Ischemia of rat stomach mobilizes ECL cell histamine

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Kitano, Masayuki, Maria Bernsand, Yosuke Kishimoto, Per Norlén, Rolf Håkanson, Yoko Haenuki, Masatoshi Kudo, and Junichi Hasegawa. Ischemia of rat stomach mobilizes ECL cell histamine. Am J Physiol Gastrointest Liver Physiol 288: G1084–G1090, 2005. First published January 20, 2005; doi:10.1152/ajpgi.00004.2004.—Microdialysis was used to study how ischemia-evoked gastric mucosal injury affects rat stomach histamine, which resides in enterochromaffin-like (ECL) cells and mast cells. A microdialysis probe was inserted into the gastric submucosa, and the celiac artery was clamped (30 min), followed by removal of the clamp. Microdialysate histamine was determined by enzyme-linked immunosorbent assay. In addition, we studied the long-term effects of ischemia on the oxyntic mucosal histidine decarboxylase activity in omeprazole-treated rats. Gastric mucosal lesions induced by the ischemia were enlarged on removal of the clamp. The microdialysate histamine concentration increased immediately on clamping (50-fold rise within 30 min) and declined promptly after the clamp was removed. In contrast, histidine decarboxylase activity of the ECL cells was lowered by the ischemia and returned to preischemic values 9 days later. Mast cell-deficient rats responded to ischemia-reperfusion much like wild-type rats with respect to histamine mobilization. Pretreatment with the irreversible inhibitor of histidine decarboxylase, α-fluoromethylhistidine, which is known to eliminate histamine from ECL cells, prevented the rise in microdialysate histamine. Pharmacological blockade of acid secretion (cimetidine or omeprazole) prevented the lesions induced by ischemia-reperfusion insult but not the mobilization of histamine. In conclusion, ischemia of the celiac artery mobilizes large amounts of histamine from ECL cells, which occurs independently of the gross mucosal lesions. The prompt reduction of the mucosal histidine decarboxylase activity in response to ischemia probably reflects ECL cell damage. The lesions develop not because of mobilization of histamine per se but because of ischemia plus reperfusion plus gastric acid. 

The gastric mucosal injury induced by ischemia-reperfusion is a useful experimental model for the study of stress ulcer formation. Clamping the celiac artery for 30 min (ischemia) followed by removal of the clamp (reperfusion) induces gross damage to the oxyntic mucosa, resulting in the formation of petechiae and sometimes ulcers deep down in the mucosa (28). Whereas histamine is needed for gastric acid secretion to occur (1, 18, 29), gastric acid secretion is required for the lesions to develop (6, 13, 15, 22, 27, 31). Reactive oxygen species probably contribute to the ischemia-reperfusion injury (3, 11, 32).

Histamine resides in two different cell types in the acid-producing part of the stomach, mast cells and enterochromaffin-like (ECL) cells (2). Mucosal mast cells are relatively few, occurring mainly at the surface of the mucosa and in the submucosa. ECL cells occur predominantly in the basal half of the mucosa. ECL cell histamine is mobilized by gastrin to stimulate acid secretion by activating histamine H2 receptors on the parietal cells (1, 18, 29). In previous studies, administration of acid inhibitory agents (including proton pump inhibitors and H2 receptor blockers) reduced the area of damaged mucosa after ischemia-reperfusion, suggesting that gastric acid contributes to the damage (6, 15). So far, there has been no attempt to study how gastric histamine responds to an insult to the gastric mucosa. The technique of gastric submucosal microdialysis was adopted in our laboratories (8, 14) to make it possible to monitor the mobilization of gastric histamine. It was soon realized that clamping of the celiac artery resulted in the mobilization of massive amounts of histamine from the stomach (present study). To identify the cellular source of the mobilized histamine, we compared wild-type rats with rats devoid of mast cells (19, 20) and examined the effects of α-fluoromethylhistidine (α-FMH), an irreversible inhibitor of histidine decarboxylase (HDC) (16), known to eliminate histamine from ECL cells but not from mast cells (1, 2). In addition, we compared the effects of arterial clamping in control rats with those observed in rats pretreated with the histamine H2 receptor antagonist cimetidine or the proton pump inhibitor omeprazole.

MATERIALS AND METHODS

Drugs

α-FMH was purchased from Sigma (St. Louis, MO). The histamine H2 receptor antagonist cimetidine was obtained from Sumitomo Pharmaceutical (Tokyo, Japan). The proton pump inhibitor omeprazole was a kind gift from AstraZeneca (Mölndal, Sweden). All other chemicals were reagent grade and commercially available.

