Substance P may attenuate gastric hyperemia by a mast cell-dependent mechanism in the damaged gastric mucosa

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Rydning, Astrid, Oddveig Lyng, Steinar Aase, and Jon Erik Grønbech. Substance P may attenuate gastric hyperemia by a mast cell-dependent mechanism in the damaged gastric mucosa. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G1064–G1073, 1999.—Calcitonin gene-related peptide (CGRP) released from sensory neurons, which are closely apposed to mast cells and blood vessels, mediates gastric hyperemia in response to acid challenge of the damaged mucosa. Substance P (SP) is coreleased with CGRP from sensory neurons, but the role of this peptide in gastric blood flow regulation is largely unknown. Chambered rat stomachs were exposed to 1.5 M NaCl and acidic saline after treatment with SP, aprotinin (serine protease inhibitor), and the mast cell stabilizers ketotifen and sodium cromoglycate (SCG). Gastric hyperemia (measured with a laser Doppler flow velocimeter) after hypertonic injury and acid challenge was nearly abolished by SP. Aprotinin infused together with SP and pretreatment with ketotifen and SCG before SP restored the gastric hyperemia. Ketotifen and SCG inhibited mast cell degranulation in SP-treated rats. Preservation of gastric hyperemia was correlated with improved mucosal repair. These data suggest that impaired hyperemia by SP during acid challenge of the gastric mucosa may be mediated by a mast cell-dependent mechanism involving the release of proteases from mast cells.

GASTRIC MUCOSAL HYPEREMIA caused by increased H+ back-diffusion after superficial mucosal injury and acid challenge is a first-line mechanism for protection and repair of the damaged mucosa (15, 31). Activation of sensory afferent neurons, located around blood vessels in the submucosa of the gastric wall, appears to play a major role as a signal system for vasoconstriction when the mucosa is challenged by acid (21, 22). Calcitonin gene-related peptide (CGRP), localized in sensory neurons of the gastric mucosa (10), appears to be an important mediator for the hyperemic response to acid back-diffusion because it is a potent vasodilator in the gastric mucosa (20) and has been shown to be an important factor in response to both selective stimulation of sensory neurons by capsaicin and acid back-diffusion (26, 27). The role of the tachykinins, substance P (SP) and neurokinin A, which are colocalized with CGRP in sensory neurons and released together with CGRP (10, 24), is less well characterized. Neurokinin A was recently shown to inhibit and a NK2 receptor antagonist to enhance the gastric hyperemic response to acid back-diffusion, whereas no effect to gastric hyperemia was observed in response to stimulation of sensory neurons by capsaicin (18, 19, 36). SP has been implicated in the aggravation of ethanol-induced damage of the gastric mucosa by stimulation of mast cells (23), which are in intimate contact with peptidergic nerves in the gastrointestinal tract (34, 35). In the skin, SP was shown to modulate CGRP-induced vasodilation (2), and, in the stomach mucosa, SP nearly abolished gastric mucosal hyperemia caused by stimulation of sensory neurons with capsaicin (12).

In the present study, we used a previously well-characterized model for evaluation of superficial gastric mucosal damage and repair (13, 14). We explored the possible influence of SP on gastric mucosal damage by 1.5 M NaCl and the ensuing repair during mucosal challenge by acid. We also studied whether SP had any influence on gastric hyperemia induced by superficial mucosal damage alone and on gastric hyperemia caused by H+ back-diffusion. These findings were correlated with changes in gastric submucosal mast cell counts caused by hypertonic injury and SP treatment. Finally, we tested the hypothesis that inhibition of proteases, which are known to be released on activation of mast cells by SP (2, 3, 7), and treatment with mast cell stabilizers could modulate both gastric blood flow responses and the mucosal repair in this model.

