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Role of capsaicin-sensitive nerves and histamine H_1, H_2, and H_3 receptors in the gastroprotective effect of histamine against stress ulcers in rats

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Abstract

It is assumed that an overproduction of gastric acid is the most important factor in the development of peptic ulcer. However, it has been also demonstrated that gastric defense mechanisms, which prevent mucosal injury, are enhanced by the same factors that increase acid secretion. The aim of this study was to examine the role of capsaicin-sensitive sensory nerves and histamine H_1, H_2, and H_3 receptors in histamine-induced gastroprotection against stress ulcers. Studies were performed on rats with intact or ablated sensory nerves. Ablation of sensory nerves was induced by neurotoxic doses of capsaicin. Gastric ulcers were induced by water immersion and restrain stress. Before exposure to stress, rats were pretreated with saline (control), histamine (10 μmol/kg), histamine H_1 receptor antagonist pyrilamine (100 μmol/kg), histamine H_2 receptor antagonist ranitidine (100 μmol/kg), histamine H_3 receptor antagonist thioperamide (100 μmol/kg), or a combination of histamine with these histamine receptor antagonists.

Results: Histamine alone reduced ulcer area evoked by stress and this effect was accompanied by an increase in gastric mucosal blood flow and mucosal DNA synthesis, as well as a decrease in serum pro-inflammatory interleukin-1β concentration. Treatment with combination of pyrilamine plus histamine caused an increase in gastric ulcer area and serum interleukin-1β above the value observed in animals treated with saline, and this effect was accompanied by a decrease in gastric mucosal DNA synthesis. Ranitidine, in combination with histamine, reduced the ulcer area and serum interleukin-1β to a minimal value, whereas gastric mucosal blood flow and DNA synthesis reached a maximal value. Pretreatment with thioperamide before histamine administration abolished the histamine-evoked reduction in gastric ulcer area. Ablation of sensory nerves increased the ulcer area in animals treated with saline or histamine, or histamine in combination with pyrilamine or ranitidine. In animals with sensory nerves ablation combined with administration of thioperamide plus histamine, the ulcer area was similar to that in saline-treated animals with intact sensory nerves. We conclude that: (1) histamine exhibits protective effect against stress-induced gastric ulcer and that this gastroprotection is related to stimulation of histamine H_1 and H_3 receptors; (2) blockade of histamine H_2 receptors exhibited beneficial effect on gastric mucosa against stress-induced gastric ulcers; and (3) ablation of sensory nerves aggravates stress-induced gastric ulcer and reduces histamine-evoked gastroprotection related to stimulation of histamine H_3 receptors.

Keywords: Histamine; Gastric ulcer; Histamine receptor antagonist; Capsaicin; Gastric mucosal blood flow; Interleukin-1β

1. Introduction

Peptic ulcer has been attributed to an imbalance between ulcer-promoting factors, such as excessive secretion of acid and pepsin, reduction in mucosal blood flow, and mucosal protective factors, such as mucus, alkaline, and prostaglandin production, or rapid mucosal cell turnover (Konturek, 1985). Since the dictum of Schwartz (1910)—"no acid–no ulcer"—overproduction of gastric acid was considered to be the most important factor in the pathogenesis of peptic ulcer and treatment of this disease was based mainly on the inhibition of gastric acid secretion (Dammann et al., 1985; Hüttemann, 1986).

On the other hand, physiological gastric secretagogues, such as gastrin (Isobe et al., 1988; Kobayashi et al., 1988;

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Konturek et al., 1995; Nishizaki et al., 1994; Tanaka et al., 1998), cholecystokinin (Konturek et al., 1995; West et al., 2003), or acetylcholine (Ichikawa et al., 1998; Kobayashi et al., 1988), have been reported to enhance the gastric defense mechanisms against injury caused by various noxious agents. The role of histamine in ulcerogenesis is unclear. Histamine given alone (Hase et al., 2003) or in combination with nonsteroidal anti-inflammatory drugs (Takeuchi et al., 1986) may induce gastric or duodenal lesions. On the other hand, administration of histamine or its analogs has shown gastroprotective effect against ulcers evoked by HCl (Isobe et al., 1988, Kobayashi et al., 1988), ethanol (Palitzsch et al., 1995), or ammonia (Warzecha et al., 2000), whereas administration of H_2 receptor antagonists to block the gastric acid secretion aggravates gastric ulcers evoked by ethanol (Tarnawski et al., 1985), acidified aspirin (Tanaka et al., 1998), or ammonia (Brzozowski et al., 1996; Warzecha et al., 2000).

Primary capsaicin-sensitive sensory nerves serve for conduction of nociceptive information to the central nervous system, but also are able to release neuromediators from the activated peripheral endings; this process is a basic for the local "axon reflex" (Holzer, 1991). Sensory fibers have a special sensitivity to capsaicin (Buck and Burks, 1986). Capsaicin, a main pungent ingredient of chili pepper, binds to specific vanilloid (capsaicin) receptors on primary sensory neurons (Caterina et al., 1997; Hayes et al., 2000). Low doses of capsaicin stimulate primary sensory nerves by opening the nonselective cation channels involved in vanilloid receptors, resulting in local release of neurotransmitters such as calcitonin gene-related peptide (CGRP) and substance P (Holzer et al., 1990; Ren et al., 1993). On the other hand, high neurotoxic doses of capsaicin lead to ablation of sensory nerves with decrease in plasma and tissue levels of CGRP (Sternini et al., 1987; Wimalawansa, 1993). Gastric mucosa is densely innervated by capsaicin-sensitive nerves (Holzer et al., 1990; Sternini et al., 1987). Sensory nerves and CGRP (which seems to be the predominant neurotransmitter of spinal afferents in the rat stomach) are involved in different aspects of the stomach pathology. The stimulation of sensory fibers, as well as the administration of exogenous CGRP, were found to exert a protective effect in different experimental models of gastric ulcers (Clementi et al., 1993; Holzer and Lippe, 1988), whereas the ablation of sensory nerves aggravated gastric mucosal lesions induced by various noxious factors (Brzozowski et al., 1995; Szolcsányi and Barthó, 1981), inhibited gastric mucosal growth (Dembiński et al., 1995), and prolonged gastric ulcer healing (Takeuchi et al., 1994; Warzecha et al., 1996).

