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Role of gastric acid secretion in progression of acute gastric erosions induced by ischemia–reperfusion into gastric ulcers

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Abstract

Ischemia followed by reperfusion is known to produce gastric lesions due to oxidative stress, but the role of gastric H^+ secretion in the formation of this mucosal injury remains unknown. We studied alterations in gastric acid secretion and gastric histamine content, as well as the expression of histidine-decarboxylase and interleukin-1β during the mucosal recovery from ischemia–reperfusion erosions. Gastric secretion was studied in rats (series A) with gastric fistula before, during and after the ischemia induced by clamping of celiac artery for 0.5 h followed by reperfusion in animals pretreated with vehicle (saline), omeprazole, a proton pump inhibitor, or ranitidine, a histamine (H_2) receptor antagonist. In series B, the animals were submitted to 0.5 h of ischemia followed by 1 h of reperfusion and then anesthetized at 0, 3, 12 and 24 h or 3, 5, 10 or 15 days after the end of ischemia–reperfusion to determine gastric blood flow by H_2-gas clearance technique, area of gastric lesions, plasma gastrin and interleukin-1β levels, histamine content by radioimmunoassay (RIA) and expression of histidine-decarboxylase and interleukin-1β mRNA by reverse transcription polymerase chain reaction. Clamping of celiac artery caused cessation of gastric blood flow and almost complete suppression of basal gastric acid secretion (series A) that returned gradually to the control value at day 3 after ischemia–reperfusion, accompanied by the rise in plasma gastrin levels, pronounced expression of histidine-decarboxylase mRNA and increased mucosal histamine content. Ischemia, followed by 1 h of reperfusion, produced gastric erosions (series B) that reached maximum at 12 h, but then declined at 24 h. These erosions progressed at day 3 into deeper ulcers whose area declined progressively within the next 5–15 days. The gastric blood ceased to flow (series B) during 30 min of clamping and was reduced throughout the period of healing of acute erosions, being accompanied by a gradual rise in mucosal interleukin-1β mRNA content and in plasma interleukin-1β levels. Treatment with omeprazole or ranitidine, which completely suppressed gastric acid secretion and significantly raised plasma gastrin level, greatly reduced the formation of erosive lesions preventing the progression of these lesions to chronic gastric ulcers, and this was accompanied by the rise in gastric blood flow and plasma gastrin levels and the significant attenuation of plasma interleukin-1β levels. The ranitidine and omeprazole-induced suppression of ischemia–reperfusion erosions were abolished by the instillation of exogenous 0.2 N HCl into the stomach of these rats. The histidine-decarboxylase was faintly expressed in the intact gastric mucosa, but strongly upregulated during mucosal recovery from the damage induced by ischemia–reperfusion. We conclude that following ischemia–reperfusion: (1) gastric acid secretion, gastric microcirculation and histamine production markedly decline, while interleukin-1β release significantly increases, probably playing an important role in the progression of acute lesions into chronic gastric ulcerations; (2) the suppression of gastric acid secretion by omeprazole and ranitidine, that induces hypergastrinemia, prevents the progression of gastric erosions into ulcers; and (3) the addition of exogenous acid restores the progression of the acute lesions into gastric ulcers, indicating that gastric acid plays a key role in ulcerogenesis induced by ischemia–reperfusion.

Keywords: Ischemia–reperfusion; Gastric secretion; Omeprazole; Ranitidine; Ulcer healing; Gastric blood flow; Interleukin-1β; Gastrin; Somatostatin

1. Introduction

Ischemia and reperfusion are known to induce gastric lesions predominantly due to excessive formation of reac-

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tive oxygen metabolites, adhesion of neutrophils to endothelial cells and microvascular dysfunction (Yoshikawa et al., 1992; Andrews et al., 1994; Kawai et al., 1994), but the role of gastric acid secretion, endogenous histamine and gastrin release in the pathogenesis of these lesions has not been fully explored. Previous studies documented that ischemia weakens gastric mucosal barrier and increases the acid back-diffusion predisposing the gastric mucosa to the damage (Kawai et al., 1994; Itoh and Guth, 1985). Recent evidence suggests that enhanced release of endothelin-1 that causes microvascular dysfunction and alteration in gastric motility is an initiating event in the development of ischemia–reperfusion-induced mucosal damage (Wood et al., 1995; Hassan et al., 1997). After the reperfusion, the reactive oxygen species are generated, especially from xanthine–xanthine oxidase system and activated neutrophils, leading to tissue lipid peroxidation, which, in combination with gastric acid secretion, result in cellular death and mucosal injury (Yoshikawa et al., 1992; Andrews et al., 1994; Smith et al., 1996; Wada et al., 1996).

Gastric acid is considered as an important aggressive factor in the stomach and is known to produce gastric injury when introduced exogenously in excessive amounts into normal stomach. Previous studies (Seno et al., 1995; Nakamoto et al., 1998) documented that ischemia itself, while inducing gastric lesions, actually suppresses gastric acid secretion parallel to the fall in gastric microcirculation, suggesting that endogenous acid and limitation of the blood flow are important factors in the ischemia–reperfusion-induced gastric ulcerogenesis (Kawai et al., 1994). In some studies, enhancement of gastric lesions was achieved by the addition of exogenous acid into the stomach during ischemia–reperfusion, but this excluded the possibility of determination of the involvement of endogenous acid in the pathogenesis of these lesions. Anyway, these studies demonstrated that the presence of acid in the stomach is a prerequisite of the ischemia–reperfusion-induced gastric damage (Kawai et al., 1994), but little information is available regarding the real importance of endogenous acid in pathogenesis of this damage.

Using the model of acute gastric erosions induced by ischemia–reperfusion that are restricted predominantly to the stomach and do not require exogenous acid, as originally proposed by Wada et al. (1996), we attempted to determine the contribution of gastric acid secretion and the effects of suppression of this secretion by antisecretory agents with or without addition of exogenous acid on the progression of acute erosions into ulcers following ischemia–reperfusion. The alterations in gastric secretion, gastric blood flow, mucosal histamine content and plasma gastrin levels were determined during the mucosal recovery from gastric erosions induced by ischemia–reperfusion. Since proinflammatory cytokines such as interleukin-1β were shown to influence both gastric secretion, gastrin release and ulcer healing (Robert et al., 1991), we decided to study the expression of interleukin-1β and plasma levels of this cytokine. Also, the expression of mucosal histidine-decarboxylase mRNA, the key enzyme in the synthesis of histamine, the final chemostimulant of gastric secretion, was studied along the time-course of the recovery of gastric mucosa from the ischemia–reperfusion damage. Another attempt has been made to determine the involvement of gastrin in the healing of ischemia–reperfusion damage since this hormone was shown to exert the gastroprotective activity against lesions induced by corrosive substances such as absolute ethanol (Konturek et al., 1995). Furthermore, the hypergastrinemia was proposed previously to contribute to the healing of pre-existing gastric ulcers in rats (Li and Helander, 1996), but the possibility that gastrin may be involved in the recovery from acute lesions and subsequent chronic ulcers induced by ischemia–reperfusion has not been explored.

