Contribution of capsaicin-sensitive sensory neurons to stress-induced increases in gastric tissue levels of prostaglandins in rats

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Harada, Naoaki, Kenji Okajima, Mitsuhiro Uchiba, Takeshi Katsuragi, Contribution of capsaicin-sensitive sensory neurons to stress-induced increases in gastric tissue levels of prostaglandins in rats. Am J Physiol Gastrointest Liver Physiol 285: G1214–G1224, 2003. First published July 31, 2003; 10.1152/ajpgi.00364.2002.—We examined whether capsaicin-sensitive sensory neurons might be involved in the increase in the gastric tissue level of prostaglandins, thereby contributing to the reduction of water immersion restraint stress (WIR)-induced gastric mucosal injury in rats. Gastric tissue levels of calcitonin gene-related peptide (CGRP), 6-keto-PGF1α, and PGE2 were transiently increased 30 min after WIR. These increases were significantly inhibited by subcutaneous injection of capsazepine (CPZ), a vanilloid receptor antagonist, and by functional denervation of capsaicin-sensitive sensory neurons, and CGRP-(8–37) significantly increased gastric tissue levels of prostaglandins in rats subjected to WIR. Such activation of capsaicin-sensitive sensory neurons might contribute to the reduction of WIR-induced gastric mucosal injury mainly by inhibiting neutrophil activation.

calcitonin gene-related peptide; endothelial cells; gastric mucosal injury; neutrophils.

CAPSAICIN-SENSITIVE SENSORY neurons, nociceptive neurons, are activated by a wide variety of noxious stimuli (7) and they have been shown to play an important role in gastric cytoprotection by increasing gastric mucosal blood flow through the release of calcitonin gene-related peptide (CGRP) (21). Because chemical ablation of the sensory fibers resulted in a marked increase in the severity of inflammation (39), capsaicin-sensitive sensory neurons might contribute to the reduction of tissue injury by regulating the inflammatory responses. PGL2 plays an important role in the gastric cytoprotection that prevents gastric mucosal injury induced by various noxious stimuli (24, 34). Activated neutrophils play critical roles in the development of the gastric mucosal injury induced by stress (13), nonsteroidal anti-inflammatory drugs (43), and hemorrhagic shock (27) by inducing local inflammation (39, 44, 45). Because PGL2 inhibits neutrophil activation by increasing the intracellular concentration of cAMP (22, 38), PGL2 might play a role in the gastric cytoprotection by inhibiting neutrophil activation. Consistent with this hypothesis are our previous findings showing that gastric tissue levels of PGL2 were significantly increased in rats subjected to water immersion restraint stress (WIR), which contributed to the prevention of stress-induced gastric mucosal injury mainly by inhibiting neutrophil activation (15, 16). However, the mechanism(s) by which the gastric tissue level of PGL2 increases in rats subjected to WIR are not known. Because capsaicin reduced the WIR-induced gastric mucosal injury by increasing the gastric tissue level of CGRP (33) and because CGRP has been shown to increase the endothelial production of PGL2 in vitro (6), activation of capsaicin-sensitive sensory neurons might contribute to gastric cytoprotection by increasing the gas-

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tric tissue level of PGI₂, which might reduce the gastric mucusal injury by inhibiting neutrophil activation.

PGE₂ is a well-known gastric cytoprotective agent synthesized in endothelial cells from PGH₂, a common precursor of PGI₂ (11). Because PGE₂ has been shown to possess biological activities similar to those of PGI₂, (46), activation of capsaicin-sensitive sensory neurons might increase the gastric tissue levels of PGE₂ as well as PGI₂, thereby contributing to the reduction of gas-

tric mucosal injury.

Recent studies have demonstrated that CGRP increases endothelial production of nitric oxide (NO) (3) and that NO, in turn, activates cyclooxygenase (COX)-1, thereby increasing endothelial production of prostaglandins (4). Taken together, these observations strongly suggest that CGRP might increase endothelial production of PGI₂ and PGE₂ through NO-mediated activation of COX-1.

