ELSEVIER

European Journal of Pharmacology 432 (2001) 203–210

ejp

www.elsevier.com/locate/ejphar

Effects of vanilloid receptor agonists and antagonists on gastric antral ulcers in rats

Hisashi Yamamoto [a], Syunji Horie [a,*], Masayuki Uchida [b], Shizuko Tsuchiya [a], Toshihiko Murayama [a], Kazuo Watanabe [a]

[a] *Laboratory of Chemical Pharmacology, Department of Drug Evaluation and Toxicological Sciences, Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*

[b] *Food Science and Evaluation Department, Food Functionality Research Institute, Meiji Milk Products Co., Ltd., Kanagawa, Japan*

Received 7 June 2001; received in revised form 17 October 2001; accepted 19 October 2001

Abstract

Defunctionalization of capsaicin-sensitive afferent nerves by pretreatment with a neurotoxic dose of capsaicin aggravates gastric ulcers in rats. In the present study, we investigated the roles of vanilloid receptors in gastric antral ulcers, using vanilloid receptor agonists and antagonists. Gastric antral ulcers were induced by a combination of diethyldithiocarbamate and 1 N HCl in refed rats. The administration of ruthenium red (1.5 mg/kg, s.c., twice daily) aggravated gastric antral ulcers (ulcer index: control, 33.7 ± 13.7 mm^2; ruthenium red, 99.9 ± 11.0 mm^2). A similar result was observed in rats pretreated with a neurotoxic dose of capsaicin. On the other hand, capsaicin (1–10 mg/kg, p.o., twice daily) inhibited antral ulcer formation (ulcer index: control, 99.2 ± 20.6 mm^2; capsaicin 10 mg/kg, 37.0 ± 11.7 mm^2). A similar effect was obtained in rats treated with the novel antiulcer drug, lafutidine (3–10 mg/kg, p.o., twice daily), which has gastroprotective activity mediated by capsaicin-sensitive afferent nerves. The antiulcer effects of capsaicin and lafutidine were abolished by ruthenium red and by pretreatment with a neurotoxic dose of capsaicin. These results suggest that vanilloid receptors play a gastroprotective role in gastric antral ulcers. In addition, treatment with ruthenium red may be an alternative tool for defunctionalization of capsaicin-sensitive afferent nerves.

Keywords: Capsaicin; Gastric antral ulcer; Ruthenium red; Lafutidine; Vanilloid receptor

1. Introduction

Capsaicin-sensitive afferent nerves have long been a focus of studies on gastroprotection in the gut. Caterina et al. (1997) reported that capsaicin, the main pungent ingredient of chili peppers, binds to specific receptors, the vanilloid receptors. Vanilloid receptors are ionotropic receptors on primary afferent nerves. Capsaicin stimulates primary afferent 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, 1998; Abdel-Salam et al., 1997; Mózsik et al., 1997). On the other hand, the function of capsaicin-sensitive afferent nerves is lost after a few days on pretreatment with a neurotoxic dose of capsaicin (Holzer, 1991; Abdel-Salam et al., 1997; Mózsik et al., 1997).

It was reported that capsaicin inhibits the formation of lesions in the stomach via the activation of gastroprotective mechanisms such as an increase in gastric mucosal blood flow (Holzer and Lippe, 1988). Furthermore, it was reported that a neurotoxic dose of capsaicin potentiates the formation and delays the healing of gastric lesions through degeneration of capsaicin-sensitive afferent nerves (Esplugues and Whittle, 1990; Takeuchi et al., 1994a,b).

In the present study, we tried to elucidate the role of vanilloid receptors in the gastroprotective action of capsaicin by using the so-called functional vanilloid receptor agonists and antagonists. Ruthenium red and capsazepine are functional antagonists of vanilloid receptors. Ruthenium red is shown to block nonselective cation channels involved in vanilloid receptors (Maggi et al., 1988, 1989; Wood et al., 1988; Szallasi and Blumberg, 1999). On the other hand, capsazepine binds to vanilloid receptors and competitively antagonizes the effect of capsaicin (Dickenson and Dray, 1991). In the present study, we used ruthenium red as a functional antagonist of vanilloid receptors. Capsazepine could have been used as an antagonist in our investigation.

* Corresponding author. Tel.: +81-43-290-2924; fax: +81-43-290-3021.
E-mail address: syunji@p.chiba-u.ac.jp (S. Horie).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01481-9

Another study we performed showed that capsazepine inhibited capsaicin-induced gastroprotection at a dose 10 times higher than that of ruthenium red against HCl-induced lesions (to be submitted). Rudd and Wai (2001) reported that ruthenium red is 100 times more potent than capsazepine to inhibit the emetic action of capsaicin. Thus, the usefulness of capsazepine is limited by its moderate potency (Szallasi and Blumberg, 1999). One should be careful when using capsazepine at relatively higher doses as a vanilloid receptor antagonist because the drug blocks voltage-dependent calcium channels and nicotinic acetylcholine receptors (Szallasi and Blumberg, 1999). Therefore, capsazepine is not necessarily a better tool than ruthenium red as an antagonist of vanilloid receptors. In addition, it would have been too costly to study the effect of capsazepine administered twice daily for 3 days on formation of antral ulcers in our investigation. Therefore, ruthenium red was used instead of capsazepine.

Defunctionalization of capsaicin-sensitive afferent nerves by pretreatment with a neurotoxic dose of capsaicin has been used in the study of capsaicin-sensitive afferent nerves, but this involves time-consuming and labour-intensive animal care (Holzer, 1991; Abdel-Salam et al., 1997; Mózsik et al., 1997). We, thus, investigated whether administration of ruthenium red could be substituted for pretreatment with a neurotoxic dose of capsaicin.

