Mast cells are involved in the gastric hyperemic response to acid back diffusion via release of histamine

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Rydning, Astrid, Oddveig Lyng, Birgitte Lid Adamsen, Sture Falkmer, Arne K. Sandvik, and Jon Erik Grønbech. Mast cells are involved in the gastric hyperemic response to acid back diffusion via release of histamine. Am J Physiol Gastrointest Liver Physiol 280: G1061–G1069, 2001.—Acid back diffusion into the rat stomach mucosa leads to gastric vasodilation. We hypothesized that histamine, if released from the rat mucosa under such conditions, is mast cell derived and involved in the vasodilator response. Gastric blood flow (GBF) and luminal histamine were measured in an ex vivo chamber. Venous histamine was measured from totally isolated stomachs. Mucosal mast cells (MMC), submucosal connective tissue mast cells (CTMC), and chromogranin A-immunoreactive cells (CgA IR) were assessed morphometrically. After mucosal exposure to 1.5 M NaCl, the mucosa was subjected to saline at pH 5.5 (control) or pH 1.0 (H+ back diffusion) for 60 min. H+ back diffusion evoked a marked gastric hyperemia, increase of luminal and venous histamine, and decreased numbers of MMC and CTMC. CgA IR cells were not influenced. Depletion of mast cells with dexamethasone abolished (and stabilization of mast cells with ketotifen attenuated) both hyperemia and histamine release in response to H+ back diffusion. GBF responses to H+ back diffusion were attenuated by H1 receptor blockers and abolished by H2 receptor blockers. Our data conform to the idea that mast cells are involved in the gastric hyperemic response to acid back diffusion via release of histamine.

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It is known that tachykinins, such as substance P, contained in sensory neurons are released on activation of these afferent nerves (27) and that a specific bradykinin antagonist attenuates gastric mucosal vasodilation caused by acid back diffusion (35). These observations are of particular interest because both tachykinins and bradykinin are very active in the release of histamine from mast cells (17, 28, 33, 34).

Release of histamine from the rat and dog stomach mucosa in response to acetic acid or salicylic acid damage was reported more than 30 years ago (23, 24). Exogenously administered histamine is known to have a potent vasodilatory effect on the gastric vasculature (7, 30), an effect that Bruggeman et al. (7) showed to be attenuated by an H1 receptor antagonist.

More recently, Gislason et al. (12) reported that acid back diffusion into the cat stomach mucosa in hyperosmolar-injured stomachs was followed by a marked increase of histamine in portal blood and that gastric mucosal hyperemia under such conditions was partly attenuated by a combination of H1 and H2 receptor blockers (12). However, the cellular source of histamine release was not documented in these studies, and apart from the study by Gislason et al. (12), the possible role of endogenously released histamine from the mucosa in regulation of local blood flow during acid back diffusion after mucosal barrier disruption has not been assessed before.

In the present study, we decided to evaluate the role of mast cells in the stomach in response to acid back diffusion. We tested the hypotheses that 1) release of histamine from the mucosa is due to degranulation of mast cells in the stomach wall and that 2) the proposed histamine release is involved in the local vasodilation seen under such conditions.

MATERIALS AND METHODS

Animal Preparation

Ex vivo chambered experiments. Male Wistar rats (mean weight 286 ± 4 g; Mallegaard Breeding and Research Centre, Skensved, Denmark) were kept on standard laboratory chow.
with 1 ml/min saline (pH 5.5) and gassed with 100% O2 for a
membrane oxygenator. The gastric lumen was perfused
through the aorta with 2 ml/min of a Krebs-Ringer
buffer. The stomachs were trans-
collected.