In the present study, we examined whether activation of capsaicin-sensitive sensory neurons reduces the stress-induced gastric mucosal injury by inhibiting neutrophil activation in rats through increase in the endothelial production of prostaglandins. Furthermore, possible involvement of NO in the process leading to the increase of prostaglandin production was also examined.

**MATERIALS AND METHODS**

**Reagents.** Capsaicin; capsazepine (CPZ), an antagonist of capsaicin; N⁵-nitro-L-arginine methyl ester (l-NNAME), a nonselective inhibitor of NO synthase (NOS); l-NAME; a selective inhibitor of inducible form of NOS; and indomethacin, a nonselective inhibitor of COX, were purchased from Sigma (St. Louis, MO). NS-398, a selective inhibitor of COX-2, was a generous gift from Taisho Pharmaceutical (Saitama, Japan). Rats α-CGRP and human CGRP (8–37) were purchased from Peptide Institute (Osaka, Japan). All other reagents were of analytical grade.

**WIR-induced gastric mucosal lesion formation in rats.** Adult male Wistar rats (Nihon, Hamamatsu, Japan) weighing 280–320 g were used in each experiment. The care and handling of the animals were in accordance with the National Institutes of Health guidelines. All experimental procedures described below were approved by the Kumamoto University Animal Care and Use Committee. Before each experiment, rats were deprived of food but not water for 24 h. The animals were then placed in a restraint cage and im-

munized against capsaicin, which might increase the gastric tissue levels of PGE₂ as well (46), activation of capsaicin-sensitive sensory neurons reduces the gastric visceral afferent nerves was accomplished by high-dose capsaicin administration as previously described (8, 23, 29, 33). Rats received a total dose of 125 mg/kg capsaicin administered subcutaneously in divided doses over 2 days. Two weeks after treatment with high-dose capsaicin, animals were subjected to stress. To determine the effectiveness of the sensory afferent nerve denervation procedure, a drop of 0.001% capsaicin in saline was instilled into either eye of the rats, and their protective wiping movements were observed. Capsaicin-treated rats that showed any wiping movement were excluded from the study. Control animals were injected subcutaneously with 1 ml of 10% Tween 20/10% ethanol (10%) with normal saline.

**Determination of gastric CGRP level.** Gastric levels of CGRP were determined in animals before and during WIR by modification of the methods as described previously (10). Briefly, the stomachs were weighed and then homogenized in 3 ml of 2 N acetic acid. The homogenates were bathed in 90°C water for 20 min and then centrifuged at 4,500 g for 20 min. The supernatant was then added to 5 ml of 10% acetic acid and the solvent was evaporated under a stream of nitrogen gas. The concen-

tration of CGRP was assayed by using a specific enzyme immunoassay kit (SPI-BIO, Massey Cedex, France). The sensitivity of the CGRP assay was 10 pg/ml. The antisera were cross-reacted 100% of rat α- and β-CGRP according to the manufacturer's data sheet. Results are expressed as micrograms of CGRP per gram of tissue.

**Immunohistochemical staining of CGRP in the stomach.** The peroxide-antiperoxide technique was used for immunohistochemical staining of the stomach with anti-CGRP antibody according to methods described previously with slight modification (38). The unfixed tissue blocks of rat stomach were washed in dry ice-cooled optimum cutting temperature compound (Tissue Tek; Miles, Elkhart, IN). Sections (6–8 μm thick) were mounted on glass slides, immersed in absolute acetone at −20°C for 5 min, rinsed in PBS five times for 5 min each, and then incubated for 20 min with 10% normal rat antiserum for 1 h at 37°C with rabbit anti-CGRP polyclonal antibody at 1:100 dilution. After five rinses in PBS, the sections were treated