In most gastric ulcer models in animals, gastric lesions are induced in the corpus of the stomach. Oi et al. (1968, 1969) reported that human gastric ulcers are located in the antrum of the stomach in most cases. We studied animal gastric antral ulcer models and produced gastric ulcers selectively in the antrum of the stomach in rats (Uchida et al., 1991a,b; 1999). Rat gastric antral ulcers are similar to human ulcers with regard to location and histology. We studied the involvement of vanilloid receptors in gastroprotection using this method.

Recently, a novel histamine H_2 receptor antagonist, lafutidine ((±)-2-(furfurylsulfinyl)-*N*-[4-[4-(piperidinomethyl)-2-pyridyl]oxy-(*Z*)-2-butenyl] acetamide), which protects gastric mucosa through activation of capsaicin-sensitive afferent nerves (Yamaura et al., 1992; Shibata et al., 1993; Onodera et al., 1995), has come into clinical use for peptic ulcers. We chose it as an activator of the vanilloid receptor-mediated pathway. In the present study, we investigated the involvement of vanilloid receptors in lafutidine-induced gastroprotection, using ruthenium red. The effect was compared with that of famotidine, another histamine H_2 receptor antagonist.

2. Materials and methods

2.1. Animals

Seven-week-old male Sprague–Dawley rats (160–290 g, Charles River, Japan) were used after preliminary rearing on tap water and a pellet diet in cages with raised mesh floors. The experiments were carried out in strict accordance with Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and the guideline approved by the Ethical Committee on Animal Care and Animal Experiment of the Faculty of Pharmaceutical Sciences, Chiba University.

2.2. Chemicals

Capsaicin (Wako, Osaka, Japan) was dissolved in a solution of 10% ethanol, 10% Tween 80 (Kanto Chemical, Tokyo, Japan) and 80% physiological saline for subcutaneous administration or in 5% ethanol plus 0.1% Tween 80 solution for oral administration. Famotidine (Sigma, St. Louis, MO, USA) and lafutidine were suspended in 5% gum arabic solution. Ruthenium red (Sigma), diethyldithiocarbamate (Wako), terbutaline (Sigma) and aminophylline (Sigma) were dissolved in saline. Each agent was given orally in a volume of 0.5 ml/100 g body weight, subcutaneously in a volume of 0.2 ml/100 g and intramuscularly in a volume of 0.05 ml/100 g. Lafutidine was the kind gift of Taiho Pharmaceutical (Tokyo, Japan).

2.3. Gastric antral ulcers

Gastric antral ulcers were induced by treatment with 1 N HCl in refed rats as described previously (Uchida et al., 1999). Briefly, rats were deprived of food but allowed free access to tap water for 48 h before the experiment. The fasted rats were refed later for 1 h, then injected subcutaneously with diethyldithiocarbamate at a dose of 250 mg/kg. One hour later, 1 N HCl was administered orally in a volume of 0.5 ml/100 g. The rats were deprived of food but allowed free access to tap water for 6 h after HCl administration. Capsaicin (1, 3 or 10 mg/kg), famotidine (3 mg/kg) or lafutidine (3 or 10 mg/kg) was given orally twice daily for 3 days after the HCl treatment. Ruthenium red (1.5 mg/kg) or diethyldithiocarbamate (250 mg/kg) was injected subcutaneously 30 min before treatment with the gastroprotective drugs twice daily for 3 days from day 1. On day 4 after HCl administration, the animals were killed by deep chloroform anesthesia for evaluation of gastric ulcers.

2.4. Gross morphological evaluation of gastric antral ulcers

After the rats were killed, the stomach was excised and the pylorus and cardia of the stomach were ligated. Seven milliliters of saline was instilled into the stomach and the outside of the stomach was fixed with 5% formalin for 20 min. The stomach was rinsed with saline and cut along the greater curvature. Gastric antral ulcers were evaluated using a software for image analysis (NIH Image version 1.55, Wayne Rasband, National Institutes of Health,

Bethesda, MD, USA). The areas of ulcerated gastric antrum (mm^2) were expressed as the ulcer index. Animals who died during the experiments were excluded from the analysis.

2.5. Histological studies

After assessment of gross damage, tissues from several animals were fixed in 5% formalin and subsequently embedded in paraffin for histological examination. Sections were cut at 4 μm and stained with hematoxylin and eosin.

2.6. Defunctionalization of the capsaicin-sensitive afferent nerves

Rats received capsaicin (125 mg/kg, s.c.) over 2 days, with doses of 25 mg/kg in the morning and 50 mg/kg in the afternoon on the first day and 50 mg/kg in the morning on the second day. This treatment is known to elicit permanent degeneration of unmyelinated afferent neurons (Holzer, 1991; Mózsik et al., 1997). Control animals received an equal volume of vehicle (10% ethanol, 10% Tween 80 and 80% physiological saline) in the same way. Capsaicin was given under ether anesthesia. 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. In order to check the effectiveness of the treatment, a drop of a 0.1% solution of capsaicin was instilled into either eye of the rats and their protective wiping movements were counted. The capsaicin-treated animals that showed any wiping movement were excluded from the study. The rats were used for the experiment 10–14 days after the treatment with capsaicin.

2.7. Statistical analyses

Data are presented as means ± S.E.M. Statistical analyses were performed by two-tailed Student's *t*-test for comparison of two groups and by one-way analysis of variance followed by Dunnett's multiple comparison test for comparison of more than three groups. Values of $P < 0.05$ were regarded as significant.