Stress-related gastric ulcer is observed in patients in intensive care units and can result in clinically important bleeding, which is associated with increased mortality (Steinberg, 2002). Stress ulcer develops usually within a few hours after burns, polytrauma, central nervous system lesions, shock, large operations, or severe infection (Schiessel, 1989). Our previous experimental study (Warzecha et al., 2001) has shown that treatment with histamine protects the gastric mucosa and enhances the gastroprotective effect of ranitidine and omeprazole against stress-induced gastric lesions; however, mechanisms involved in this gastroprotective effect of histamine are not clear. Therefore, the aim of our present investigation was to determine the role of histamine H_1, H_2, and H_3 receptors and capsaicin-sensitive sensory nerves in histamine-induced protection against water immersion and restrain stress-evoked gastric ulcers.

2. Materials and methods

2.1. Animals and treatment

The study was performed on 112 Wistar male rats weighing 200–250 g fasted for 24 h prior to the experiment with the unlimited access to water. The study was conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University.

Acute gastric ulcers were induced by immobilization of animals in special individual cages and immersion of these animals in water at 23 °C for 3.5 h to the level of xyphoid process (water immersion and restraint stress) as described originally by Takagi et al. (1964). Before exposure to stress, rats were divided randomly into 14 groups (eight animals in each group). Rats with sensory nerve ablation were divided into five groups and pretreated as follows: (1) saline; (2) histamine [30 min before stress, 10 μmol/kg, subcutaneously (s.c.)]; (3) pyrilamine in combination with histamine (pyrilamine 1 h before stress, 100 μmol/kg, s.c.; histamine 30 min before stress, 10 μmol/kg, s.c.); (4) ranitidine in combination with histamine (ranitidine 1 h before stress, 100 μmol/kg, s.c.; histamine 30 min before stress, 10 μmol/kg, s.c.); and (5) thioperamide in combination with histamine (thioperamide 1 h before stress, 100 μmol/kg, s.c.; histamine 30 min before stress, 10 μmol/kg, s.c.). Rats with intact sensory nerves were randomly divided into nine groups. Five groups of rats were treated before exposure to water immersion and restrain stress as animals with ablation of sensory nerves. Additional three groups were treated with: (1) pyrilamine alone (1 h before stress, 100 μmol/kg, s.c.); (2) ranitidine alone (1 h before stress, 100 μmol/kg, s.c.); or (3) thioperamide alone (1 h before stress, 100 μmol/kg, s.c.). One group of animals with intact sensory nerves was treated subcutaneously with saline without exposure to stress and served as the control group.

Sensory nerve ablation was induced by capsaicin (Fluka, Buchs, Switzerland) injected s.c. at the total dose of 100 mg/kg over three consecutive days as described previously (Dembiński et al., 1996). Two injections per day were performed under ether anesthesia to prevent pain reaction and respiratory impairment associated with capsaicin

injection. After the last capsaicin injection, a recovery period of 10 days was allowed before the final experiments. To assess the effectiveness of sensory nerve ablation, 1 day before induction of ulcers, a drop of 0.33 mM solution of capsaicin was installed into the eye of each rat and the presence of the wiping movements was examined. All animals pretreated with capsaicin showed negative wiping movement test, thus confirming functional deactivation of capsaicin-sensitive nerves.

2.2. Determination of gastric blood flow and gastric mucosal lesions

After 3.5 h of water immersion and restrain, stress animals were anesthesized with ketamine (50 mg/kg i.p.; Bioketan, Biowet, Gorzów, Poland) and the abdomen was opened by a midline incision. The stomach was exposed and the gastric mucosal blood flow was measured using laser Doppler flowmeter (PeriFlux 4001 Master monitor; Perimed, Järfälla, Sweden). Gastric mucosal blood flow was measured in five areas of the oxyntic portion of the stomach and the mean value of five recordings was presented as percent of the gastric mucosal flow recorded in control rats with intact sensory nerves and without exposure to stress. After measurement of gastric mucosal blood flow, the number and the area of necrotic lesions in the oxyntic mucosa were measured, using computerized planimeter (Morphomat; Carl Zeiss, Berlin, Germany) as described previously (Konturek et al., 1988a). The measurement was made by personnel blinded to the origin of coded specimens. The stress lesion was defined as a round or linear mucosal black or red defect of at least 0.1 mm in diameter.

2.3. Determination of mucosal DNA synthesis

The rate of DNA synthesis in the mucosa scraped from the oxyntic gland area was determined as described previously (Dembiński et al., 1991). Briefly, the mucosa was incubated at 37 °C for 45 min in 2 ml of medium containing 8 Ci/ml [^{3}H]thymidine ([6-^{3}H]thymidine, 20–30 Ci/mmol; Institute for Research, Production, and Application of Radioisotopes, Prague, Czech Republic). The reaction was stopped with 0.4 N perchloric acid. DNA content of the samples was determined by Giles and Myers procedure (1965). The incorporation of [^{3}H]thymidine into DNA was determined by counting 0.5 ml of DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as [^{3}H]thymidine disintegrations per minute per microgram of DNA (dpm/μg DNA).

2.4. Determination of serum interleukin-1β concentration

After measurement of the gastric lesion area, venous blood was taken from aorta and serum was collected for later determination of interleukin-1β concentrations. Serum interleukin-1β was measured in duplicate using the BioSource Cytoscreen rat interleukin-1β kit based on a solid-phase sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) (BioSource International, Camarillo, CA, USA). Concentration of interleukin-1β was expressed as picograms per milliliter.

