Histamine is involved in gastric vasodilation during acid back diffusion via activation of sensory neurons

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Received 18 December 2001; accepted in final form 9 May 2002

Rydning, Astrid, Oddveig Lyng, Sture Falkmer, and Jon Erik Grønbech. Histamine is involved in gastric vasodilation during acid back diffusion via activation of sensory neurons. Am J Physiol Gastrointest Liver Physiol 283: G603–G611, 2002.—Protective vasodilation during acid back diffusion into the rat gastric mucosa depends on activation of sensory neurons and mast cell degranulation with histamine release. We hypothesized that these two mediator systems interact and that histamine partly exerts its effect via sensory nerves. Gastric blood flow (GBF) and luminal histamine were measured in chambered stomachs, and mast cell numbers were assessed by morphometry. Ablation of sensory neurons and depletion of mast cells were produced by pretreatment with capsaicin or dexamethasone, respectively. Mucosal exposure to 1.5 M NaCl and then to pH 1.0 saline in ablated and control rats caused increased luminal histamine release and reduced numbers of mast cells. Enterochromaffin-like cell marker pancreastatin remained unchanged. Only control rats responded with an increase in GBF. Capsaicin stimulation (640 μM) of the undamaged mucosa induced identical increase in GBF and unchanged mast cell mass in normal and dexamethasone-treated rats. Increase in GBF after topical exposure to histamine (30 mM) in rats pretreated with capsaicin or a calcitonin gene-related peptide (CGRP)1 antagonist human CGRP8–37 or exposed to the calcium pore blocker ruthenium red was less than one-half of that in control rats. These data suggest that mast-cell-derived histamine is involved in gastric vasodilatation during acid back diffusion partly via sensory neurons.

CONVINCING EVIDENCE has been provided showing that, in the rat stomach, sensory afferent neurons signal for vasodilation in response to acid back diffusion after disruption of the gastric mucosal barrier (17, 19, 20). This type of vasodilation protects the mucosa against further acid injury (15, 20, 36) and appears to be mediated via release of calcitonin gene-related peptide (CGRP) from sensory afferent nerve fibers, which in turn stimulate the formation of nitrogen monoxide, acting as the final vasodilator messenger (28, 29, 42). Mechanisms by which H+ back diffusion conveys signals to the sensory neurons are less clear, although some evidence suggests that the nerves may respond directly to increased concentrations of protons (7, 11). We (38) recently showed that histamine released from mast cells is involved in the vasodilator response to acid back diffusion in the chambered stomach of the rat. The reason for this contention was that rats with either pharmacologically depleted or stabilized mast cell mass showed almost abolished release of histamine and hyperemia in response to acid back diffusion into the mucosa. Furthermore, blockade of histamine receptors with H1 and H3 receptor antagonists was followed by a substantial attenuation of gastric hyperemia caused by acid back diffusion.

Against this background, it appears that both preserved integrity of sensory neurons and release of histamine from the stomach mucosa are necessary for acid back diffusion-induced hyperemia to occur (17, 19, 20). Because it is also known that histamine in other parts of the gastrointestinal tract is able to activate sensory neurons (1, 24) and to influence vasoconstrictor tone via nerves (4), we tested the hypothesis in the present study that mast cell-derived histamine released from the stomach mucosa during acid back diffusion may exert part of its ability to induce mucosal hyperemia via sensory neurons.

MATERIALS AND METHODS

Animal Preparation

Male Wistar and Sprague-Dawley rats (260–320 g; from Møllegaard Breeding and Research Centre, Skensved, Denmark) were kept on standard laboratory chow and a 12:12-h light-dark cycle. The Norwegian State Commission for Animal Experimentation approved the protocol. The rats were deprived of food but not water for 16–20 h before surgical manipulation. Anesthesia was given by subcutaneous administration of 0.25 ml/100 g body wt of a combination of (in mg/ml): 1.25 midazolam (Dormicum, Hoffman La Roche, Basel, Switzerland), 2.5 fluanisone, and 0.05 fentanyl (Hypnorm, Janssen Cilag, Buckinghamshire, UK). To perform ex vivo chamber (32) experiments, the animals were prepared as previously described (14, 37). After laparotomy, the esophagus was exposed and divided between ligatures, and the pylorus was also ligated. Care was taken to avoid damage to the vagus nerve trunks and blood vessels.