The mucosa, forming a chamber (32). The chamber was there-
united by addition of 0.5 M HCl; and
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Test Solutions

Three types of chamber solutions were made up in batches
and used throughout the experiments: 1) isotonic saline (154
mM NaCl, pH 5.6); 2) saline that had the pH changed to 1.0
by addition of 0.5 M HCl; and 3) hypertonic NaCl (1.5 M),
prepared with deionized water, at pH ~ 6.4. All solutions
were prewarmed to 37°C before use. Ketotifen, pyrilamine,
and cimetidine were purchased from Sigma Chemical (St.
Louis, MO). Thiopeiramide was purchased from Tocris (Bris-
tol, UK). These drugs were dissolved in Ringer solution,
and the final concentration of these drugs was made consistent
with an intravenous infusion rate of 10 ml·kg⁻¹·h⁻¹. Dexam-
ethasone (Merck, Harlem, The Netherlands) was dissolved in
deionized water to make a solution of 1 mg/ml.

Blood Flow Measurements

Gastric blood flow was measured with the laser-Doppler
velocimetry technique using the Periflux 4001 Master instru-
ment (Perimed, Stockholm, Sweden) as previously described
(14, 15). Briefly, the instrument was operated in the 12-kHz
band mode using the PF 403 probe. This probe was fastened
to a micromanipulator and thereafter lowered until it kept a
distance of 1 mm from the mucosal surface. This arrange-
ment allowed recordings from the same spot on the mucosa
throughout the entire experiment. The recordings were con-
tinuously sampled to a personal computer during the whole
experiment by using a dedicated data acquisition software
(PeriSoft, Perimed), which was used for recording and anal-
ysis. Results were given as mean perfusion units for the
period. Baseline recordings of gastric blood flow were collected
for 12 min after 28 min of stabilization. All later changes in blood flow caused by change of solution in the
chamber or by drugs given intravenously were calculated as
percentage of the baseline recording.

Light Microscopic Technique

At the end of each experiment, the stomach was gently
removed from the chamber and divided into two equal halves
along the greater curvature. One half was mainly used for
assessing mast cells; it was fixed in 2% buffered glutaralde-
hyde and embedded in Epon-Araldite. The other half was
mainly used for assessing neuroendocrine (NE) cells; it was
fixed in 10% buffered formalin and embedded in paraffin.
The sections were made for at least 24 h and 5-μm-thick strips of the fundus were cut with their long axes
parallel to the limiting ridge and 4–5 mm distal to it.

The reason for including the NE cells in the investiga-
tions was that one type of these cells, i.e., the enterochromaffin-
like (ECL) cells, are known to produce histamine (43). In the
rat oxyntic mucosa, the ECL cells constitute 65–75% of the
NE cells (43). They are known to synthesize and store chro-
mogranin A (CgA). Consequently, they are readily visualized
in histological sections by means of their strong immunore-
activity (IR) with antisera raised against CgA (43). Although
CgA is a commonly used NE marker, thus able to visualize at
least some of the other 3–4 kinds of NE cells that occur in the
rat oxyntic mucosa, it is, nevertheless, reasonable to assume
that the majority of the CgA IR cells in the oxyntic mucosa of
the rats in the present investigation consist of ECL cells (43).

The quantitative assessments of the submucosal connective
tissue mast cells (CTMC) were made on ultramicrotome-cut
sections (3 μm thick) conventionally stained with toluidine
blue. They were readily identified by their characteristic size
and structure with their large, distinctly metachromatic,
cytoplasmic granules. The mucosal mast cells (MMC), on the
other hand, which were smaller and equipped with less
distinctly visible cytoplasmic granules, were not so readily
identified. Here it was found that their visualization became
improved by a slight modification of the histological tech-
nique. At deplastification (31), the semithin sections were
submerged for 10–15 min into a solution of saturated NaOH
in absolute ethanol (i.e., sodium ethoxide), diluted 1:1 with
fresh absolute ethanol. After being removed from the Epon
and subsequently rinsed in water and conventionally stained
with toluidine, the sections were mounted by means of a
glycerol water medium. By using this technique, it was found
that the sections were sensitive to long exposures to light.
Consequently, they were all assessed promptly, at the latest
within 24 h.
The mast cell countings were made at high power magnifications (×40 objective) by using an ocular micrometer, consisting of a square lattice with one edge aligned parallel to the muscularis mucosae layer included in the optical field of the section to be examined. The number of mast cells was assessed for the full length of the section. Only cells with a distinctly visible nucleus and a cytoplasm with metachromatic granules were counted. Then the mean for one unit of the square lattice was calculated. The slides were numbered randomly to hide the identity of the experimental groups from the investigator examining the stained sections.