2.5. Statistical analysis

Results were expressed as mean±S.E.M. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Neuman–Keuls multiple comparison test using GraphPadPrism. A difference with P value less than 0.05 was considered statistically significant.

3. Results

3.1. Gastric lesions

Fig. 1 shows the effect of subcutaneous pretreatment with histamine and histamine H_1 receptor antagonist, pyrilamine, on the formation of acute gastric mucosa lesion induced by water immersion and restrain stress in animals with intact or deactivated sensory nerves. Exposure to water immersion and restrain stress for 3.5 h resulted in appearance of multiple erosions in oxyntic mucosa. The mean lesion area in saline-treated animals with intact sensory nerves reached 8.2±0.3 mm^2. In animals with intact sensory nerves, pretreatment with histamine significantly reduced the ulcer area by 66%, whereas pretreatment with pyrilamine alone did not significantly affect gastric mucosal damage evoked by stress. Administration of a

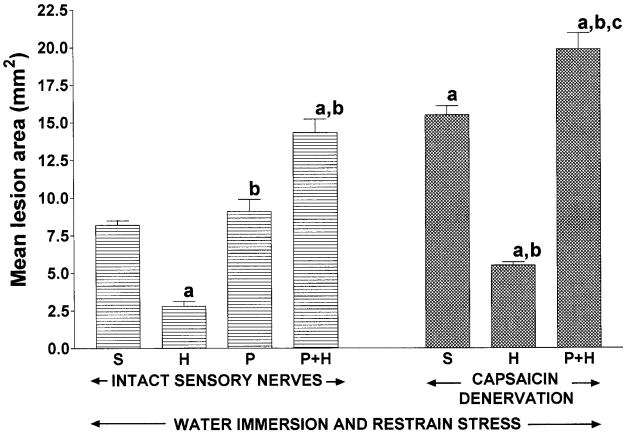

Fig. 1. Effect of treatment with saline (S), histamine (H), pyrilamine (P), or a combination of pyrilamine plus histamine (P+H) on the water and restraint stress-induced gastric lesions in rats with or without capsaicin denervation. Mean±S.E.M. N=8 in each group of animals. [a]P<0.01 compared with saline (S)+stress+intact sensory nerves. [b]P<0.01 compared with histamine (H)+stress+intact sensory nerves. [c]P<0.001 compared with pyrilamine+histamine (P+H)+stress and intact sensory nerves.

combination of pyrilamine plus histamine prior to stress caused a marked increase in the area of gastric lesions over the value observed in saline- or histamine-treated rats (Fig. 1). Ablation of sensory nerves by capsaicin significantly increased the ulcer area in animals treated with saline, histamine alone, or a combination of pyrilamine plus histamine by 89%, 96%, or 39%, respectively.

In animals with intact sensory nerves, treatment with histamine H_2 receptor antagonist, ranitidine, alone (Fig. 2) decreased gastric mucosal damage and the mean ulcer area in this group of animals was significantly lesser than that observed in animals treated with saline or histamine alone (1.4 ± 0.1 vs. 8.2 ± 0.3 or 2.8 ± 0.3 mm^2). Pretreatment with ranitidine before histamine administration maximally reduced gastric mucosal damage (0.7 ± 0.2 mm^2), but a difference in mean ulcer area between animals treated with ranitidine alone and animals treated with a combination of ranitidine plus histamine was not statistically significant. Ablation of sensory nerves by capsaicin significantly increased the ulcer area in animals treated with a combination of ranitidine plus histamine; however, the mean gastric lesion area was still smaller than the area of lesions found in animals with intact sensory nerves and treated with histamine alone (Fig. 2).

In animals with intact sensory nerves, administration of histamine H_3 receptor antagonist, thioperamide, alone was without effect on gastric mucosal damage evoked by stress (Fig. 3). Pretreatment with thioperamide before histamine administration abolished the histamine-evoked reduction in gastric ulcer area (6.9 ± 0.9 vs. 2.8 ± 0.3 mm^2). Ablation of sensory nerves by capsaicin did not significantly affect gastric damage area in animals treated with thioperamide plus histamine. In this group of animals, the ulcer area (7.8 ± 1.5 mm^2) was similar to that observed in saline-treated rats with intact sensory nerves (8.2 ± 0.3 mm^2).

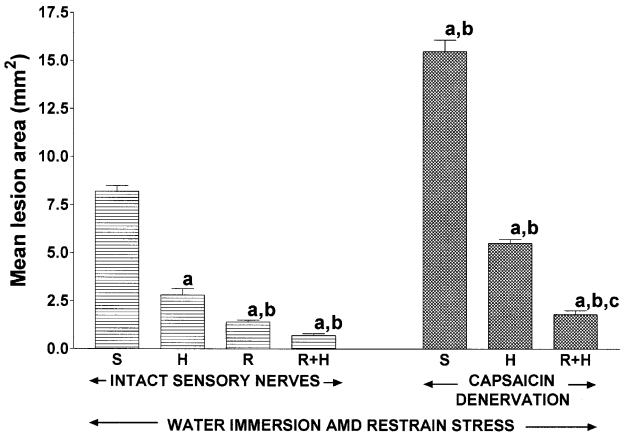

Fig. 2. Effect of treatment with saline (S), histamine (H), ranitidine (R), or a combination of ranitidine plus histamine (R+H) on the water and restraint stress-induced gastric lesions in rats with or without capsaicin denervation. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with saline (S)+stress and intact sensory nerves. [b]P<0.05 compared with histamine (H)+stress+intact sensory nerves. [c]P<0.05 compared with ranitidine+histamine (R+H)+stress+intact sensory nerves.