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The forestomach and the anterior two-thirds of the glandular stomach were opened and pulled up through an oval hole in an overlying Plexiglas plate unfolded with the mucosal side up. A 25-mm-diameter cylinder was then clamped onto the mucosa forming a chamber (32). The chamber was filled with 10 ml of isotonic saline, and the animals were allowed to stabilize for 28 min before baseline recordings were collected.

8-Methyl-N-vanillyl-nonanamide (capsaicin; Sigma, St. Louis, MO) was used to produce functional ablation of capsaicin-sensitive sensory neurons (31, 45). Capsaicin stock solution was prepared to 40 mM by dissolving it in a vehicle consisting of 10% ethanol (100%), 10% Tween 80, and 80% saline (vol/vol/vol). The rats were treated with increasing doses of capsaicin (20, 30, and 50 mg/kg body wt sc) on three consecutive days, in a regimen shown to deplete neuropeptides in primary afferent neurons in adult rats (31, 45). All capsaicin injections were made under anesthesia with 0.2 ml of a combination of midazolam, fluanisone, and fentanyl, and, to counteract any respiratory impairment associated with the administration of capsaicin, the rats were pretreated with terbutaline (0.1 mg/kg im) and aminophylline (10 mg/kg im) before the capsaicin injection (45). These animals were used 2 wk after capsaicin treatment. In control studies, animals received the same schedule of treatment and vehicle for capsaicin. In the initial phase of the study, we planned to use Wistar rats for all the experiments in this study. However, because of ~80% mortality associated with the regimen of capsaicin treatment outlined above, we had to change to Sprague-Dawley rats. This strain of rats appeared to tolerate capsaicin well and showed no mortality from the treatment. We were not able to identify any particular reason for this discrepancy. Sprague-Dawley rats were chosen for further experiments that involved systemic treatment with capsaicin, and corresponding control experiments were repeated using the same strain of rats (see below). On the day of the experiment, effectiveness of sensory nerve ablation was assessed by topical instillation of one drop of capsaicin (0.1 mM) onto the eye of the rat. Vehicle-treated rats reacted by wiping the eye with the paw, whereas capsaicin-treated rats did not. The eye was immediately rinsed with water.

In some experiments rats were treated with dexamethasone (1 mg/kg ip) 48 and 24 h before the experiment. This regimen has been shown to cause depletion of mast cells within the gastrointestinal tract in rats (35, 39, 44) due to phagocytosis by macrophages (39).

Test Solutions

Five chamber solutions were made up in batches and used throughout the experiments: 1) isotonic saline (154 mM NaCl), pH 5.5; 2) saline with the pH changed to 1.0 by adding 0.5 M HCl; 3) hypertonic NaCl of 1.5 M, prepared using deionized water, with pH of ~6.4; 4) capsaicin, diluted from stock solution to 640 µM in saline (14); and 5) histamine (Sigma, St. Louis, MO) dissolved in saline to a concentration of 30 mM. Preliminary experiments indicated that histamine (30 mM) could produce a consistent hyperemic response in the gastric mucosa as measured with the laser Doppler velocimetry technique. All solutions were prewarmed to 37°C before use. Dexamethasone (Merck, Sharp & Dohme, Harlem, Netherlands) was dissolved in deionized water to make a solution of 1 mg/ml. Terbutaline (AstraZeneca, Oslo, Norway), aminophylline, papaverine (Nycomed Pharma, Oslo, Norway), and ruthenium red (Sigma) were dissolved in saline. Human (h) CGRP8–37 (Tocris Cookson, Avonmouth, UK) was dissolved in 0.1% BSA containing 0.9% saline.