**Administration of various agents.** Capsaicin and CPZ were dissolved in 10% Tween 20/10% ethanol (10%) with normal saline. capsaicin (1 mg/kg) was administered orally 1 h before stress as described previously (2). CPZ (15 mg/kg) was in-

jected subcutaneously 30 min before stress as described previously (31). CGRP (10 μg/kg) and CGRP (8–37) (100 μg/kg) were dissolved in sterile distilled water and injected intrave-

nously immediately before stress as described previously (23). l-NNAME (5 mg/kg) and aminoguanidine (40 mg/kg) were dissolved in normal saline solution and administered subcutaneously 30 min before the experiment as described previously (12, 29). Indomethacin (5 mg/kg) was suspended in bicarbonate-buffered saline and injected subcutaneously 30 min before the experiment as described previously (15). NS-398 (30 mg/kg) was suspended in a 0.5% carboxymethyl cellulose aqueous suspension and administered orally 1 h before the experiment as described previously (1). Solutions were prepared immediately before the experiments. Each control animal received the vehicle in these experiments. However, because results in control experiments using the vehicle of each solution were not significantly different from those obtained by using saline (data not shown), we used as a representative control the data obtained by using saline in the present study.

**Determination of primary sensory nerves by capsaicin.** Ablation of gastric visceral afferent nerves was accomplished by high-dose capsaicin administration as previously described (8, 23, 29, 33). Rats received a total dose of 125 mg/kg capsaicin administered subcutaneously in divided doses over 2 days. Two weeks after treatment with high-dose capsaicin, animals were subjected to stress. To determine the effectiveness of the sensory afferent nerve denervation procedure, a drop of 0.001% capsaicin in saline was instilled into either eye of the rats, and their protective wiping movements were observed. Capsaicin-treated rats that showed any wiping movement were excluded from the study. Control animals were injected subcutaneously with 1 ml of 10% Tween 20/10% ethanol (10%) with normal saline.

**Administration of various agents.** Capsaicin and CPZ were dissolved in 10% Tween 20/10% ethanol (10%) with normal saline. capsaicin (1 mg/kg) was administered orally 1 h before stress as described previously (2). CPZ (15 mg/kg) was in-
with horseradish peroxidase-conjugated anti-rabbit IgG (MBL, Nagoya, Japan) at 1:2,000 dilution for 1 h at 37°C. Reaction products were developed by immersing the sections in 3,3′-diaminobenzidine tetrahydrochloride solution containing 0.03% hydrogen peroxide. The control for immunostaining was performed by nonimmune rabbit serum as the first step in place of primary antiserum and omission of the first step or use of the first antiserum preabsorbed with an excess of the homologous antigen. Samples were mounted with Entellan onto glass slides, examined, and photographed under a light microscope.

**Determination of gastric tissue levels of 6-keto-PGF1α and PGE2.** Gastric levels of 6-keto-PGF1α and PGE2 were determined in animals before and during WIR according to the methods described previously (30, 32). Briefly, stomachs were weighed and then homogenized in 5 ml of 0.1 M PBS (pH 7.4) at 5°C. The homogenates were centrifuged at 2,000 g for 10 min to remove the tiny amounts of solid tissue debris. The supernatant was then acidified with 1 M HCl. 6-keto-PGF1α and PGE2 were extracted from the supernatant by using columns packed with ethyl-bonded silica gel (ethyl C2; Amersham). Columns were prepared by washing with 2 ml of methanol, followed by 2 ml of water. The acidified supernatant was applied onto the column followed by washing sequentially with 5 ml of 10% ethanol and 5 ml of hexane. 6-keto-PGF1α and PGE2 were eluted with 5 ml of methyl formate, and the solvent was evaporated under a stream of nitrogen gas. The evaporated supernatant was reconstituted with the buffer equipped in specific enzyme immunoassay kits for the determination of the concentration of 6-keto-PGF1α and PGE2 (Amersham). The cross-reactivities of the assay for 6-keto-PGF1α with PGE2, PGF2α, thromboxane B2, and arachidonic acid were 2.8, 1.4, 0.03, and 0.01%, respectively, according to the manufacturer’s data sheet. The cross-reactivities of the assay for PGE2 with PGE1, PGF2α, 6-keto-PGF1α, and thromboxane B2 were 7.0, 4.3, 5.4, and < 0.1%, respectively, according to the manufacturer’s data sheet. Results are expressed as micrograms of 6-keto-PGF1α or PGE2 per gram of tissue.

