Gastroprotective Activity of FRG-8813, a Novel Histamine H2-Receptor Antagonist, in Rats

Sadayoshi Onodera, Masahiro Shibata*, Masato Tanaka, Niro Inaba, Tetsuaki Yamaura and Haruo Ohnishi

Pharmaceuticals Research Laboratories, Fujirebio, Inc., 51, Komiya-cho, Hachioji, Tokyo 192, Japan

Received October 26, 1994 Accepted March 10, 1995

ABSTRACT—FRG-8813 ((±)-2-(furfurylsulfinyl)-N-[4-[4-(piperidinomethyl)-2-pyridyloxy-(Z)-2-butenyl]acetamide) is a novel histamine H2-receptor antagonist with gastric antisecretory and gastroprotective activities. The present study was designed to investigate the property of gastroprotective action. The oral ED50 values for inhibition of mucosal lesions against 1% NH3-, 60% ethanol in 0.15 N HCl-, 100% ethanol-, 0.6 N HCl- and sodium taurocholate in 0.4 N HCl-induced damage were 3.3, 11.1, 14.9, 23.3 and 23.1 mg/kg, respectively. FRG-8813 was gastroprotective despite pretreatment with omeprazole, suggesting that the protective effect is independent of its antisecretory activity. It is unlikely that FRG-8813 works as a mild irritant because it showed a gastroprotective effect after intraperitoneal injection, but oral administration itself did not influence the rat gastric mucosa. Although pretreatment with indomethacin or N-ethylmaleimide did not affect the gastroprotection of FRG-8813, chemical deafferentation induced by capsaicin abolished the gastroprotection. Furthermore, prior administration of tetrodotoxin, the calcitonin gene-related peptide (CGRP) antagonist hCGRP8-37 or N^-nitro-L-arginine attenuated the gastroprotection of FRG-8813 as well as that of capsaicin. In contrast to capsaicin, repeated administration of FRG-8813 neither enhanced the susceptibility of the mucosa to damage nor affected the gastroprotective action of short-term treatment. In conclusion, these results suggest that FRG-8813 has gastroprotective activity independently of acid antisecretory activity and that capsaicin-sensitive nerves may be partially or fully involved in the gastroprotective mechanisms of FRG-8813.

Keywords: FRG-8813, Gastroprotection, Histamine H2-receptor antagonist, Capsaicin-sensitive nerve, Antiulcer drug

Successful peptic ulcer therapy depends on the restoration of the compromised integrity of the gastroduodenal mucosa by either reducing the aggressive factors or enhancing the defensive process in the mucosa. This has been mainly accomplished by reducing intraluminal acid with an histamine H2-receptor antagonist for more than 10 years. The histamine H2-receptor antagonists are among the most frequently employed drugs worldwide, and their leading position can be explained by their proven high antisecretory potency (1, 2). However, numerous reports showed that the incidence of ulcer relapse after discontinuation of the histamine H2-receptor antagonist reaches the same level as that in patients receiving a placebo (3–5). From these viewpoints, further improvement in ulcer therapy, with regard to quality of ulcer healing and/or ulcer relapse, might be achieved if the agent could enhance the mucosal defensive capacity in addition to decreasing gastric acid secretion.

The property of agents that protect the gastric mucosa against necrotizing agents such as ethanol was defined as so-called “cytoprotection” by Robert et al. (6). Subsequent observations demonstrated that although the gastric cells localized deep in the mucosa were protected, cytoprotective agents did not prevent disruption of the surface epithelium by necrotizing agents (7, 8). Therefore, this beneficial effect of prostaglandins and other compounds was appropriately referred to as gastroprotection or mucoprotection. Although the exact mechanisms of the protection are unknown, it is presumably considered to involve one or more of the naturally occurring defensive factors within the mucosa. So, this experimental procedure has been used for evaluating gastroprotective activity by a drug and as a model of acute gastric mucosal lesions.

* To whom correspondence should be addressed.
FRG-8813 ((±)-2-(furfurylsulfinyl)-N-[4-[4-(piperidinomethyl)-2-pyridyl]oxy-(Z)-2-butenyl]acetamide) is a new histamine H₂-receptor antagonist synthesized by Fujirebio, Inc. This compound has potent and long-lasting acid antisecretory activities in rats and dogs (9-11). The trials carried out in normal human volunteers revealed that FRG-8813 given orally produced prolonged inhibition of gastric acid secretion like it did in animals (Mori et al., unpublished data). Besides the antisecretory effect, FRG-8813 exerts potent gastroprotective effect in the dose range showing antisecretory activity, and hence this study was designed to investigate the property and mechanisms of the gastroprotection.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats weighing 160–340 g (Charles River, Inc., Tokyo) were used. The animals, which were kept in individual cages with raised mesh bottoms to prevent coprophagia, were deprived of food but allowed free access to water for 18 hr before the experiments.

Drugs

FRG-8813, famotidine and N⁶-nitro- L-arginine (L-NNA) were synthesized by the Pharmaceuticals Research Laboratories of Fujirebio, Inc. Other drugs used were as follows: cimetidine (Industrie Chimiche Farmaceutiche Italian, Milan, Italy); absolute ethanol, 1 N NaOH, 1 N HCl, 25% ammonia solution, N-ethylmaleimide, 2-deoxy-D-glucose (2-DG), tetrodotoxin (TTX) and capsaicin (Wako, Osaka); sodium taurocholate (Hoechst, Frankfurt, Germany); indomethacin, terbutaline, guanethidine sulfate, aminophylline and bovine serum albumin (BSA) (Sigma, St. Louis, MO, USA); rat calcitonin gene-related peptide (CGRP) and human (h) CGRP₈₋₃₇ (Peptide Institute, Minoh); omeprazole (Yoshitomi, Osaka), methoxamine hydrochloride (Nippon Shinyaku, Kyoto); acetylcholine chloride (Daichi Selyaku, Tokyo); and polyoxyethylene (60) hydrogenated castor oil (HCO60; Nikko Chemicals, Tokyo).

FRG-8813, famotidine, cimetidine, omeprazole and capsaicin were suspended in 5% arabic gum solution when orally applied. FRG-8813 was dissolved in 0.1 N HCl and neutralized by 0.1 N NaOH for i.p. injection. Omeprazole was suspended in 1% arabic gum solution containing 0.2% NaHCO₃ for s.c. injection. In the case of chemical deafferentation, capsaicin was dissolved in HCO60-ethanol solution (10% ethanol, 10% HCO60, 80% saline). L-NNA was prepared in 0.01 N HCl before being neutralized to pH 7.0. Indomethacin and sodium taurocholate were dissolved in 4% NaHCO₃ solution and 0.4% HCl, respectively. Rat CGRP was dissolved in phosphate-buffered saline containing 1% BSA, and then the stock solutions were freshly diluted with phosphate-buffered saline. Other chemicals were dissolved in saline. Each agent was prepared immediately before use and given in a volume of 2 ml/kg, except for the oral administration of noxious agents in a volume of 1 ml/rat.

