect the results. However, the results suggest that the impact of the inflammatory response is minor and does not affect the results of the experiments. 2) Histamine may be degraded before reaching the interior of the probe, resulting in misleadingly low values. Indeed, blockade of histamine-degrading enzymes doubled the recovery of histamine.

Although the "true" submucosal histamine concentration can be calculated from the microdialysate histamine concentration, determination of the submucosal histamine concentration will not reveal the "true" histamine concentration around the parietal cells. Nonetheless, the results

provide support for the view that histamine from the ECL cells can reach adjacent target cells (e.g. parietal cells) by diffusion.

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