Animals

Male Wistar rats were purchased from SLC (Shizuoka, Japan) or from B&K (Sollentuna, Sweden). They weighed 200–260 g at the time of the experiment. The Wistar rats are referred to as wild type (WT). Ws-RC (Ws/Ws) rats are deficient in mast cells (19, 20). α-FMH was administered via osmotic minipumps (Alzet 2 MLI; Alza, Palo Alto, CA) implanted subcutaneously in the neck under diethyl ether anesthesia. The studies were approved by the Guidelines for Animal Experimentation in the Faculty of Medicine, Tottori University, and the local Animal Welfare Committee of Lund/Malmö. All rats were fasted for 18–24 h (free access to water) before the experiments unless otherwise stated.

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Gastric Submucosal Microdialysis

Rats were anesthetized with pentobarbital (50 mg/kg ip) during the experiments. The abdomen was opened by a midline incision, and the microdialysis probe was implanted into the gastric submucosa (8, 14). A flexible microdialysis probe (length 8 mm, outer diameter 0.22 mm, cut-off 50 kDa; Pt-200–08-PW; Eicom, Kyoto, Japan) was used. The serosa of the dorsal aspect of the acid-producing part of the stomach was tangentially punctured by a needle (22 gauge), and a tunnel (12–15 mm) was made in the submucosa from the greater to the lesser curvature. The probe was gently inserted into the tunnel and kept in place with sutures. The inlet tube was connected to a microinfusion pump, and the outlet was allowed to drain into 0.3-ml polystyrene vials. Immediately after implantation, the microdialysis probes were perfused with degassed saline (1.2 μl/min). Sampling of microdialysate started after 60 min of equilibration. Samples were then collected every 10 min throughout the experiment.

Ischemia-Reperfusion

Gastric mucosal injury was induced by the ischemia-reperfusion technique of Wada et al. (28). The celiac artery was occluded with a small clamp (Sugita standard aneurysm clip, holding force 145 g; Mizuho Ikakogyo, Tokyo, Japan). Reperfusion was initiated 30 min later by removal of the clamp. For comparison, some rats underwent sham operation (surgery but no clamping) or reperfusion (80 min) after a shorter duration (10 min) of ischemia. If not otherwise stated, the rats were killed (exsanguination from the abdominal aorta) after 30 min of arterial clamping and 60 min of reperfusion (Fig. 1). The stomach was removed and opened along the major curvature. The mucosa was exposed, the lesions were photographed, and the injury score was calculated as the total area of erosions with a computer system of imaging analysis (28).

Mast Cell-Deficient Rats

To examine the role of mast cells in the pathogenesis of the ischemia-reperfusion injury, experiments were performed on mutant rats deficient in mast cells (Ws/Ws rats) (19, 20). Ws/Ws rats and WT rats were compared with respect to changes in the microdialysate histamine concentration and in the area of gastric mucosal lesions (60 min after reperfusion).

Treatment with Antisecretory Drugs

α-FMH. WT rats and Ws/Ws rats received saline or α-FMH (3 mg·kg⁻¹·h⁻¹) via osmotic minipumps implanted 4 days before the ischemia-reperfusion experiments. This dose of α-FMH is known to abolish histamine formation and to deplete histamine from ECL cells but not from mast cells (1, 2). The α-FMH treatment lowers basal acid secretion and prevents gastrin from stimulating acid secretion (1) as a consequence of impaired ECL cell histamine mobilization (14, 21). Microdialysis was conducted during ischemia-reperfusion, and the total area of mucosal damage was measured 60 min after start of reperfusion.

Histamine H₂ receptor antagonist. Cimetidine was dissolved in 0.9% saline and given by subcutaneous injection at doses of 0.1, 1, 3, 10, 30, and 100 mg/kg. The purpose of the experiment was to produce dose-dependent H₂ receptor blockade with consequent gastric acid inhibition (5, 7). Ischemia-reperfusion of the celiac artery was performed in rats pretreated with the different doses of cimetidine. The celiac artery was clamped 70 min after the injection of cimetidine. The total area of erosions was measured 60 min after removal of the clamp.