Gastroprotective activity

Gastroprotective studies were conducted after the method of Robert et al. (6). The histamine H₂-receptor antagonists or vehicle of 5% arabic gum solution was orally given. Thirty minutes later, acute gastric mucosal lesions were induced by five different noxious agents in a volume of 1 ml: 100% ethanol, 0.6 N HCl, acidified ethanol (60% ethanol in 0.15 N HCl), acidified sodium taurocholate (100 mg in 0.4 N HCl) and 1% NH₃ solution. One hour afterwards, the animals were sacrificed. The stomach was removed and inflated by injection of 10 ml of 2% formalin. Subsequently, the stomach was incised along the greater curvature and examined for macroscopic hemorrhagic damage under a dissecting microscope (×10). The area of each lesion (mm²) was measured, and the sum of the area was regarded as the lesion index.

In some experiments, the stomach was excised 30 min after the oral administration of FRG-8813 at a dose of 10 or 30 mg/kg or vehicle alone. After slightly fixing the stomach by instilling 10 ml of Tyrode solution containing 10% formalin, the stomach was opened along the greater curvature. The Tyrode solution had the following composition: 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 0.4 mM NaH₂PO₄, 12 mM NaHCO₃ and 5.6 mM glucose. Then the tissue samples were processed for routine light microscopy and stained with periodic acid-Schiff and alcian blue.

After a dose-dependent study, we chose 1% NH₃ solution as a noxious agent and FRG-8813, p.o. or i.p., at a dose of 10 mg/kg for the subsequent studies in conscious rats.

Effect of gastric acid suppression by omeprazole on the gastroprotection of orally-administered FRG-8813 and omeprazole

To examine whether or not the gastroprotective effect of FRG-8813 is independent of its antisecretory activity, rats were s.c. pretreated with omeprazole (30 mg/kg), a H⁺-K⁺ ATPase inhibitor, to suppress gastric acid secretion. One hour later, they were orally given omeprazole (30 mg/kg), FRG-8813 or its vehicle alone; and 30 min later, they were given 1 ml of 1% NH₃ solution for induction of gastric damage. One hour later, they were sacrificed to determine the gastric ulcer index.

The antisecretory effect of omeprazole (30 mg/kg, s.c.)
on 2-DG-stimulated acid secretion was examined in pylorus-ligated rats. Rats were deprived of food for 18 hr before the experiment. While under ether anesthesia, the abdomen was opened and the pylorus was ligated according to the method of Shay et al. (12). At the time of ligation, omeprazole (30 mg/kg) and 2-DG (100 mg/kg) for stimulating acid secretion were injected s.c. and i.v., respectively, in a volume of 2 ml/kg. Pylorus ligation was performed for 2.5 hr in order to match the time-course of the gastroprotective experiment carried out in the s.c. treatment with omeprazole. Then, the rats were sacrificed to collect the gastric contents. After centrifugation of the gastric juice, the volume of the supernantant was measured. The acid concentration was determined by titration of a 0.5-ml aliquot against 0.01 N NaOH to pH 7.0. Total acid output (μEq/2.5 hr) was calculated as the volume times the acid concentration.

**Onset of action and duration of action**

To study the onset and duration of gastroprotection, FRG-8813 (10 mg/kg) was given p.o. or i.p. at various times up to 5 hr before oral gavage of 1 ml of 1% NH₃ solution. Control animals received vehicle, 5% arabic gum solution or saline, at the same time intervals before oral administration of 1 ml of 1% NH₃ solution. The animals were sacrificed 1 hr after receiving NH₃ solution to determine the ulcer index.

**Studies of the gastroprotective mechanisms of FRG-8813**

**Effect of indomethacin, N-ethylmaleimide, chemical deafferentation and L-NNA: Indomethacin at a dose of 10 mg/kg was injected s.c. 2 hr before oral administration of FRG-8813 (10 mg/kg) to determine whether mucosal protection by FRG-8813 is dependent on the synthesis of prostaglandins. The dose (10 mg/kg, s.c.) of indomethacin was selected to ensure the reduction of mucosal prostaglandins contents, because indomethacin at 5 mg/kg, s.c. has been reported to induce near-maximal inhibition of formation of mucosal prostaglandins (13–15). Rats were s.c. treated with N-ethylmaleimide at a dose of 5 mg/kg 15 min before oral administration of FRG-8813 (10 mg/kg) to investigate the involvement of a sulfhydryl-containing substance of the mucosa in gastroprotection. Rats received capsaicin by s.c. injection once daily for three consecutive days (20, 30 and 50 mg/kg) for the induction of chemical deafferentation at the age of 6 weeks (16). The rats were used for the experiments 10 to 14 days later. All capsaicin injections were done under ether anesthesia, and the rats were pretreated intramuscularly with terbutaline (0.1 mg/kg) and aminophylline (10 mg/kg) to counteract the respiratory impairment associated with capsaicin injection. The effectiveness of the treatment was tested by examining the protective wiping movement of the eye. To investigate the involvement of nitric oxide (NO) formation in the gastroprotection, L-NNA at 10 mg/kg, an inhibitor of endogenous NO formation, was injected intravenously 10 min before oral administration of FRG-8813 (10 mg/kg). The dose of L-NNA was selected to induce a marked increase of arterial blood pressure (unpublished data, S. Onodera et al.). Rats were orally given 1 ml of 1% NH₃ solution 30 min after receiving the vehicle or FRG-8813 and sacrificed 1 hr later to determine the ulcer index. Capsaicin at 30 mg/kg, p.o. was used as a reference gastroprotective drug in the experiments of chemical deafferentation and L-NNA.

**Effect of intra-arterial infusion of TTX or hCGRP₈₋₃₇:** After the induction of anesthesia with urethane (1.2 g/kg, i.p.), the rat was fitted with a tracheal cannula to facilitate spontaneous respiration. A polyethylene catheter was inserted retrogradely into the splenic artery close to the celiac artery for intra-arterial infusion. A soft catheter for an inflow was inserted into the stomach through the oesophagus and gently tied in the cervical oesophagus. A second polyethylene cannula for an outflow was inserted into the stomach via an incision in the duodenum; it was held in place by a ligature. The tip of the outflow cannula was placed 3 cm above the stomach over the experimental period. After completion of the surgery, the gastric lumen was perfused with saline (37°C) for 60 min at a rate of 1 ml/min. After that, the rats received an intra-arterial infusion of TTX (50 ng/min), hCGRP₈₋₃₇ (2 μg/min) or saline (0.2 ml/hr) for 30 min. Ten minutes after starting the infusion, FRG-8813 (30 mg/kg), capsaicin (3 mg/kg) or its vehicle (4 ml of 5% arabic gum solution) was introduced into the gastric lumen. Ten minutes later, 10 ml of 0.25% NH₃ solution was introduced into the stomach for induction of gastric lesions by the same route, this procedure allowing about 4 ml of the NH₃ solution to be left in the lumen and about 6 ml to be drained away from the outflow cannula. Ten minutes later, the NH₃ solution in the lumen was exchanged with saline, and the stomach was perfused with saline for another 60 min and removed to assess gastric lesions (mm²).