MATERIALS AND METHODS

Animal Preparation

Male Wistar rats (mean weight 310 ± 4 g, Møllegaard Breeding and Research Centre, Skensved, Denmark) were kept on standard laboratory chow and 12:12-h light-dark cycle. The protocol was approved by the Norwegian State Commission for Animal Experimentation. The rats were deprived of food but not water for 16–20 h before surgical manipulation. Anesthesia with 0.3 ml/100 g body wt of a combination of (per ml) 1.25 mg midazolam (Dormicum, F. Hoffmann La Roche, Basel, Switzerland), 2.5 mg fluanisone, and 0.05 mg fentanyl (Hypnorm, Janssen Cilag, Buckinghamshire, UK) given subcutaneously was chosen because in a previous study this anesthesia was shown to be suitable for long-term experiments with rats (33). It was also shown that this type of anesthesia yields tissue perfusion similar to pentobarbital anesthesia in most organs, among them the gastric mucosa, even if the mean aortic blood pressure (MAP) is lowered as a consequence of α-receptor blockade by fluanisone (33). The animals underwent tracheotomy; a catheter (PE-50) was introduced into the carotid artery and connected to a Marquette Tram Rac 4 transducer allowing continuous measurements of MAP, which was displayed on a Marquette Tramscpe 12C (Marquette Electronics, Milwaukee, WI). Another catheter (PE-50) was placed in the right femoral vein and, by a constant rate pump (model 351, Orion syringe...
pump, Sage Instruments, Orion Research, Boston, MA), was used for continuous infusion of Ringer solution (Kabi Pharmacia, Stockholm, Sweden) at 10 ml·kg\(^{-1}·h^{-1}\) to maintain stable hydration status and infusion of SP, aprotinin, ketotifen, and sodium cromoglicate (SCG) (Sigma Chemical, St. Louis, MO) as described later. The animals were placed on a heating pad. After laparotomy, the esophagus was exposed and divided between ligatures, and the pylorus was also ligated. In both instances, care was taken to avoid damage to the vagus nerve trunks and blood vessels. 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 mucosal side up, and pinned along the edges. A 25-mm-diameter cylinder was then clamped onto the mucosa forming a chamber (29). The chamber was then filled with 10 ml of isotonic saline, and the animals were allowed to stabilize for 30 min before baseline recordings were collected.

Test Solutions

Three types of chamber solutions were made up in batches and used throughout the experiments: 1) isotonic saline (154 mM NaCl, pH of ~5, 2) saline that had the pH changed to 1.0 by addition of 0.5 M HCl, and 3) hypertonic NaCl of 1.5 M, prepared using deionized water, with pH of ~6.4. All solutions were prewarmed to 37°C before use.

Stock solution of SP (0.1 mM) was prepared by dissolving it in 0.1 M acetic acid. Aprotinin and ketotifen were dissolved in Ringer solution to make stock solutions of 100 tryptase inhibitory units (TIU)/ml and 16 mg/ml, respectively. SCG was dissolved in deionized water to make a stock solution of 30 mg/ml.

Shortly before use, drugs infused intravenously were diluted with Ringer solution, and acidity of the final solution or the vehicle was adjusted to pH 5.5. The final concentration of the drugs was made consistent with an intravenous infusion rate of 10 ml·kg\(^{-1}·h^{-1}\).

SP, aprotinin, ketotifen, and SCG were purchased from Sigma Chemical.

Blood Flow Measurements

Gastric blood flow was measured with the laser Doppler velocimetry technique using the Periflux PF-2B instrument (Perimed, Stockholm, Sweden) as previously described (12, 16, 32). Briefly, the instrument was operated in the 12-kHz band mode using the PF-308 standard probe. This probe was fastened to a micromanipulator and thereafter lowered until it lightly touched the mucosal surface. This arrangement allowed recordings from the same spot of the mucosa throughout the entire experiment. The recordings were displayed on a servograph. Baseline recordings of gastric blood flow were collected for 15 min by calculating the mean blood flow for this period. All later changes in blood flow caused by change of solutions in the chamber or by drugs given intravenously were calculated as percent of the baseline recording.