Blood Flow Measurements

Gastric blood flow (GBF) was measured with laser Doppler velocimetry technique using the Periflux 4001 Master instrument (Perimed, Stockholm, Sweden) as previously described (14, 37). 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 arrangement allowed recordings from the same spot of the mucosa throughout the entire experiment. Recordings were continuously sampled to a personal computer during the whole experiment by using a dedicated data software program (PeriSoft; Perimed, Stockholm, Sweden), for recording and analysis. Results were given as mean perfusion units for the period. Baseline recordings of GBF were collected for 12 min, after 28 min of stabilization. All subsequent 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 halves. One-half was used for assessing mast cells; it was fixed in Carnoy’s fixative for 2 h (3) before transfer to 80% ethanol (3, 41). The other one-half was fixed in 4% neutral formaldehyde. All specimens were embedded in paraffin. The gross examination of the fixed specimens, with the trimming of them, was made in a standardized way. Strips of the fundus (2–3 mm thick) were cut with their long axes parallel to the limiting ridge. The quantitative assessments of the submucosal connective tissue mast cells (CTMC) and mucosal mast cells (MMC) were made on 3-µm-thick sections stained with Astra blue/safranin (41). The CTMC were readily identified due to their characteristic size and structure with their large, distinctly metachromatic cytoplasmic granules. The MMC were also readily identified; they were smaller than the CTMC and equipped with less distinctly visible cytoplasmic granules.

Mast cell counting was made at high-power magnification (>40 objective), using an ocular micrometer, consisting of a square lattice with one edge aligned parallel to the mucosal muscular layer (for assessing CTMC) or to the mucosal surface (for assessing MMC), 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. Slides were numbered randomly to hide the identity of the experimental groups for the investigator examining the stained sections.

Morphometrical analyses of the surface epithelium were made on paraffin-embedded slides using a modification of the Lacy and Ito (26) classification. The ocular micrometer was aligned parallel to and just above the mucosal surface at the length of the slide, and the superficial mucosal epithelium was divided into four categories. Category 1 consisted of normal epithelium. Category 2 was in situ changed epithelium in which the cells remained adherent to the basal lamina. Cells in this category were found to show extensive vacuolization and could comprise cells covering the basal lamina displaying a low cuboidal or even a squamous structure. Thereby, the cells of this category fulfilled the criteria of restitution. Category 3 comprised epithelium with superficial lesions, embracing also necrotic damage or tissue loss in the superficial third part of the mucosa. Category 4 consisted of

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epithelium with erosive mucosal lesions with hemorrhages penetrating deeper than just the superficial third of the mucosa.

**Histamine and Pancreastatin Analysis**

Histamine analysis was performed with a previously evaluated commercial histamine radioimmunoassay kit (Immunotech, Marseilles, France) (43). It is specifically high and has a sensitivity for histamine of 0.5 nM and a coefficient of variation of 6.4% (43). All samples were boiled for 5 min, and acidic samples were neutralized with 0.1 M NaOH. Pancreastatin analysis was performed with a commercial pancreastatin radioimmunoassay kit (Bachem/Peninsula Laboratories, San Carlos, CA).

**Experimental Protocol**

As mentioned above, in all experiments involving pretreatment with capsaicin and corresponding control experiments, Sprague-Dawley rats were used.

**Gastric mucosal injury and exposure to acid.** To evaluate the effect of acid back diffusion on mast cell density and release of histamine from the gastric mucosa in rats with ablated sensory neurons, chambered stomachs of rats pretreated with capsaicin were exposed first to saline with pH 5.5 for a baseline period of 12 min, next to hyperosmolar mucosal injury with 1.5 M NaCl for 10 min, and finally to saline with pH 1.0. The chamber solution was exchanged every 12 min for 60 min. Such rats were compared with control rats, which had been pretreated with vehicle for capsaicin. To assess whether increased luminal release of histamine in response to acid back diffusion in the present experiments was related to activated mast cells or enterochromaffin-like (ECL) cells, luminal release of pancreastatin was also measured in some experiments. Pancreastatin, a peptide fragment of chromogranine A, is stored in ECL cells in the rat stomach (22). It is reported to be coreleased with histamine in response to acid back diffusion in the present study to separate the effect of acid back diffusion on mast cell density after baseline exposure to saline.