**Measurement of gastric MPO activity.** After animals were immersed for the indicated period of stress, all were immediately killed. Their stomachs were quickly removed and opened along the greater curvature. In some experiments, leukocyte infiltration in gastric mucosa was assessed by determining tissue activity of MPO, an enzyme used as a marker for leukocyte infiltration in a variety of tissues including rat gastric mucosa (5, 41, 42). MPO activity was determined by a modification of the method as described previously (26). Briefly, the stomachs were weighed and suspended in 6 ml of 50 mM phosphate buffer (pH 6.0) containing 1% hexadecyltrimethylammonium bromide. Samples were homogenized; the homogenate was sonicated, freeze-thawed, and then centrifuged (4,500 g for 15 min at 4°C). MPO activity was determined in the supernatant (0.1 ml) after the addition of 0.6 ml of PBS (pH 6.0) containing 0.05 ml of 1.25 mg/ml of o-dianisidine dihydrochloride and 0.05 ml of 0.05% hydrogen peroxide. The change in absorbance at 460 nm over a 6.5-min period was measured in a spectrophotometer (model DU-54; Beckman, Irvine, CA). One unit of MPO activity is defined as the amount of enzyme that will reduce 1 µmol peroxidase/min. Results are expressed as units of MPO activity per gram of tissue.

**Statistical analysis.** Data are expressed as means ± SD. Results were compared by using either an ANOVA followed by Scheffé’s post hoc test for multiple comparisons or a Student’s t-test for single comparison. A level of P < 0.05 was considered statistically significant.

**RESULTS**

Changes in gastric tissue levels of CGRP, 6-keto-PGF1α, and PGE2 and immunohistochemical staining of CGRP in the stomach in rats subjected to WIR. Gastric tissue levels of CGRP were significantly increased 30 min after WIR compared with the pre-WIR levels (Fig. 1A). These levels were decreased rapidly to the pre-WIR levels at 1 h of WIR and were not changed during 1 to 8 h of WIR (Fig. 1A). Immunohistochemical staining of CGRP in the rat gastric mucosa 30 min after WIR was increased in the lamina propria of the gastric mucosa (Fig. 2B) compared with that of non-

![Fig. 1. Changes in gastric levels of calcitonin gene-related peptide (CGRP; A), 6-keto-PGF1α (B), and PGE2 (C) in rats subjected to water immersion restraint stress (WIR). Values are expressed as means ± SD derived from 5 animal experiments. *P < 0.05 vs. pre-WIR; §P < 0.01 vs. pre-WIR.](http://ajpgi.physiology.org/)
stressed rats (Fig. 2A). Gastric tissue levels of both 6-keto-PGF$_{1\alpha}$ and PGE$_2$ were also significantly increased 30 min after WIR compared with the pre-WIR levels (Fig. 1, B and C). These levels began to decrease after 1 h of WIR and were significantly decreased to less than pre-WIR levels after 6 and 8 h of WIR (Fig. 1, B and C).

Effects of CPZ, the functional denervation of capsaicin-sensitive sensory neurons and capsaicin on the increases in gastric tissue levels of CGRP, and immunohistochemical staining of CGRP in the stomach 30 min after WIR. Both CPZ and the functional denervation of capsaicin-sensitive sensory neurons significantly inhibited the increases in gastric tissue levels of CGRP (Fig. 3) and the increase in the gastric mucosal immunohistochemical staining of CGRP 30 min after WIR (Fig. 2, C and D). The administration of capsaicin significantly enhanced the WIR-induced increase in the gastric tissue level of CGRP 30 min after WIR (Fig. 3). Each control animal that received the vehicle or saline as shown in MATERIALS AND METHODS showed the increase in the gastric mucosal immunohistochemical staining of CGRP (data not shown).