Microscopy

At the end of each experiment, the stomach was gently removed from the chamber and divided into two halves along the greater curvature. One-half was immersed in fixative consisting of 2% glutaraldehyde made up in 0.1 M Sørensen sodium phosphate buffer. The specimens remained in fixative for at least 24 h and were kept refrigerated. The tissue was dehydrated in ethanol and embedded in Epon-Araldite (Ladd Research Industries, Burlington, VT). Three-micrometer-thick sections were mounted on glass slides, stained with alkalized toluidine blue, and studied with a Zeiss Axioskop light microscope (Carl Zeiss). The microscopic registrations were carried out with ocular magnification of ×10 and an objective of ×40/0.65. Slides were randomly numbered to hide the identity of the experimental groups from the investigator reading the slides. An ocular micrometer consisting of a square lattice was aligned with one edge parallel to and just including the muscularis mucosa in the light microscope. The number of mast cells in the submucosa was counted for the full length of the slide, and the mean number in one unit of the square lattice was calculated.

The other half of each specimen was immersed in 4% formaldehyde buffered in sodium dihydrogen phosphate (0.45%) and disodium phosphate (0.83%) and then embedded in paraffin. Three-micrometer-thick slides were cut and stained by hematoxylin-erythrosin-safranin. Trimming of both halves was performed in a standardized way; strips of fundus (2–3 mm wide) were cut with the long axes parallel to and 4–5 mm distal to the limiting ridge.

Morphometry was accomplished on the paraffin-embedded slides using a modification of the classification by Lacy and La"ito (25). 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 as follows. Category 1 included normal epithelium. Category 2 included in situ-changed epithelium in which the cells remained adherent to the basal lamina. Cells in this category may show extensive vacuolization or may include cells covering the basal lamina that show a low cuboidal or a squamous morphology, thereby fulfilling the criteria of restitution. This category also included necrotic surface epithelium, but the cells still remained adherent to the basal lamina. Category 3 included epithelium with superficial lesions, which included necrotic damage or tissue loss in the superficial third part of the mucosa. Category 4 included epithelium with erosions, that is, mucosal lesions with hemorrhage, going deeper than the superficial third of the mucosa.

Experimental Protocol

Gastric mucosal injury and exposure to acid alone. After a stabilization period, one group of rats had neutral saline in the chamber for a baseline period of 30 min. The solution was then changed to acidic saline. The chamber was gently emptied, and saline at pH 1.0 replaced the neutral solution for the remaining 60 min of the experiment, except for replacement with fresh acidic solution during the middle of this period. To assess the effect of SP and aprotinin (a serine protease inhibitor), two groups of rats were also given SP (200 pmol/min iv) or aprotinin (1,466 TIU/min iv), respectively, starting 5 min before and ending 5 min after the chamber solution was changed from neutral to acidic saline.

Gastric mucosal injury and exposure to acid. After the baseline period, one group of rats had their mucosa exposed to 1.5 NaCl by filling the chamber with this solution for 10 min. After removal of the hypertonic saline, the mucosa were exposed to acidic saline at pH 1.0 for 60 min as described above. In another set of experiments, SP (200 pmol/min iv) was given for 10 min starting 5 min before and terminating 5 min after exposure of the gastric mucosa to 1.5 M NaCl. To assess the effect of a protease inhibitor, aprotinin (1,466 TIU/min iv) was given either together with SP or alone but with the same time schedule as SP.

To evaluate the effect of pharmacological mast cell stabilization, groups of rats were pretreated with either ketotifen or SCG. Ketotifen (40 μg/min iv) was given for 10 min immediately before infusion of SP. Because this dose did not appear to preserve submucosal mast cells under the present experi-
mental conditions, the dose of ketotifen was increased threefold (120 µg/min) in another set of experiments and was given to rats both with and without SP treatment. SCG was given intravenously (total dose of 80 µg/kg) to corresponding groups of rats with the same time schedule as ketotifen-treated rats.

Short-term experiments. Separate experiments were performed to obtain information about early changes in submucosal mast cell counts and morphology of the mucosal surface caused by the hypertonic injury alone and concomitant treatment with the drugs under study. Such animals were prepared identically to the above-mentioned protocol, but the experiments were terminated before mucosal exposure to acidic saline for 60 min.