3. Results

3.1. Effects of ruthenium red, a blocker of cation channels involved in vanilloid receptors, on gastric antral ulcers

HCl and diethyldithiocarbamate were administered to refed rats to induce gastric antral ulcers. On day 4 after the administration of HCl, severe gastric ulcers were observed in the antral area of the stomach as indicated by an arrow in Fig. 1A. Histological studies showed the apparent pathology of gastric ulcers (Fig. 2A). The ulcers had penetrated the muscularis mucosae and the thickness of the mucosa was markedly diminished, with loss of structure of the gastric glands. On the other hand, no lesions were

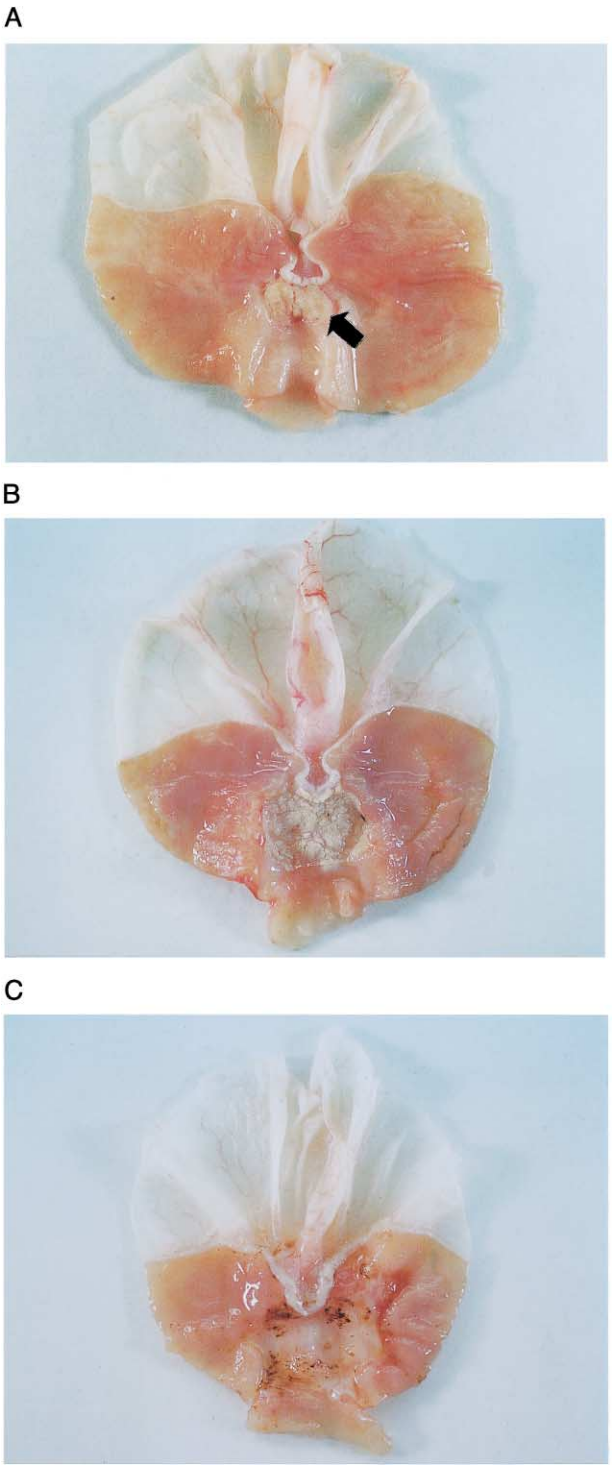

Fig. 1. Aggravation of gastric antral ulcers by repeated administration of diethyldithiocarbamate or ruthenium red on day 4 after HCl treatment. (A) Control: antral ulcer induced by the combination of diethyldithiocarbamate (250 mg/kg, s.c.) and 1 N HCl in refed rat. (B) Repeated treatment with diethyldithiocarbamate (250 mg/kg, s.c.). (C) Repeated treatment with ruthenium red (1.5 mg/kg, s.c.).

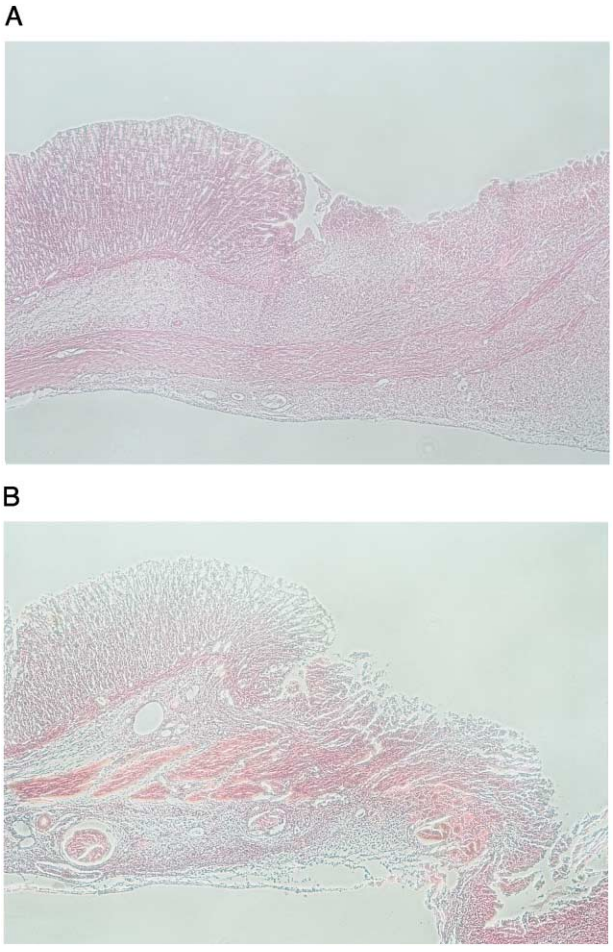

Fig. 2. Aggravation of gastric antral ulcers due to repeated administration of ruthenium red induced on day 4 after HCl treatment in refed rat. Gastric tissues were histologically examined for ulcers on day 4 (stained with hematoxylin and eosin, ×40). (A) Control: antral ulcer induced by the combination of diethyldithiocarbamate (250 mg/kg, s.c.) and 1 N HCl (5 ml/kg, p.o.) in refed rat. (B) Repeated treatment with ruthenium red (1.5 mg/kg, s.c.).