The dose of TTX was selected to block the 2-DG (200 mg/kg, i.v.)-induced increase in gastric motility, but not to affect mean arterial pressure and heart rates, over a 30 min period in anesthetized rats. For the selection of the dose of hCGRP₈₋₃₇, the experiment of perfusion pressure in rat gastric vascular bed with active tone by methoxamine was performed. Gastric vascular bed in situ was perfused with methoxamine (10 μM) through a cannula to the celiac artery, and the perfusion pressure was monitored continuously. CGRP (10 pmol) and acetylcholine (0.1 nmol), applied into the perfusate, reduced the pressure by 37.4±4.8% (n=6) and 38.8±5.0% (n=6), respectively, in a volume of 2 ml/kg. Pylorus ligation was performed for 2.5 hr in order to match the time-course of the gastroprotective experiment carried out in the s.c. treatment with omeprazole. Then, the rats were sacrificed to collect the gastric contents. After centrifugation of the gastric juice, the volume of the supernantant was measured. The acid concentration was determined by titration of a 0.5-ml aliquot against 0.01 N NaOH to pH 7.0. Total acid output (μEq/2.5 hr) was calculated as the volume times the acid concentration.
respectively, of methoxamine-induced tone. CGRP- and acetylcholine-induced reductions of perfusion pressure were 18.7 ± 4.5% (n = 6) and 44.4 ± 10.7% (n = 6), respectively, when hCGRP8-37 (2 µg/min) was infused into the perfusate, indicating that the dose of hCGRP8-37 was able to selectively antagonize the response to CGRP. Therefore, 2 µg/min of hCGRP8-37 was used for the gastroprotective study.

Effect of repeated administration on the gastroprotection

The influence of the repeated administration of FRG-8813 (10 mg/kg), capsaicin (30 mg/kg) or its vehicle (5% arabic gum solution) on the mucosal protective effect of short-term FRG-8813 (10 mg/kg, p.o.) or capsaicin (30 mg/kg, p.o.) was examined. The rats were given FRG-8813 or capsaicin twice a day for 6 days and fasted overnight. The last administration of the drug was performed in the morning of the 7th day. The gastroprotective effect was evaluated 6 hr after the last administration. The animals received orally 1% NH₃ solution, and the stomach was removed 1 hr later to assess the lesions. FRG-8813, capsaicin or its vehicle was given 30 min before the NH₃ treatment.

Statistical analyses

Results are expressed as the mean ± standard error (S.E.). Statistical differences between the treatment groups were determined by Student’s t-test or Aspine-Welch’s test. The fifty percent effective doses (ED₅₀) were obtained by the single regression line method, and 95% confidence limits were calculated according to Filler’s theorem (17). Values of P < 0.05 were regarded as significant.

RESULTS

Gastroprotective activity

Oral administration of 1% NH₃ solution produced hemorrhagic band-like lesions in the gastric mucosa of rats, and the control ulcer index was 53.1 ± 10.0 (mm²) (Table 1). When rats were orally pretreated with FRG-8813 at doses of 1, 3, 10 and 30 mg/kg, respectively, 30 min before the NH₃ treatment, the production of mucosal injury was inhibited in a dose-dependent manner. The oral ED₅₀ value for inhibition of the NH₃-induced lesion formation was 3.3 mg/kg. Oral treatment with cimetidine at doses of 30, 100 and 300 mg/kg, respectively, also reduced the NH₃-induced lesions in a dose-dependent manner, and the ED₅₀ value was 111.6 mg/kg. When famotidine at a high dose of 150 mg/kg was orally administered, the formation of the NH₃-induced lesions was slightly inhibited.

The other four noxious agents, acidified ethanol (60% ethanol in 0.15 N HCl), 100% ethanol, 0.6 N HCl and acidified sodium taurocholate (100 mg/kg sodium taurocholate in 0.4 N HCl), caused hemorrhagic band-like lesions like those caused by 1% NH₃ solution in macroscopical appearance. The mean ulcer indexes of control gastric lesions induced by acidified ethanol, 100% ethanol, 0.6 N HCl and acidified sodium taurocholate were 35.9 ± 7.4, 41.9 ± 11.0, 30.0 ± 5.2 and 59.8 ± 7.1 (mm²), respectively. FRG-8813 given orally also prevented the formation of these mucosal injuries in a dose-dependent fashion, but famotidine did not at any dose. The respective oral ED₅₀ values of FRG-8813 were 11.1, 14.9, 23.3 and 23.1 mg/kg against acidified ethanol-, 100% ethanol-, 0.6 N HCl- and acidified sodium taurocholate-induced lesions, respectively.

| Noxious agents | FRG-8813 | Famotidine | Cimetidine |
|----------------|----------|------------|------------|
| 1% NH₃         | 3.3      | 150 <      | 111.6      |
| 0.15 N HCl + 60% EtOH | 11.1      | 10 <     | 112.2      |
| 100% Ethanol   | 14.9     | 150 <     | 135.6      |
| 0.6 N HCl      | 23.3     | 150 <     | 172.1      |
| 0.4 N HCl + Na taurocholate | 23.1     | 150 <     | 75.9       |

N = 9–10/each dose. Rats were orally given vehicle or the H₂-antagonists. Thirty minutes later, the rats were orally given 1 ml of the noxious agent. The animals were sacrificed to determine the ulcer index 1 hr after the application of the noxious agent. ED₅₀ values were calculated by using Filler’s theorem.
Fig. 1. Microscopic observation of the rat gastric mucosa excised 30 min after oral administration of FRG-8813. A: Vehicle (5% arabic gum solution). B: FRG-8813, 10 mg/kg, p.o. C: FRG-8813, 30 mg/kg, p.o. Note that no histological changes in gastric mucosa can be seen after oral administration of FRG-8813. (Periodic acid-Schiff and alcian blue staining) (×200).
induced lesions (Table 2). The oral protective effect of cimetidine was also observed dose-dependently, with the ED₅₀ values of 112.2, 135.6, 172.1 and 75.9 mg/kg for inhibition of acidified ethanol-, 100% ethanol-, 0.6 N HCl and acidified sodium taurocholate-induced lesions, respectively (Table 2).

Macroscopically, no damage was observed in the gastric mucosa 30 min after oral treatment with FRG-8813 at doses of 10 and 30 mg/kg. Microscopic observation also showed that there was no damage in the mucosa after FRG-8813 as well as after vehicle (Fig. 1).

**Effect of gastric acid suppression by omeprazole on the gastroprotection of orally-administered FRG-8813 and omeprazole**

Both FRG-8813 (10 mg/kg, p.o.) and omeprazole (30 mg/kg, p.o.) significantly reduced the 1% NH₃-induced mucosal lesions in vehicle-treated rats. In the pretreatment with omeprazole (30 mg/kg, s.c.), oral FRG-8813 was also effective in reducing the mucosal damage, while oral omeprazole was ineffective (Fig. 2).

The antisecretory effect of omeprazole (30 mg/kg, s.c.) on 2-DG (100 mg/kg, i.v.)-stimulated gastric acid secretion was examined in 2.5 hr pylorus-ligated rats. Omeprazole significantly suppressed acid output by about 90%: 2-DG, 262.9 ± 39.8 μEq/2.5 hr; 2-DG + omeprazole, 28.4 ± 3.1 μEq/2.5 hr.