Statistics
Student's t-test (two-tailed) for unpaired data was used to test differences in mast cell counts and morphology. Two-way ANOVA for repeated measurements was performed for testing changes in MAP, gastric blood flow, and gastric vascular resistance. Significant interaction effects were further explored according to the method described by Winer (40). When justified by preceding ANOVA, contrast tests (Newman-Keuls) were used to calculate probabilities within and between groups. 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
Hemodynamics
Gastric mucosal exposure to acid alone. The basal MAP in rats before intravenous treatment with vehicle, SP, or aprotinin was 80 ± 2 mmHg. Neither SP nor aprotinin appeared to influence MAP because no differences in MAP between these groups were noted throughout the experiments (Fig. 1A). However, all groups showed, during the course of the experiment, a gradual increase of MAP to 90 ± 2 mmHg at the end of the experiment (P < 0.001 for all groups, compared with baseline values).

Exposure of the gastric mucosa to acidic saline was associated with a modest and similar increase in gastric blood flow in rats treated with vehicle, SP, and aprotinin (Fig. 1B). The increase in blood flow was significantly evident at 30 min after mucosal exposure to acidic saline and for the remaining part of the experiment (P at least <0.05 for all time points).

Because there was a significant elevation of MAP toward the end of the experiments and in most animals also a transient effect on MAP after infusion of SP, we also calculated gastric vascular resistance to assess the influence of MAP on local gastric blood flow. Vascular resistance in this study is defined as MAP divided by relative changes in gastric blood flow at corresponding time points. Rats with mucosal exposure to acid alone did not show significant changes of vascular resistance in vehicle-, SP-, or aprotinin-treated animals throughout the experiment, which may indicate that the increased gastric blood flow was merely a result of increased MAP rather than a true response to acid (Fig. 1C).

Gastric mucosal injury and exposure to acid. Rats that were subjected to gastric mucosal damage with 1.5 M NaCl showed a gradual increase of MAP during the subsequent 60 min of mucosal exposure to acidic saline, with MAP being 90 ± 4 mmHg at the end of the experiment compared with 74 ± 3 mmHg at baseline (P < 0.001) (Fig. 2A).

Damage of the gastric mucosa with 1.5 M NaCl for 10 min caused an immediate and marked (85 ± 16%) increase in gastric blood flow (P < 0.01) (Fig. 2B). Postdamage exposure of the mucosa to acidic saline was associated with a sustained and even more pro-
nounced elevation of gastric blood flow for the remaining part of the experiment (P < 0.001 for all time points).

Calculation of vascular resistance showed that the increased gastric blood flow in response to mucosal damage and acid challenge was reflected in a corresponding and highly significant fall in vascular resistance (P < 0.001 for all time points) (Fig. 2C).

Effect of SP. Infusion of SP (200 pmol/min) caused an immediate fall in MAP of ~7 mmHg (P < 0.001, compared with baseline values). However, the drop of MAP lasted for <10 min, and, later in the experiment, MAP was not different from that observed in rats receiving infusion of vehicle at any time point (Fig. 2A). SP-treated rats showed an apparent increase in gastric blood flow in response to mucosal exposure to 1.5 M NaCl (Fig. 2B). However, ANOVA showed that this increase was not significantly different from baseline values, and gastric blood flow in SP-treated rats was, during the subsequent mucosal exposure to acidic saline, much lower than that for vehicle-treated rats (P < 0.001 for all time points). Similar observations were made with regard to vascular resistance, which did not show significant changes throughout the experiment (Fig. 2C).

Effect of aprotinin. Rats that received infusion of aprotinin together with SP showed a short-lived drop in MAP in response to SP infusion similar to that observed in corresponding rats treated with SP alone (Fig. 2A). However, aprotinin per se did not appear to influence MAP because MAP in these rats showed a pattern very similar to that observed in corresponding rats, which were treated with either vehicle or SP alone.