Effects of CPZ, CGRP-(8–37), functional denervation of capsaicin-sensitive sensory neurons, capsaicin, and CGRP on increases in gastric tissue levels of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ 30 min after WIR. Gastric tissue levels of 6-keto-PGF$_{1\alpha}$ 30 min after WIR in animals pretreated with CPZ and CGRP-(8–37) and in those with the functional denervation of capsaicin-sensitive sensory neurons (2.03 ± 0.28, 2.06 ± 0.15, and 1.95 ± 0.22 μg/g tissue, respectively; n = 5 in each group) were significantly lower than those in control animals (2.76 ± 0.21 μg/g tissue; n = 5, P < 0.01). However, these levels in animals pretreated with capsaicin and CGRP (6.05 ± 0.90 and 5.90 ± 0.52 μg/g tissue, respectively; n = 5 in each group) were significantly higher than those in control animals (P < 0.01). Gastric tissue levels of PGE$_2$ 30 min after WIR in animals pretreated with CPZ or CGRP-(8–37) and in those with the functional denervation of capsaicin-sensitive sensory neurons (2.62 ± 0.40, 2.56 ± 0.31, and 2.66 ± 0.25 μg/g tissue, respectively; n = 5 in each group) were significantly lower than those in control animals (5.96 ± 0.48 μg/g tissue; n = 5, P < 0.01). These levels in animals...
pretreated with capsaicin and CGRP (12.40 ± 0.38 and 11.15 ± 0.58 μg/g tissue, respectively; n = 5 in each group) were significantly higher than those in control animals (P < 0.01).

Effects of L-NAME, aminoguanidine, indomethacin, and NS-398 on increases in gastric tissue levels of 6-keto-PGF1α and PGE2 30 min after WIR. Gastric tissue levels of 6-keto-PGF1α 30 min after WIR in animals pretreated with L-NAME and indomethacin (1.34 ± 0.36 and 1.26 ± 0.28 μg/g tissue, respectively; n = 5 in each group) were significantly lower than those in control animals (2.76 ± 0.21 μg/g tissue; n = 5, P < 0.01). In contrast, these levels were not affected by pretreatment with aminoguanidine and NS-398 (2.16 ± 0.46 and 2.40 ± 0.58 μg/g tissue, respectively; n = 5 in each group). Gastric tissue levels of PGE2 30 min after WIR in animals pretreated with L-NAME and indomethacin (1.90 ± 0.25 and 2.11 ± 0.20 μg/g tissue, respectively; n = 5 in each group) were significantly lower than those in control animals (5.96 ± 0.48 μg/g tissue; n = 5, P < 0.01). However, these levels were not affected by pretreatment with aminoguanidine and NS-398 (5.74 ± 0.68 and 5.68 ± 0.75 μg/g tissue, respectively; n = 5 in each group).

Effects of L-NAME, aminoguanidine, indomethacin, and NS-398 on capsaicin- or CGRP-induced increases in gastric tissue levels of 6-keto-PGF1α and PGE2 30 min after WIR. Pretreatment with L-NAME inhibited the capsaicin- or CGRP-induced increases in gastric tissue levels of 6-keto-PGF1α (Fig. 4A) and PGE2 (Fig. 4B) 30 min after WIR, whereas that with aminoguanidine did not affect these levels (Fig. 4, A and B). Although pretreatment with indomethacin inhibited the capsaicin- or CGRP-induced increases in gastric tissue levels of 6-keto-PGF1α (Fig. 4A) and PGE2 (Fig. 4B) 30 min after WIR, that with NS-398 did not inhibit these increases (Fig. 4, A and B).

Effects of CPZ, functional denervation of capsaicin-sensitive sensory neurons, CGRP-(8–37), capsaicin, and CGRP on the WIR-induced changes in gastric accumulation of neutrophils and gastric mucosal injury. Gastric accumulation of neutrophils as evaluated by measuring the gastric MPO activity increased 8 h after WIR compared with pre-WIR levels (15). The gastric lesion index was significantly increased 4 h after WIR compared with the pre-WIR level, and it reached the maximum 8 h after WIR (15). To clarify whether activation of capsaicin-sensitive sensory neurons contributes to the reduction of WIR-induced gastric mucosal injury by limiting gastric neutrophil accumulation, we examined the effects of CPZ, CGRP-(8–37), and the functional denervation of capsaicin-sensitive sensory neurons on the changes in gastric MPO activity and gastric lesion index 4 h after WIR when these variables did not reach their maximum values. Pretreatment with CPZ and CGRP-(8–37), and the functional denervation of capsaicin-sensitive sensory neurons significantly increased both the gastric

