Role of Capsaicin-Sensitive Sensory Neurons and Nitric Oxide in the Protective Effect of Lansoprazole, a Proton Pump Inhibitor, on the Gastric Mucosa in Rats

Izumi Murakami, Hiroshi Satoh, Shoichi Asano and Rika Maeda

Pharmaceutical Research Laboratories III, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd.,
2-17-85 Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

ABSTRACT—The mucosal protective effect of lansoprazole, a proton pump inhibitor, was examined in ethanol- and acidified taurocholate-induced rat gastric lesion models. The formation of gastric lesions was markedly inhibited by prostaglandin E_2 but hardly inhibited by cimetidine, ranitidine and famotidine. Lansoprazole (3–30 mg/kg, p.o.) inhibited the formation of gastric lesions in a dose-dependent manner, with ID_{50} values of 8.5 (ethanol) and 4.1 mg/kg, p.o. (acidified taurocholate). The protective effect of lansoprazole was significantly decreased by functional ablation of capsaicin-sensitive sensory neurons or prior administration of indomethacin or $N\omega$-nitro-L-arginine methyl ester (L-NAME), a selective inhibitor of nitric oxide (NO) synthesis. The inhibitory effect of L-NAME was antagonized by prior administration of L-arginine, a substrate of endogenous NO, but not D-arginine. The antisecretory effect of lansoprazole on the basal acid secretion in pylorus-ligated rats was not affected by any of these treatments. Lansoprazole (5 and 15 mg/ml) administered directly into the gastric chamber obviously increased both the production of NO in the mucosa and mucosal blood flow, which was prevented by pretreatment with L-NAME. These results suggest that capsaicin-sensitive sensory neurons, NO and prostaglandins are involved in the mucosal protection afforded by lansoprazole possibly via an increase in mucosal blood flow, but are not involved in the antisecretory action of lansoprazole.

Keywords: Lansoprazole, Proton pump inhibitor, Capsaisin-sensitive sensory neuron, Nitric oxide, Mucosal protection

Lansoprazole is a novel antiulcer drug that has both antisecretory and mucosal protective actions (1–3). The antisecretory action has been explained by the compound's inhibitory effect on the proton pump in parietal cells (4, 5), but the mode of action responsible for the mucosal protective effect has yet to be fully elucidated. It is reported that the other proton pump inhibitors, omeprazole (6–8) and NC-1300 (8), also have mucosal protective actions, although their mode of action has still not been fully clarified. Most of the previous work on mucosal protection centered on prostaglandins (PGs), but recently, it has been reported that both capsaicin sensitive sensory neurons (9) and nitric oxide (NO) (10, 11), a potent endothelium-derived relaxing factor, play an important role in gastric mucosal protection.

In the present study, we examined the effects of lansoprazole on ethanol- and acidified taurocholate-induced gastric lesions in the rat, and the results were compared with those obtained with omeprazole, prostaglandin E_2 (PGE$_2$) and histamine H_2-receptor antagonists. We also investigated the mode of the protective actions of lansoprazole with relation to capsaicin-senstive sensory neurons, endogenous nitric oxide, prostaglandins and mucosal blood flow.

MATERIALS AND METHODS

Animals

Seven-week old male Sprague-Dawley rats (Japan Clea, Ishibe) weighing 185 to 245 g were used. The animals were fasted for 24 hr but had free access to water.

Acute gastric lesion formation

Gastric lesions were induced by oral administration of 1 ml of absolute ethanol or acidified taurocholate (0.2 N HCl and 40 mM sodium taurocholate). The animals were

sacrificed by CO_2 asphyxiation 1 hr after receiving ethanol or acidified taurocholate. The stomachs were removed, filled with 10 ml of a 1% formalin solution and then immersed in the same formalin solution for 10 min. The stomachs were opened along the greater curvature. The length (mm) of the individual lesions in the corpus was measured under a dissecting microscope with a 1-mm square grid eyepiece ($\times$ 10), and the sum of the lengths of all the lesions in each stomach was used as the lesion index. Drugs were administered orally 30 min before ethanol or acidified taurocholate.

Functional ablation of capsaicin-sensitive sensory neurons

Functional ablation of capsacin-senstive sensory neurons (CSN) was performed as described by Takeuchi et al. (12). Rats were treated with a subcutaneous injection of capsaicin (20, 30 and 50 mg/kg, s.c.) for 3 consecutive days 2 weeks before the experiment. Capsaicin injections were performed under ether anesthesia. The animals were pretreated with terbutaline (0.1 mg/kg, intramuscularly) just before capsaicin injection to prevent the respiratory impairment associated with capsaicin injection. The functional ablation of CSN was confirmed by the lack of protective wiping movements after the instillation of a drop of capsaicin (0.1 mg/ml) into one eye just before the experiment.

Determination of the role of endogenous NO and PGs

To examine the possible involvement of endogenous NO in the gastric mucosal protective action of lansoprazole, 3 mg/kg of $N\omega$-nitro-L-arginine methyl ester (L-NAME), a selective inhibitor of NO synthesis, was administered intravenously 15 min before oral administration of lansoprazole.

In addition, to confirm the participation of endogenous NO in the effect of L-NAME, L-arginine or D-arginine (300 mg/kg each) was given intravenously 10 min before the administration of L-NAME, and the effect on L-NAME-induced inhibition was examined.

To elucidate the role of endogenous PGs in the gastric mucosal protective action of lansoprazole, indomethacin (10 mg/kg) was injected subcutaneously 60 min before the administration of lansoprazole.