Coadministration of aprotinin with SP caused restoration of the hyperemic response to both mucosal damage by 1.5 M NaCl and the subsequent challenge by acid (Fig. 2B). Gastric blood flow in these rats was similar to that for rats treated with vehicle and rats treated with aprotinin alone before mucosal damage but highly different from rats pretreated with SP alone before they had mucosal damage by 1.5 M NaCl and subsequent acid challenge (P < 0.001 for all time points). Analysis of vascular resistance confirmed these observations (Fig. 2C).

Effect of mast cell stabilizers. Pretreatment with the mast cell stabilizer ketotifen or SCG before infusion of SP or vehicle did not produce significant changes in MAP in response to these drugs alone (Fig. 3A). However, as observed in the preceding experimental groups, infusion of SP was followed by a transient and short fall in MAP, although this fall did not reach statistical significance (ANOVA). Also, all of these experimental groups showed a similar and significant increase of MAP toward the end of the experiment (P at least <0.005) from the 36- to 60-min time point for all groups compared with baseline values.

Neither ketotifen nor SCG appeared to influence basal gastric blood flow (Fig. 3B). Pretreatment with ketotifen at a dose of 40 µg/min (n = 7) for 10 min before SP infusion was followed by an apparent but not significant increased blood flow in response to mucosal damage with 1.5 M NaCl and subsequent acid challenge of the mucosa, flow being 151 ± 15% and 134 ± 13% of basal values during the first and second 30-min period of mucosal acid challenge, respectively (data not
shown in Fig. 3). However, pretreatment with ketotifen at a dose of 120 µg/min as well as SCG (8 mg·kg⁻¹·min⁻¹) for 10 min before SP or vehicle infusion resulted in blood flow levels from 160 to 220% of basal values during the 60-min period of acid challenge after damage of the mucosa (Fig. 3B). The increased blood flow in this time period was evident compared with the baseline level (P at least <0.005 for all time points) for both the ketotifen and SCG groups and compared with corresponding rats treated with SP alone (P at least <0.05 for the 24- to 60-min time points for both the ketotifen and SCG groups), except for rats pretreated with SCG alone in which the difference compared with rats treated with SP alone did not reach statistical significance at 60 min.

Vascular resistance showed a drop during mucosal acid challenge in all groups of rats pretreated with either ketotifen (120 µg/min) or SCG (8 mg·kg⁻¹·min⁻¹) for 10 min before SP or vehicle infusion compared with baseline (P, 0.001 for all groups) and compared with rats treated with SP alone (P at least <0.05 for the 24- to 60-min time points) for all groups (Fig. 3C).

**Microscopy**

Mast cells. In the present study, we chose to count connective tissue mast cells (submucosal mast cells) only because they are located close to the arterioles that penetrate the muscular mucosa. These vessels are known to be instrumental in regulation of gastric mucosal blood flow (16).

Connective tissue mast cells in the gastric submucosa were identified by their strongly metachromatic toluidine blue staining and round cytoplasmic granules. They are particularly abundant adjacent to the deeper margin of the muscularis mucosae and major vessels that penetrate this boundary (Fig. 4). These cells were counted on coded slides, and the results are summarized in Fig. 5.

**SHORT-TERM EXPERIMENTS.** Intravenous treatment with SP did not produce significantly reduced mast cell numbers, as observed immediately after this treatment, either in rats with undamaged mucosa or in rats with damaged mucosa (Fig. 5, A and B). The gastric mucosa of rats exposed to 1.5 M NaCl for 10 min immediately showed ~35% fewer submucosal mast cells than corresponding control rats with undamaged mucosa (P < 0.05).

**LONG-TERM EXPERIMENTS.** Exposure of the gastric mucosa to acidic saline alone for 60 min did not produce reduction of visible mast cells. However, treatment of such rats with SP was associated with reduced submucosal mast cell numbers of ~35% (P < 0.001) (Fig. 5C).