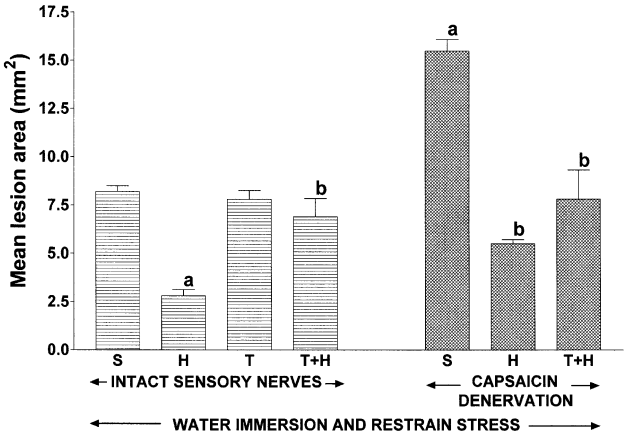

Fig. 3. Effect of treatment with saline (S), histamine (H), thioperamide (T), or a combination of thioperamide plus histamine (T+H) on the water and restraint stress-induced gastric lesions in rats with or without capsaicin denervation. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared saline (S)+stress and intact sensory nerves. [b]P<0.05 compared with histamine (H)+stress and intact sensory nerves.

3.2. Gastric blood flow

In control animals with intact sensory nerves and without exposure to water immersion and restrain stress, gastric mucosal blood flow reached 49.6 ± 1.2 ml/100 g tissue/min—this value was recognized as 100% of gastric mucosal blood flow (Fig. 4). In animals with intact sensory nerves, the exposure to stress reduced gastric mucosal blood flow by 52.5%. Treatment with histamine partly, but significantly, reversed this effect. In this group of animals, gastric mucosal blood flow reached 67.5% of control value. Pretreatment with pyrilamine alone additionally reduced gastric mucosal blood flow in animals exposed to stress. Administration of pyrilamine in

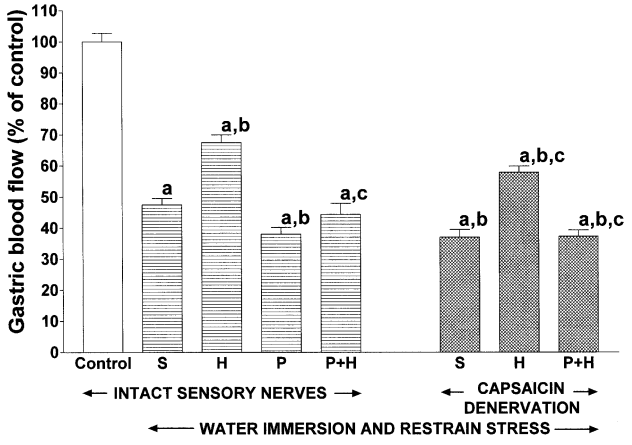

Fig. 4. Effect of treatment with saline (S), histamine (H), pyrilamine (P), or a combination of pyrilamine plus histamine (P+H) on gastric mucosal blood flow in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.05 compared with saline (S)+stress+intact sensory nerves. [c]P<0.05 compared with histamine (H)+stress+intact sensory nerves.

combination with histamine completely abolished the histamine-induced increase in gastric mucosal blood flow. Ablation of sensory nerves by a neurotoxic dose of capsaicin led to the additional and significant reduction in gastric mucosal blood flow in animals exposed to stress and treated with saline or histamine. In animals treated with a combination of pyrilamine plus histamine, ablation of sensory nerves tended to reduce gastric mucosal blood flow; however, this effect was not statistically significant (Fig. 4).

In animals with intact sensory nerves and exposed to stress, treatment with ranitidine alone was without significant effect on gastric circulation (Fig. 5), whereas administration of ranitidine in combination with histamine caused maximal restoration of gastric mucosal blood flow, which reached 80.2±2.5% of control value. Gastric blood flow in this group of animals was significantly higher than in animals treated with histamine or ranitidine alone. Ablation of sensory nerves by capsaicin caused the additional and significant reduction in gastric mucosal blood flow in animals exposed to stress and treated with a combination of ranitidine plus histamine (Fig. 5).

In animals with intact sensory nerves and exposed to stress, treatment with thioperamide significantly reduced gastric mucosal blood flow (39.5±1.5 vs. 47.5±2.0) (Fig. 6). Also administration of thioperamide prior to histamine abolished the histamine-evoked increase in gastric blood flow. Ablation of sensory nerves by a neurotoxic dose of capsaicin was completely without effect on gastric mucosal blood flow in animals treated with a combination of thioperamide plus histamine (Fig. 6).

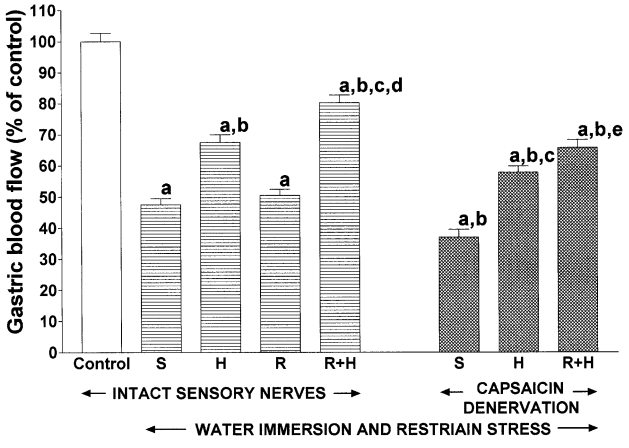

Fig. 5. Effect of treatment with saline (S), histamine (H), ranitidine (R), or a combination of ranitidine plus histamine (R+H) on gastric mucosal blood flow in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.01 compared with saline (S)+stress+intact sensory nerves. [c]P<0.05 compared with histamine (H)+stress+intact sensory nerves. [d]P<0.001 compared with ranitidine (R)+stress and intact sensory nerves. [e]P<0.001 compared with ranitidine+histamine (R+H)+stress and intact sensory nerves.