The submucosal CTMC were found to be particularly abundant in the areas situated close to the deeper margin of the muscularis mucosae layer with its blood vessels. The MMC occurred in the superficial layers of the mucosa, particularly close to the lamina propria in the interstitial space between the tops of the glands. Only rarely did they appear close to the bottom of the glands.

The quantitative assessment of the CgA IR cells essentially followed the technique used for the mast cells. They were easily identified as NE cells of both open and closed type. They were located together with the excocrine parenchymal cells of the gland. Most of them occurred as disseminated cells in their deeper parts. Thus their structure and location conformed well to the assumption that most of them actually were ECL cells (5, 43). The CgA IR cells were counted in 20 consecutive squares (only cells with a distinctly visible nucleus were included), and the mean number of them in one square was calculated. Despite the fact that mast cells sometimes can become falsely immunostained by the technique we used (41), it was found that there was actually no risk of misinterpretation as soon as the investigators responsible for the light microscopic examinations (A. Rydning and S. Falkmer) were aware of this source of error.

**Histamine Analysis**

Histamine analysis was performed with a previously evaluated (42) commercial histamine RIA kit (Immunotech, Marseille, France). It is highly specific and has a sensitivity for histamine of 0.5 nM and a coefficient of variation of 6.4% (42).

All samples were boiled for 5 min, and acidic samples were neutralized with 0.1 M NaOH.

**EX VIVO CHAMBERED EXPERIMENTS.** GASTRIC MUCOSAL EXPOSURE TO SALINE AT pH 5.5 OR 1.0. After the stabilization period, the rats were given pH 5.5 saline in the chamber for a baseline period of 12 min. Thereafter, the mucosa was exposed to pH 5.5 or 1.0 saline for 60 min. During the 40 min of stabilization and baseline period, the chamber solution was exchanged twice, and during the subsequent 60-min period the solution was replaced five times to obtain samples for histamine measurements.

**GASTRIC MUCOSAL INJURY AND EXPOSURE TO ACID.** After the baseline period, the mucosa was subjected to injury with 1.5 M NaCl by filling the chamber with this solution for 10 min. After removal of the hypertonic saline, the mucosa was exposed to either pH 5.5 or 1.0 saline, as described above. To assess the effect of mast cell depletion, one group of rats was pretreated with dexamethasone (1 mg/kg ip 48 and 24 h before the experiment) (39) but otherwise treated like rats with postdamage exposure to pH 1.0 saline. In another set of experiments, the effect of pharmacological mast cell stabilization in rats with postdamage exposure to acid was evaluated by treatment with ketotifen (120 μg/min iv) for 10 min starting 5 min before mucosal exposure to hypertonic saline (37). Histamine receptor blockade was evaluated in rats with postdamage exposure to acid by infusion of H1 (pyrilamine, 3 mg/kg), H2 (cimetidine, 4 mg/kg loading, followed by 1.4 mg·kg⁻¹·h⁻¹ for the rest of the experiment), or H3 (thioperamide, 2 mg/kg) receptor antagonists, given intravenously on the same schedule as ketotifen. These doses have been shown to be effective and selective for H1, H2, and H3 receptor-mediated responses in rats (2, 4, 6, 7, 8, 26).

**SHORT-TERM EXPERIMENTS.** Separate experiments were performed to obtain information about early changes in MMC and submucosal CTMC caused by mounting the stomach in the ex vivo chamber and by pretreatment with dexamethasone. Such animals were pretreated identically to the above-mentioned protocol, but the experiments were terminated before mucosal exposure to hypertonic saline or acidic saline for 60 min.