In addition, rats that were subjected to hypertonic mucosal damage and subsequent acid challenge for 60 min showed reduced mast cell numbers compared with corresponding rats without hypertonic damage. Furthermore, treatment of such rats with SP resulted in a further reduction of the mast cell numbers beyond that caused by hypertonic damage alone (P < 0.025) (Fig. 5D). Rats that were treated with aprotinin in addition to SP showed a similar reduction of mast cells,
although the difference did not reach statistical significance compared with corresponding rats without SP plus aprotinin treatment \( (P = 0.09) \). Treatment with ketotifen before SP infusion in rats with mucosal damage and subsequent acid challenge did not preserve the mast cells when the lower dose of ketotifen \( (40 \mu\text{g/min}) \) was used \( (0.75 \pm 0.07 \text{ mast cells/square in the counting lattice; data not shown in Fig. 5D}) \). However, the higher dose of ketotifen \( (120 \mu\text{g/min}) \) preserved the mast cells in both rats subjected to hypertonic injury alone and rats that were in addition treated with SP. At this higher dose, the mast cell counts were similar to those for rats with undamaged mucosa or rats with mucosal exposure to acid alone \( (\text{Fig. 5, A, C, and D}) \). Also, treatment with SCG before SP infusion in rats with mucosal damage preserved the mast cells at a significantly higher level than corresponding rats treated with SP alone \( (P < 0.01) \). Infusion of SCG before hypertonic injury alone produced mast cell numbers that were similar to ketotifen-treated rats.

Mucosal damage. Figure 6 summarizes the morphological changes apart from the mast cell counts.

ACID ALONE. Rats that had the mucosa exposed to acidic saline for 60 min without preceding hypertonic damage showed mostly normal superficial and deep structure of the mucosa, and no difference was observed between rats with or without SP treatment \( (\text{Fig. 6A}) \).

HYPTERTONIC INJURY. Mucosal exposure to 1.5 M NaCl for 10 min caused mucosal damage comprising more than 90% of the surface, which was observed immediately after this treatment \( (\text{Fig. 6B}) \). The main part of the damage consisted of “in situ damage,” whereas about one-third consisted of “superficial lesions” \( (\text{see MATERIALS AND METHODS}) \). No difference was found in
extent or depth of the damage when comparing rats with or without SP treatment.

**Hypertonic Injury and Acid Challenge.** In stomachs in which the mucosa had been subjected to hypertonic injury and subsequent exposure to acidic saline for 60 min, ~35% of the surface epithelium appeared normal (Fig. 6C). This finding indicates that a considerable restitution had taken place because corresponding rats killed immediately after hypertonic injury showed only ~5% normal surface epithelium (Fig. 6B). In contrast, SP-treated rats showed much less normal surface epithelium than corresponding rats with hypertonic injury and postdamage acid challenge alone. Adding aprotinin to the SP infusion reversed this situation because the rats showed significantly more normal epithelium, more in situ damage, and less severe damage (superficial lesions) compared with rats treated with SP alone (Fig. 6C). A similar observation was made in rats pretreated with ketotifen and SCG before SP infusion because rats treated with both the low and high dose of ketotifen and SCG showed more normal surface epithelium than rats with SP treatment alone; the morphological pattern in these rats was indistinguishable from corresponding rats without SP treatment. Data for ketotifen (40 µg/min) is not shown in Fig. 6.

**Discussion**

Consistent with a recent study that used an experimental model identical to that used in this study, we found that exposure of the gastric mucosa to 1.5 M NaCl for 10 min caused a uniform superficial damage comprising nearly 100% of the surface area (14). Treatment with SP did not appear to influence the degree of damage caused by 1.5 M NaCl either in depth or extent of the mucosal surface, as observed immediately after removal of the hypertonic saline. When the mucosa of rats damaged by 1.5 M NaCl was further exposed to acidic saline for 60 min, our microscopic evaluation at that time revealed that ~35% of the surface area showed normal epithelium. This finding indicates that a substantial restitution had taken place even in a relatively acidic environment. This observation is in agreement with several previous studies using different species and experimental models (5, 11, 14, 25, 37).