found in the corpus area (Fig. 1A). Antral ulcers present on day 1 reached a maximum on days 3–4 and thereafter decreased gradually as reported previously (Uchida et al., 1999).

Diethyldithiocarbamate, which was administered to rats twice daily for 3 days from day 1, greatly aggravated the gastric antral ulcers (Fig. 1B). Diethyldithiocarbamate is known to inhibit superoxide dismutase (Heikkila et al., 1976; Takeuchi et al., 1991). The ulcers were twice as large as those of the control group (Fig. 3). Ruthenium red, which was administered twice daily for 3 days from day 1, markedly increased the ulcers (Fig. 1C). The ulcer index was almost identical to that obtained with diethyldithiocarbamate treatment (Fig. 3). Deep histological injury was observed in the stomach of ruthenium red-treated rats (Fig. 2B). The ulcers had penetrated the tunica muscularis and reached the serosal side. Administration of ruthenium red had no obvious effect on the behaviour of the rats.

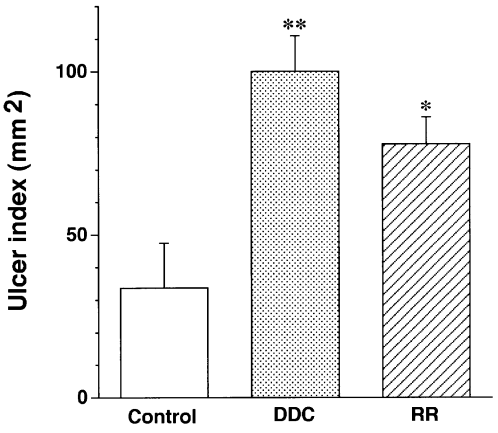

Fig. 3. Effects of repeated administration of diethyldithiocarbamate (DDC) and ruthenium red (RR) on gastric antral ulcers. Gastric antral ulcers were induced by the combination of DDC and 1 N HCl in refed rats. DDC (250 mg/kg, s.c.) and RR (1.5 mg/kg, s.c.) were administered twice daily for 3 days after HCl treatment. Each value represents the mean ± S.E.M. of data obtained from 6–11 rats. $*P<0.05$, $**P<0.01$, significant difference from the control group.

Reflex responses such as the wiping response were abolished in rats treated with ruthenium red.

Defunctionalization of the capsaicin-sensitive afferent nerves also induced a significant increase in the ulcer index (Fig. 4).

3.2. Effects of vanilloid receptor agonists on gastric antral ulcers

We examined the effect of capsaicin on gastric antral ulcers in rats. A low dose of capsaicin inhibited the formation of antral ulcers aggravated by successive administrations of diethyldithiocarbamate (Fig. 5). The effect was dependent on dosage (1–3 mg/kg, p.o., twice a day for 3 days) (Fig. 6). The maximum effect was obtained at

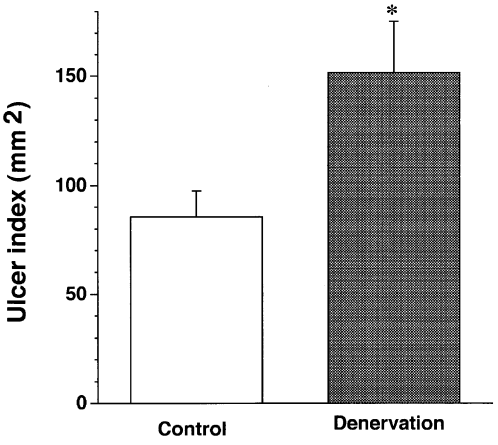

Fig. 4. Effect of capsaicin-sensitive afferent nerve degeneration on gastric antral ulcers induced by the combination of diethyldithiocarbamate and 1 N HCl in refed rats. Each value represents the mean ± S.E.M. of data obtained from 9–11 rats. $*P<0.05$, significant difference from the control group.