**Onset of action and duration of action**

The onset and duration of the gastroprotection after oral or i.p. FRG-8813 administration were examined by using 1% NH₃ solution as a noxious agent. Oral administration of FRG-8813 (10 mg/kg) tended to reduce the lesion formation 5 min before the NH₃ treatment, although this did not reach any statistical significance (Fig. 3). A significant inhibitory effect was observed from 10 to 180

![Graph](image-url)

**Fig. 2.** Effect of gastric acid suppression by omeprazole on gastroprotection of oral-administered FRG-8813 and omeprazole against 1% NH₃-induced gastric mucosal lesions in rats. Rats were subcutaneously treated with omeprazole (30 mg/kg) or its vehicle. One hour later, the rats were orally given omeprazole (30 mg/kg), FRG-8813 (10 mg/kg) or its vehicle alone; and 30 min later, the animals were given 1 ml of 1% NH₃ solution. One hour later, the animals were sacrificed to determine the gastric ulcer index. Each column represents the mean ± S.E. of 9 animals. *P < 0.05, **P < 0.01, Significant difference from the corresponding control value. □ Control; ■ FRG-8813, 10 mg/kg; □ Omeprazole, 30 mg/kg.

![Graph](image-url)

**Fig. 3.** Onset and duration of the gastroprotective effect of FRG-8813 given orally or intraperitoneally on 1% NH₃-induced gastric mucosal lesions in rats. Rats were orally or intraperitoneally given vehicle or FRG-8813. Five to 300 min later, respectively, the rats were orally given 1 ml of 1% NH₃ solution. The animals were sacrificed to determine the ulcer index 1 hr after the 1% NH₃ treatment. Each column represents the mean ± S.E. of 8–10 animals. *P < 0.05, Significant difference from the corresponding control value. □ Control; ■ FRG-8813, 10 mg/kg.
min, and the protective effect disappeared by 300 min.

By the i.p. route, FRG-8813 (10 mg/kg) did not produce any gastroprotective effect 5 min before the NH3 treatment, and it significantly inhibited the lesions at 30 and 60 min (Fig. 3). Although there was the tendency for i.p. injection to decrease the lesion formation 10 and 120 min before the NH3 treatment, the degree of the inhibitory effect did not reach a significant level.

Studies of the gastroprotective mechanisms of FRG-8813 Effect of indomethacin, N-ethylmaleimide and chemical deafferentation: Pretreatment with indomethacin (10 mg/kg, s.c.) by itself did not alter the formation of the 1% NH3-induced lesions (Fig. 4). FRG-8813 given orally (10 mg/kg) significantly inhibited the lesion formation by 56% and 65%, respectively, in vehicle- and indomethacin-treated rats. In the experiment on N-ethylmaleimide, the lesions were significantly aggravated by the pretreatment with N-ethylmaleimide (5 mg/kg, s.c.). FRG-8813 was also effective in reducing the lesion formation by 55% and 56%, respectively, in vehicle- and N-ethylmaleimide-treated rats.

Chemical deafferentation by high doses of capsaicin (total dose 100 mg/kg, s.c.) tended to aggravate the 1% NH3-induced lesions. The gastroprotective effect of oral FRG-8813 was completely abolished in the deafferented rats (Fig. 4). Similarly, gastroprotective effect of acute capsaicin (30 mg/kg) given orally on the 1% NH3-induced lesions was substantially attenuated in the deafferented rats. By the i.p. route, FRG-8813 (10 mg/kg) did not produce any gastroprotective effect 5 min before the NH3 treatment, and it significantly inhibited the lesions at 30 and 60 min (Fig. 3). Although there was the tendency for i.p. injection to decrease the lesion formation 10 and 120 min before the NH3 treatment, the degree of the inhibitory effect did not reach a significant level.

Fig. 4. Effect of indomethacin, N-ethylmaleimide and chemical deafferentation on the gastroprotection of FRG-8813 against 1% NH3-induced gastric mucosal lesions in rats. Rats were subcutaneously treated with indomethacin (10 mg/kg) and N-ethylmaleimide (5 mg/kg) at 2.5 hr and 10 min, respectively, before oral administration of FRG-8813 or vehicle. Chemical deafferentation was performed by consecutive injections of capsaicin (total dose: 100 mg/kg, s.c.) two weeks before the experiment. The rats were orally given 1 ml of 1% NH3 solution 30 min after the oral administration of FRG-8813 or vehicle. One hour later, the animals were sacrificed to determine the ulcer index. Each column represents the mean ± S.E. of 8-10 animals. *P<0.05, Significant difference from the corresponding control value. #P<0.05, Significant difference from the vehicle-control value. □ Control; ■ FRG-8813, 10 mg/kg. IND: Indomethacin, NEM: N-ethylmaleimide, Cap: Capsaicin.

Fig. 5. Effect of N°-nitro-L-arginine on the gastroprotection of FRG-8813 or capsaicin against 1% NH3-induced gastric mucosal lesions in rats. N°-nitro-L-arginine (10 mg/kg) or its vehicle was intravenously injected into rats. Ten minutes later, FRG-8813 (10 mg/kg), capsaicin (30 mg/kg) or vehicle was orally administered. The rats were orally given 1 ml of 1% NH3 solution 30 min after oral administration of FRG-8813 or capsaicin and sacrificed 1 hr later to determine the ulcer index. Each column represents the mean ± S.E. of 9-10 animals. **P<0.01, Significant difference from the corresponding control value. #P<0.01, Significant difference between the groups. □ Control; ■ FRG-8813, 10 mg/kg; ® Capsaicin, 30 mg/kg. L-NNA: N°-nitro-L-arginine.
rats. The ulcer indices after vehicle and oral capsaicin treatments were 56.8±6.3 and 18.1±5.5 (P<0.05), respectively, in normal rats, and 107.9±15.7 and 126.1±17.4, respectively, in the deafferentated rats.

**Effect of L-NNA:** Intravenous injection of L-NNA (10 mg/kg) significantly worsened the lesion formation induced by 1% NH₃ solution in vehicle-treated rats. Furthermore, L-NNA diminished the gastroprotective effect of FRG-8813 (10 mg/kg, p.o.) as well as that of capsaicin (30 mg/kg, p.o.) (Fig. 5).

**Effect of TTX and hCGRP₈₋₃₇:** Intraluminal instillation of NH₃ solution (0.25%) produced relatively broad and vague-outlined lesions in anesthetized rats as compared with those in conscious rats. Prior administration of FRG-8813 (30 mg/kg, i.g.) or capsaicin (3 mg/kg, i.g.) significantly reduced the lesions. Local intra-arterial infusion of TTX (50 ng/min) for 30 min significantly augmented the mucosal lesion formation. The gastroprotective effect of FRG-8813 and capsaicin was completely blocked in TTX-treated rats (Table 3).

Intra-arterial infusion of hCGRP₈₋₃₇ (2 µg/min) for 30 min by itself did not influence the lesion formation caused by NH₃ solution. However, the gastroprotective effect of FRG-8813 and capsaicin was antagonized by the treatment with hCGRP₈₋₃₇ (Fig. 6).