2. Materials and methods

Male Wistar rats, weighing 220–250 g, fasted for 24 h before the study, were used in all studies.

2.1. Studies on gastric secretion

Gastric secretion was studied in 60 rats equipped surgically (about 4 weeks before secretory studies) with the cannula (5 mm in diameter) placed in the distal portion of the stomach to form gastric fistula as described previously (Brzozowski et al., 1996). For gastric secretory studies performed under the conditions of ischemia–reperfusion and subsequent recovery, conscious gastric fistula rats were used with clamping device placed around the celiac artery. Both the cannula of the gastric fistula and the polyethylene tube containing the occluding wire loop were brought out through the abdominal wall via two separate small incisions. These procedures successfully allowed us to collect gastric juice in conscious rats, with or without the occlusion of the blood flow to the stomach through the celiac artery. Before the secretory test, rats were fasted for 24 h in separate cages preventing coprophagy, but with access to water, and then, the cannula of gastric fistula was opened, the stomach washed out with 5 ml of saline and collection of basal secretion was started. After a 0.5-h period of the collection of basal gastric secretion, animals were subjected to standard period of 0.5 h of gastric ischemia achieved by occluding the celiac artery with device mentioned above. Then the clump was released, allowing for gastric reperfusion as described previously (Brzozowski et al., 1999a). During ischemia, the gastric fistula was closed and the rats received subcutaneously (s.c.) vehicle (saline), ranitidine (40 mg/kg s.c.) or omeprazole (20 mg/kg s.c.) and this treatment was then repeated every 6 h up to 24 h after the end of ischemia–reperfusion. The gastric secretion was collected immediately (time 0) and at 3, 12, 24 h and at day 3, 5, 10 and 15 after

the termination of ischemia–reperfusion. The gastric samples were collected from gastric fistula rats and the acid and pepsin concentrations were measured in each 30-min collected aliquot and expressed as mean acid output per 30 min.

2.2. Production of ischemia–reperfusion lesions

Ischemia–reperfusion erosions were produced in 120 rats by the method originally proposed by Wada et al. (1996). Briefly, under pentobarbital anesthesia (50 mg/kg i.p.), the celiac artery was clamped with a small device for 0.5 h. Then the clamping device was removed to obtain reperfusion. Erosions were determined already after 1 h of reperfusion (time 0) and further at 3, 12 or 24 h after the termination of ischemia–reperfusion in rats treated with vehicle (saline-control), ranitidine (40 mg/kg s.c.) and omeprazole (20 mg/kg i.g.) applied in a total volume of 1 ml about 0.5 h before ischemia–reperfusion and then repeated at days 1 and 3 after the termination of ischemia–reperfusion. Both inhibitors at the doses used twice daily in this study were shown to induce achlorhydria in the rat stomach (Konturek et al., 1981, 1983). In addition, omeprazole in the dose used in this study was shown originally to attenuate significantly the formation of acute gastric lesions induced by necrotizing agents (Konturek et al., 1983). In separate groups of rats, longer observation periods were used after ischemia–reperfusion, starting from day 3 up to day 15 upon ischemia–reperfusion, in order to determine up to what extent does the suppression of gastric acid with ranitidine or omeprazole affect the time-course recovery of gastric mucosa from the lesions induced by ischemia–reperfusion.

2.3. Determination of gastric blood flow, plasma interleukin-1β, histamine and gastrin concentrations

To evaluate the effect of ischemia–reperfusion on gastric blood flow, the groups of animals were anesthetized with ether and the abdomen was opened by midline incision. The stomach was exposed to assess the blood flow using H_2-gas clearance technique as described previously (Brzozowski et al., 1997). Briefly, the gastric blood flow was measured in the intact vehicle-treated rats and those subjected to ischemia–reperfusion immediately after 0.5 h of ischemia, following 0, 3, 12, 24 h and then at day 3, 5, 10 and 15 after the end of clamping of celiac artery using double electrodes of electrolytic regional blood flowmeter (Biotechnical Science, Model RBF-2, Osaka, Japan) inserted through the serosa into the mucosa. One of these electrodes was used for the local generation in the mucosa of H_2 and another for measurement of tissue H_2. With this method, the H_2 generated locally is carried out by flow of blood, while the polarographic current detector reads out decreasing tissue H_2. The clearance curve of tissue H_2 was used to calculate an absolute blood flow rate (ml/min/100 g) in the oxyntic gland area as described previously (Brzozowski et al., 1997; Konturek et al., 1998). The measurement was made in three areas of the gastric oxyntic mucosa and the mean values of these measurements were calculated and expressed as percent changes from those recorded in vehicle-treated control animals not exposed to ischemia–reperfusion.

Immediately after measurement of blood flow, a venous blood sample was withdrawn from the vena cava into the EDTA containing vials and used either for the determination of plasma gastrin by radioimmunoassay (RIA) as described previously (Konturek et al., 1992) and the plasma interleukin-1β concentrations by specific enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's recommendation (Endogen, Cambridge, MA, USA). For the determination of histamine content in the rat gastric mucosa, the oxyntic mucosa (about 300 mg) was scraped off using glass slide, then homogenized in 1 ml of phosphate-buffered saline (pH 7.4) and supernatant stored in −80°C until RIA-histamine using commercially available RIA kit (IBL, Hamburg, Germany). The antiserum used in this measurement recognized only *N*-acylhistamine and did not cross-react with *N*-methylhistamine, L-histidine, serotonin, imidazole, acetic acid or 5-hydroxy-indole acetic acid. The detection limit of the assay was 0.05 ng/ml and interassay and intraassay variations were 3% and 5%, respectively.

2.4. Histological evaluation of gastric mucosa in rats exposed to ischemia–reperfusion

The samples of the gastric mucosa with or without ischemia–reperfusion were excised for histological examination in each rat at various time intervals after the end of ischemia and following the ischemia–reperfusion procedure at the time when the stomach was removed and animals were killed. The samples were fixed in 10% buffered formalin and embedded in paraffin (Konturek et al., 1998). The paraffin sections were cut at a thickness of 5 mm and stained with hematoxylin and eosin. Histological examination was performed on coded slides by two experienced pathologists unaware of the treatment given.