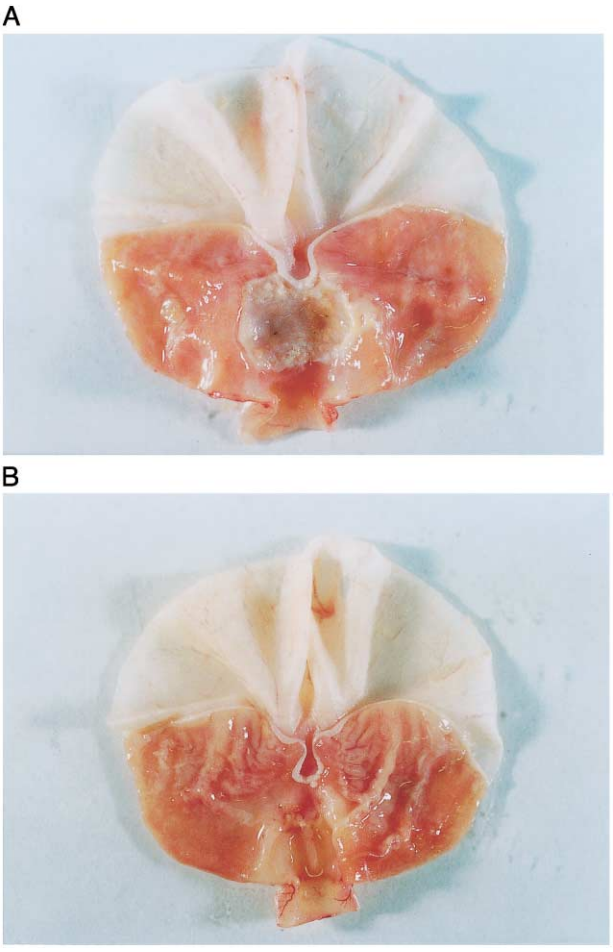

Fig. 5. Macroscopic effect of capsaicin on gastric antral ulcers in rats treated with repeated administration of diethyldithiocarbamate. (A) Control: repeated treatment with diethyldithiocarbamate (250 mg/kg, s.c.). (B) Repeated treatment with diethyldithiocarbamate (250 mg/kg, s.c.) and capsaicin (3 mg/kg p.o.).

3 mg/kg. The antiulcer effect of capsaicin at 10 mg/kg was approximately equipotent with that at 3 mg/kg. Lafutidine, a novel histamine H_2 receptor antagonist, also inhibited antral ulcer formation (3–10 mg/kg, p.o., twice a day for 3 days) (Fig. 6). The inhibitory effect of lafutidine at 10 mg/kg was more potent than that at 3 mg/kg. On the other hand, 3 mg/kg famotidine, another histamine H_2 receptor antagonist that does not activate capsaicin-sensitive afferent nerves, induced a slight, but not significant, decrease in the ulcer index (Fig. 6). The preliminary experiment showed that the antiulcer effect of 10 mg/kg famotidine (ulcer index 80.0 mm^2) was almost equipotent with that at 3 mg/kg. The maximum effect was obtained at 3 mg/kg.

3.3. Effects of ruthenium red on gastroprotection induced by capsaicin and lafutidine

Gastric antral ulcers induced by combination of HCl and diethyldithiocarbamate in refed rats were aggravated

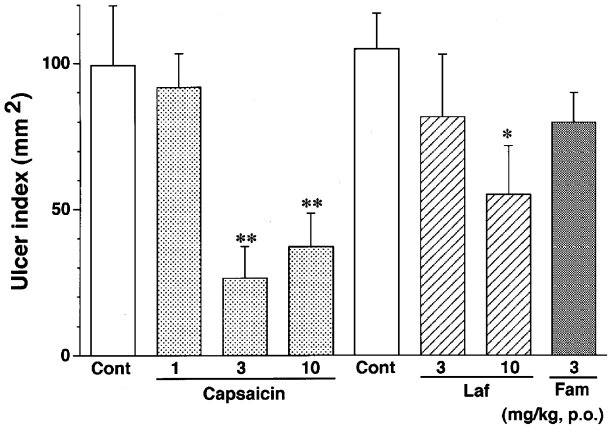

Fig. 6. Effects of capsaicin, lafutidine (Laf) and famotidine (Fam) on gastric antral ulcers in diethyldithiocarbamate-treated rats. Diethyldithiocarbamate (250 mg/kg, s.c.) was administered twice daily for 3 days after HCl treatment. Capsaicin, Laf or Fam was administered orally twice daily for 3 days after HCl treatment. Each value represents the mean ± S.E.M. of data obtained from 4–6 rats. One rat died in the course of the experiment in each group of control and capsaicin 1 mg/kg. Animals who died during the experiments were excluded from the analysis. Cont: control group. $*P<0.05$, $**P<0.01$, significant difference from the corresponding control group.

by ruthenium red (1.5 mg/kg, s.c., twice daily for 3 days). The antral ulcer index was 76.2 ± 16.3 mm^2. The gastroprotective effects of capsaicin on the antral ulcers were abolished in ruthenium red-treated rats (Fig. 7). Lafutidine also failed to inhibit gastric antral ulcer formation in ruthenium red-treated rats (Fig. 7). In addition, the effects of capsaicin and lafutidine on antral ulcers were studied in

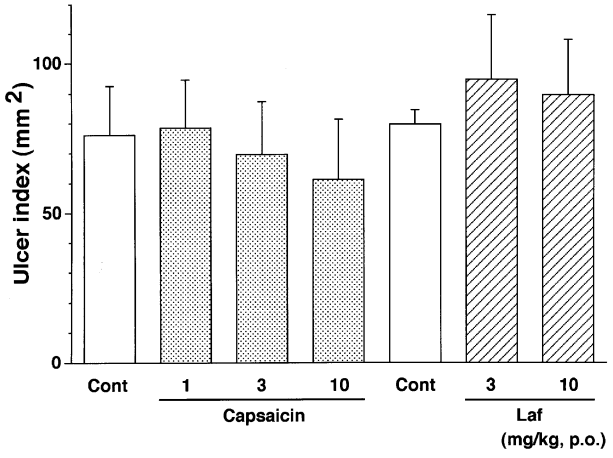

Fig. 7. Effects of capsaicin and lafutidine (Laf) on gastric antral ulcers in ruthenium red-treated rats. Ruthenium red (1.5 mg/kg, s.c.) was administered twice daily for 3 days after HCl treatment. Capsaicin or Laf was orally administered twice daily for 3 days after HCl treatment. Each value represents the mean ± S.E.M. of data obtained from 5–6 rats. One rat died in the course of the experiment in each group of capsaicin 3 and 10 mg/kg. Animals who died during the experiments were excluded from the analysis. Cont: control group.