In contrast, SP-treated rats showed much less normal surface epithelium when subjected to acid challenge for 60 min after hypertonic damage, indicating a substantial retardation of the repair process by this neuropeptide. A likely explanation for the retarded repair process in SP-treated rats is the much less pronounced vasodilation in the stomach mucosa both in response to hypertonic damage alone and in response to subsequent acid challenge and thereby acid back-diffusion into the mucosa. This view is supported by studies showing similar increases of damage when constriction of the celiac artery (15, 17) or chemical ablation of sensory neurons (14, 21) was used to hamper the hyperemic response to acid back-diffusion after disruption of the stomach mucosal barrier.

The local concentration of SP close to the mast cells and vessels that may be achieved when sensory neurons of the stomach mucosa are stimulated by acid back-diffusion is not known. Given this shortcoming, we considered it important not to exceed an exogenous dose of SP that may have significant or prolonged...
effects on systemic blood pressure and blood flow in the normal stomach mucosa. The dose of SP used in the present study is similar to or lower than that previously reported to be without effect on these parameters (12, 20). This finding was confirmed in the current study because only a modest effect on the blood pressure that lasted for \(<10\) min was observed in response to SP treatment in some of the experimental groups. No significant effect on gastric blood flow was observed in response to SP treatment in animals subjected to mucosal exposure to acidic saline alone (Fig. 1) or during baseline conditions before exposure to 1.5 M NaCl (Fig. 2). It should also be noted that exposure of the gastric mucosa to acidic saline with pH 1.0 alone and coadministration of SP in such experiments produced similar and only negligible morphological changes of the gastric mucosa.

Exposure of the gastric mucosa to 1.5 M NaCl caused by itself degranulation of submucosal mast cells, as shown by our microscopic evaluation both immediately after mucosal damage and after 60 min with further acid challenge. This observation is consistent with another study showing that topical administration of 40% ethanol also caused degranulation of mast cells in the rat stomach (1). Although treatment with SP alone did not produce a detectable reduction of submucosal mast cells as observed immediately after termination of this treatment, rats that were killed 60 min after receiving SP showed \(<35\)% reduction of visible mast cells. This finding was evident when comparing these rats with corresponding rats that had the mucosa exposed to acidic saline for 60 min and rats from short-term experiments without mucosal damage with hypertonic saline. Furthermore, rats that were treated with SP and thereafter subjected to mucosal hypertonic damage and acid challenge for 60 min showed a further reduction of mast cells beyond that caused by hypertonic damage alone.

The question then arises as to whether the degranulation of mast cells by SP could also be responsible for the impaired hyperemic response to increased H+ back-diffusion after disruption of the mucosal barrier seen in our study. This hypothesis is supported by one report showing that intradermal coadministration of SP with CGRP abolished the local hyperemic response in the skin normally evoked by administration of CGRP alone (2). In another report, infusion of SP abolished vasodilatation in the gastric mucosa in response to selective stimulation of sensory neurons with capsaicin (12). In both of these studies, the effect could be explained by SP-induced release of proteases that again cleave and inactivate CGRP. In the present study, which was designed to test this hypothesis in a model of gastric mucosal damage and repair, we used a pharmacological approach that was very similar to that previous study (12). Aprotinin, a serine protease inhibitor that has been shown to be particularly effective in inhibition of cleavage of CGRP by mast cell lysates and purified mast cell tryptase (38), was infused together with SP. With this treatment, the hyperemic response to mucosal barrier disruption and acid challenge was restored in rats subjected to infusion of SP.