**Effect of repeated administration on the gastroprotection**

After repeated administration of capsaicin (30 mg/kg, p.o.) for 6.5 days, the ulcer indices of the NH₃-induced damage were significantly greater than those in vehicle-treated rats. Furthermore, the protective effect of short-term capsaicin (30 mg/kg, p.o.) disappeared in animals treated with long-term capsaicin. In contrast, long-term

### Table 3. Effect of intra-arterial infusion of tetrodotoxin on the gastroprotection of FRG-8813 or capsaicin against 0.25% NH₃-induced gastric mucosal lesions in anesthetized rats

| Treatment          | No. of rats | Ulcer index (mm²) | Inhibition (%) |
|--------------------|-------------|-------------------|----------------|
| **Vehicle**        |             |                   |                |
| Control            | 8           | 73.9±4.9          |                |
| FRG-8813, 30 mg/kg| 8           | 37.0±4.2***       | 49.9           |
| Capsaicin, 3 mg/kg | 8           | 23.6±4.3***       | 68.0           |
| **Tetrodotoxin infusion** |           |                   |                |
| Control            | 8           | 115.4±10.2#       |                |
| FRG-8813, 30 mg/kg| 8           | 97.0±10.8         | 15.9           |
| Capsaicin, 3 mg/kg | 8           | 86.5±11.6         | 25.0           |

*After equilibration, tetrodotoxin (50 ng/min) or saline (0.2 ml/hr) was infused for 30 min through a cannula inserted in the splenic artery. Ten minutes after initiation of tetrodotoxin infusion, the stomach was treated with capsaicin (3 mg/kg) or FRG-8813 (30 mg/kg) for 10 min, and then the gastric mucosa was washed and perfused for 60 min with warm saline. Rats were sacrificed to determine the ulcer index (mm²) after the perfusion. Each value of ulcer index represents the mean±S.E. of 8 animals. **P<0.001, Significant difference from the corresponding control value. #P<0.01, Significant difference from the vehicle-control value.*

![Fig. 6. Effect of intra-arterial infusion of hCGRP₈₋₃₇ on the gastroprotection of FRG-8813 or capsaicin against 0.25% NH₃-induced gastric mucosal lesions in anesthetized rats. After equilibration, hCGRP₈₋₃₇ (2 µg/min) or saline (0.2 ml/hr) was infused through a cannula inserted in the splenic artery for 30 min. Ten minutes after initiation of hCGRP₈₋₃₇ infusion, the stomach was treated with FRG-8813 (30 mg/kg) or capsaicin (3 mg/kg) for 10 min, and then the gastric mucosa was exposed to 0.25% NH₃ solution for another 10 min. After that, the gastric mucosa was washed and perfused for 60 min with warm saline. Rats were sacrificed to determine ulcer index (mm²) after the perfusion. Each column shows the mean±S.E. of 10 animals. *P<0.05, Significant difference from the corresponding control value. □ Control; ■ FRG-8813, 30 mg/kg; □ Capsaicin, 3 mg/kg.**
treatment with FRG-8813 (10 mg/kg, p.o.) for 6.5 days did not affect either the basal lesion formation or the protective effect of short-term FRG-8813 (10 mg/kg) (Fig. 7).

DISCUSSION

FRG-8813 is a novel histamine H2-receptor antagonist with potent and long-lasting gastric acid antisecretory activities. The present studies showed that FRG-8813, besides its antisecretory activity, has gastroprotective activity, as it dose-dependently reduced the appearance of hemorrhagic lesions in the gastric mucosa of rats after oral gavage of several noxious agents. Comparative study of gastroprotective ED50 values revealed that the protective effect was most pronounced when a 1% NH3 solution was given to rats. In the present report (11), the antisecretory ED50 values for FRG-8813 and cimetidine given intraduodenally were determined to be 6.1 mg/kg and 14.1 mg/kg, respectively, in conscious pylorus-ligated rats for 4 hr, which means that FRG-8813 exerts both antisecretory and gastroprotective activities in the same dose ranges. In contrast, oral pretreatment with famotidine failed to prevent the formation of these lesions at any dose, and the doses of oral cimetidine required to produce the gastroprotective effect were rather high compared with those needed to suppress gastric acid secretion. Because FRG-8813 is a racemic compound of two enantiomers, it is necessary to determine whether or not the enantiomers show differences in gastroprotection. Our previous data revealed that when (±)-, (+)- or (−)-FRG-8813 at an oral dose of 10 mg/kg was given 30 min before the application of 1% NH3 solution, almost the same inhibitory percentage of the 1% NH3-induced mucosal lesions were observed among the three compounds: (±) 64%, (+) 61%, (−) 61%. These findings clearly distinguish FRG-8813 from conventional histamine H2-receptor antagonists such as cimetidine and famotidine in aspects of gastroprotective activity.

It is also important that the gastroprotective effect is not caused by the antisecretory activity. In the present studies, a sufficient amount of exogenous acid (60% ethanol in 0.15 N HCl, 0.6 N HCl, sodium taurocholate in 0.4 N HCl) was used to exclude the possibility that the antisecretory activity may be responsible for the protective effect. In addition, FRG-8813 also proved to be effective in preventing the lesion formation even when the rats were pretreated with omeprazole (30 mg/kg, s.c.) at the dose required to suppress 2-DG-stimulated acid secretion by about 90%. In contrast, the gastroprotective effect of oral omeprazole used as a reference drug was completely attenuated by the same treatment, and this result is consistent with the previous reports (18, 19). Since omeprazole is readily degraded by acidic pH (20), the degraded compounds produced in the gastric juice of the lumen are considered to exert the gastroprotective effect by a local action from the luminal side. Therefore, omeprazole loses the ability to exert a gastroprotective effect in the absence of luminal acid. Although the precise mechanisms of the
gastroprotection are not fully understood, Holm (21) showed that the increase in gastric mucosal blood flow was observed only in the case of oral administration, not in the parenteral injection; and Okabe et al. (22) suggested that endogenous sulfhydryl compounds are, in part, involved in the mechanisms of the action of omeprazole. Taken together, these results suggest that the gastroprotective action of FRG-8813 on the gastric mucosa is due to some mechanism other than inhibition of acid secretion or histamine H2-receptor antagonism.

The studies of the onset and duration of gastroprotective activities revealed that the oral protective effect, though not statistically significant, tended to occur even at 5 min following the administration and continued at least for 3 hr. A significant protective effect was also observed after i.p. injection at the same dose as in the oral administration, but the onset was longer and the duration was shorter when compared with oral administration. These results have two implications. First, since both oral and i.p. treatments with FRG-8813 were effective, the protective effect is not the result of "adaptive cytoprotection", which occurs following the application of a mild irritant to the gastric mucosa and which may be mediated by increased levels of tissue prostaglandin (23, 24). Our studies also showed that pretreatment with indomethacin (10 mg/kg, s.c.) did not affect the gastroprotection of FRG-8813, indicating that the presence of endogenous prostaglandins is not essential to the protective activity by FRG-8813. Besides, histological examination revealed that intragastric FRG-8813 (10 and 30 mg/kg) did not produce any mucosal damage, although it is well known that a mild irritant causes damage in the superficial epithelial cells (25-27). Second, the facts that the gastroprotection can take place 5 min after oral administration of FRG-8813, at which time the protective effect was not seen yet after i.p. injection, and that the gastroprotective efficacy by oral route was somewhat more potent than that by the i.p. route may point to a local protective action on the gastric mucosa through mechanisms distinct from those of mild irritants. Therefore, FRG-8813 is gastroprotective after reaching the gastric mucosa from either the luminal or the serosal side, the luminal pathway being more efficient.