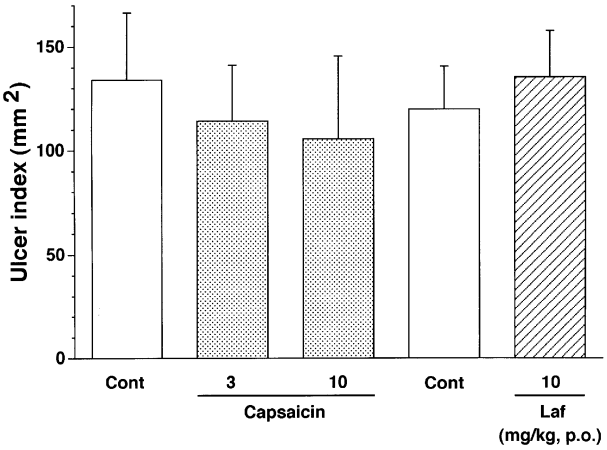

Fig. 8. Effects of capsaicin and lafutidine (Laf) on gastric antral ulcers in rats with degenerated capsaicin-sensitive afferent nerves. Capsaicin or Laf was administered orally twice daily for 3 days after HCl treatment. Each value represents the mean ± S.E.M. of data obtained from 4–7 rats. One rat died in the course of the experiment in each group of control, capsaicin 3 and 10 mg/kg and lafutidine 10 mg/kg. Animals who died during the experiments were excluded from the analysis. Cont: control group.

chemically deafferentated rats and again did not show gastroprotection (Fig. 8).

4. Discussion

4.1. Gastric antral ulcer model

The majority of human gastric ulcers occurs in the antrum of the stomach (Oi et al., 1968, 1969). Accordingly, gastric ulcer models that are induced selectively in the antral area in rats are important for studying the gastroprotective effects of antiulcer drugs. Satoh et al. (1981) and Maeda-Hagiwara and Watanabe (1983) established animal models for gastric antral ulcers, but the effect of antiulcer drugs cannot be clearly demonstrated in these models because of low frequency and small ulcer formation. We succeeded in inducing large antral ulcers in rats by refeeding and administering HCl only (Uchida et al., 1998, 1999). This combination of refeeding and HCl with administration of diethyldithiocarbamate elicited more severe antral ulcers in almost all rats, thus, providing an animal model of antral ulcers with which to evaluate effects of antiulcer drugs. The administration of diethyldithiocarbamate twice daily for 3 days after ulceration significantly aggravated the gastric antral ulcers. We used these antral ulcer models to study the roles of vanilloid receptors in gastroprotection.

4.2. Aggravation of gastric antral ulcers by ruthenium red

Capsaicin-sensitive afferent nerves play important roles in the physiological function of the stomach and in defense mechanisms of the gastric mucosa. Capsaicin stimulates the capsaicin-sensitive afferent nerves to enhance the defensive mechanisms of the gastric mucosa (Holzer and Lippe, 1988). When defunctionalization of capsaicin-sensitive afferent nerves is accomplished by pretreatment with a neurotoxic dose of capsaicin over a few days, gastric lesions in the corpus of the stomach are aggravated compared to those of sham-pretreated rats (Esplugues and Whittle, 1990). We also reported that absolute ethanol-induced lesions in the antrum are aggravated by defunctionalization of capsaicin-sensitive afferent nerves (Uchida et al., 1993).

In the present study, we used our newly established model of gastric antral ulcers to study the roles of capsaicin-sensitive afferent nerves in gastroprotective mechanisms. Ruthenium red aggravated gastric antral ulcers induced by the combination of refeeding, HCl and diethyldithiocarbamate by blocking cation channels involved in vanilloid receptors. Thus, the blockade of vanilloid receptors results in the weakening of gastroprotective mechanisms in the gastric antrum. The functional ablation of capsaicin-sensitive afferent nerves also increased the ulcer index. Defunctionalization of capsaicin-sensitive afferent nerves depletes afferent neuron-derived neuropeptides such as CGRP and substance P from the stomach, leading to aggravation of gastric antral ulcers due to decreased gastric mucosal blood flow (Holzer, 1998). These results also demonstrated the protective role of vanilloid receptors in the antrum. It is likely that luminal proton or unidentified endogenous agonists for vanilloid receptors protect the gastric mucosa under physiological and pathological conditions.

Defunctionalization of capsaicin-sensitive afferent nerves induced by a neurotoxic dose of capsaicin has been used as a tool for studies on capsaicin-sensitive afferent nerves, but the treatment is time-consuming and labour-intensive. On the other hand, ruthenium red is known to block the effects mediated by vanilloid receptors and to inactivate capsaicin-sensitive afferent nerves (Maggi et al., 1988; Wood et al., 1988). The present results suggest that treatment with ruthenium red can be an alternative for pretreatment with a neurotoxic dose of capsaicin in the experiments with experimental ulcer models. Ruthenium red was used for a similar purpose in analgesic experiments (Szolcsányi et al., 1993). Pretreatment of rats with a neurotoxic dose of capsaicin leads to defunctionalization of capsaicin-sensitive afferent nerves which is accompanied by the depletion of sensory neuropeptides (Holzer, 1998). Thus, treatment with ruthenium red may not completely replace for the pretreatment of rats with a neurotoxic dose of capsaicin.