Measurment of gastric mucosal blood flow and NO production

Under urethane (1.2 g/kg, i.p.) anesthesia, the stomach was exposed by laparotomy, and an incision was made into the forestomach. The stomach was then mounted on a Lucite chamber (2 cm in diameter) as described by Takeuchi et al. (12). Saline (0.9%) warmed to 37°C was placed inside this chamber in contact with the mucosal surface of the corpus. A laser probe was placed on the corpus mucosa using a balancer, and gastric mucosal blood flow was measured by a laser Doppler flowmeter (Model ALE-2100; Advance, Tokyo). NO production was measured according to the methods described by Ichimori et al. (13) and Mitsuhata et al. (14) with an NO-sensitive electrode (Intermedical Co., Tokyo) inserted into the gastric corpus mucosa. After the blood flow and NO production were stabilized, each drug was given intravenously or introduced directly into the chamber. In addition, to examine the possible involvement of endogenous NO synthesis in the gastric mucosal protection afforded by lansoprazole, 3 mg/kg of L-NAME was administered intravenously 30 min before application of lansoprazole. Changes in gastric mucosal blood flow are represented as the percentage (%) of the basal value just before the administration of each drug. Changes in NO prodution are represented by the pA-order redox current.

Determination of gastric acid secretion

After laparotomy under light ether anesthesia, the pylorus was ligated, and the abdomen was closed by suturing. Lansoprazole (10 mg/kg) or vehicle was given intraduodenally just after the pylorus was ligated. The animals were sacrificed 3 hr later by CO_2 asphyxiation, and the stomachs were removed. The gastric contents were collected and centrifuged at 1,500$\times g$ for 10 min. The volume of supernatant was measured, and the acid concentration was determined by automatic titration (TTA81; Radiometer, Copenhagen, Denmark) to pH 7.0 with 0.1 N NaOH. The total acid output during the 3-hr period was calculated.

Drugs

The following drugs were used: taurocholic acid, terbutaline, L-NAME, D-arginine, L-arginine, indomethacin, sodium nitroprusside (SNP), famotidine, ranitidine and cimetidine (Sigma, St. Louis, MO, USA); capsaicin (Wako Pure Chemical, Osaka); PGE_2 (Fuji Chemical, Toyama). Lansoprazole and omeprazole were synthesized in our division. Taurocholic acid was dissolved in distilled water. Capsaicin was dissolved in a saline containing 10% ethanol and 10% Tween 80 (Wako Pure Chemical) for subcutaneous injection and was suspended in a 0.5% methylcellulose solution for oral administration. Terbutaline, L-NAME, D-arginine, L-arginine and SNP were dissolved in saline. Indomethacin was suspended in a 0.5% methylcellulose solution. Lansoprazole, omeprazole, famotidine, ranitidine, cimetidine and PGE_2 were suspended in a 0.5% methylcellulose solution containing 1% $NaHCO_3$ for oral administration and in a 0.5% methylcellulose solution for intraduodenal administration and direct application to the gastric chamber. Each

drug was prepared just beforethe experiment and given orally, intraduodenally, intravenously or subcutaneously in a volume of 0.2 ml/100 g body wt.

Statistics

In the study on gastric lesions, treatment with drugs in each animal was designed in a randomized fashion, and the gastric lesions were measured by a person who did not know which treatment the animal received. Data are expressed as the mean values with the standard error. Statistical significance among groups was determined by Dunnett's test or by Student's t-test for unpaired values. ID_{50} values were calculated from the dose-inhibition relationships by the method of least squares. Fiducial limits of ID_{50} values were calculated according to Fieller's theorem (15).

RESULTS

Effect on ethanol-induced gastric mucosal lesions

Administration of absolute ethanol produced severe band-like lesions with congestion in the corpus mucosa; the mean lesion index in the control group was 83.6 ± 14.5 mm (n = 12). Both lansoprazole (3 – 30 mg/kg, p.o.) and omeprazole (3 – 30 mg/kg, p.o.) inhibited the formation of gastric lesions dose-dependently; the ID_{50} values were 8.5 and 15.3 mg/kg, p.o., respectively (Fig. 1). The effects of lansoprazole in doses of 10 and 30 mg/kg and
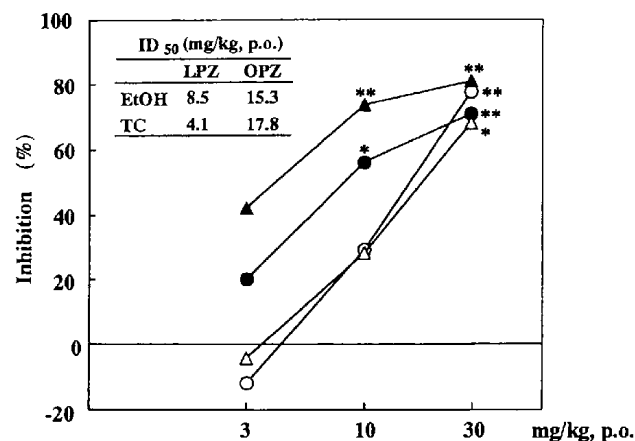
Fig. 1. Effects of lansoprazole (LPZ) and omeprazole (OPZ) on gastric lesions induced by absolute ethanol (EtOH) or acidified taurocholate (TC) in rats. EtOH-induced gastric lesions: ●, LPZ; ○, OPZ; TC-induced gastric lesions: ▲, LPZ; △, OPZ. Each drug or vehicle was administered orally after a 24-hr fast, and 30 min later, 1 ml of EtOH or TC (0.2 N-HCl and 40 mM-sodium taurocholate) was administered by gastric gavage. The animals were sacrificed 1 hr later, and gastric mucosal lesions in the corpus were measured. The results are expressed as percentage inhibition with respect to the lesion index in the vehicle group. Each point represents the mean value for 12 rats. *P < 0.05, **P < 0.01 vs vehicle.

omeprazole at a dose of 30 mg/kg were significant (Fig. 1). The formation of lesions was inhibited by 92% by PGE_2 (0.1 mg/kg, p.o.) (Fig. 2A), but hardly affected by cimetidine, ranitidine and famotidine even at a dose as high as 100 mg/kg, p.o. (data not shown).