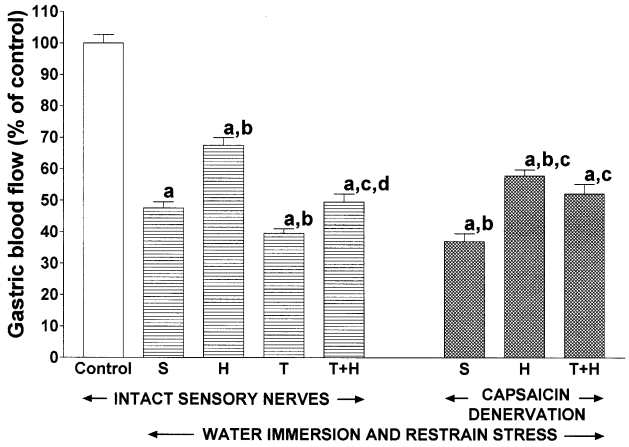

Fig. 6. Effect of treatment with saline (S), histamine (H), thioperamide (T), or a combination of thioperamide plus histamine (T+H) on gastric mucosal blood flow in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.05 compared with saline (S)+stress+intact sensory nerves. [c]P<0.01 compared with histamine (H)+stress+intact sensory nerves. [d]P<0.05 compared with thioperamide (T)+stress+intact sensory nerves.

3.3. Gastric mucosal DNA synthesis

Fig. 7 demonstrates the effect of treatment with saline, histamine, or pyrilamine given alone or in their combination on gastric mucosal DNA synthesis in animals with or without ablation of sensory nerves and exposed to water immersion and restrain stress. In control animals with intact sensory nerves and without exposure to stress, gastric mucosal DNA synthesis reached 45.2±1.7 dpm/μg DNA. In this group of animals, the exposure to stress reduced gastric mucosal

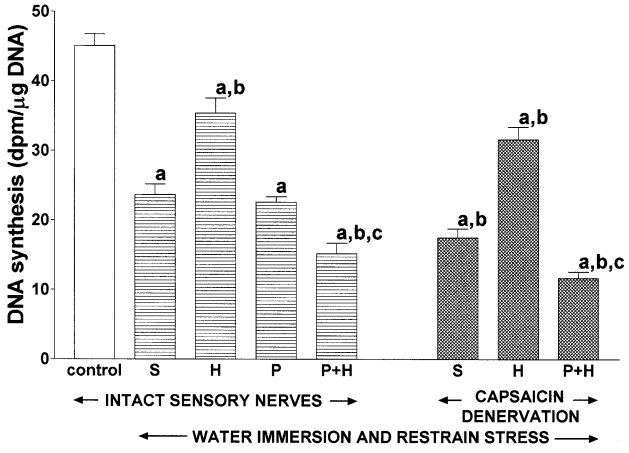

Fig. 7. Effect of treatment with saline (S), histamine (H), pyrilamine (P), or a combination of pyrilamine plus histamine (P+H) on gastric mucosal DNA synthesis in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.05 compared with saline (S)+stress+intact sensory nerves. [c]P<0.001 compared with histamine (H)+stress+intact sensory nerves.

DNA synthesis by 48%. In animals with intact sensory nerves, treatment with histamine partly, but significantly, reversed stress-evoked decrease in gastric mucosal DNA synthesis—gastric mucosal DNA synthesis reached 35.4±2.0 dpm/μg DNA. Administration of pyrilamine alone was without effect on gastric mucosal DNA synthesis in animals with intact sensory nerves and exposed to stress. In histamine-treated rats, pyrilamine significantly decreased gastric mucosal DNA synthesis below a value observed in animals exposed to stress and treated with saline or histamine alone. Ablation of sensory nerves by capsaicin tended to reduce gastric mucosal DNA synthesis in all animals exposed to stress, but this effect was statistically significant only in animals treated with saline alone (Fig. 7).

Treatment with ranitidine alone significantly increased gastric mucosal DNA synthesis in animals with intact sensory nerves and exposed to stress (Fig. 8). In animals with intact sensory nerves and exposed to stress, a combination of ranitidine plus histamine caused an additional increase in gastric mucosal blood flow, reaching a value significantly higher than observed in animals treated with histamine or ranitidine alone. Ablation of sensory nerves by capsaicin tended to reduce gastric mucosal DNA synthesis in animals exposed to stress and treated with histamine or a combination of ranitidine plus histamine, but this effect was not statistically significant (Fig. 8).

Administration of thioperamide prior to water immersion and restrain stress was without effect on gastric mucosal DNA in animals with intact sensory nerves and treated with saline (Fig. 9), but thioperamide completely abolished the histamine-induced increase in gastric mucosal DNA synthesis. Ablation of sensory nerves by capsaicin was without effect on gastric mucosal DNA synthesis in animals exposed to stress and treated with combination of thioperamide plus histamine (Fig. 9).

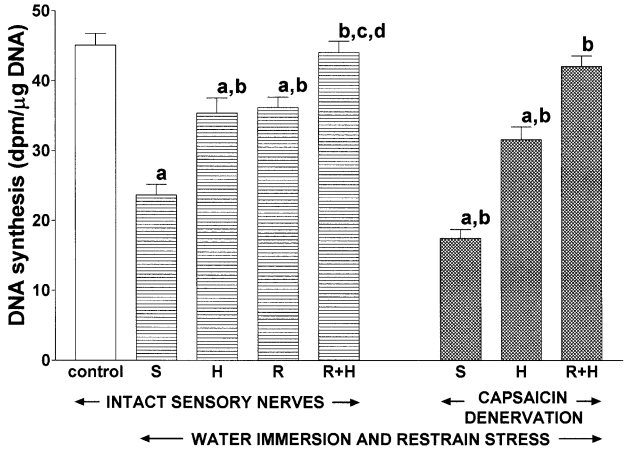

Fig. 8. Effect of treatment with saline (S), histamine (H), ranitidine (R), or a combination of ranitidine plus histamine (R+H) on gastric mucosal DNA synthesis in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.01 compared with control without exposure to stress. [b]P<0.05 compared with saline (S)+stress+intact sensory nerves. [c]P<0.01 compared with histamine (H)+stress+intact sensory nerves. [d]P<0.01 compared with ranitidine (R)+stress+intact sensory nerves.