4.3. Gastroprotection by capsaicin and lafutidine against gastric antral ulcers

Capsaicin has gastroprotective effects against gastric antral ulcers. Capsaicin inhibits the aggravation of gastric

antral ulcers induced by successive administration of diethyldithiocarbamate, probably due to the elevated gastric mucosal blood flow produced via sensory neuropeptides such as CGRP released by capsaicin. It is reported that CGRP-(8-37), a CGRP receptor antagonist, and a monoclonal antibody to CGRP abolish capsaicin-induced gastroprotection in acute gastric corpus lesions (Lambrecht et al., 1993; Peskar et al., 1993). Capsaicin also promotes the healing of acetic acid-induced gastric ulcers in rats (Kang et al., 1996). These findings provide evidence that capsaicin-sensitive afferent nerves play an important role in gastroprotection against gastric antral ulcers.

Lafutidine is a novel antiulcer drug possessing a long-lasting gastric antisecretory effect due to blockade of histamine H_2 receptors (Shibata et al., 1993). In addition, lafutidine also has gastroprotective activity due to activation of capsaicin-sensitive nerves (Onodera et al., 1995; Umeda et al., 1999). Lafutidine has potent antiulcer effects on indomethacin-induced gastric corpus lesions in fasted rats (Yamaura et al., 1992) and on antral ulcers in refed rats (Onodera et al., 1999). Further, the present study also showed that lafutidine significantly inhibits gastric antral ulcer formation at a dose of 10 mg/kg. The antiulcer effect of lafutidine was compared with that of famotidine, another histamine H_2 receptor antagonist. Famotidine at a dose of 3 mg/kg did not significantly inhibit gastric antral ulcer formation. Famotidine is known to inhibit gastric acid secretion 10 times more potently than lafutidine (Yamaura et al., 1992; Shibata et al., 1993). These data confirm that lafutidine has a gastroprotective activity unrelated to its antisecretory activity and famotidine has no gastroprotective activity even at its higher doses (Inatomi et al., 1991; Onodera et al., 1995).

4.4. Involvement of vanilloid receptors in capsaicin-elicited gastroprotection

We studied the involvement of capsaicin-sensitive afferent nerves and vanilloid receptors in the gastroprotective effects of capsaicin and lafutidine using ruthenium red. The gastroprotective effects of capsaicin and lafutidine were abolished by treatment with ruthenium red. In addition, capsaicin and lafutidine also failed to inhibit gastric antral ulcer formation in chemically deafferentated rats. These results suggest that the protective effects of capsaicin and lafutidine are elicited mainly via activation of vanilloid receptors on capsaicin-sensitive afferent nerves.

4.5. Summary

In summary, we found that the vanilloid receptor agonist, capsaicin, inhibited gastric antral ulcer formation in rats, whereas ruthenium red, which blocks cation channels involved in vanilloid receptors, aggravated gastric antral ulcers. This is the first demonstration that vanilloid receptors play protective roles in the etiology of gastric antral ulcer in rats. Additionally, treatment with ruthenium red may be an alternative for the pretreatment of rats with a neurotoxic dose of capsaicin.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. The authors are grateful to the Tokushima Research Center of Taiho Pharmaceutical (Tokushima, Japan) for the kind gift of lafutidine.

References

Abdel-Salam, O.M.E., Szolcányi, J., Mózsik, G., 1997. Capsaicin and the stomach. A review of experimental and clinical data. J. Physiol. (Paris) 91, 151–171.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Dickenson, A.H., Dray, A., 1991. Selective antagonism of capsaicin by capsazepine: evidence for a spinal receptor site in capsaicin-induced antinociception. Br. J. Pharmacol. 104, 1045–1049.

Esplugues, J.V., Whittle, B.J.R., 1990. Morphine potentiation of ethanol-induced gastric mucosal damage in the rat. Gastroenterology 98, 82–89.

Heikkila, R.E., Cabbat, F.S., Cohen, G., 1976. In vitro inhibition of superoxide dismutase in mice by diethyldithiocarbamate. J. Biol. Chem. 251, 2182–2185.

Holzer, P., 1991. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol. Rev. 43, 143–201.

Holzer, P., 1998. Neural emergency system in the stomach. Gastroenterology 114, 823–839.

Holzer, P., Lippe, I.T., 1988. Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa. Neuroscience 27, 981–987.

Inatomi, N., Nagaya, H., Takami, K., Shino, A., Satoh, H., 1991. Effects of a proton pump inhibitor, AG-1749 (lansoprazole), on reflux esophagitis and experimental ulcers in rats. Jpn. J. Pharmacol. 55, 437–451.

Kang, J.Y., Teng, C.H., Chen, F.C., 1996. Effect of capsaicin and cimetidine on the healing of acetic acid induced gastric ulceration in the rat. Gut 38, 832–836.

Lambrecht, N., Burchert, M., Respondek, M., Muller, K.M., Peskar, B.M., 1993. Role of calcitonin gene-related peptide and nitric oxide in the gastroprotective effect of capsaicin in the rat. Gastroenterology 104, 1371–1380.

Maeda-Hagiwara, M., Watanabe, K., 1983. Gastric antral ulcers produced by the combined administration of indomethacin with 2-deoxy-d-glucose in the rat. Eur. J. Pharmacol. 89, 243–250.

Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Geppetti, P., Meli, A., 1988. Protective action of ruthenium red toward capsaicin desensitization of sensory fibers. Neurosci. Lett. 88, 201–205.

Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Bianco, E.D., Geppetti, P., Meli, A., 1989. The 'efferent' function of capsaicin-sensitive nerves: ruthenium red discriminates between different mechanisms of activation. Eur. J. Pharmacol. 170, 167–177.