Effects on acidified taurocholate-induced gastric lesions

Administration of either 0.2 N HCl or 40 mM taurocholate caused only reddening of the corpus mucosa. However, acidified taurocholate (0.2 N HCl and 40 mM taurocholate) produced many linear lesions in the corpus mucosa; the lesion index in the control group was 69.6 ± 11.5 mm (n = 7). Both lansoprazole (3 – 30 mg/kg, p.o.) and omeprazole (3 – 30 mg/kg, p.o.) dose-dependently prevented the formation of gastric lesions; the ID_{50} values were 4.1 and 17.8 mg/kg, p.o., respectively (Fig. 1). PGE_2 (0.1 mg/kg, p.o.) inhibited the formation of gastric lesions by 84% (Fig. 2B). On the other hand, the lesions were not significantly inhibited by oral administration of 100 mg/kg of cimetidine, ranitidine or famotidine (data not shown).
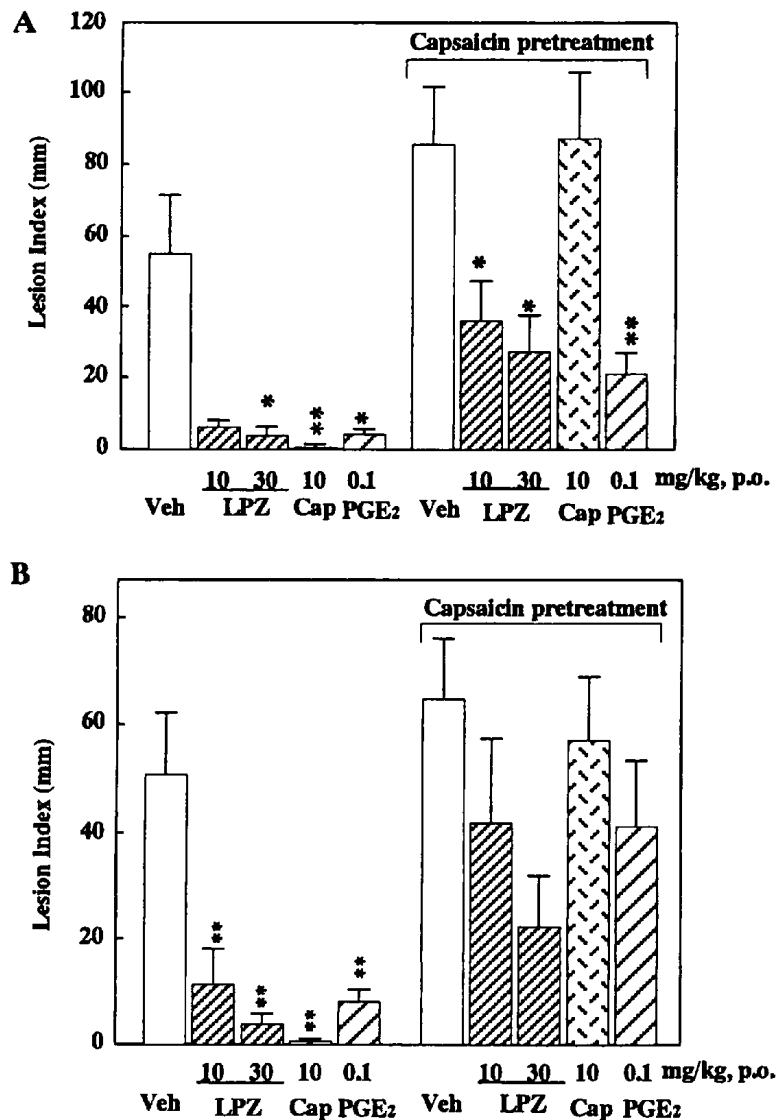
Effects of functional ablation of CSN on the mucosal protection afforded by lansoprazole

Ethanol-induced gastric mucosal lesions: The formation of gastric lesions induced by absolute ethanol was markedly inhibited by oral administration of lansoprazole (10 and 30 mg/kg), capsaicin (10 mg/kg) or PGE_2 (0.1 mg/kg) (Fig. 2A). Pretreatment with capsaicin caused an increase in the gastric mucosal lesions induced by ethanol, but the effect was not significatly as compared with the non-treated group (Fig. 2A). The inhibitory effect of capsaicin was almost completely abolished in the rats pretreated with capsaicin (Fig. 2A). Pretreatment with capsaicin reduced the protective effect of lansoprazole on the gastric mucosa and partially counteracted that of PGE_2 (Fig. 2A).

Acidified taurocholate-induced gastric lesions: The formation of gastric lesions induced by acidifid taurocholate was significantly inhibited by oral administration of lansoprazole (10 and 30 mg/kg), capsaicin (10 mg/kg) or PGE_2 (0.1 mg/kg) (Fig. 2B). Pretreatment with capsaicin did not affect the gastric mucosal lesions induced by acidified taurocholate as compared with the non-treated group. Pretreatment with capsaicin completely abolished the inhibitory effect of capsaicin and obviously reduced the effects of lansoprazole and PGE_2 (Fig. 2B).

Effects of L-NAME on the mucosal protective effect of lansoprazole

Ethanol-induced gastric lesions: Pretreatment with L-NAME (3 mg/kg, i.v.) did not affect the lesion index of the lesions induced by absolute ethanol, but increased the

Fig. 2. Effect of functional ablation of capsaicin-sensitive sensory neurons on inhibitory actions of lansoprazole (LPZ), cap-saicin (Cap) and PGE$_2$ against absolute ethanol (A)- or acidified taurocholate (B)-induced gastric lesions in rats. Capsaicin (20, 30 and 50 mg/kg) or vehicle was given s.c. for 3 consecutive days 2 weeks before the animals were used for the experiment. Each drug or vehicle was administered orally 30 min before the administration of absolute ethanol or acidified taurocholate, and gastric lesions were measured 1 hr later. Each column and bar represents the mean value and the S.E. for 6 rats. *P<0.05, **P<0.01 vs vehicle.

depth of the lesions. Pretreatment with L-NAME mark-edly reduced the protective effect of lansoprazole (10 and 30 mg/kg, p.o.) and partially prevented the effect of capsaicin at 10 mg/kg, but did not affect that of PGE$_2$ (0.1 mg/kg, p.o.) (Fig. 3A).