**Totally isolated rat stomachs.** After a stabilization period of 35 min, the mucosa was exposed to pH 1.0 saline. In another set of experiments, the mucosa was, after the stabilization period, exposed to 1.5 M NaCl for 10 min and thereafter to pH 1.0 saline.

**Statistics**

One-way ANOVA was used to test differences in numbers of MMC, CTMC, and CgA IR cells. Two-way ANOVA for repeated measurements was performed for testing changes in MAP, gastric blood flow, and histamine release (SPSS, Chicago, IL). Contrast tests (Neuman-Keuls) were used to calculate probabilities within and between groups when justified by a preceding ANOVA. Values of P < 0.05 were considered significant. Interaction effects were, however, also further explored when P < 0.1. Data are expressed as means ± SE.

**RESULTS**

**Gastric Mucosal Exposure to pH 5.5 or 1.0 Saline Alone**

Baseline MAP in these experiments was 67 ± 2 mmHg. Both groups of rats showed, during the course of the experiment, a similar and gradual increase of MAP to 88 ± 3 mmHg at the end of the experiment (P < 0.001 for both groups vs. baseline values; Fig. 1A). Neither gastric blood flow nor luminal release of histamine from the mucosa appeared to be influenced by acidity of the saline in the ex vivo chamber because no difference between rats exposed to pH 5.5 or 1.0 saline was detected with regard to these variables throughout the experiment (Fig. 1, B and C). Both groups showed a modest fall in luminal histamine release that was evident from time 0 (P < 0.01) and for the remaining part of the experiment (Fig. 1C). Also, the numbers of MMC, CTMC, and CgA IR cells were similar in mucosa exposed to pH 5.5 or 1.0 saline (Table 1).

**Gastric Mucosal Injury and Exposure to Acid**

Rats that were subjected to gastric mucosal damage with 1.5 M NaCl for 10 min showed a gradual increase of MAP during the subsequent 60 min of exposure to both pH 5.5 and 1.0 saline, MAP being 85 ± 3 mmHg at the end of the experiment compared with 75 ± 2 mmHg at baseline (P < 0.001) (Fig. 2A). Damage of the gastric mucosa with 1.5 M NaCl caused an immediate (50 ±
13%) increase in gastric blood flow ($P < 0.001$; Fig. 2B). Postdamage exposure of the mucosa to pH 1.0 saline was associated with a further increase of gastric blood flow for the remainder of the experiment. In contrast, rats with mucosal exposure to pH 5.5 saline showed a gradual fall in gastric blood flow during the last 40 min of the experiments to a level that was significantly lower than in rats with mucosal exposure to pH 1.0 saline ($P < 0.001$ at the end of the experiment).

As shown in Fig. 2C, rats with postdamage mucosal exposure to pH 1.0 saline responded with an immediate and marked increase of luminal histamine, whereas such a response was not observed in rats with postdamage mucosal exposure to pH 5.5 saline. This observation was further confirmed using totally isolated vascularly perfused rat stomachs in which histamine in the venous effluent was markedly increased in response to mucosal exposure to pH 1.0 saline after mucosal disruption with 1.5 M NaCl ($P < 0.001$), whereas such a response was not observed when the mucosa was exposed to pH 1.0 saline alone (Fig. 3).

The light microscopic analysis revealed that the number of submucosal CTMC was reduced to $\sim 30$–$40\%$ in rats with postdamage exposure to pH 1.0 saline compared with corresponding rats exposed to pH 5.5 saline ($P = 0.01$), whereas the reduction of MMC did not completely reach statistical significance ($P = 0.06$) (Fig. 4). The number of CgA IR cells did not show any changes when the data from rats with postdamage mucosal exposure to pH 1.0 saline were compared with those from rats with exposure to pH 5.5 saline.