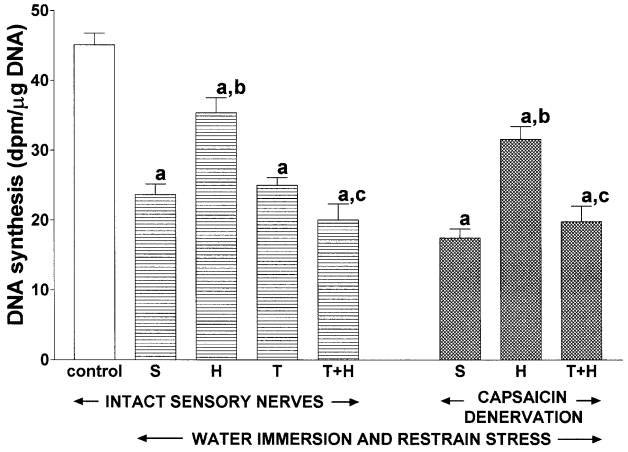

Fig. 9. Effect of treatment with saline (S), histamine (H), thioperamide (T), or a combination of thioperamide plus histamine (T+H) on gastric mucosal DNA synthesis in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.001 compared with saline (S)+stress+intact sensory nerves. [c]P<0.001 compared with histamine (H)+stress+intact sensory nerves.

3.4. Serum interleukin-1β concentration

In control animals treated with saline without exposure to water immersion and restrain stress, serum interleukin-1β concentration reached a value 44.0±2.0 pg/ml (Fig. 10). The exposure of these animals to stress led to a threefold

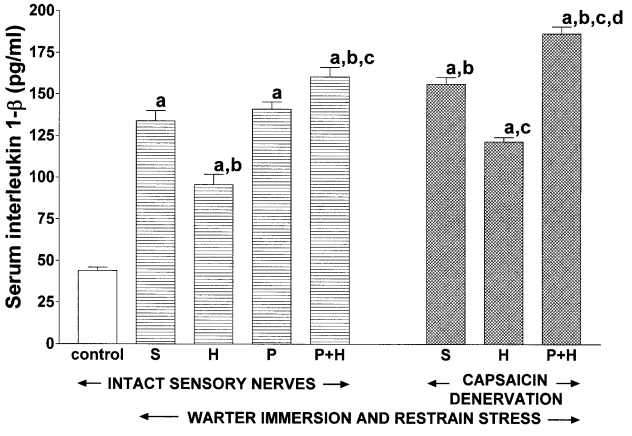

Fig. 10. Effect of treatment with saline (S), histamine (H), pyrilamine (P), or a combination of pyrilamine plus histamine (P+H) on serum interleukin-1β concentration in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.001 compared with saline (S)+stress+intact sensory nerves. [c]P<0.001 compared with histamine (H)+stress+intact sensory nerves. [d]P<0.001 compared with pyrilamine+histamine (P+H)+stress and intact sensory nerves.

increase in serum interleukin-1β concentration. Treatment with histamine significantly attenuated the stress-evoked increase in serum interleukin-1β concentration by 28%. Administration of pyrilamine alone was without significant effect on serum concentration of interleukin-1β, whereas pyrilamine given prior to histamine completely abolished the histamine-induced reduction in serum interleukin-1β concentration. In this group of animals, serum interleukin-1β concentration reached 160.5±5.8 pg/ml and this value was significantly higher than the value observed in animals exposed to stress and treated with saline or histamine alone. Ablation of sensory nerves by capsaicin led to a significant increase in serum interleukin-1β concentration in animals treated with saline, histamine, or a combination of pyrilamine plus histamine (Fig. 10).

Administration of ranitidine alone decreased serum interleukin-1β in animals with intact sensory nerves and exposed to stress (Fig. 11). Combination of ranitidine plus histamine additionally reduced serum interleukin-1β concentration, reaching a value significantly lower than that observed in animals exposed to stress and treated with histamine. Ablation of sensory nerves by capsaicin led to a significant increase in serum interleukin-1β concentration in animals treated with ranitidine in combination with histamine (Fig. 11).

Administration of thioperamide alone was without effect on serum interleukin-1β concentration in rats with intact sensory nerves and exposed to stress. Pretreatment with thioperamide before administration of histamine significantly reduced the histamine-induced decrease in serum interleukin-1β concentration. Ablation of sensory nerves by capsaicin was without significant effect on serum interleukin-1β concentration in animals treated with thioperamide plus histamine and exposed to stress (Fig. 12).

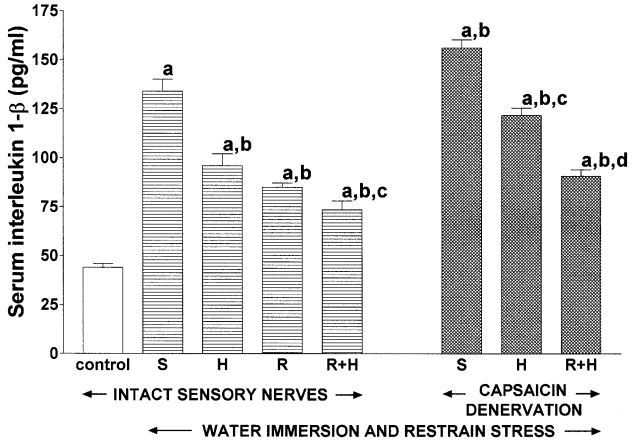

Fig. 11. Effect of treatment with saline (S), histamine (H), ranitidine (R), or a combination of ranitidine plus histamine (R+H) on serum inerleukin-1β concentration in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.05 compared with saline (S)+stress+intact sensory nerves. [c]P<0.01 compared with histamine (H)+stress and intact sensory nerves. [d]P<0.05 compared with ranitidine+histamine (R+H)+stress and intact sensory nerves.