To examine the possibility that endogenous NO biosyn-thesis is involved in the effect of lansoprazole, the effect of L-arginine or D-arginine (300 mg/kg, i.v.) on the inhib-itory action of L-NAME was examined. As shown in Fig. 4, the inhibitory effect of L-NAME on the protective ac-tion of lansoprazole was reversed by the administration

of L-arginine but not D-arginine.

Acidified taurocholate-induced gastric lesions: Pre-treatment with L-NAME (3 mg/kg, i.v.) did not affect the gastriclesion formation induced by acidified tauro-cholate as compared with the group given vehicle. Pre-treatment with L-NAME reduced the protective action of lansoprazole (10 and 30 mg/kg), capsaicin (10 mg/kg) and PGE$_2$ (0.1 mg/kg) (Fig. 3B).

Effects of indomethacin on the mucosal protective effect of lansoprazole

Ethanol-induced gastric lesions: Pretreatment with indomethacin (10 mg/kg, s.c.) did not affect the leson index of the lesions induced by absolute ethanol, but slightly increased the depth of the lesions as compared with the group given the vehicle. Pretreatment with indomethacin reduced the protective action of lansoprazole (10 and 30 mg/kg, p.o.), but did not affect that of PGE$_2$ (0.1 mg/kg) (Fig. 5A).

Acidified taurocholate-induced gastric lesions: Pretreatment with indomethacin (10 mg/kg, s.c.) increased the gastric lesion index induced by acidified taurocholate, but the effect was not significatly as compared with the group given vehicle. As seen with the ethanol-induced gastric lesions, the protective effect of lansoprazole was reduced by indomethacin-treatment, but the protective action of PGE$_2$ was not affected (Fig. 5B).