It has been also reported that endogenous sulphydryl compounds may play an important role in gastroprotection (28-30). Pretreatment with N-ethylmaleimide, a sulphydryl blocker, by itself significantly aggravated the NH3-induced lesions, and these results are consistent with those of Takeuchi et al. (31). They suggested that the enhancement of vascular permeability by N-ethylmaleimide may account for the aggravation of the lesions by using absolute ethanol. However, the treatment with N-ethylmaleimide did not counteract the protective effect of FRG-8813, which means that endogenous sulphydryls are not involved in the mechanisms of gastroprotection afforded by FRG-8813.

Capsaicin-sensitive afferent nerves have recently been suggested to be closely involved in gastric mucosal defense mechanisms (32, 33). In the rat stomach, the predominant neuropeptide in capsaicin-sensitive nerves is CGRP, and such nerves are found in close proximity to the submucosal microvasculature (34-36). There is pharmacological evidence that stimulation of these nerves causes release of CGRP into the vascular bed of the stomach (37) and that CGRP exerts its vasodilating effect (37, 38), which is suggested to be a gastroprotective mechanism, by a direct mechanism or indirectly by promoting the local release of a further vasodilating substance such as NO. In our study, both functional ablation of the afferent nerve and intra-arterial treatment with TTX, a drug that specifically blocks nerve conduction, aggravated the 1% NH3-induced mucosal lesions, although the aggravation induced by chemical deafferentation did not reach the level of statistical significance. The aggravation is consistent with previous reports that chronic capsaicin treatment (39, 40) or intra-arterial administration of TTX (33) worsens the injury induced by a variety of damaging agents. These observations suggest the nervous system, probably capsaicin-sensitive nerves, in the mucosa plays an important role in gastric defense mechanisms. The gastroprotective effect of FRG-8813 was blocked by chemical deafferentation as well as by intra-arterial infusion of TTX or the CGRP antagonist hCGRP6-37 in a similar fashion to that of capsaicin, indicating the close involvement of capsaicin-sensitive nerves in the mechanisms of the action of FRG-8813. Recent studies showed that NO participates in the regulation of gastric mucosal integrity (41, 42) and is involved in the vasodilating and gastroprotective actions of CGRP (43, 44). Therefore, the influence of L-NNA, an inhibitor of NO biosynthesis, on the gastroprotective effect of FRG-8813 was examined. Intravenous injection of L-NNA significantly increased the susceptibility of mucosa to damage by NH3 solution and abolished the gastroprotection by capsaicin. These data are compatible with previous reports (45). The gastroprotective effect of FRG-8813 was also attenuated by the treatment with L-NNA, further suggesting that the mechanisms of gastroprotection of FRG-8813 are related to capsaicin-sensitive nerves.

It is still controversial whether capsaicin action depends on the presence of endogenous prostaglandins (PGs) (46, 47). Our study showed that the protective action of FRG-8813 was abolished by sensory denervation, but not affected by prior administration of indomethacin. In our preliminary study concerning the effect of indomethacin on capsaicin-induced gastroprotection, we had two opposite results. When indomethacin was treated s.c.
Recently, much attention has been paid to NH3-in-
deafferentation when administered repeatedly. However,
gastric capsaicin acts as a neurotoxin to cause sensory
of capsaicin (56). These observations indicate that intra-
irreversibly defunctionalized by repeated administration
treatment with capsaicin. The primary afferent nerves are
NH3 solution. Furthermore, the same treatment complete-
6.5 days significantly aggravated the mucosal damage by
superiority in the NH3-induced mucosal lesions.
Thus, efforts continue in our laboratory to investigate the
protection against the NH3-induced mucosal damage.

30 min before the application of capsaicin, capsaicin-in-
duced gastroprotection disappeared. On the other hand,
significant gastroprotective effect by capsaicin was ob-
served when indomethacin treatment was performed 2 hr
before capsaicin administration. Since about 90% redu-
tion of mucosal PGs by indomethacin (5 mg/kg) was ob-
served at least up to 4 hr (14), it might follow that capsai-
cin action is independent of endogenous PGs. However,

Further studies are being carried to clarify the relationship
between capsaicin-sensitive nerves and endogenous PGs
by using other experiments such as measurement of transmucosal potential difference to rule out the interfer-
eence of enhanced gastric motility by indomethacin.

Recently, much attention has been paid to NH3-in-
duced gastric mucosal damage (48, 49), since it has been
reported that NH3 generated by Helicobactor pylori in
the mucosa may be one of the causative factors in the pathophysiology of gastroduodenal disease (50–53). In
view of this point, the fact that the potency of gastro-
protection of FRG-8813 for NH3-induced mucosal le-
sions is relatively high as compared with that for other
noxious agents-induced mucosal lesions might suggest
that FRG-8813 would be beneficial in Helicobactor pylori-related gastroduodenal disease. The mechanisms
by which the gastroprotective action against the NH3-
induced lesions was greater than that against lesions
induced by other agents remains unclear. However, our
previous studies showed that although NH3 solution is a
damaging agent, NH3 solution applied on the gastric mu-
cosa transiently causes the stimulation of capsaicin-sensi-
tive nerves to some extent in contrast to ethanol, resulting
in a rapid increase in gastric mucosal blood flow, and that
oral pretreatment with diluted NH3 solution protects
against ethanol-induced mucosal damage independently
of endogenous PGs (54). Furthermore, when NH3
solution was applied with intra gastric FRG-8813, the
sustained increase in gastric mucosal blood flow was
observed (55). Therefore, the stimulating or potentiating
action of FRG-8813 on capsaicin-sensitive nerves might
possibly be enhanced when NH3 solution was used as a
damaging agent, resulting in the superiority in gastro-
protection against the NH3-induced mucosal damage.

Thus, efforts continue in our laboratory to investigate the
superiority in the NH3-induced mucosal lesions.

Repeated administration of intragastric capsaicin for
6.5 days significantly aggravated the mucosal damage by
NH3 solution. Furthermore, the same treatment complete-
ly attenuated the mucosal protective action of short-term
treatment with capsaicin. The primary afferent nerves are
irreversibly defunctionalized by repeated administration
of capsaicin (56). These observations indicate that intra-
gastric capsaicin acts as a neurotoxin to cause sensory
deafferentation when administered repeatedly. However,
daily administration of FRG-8813 did not worsen the
NH3-induced lesions at all, and even after long-term treat-
ment with FRG-8813, short-term treatment with FRG-
8813 produced significant gastroprotection. These results
showed that although there is the close relationship be-
tween the gastroprotective mechanisms of FRG-8813 and
capsaicin, administered FRG-8813 does not act as a neu-
rotoxin in contrast to capsaicin. Further studies are being
conducted to clarify the difference between the stimulat-
ning mechanisms of FRG-8813 and capsaicin on the
nerves.