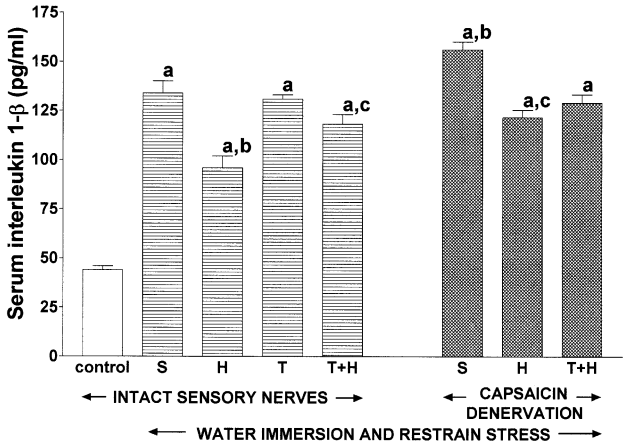

Fig. 12. Effect of treatment with saline (S), histamine (H), thioperamide (T), or a combination of thioperamide plus histamine (T+H) on serum inerleukin-1β concentration in rats with intact or ablated sensory nerves (capsaicin denervation) and exposed to water and restraint stress. Mean±S.E.M. N=8 in each group of animals. [a]P<0.001 compared with control without exposure to stress. [b]P<0.001 compared with saline (S)+stress+intact sensory nerves. [c]P<0.001 compared with histamine (H)+stress+intact sensory nerves.

4. Discussion

This study confirms and extends our previous observation (Warzecha et al., 2001) that histamine administration prior to water immersion and restrained stress leads to a significant reduction in the gastric lesion area. This gastroprotective effect of treatment with histamine was accompanied by increase in gastric blood flow and mucosal DNA synthesis, and by decrease in serum level of pro-inflammatory interleukin-1β. The relation between the histamine-induced protection of gastric mucosa and the increase in gastric mucosal blood flow is in agreement with observations of previous studies (Sorbye and Svanes, 1994; Warzecha et al., 2000, 2001; West et al., 2003). Gastric mucosal blood flow plays an important role in protection of gastric mucosa. Numerous experimental studies show that exposure of gastric mucosa to potentially noxious environment results in little or no damage, as long as adequate blood flow is maintained, whereas reduction in mucosal blood flow leads to severe gastric injury (Sorbye and Svanes, 1994). Blood flow contributes to protection by supplying the mucosa with oxygen, bicarbonate, and nutritious substances, and by removing carbon dioxide, hydrogen ions, and toxic agents diffusing from the gastric lumen (Sorbye and Svanes, 1994). Gastric hypoxia, resulting in accumulation of H^+ within the gastric mucosa, leads to mucosal acidification and development of gastric ulcers (Allen et al., 1993). In stress ulcers, the fall of gastric intramucosal pH is an important predictor of risk of mucosal bleeding (Fiddian-Green et al., 1983). On the other hand, the

relationship between blood flow and gastric mucosal damage seems to be bidirectional. Improvement of gastric mucosal blood flow reduces gastric mucosal damage, but also the reduction in gastric mucosal damage leads to improvement of gastric mucosal circulation.

In the present study, we have examined, using specific histamine receptor antagonists, the role of histamine H_1, H_2, and H_3 receptors in the gastroprotective effect of histamine administration against stress ulcer. Administration of pyrilamine, a histamine H_1 receptor antagonist, was without effect on gastric mucosal damage in saline-treated rats; but pyrilamine administered in combination with histamine abolished the gastroprotective effect of histamine and aggravated damage of the gastric mucosa evoked by stress. This deleterious effect was accompanied by the fall in gastric blood flow and mucosal DNA synthesis and by increase in serum level of pro-inflammatory interleukin-1β. The vascular effect of pyrilamine administration observed in the present study is in agreement with previous findings. Histamine H_1 receptors are present in vascular smooth muscle and vascular endothelial cells (Hill et al., 1997). Stimulation of histamine H_1 receptors in vascular endothelial cells leads to several cellular responses such as increase in vascular permeability, prostacyclin synthesis, and release of nitric oxide (Hill et al., 1997). Histamine H_1 receptors are involved in pentagastrin-induced gastric hyperemia, and the administration of histamine H_1 receptor antagonist, diphenhydramine, blocks the primary hyperemic response to pentagastrin (Gerkens et al., 1977). Also histamine H_1 receptor antagonist administration was found to inhibit ethanol-induced gastric hyperemia (Oates and Hakkinen, 1988).

The other mechanism of detrimental effect of pyrilamine administration on gastric mucosa may depend on its influence on gastric mucosa growth. The exposure to stress leads to inhibition of DNA synthesis and cell renewal of gastric mucosa (Greant et al., 1988). The same effect has been observed in our present study. Also, we have found that administration of histamine partly reversed this effect; whereas pyrilamine, given in combination with histamine, significantly decreased gastric mucosal DNA synthesis below the value observed in animals exposed to stress and treated with saline or histamine alone. These findings are in agreement with the study of Andre et al. (1985). They have found that histamine and histamine H_1 receptor agonist (betahistine) stimulate thymidine uptake in the gastric mucosa in the model of gastric ulceration induced by mucosal anaphylaxis (Andre et al., 1985).

Our observation of deleterious effects of pyrilamine on gastric mucosa exposed to stress is also consistent with the study performed by Palitzsch et al. (1995). They have found that administration of histamine or histamine H_1 receptor agonist (betahistine) reduces the area of ethanol-induced gastric haemorrhagic lesions. On the other hand, there are some controversies concerning the gastroprotective properties of histamine H_1 receptor agonists and deleterious properties of histamine H_1 receptor antagonists. In the histamine-induced model of gastric ulcer, Del Soldato (1984), as well as Amagase and Okabe (2003) have found that histamine H_1 and H_2 receptor antagonists reduce gastric lesion formation. These differences concerning effects of histamine H_1 antagonist administration in our and their studies suggest that stress and an overdose of histamine may involve dissimilar ulcerogenic mechanisms. These discrepancies can be also caused by differences of histamine dose used in the studies. In our study, we have used a model of stress-induced gastric lesions and histamine was administered at the low gastroprotective dose. In the model of histamine-induced gastric lesions, histamine has been used in the toxic doses, which are 40-fold higher than the dose used by us.