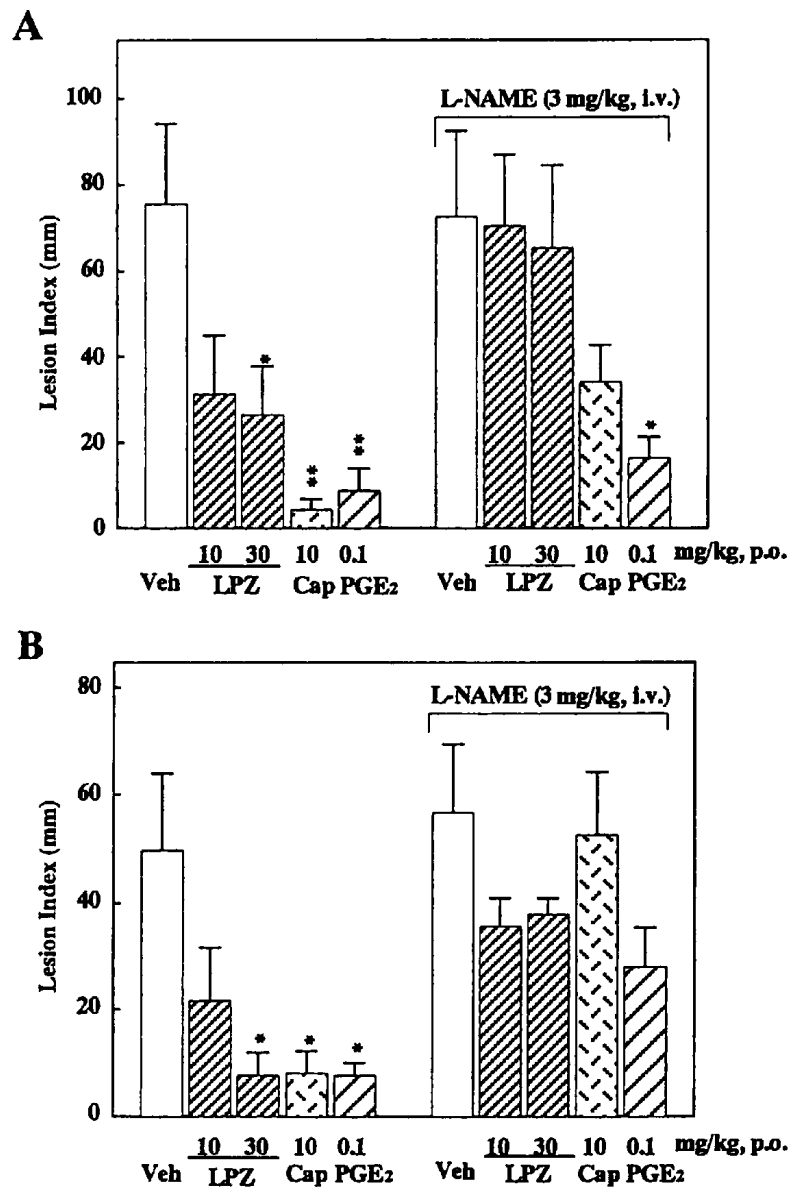
Fig. 3. Effect of L-NAME on the inhibitory actions of lansoprazole (LPZ), capsaicin (Cap) and PGE$_2$ against absolute ethanol (A)- or acidified taurocholate (B)-induced gastric lesions in rats. Each drug (LPZ, Cap or PGE$_2$) was administered orally 30 min before the administration of absolute ethanol or acidified taurocholate, and gastric lesions were measured 1 hr later. L-NAME (3 mg/kg) or vehicle was given i.v. 15 min before the administration of LPZ, Cap or PGE$_2$. Each column and bar represents the mean value and the S.E. for 6 rats. *P<0.05, **P<0.01 vs vehicle.
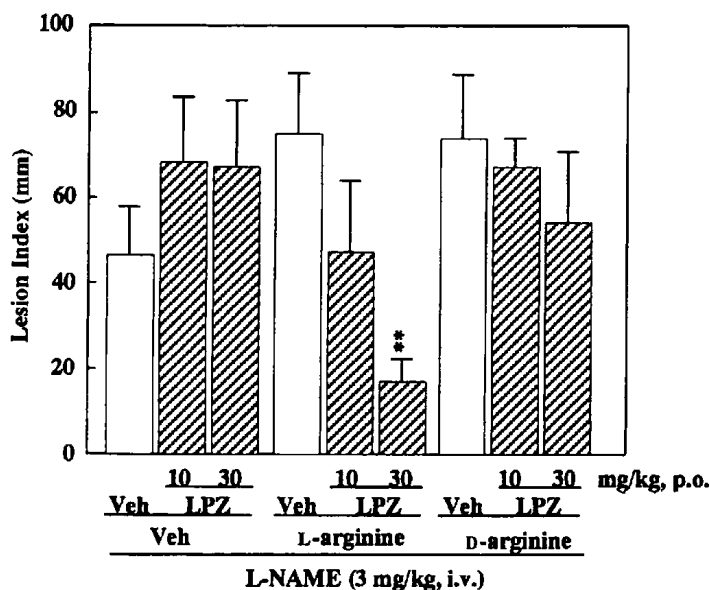
Fig. 4. Effect of L- and D-arginine on the inhibitory action of L-NAME against the protective effect of lansoprazole (LPZ) against ethanol-induced gastric lesions in rats. LPZ was administered orally 30 min before the administration of absolute ethanol, and gastric lesions were measured 1 hr later. L-NAME (3 mg/kg) was given i.v. 15 min before the administration of LPZ, and L-arginine (300 mg/kg), D-arginine (300 mg/kg) or vehicle was given i.v. 10 min before the administration of L-NAME. Each column and bar represents the mean value and the S.E. for 6 rats. **P < 0.01 vs vehicle.
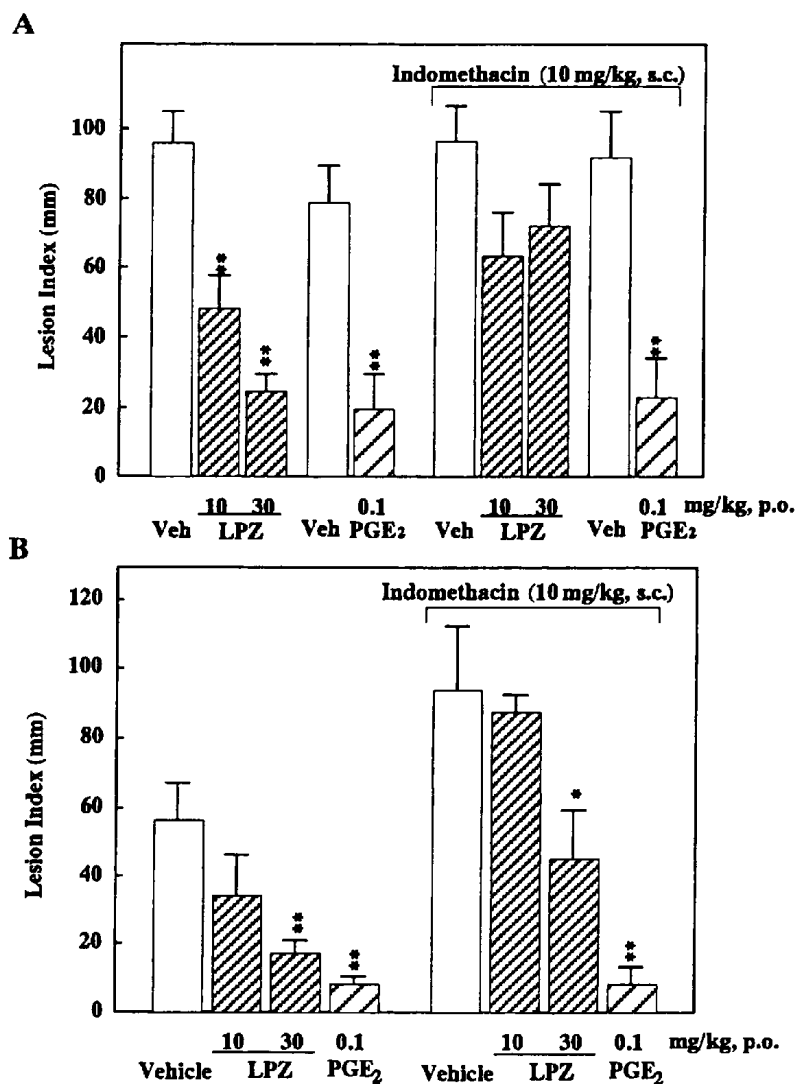