![Fig. 4. Effects of pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME), aminoguanidine (AG), indomethacin (IM), and NS-398 on capsaicin- or CGRP-induced changes in the gastric tissue levels of 6-keto-PGF1α (A) and PGE2 (B) 30 min after WIR. Capsaicin (1 mg/kg) was administered orally 1 h before WIR. CGRP (10 μg/kg) was injected intravenously immediately before WIR. L-NAME (5 mg/kg), AG (40 mg/kg), and IM (5 mg/kg) were injected subcutaneously 30 min before capsaicin or CGRP administration. NS-398 (30 mg/kg) was administered orally 1 h before capsaicin or CGRP administration. Values are expressed as means ± SD. ΔP < 0.01 vs. WIR + capsaicin; ¶P < 0.01 vs. WIR + CGRP.
MPO activity and the gastric lesion index 4 h after WIR (Fig. 5, A and B). Although the administration of capsaicin and CGRP had no effect on the gastric MPO activity, it reduced the gastric lesion index 4 h after WIR (Fig. 5, A and B). CPZ, CGRP-(8–37), and the functional denervation of capsaicin-sensitive sensory neurons did not affect the WIR-induced increases in either the gastric MPO activity or the gastric lesion index 8 h after WIR (Fig. 5, C and D), whereas capsaicin and CGRP significantly inhibited these increases (Fig. 5, C and D).

Effects of L-NAME, aminoguanidine, indomethacin, and NS-398 on the WIR-induced changes in gastric accumulation of neutrophils and gastric mucosal injury. Pretreatment with L-NAME and indomethacin significantly increased gastric MPO activities and gastric mucosal injury 4 h after WIR (Fig. 6); that with aminoguanidine and NS-398 had no effects on these values (Fig. 6). Pretreatment with L-NAME, aminoguanidine, indomethacin, and NS-398 did not affect these variables seen 8 h after WIR (data not shown).

Effects of L-NAME, aminoguanidine, indomethacin, and NS-398 on the capsaicin- and CGRP-induced effects on gastric accumulation of neutrophils and gastric mucosal injury. Pretreatment with L-NAME and indomethacin significantly increased gastric MPO activities in animals given capsaicin and CGRP 4 h after WIR; that with aminoguanidine and NS-398 did not affect these values (Fig. 7A). Although pretreatment with L-NAME and indomethacin abrogated both capsaicin- and CGRP-induced reduction of the gastric lesion index 4 h after WIR, that with aminoguanidine and NS-398 had no affect on these variables (Fig. 7B). Inhibition of gastric MPO activities induced by capsaicin and CGRP 8 h after WIR were not observed when animals were pretreated with L-NAME and indomethacin, whereas these inhibitory effects were unaffected in animals pretreated with aminoguanidine and NS-398 (Fig. 7C). Although reduction of the gastric lesion index induced by capsaicin or CGRP seen 8 h after WIR was not observed in animals pretreated with L-NAME and indomethacin, such effects were unaffected by pretreatment with aminoguanidine and NS-398 (Fig. 7D).

**DISCUSSION**

As shown in the present study, both the gastric tissue level and the immunohistochemical staining of CGRP were significantly increased in rats 30 min after WIR. Although the immunohistochemical staining of CGRP in the gastric tissue was apparently observed in
the surface epithelium as well as in the lamina propria (Fig. 2), distribution of the sensory neurons was not shown in the surface epithelium of the stomach (19), suggesting that staining in the surface epithelium might be an artifact. Increases in the gastric tissue level and the immunohistochemical staining of CGRP were inhibited by pretreatment with CPZ and the functional denervation, and the increase in the gastric tissue level of CGRP was enhanced by pretreatment with capsaicin, suggesting that capsaicin-sensitive sensory neurons could be activated in the stomach of rats subjected to WIR.