**Histamine mucosal exposure to histamine.** To evaluate a potential effect of histamine on GBF mediated by sensory neurons, chambered stomachs were exposed to pH 5.5 saline for 40 min and thereafter, to saline with histamine (30 mM, final concentration) for 60 min. Such rats were compared with 1) rats pretreated with capsaicin to ablate sensory neurons; 2) rats treated with the CGRP receptor antagonist hCGRP8-37 (15 µg/kg bolus iv) 10 min before mucosal exposure to histamine and thereafter, to 3 µg·kg⁻¹·h⁻¹ iv for the remaining part of the experiment (9, 23); or 3) rats treated with ruthenium red added to the chamber solution to a final concentration of 0.1%, starting 10 min before mucosal exposure to histamine. Ruthenium red is a calcium pore blocker reported to antagonize activity of sensory neurons (2, 44). To reduce the potential formation of an unstirred layer of mucus in close vicinity to the mucosa and thereby possible interference with penetration of histamine into the mucosa, the solution in the chamber was stirred by means of a mechanical paddle, mounted on a firm stand and rotating at 20 rpm during the last 5 min of pH 5.5 saline in the chamber and for the rest of the experiment. Stirring did not influence resting blood flow (flow after stirring was 6.0 ± 2.1% above baseline; P = 0.18 for 28 observations). The chamber solution was gently withdrawn and replaced with a fresh one every 12 min.

To ensure that pretreatment of rats with capsaicin did not alter the ability of the gastric microcirculation to dilate in response to vasodilators in general, chambered stomachs of such rats and corresponding controls were exposed to saline with papaverine (1 mg/ml, final concentration) (25). The experiments were conducted identically to the above-mentioned ones except that histamine was replaced with papaverine.

**Statistics**

One-way ANOVA or two-tailed Student’s t-test was used to test differences in numbers of MMC and CTMC and in degree of morphometrically structural damage. Two-way ANOVA for repeated measurements was performed for testing changes in mitogen-activated protein (MAP), GBF, and histamine release (SPSS, Chicago, IL). Contrast tests (Newman-Keul) were used to calculate probabilities within and between groups when justified by preceding ANOVA. Values of P < 0.05 were considered significant. Interaction effects were, however, also further explored when P was <0.1. Data are expressed as means ± SE.

**RESULTS**

**Effect of Acid Back Diffusion**

When the rats, both those with and without ablation of sensory neurons, were subjected to gastric mucosal damage by 1.5 M NaCl for 10 min, they showed a gradual increase of MAP during the subsequent 60 min of exposure of pH 1.0 saline. MAP was 83 ± 3 mmHg at the end of the experiment and 71 ± 3 mmHg at baseline (P < 0.05) (Fig. 1A).

Damage of the gastric mucosa with 1.5 M NaCl caused an immediate (~50%) increase in GBF, both in control rats and in rats with destroyed (ablated) sensory neurons after capsaicin pretreatment (P < 0.005 treated with dexamethasone or its vehicle were performed but terminated before mucosal exposure to capsaicin or its vehicle to obtain information about mast cell density after baseline exposure to saline.
for both groups). Postdamage exposure of the mucosa to pH 1.0 saline was associated with a sustained increase of GBF for the remainder of the experiment in control rats ($P < 0.001$ for all time points), whereas rats with ablation of sensory neurons showed an immediate fall in GBF to baseline level (Fig. 1B).

Both control rats and rats with ablated sensory neurons responded to postdamage exposure to pH 1.0 saline with an immediate and marked luminal release of histamine. It was sustained for 48 min of the experiment and was even higher in ablated rats at 24 min of acid back diffusion (Fig. 1C).