Fig. 5. Effect of indomethacin on the inhibitory actions of lansoprazole (LPZ) and PGE$_2$ against absolute ethanol (A)- or acidified taurocholate (B)-induced gastric lesions in rats. LPZ, PGE$_2$ or vehicle was administered orally 30 min before the administration of absolute ethanol or acidified taurocholate, and gastric lesions were measured 1 hr later. Indomethacin (10 mg/kg) was given s.c. 60 min before the administration of each drug or vehicle. Each column and bar represents the mean value and the S.E. for 6 rats. *P < 0.05, **P < 0.01 vs vehicle.

Effects on NO production and blood flow in the gastric mucosa

Intravenous administration of sodium nitroprusside (0.3−3 mg/kg) caused a dose-dependent and sustained increase in both NO production and blood flow in the gastric mucosa. As shown in Fig. 6, the maximal responses in NO production and blood flow induced by 1 mg/kg of SNP were obtained 10 min and 15 min after drug administration, and the values were 432.9±55.5 pA and 142.7±10.5% (n=6), respectively. Topical application of lansoprazole (5 and 15 mg/ml) also dose-depend-

ently increased NO production in the mucosa after a transient decrease as shown in Fig. 7 (data of 5 mg/ml are not shown). The maximal increase in NO production induced by 15 mg/ml was seen 8 min after drug administration, and the value was 201.7±82.6 pA (n=6). The mucosal blood flow was also increased by lansoprazole (Fig. 7B). The maximal response was obtained 15 min after application of lansoprazole, and the value was 81.1±30.7% (n=6). Pretreatment with L-NAME (3 mg/kg, i.v.) significantly reduced the increase in NO production and blood flow in the gastric mucosa induced
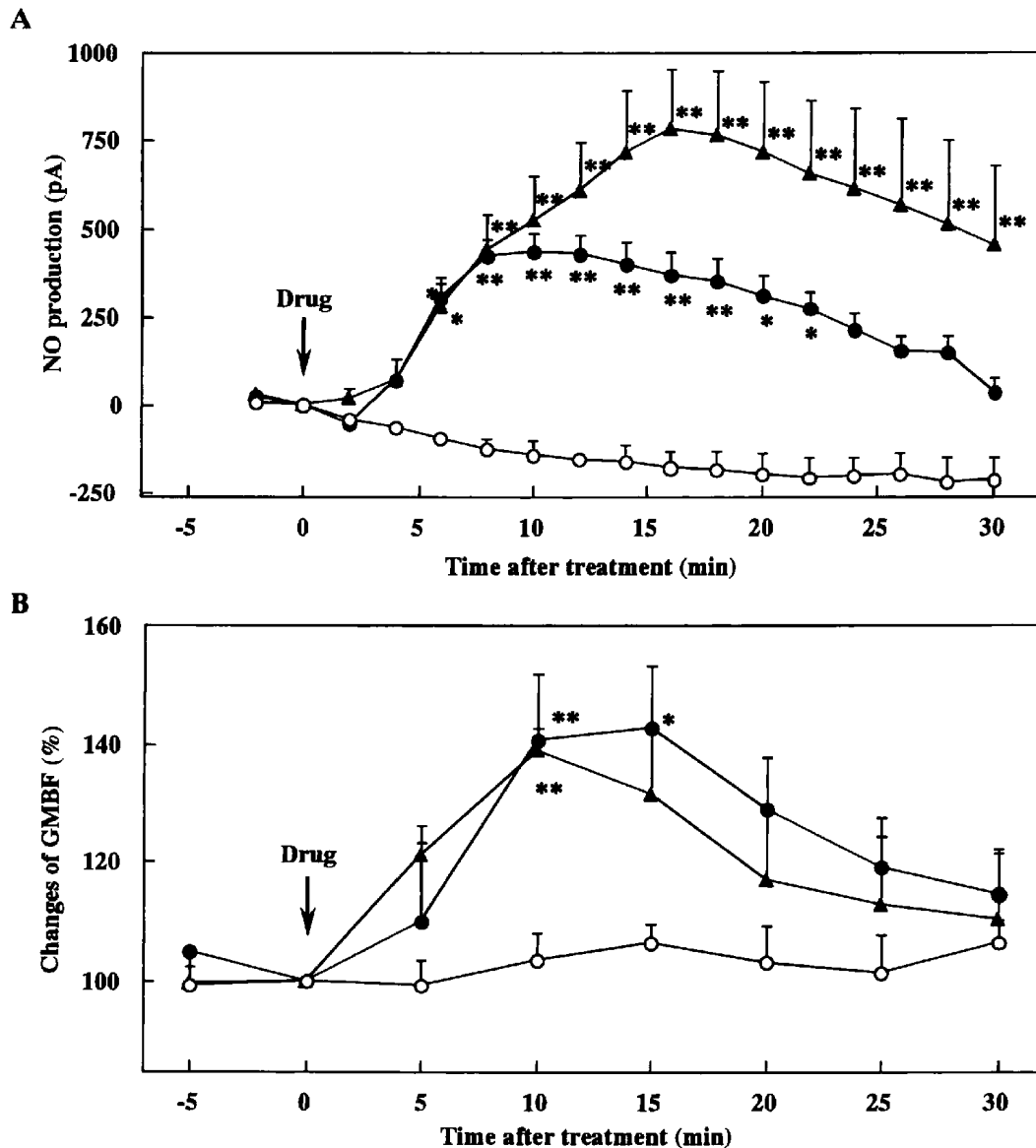
Fig. 6. Effect of sodium nitroprusside (SNP) on NO production (A) and blood flow (B, GMBF) in the gastric mucosa in rats. ○, vehicle; ●, SNP; ▲, L-NAME+SNP. NO production and blood flow in the gastric mucosa were measured using an NO-sensitive electrode and laser Dopplar flowmeter, respectively. SNP (1 mg/kg) or vehicle was given i.v. L-NAME (3 mg/kg) was given i.v. 30 min before the administration of SNP. Each point represents the mean value and the S.E. for 6 (SNP), 10 (vehicle) and 7 (L-NAME+SNP) rats. *P<0.05, **P<0.01 vs vehicle.

by lansoprazole (Fig. 7), but did not affect the increases induced by SNP (1 mg/kg, i.v.; Fig. 6).

Effects of functional ablation of CSN, L-NAME and in-domethacin on the antisecretory action of lansoprazole

Pretreatment with high doses of capsaicin markedly increased basal gastric secretion both in volume and acidity. Lansoprazole (10 mg/kg, i.d.) inhibited the acid secretion by 98% in the normal rat, and the effect was not affected by pretreatment with capsaicin (Table 1).

The antisecretrory effect of lansoprazole (10 mg/kg, i.d.) was not prevented by pretreatment with L-NAME (3 mg/kg, i.v.) or indomethacin (10 mg/kg, s.c.) (Table 1).