Ren et al. (33) demonstrated that high-dose capsaicin treatment markedly decreased gastric CGRP levels in rats subjected to WIR to ~25% of the levels of rats subjected to WIR alone. Although we treated rats with high-dose capsaicin according to the method described by Ren et al. (33), gastric CGRP levels in the rats subjected to WIR were decreased to ~40% of the levels of rats subjected to WIR alone. In the present study, we excluded animals that showed protective wiping movements after instillation of 0.001% capsaicin into either eye to select the animals with successful functional denervation.

Gastric tissue levels of CGRP were not significantly decreased during WIR compared with the pre-WIR levels as shown in the present study. However, recent studies have demonstrated that gastric tissue levels of CGRP were significantly decreased in rats after 3–4 h of WIR compared with the pre-WIR levels (8, 33). Because a range of physical and chemical stimuli can promote the release of CGRP from the sensory nerve endings (7), the tissue level of CGRP after the insult might be dependent on the magnitude of the insult. Consistent with this hypothesis, the gastric mucosal lesion indexes after WIR in these two reports were about three times higher than those observed in the present study, explaining why the gastric tissue levels of CGRP were decreased in these two previous reports.

Gastric tissue levels of 6-keto-PGF1α, a stable metabolite of PGI2, and PGE2 were increased 30 min after WIR, which contributed to prevent the WIR-induced gastric mucosal injury mainly by inhibiting neutrophil activation (9, 12, 13, 18). However, the mechanisms underlying the increase in the gastric tissue level of PGI2 and PGE2 have not been fully clarified.

Because CGRP is released from the nerve endings of capsaicin-sensitive sensory neurons located around submucosal arterioles (19), the released CGRP could interact with endothelial cells. CGRP has been shown to increase the endothelial production of PGI2 in vitro (6), suggesting that the released CGRP in the gastric mucosa might increase the gastric tissue level of PGI2 in rats subjected to WIR. Consistent with this hypothesis are the present observations showing that the increase in the gastric tissue level of 6-keto-PGF1α was enhanced by capsaicin and CGRP and was inhibited by CPZ, the functional denervation of capsaicin-sensitive sensory neurons and CGRP-(8–37). Furthermore, because the increase in the gastric tissue level of PGE2 was enhanced by capsaicin and CGRP and was inhibited by CPZ, it is possible that WIR-induced activation of capsaicin-sensitive sensory neurons might also increase the gastric PGE2 production by releasing CGRP. We previously reported that pretreatment with indomethacin inhibited the WIR-induced increase in the gastric tissue level of 6-keto-PGF1α, whereas that with NS-398, a selective inhibitor of COX-2, did not affect the increase (16). Because the WIR-induced increases in the gastric tissue levels of 6-keto-PGF1α and PGE2 were inhibited by indomethacin, but were not affected by NS-398, the capsaicin-sensitive sensory neuron-mediated increase in the gastric production of PGI2 and PGE2 might be mediated by COX-1. Consistent with these observations are previous studies reporting that CGRP has been shown to increase the endothelial production of NO that activates COX-1 selectively.
Fig. 7. Effects of pretreatment with L-NAME, AG, IM, and NS-398 on capsaicin- or CGRP-induced changes in gastric MPO activity and the gastric lesion index after 4 h (A and B) or 8 h (C and D) of WIR. Capsaicin (1 mg/kg) was administered orally 1 h before WIR. CGRP (10 μg/kg) was injected intravenously immediately before WIR. L-NAME (5 mg/kg), AG (40 mg/kg), and IM (5 mg/kg) were injected subcutaneously 30 min before capsaicin or CGRP administration. NS-398 (30 mg/kg) was administered orally 1 h before capsaicin or CGRP administration. Values are means ± SD. ‡P < 0.01 vs. WIR + capsaicin; ¶P < 0.01 vs. WIR + CGRP.
WIR-induced increases in gastric tissue levels of 6-keto-PGF$_{1a}$ and PGE$_2$ were inhibited by pretreatment with L-NAME, a nonselective inhibitor of NOS, but not by pretreatment with aminoguanidine, a selective inhibitor of an inducible isoform of NOS. These observations suggest that the constitutive form of NOS could be importantly involved in the capsaicin-sensitive sensory neuron-mediated increases in the gastric tissue levels of PGI$_2$ and PGE$_2$ in rats subjected to WIR. Consistent with these observations is our previous report showing that activation of capsaicin-sensitive sensory neurons increased the hepatic production of PGI$_2$ in rats subjected to hepatic ischemia/reperfusion by activating endothelial NOS (17).