At baseline, luminal release of pancreastatin (ECL cell marker) was $10.9 \pm 0.4$ pg·ml$^{-1}$·min$^{-1}$ and $9.5 \pm 1.5$ pg·ml$^{-1}$·min$^{-1}$ in ablated ($n = 3$) and control rats ($n = 5$), respectively. No change from baseline level was noted during mucosal exposure to saline at pH 1.0; the release at 24 min after hypertonic injury was $10.8 \pm 0.8$ pg·ml$^{-1}$·min$^{-1}$ in ablated rats and $14.0 \pm 5.4$ pg·ml$^{-1}$·min$^{-1}$ in control rats.

At the microscopic examination, pretreatment of rats with capsaicin was found not to have any influence on the numerical density of either CTMC or MMC in the stomach wall (Fig. 2). The submucosal CTMC were found to be particularly abundant in the areas situated close to the deeper margin of the mucosal muscular 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 exceptionally did they appear close to the bottom of the glands. Hypertonic injury of the gastric mucosa with 1.5 M NaCl, followed by exposure to saline at pH 1.0 for 60 min, was associated with a reduction of both CTMC and MMC to $40\%$ of pre-damage level, both in control rats and ablated ones ($P < 0.001$ for both groups) (Fig. 2).

As mentioned above, some initial experiments were performed on Wistar rats to examine whether mast cell degranulation was caused by acid back diffusion or hypertonic injury. Exposure of chambered rat stomachs to pH 5.5 saline for 60 min after hypertonic injury did not reveal any difference in the numerical density.
of either MMC or CTMC when compared with exposure to pH 5.5 saline alone (Table 1). In contrast, exposure of stomach mucosa to pH 1.0 saline after hypertonic injury caused a reduction in the numerical density of CTMC to 35% and MMC to 60% of the mast cells found in rats exposed either to pH 5.5 saline alone or to pH 5.5 saline after hypertonic injury (P < 0.006 for MMC and P < 0.02 for CTMC compared with both pH 5.5 saline alone and hypertonic injury and, thereafter, pH 5.5 saline).

Figure 3 summarizes the histopathological changes of the gastric mucosa in addition to those of the mast cell numbers. Control rats with hypertonic injury and subsequent challenge with pH 1.0 saline for 60 min showed only a negligible (2 ± 2%) percentage of erosions, and most (46 ± 6%) of the surface epithelium was normal. In contrast, the gastric mucosa in rats with ablated sensory neurons showed many more erosions (31 ± 4%; P = 0.001), and lower incidences of normal surface epithelium (12 ± 3%; P = 0.003) than those observed in control rats.

Effect of Activation of Sensory Neurons with Capsaicin

As expected, pretreatment with dexamethasone reduced the numerical density of CTMC to 43% and that of MMC to 13% of the mast cell density in the gastric mucosa of the control rats (P = 0.001) (Fig. 4). Exposure of chambered stomachs to 640 μM capsaicin for 60 min did not have any influence on the numerical density of CTMC or MMC in control rats or in the dexamethasone-pretreated rats.

MAP in dexamethasone-pretreated rats was higher than in control rats, the difference being statistically significant at baseline and during the first 20 min of mucosal exposure to capsaicin but not at the end of the experiment (Fig. 5A). Within-group analysis (ANOVA) did not reveal any change in MAP during the course of the experiment in dexamethasone-treated rats or control rats.

As shown in Fig. 5B, gastric mucosal exposure to capsaicin caused an immediate increase in GBF, sustained throughout the experiment, both in dexamethasone-pretreated rats and in control rats (P < 0.001 for all time points). Dexamethasone-pretreated rats and control rats had a similar release of histamine at baseline (Fig. 5C). Both groups showed a small drop (P < 0.025) to almost identical levels of histamine release during 48 min of mucosal exposure to capsaicin.