DISCUSSION

We have already reported that lansoprazole has both antisecretory and gastric mucosal protective actions (1), but the mode of responsible for the protective activity has yet to be fully elucidated. In the present study, we investigated the mechanism of the gastric mucosal protective effect of lansoprazole using two experimental models, i.e., gastric lesions induced in rats by absolute ethanol or acidified taurocholate. The lesion formation in these two
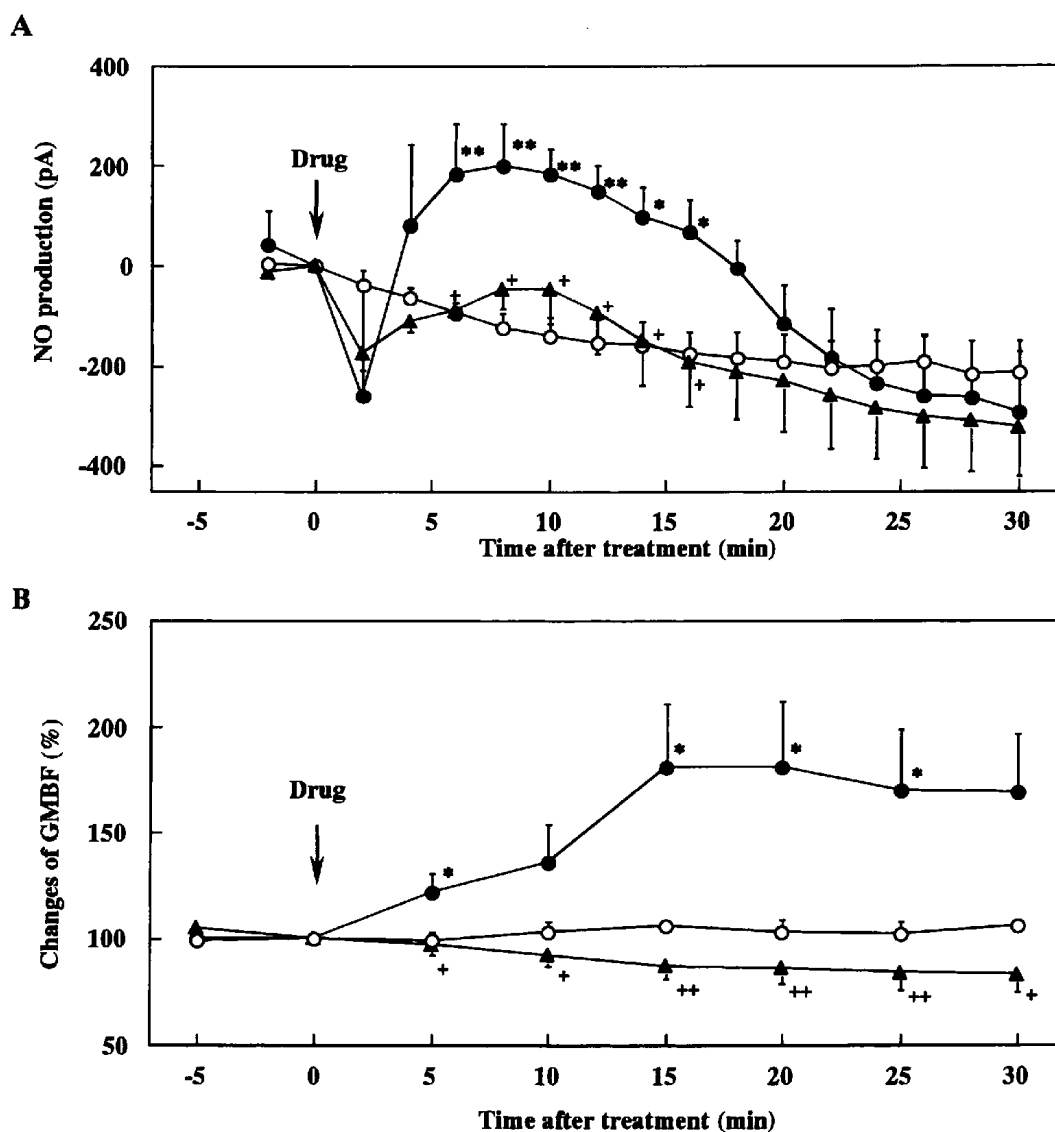
Fig. 7. Effect of lansoprazole (LPZ) on NO production (A) and blood flow (B, GMBF) in the gastric mucosa in rats. ○, vehicle; ●, LPZ; ▲, L-NAME+LPZ. NO production and blood flow in the gastric mucosa were measured using an NO-sensitive electrode and laser Dopplar flowmeter, respectively. LPZ (15 mg/ml) or vehicle was introduced directly into the chamber. L-NAME (3 mg/kg) was given i.v. 30 min before the administration of LPZ. Each point represents the mean value and the S.E. for 6 (LPZ), 10 (vehicle) and 7 (L-NAME+LPZ) rats. $*P<0.05$, $**P<0.01$ vs vehicle, $^-P<0.05$, $^{++}P<0.01$ vs LPZ.

Table 1. Effects of functional ablation of capsaisin-sensitive sensory nerves, L-NAME and indomethacin on the antisecretory action of lansoprazole in pylorus-ligated rats

Pretreatment	Treatment	No. of rats	Total acid output (μEq/3 hr)	Inhibition (%)
Vehicle	Vehicle	6	196.3±42.6	—
Vehicle	Lansoprazole	6	8.3± 4.5**	96
Capsaicin[a]	Vehicle	6	633.8±76.5[++]	—
Capsaicin	Lansoprazole	6	6.0± 3.8**	99
Vehicle	Vehicle	6	161.8±27.9	—
Vehicle	Lansoprazole	6	26.5± 5.6**	84
L-NAME[b]	Vehicle	6	148.8±28.3	—
L-NAME	Lansoprazole	6	42.5± 6.4*	71
Vehicle	Vehicle	6	123.0±38.6	—
Vehicle	Lansoprazole	6	22.1± 7.3**	82
Indomethacin[c]	Vehicle	6	303.8±84.2	—
Indomethacin	Lansoprazole	6	13.7± 6.4*	95

[a] Capsaicin (20, 30 and 50 mg/kg) or vehicle was given s.c. for 3 consecutive days 2 weeks before the animals were used for the experiment. [b] L-NAME (3 mg/kg) or vehicle was given i.v. 15 min before the administration of LPZ. [c] Indomethacin (10 mg/kg) or vehicle was given s.c. 60 min before the administration of LPZ. LPZ (10 mg/kg) or vehicle was given i.d. just after the pylorus was ligated. Data show the mean values and the S.E. *P<0.05, **P<0.01 vs vehicle, [++]P<0.01 vs normal control (vehicle+vehicle).

models was markedly inhibited by low doses of PGE$_2$, but hardly inhibited by doses of cimetidine, ranitidine and famotidine sufficient to inhibit gastric acid secretion. In both these models, lansoprazole and omeprazole inhibited the formation of lesions in a dose-dependent manner, and the protective effects of lansoprazole were 2 and 4 times as strong as those of omeprazole. These results support the previous findings that lansoprazole has a protective action on the gastric mucosa in addition to its antisecretory action (1).