**Effect of Mast Cell Depletion**

Pretreatment with dexamethasone reduced CTMC to $\sim 39\%$ and MMC to $\sim 23\%$ of the mast cell mass found in corresponding control rats ($P < 0.001$) (Fig. 5). When dexamethasone-pretreated rats were subjected to mucosal damage with 1.5 M NaCl and subsequently exposed to pH 1.0 saline for 60 min, no further reduction, either in CTMC or MMC, was detected (Fig. 5). Baseline MAP in dexamethasone-treated rats (data obtained for only 6 rats due to failure of technical equipment) showed a tendency toward a higher baseline level than corresponding control rats, but they showed a similarly gradual increase of MAP during the course of the experiment, MAP at the end of the experiment being $100 \pm 4$ mmHg compared with $83 \pm 5$ at baseline ($P < 0.001$) (Fig. 6A). As shown in Fig. 6B, the hyperemic response to acid challenge after mucosal barrier disruption was abolished when such rats were compared with corresponding control rats. Also, luminal histamine release in mast cell-depleted rats was not elevated in response to mucosal damage and subsequent acid challenge, histamine release being significantly lower than in rats without dexamethasone pretreatment at 12, 24, and 48 min after mucosal injury ($P = 0.035$) (Fig. 6C).

**Effect of Mast Cell Stabilization**

Intravenous infusion of ketotifen before gastric mucosal exposure to 1.5 M NaCl and the subsequent

<table>
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<th>Saline pH</th>
<th>CTMC</th>
<th>MMC</th>
<th>CgA IR cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>1.28 ± 0.11 (6)</td>
<td>9.1 ± 0.7 (5)</td>
<td>23.6 ± 1.6 (4)</td>
</tr>
<tr>
<td>1.0</td>
<td>1.26 ± 0.11 (6)</td>
<td>11.4 ± 2.2 (3)</td>
<td>21.5 ± 2.8 (3)</td>
</tr>
</tbody>
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Values are means ± SE per square in a counting lattice. Numbers in parentheses indicate number of experiments. CTMC, connective tissue mast cells; MMC, mucosal mast cells; CgA IR cells, chromogranin A-immunoreactive neuroendocrine cells.
exposure to pH 1.0 saline for 60 min preserved both the CTMC and MMC at a level that was not different from rats with mucosal exposure to pH 5.5 saline for 30 min alone (Fig. 5). Infusion of ketotifen caused an immediate increase in MAP of 20 mmHg (P < 0.025). However, the increase in MAP lasted for <30 min, and later in the experiment MAP was not different from that observed in corresponding rats without infusion of ketotifen at any time point (Fig. 6A). Ketotifen-treated rats showed an immediate increase of gastric blood flow in response to mucosal injury with 1.5 M NaCl (P < 0.05). However, during the subsequent mucosal exposure to pH 1.0 saline, gastric blood flow was not significantly elevated when compared with baseline, and at 60 min after mucosal damage blood flow was clearly lower than in corresponding rats without ketotifen treatment (P < 0.009) (Fig. 6B). Also, ketotifen-treated rats responded to acid challenge of the mucosa after mucosal damage with 1.5 M NaCl only by a modest increase of luminal histamine release, the release being elevated above baseline level at 12 and 24 min after mucosal injury (P < 0.05). However, the release was less pronounced than in corresponding rats not treated with ketotifen (P ≤ 0.05 at all time points after mucosal injury except for at 36 min; Fig. 6C).
**Effect of Histamine Receptor Blockade**

Rats subjected to histamine receptor blockade displayed a gradual and similar increase in MAP throughout the experiment, MAP being 84 ± 2 mmHg at the end of the experiment compared with 76 ± 2 mmHg at baseline (P < 0.01) (Fig. 7A). Gastric blood flow in rats with histamine blockade showed an increase in response to mucosal injury with 1.5 M NaCl that was not significantly different from rats without histamine receptor blockade (Fig. 7B). However, during the subsequent time period with mucosal exposure to acid, gastric hyperemia in rats with H1 and H2 receptor blockade was clearly less pronounced than in rats without histamine blockade both at 40 min (P < 0.005 for both groups) and at 60 min (P < 0.005 for both groups). In contrast, rats with H2 receptor blockade displayed a gastric blood flow response to acid challenge after mucosal injury that was indistinguishable from that of corresponding control rats (Fig. 7B).