The basal gastric acid output was measured by luminal perfusion of the rat stomach (15). Briefly, a soft catheter was inserted into the esophagus, and another cannula was inserted into the stomach via an incision in the duodenum at a distance of 1 cm from the pylorus. The gastric lumen was perfused with physiological saline solution (NaCl 0.15 mol/l, pH 7.0, 37°C) at a rate of 1 ml/min with a peristaltic pump. Seventy minutes after the subcutaneous injection of different doses of cimetidine (0.1, 1, 3, 10, 30, 100 mg/kg), the perfusate was collected for 30 min and the acid in the perfusate was determined by titration with NaOH (5 mmol/l).

In another experiment, gastric submucosal microdialysis was conducted during ischemia-reperfusion in rats pretreated with a maximally effective dose of cimetidine (100 mg/kg) given subcutaneously 70 min before the ischemia. Controls received vehicle (saline).

Proton pump inhibitor. Omeprazole (a proton pump inhibitor) was dissolved in 0.25% Methocel and given in a dose of 400 μmol/kg once daily by oral gavage for 4 days. This dose is known to induce sustained inhibition of gastric acid secretion (24). The rats were fasted overnight, and omeprazole was given in the morning 2 h before clamping. In one experiment, treatment with omeprazole continued for as long as 9 days after clamping.

Fig. 1. Protocol of the ischemia-reperfusion experiments. Microdialysis samples were collected every 10 min after 1 h of equilibration. After 3 samples were collected, the celiac artery was occluded for 30 min (ischemia) and reopened for 60 min (reperfusion), i.e., until the experiment was terminated.
Determination of Microdialysate Histamine

Histamine in the microdialysate was measured by enzyme-linked immunosorbent assay with a commercially available kit (Immunotech, Paris, France). The histamine concentration was expressed as picomoles per milliliter. The sensitivity of this method is 0.1 pmol/m1, and the intra-assay variation was 15%.

Determination of Oxyntic Mucosal HDC Activity and Histamine Concentration

Mucosa was scraped off the acid-producing part of the stomach, weighed, and homogenized in ice-cold 0.1 mol/l sodium phosphate buffer, pH 7.4, to a concentration of 100 mg wet wt/ml. Aliquots (80 μl) were incubated with [1-14C]histidine (0.02 mCi/ml, specific activity 50 mCi/mmol), 5 × 10^-4 M l-histidine, and 10^-5 M pyridoxal 5'-phosphate in a total volume of 160 μl at 37°C for 1 h under nitrogen (17). HDC activity was expressed as picomoles of 14CO2 per milligram per hour. For determination of histamine, the mucosal homogenates were diluted 1:10 with redistilled water and heated in boiling water for 10 min to release bound histamine. The homogenates were then centrifuged at 6,000 g for 10 min; histamine in the supernatant was measured spectrophotofluorometrically (23).

Statistical Analysis

Results are expressed as means ± SE. Student’s t-test was used to evaluate the significance of differences between pairs of data. With multiple comparisons, the statistical significance of the differences was determined by one-way analysis of variance followed by Dunnett’s test. P < 0.05 was considered significant.

RESULTS

Time Course of Gastric Mucosal Damage and Mobilization of Gastric Histamine in Response to Ischemia-Reperfusion

Ischemia alone (30 min) produced macroscopically visible lesions on the surface of the gastric (oxyntic) mucosa. Ninety minutes of ischemia did not produce more mucosal damage than thirty minutes of ischemia (35.1 ± 10.8 mm² vs. 48 ± 20 mm²). Reperfusion for 30 or 60 min greatly increased the total area of the erosions (Fig. 2A).

Immediately after the start of the ischemia, the microdialysate histamine concentration began to rise, increasing 15- and 50-fold after 10 and 30 min, respectively (Fig. 2B); it declined promptly on removal of the clamp. Within 30 min of reperfusion the microdialysate histamine concentration had returned to preischemia levels.

Effect of Ischemia-Reperfusion on Gastric Mucosal Erosions and Microdialysate Histamine in Mast Cell-Deficient Rats

The gastric mucosa displayed macroscopically visible damage in response to 30 min of arterial clamping and 60 min of reperfusion in both WT rats and Ws/Ws rats (Fig. 3, A and B). There was no statistically significant difference in gastric lesions between the two groups of rats (Fig. 3C). As in the WT rats, the microdialysate histamine concentration in the Ws/Ws rats increased in response to the ischemia. The concentration reached the same level in both strains of rats. (Fig. 3D).