### Table 1. Numerical density of metachromatically identified mast cells in chambered rat stomachs

<table>
<thead>
<tr>
<th></th>
<th>pH 5.5 Saline, 40 min</th>
<th>1.5 M NaCl + pH 5.5 Saline</th>
<th>pH 5.5 Saline No. 1.5 M NaCl</th>
<th>1.5 M NaCl + pH 1.0 Saline</th>
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<tr>
<td></td>
<td>No. 1.5 M NaCl</td>
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<tr>
<td>MMC</td>
<td>21.2 ± 3.6</td>
<td>17.4 ± 3.2</td>
<td>6.6 ± 0.6*</td>
<td>6.6 ± 0.6*</td>
</tr>
<tr>
<td>CTMC</td>
<td>1.39 ± 0.16</td>
<td>1.25 ± 0.10</td>
<td>0.86 ± 0.07*</td>
<td>0.86 ± 0.07*</td>
</tr>
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</table>

Values are means ± SE per square in a counting lattice. No. = no. of rats. Mucosal mast cell (MMC) and connective tissue mast cell (CTMC) counts of chambered stomachs exposed to pH 5.5 saline alone for 40 min, and 1.5 M NaCl for 10 min, and subsequently, to pH 5.5 or pH 1.0 saline for 60 min. *Significant difference from pH 5.5 saline for 40 min and 1.5 M NaCl for 10 min and subsequently to pH 5.5 saline for 60 min.
Histopathologically, the surface epithelium of the gastric mucosa did not reveal any significant lesions. Thus, 95% of the surface epithelium was normal both in control and in dexamethasone-pretreated rats (data not shown).

Effect of Histamine

Exposure of chambered stomachs of rats with ablated sensory neurons and those of control rats to 30 mM histamine caused a fall in MAP of ~10 mmHg at 20 min, but MAP was restored to baseline level 40 min after exposure to histamine (Fig. 6A).

GBF increased markedly (>100%) in response to mucosal exposure to histamine in control rats (P < 0.005) (Fig. 6B). Also, rats with ablated sensory neurons treated with the CGRP_1 receptor antagonist hCGRP_8–37 or exposed to the calcium pore blocker ruthenium red responded to gastric mucosal exposure to histamine with increased GBF (P < 0.05 for all time points), but the hyperemia was less than one-half that of control rats, similar to the three groups, and the difference between these groups and control rats was significant for the time points at 40 and 60 min of exposure to histamine (P < 0.05). Mast cell number density in the stomach wall and integrity of the surface epithelium did not appear to be influenced by gastric mucosal exposure to histamine, either in control rats or in rats pretreated with capsaicin (data not shown).

Exposure of the chambered gastric mucosa to papaverine (25) in rats with intact sensory neurons (control) and in rats with ablated neurons after pretreatment with capsaicin resulted in a similar increase in GBF in both groups (P < 0.01 for all time points; Table 2).

DISCUSSION

The main finding of the present study is that the vascular responsiveness to histamine to a significant degree depends on preserved integrity of sensory afferent neurons in the stomach wall. Evidence supporting...
this conclusion is apparent from our experiments, showing a hyperemic response to topical exposure of the stomach mucosa to histamine in rats 1) with ablated sensory neurons, 2) treated with a CGRP1 receptor blocker, or 3) exposed to ruthenium red, being less than one-half of that observed in control rats.

We have recently shown (38) that acid back diffusion into the rat stomach mucosa caused by exposure of the mucosa to hypertonic saline and subsequently to acidic saline is a strong stimulus for both degranulation of mast cells and release of histamine from the stomach mucosa. Experiments directed at stabilization of mast cells or depletion of this cell type abolished both the gastric hyperemia and histamine release in response to acid back diffusion. Blockade of H1 or H3 receptors, either attenuated or abolished, respectively, the hyperemic response to acid back diffusion. These findings led to the conclusion that mast cells are involved in the gastric hyperemic response to acid back diffusion via release of histamine (38). However, the question still remains whether the mast cells are directly influenced by back-diffusing protons or whether they are degranulated by, for example, activated sensory neurons. The reason is that it has been shown that stimulation of sensory neurons alone may activate mast cells (3, 44) by release of neuropeptides like substance P (31), which are known to be highly active in releasing histamine from mast cells (33, 34).