2.5. RNA extraction and reverse transcriptase polymerase chain reaction to detect messenger RNA (mRNA) for histidine-decarboxylase, interleukin-1β and GAPDH

When the stomachs were removed from rats with intact gastric mucosa and from those exposed to ischemia–reperfusion at various time intervals after ischemia and ischemia–reperfusion–reperfusion, mucosal specimens were scraped off using a slide glass and immediately snap frozen in liquid nitrogen and stored at −80°C until analysis. Total RNA was extracted from mucosal samples by a guanidinum isothiocyanate/phenol chloroform method using kit from Stratagene® (Heidelberg, Germany) (Chomc-

zynski and Sacchi, 1987). The total RNA concentration in each sample was determined by 1% agarose-formaldehyde gel electrophoresis and ethidium bromide staining. Aliquoted RNA samples were stored at −80°C until analysis.

Single-stranded complementary DNA (cDNA) was generated from 5 μg of total cellular RNA using StrataScript reverse transcriptase (Stratagene) and oligo-(dT)-primers (Stratagene). Briefly, 5 μg of total RNA was uncoiled by heating (65°C for 5 min) and then reverse by transcription into cDNA in a 50-μl reaction mixture that contained 50 U of Moloney murine leukemia virus reverse transcriptase (MMLV-RT), 0.3 mg oligo-(dT)-primer, 1 ml RNase Block Ribonuclease Inhibitor (40 U/μl), 2 ml of a 100 mmol/l mixture of deoxyadenosine triphosphate (dATP), deoxyribothymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 5 ml 10× RT buffer (10 mM/l Tris–HCl, pH = 8.3, 50 mM/l KCl, 5 mM/l $MgCl_2$). The resultant cDNA (2 μl) was amplified in a 50-μl reaction volume containing 0.3 ml (2.5 U) Taq polymerase, 200 mM/l (each) dNTP (Pharmacia, Germany), 1.5 mM/l $MgCl_2$, 5 ml 10× polymerase chain reaction buffer (50 mM/l KCl, 10 mM/l Tris–HCl, pH = 8.3) and primers used at final concentration of 0.5 mM/l. The mixture was overlaid with 25 μl of mineral oil to prevent evaporation. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT) and the nucleotide sequence of the primers for interleukin-1β and GAPDH mRNA was based on the basis of the published cDNA encoding interleukin-1β and GAPDH, respectively (Brzozowski et al., 1999b). The nucleotide sequence of the primers for histidine-decarboxylase were as follows: sense ′5 ATG CTG ATG AGT CCT CTC TG3′; antisense 5′ GCT TGT ACT TGT CCT TGA CC3′. The primers were synthesized by GIBCO BRL/Life Technologies, Eggenstein, Germany.

Polymerase chain reaction products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of a predicted products was confirmed by using GIBCO 100-bp ladder as a standard size marker.

The intensity of bands was quantified using densitometry (LKB Ultrascan, Pharmacia, Sweden) as described in details in our previous studies (Konturek et al., 1998; Brzozowski et al., 1999b). The interleukin-1β and histidine-decarboxylase mRNA signals were standardized against the GAPDH mRNA signal for each sample and results were expressed as histidine-decarboxylase and interleukin-1β/GAPDH mRNA ratio.

2.6. Statistical analysis

Results are expressed as means ± S.E.M. For statistical analysis, the nonparametrical Mann–Whitney *U* and Kruskal–Wallis tests for unpaired comparisons were applied where appropriate with p values less than 0.05 taken as statistically significant.

3. Results

3.1. Effect of gastric acid inhibitors or vehicle on gastric secretion

Fig. 1 shows the basal acid output in conscious gastric fistula rats treated with vehicle (saline) and those subjected to the standard period of ischemia–reperfusion with or without the treatment with ranitidine (40 mg/kg day s.c.) or omeprazole (20 mg/kg day s.c.). The basal gastric acid output in vehicle-treated control rats before ischemia–reperfusion reached the value of 132 ± 16 μmol/h (Fig. 1). At 1 h after the end of ischemia–reperfusion, the gastric secretion was completely suppressed and this inhibition lasted up to 3 h post ischemia–reperfusion in all three groups of animals (treated with vehicle, ranitidine or omeprazole). In vehicle-treated controls, the gastric acid secretion was still significantly inhibited within 12–24 h after ischemia–reperfusion, but then returned to the value similar to that obtained in these animals before the ischemia–reperfusion procedure. Within the period of 3–15 days following ischemia–reperfusion, the gastric acid secretion remained at the level not significantly different from that observed in vehicle-treated control animals without ischemia–reperfusion. Omeprazole or ranitidine inhibited significantly the gastric acid secretion immediately (0 h) after the end of ischemia–reperfusion and this suppres-

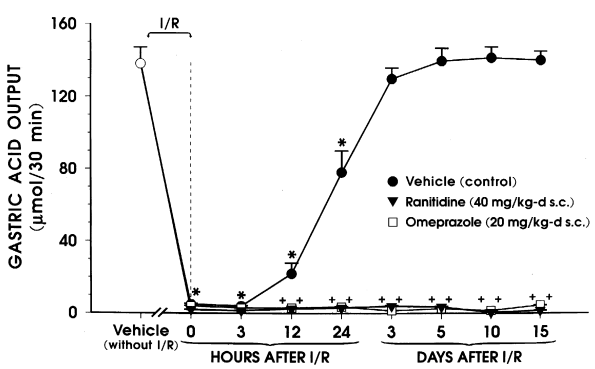

Fig. 1. Gastric acid secretion in rats equipped with gastric fistula and exposed to standard ischemia (0.5 h) followed by reperfusion with or without the treatment with vehicle (saline), ranitidine (40 mg/kg s.c.) or omeprazole (20 mg/kg s.c.) and determined immediately after the end of ischemia–reperfusion (0 h) or at 3, 12 and 24 h and at 3, 5, 10 or 15 day after the termination of ischemia–reperfusion (I/R). Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-treated rats before clamping celiac artery to induce ischemia and then reperfusion. Cross indicates a significant change as compared to the value obtained in vehicle-treated control rats before ischemia–reperfusion. Cross indicates significant decrease below the value obtained in vehicle-treated rats at various time intervals after ischemia–reperfusion.

sion of gastric acid was sustained at all time intervals tested until the end of observation period (Fig. 1).

3.2. Mucosal recovery from ischemia–reperfusion damage and alteration in the gastric blood flow and gastric histamine content

The area of gastric erosions induced by 0.5 h ischemia followed by 1 h reperfusion in rats treated with vehicle or with suppressed gastric secretion by omeprazole and ranitidine is presented in Figs. 2–4. Immediately after clamping of celiac artery, no gastric lesions were observed in any of the groups tested (these results are not included for the sake of simplicity), but after 1 h reperfusion, the area of gastric erosions were recorded in all tested stomachs. The area of these erosions was significantly increased at 3 h and peaked at 12 h, but then declined at 24 h after ischemia–reperfusion (Fig. 2, upper panel). After 24 h, these erosions progressed into deep ulcers (as assessed macroscopically and histologically) and the area of these lesions reached the maximum at day 3. Then the area of ulcers gradually declined at days 5 and 10 to almost completely disappear at day 15 upon ischemia–reperfusion (Fig. 2, lower panel). Examples of typical histological appearance of acute gastric lesions observed at 3 h and at day 3 following ischemia–reperfusion are presented in Fig. 3a and b. At 3 h after the termination of ischemia–reperfusion, the desquamation of the surface epithelium and acute necrotic and bleeding erosions developed in all stomachs being localized mostly in the oxyntic gland area (Fig. 3a).