Fig. 3. A and B: photographs of oxyntic mucosa of wild-type (WT; A) and mast cell-deficient (Ws/Ws; B) rats, displaying lesions caused by 30 min of ischemia and 60 min of reperfusion. C: comparison of WT rats and Ws/Ws rats with respect to area of mucosal lesions. D: time course of change in the microdialysate histamine concentration in WT and Ws/Ws rats during ischemia-reperfusion. Values are means ± SE; n = 12. NS, not statistically significant difference.
Effect of Ischemia-Reperfusion on Gastric Mucosal Lesions and Microdialysate Histamine after Pharmacological Acid Blockade

α-FMH. Pretreatment with α-FMH greatly reduced the area of ischemia-reperfusion-evoked mucosal lesions and virtually abolished the rise in microdialysate histamine in both WT and Ws/Ws rats (Fig. 4).

Histamine H2 receptor antagonist. Cimetidine dose-dependently reduced the area of mucosal damage induced by ischemia-reperfusion in parallel with the reduction in basal acid output (Fig. 5A). There was no difference in the microdialysate histamine concentration between rats treated with cimetidine (maximally effective dose) and controls treated with vehicle (Fig. 5B).

Proton pump inhibitor. Acid blockade by omeprazole greatly reduced the ischemia-reperfusion-evoked mucosal damage without suppressing the concurrent histamine mobilization (Fig. 6, A and B). In contrast, histamine mobilization tended to be greater in omeprazole-treated rats than in vehicle-treated rats ($P = 0.14$).

Recovery of Gastric Mucosal HDC and Histamine after Ischemia-Reperfusion

The effect of ischemia on gastric mucosal HDC activity was studied in omeprazole-treated rats. HDC is a hallmark feature of rat stomach ECL cells (33). The enzyme is known to be activated by omeprazole treatment (because of the hypergastrinemia; Refs. 17, 33). Ischemia (30 min) of the stomach of omeprazole-treated rats promptly lowered the HDC activity (Fig. 6C). However, the HDC activity started to return to preischemic values after 2 days and was back to normal after 9 days (continued omeprazole treatment).

The histamine concentration was reduced by ~50% after ischemia; the concentration was back to normal after 4 days (Fig. 6D).

DISCUSSION

Ischemia-reperfusion of the stomach is known to produce mucosal lesions (28), and it is also known that such lesions can be prevented by pharmacological acid blockade (15). The present study made use of the in vivo microdialysis technique to show that large amounts of gastric histamine are mobilized in response to ischemia. The microdialysis technique can be used to monitor the precise time course of gastric histamine release into the submucosal compartment in response to a great variety of stimuli and pharmacological treatments (8, 14). Ischemia-reperfusion is known to release histamine in a number of organs. Thus the histamine content in the mesenteric lymph nodes increased after occlusion of the superior mesen-

![Fig. 4](image-url). Effects of ischemia-reperfusion on oxyntic mucosal erosions (A and C) and microdialysate histamine (B and D) after pretreatment with α-fluoromethylhistidine (α-FMH) or vehicle in WT (A and B) and Ws/Ws (C and D) rats. Values are means ± SE; $n = 6$. 

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teric artery (10). Occlusion followed by reperfusion of the renal vessels raised the plasma histamine levels in the renal vein (12). Cardiac mast cells degranulate after myocardial ischemia, releasing histamine (9). However, in all these reports, histamine was released in moderate amounts. In the present study, ischemia was found to raise the microdialysate histamine concentration 50 times, suggesting that the stomach, which is a rich source of histamine, is a notably sensitive target for ischemia-evoked signals.

Ischemia Increases Histamine in Submucosal Compartment of Stomach

Microdialysis is a useful technique to monitor the mobilization of histamine from an intracellular to an extracellular compartment. Normally, histamine mobilized from ECL cells will be washed away promptly, only to appear in the portal vein. However, the clamping of the gastric artery will prevent such washout. Hence, the greatly elevated histamine concentration in the gastric submucosa following ischemia probably reflects not only mobilization but also accumulation/entrapment of the amine in the tissue.

Origin of Mobilized Gastric Histamine

To identify the source of the histamine that is mobilized by ischemia of the stomach, we compared mast cell-deficient rats (Ws/Ws rats) and WT rats. First, the area of the gastric mucosal erosions did not differ between Ws/Ws rats and WT rats, suggesting that mast cells do not contribute to the mucosal...

Fig. 5. A: effect of different doses of the histamine H₂ receptor antagonist cimetidine (given by subcutaneous injection 70 min before start of ischemia) on mucosal erosions induced by 30 min of ischemia and 60 min of reperfusion and basal acid output. B: time course of change in microdialysate histamine during ischemia-reperfusion after pretreatment with vehicle or a maximally effective dose of cimetidine (100 mg/kg). Values are means ± SE; n = 6.