The gastroprotective action of histamine H2-receptor
antagonists such as cimetidine, ranitidine and famotidine
remains controversial, and it seems to be related, at least
in part, to the inhibition of gastric acid secretion. In fact,
several studies reported that the histamine H2-receptor an-
tagonists did not reduce ethanol-induced gastric damage,
which is the most appropriate model for studying gas-
 troprotection unrelated to the inhibition of gastric acid
secretion in animals (57–59) and in humans (60), suggest-
ing that ordinary histamine H2-receptor antagonists are
deprived of gastroprotective activity. On the other hand,
FRG-8813 has gastroprotective activity independent of an-
tisecretry action, and capsaicin-sensitive afferent nerves
partially or fully contribute to the mechanisms of the
gastroprotective action by FRG-8813. Furthermore,
Ichikawa et al. (61) showed that FRG-8813 has the ability
to potently stimulate mucin biosynthesis in rat gastric
mucosa by using an organ culture technique. We previously
reported that FRG-8813 has potent and long-lasting anti-
secretory effects on gastric acid secretion as a histamine
H2-receptor antagonist, the antisecretry potency being
much greater than that of cimetidine. In the management
of peptic ulceration, even with the strong inhibitors of
gastric acid secretion, however, relapse of ulcers could not
be prevented. In this regard, great attention has recently
been paid to the defensive mechanisms of gastric mucosa.

Taking these facts into consideration, FRG-8813 may
be expected to be a promising antiulcer drug in the therapy
of peptic ulcer as compared with ordinary histamine H2-
receptor antagonists. Furthermore, FRG-8813 may exert
a positive effect on acute gastric mucosal lesions and gas-
tritis.

REFERENCES
1 Dammann HG, Dau B and Dreyer M: H2-Rezeptorantagonisten
in der Therapie der peptischen Ulkuserkrankung. Z Gastro-
enterol 25, Supp 3, 136–145 (1987) (Abstr in English)
2 Jones DB, Howden CW, Burget DW, Kerr GD and Hunt RH:
Acid suppression in duodenal ulcer: a meta-analysis to define
optimal dosing with antisecretory drugs. Gut 28, 1120–1127
(1987)
3 Dronfield MW, Batchelor AJ, Larkworthy W and Langman
MJS: Controlled trial of maintenance cimetidine treatment in healed duodenal ulcer: short and long-term effects. Gut 20, 526–530 (1979)
4 Martin DF, Hollanders D, May SJ, Ravenscroft MM, Tweedle DE and Miller JP: Difference in relapse rate of duodenal ulcer after healing with cimetidine or tripotassium dichromate bismuthate. Lancet 1, 7–10 (1981)
5 Rune SJ, Greibe J, Mollman KM, Madsen JR, Rahbek I, Willumsen L and Wulff HR: Recurrence of duodenal ulcer pain after treatment with cimetidine for four and eight weeks. Gut 21, 151–153 (1980)
6 Robert A, Nezamis JE, Lancaster C and Hancher AJ: Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroenterology 77, 433–443 (1979)
7 Lacy ER and Ito S: Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandin. Gastroenterology 83, 619–625 (1982)
8 Robert A, Lancaster C, Davis JP, Field SO, Wickrema Sinha AJ and Thornburgh BA: Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroenterology 83, 328–333 (1985)
9 Yamaura T, Shibata M, Inaba N, Onodera S, Chida Y and Ohnishi H: Effects of FRG-8813, a new type histamine H2 receptor antagonist, on various experimental gastric and duodenal lesions in rats. Folia Pharmacol Jpn 99, 401–410 (1992) (Abstr in English)
10 Yamaura T, Shibata M, Chida Y, Inaba N, Onodera S and Ohnishi H: Effects of FRG-8813, a new type histamine H2 receptor antagonist, on the healing of gastric and duodenal ulcer in rats and spontaneously ulcerative mice. Folia Pharmacol Jpn 99, 411–420 (1992) (Abstr in English)
11 Shibata M, Yamaura T, Inaba N, Onodera S, Chida Y and Ohnishi H: Gastric antisecretory effect of FRG-8813, a new H2 receptor antagonist, in rats and dogs. Eur J Pharmacol 235, 245–253 (1993)
12 Shay H, Sun DC and Gruenstein M: A quantitative method for measuring spontaneous gastric secretion in the rat. Gastroenterology 26, 906–913 (1954)
13 Whittle BJR: The potentialization of taurocolate-induced rat gastric erosions following parenteral administration of cyclooxygenase inhibitors. Br J Pharmacol 80, 545–551 (1983)
14 Okada M, Niida H, Takeuchi K and Okabe S: Role of prostaglandin deficiency in pathogenetic mechanism of gastric lesions induced by indomethacin in rats. Dig Dis Sci 34, 694–702 (1989)
15 Ligumsky M, Hansen DG and Kauffman GL Jr: Salicylic acid blocks indomethacin- and aspirin-induced cyclo-oxygenase inhibition in rat gastric mucosa. Gastroenterology 83, 1043–1046 (1982)
16 Matsumoto J, Takeuchi K and Okabe S: Characterization of gastric mucosal blood flow response induced by intragastric capsaicin in rats. Jpn J Pharmacol 57, 205–213 (1991)
17 Finney DJ: Fieller’s theorem. In Statistical Method in Biochemical Assay, pp 27–35, Charles Griffin and Company, London (1964)
18 Arakawa T, Fukuda T, Higuchi K, Koike K, Satoh H and Kobayashi K: NC-1300, a proton-pump inhibitor, requires gastric acid to exert cytoprotection in rat gastric mucosa. Jpn J Pharmacol 61, 299–302 (1993)
19 Konturek SJ, Brzozowski T and Radecki T: Protective action of omeprazole, a benzimidazole derivative, on gastric mucosal damage by aspirin and ethanol in rats. Digestion 27, 159–164 (1983)
20 Regardh CG, Gabrielson M, Hoffman KJ, Löfberg I and Skanberg I: Pharmacokinetics and metabolism of omeprazole in animals and man—an overview. Scand J Gastroenterol 20, Supp 108, 79–94 (1985)
21 Holm L: Gastric mucosal blood flow and mucosal protection. J Clin Gastroenterol 10, Supp S114–S119 (1988)
22 Okabe S, Miyake H and Awane Y: Cytoprotective effects of NC-1300 and omeprazole on HCl-ethanol-induced gastric lesions in rats. Jpn J Pharmacol 42, 123–133 (1986)
23 Robert A, Nezamis JE, Lancaster C, Davis JP, Field SO and Hanchar J: Mild irritants prevent gastric necrosis through “adaptive cytoprotection” mediated by prostaglandins. Am J Physiol 245, G113–G121 (1983)
24 Konturek SJ, Brzozowski T, Piastucki I, Radecki T, Dembinski A and Dembinske-Kiec A: Role of locally generated prostaglandins in adaptive gastric cytoprotection. Dig Dis Sci 27, 967–971 (1982)
25 Hawkey CJ, Kemp RT, Walt RP, Bhaskar NK, Davies J and Fallowfield B: Evidence that adaptive cytoprotection in rats is not mediated by prostaglandins. Gastroenterology 94, 948–954 (1988)
26 Takeuchi K, Nishiwaki H, Osano H, Ebara S and Okabe S: Repair of mucosal damage induced by ethanol in the rat stomach. Digestion 40, 1–10 (1988)
27 Lacy ER: Gastric mucosal resistance to a repeated ethanol insult. Scand J Gastroenterol 20, Supp 110, 63–72 (1985)
28 Szabo S, Trier JS and Frankel PW: Sulphydryl compounds may mediate gastric cytoprotection. Science 214, 200–202 (1981)
29 Szabo S and Trier JS: Pathogenesis of acute gastric mucosal injury: sulphydryls as a protector, adrenal cortex as modulator, and vascular endothelium as target. In Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract, Edited by Allen J, Flemstrom G, Garner A, Silen W and Turnberg LA, pp 287–293, Raven Press, New York (1984)
30 Möszik GY, Pihan G, Szabo S, Javor T, Czeglédi B, Tigyai A, Tárnok F and Zsoldos T: Free radicals, nonsulphydryl antioxidants, drugs, and vitamins in acute gastric mucosal injury and protection. In New Pharmacology of Ulcer Disease, Edited by Szabo S and Möszik GY, pp 197–207, Elsevier, New York (1987)
31 Takeuchi K, Okada M, Niida H and Okabe S: Role of sulphydryls in mucosal injury caused by ethanol: Relation to microvascular permeability, gastric motility and cytoprotection. J Pharmacol Exp Ther 248, 836–841 (1989)
32 Holzer P and Lippe ITh: Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa. Neurosci 27, 981–987 (1987)
33 Holzer P, Livingston EH and Guth PH: Sensory neurons signal for an increase in the rat gastric mucosal blood flow in the face of pending acid injury. Gastroenterology 101, 416–423 (1991)
34 Ekblad E, Ekelund M, Graffner H, Hakanson R and Sundler F: Sensory neurons signal for an increase in the rat gastric mucosal blood flow in the face of pending acid injury. Gastroenterology 101, 416–423 (1991)
35 Su HC, Bishop AE, Power RF, Hamada Y and Polak JM: Dual intrinsic and extrinsic origins in CGRP- and NPY-immunoreactive nerves of rat gut and pancreas. J Neurosci 7, 2674–2687
36 Sternini C, Reeve JR and Brecha N: Distribution characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats. Gastroenterology 93, 852–862 (1987)