Histamine H_3 receptors have been primary identified in the central nervous system where they are located presynaptically and negatively control the synthesis and release of histamine from histaminergic neurons (Arrang et al., 1983). Previous studies have shown that the use of high doses of histamine H_3 receptor-selective agonists (10–100 mg/kg) inhibits the development of gastric lesions induced by ethanol (Morini et al., 1995) or 0.6 N HCl (Morini et al., 2002b). Also, gastric mucosal damage induced by stress has been reduced by stimulation of H_3 receptors (Belcheva et al., 1997). Our present study confirms and extends these findings. We have found that administration of thioperamide, a selective histamine H_3 antagonist, almost completely abolishes the gastroprotective effect of histamine administration against stress-induced gastric ulcers. Moreover, pretreatment with thioperamide has abolished the histamine-induced increase in gastric mucosal DNA synthesis and gastric mucosal blood flow. These data are additionally supported by observations of Morini et al. (2002a). They have found that the histamine H_3 receptor agonist, (*R*)-alpha-methylhistamine, stimulates cell proliferation and accelerates differentiation towards pit cells and their outward migration. These effects were reversed by administration of histamine H_3 receptor antagonists.

Another finding of our present study is the observation that administration of ranitidine in combination with histamine maximally reduced the gastric lesion area. This effect was accompanied with maximal improvement of gastric mucosal blood flow and mucosal DNA synthesis. We have also found that administration of ranitidine plus histamine maximally reduces the serum interleukin-1β concentration. Interleukin-1β is a well-known component of acute inflammation and plays a crucial role in the induction of systemic acute phase response and in the release of other members of the cytokine cascade (Dinarello, 1991). Interleukin-1β has been found to stimulate the production of inflammation mediators such as: tumor necrosis factor, platelet-activating factor, prostaglandins, and pro-inflammatory interleukins as interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, and interleukin-8 (Dinarello, 1991; Dinarello and

Wolff, 1993). The fundamental role of interleukin-1β in the development of inflammation is also evidenced by the observation that the blockage of its action by interleukin-1 receptor antagonist prevents the synthesis and release of pro-inflammatory mediators and reduces the severity of inflammation (Dinarello and Wolff, 1993). These data are in agreement with our present observation and we have found a close correlation between reduction in serum pro-inflammatory interleukin-1β and reduction of gastric mucosal damage.

In our present study, treatment with a combination of ranitidine plus histamine maximally reduced gastric stress-induced damage, whereas treatment with pyrilamine or thioperamide reduced the beneficial effect of histamine on gastric mucosa. These findings, taken together, indicate that the gastroprotective effect of histamine against stress ulcers is dependent on the stimulation of histamine H_1 and H_3 receptors.

Previous studies have shown that capsaicin-sensitive sensory nerves and their predominant neurotransmitter, CGRP, play an important role in the protection of gastric mucosa against damage evoked by various noxious agents (Brzozowski et al., 1995; Clementi et al., 1993; Holzer and Lippe, 1988; Szolcsányi and Barthó, 1981; Takeuchi et al., 1994). Our present study confirms and extends these findings. In saline- or histamine-treated rats, ablation of sensory nerves by capsaicin increased the ulcer area almost twofold. This effect was well correlated with the reduction in gastric mucosal blood flow and DNA synthesis and the increase in serum interleukin-1β concentration, but the influence of sensory nerves ablation on these parameters was less pronounced. This observation indicates that deleterious effects of sensory nerve ablation on gastric mucosa depend, in part, on changes in gastric mucosal blood flow, DNA synthesis, and release of pro-inflammatory interleukin-1β, but also suggest the involvement of other factors in noxious effects of sensory nerve ablation. In the study performed by Harada et al. (2003), it was shown that capsaicin-sensitive sensory nerves are involved in gastric release of prostaglandin I_2 and prostaglandin E_2. Both prostaglandins are vasodilators and exhibit a strong gastroprotective effect, which is related and unrelated to their vascular action. Deleterious effects of sensory nerve ablation on gastric mucosa may also be dependent on the release of endogenous epidermal growth factor (EGF). EGF exhibits a well-known gastroprotective effect (Konturek et al., 1988b) and, in the study performed by Ma et al. (2000), it was shown that ulcer induction dramatically elevates EGF in salivary glands and serum, whereas ablation of sensory nerves completely abolishes this effect.

In animals treated with a combination of thioperamide plus histamine, ablation of sensory nerves by neurotoxic doses of capsaicin has not aggravated gastric damage; the ulcer area, gastric mucosal blood flow, and mucosal DNA synthesis were similar to those observed in rats with intact sensory nerves and treated with saline or a combination of thioperamide plus histamine. The lack of significant deleterious effect of sensory nerve ablation on gastric mucosa in animals treated with a combination of thioperamide plus histamine suggests that the gastroprotective effect of histamine H_3 agonists in the stomach depends mainly on the stimulation of histamine H_3 receptors present on afferent capsaicin-sensitive sensory nerves. The study performed by Coruzzi et al. (2001) upholds support for this concept. They have found the presence of histamine H_3 receptors in cholinergic and nonadrenergic noncholinergic (NANC) nerves of the myenteric plexus, and in endocrine and/or paracrine cells of the gastric mucosa.

Finally, our present study demonstrates that: (1) histamine exhibits a protective effect against stress-induced gastric ulcer and this effect is related to the stimulation of histamine H_1 and H_3 receptors; (2) blockade of histamine H_2 receptors enhances the gastroprotective effect of histamine against stress-induced gastric ulcers; and (3) ablation of sensory nerves aggravates stress-induced gastric ulcer and reduces the gastroprotective effect of histamine administration, and this effect seems to be mainly dependent on histamine H_3 receptor deactivation.

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