The light microscopic analysis of histamine receptor blockade-treated rats showed that MMC counts were 6.51 ± 0.89, 7.14 ± 0.55, and 5.37 ± 0.51 with H1, H2, and H3 receptor blockade, respectively. The numbers of CTMC were 0.81 ± 0.04, 0.81 ± 0.08, and 0.84 ± 0.06, respectively. No difference was detected when these mast cell counts were compared with those in corresponding rats without histamine receptor blockade.

**DISCUSSION**

It is reasonable to assume that a numerical decrease of the mast cells in the histological sections can be interpreted as a sign of degranulation of these cells. If so, the observations made in the present study are in good conformity with the hypothesis that mast cells in the stomach wall are essential for the gastric hyperemic response to acid back diffusion after disruption of the mucosal barrier. The evidence supporting this conclusion is that H+ back diffusion by itself caused a numerical decrease (i.e., a degranulation) of both CTMC and MMC. This event was accompanied by a substantial increase of histamine release from the stomach mucosa and, at the same time, a marked hyperemia of the mucosa. Pharmacological stabilization of the mast cells markedly attenuated, and a pharmacological depletion of this cell type abolished, both the gastric hyperemia and the histamine releases in response to acid back diffusion. Furthermore, blockade of the H1 receptor partially attenuated, and a blockade of the H3 receptor abolished, the hyperemic response to acid back diffusion.

We have repeatedly shown that in our experimental model an exposure of the rat mucosa to hyperosmolar NaCl in the concentration range of 1–2 M produces damage that is confined to the surface epithelium and most superficial parts of the mucosa (14, 37). Exposure of such a mucosa to acid leads to sustained H+ back diffusion and mucosal hyperemia (14). In the present study, we have shown that, in this model, acid back diffusion after barrier disruption is associated with an increased release of histamine, whereas this was not observed either in rats with the mucosa exposed to neutral saline after mucosal damage or during exposure to acidic saline without preceding damage by 1.5 M NaCl. Experiments with totally isolated vascularly perfused rat stomachs confirmed these observations because increased histamine release, as measured in the venous effluent, was only detected when the stomach lumen was perfused with acidic saline after mucosal damage with 1.5 M NaCl and the time course of histamine release corresponded closely to the luminal release observed in chambered stomachs.

Rats whose stomach mucosa was exposed to acidic saline after mucosal barrier disruption showed a substantial numerical decrease of both MMC and CTMC. When such rats were pretreated with ketotifen in a dose that effectively prevented mast cell degranulation, they showed significantly lower release of histamine than corresponding controls. A similar observation was made in rats in which the mast cells of the gastric wall were reduced to less than one-third of the normal level by pretreatment with dexamethasone. This latter effect has been shown to be related to phagocytosis of mast cells by macrophages (39). These findings, together with our observation that the CgA IR cells were not influenced by H+ back diffusion, provide strong support for the view that mast cells were the main source of histamine released in response to H+ back diffusion in the present study.

The question then arises as to whether mast cell degranulation and release of histamine could be responsible for the gastric mucosal hyperemia in response to H+ back diffusion as observed in the present
study. Our findings of a similar hyperemic response to topical application of hypertonic saline to the mucosa, irrespective of interventions directed at stabilization of mast cells or histamine receptor blockade, are in agreement with the observations made by Endoh et al. (9). They showed that gastric hyperemia in response to 2 M NaCl was unaffected by ablation of sensory neurons with capsaicin, H₁ receptor blockade, and blockade of nitric oxide but was abolished by pretreatment with indomethacin, suggesting an important role of the prostaglandins. However, in our study, during the period of mucosal exposure to acidic saline after mucosal damage gastric blood flow showed a consistent correlation with both mast cell degranulation and release of histamine from the mucosa. Furthermore, blockade of the H₁ and H₂ receptors significantly attenuated the gastric mucosal hyperemia caused by H⁺ back diffusion.