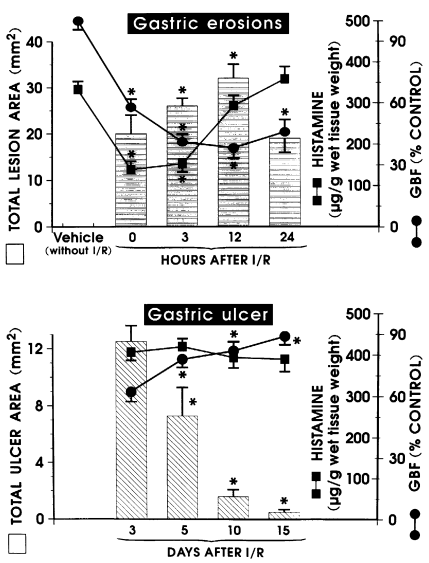

Fig. 2. Area of acute gastric erosions and gastric ulcers, gastric blood flow (GBF) and histamine content in the gastric mucosa of rats exposed to standard ischemia–reperfusion and measured immediately (time 0) and 3, 12 and 24 h or at day 3, 5, 10 or 15 after the end of this procedure. Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared with the value obtained immediately after ischemia–reperfusion (upper panel) or at day 3 after the termination of this procedure (lower panel).

With extension of the observation time, these acute lesions progressed into chronic ulcerated lesions with extensive regeneration at the margin recorded both macroscopically and microscopically already at day 3 after ischemia–reperfusion (Fig. 3b).

The gastric blood flow in intact mucosa of vehicle-treated rats averaged 48 ± 6 ml/min 100 g (taken as 100%), and clamping of the celiac artery resulted in an immediate cessation of gastric blood flow, and this has not been included for the sake of clarity. As shown in Fig. 2, at 1 h after removal of the clamp, the gastric blood flow was reduced by about 40% as compared to initial value (before ischemia–reperfusion) recorded in vehicle-treated controls. The reduction in gastric blood flow was still observed after 3, 12 and 24 h upon termination of the ischemia–reperfusion experiment. The gastric blood flow was still reduced significantly at day 3, but at days 5–15, it reached the values not significantly different from that in vehicle-control rats.

As shown in Fig. 2, the gastric mucosal histamine content in intact gastric mucosa averaged 382 ± 28 μg/g of wet tissue weight. In vehicle-pretreated rats exposed to standard ischemia–reperfusion, a significant decrease in gastric histamine content was observed immediately after ischemia–reperfusion and this remained significantly reduced at 3 h after ischemia–reperfusion. The mucosal histamine content rose significantly starting from 12 h after the end of ischemia–reperfusion and remained elevated and not significantly different from that in intact mucosa at 24 h and then at days 3–15 after this procedure.

3.3. Effect of gastric acid inhibitors on mucosal recovery from ischemia–reperfusion damage and gastric blood flow

The area of gastric lesions in rats treated with omeprazole or ranitidine was markedly reduced as compared to that recorded in vehicle-treated animals at 0–24 h after the end of ischemia–reperfusion and the rate of the healing of these erosions expressed in percent of initial value of damaged area, was two- to threefold higher with ranitidine (45%) or omeprazole (76%) as compared to vehicle (Figs. 4 and 5). At day 3 after ischemia–reperfusion, when erosions progressed into ulcers, the area of these ulcers in rats treated with omeprazole or ranitidine also remained significantly smaller than that measured in vehicle-treated controls (Figs. 4 and 5). This decrease in the area of gastric ulcers of rats treated with the gastric acid inhibitors was sustained during all tested time intervals (3–15 days) after ischemia–reperfusion, but the rate of ulcer healing with ranitidine or omeprazole was not significantly different from that observed in vehicle control (Figs. 4 and 5).

In rats treated with ranitidine and omeprazole, the increase of the gastric blood flow was significantly higher than in vehicle controls at all time periods following ischemia–reperfusion, except at day 15 when this flow