In the present study, we used our experimental model consisting of exposure of the gastric mucosa to 1.5 M NaCl, which produces damage confined to the surface epithelium and most superficial parts of the mucosa (13, 37). Exposure of such a mucosa to acid leads to sustained H+ back diffusion and mucosal hyperemia (13). Pretreatment of rats with capsaicin to ablate sensory neurons led to almost abolished hyperemic response to acid back diffusion in this model and to much more damage of the stomach mucosa than in control animals. These observations confirm previous studies in various species on the importance of the gastric hyperemic response as a protective factor against acid injury, and attest to the instrumental role of sensory neurons as a mediator system for hyperemia in response to acid back diffusion (12, 13, 16, 20).

As in our recent study (38), exposure of the gastric mucosa to saline pH 1.0 after hypertonic injury led to a substantial reduction of stainable mast cells, an effect that could be attributed to acid back diffusion, because it was not evident when the mucosa was exposed to neutral saline after hypertonic injury (Table 1). It might be pertinent to consider whether or not the decrease of stainable mast cells could be a result of damage of the mucosa and thereby lysis of mast cells. However, rats with ablated neurons, which had much more damage of the mucosa after hypertonic injury and exposure to acid than that of control rats, had a pre-damage level of mast cells and showed a reduction of both CTMC and MMC identical to that of control rats (Fig. 2). Furthermore, in two recent studies (37, 38) using the same experimental model as in the present investigations, we showed that mast cell-stabilizing ketotifen and sodium cromoglycate, which, to our knowledge, cannot protect against lysis, were able to completely protect against reduction of the mast cell mass associated with acid back diffusion. Therefore, it is reasonable to suggest that the reduction of stainable mast cells could be attributed to degranulation, although we acknowledge the possibility that lysis of mast cells could be part of it. It is also reasonable to assume that the increased luminal release of histamine in response to acid back diffusion in the present study at least mainly comes from mast cells because: 1) a detailed morphometric analysis of ECL cells (evaluated as chromogranin-A immunoreactive cells) in our recent study (38) using the same experimental model did not reveal any changes whatsoever during acid back diffusion; 2) luminal release of histamine associated with acid back diffusion was closely correlated with preservation of stainable mast cells by a mast cell-stabilizing agent (38); and 3) in the present study no increase of the ECL cell marker pancreastatin was detected during acid back diffusion.

In the present investigation, release of histamine from the stomach mucosa in response to acid back diffusion was similar or even more pronounced in rats pretreated with capsaicin than in controls. These observations strongly suggest that in our experiments degranulation of mast cells, and thereby release of histamine, was not mediated by activated sensory neurons. In addition, the findings may lend support to the concept that intact sensory neurons are necessary for histamine to fully exert its action on the vessels in the stomach wall.

Another set of experiments in the present study was designed to assess whether direct stimulation of sensory neurons of the gastric mucosa with capsaicin in our experimental model could lead to degranulation of mast cells and increased luminal release of histamine. Because there is some evidence to suggest that gastric hyperemia in response to topical exposure of the mucosa to capsaicin partly could be explained by release of histamine from degranulated mast cells by either a direct effect of capsaicin on mast cells or indirectly via activation of sensory neurons and thereby release of neuropeptides like substance P (44), such rats were compared with rats pretreated with dexamethasone to deplete the mast cell mass. As shown in Fig. 4, topical exposure of the mucosa to capsaicin did not appear to

| Table 2. Changes in gastric blood flow in chambered rat stomachs exposed to papaverine for 60 min |
|---------------------------------|--------|--------|--------|--------|
|                                | Saline | 20 min | 40 min | 60 min |
| Control                        | 4.2±5.0| 21.1±10.3*| 29.6±11.8*| 47.7±13.4*|
| Ablated                        | 7.3±6.4| 34.7±11.6*| 35.5±8.4*| 37.1±9.2*|