We (15, 16) previously reported that gastric PGI$_2$ contributed to prevent stress-induced gastric mucosal injury mainly by inhibiting neutrophil activation. Gastric PGE$_2$ has also been shown to prevent indomethacin-induced gastric mucosal injury by inhibiting neutrophil activation (35). Thus activation of capsaicin-sensitive sensory neurons might contribute to the reduction of the gastric mucosal injury by inhibiting neutrophil activation through increasing the gastric tissue levels of PGI$_2$ and PGE$_2$. The administration of capsaicin and CGRP significantly inhibited the 8-h WIR-induced increases in both the gastric MPO activity and the gastric mucosal injury. Furthermore, neither capsaicin- nor CGRP-induced effects were observed in animals pretreated with L-NAME and indomethacin, but these effects were unaffected in animals pretreated with aminoguanidine and NS-398. These observations strongly suggest that activation of capsaicin-sensitive sensory neurons might lead to the reduction of gastric mucosal injury by inhibiting neutrophil activation through promotion of the constitutive form of NOS- and COX-1-mediated production of PGI$_2$ and PGE$_2$ in rats subjected to WIR.

Capsaicin-sensitive sensory neurons in the gastric mucosa could be activated in rats subjected to WIR as shown in the present study. However, the mechanisms by which capsaicin-sensitive sensory neurons could be activated in rats subjected to WIR are not fully understood. Preliminary studies showed that pretreatment of rats with omeprazole, a proton pump inhibitor, and famotidine, an H$_2$-receptor antagonist, both of which potently inhibit acid secretion, significantly inhibited the WIR-induced increases in gastric tissue levels of both CGRP and 6-keto-PGF$_{1a}$. These observations suggest that gastric acid back diffused to the gastric mucosa might play a role in the stimulation of the capsaicin-sensitive sensory neurons in rats subjected to WIR. These observations are consistent with previous observations that showed that capsaicin-sensitive sensory neurons could be activated by an acidic environment (19) and that an acid-induced increase in the gastric mucosal blood flow could be mediated by capsaicin-sensitive sensory neuron activation (20).

Because CGRP, NO, PGI$_2$, and PGE$_2$ have been shown to increase the gastric mucosal blood flow (15, 19, 21, 25), activation of capsaicin-sensitive sensory neurons, which leads to CGRP release and a subsequent increase in the endothelial production of NO, PGI$_2$, and PGE$_2$, might contribute to maintain the gastric mucosal integrity by increasing gastric mucosal blood flow as well as by inhibiting neutrophil activation (14, 19). Thus capsaicin-sensitive sensory neurons in the gastric mucosa might play a role not only in the sensory nervous system but in the cytoprotective system, which could increase the gastric mucosal blood flow and attenuate the local inflammatory responses.

Hecker et al. (18) demonstrated that, although PGE$_2$ inhibited N-formyl-methionyl-leucyl-phenylalanine-induced cytotoxic enzyme release from human neutrophils by increasing intracellular levels of cAMP, neither PGI$_2$ nor iloprost, a stable analog of PGI$_2$, had any effect. In contrast, Kainoh et al. (22) reported that both PGE$_2$ and PGI$_2$ inhibited oxygen free radical production by increasing intracellular cAMP levels in rat polymorphonuclear leukocytes. Although inhibition of neutrophil activation by PGI$_2$ in vitro is still controversial, iloprost prevented WIR-induced gastric mucosal injury by inhibiting accumulation of neutrophils in rats (15). Therefore, PGI$_2$ might contribute to prevent stress-induced gastric mucosal injury by inhibiting neutrophil activation in our animal model of gastric mucosal injury.

Figure 8 shows the possible mechanism by which activation of capsaicin-sensitive sensory neurons reduced the gastric mucosal injury by increasing the
gastric tissue levels of prostaglandins in rats subjected to WIR.

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