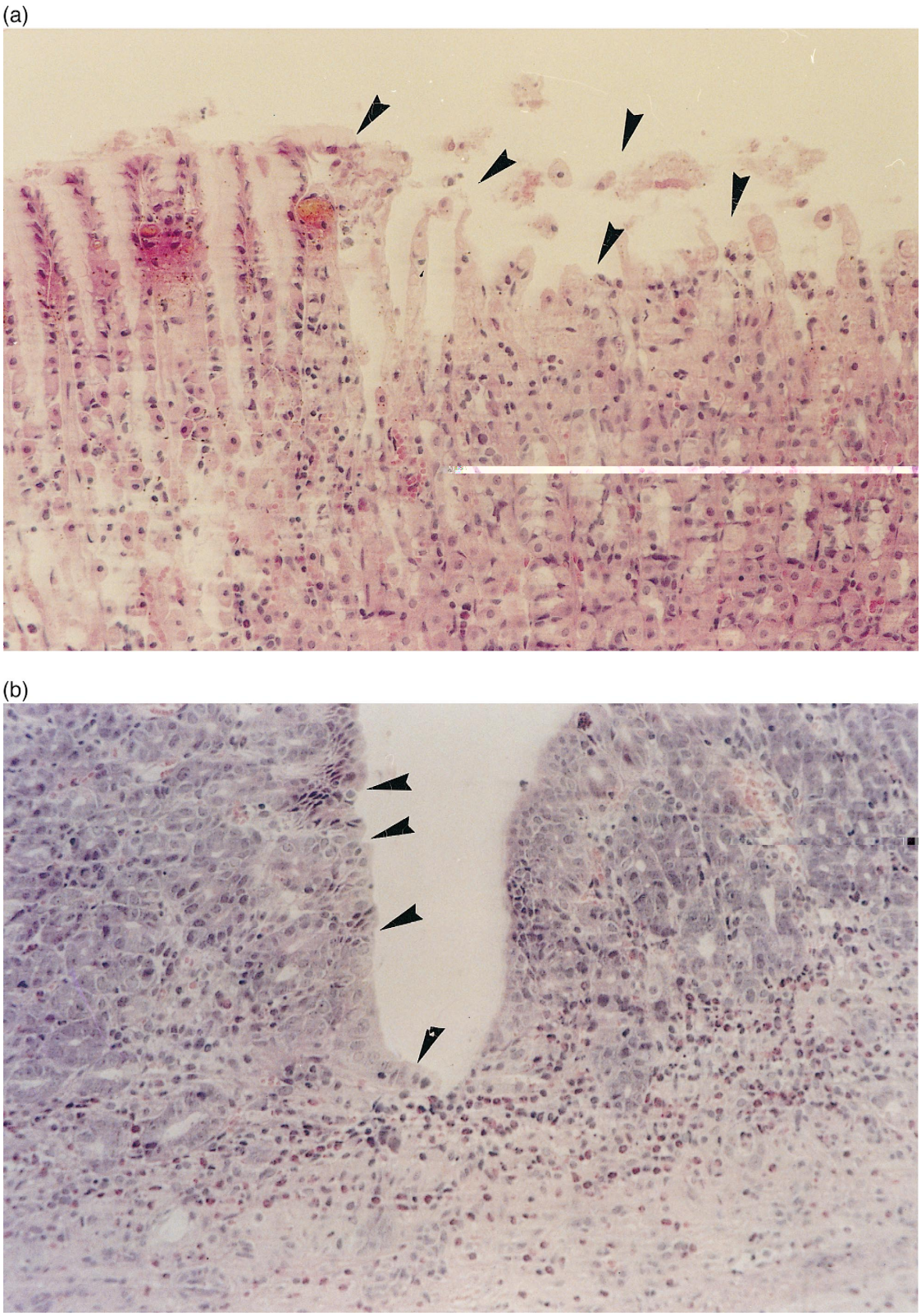

Fig. 3. (a and b) Microscopic appearance of gastric mucosa in vehicle-treated animals exposed to standard ischemia–reperfusion and sacrificed at 3 h after this procedure (a) or at day 3 after the termination of this procedure (b). Note that at 3 h following ischemia–reperfusion, the gastric mucosa exhibits a discontinuation of the surface epithelium (arrows) and the massive acute necrosis of the gastric oxyntic mucosa involving upper half of the mucosal thickness. Necrotic tissue is denuded of surface epithelium. Hematoxylin and eosin, magnification 260× (a). At day 3 after the ischemia–reperfusion, chronic mucosal ulceration is observed. There is less necrotic tissue surrounding ulcer area and an early regeneration is present at the margin (arrows). Hematoxylin and eosin, magnification 150× (b).

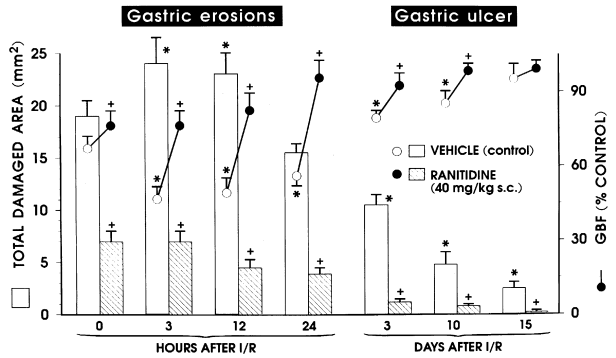

Fig. 4. Area of gastric erosions and deep gastric ulcers and gastric blood flow (GBF) in the gastric mucosa of rats exposed to 0.5 h of ischemia followed by 1 h of reperfusion with or without treatment with vehicle or ranitidine (40 mg/kg s.c.). Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared to the value recorded in vehicle-treated rats immediately (0 h) after the end of ischemia–reperfusion. Cross indicates a significant change as compared with the value obtained in vehicle-treated control rats at each time intervals after the termination of ischemia–reperfusion.

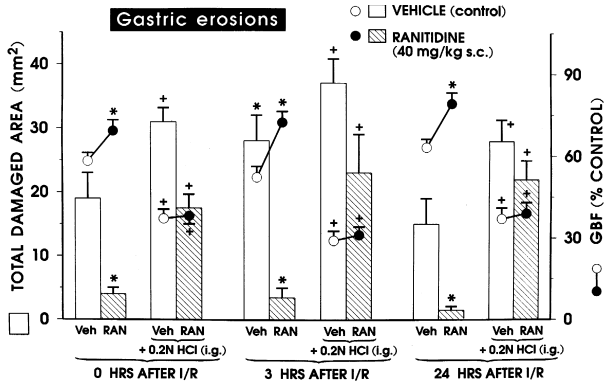

Fig. 6. Effect of vehicle or ranitidine (40 mg/kg s.c.) with or without addition of 0.2 N HCl (2 ml/day) on the area of gastric lesions and accompanying changes in the GBF induced by the exposure to standard ischemia–reperfusion and determined at 0, 3 and 24 h after the end of this procedure. Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared with the value obtained in vehicle-control gastric mucosa at different time intervals after the end of ischemia–reperfusion. Cross indicates a significant change as compared with the value obtained in rats without HCl administration.

reached the level similar to that recorded in the vehicle-treated rats (Figs. 4 and 5). Thus, the progression of acute lesions into chronic ulcers after 3–10 days upon ischemia–reperfusion in rats pretreated with omeprazole or ranitidine was accompanied by the significantly greater rise in the gastric blood flow compared to that in vehicle controls, except at day 15 after ischemia–reperfusion when the ulcers almost completely disappeared and the blood flowed in these rats. Also, this progression did not differ from that in vehicle-control animals and approached the value similar to that recorded in intact gastric mucosa (Figs. 4 and 5).

The area of lesions caused by ischemia–reperfusion at various time intervals after ischemia–reperfusion and the accompanying changes in the gastric blood flow in rats treated with vehicle, ranitidine or omeprazole with or without intragastric addition of 0.2 N HCl are presented in Figs. 6–9. In vehicle-treated controls, typical healing patterns of acute and then chronic ulcers were observed and they were accompanied by the usual rise of gastric blood flow at 0, 3 and 24 h and at 3, 5 and 10 days of the recovery from ischemia–reperfusion-induced gastric lesions. When ranitidine or omeprazole was applied in the

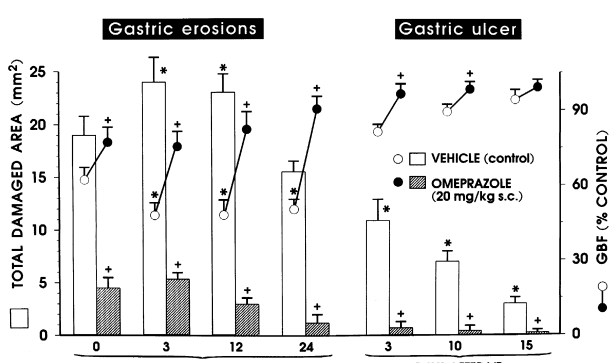

Fig. 5. Area of gastric erosions and deep gastric ulcers and gastric blood flow (GBF) in the gastric mucosa of rats exposed to 0.5 h of ischemia followed by 1 h of reperfusion with or without treatment with vehicle or omeprazole (40 mg/kg s.c.). Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared to the value recorded in vehicle-treated rats immediately (0 h) after ischemia–reperfusion. Cross indicates a significant change as compared with the value obtained in vehicle-control rats at each time intervals after the termination of ischemia–reperfusion.