Mózsik, G., Abdel-Salam, O.M.E., Szolcányi, J., 1997. Experimental gastric ulcer studies. In: Mózsik, G., Abdel-Salam, O.M.E., Szolcányi, J. (Eds.), Capsaicin-Sensitive Afferent Nerves in Gastric Mucosal Damage and Protection. Akadémiai Kiadó, Budapest, Hungary, pp. 41–94.

Oi, M., Oshida, K., Sugimura, S., 1968. The location of gastric ulcer. Gastroenterology 54, 740–741, suppl.

Oi, M., Ito, Y., Kumagai, F., Yoshida, K., Tanaka, Y., Yoshikawa, K., Miho, O., Kijima, M., 1969. A possible dual control mechanism in the origin of peptic ulcer. Gastroenterology 57, 280–293.

Onodera, S., Shibata, M., Tanaka, M., Inaba, N., Yamaura, T., Ohnishi, H., 1995. Gastroprotective activity of FRG-8813, a novel histamine H_2-receptor antagonist, in rats. Jpn. J. Pharmacol. 68, 161–173.

Onodera, S., Tanaka, M., Aoyama, M., Arai, Y., Inaba, N., Suzuki, T., Nishizawa, A., Shibata, M., Sekine, Y., 1999. Antiulcer effect of lafutidine on indomethacin-induced gastric antral ulcers in refed rats. Jpn. J. Pharmacol. 80, 229–235.

Peskar, B.M., Wong, H.C., Walsh, J.H., Holzer, P., 1993. A monoclonal antibody to calcitonin gene-related peptide abolishes capsaicin-induced gastroprotection. Eur. J. Pharmacol. 250, 201–203.

Rudd, J.A., Wai, M.K., 2001. Genital grooming and emesis induced by vanilloids in *Suncus murinus*, the house musk shrew. Eur. J. Pharmacol. 422, 185–195.

Satoh, H., Inada, I., Hirata, T., Maki, Y., 1981. Indomethacin produces gastric antral ulcers in the refed rat. Gastroenterology 81, 719–725.

Shibata, M., Yamaura, T., Inaba, N., Onodera, S., Chida, Y., Ohnishi, H., 1993. Gastric antisecretory effect of FRG-8813, a new histamine H_2 receptor antagonist, in rats and dogs. Eur. J. Pharmacol. 235, 245–253.

Szallasi, A., Blumberg, M., 1999. Vanilloid (Capsaicin) receptors and mechanisms. Pharmacol. Rev. 51, 159–211.

Szolcsányi, J., Nagy, J., Pethö, G., 1993. Effect of CP-96,345 a non-peptide substance P antagonist, capsaicin, resiniferatoxin and ruthenium red on nociception. Regul. Pept. 46, 437–439.

Takeuchi, K., Nishiwaki, H., Niida, H., Ueshima, K., Okabe, S., 1991. Duodenal ulcers induced diethyldithiocarbamate, a superoxide dismutase inhibitor, in rats: role of antioxidative system in the pathogenesis. Jpn. J. Pharmacol. 57, 299–310.

Takeuchi, K., Ohuchi, T., Okabe, S., 1994a. Capsaicin-sensitive sensory neurons in healing gastric lesions induced by HCl in rats. Dig. Dis. Sci. 39, 2543–2546.

Takeuchi, K., Ueshima, K., Ohuchi, T., Okabe, S., 1994b. The role of capsaicin-sensitive sensory neurons in healing of HCl-induced gastric mucosal lesions in rats. Gastroenterology 106, 1524–1532.

Uchida, M., Yano, S., Watanabe, K., 1991a. A reliable method for the production of antral gastric ulcer by a combination of 2-deoxy-d-glucose, aspirin and ammonia in rats. Jpn. J. Pharmacol. 56, 475–481.

Uchida, M., Yano, S., Watanabe, K., 1991b. Aggravation by the capsaicin-treatment of gastric antral ulcer induced by the combination of 2-deoxy-d-glucose, aspirin and ammonia in rats. Jpn. J. Pharmacol. 57, 377–385.

Uchida, M., Yano, S., Watanabe, K., 1993. Involvement of CGRP, substance P and blood circulation in aggravating mechanism of absolute ethanol-induced antral lesions by capsaicin treatment in rats. Jpn. J. Pharmacol. 62, 123–129.

Uchida, M., Kato, Y., Takayama, M., Tsuchiya, S., Horie, S., Yano, S., Watanabe, K., 1998. Causative factors on the formation of gastric antral ulcer in refed rats and aggravation of the antral ulcer by the combination with diethyldithiocarbamate. Ulcer Res. 25, 167–170.

Uchida, M., Takayama, M., Kato, Y., Tsuchiya, S., Horie, S., Watanabe, K., 1999. A novel method to produce extensive gastric antral ulcer in rats: pharmacological factors involved in the etiology of antral ulceration. J. Physiol. (Paris) 93, 437–442.

Umeda, M., Fujita, A., Nishiwaki, H., Takeuchi, K., 1999. Effect of lafutidine, a novel histamine H_2-receptor antagonist, on monochloramine-induced gastric lesions in rats: role of capsaicin-sensitive sensory neurons. J. Gastroenterol. Hepatol. 14, 859–865.

Wood, J.N., Winter, J., James, I.F., Rang, H.P., Yeats, J., Bevan, S., 1988. Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. J. Neurosci. 8, 3208–3220.

Yamaura, T., Shibata, M., Inaba, N., Onodera, S., Chida, Y., Ohnishi, H., 1992. Effects of FRG-8813, a new type histamine H_2 receptor antagonist, on various experimental gastric and duodenal lesions in rats. Folia Pharmacol. Jpn. 99, 401–410.