Fig. 6. Effect of gastric ischemia in rats pretreated with omeprazole or vehicle per os for 4 days before clamping of the celiac artery. A and B: area of oxyntic mucosal erosions in fasted rats killed after 30 min of ischemia and 60 min of reperfusion (A) and integrated microdialysate histamine response to 30 min of ischemia (B). Values are means ± SE; n = 4–11. C and D: oxyntic mucosal histidine decarboxylase (HDC) activity (C) and histamine concentration (D) 1 h, 2 days, 4 days, or 9 days after 30 min of clamping (continued treatment with omeprazole and free access to food at all times). Omeprazole-treated controls were killed 13 days after start of drug treatment. Values are means ± SE; n = 7–9. Statistical comparison is made with the starting value (before ischemia): **P < 0.01, *P < 0.05 (1-way ANOVA followed by Dunnett’s test).
damage. Second, the rise in microdialysate histamine concentration (and hence in the submucosal histamine concentration; Ref. 8) was the same in the Ws/Ws rats and the WT rats, suggesting that mast cells do not contribute importantly to the histamine response to ischemia. After pretreatment of the two strains of rats with α-FMH, which is known to deplete histamine from ECL cells but not from mast cells (1, 2), the ischemia-induced histamine response was virtually abolished (a few percent remaining), in support of the view that the histamine that is mobilized by ischemia comes mainly from the ECL cells.

Mechanism Behind Mobilization of Histamine by Ischemia-Reperfusion

The precise mechanism behind the ischemia-evoked histamine mobilization remains unknown. It is possible but not very likely that the histamine mobilization reflects the death of ECL cells because of damage. This is unlikely because 1) omeprazole-treated rats responded to ischemia-reperfusion with histamine mobilization but not with mucosal damage and 2) clamping of the celiac artery in omeprazole-treated rats reduced the oxyntic mucosal histamine concentration and HDC activity only transiently; the histamine concentration and HDC activity were in fact back to normal 4–9 days later. Because ECL cells have a long life span (25, 26), the latter findings are in line with the view that although the cells are damaged by 30 min of ischemia, they are able to recover within a matter of days.

Is Mobilized Gastric Histamine Responsible for Gastric Lesions After Ischemia-Reperfusion?

Pretreatment with the histamine H2 receptor antagonist cimetidine or the proton pump inhibitor omeprazole prevented or greatly reduced the gastric mucosal injury in response to ischemia-reperfusion without lowering the amount of histamine mobilized from ECL cells. This is in line with the view that ischemia-evoked mobilization of histamine and development of gastric mucosal injury are independent phenomena. The major cause of the mucosal injury would be ischemia-evoked anoxia in combination with acid secretion. Without acid secretion there is no mucosal damage. The acid output is reduced by 80% after 30 min of ischemia (15). Still, local histamine is probably needed (in small quantities) to stimulate the parietal cells to produce acid. In fact, the elimination of acid by luminal perfusion of the stomach with saline (1 ml/min) protected the gastric mucosa from the ischemia-reperfusion insult, suggesting that luminal acid contributes to the mucosal damage (15). However, it cannot be excluded that it is the energy-demanding process of acid secretion rather than gastric acidity per se that is responsible for the lesions (30). The massive release of histamine in response to ischemia does not in itself cause or aggravate the mucosal damage. In a parallel study, endothelin was administered by submucosal microinfusion, causing local mucosal lesions and local release of spectacular amounts of ECL cell histamine (4), in a manner reminiscent of the events taking place after gastric ischemia. The endothelin-induced mucosal lesions could be prevented by systemic but not by local administration (via the microdialysis probe) of the histamine H2 receptor antagonist ranitidine (4), suggesting that acid per se (or the metabolic demands of acid secretion) is responsible for the damage and that histamine per se is not. Indeed, the failure of local administration of histamine (4) to induce gastric mucosal lesions is in line with this interpretation.

In conclusion, ischemia-reperfusion damages the oxyntic mucosa and causes spectacular histamine release. The lesions do not develop because of the histamine mobilization but because of ischemia plus reperfusion plus gastric acid per se. Histamine comes from ECL cells, and mast cells do not contribute to either the mucosal damage or the histamine output. ECL cell histamine is mobilized by ischemia, independently of the mucosal damage.

GRANTS

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