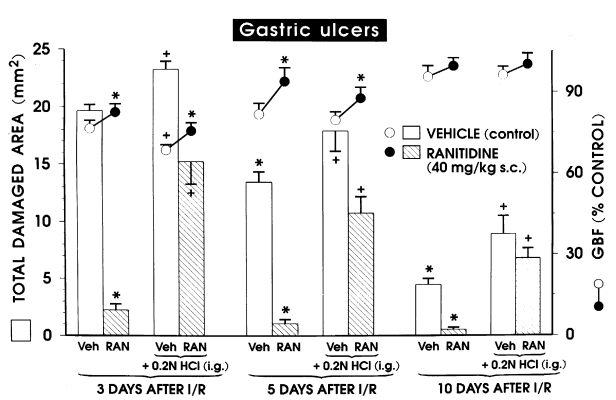

Fig. 7. Effect of vehicle or ranitidine (40 mg/kg s.c.) with or without addition of 0.2 N HCl (2 ml/day) on the area of deeper gastric ulcers and accompanying changes in the GBF induced by the exposure to standard ischemia–reperfusion and determined at 3, 5 and 10 days after the end of this procedure. Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared with the value obtained in vehicle-control gastric mucosa at different time intervals after the end of ischemia–reperfusion. Cross indicates a significant change as compared with the value obtained in rats without HCl administration.

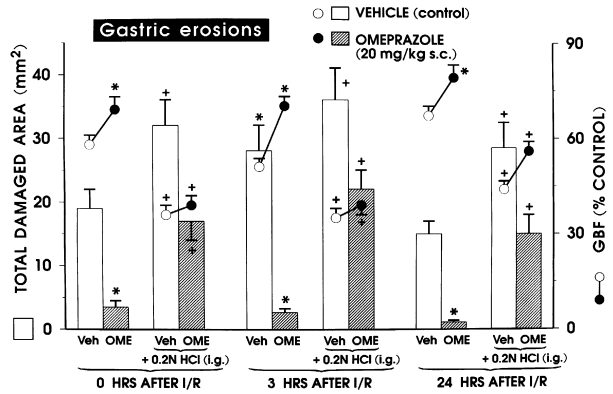

Fig. 8. Effect of vehicle or omeprazole (20 mg/kg s.c.) with or without addition of 0.2 N HCl (2 ml/day) on the area of gastric lesions and accompanying changes in the GBF induced by the exposure to standard ischemia–reperfusion and determined at 0, 3 and 24 h after the end of this procedure. Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared with the value obtained in vehicle-control gastric mucosa at different time intervals after the end of ischemia–reperfusion. Cross indicates a significant change as compared with the value obtained in rats without HCl administration.

dose that caused achlorhydria (in gastric fistula rats), usual attenuation of the total damaged area was observed and a greater increase in the gastric blood flow were recorded as described above. The addition of exogenous HCl into the stomach of ranitidine (Fig. 6) or omeprazole (Fig. 7)-treated animals resulted in a significant augmentation of the acute gastric lesions provoked by ischemia–reperfusion and a significant delay of the recovery from these lesions, as well as a significant delay in the healing of chronic ulcers, when these changes were compared with those induced by treatment with either ranitidine or omeprazole alone without instillation of exogenous HCl (Figs. 6–9).

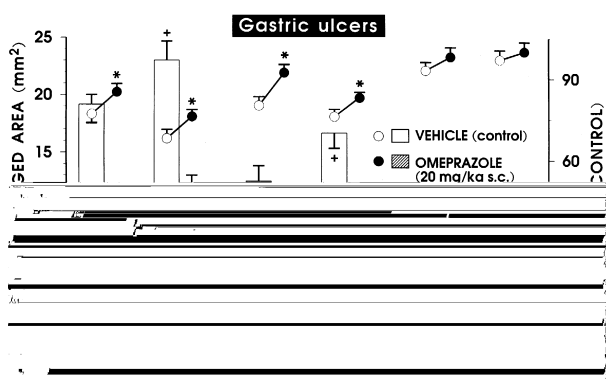

Fig. 9. Effect of vehicle or omeprazole (20 mg/kg s.c.) with or without addition of 0.2 N HCl (2 ml/day) on the area of deeper gastric ulcers and accompanying changes in the GBF induced by the exposure to standard ischemia–reperfusion and determined at 3, 5 and 10 days after the end of this procedure. Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared with the value obtained in vehicle-control gastric mucosa at different time intervals after the end of ischemia–reperfusion. Cross indicates a significant change as compared with the value obtained in rats without HCl administration.

Fig. 10. Effect of vehicle, ranitidine (40 mg/kg s.c.) or omeprazole (20 mg/kg s.c.) on the area of gastric erosions and deeper gastric ulcers and accompanying changes in the GBF and plasma IL-1β and gastrin levels caused by the exposure to standard I/R and determined at 3 h or at day 3 after the end of ischemia–reperfusion. Results are means ± S.E.M. of six to eight rats. Asterisk indicates a significant change as compared with the value obtained in intact gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats treated with vehicle and exposed to standard ischemia–reperfusion.

3.4. Effect of vehicle, omeprazole and ranitidine on plasma interleukin-1β and gastrin levels

The results of detection of plasma interleukin-1β and gastrin levels in intact rats and at 3 h and 3 days after the end of ischemia–reperfusion in vehicle-, ranitidine- or omeprazole-treated rats are shown in Fig. 10. In intact animals, the plasma interleukin-1β concentration was neg-

Fig. 11. (A–C) Messenger RNA expression for GAPDH (A), histidine-decarboxylase (B) and interleukin (IL)-1β mRNA (C) in intact gastric mucosa (lane 1) and at 0, 3, 12 and 24 h or 3, 10 and 15 days after the end of ischemia–reperfusion in rats treated with vehicle and exposed to standard period of this ischemia–reperfusion (lanes 2–8). NC is negative control (water). M — size marker DNA; Arrow — expected polymerase chain reaction product (bp).

ligible, but following ischemia–reperfusion, it rose significantly already at 3 h after this procedure. At day 3 after ischemia–reperfusion, the interleukin-1β level remained significantly elevated as compared to that recorded in intact animals. Treatment with ranitidine or omeprazole significantly reduced the plasma interleukin-1β levels as compared to that recorded in vehicle-treated rats. Plasma gastrin level was significantly increased immediately after ischemia–reperfusion, and this increment in plasma gastrin was even further significantly elevated in rats treated with omeprazole or ranitidine both at 3 h or at day 3 upon the termination of ischemia–reperfusion (Fig. 10).