In our further attempt to elucidate the mechanisms by which SP retarded the repair process after gastric mucosal damage, we used ketotifen and SCG, which are mast cell stabilizers (1, 4, 9, 30). Pretreatment with both types of mast cell stabilizers before infusion of SP was associated with a restoration of the hyperemic response after mucosal injury and acid challenge to a level that was not significantly different from corresponding rats not treated with SP. Both SCG and ketotifen appeared to protect against the further degranulation of mast cells caused by SP in rats subjected to mucosal injury. Ketotifen even protected against the combined degranulation effect of hypertonic injury and SP. In initial experiments, we used a dose of ketotifen that was only one-third of that used later. This dose of ketotifen failed to protect against degranulation of mast cells caused by hypertonic injury and SP, and the hyperemic response to acid challenge of the mucosa was not significantly different from the response shown in rats treated with SP alone. However, even this dose of ketotifen appeared to improve repair of the surface epithelium when these rats were compared with those treated with SP alone. This observation may be related to the fact that ketotifen is not entirely specific for mast cells but may also inhibit the release of potentially harmful mediators such as oxidants, platelet-activating factor, and leukotrienes from eosinophils and neutrophils. (9). A tendency toward less-efficient restoration of the hyperemic response by mast cell stabilizers compared with aprotinin was noted in SP-treated rats that had mucosal injury. This observation may not be surprising. Although ketotifen and SCG provided stabilization of mast cells as judged morphologically, it is still possible and even likely that SP could cause some activation and even partial degranulation of mast cells that are undetectable by histological means. If release of proteases from mast cells is a key factor for the loss of hyperemic response in SP-treated rats, then pharmacological inhibition of such proteases would be expected to be more efficient than mast cell stabilization.

Restoration of the gastric hyperemia in response to mucosal barrier disruption and subsequent acid challenge by aprotinin, SCG, and ketotifen was accompanied by significantly more normal surface epithelium and less damage of the mucosa than observed in rats treated with SP alone. Together, these data suggest that the impaired repair of the mucosa brought about by SP in the present study was mediated via a mast cell-dependent mechanism that most likely involved the release of proteases that again hamper the hyperemic response to acid back-diffusion.

Apart from releasing mast cell-derived proteases (3, 7), SP is known to cause the immediate release of a variety mediators from the mast cells (6, 8). Some of these mediators may have distinct, although often opposite, effects on the vessels in the stomach wall. Histamine is known to cause vasodilation (28), whereas other mediators such as leukotrienes are mainly vasoconstrictors (39). It is therefore pertinent to consider...
whether the release of vasoconstrictor types of mast cell-derived mediators could be responsible for the attenuated hyperemic response to acid challenge caused by SP in our study. However, our data from experiments in which the stomachs were undamaged may argue against this view because in these experiments no effect on blood flow in response to infusion of SP was observed either when the mucosa was exposed to neutral saline or to acidic saline alone.

In summary, the present study shows, in accordance with the study by Holzer and Guth (20), that intravenous infusion of a small dose of SP, although with only a negligible influence on systemic blood pressure and gastric blood flow in the normal gastric mucosa, produced impaired repair after superficial damage and acid challenge of the rat stomach mucosa. This observation was accompanied by an almost complete attenuation of the vasodilatation in the mucosa normally seen without SP treatment under such experimental conditions. Our study provides evidence suggesting that the impaired hyperemia by SP during acid challenge of the mucosa may be mediated by a mast cell-dependent mechanism involving the release of proteases. This finding is of particular interest because in sensory neurons of the stomach wall SP is localized together with CGRP, another neuropeptide that again has been shown to be instrumental for protective vasodilation with SP during acid challenge of the rat stomach. This observation is of particular interest because in sensory mechanism involving the release of proteases. This mucosa may be mediated by a mast cell-dependent impaired hyperemia by SP during acid challenge of the rat stomach mucosa. This observation was accompanied by an almost complete attenuation of the vasodilatation in the mucosa normally seen without SP treatment under such experimental conditions. Our study provides evidence suggesting that the impaired hyperemia by SP during acid challenge of the mucosa may be mediated by a mast cell-dependent mechanism involving the release of proteases. This finding is of particular interest because in sensory neurons of the stomach wall SP is localized together with CGRP, another neuropeptide that again has been shown to be instrumental for protective vasodilation during acid challenge of the stomach mucosa.

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