In agreement with a previous study on feline stomachs (13), H₂ receptor blockade did not appear to influence the gastric hyperemic response to acid back diffusion. Our finding that H₁ receptor blockade with pyrilamine attenuated the hyperemic response to acid...
challenge after mucosal barrier disruption is at variance with the study by Holzer et al. (20), who reported that hyperemia in response to luminal perfusion of the rat stomach with 15% ethanol in 0.15 N HCl was unaffected by pretreatment with pyrilamine. The reason for this discrepancy is uncertain, but in the study referred to, the dose was lower and the time schedule for treatment with pyrilamine was different from the protocol of our study. Furthermore, they reported blood flow measurements in pyrilamine-treated rats at 15–30 min after mucosal barrier disruption, whereas in the present study the attenuated blood flow responses in pyrilamine-treated rats were evident only from 40 to 60 min after mucosal barrier disruption (Fig. 7).

Even superficial damage to the gastric mucosa is probably associated with the release of some mediators, which may have vasoconstrictor properties, for example, leukotrienes and platelet-activating factor (PAF). Such events are likely to occur particularly in the initial phase after mucosal damage. These considerations are relevant because they may provide one explanation for the delay between the peak response of histamine release and the rise in blood flow observed in the present study (Fig. 2). Our findings are also consistent with the study by Feldman et al. (10), who reported a time pattern of luminal histamine release and rise in blood flow in response to acid challenge of the opossum esophageal mucosa, very similar to our study of the rat stomach mucosa.

Although we did not measure histamine release in experiments with histamine receptor blockade, it should be noted that in these experiments the numbers of mast cells were reduced in response to H+ back diffusion to the same level as in control animals. Therefore, we feel confident in concluding that degranulation of mast cells in the stomach wall and subsequent release of histamine could account for the gastric hyperemia that was observed during H+ back diffusion in the present study.

Although the present data suggest that mast cell-derived histamine is involved in the hyperemic response to H+ back diffusion after hyperosmolar injury in the rat stomach mucosa, our study was not designed to assess the precise mechanisms by which histamine exerts its effect. In a series of studies, Holzer et al. (19–21) showed that sensory afferent neurons were instrumental in mediation of gastric mucosal hyperemia caused by acid back diffusion in ethanol-injured stomachs, and these observations were also confirmed by using an experimental model almost identical to that of the present study (14). It is, therefore, pertinent to consider whether histamine released from the mucosa under the present experimental conditions may have interacted with afferent neurons known to be located in close apposition to vessels and mast cells in the stomach (40). Histamine has been shown to stimulate sensory afferent nerve fibers outside the gastrointestinal tract, for example in the rat skin and trachea (22, 29). Moreover, it was recently shown that histamine was able to activate sensory afferent nerves in the cat small intestine (1) and mesenteric afferent nerves of the rat jejunum (26), an effect that in the latter study was abolished by pretreatment with pyrilamine. Our finding that blockade of the H3 receptors resulted in inhibition of the gastric vasodilator response to acid back diffusion may deserve particular attention because, to the best of our knowledge, no data are available on the role of this receptor in the regulation of blood flow in the stomach.

Our data are consistent with the results of a recent study on the vasodilatory mechanism by histamine in the guinea pig ileum (3). In that study, which used the same H1 and H3 receptor blockers as the present one, the authors showed that histamine produced vasodilation of submucosal arterioles by activating H1 receptors through an endothelium-dependent pathway involving nitric oxide, whereas the mechanism behind the vasodilatory effect by activation of H3 receptors located on sympathetic nerve terminals was related to presynaptic inhibition of vasoconstrictor tone.

In summary, the observations made in the present study conform to the idea that mast cell-derived histamine is involved in gastric hyperemia caused by acid challenge after mucosal barrier disruption by hypertonic saline. Since preserved integrity of sensory afferent neurons in the stomach mucosa has been shown to be a prerequisite for this type of gastric hyperemia to occur, it is an attractive hypothesis that histamine may exert at least part of its action via histamine receptors located on this neuron system.

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