3.5. Expression of histidine-decarboxylase and interleukin-1β mRNA by polymerase chain reaction in gastric mucosa during the recovery from ischemia–reperfusion lesions

Fig. 11A–C shows expression of GAPDH, histidine-decarboxylase and interleukin-1β mRNA in oxyntic mucosa of intact rats treated with vehicle and not exposed to ischemia–reperfusion or those exposed to ischemia–reperfusion and killed immediately (time 0) after ischemia–reperfusion or after various time intervals during mucosal recovery from ischemia–reperfusion lesions at 3, 12 and 24 h and ulcers at days 3, 10 and 15. The expression of GAPDH mRNA was well preserved in the mucosal biopsy samples taken both from control rats treated with vehicle and exposed to ischemia–reperfusion and tested at various time intervals afterwards (Fig. 11A). The histidine-decarboxylase mRNA was detected in vehicle-treated controls as a weak signal, but in rats exposed to ischemia–reperfusion and killed at 0, 3, 12 and 24 h or at day 3, 10 and 15 after the end of ischemia–reperfusion, the histidine-decarboxylase mRNA has been traced with the strongest signal obtained at 3 h after ischemia–reperfusion (Fig. 11B). The interleukin-1β mRNA was detectable in intact gastric mucosa, but its expression markedly increased during the recovery from ischemia–reperfusion damage (Fig. 11C). The ratio of histidine-decarboxylase mRNA over GAPDH mRNA confirmed that the expression of histidine-decarboxylase peaked at 3 h after ischemia–reperfusion and then declined being still significantly stronger at all time periods after ischemia–reperfusion than that recorded in control animals (Fig. 12A). The ratio of interleukin-1β mRNA over GAPDH mRNA showed that this expression was maximal at 3 h then being sustained over 12 and 24 h after ischemia–reperfusion to decline at day 3 through day 15 to reach similar value to that recorded in the intact gastric mucosa (Fig. 12B).

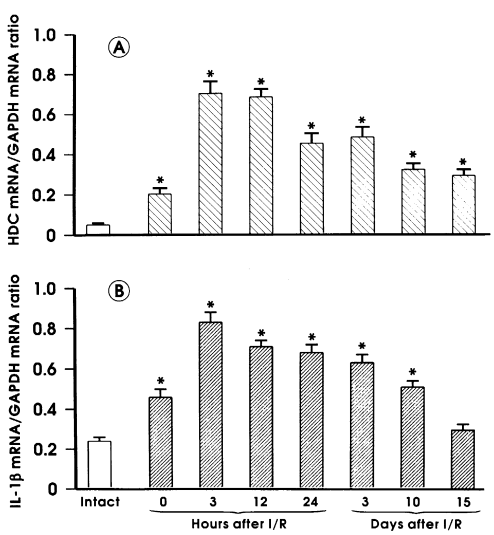

Fig. 12. (A, B) Assessment of mucosal gene expression for histidine-decarboxylase (A) and IL-1β (B) by the intensity of histidine-decarboxylase and IL-1β mRNA/GAPDH mRNA ratio in the gastric mucosa of intact rats and in those exposed to standard I/R that were recovering from ischemia–reperfusion lesions at 0, 3, 12 and 24 h or at 3, 10 and 15 days after this procedure. The asterisk indicates a significant increase above the value obtained in intact gastric mucosa.

4. Discussion

This study confirms other and our previous observations (Wada et al., 1996; Brzozowski et al., 1997, 1999a; Konturek et al., 1997) that exposure of the gastric mucosa to ischemia/reperfusion leads to the formation of acute erosions that progress within 24 h to chronic gastric ulcers. These ulcers healed progressively with time and the healing was completed in vehicle-treated controls within about 10 days after ischemia–reperfusion. The ischemia–reperfusion lesions were accompanied by an immediate decrease in the gastric blood flow, but then gastric blood flow gradually increased to reach within 10 and 15 days after ischemia–reperfusion the value similar to that recorded in vehicle-treated control animals. Mucosal histamine content was suppressed immediately after ischemia–reperfusion and at 3 h after ischemia–reperfusion, but then rose significantly with healing of acute lesions, suggesting that this amine contributes to the mucosal recovery after ischemia–reperfusion. Then, after 24 h, the histamine content stabilized indicating that the healing of chronic ulcers does not involve mucosal histamine production.

Gastric acid secretion in vehicle-treated rats was markedly suppressed after ischemia and following the end of ischemia–reperfusion, but then after 12 h, it started to gradually increase reaching the peak at day 3 after ischemia–reperfusion. Ranitidine and omeprazole, two potent gastric acid inhibitors, given at a dose that caused gastric achlorhydria and raised significantly plasma gastrin levels, greatly attenuated the formation of acute erosions induced by ischemia–reperfusion and prevented their progression into deeper ulcers at each time interval tested after ischemia–reperfusion. Such treatment with potent gastric acid suppressors, caused also marked increase in

the gastric blood flow and attenuated significantly the rise in plasma interleukin-1β levels during mucosal recovery from gastric erosions induced by ischemia–reperfusion. These protective and hyperemic effects of ranitidine or omeprazole were counteracted by the addition of exogenous acid into the stomach. The expression of histidine-decarboxylase and interleukin-1β, that was negligible in the intact gastric mucosa, was strongly upregulated during early phase of mucosal recovery from ischemia–reperfusion erosions and then significantly declined following the progression of acute erosions into deeper gastric ulcers. This study indicates that gastric acid secretion plays an important role in the progression of post ischemia–reperfusion erosions to gastric ulcers and drugs that suppress this secretion such as histamine H_2 receptor antagonists or proton pump inhibitors can be useful in acceleration of healing of these lesions.

In this study, a novel model was applied to induce gastric lesions, namely the ischemia–reperfusion that initially produced acute erosions that progressed into deeper chronic ulcers, which, like naturally occurring ulcers in humans, healed spontaneously within 15 days after ischemia–reperfusion. We confirmed original observation of Wada et al. (1996) that the exposure of the gastric mucosa to ischemia by clamping of the celiac artery followed by reperfusion does produce initially widespread acute gastric erosions that progress after 24 h into deeper chronic gastric lesions. As reported previously and confirmed by histology in this study, the initial acute lesions were superficial bleeding erosions, which then progressed into deeper ulcerated lesions that differed, however, from typical chronic gastric ulcers such as induced by acetic acid because they did not reach muscularis mucosa. These chronic ulcers reaching maximum at day 3 after ischemia–reperfusion, healed progressively with time, and the healing process was completed in vehicle-treated controls within 10–15 days after ischemia–reperfusion. These early lesions that appeared immediately after the end of ischemia–reperfusion, were accompanied by a marked fall in the gastric blood flow with no significant alteration in plasma gastrin and interleukin-1β levels, but at 3 h after ischemia–reperfusion, the significant rise in both plasma gastrin and interleukin-1β concentrations occurred. Following progression of acute gastric lesions into deeper ulcers, the gastric blood flow gradually increased and the elevated plasma interleukin-1β and gastrin levels, declined to reach, at day 15, the values similar to those recorded in intact rats without ischemia–reperfusion.