It has been reported that capsaicin, a pungent ingredient found in red pepper, exerts a selective and powerful excitatory effect on peripheral sensory nerve endings and that intragastric administration of low doses of capsaicin has gastric mucosal protective effects via inducing the release of vasoactive peptides such as calcitonin gene-related peptide, substance P and vasoactive intestinal polypeptide (16−18). On the other hand, it is reported that high doses of capsaicin cause functional ablation of CSN by exhausting the store of neurotransmitters (18). These were confirmed in the present study, i.e., the administration of a low dose of capsaicin (10 mg/kg) showed a protective effect on the gastric mucosa, and this effect was markedly inhibited by 3 consecutive days of pretreatment with high doses of capsaicin (20, 30 and 50 mg/kg). The protective effect of lansopra-

zole against ethanol- or acidified taurocholate-induced gastric lesions was obviously reduced by functional ablation of CSN by pretreatment with high doses of capsaicin. The results suggest that the gastric mucosal protective action of lansoprazole is caused, at least in part, by an excitation of CSN. However, functional ablation of CSN did not completely counteract the protective action of lansoprazole, suggesting the possibility that other mechanisms are also involved in the protective action of lansoprazole.

It has been reported that NO, a potent endothelium-derived relaxing factor, protects the gastric mucosa via increasing mucosal blood flow (10, 19). In our study, pretreatment with L-NAME, a selective inhibitor of NO synthesis, reduced the protective effects of lansoprazole on the gastric mucosa. Furthermore, the effect of L-NAME was antagonized by L-arginine, a substrate of endogenous NO, but not by pretreatment with D-arginine. These results suggest that endogenous NO plays an important role in the protective action of lansoprazole. To confirm the participation of NO in the protective effects of lansoprazole, we examined whether lansoprazole increases the production of NO in the gastric mucosa and increases the gastric mucosal blood flow in the gastric chamber method using an NO-sensitive electrode and a laser Doppler flowmeter. Introducing lansoprazole direct-

ly into the chamber increased the production of NO in the corpus mucosa and caused an increase in mucosal blood flow, although a transient decrease in NO production preceded the increase. Similar increasing effects on both NO production and blood flow were obtained when sodium nitroprusside, an NO-releasing agent, was administrated intravenously. These results strongly suggest that lansoprazole protects the gastric mucosa by increasing mucosal blood flow via increasing NO production in the gastric mucosa, although some discrepancy was observed in the time course of NO production and mucosal blood flow after treatment with lansoprazole.

It is well-known that PGs play an important role in protection of the gastrointestinal mucosa. Therefore, we examined whether PGs are involved in the protective action of lansoprazole and found that the protective effects of lansoprazole were mildly inhibited by pretreatment with indomethacin in both gastric lesion models. These results suggest that part of the protective effect of lansoprazole is caused via stimulation of endogenous PG synthesis.

There are many reports suggesting the involvement of NO and PGs in the protective action via CSN. Namely, Brzozowski et al. (20) have reported that both NO and PGs contribute to the gastroprotective action of capsaicin, but Lambrecht et al. (21) showed that NO is partly involved and PGs are not. Furthermore, Takeuchi et al. (12) and Uchida et al. (22) reported that there is a close relationship between the stimulation of CSN and endogenous PGs. In the present study, the inhibitory effects of PGE_2 on gastric lesions induced by acidified taurocholate were obviously decreased by functional ablation of CSN or by pretreatment with L-NAME, but those on ethanol-induced gastric lesions were not affected. It has been reported that ethanol causes mucosal lesions mainly by disturbing the mucosal microcirculation, followed by congestive hyperemia and increased permeability of the vessels (23), and that acidified taurocholate causes mucosal lesions by breaking down the mucosal barrier, followed by back-diffusion of acid and pepsin. It has also been reported that PGE_2 protects the gastrointestinal mucosa through several actions such as increased mucosal blood flow, mucus secretion and bicarbonate secretion (24 – 26). The results of the present study suggest that PGE_2 prevents ethanol-induced lesion formation probably by acting directly on the gastric mucosal microvessels, and that neither CSN nor NO is involved in the protective action of PGE_2 in this model. On the other hand, in acidified taurocholate-induced lesions it is suggested that PGE_2 protects the mucosa through a CSN- or NO-sensitive pathway, possibly involving an increase of bicarbonate or mucus secretion. This is partly supported by a report suggesting an important role of CSN in bicar-

bonate secretion (27). There have been several reports concerning the role of CSN in gastric secretion (28 – 30), but consistent results have not been obtained. In the present study, we observed that functional ablation of CSN markedly increased basal gastric acid secretion, indicating the inhibitory control of gastric secretion by CSN. However, capsaicin administered topically into the stomach (1 – 30 mg/kg, i.g.) did not affect basal gastric secretion (data not shown). The protective effect of lansoprazole on the stomach was significantly decreased by functional ablation of CSN and reduced by pretreatment with L-NAME or indomethacin. In addition, lansoprazole increased gastric mucosal blood flow and NO production in the mucosa. On the other hand, the antisecretory effect of lansoprazole was not affected by any of these treatments. These results suggest that capsaicin-sensitive sensory neurons, NO and prostaglandins are involved in the mucosal protection afforded by lansoprazole, probably via an increase in mucosal blood flow, but are not involved in the antisecretory action of lansoprazole. Recently we have reported that lansoprazole stimulates bicarbonate secretion via CSN in rats (31). Therefore, there is a possibility that lansoprazole causes NO production via CSN stimulation, as seen in bicarbonate secretion, although further studies are needed to confirm this.

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