Values are means ± SE of %change from baseline; n = 6 rats in each group. Chambered stomachs of control rats and rats with destroyed sensory neurons after pretreatment with capsaicin (100 mg/kg) 2 wk before the experiment (Ablated) were exposed to saline for 40 min and then to papaverine (1 mg/ml). *Significant difference from exposure to saline.
cause degranulation of submucosal CTMC or MMC. Luminal release of histamine from the mucosa in these experiments was similar in normal rats and in rats with depleted mast cell mass and was, if anything, even diminished in response to exposure to capsaicin. Furthermore, the hyperemic response to acute topical treatment of the stomach mucosa with capsaicin was identical in normal rats and rats with depleted mast cell mass. A reasonable interpretation of these data is that it is unlikely that release of histamine plays a significant role in mucosal hyperemia caused by activation of sensory neurons in the undamaged gastric wall in our model. Therefore, it is also unlikely that direct activation of sensory neurons by back-diffusing protons, if this mechanism exists, could account for the substantial degranulation of mast cells and release of histamine in response to acid back diffusion, shown to occur in the present experiments, and shown to be essential for mucosal hyperemia under such conditions in our recent study (17).

The next question that arises is whether histamine is able to stimulate sensory neurons. Outside the gastrointestinal tract, it is known that histamine is able to stimulate sensory afferent nerve fibers to release neuropeptides like CGRP, for example in the rat skin and the trachea (21, 24). Studies addressing this particular issue in the gastrointestinal tract are more sparse and their results are to some extent conflicting. Atwood et al. (3), using an in vitro model, reported that mast cell and capsaicin-sensitive nerve-evoked vasodilation mechanisms in the guinea pig ileum appeared to act independently. On the other hand, 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 (6, 24), an effect that in the latter study was abolished by pretreatment with pyrilamine and in the former reduced by pyrilamine and thioperamide.

Exposure of the normal gastric mucosa to histamine caused, in the present study, a substantial hyperemia evident even during the transient drop of blood pressure in the first part of the exposure. This is in agreement with the results of several previous studies (5, 18, 30) suggesting that this effect was partly mediated via H$_1$ receptors located on the vascular endothelium in the gastric wall. When such rats were pretreated with capsaicin to ablate sensory neurons, they responded to topical exposure of the stomach mucosa to histamine with a hyperemia less than one-half of that in control rats. That the general ability of the gastric vasculature to dilate in response to other vasodilators is essentially unchanged after pretreatment with capsaicin was supported by our experiments showing that gastric hyperemia was similar after topical exposure to papaverine in control and ablated rats. Furthermore, the hyperemic response of the stomach mucosa to hypertonic saline alone was not influenced by pretreatment with capsaicin, either in the present study or in two previous studies (10, 13). These findings strongly suggest that similar to sensory nerves of the small intestine, this population of nerves in the stomach wall also appears to be sensitive to histamine. Further evidence in support of this contention was obtained by our experiments, showing that treatment of rats with a CGRP$_1$ receptor antagonist and exposure of the gastric mucosa to ruthenium red, together with histamine, produced a reduction of gastric hyperemia, which was almost identical to that in ablated rats. It should be noted, however, that after all types of interference with sensory neurons in the present study, there still remained a significant hyperemia in response to gastric mucosal exposure to histamine. It is likely that this effect, to a large part, is mediated via histamine receptors located on the endothelium in the gastric wall.

Findings of our recent study (38), suggesting an essential role of mast cell degranulation for gastric hyperemia in response to acid back diffusion, and the findings of previous investigations (14, 19, 20), confirmed and extended in the present study on a similar role of sensory neurons in the stomach, are consistent with an interaction between these mediator systems. This hypothesis is also attractive, considering the close anatomical relationship between peptideric nerves, mast cells, and vessels, particularly in the submucosa (14, 40), because this layer of the stomach wall has been shown to be an important site for regulation of gastric mucosal blood flow (18).

In our experimental model, it seems unlikely that both a degranulation of mast cells and a release of histamine caused by acid back diffusion were a result of activation of sensory neurons. This fact and the observation that sensory nerves appear to be activated by histamine are consistent with the hypothesis that mast cells may participate as monitoring units for acid back diffusion and, by release of histamine, take part in activation of sensory neurons to induce vasodilation in the stomach wall.

This study was supported, in part, by grants from the Unimed Research Fund, the Cancer Research Foundation at St. Olavs University Hospital, and the Faculty of Medicine at the Norwegian University of Science and Technology.

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