The hypergastrinemia observed during the early period of recovery from these lesions could be attributed to the remarkable suppression of gastric acidity by the exposure to ischemia–reperfusion as demonstrated in this study. This remains in agreement with the previous observation by Kawai et al. (1994) and Nakamoto et al. (1998) that exposure to ischemia by itself produces almost complete suppression of gastric acid secretion. This gastric inhibition could also be attributed to acid-suppressing factors such as inflammatory cytokines as proposed recently (Prinz et al., 1997). Indeed, the elevation of plasma interleukin-1β concentration, which occurred in our study in rats subjected to ischemia–reperfusion indicates that the release of this proinflammatory and antisecretory cytokine might contribute to the suppression of gastric acid in these animals. Interleukin-1β has been shown to inhibit gastric secretion in vivo and to suppress the histamine synthesis and histamine stimulated gastric secretion in isolated perfused rat stomach (Kondo et al., 1994). An alternative explanation could be that exposure to ischemia–reperfusion triggers the upregulation of cyclooxygenase-2 mRNA with the subsequent release of antisecretory prostaglandins as proposed previously by Kishimoto et al. (1998) and Brzozowski et al. (1999b). Previous studies have established that PG synthesis depends upon the activity of cyclooxygenase, a rate-limiting enzyme in the synthesis of eicosanoids (Eberhart and Dubois, 1995). Cyclooxygenase-1 produces prostaglandins that are physiologically important for homeostatic functions, such as maintenance of the mucosal integrity and mucosal blood flow (Vane and Botting, 1995). In contrast, cyclooxygenase-2 is not constitutively expressed in most of tissues, but is dramatically upregulated during inflammation (Masferrer et al., 1994; Kennedy et al., 1993; Kargman et al., 1996). Our finding that interleukin-1β is overexpressed during the recovery from ischemia–reperfusion lesions is in keeping with recent observation in vitro that cyclooxygenase-2 mRNA may be activated by interleukin-1β and possible other proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) (Ristimaki et al., 1994; Feng et al., 1995).

One objective of the present study was to determine whether histidine-decarboxylase expression and histamine content are altered in response to ischemia–reperfusion. Our study demonstrates for the first time the upregulation of histidine-decarboxylase mRNA during mucosal recovery from ischemia–reperfusion erosions suggesting that vasoactive mediators such endogenous histamine released from enterochromaffin-like (ECL) cells may be involved in spontaneous healing of these lesions. This is supported by the fact that mucosal histamine, which was significantly reduced in an early period after ischemia–reperfusion, showed gradual rise already 12 h after ischemia–reperfusion and then was restored during healing of gastric ulcers. It is of interest that histidine-decarboxylase mRNA peaked at 3 h after ischemia–reperfusion when the mucosal histamine content was still suppressed and this effect coincided with potent suppression of gastric secretion. This indicates that upregulation of histidine-decarboxylase mRNA observed in mucosa injured by ischemia–reperfusion does not lead to enhanced histamine biosynthesis and this might, at least in part, explain the suppression of gastric acid secretion in an early time period after the end of ischemia–reperfusion. This overexpression of the gene for histidine-decarboxylase could be attributed to the

achlorhydria caused by ischemia–reperfusion and/or to the rise in plasma gastrin observed during recovery from ischemia–reperfusion injury. This notion is in keeping with previous observation that histidine-decarboxylase expression in the oxyntic mucosa is augmented by gastrin and suppressed by somatostatin (Ding et al., 1996). In another report (Wu et al., 1990), omeprazole in the dose that effectively inhibited gastric acid secretion in rats, produced the upregulation of gastrin mRNA with the subsequent rise in the plasma gastrin level similar to that observed in our present study. Furthermore, pretreatment with omeprazole and ranitidine, both potent gastric acid inhibitors that induced hypergastrinemia and a notable increase in the microcirculation, markedly accelerated healing of these erosions and actually prevented their progression into deeper ulcerations. This finding remains in agreement with the observation that omeprazole can induce gastric hyperemia while suppressing gastric secretion (Mattsson and Larsson, 1987). Furthermore, the drug-induced achlorhydria that enhanced serum gastrin increments was demonstrated to activate histidine-decarboxylase and ornithine decarboxylase (ODC) in the gastric mucosa (Ding et al., 1996). In fact, gastrin is considered as a trophic hormone for the oxyntic mucosa and was shown to stimulate the growth of ECL cells, which are predominant endocrine cell type in this part of the stomach (Ding et al., 1996). In addition, the continuous infusion of exogenous gastrin that mimics the plasma concentration of this hormone was found to enhance the histidine-decarboxylase activity without significant alteration in ODC activity, a key enzyme in polyamine biosynthesis.

In our study, the histidine-decarboxylase mRNA was detected as a weak signal in the intact gastric mucosa, but showed significant rise immediately after the termination of the ischemia–reperfusion procedure to reach the peak 3 h after ischemia–reperfusion and then remained elevated throughout the periods of the recovery from these lesions suggesting that this upregulation of histidine-decarboxylase mRNA with release of protective histamine, as documented in this study, could contribute to mucosal recovery from these lesions. Previous studies implicated secretagouges such as histamine, derived from histidine-decarboxylase activity, in the prevention of the mucosal injury evoked by necrotizing agents through an enhancement in mucosal generation of prostaglandin E_2 (Kobayashi et al., 1988; Brzozowski et al., 1999a). It is of interest that the expression of interleukin-1β mRNA paralleled with gene expression for histidine-decarboxylase, also appearing almost immediately after ischemia–reperfusion and reaching a maximum of 3 h after ischemia–reperfusion to remain significantly elevated at all time periods, except at 15 day when the interleukin-Iβ expression falls to the level not different from that in the intact mucosa. With the upregulation of interleukin-1β transcript, we observed the elevation of plasma interleukin-1β concentration, especially during the early phase of mucosal recovery from lesions induced by ischemia–reperfusion at the time frame when the overexpression of cyclooxyganse-2 transcript was reported previously (Kishimoto et al., 1998; Brzozowski et al., 1999b). This suggests that endogenous prostaglandin originating from the interleukin-1β activated cyclooxyganse-2 could contribute to the inhibition of gastric acid secretion observed in our study at an early period after ischemia–reperfusion and that these substances could also account for the gradual repair of the mucosa after the damage induced by ischemia–reperfusion.

With the healing of ischemia–reperfusion erosions, the decrease in expression this cytokine was evident indicating that upregulation of interleukin-1β, which may activate cyclooxygenase-2 expression, is an important event taking place during the process of the mucosal repair after mucosal damage induced by ischemia–reperfusion.

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