37 Li DS, Raybould HE, Quintero E and Guth PH: Role of calcitonin gene-related peptide in gastric hyperemic response to intragastric capsaicin. Am J Physiol 261, G657–G661 (1991)

38 Holzer P and Guth PH: Neuropeptide control of rat gastric mucosal blood flow: Increase by calcitonin gene-related peptide and vasoactive intestinal polypeptide, but not substance P and neuropeptide A. Circ Res 68, 100–105 (1991)

39 Holzer P and Sametz W: Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. Gastroenterology 91, 975–981 (1986)

40 Esplugues JV, Whittle BJR and Moncada S: Local opioid-sensitive afferent sensory neurons in the modulation of gastric damage induced by Paf. Br J PharmacoL 97, 579–585 (1989)

41 Le Creque JS and Whittle BJR: Endogenous nitric oxide as a mediator of gastric mucosal vasodilation during acid secretion. Gastroenterology 102, 168–174 (1992)

42 Lippe IT and Holzer P: Participation of endothelium-derived nitric oxide but not prostacyclin in the gastric mucosal hyperemia due to acid back-diffusion. Br J PharmacoL 105, 708–714 (1992)

43 Whittle BJR, Lopez-Belmonte J and Moncada S: Nitric oxide mediates rat mucosal vasodilation induced by intragastric capsaicin. Eur J PharmacoL 218, 339–341 (1992)

44 Brain SD, Williams TJ, Tippins JR, Morris HR and MacIntyre I: Calcitonin gene-related peptide is a potent vasodilator. Nature 313, 54–56 (1985)

45 Pesker BM, Respondek M, Müller KM and Pesker BA: A role of nitric oxide in capsaicin-induced gastroprotection. Eur J PharmacoL 198, 113–114 (1991)

46 Uchida M, Yano S and Watanabe K: The role of capsaicin-sensitive afferent nerve in protective effect of capsaicin against absolute ethanol-induced gastric lesions in rats. Jpn J PharmacoL 55, 279–282 (1991)

47 Holzer P, Pabst MA, Lippe IT, Pesker BA, Livingston EH and Guth PH: Afferent nerve mediated protection against deep mucosal damage in the rat stomach. Gastroenterology 98, 838–848 (1990)

48 Murakami M, Saita H, Teramura S, Dekigai H, Asagoe K, Kusaka S and Kita T: Gastric ammonia has a potent ulcerogenic action on the rat stomach. Gastroenterology 105, 1710–1715 (1993)

49 Tsuji M, Kawano S, Tsuji S, Fusamoto H, Kamada T and Sato N: Mechanism of gastric mucosal damage induced by ammonia. Gastroenterology 102, 1881–1888 (1992)

50 Marshall BJ: Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1, 1273–1275 (1983)

51 Marshall BJ and Langton SR: Urea hydrolysis in patients with Campylobacter pyloridis infection. Lancet 1, 965–966 (1986)

52 Graham DY, Klein PD, Evans DJ, Evans DG, Alpert LC, Opekun AR and Boulton TW: Campylobacter pylori detected noninvasively by the 13C-urea breath test. Lancet 1, 1174–1177 (1987)

53 Goodwin CS, Armstrong JA and Marshall BJ: Campylobacter pyloridis, gastritis, and peptic ulceration. J Clin Pathol 39, 353–365 (1986)

54 Shibata M, Onodera S, Tanaka M, Suzuki T, Inaba N, Fujii S and Yamamura T: Role of capsaicin sensitive afferent nerve in ammonia-induced adaptive gastroprotection. Ulcer Res 21, 230–232 (1994)

55 Onodera S, Shibata M, Tanaka M, Suzuki T, Inaba N, Fujii S and Yamamura T: Gastroprotective mechanism of a newly developed H2 receptor antagonist, FRG-8813. Ulcer Res 21, 54–57 (1994)

56 Buck SH and Burks TF: The neuropharmacology of capsaicin: review of some recent observations. Pharmacol Rev 38, 179–226 (1986)

57 Hagel J, Renner H, Hirsch G, Weig G, Kaduk B, Ruppin H and Domshke W: Gastric cytoprotection by antacids and papaervine in rats. Hepatogastroenterology 29, 271–274 (1982)

58 Tarnawski A, Hollander D, Gergely H and Stachura J: Comparison of antacid, sucralfate, cimetidine and ranitidine in protection of the gastric mucosa against ethanol injury. Am J Med 79, Supp 26, 19–23 (1985)

59 O’Brien PE, Frydman G, Holmes R, Malcontenti C and Phelan D: Evaluation of putative cytoprotective properties of antilucer drugs using quantitative histological techniques. Dig Dis Sci 35, 1130–1139 (1990)

60 Konturek SJ, Mach T, Konturek JW, Bogdal J and Stachura J: Comparison of sucralfate and ranitidine in gastroprotection against alcohol in humans. Am J Med 86 (6A), 55–59 (1989)

61 Ichikawa T, Ishihara K, Saita H and Hotta K: Effects of acid-inhibitory antilucer drugs on mucin biosynthesis in the rat stomach. Eur J PharmacoL 251, 107–111 (1994)