Possible Involvement of Non-Steroidal Anti-Inflammatory Drugs in Vagal-Mediated Gastric Acid Secretion in Rats

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Abstract—Effects of several non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin (ASA), indomethacin (IM), flurbiprofen (FP), ibuprofen (IP), phenylbutazone (PBZ) and flufenamic acid (FA) were studied on the gastric ulceration and gastric acid secretion induced by restraint and water-immersion stress (RWIS) or various secretagogues in rats. These drugs significantly increased ulcer formation. IM (1, 3 and 10 mg/kg, s.c.) reduced gastric mucosal prostaglandin (PG) content dose-dependently. There was an appreciable correlation between this decrease in the PG content of gastric tissue and associated ulceration. The gastric acid secretion induced by the peripheral secretagogues, methacholine, gastrin and histamine, was not significantly influenced by IM pretreatment. In contrast, the gastric acid secretion induced by the vagal mediated secretagogues, insulin, 2-deoxy-D-glucose (2-D-G) and RWIS, was markedly increased by IM pretreatment. These effects were not observed in vagotomized rats. By intracerebroventricular (i.c.v.) injection of IM, no influence was observed on the gastric acid secretion and ulcer formation induced by 2-D-G or RWIS. These results suggest that acidic NSAIDs potentiate the gastric acid output induced by stimulation of vagus nerve activity, and prostaglandins (PGs) may influence gastric acid output by regulating vagus nerve activity.

Prostaglandins (PGs) inhibit gastric acid secretion (1–3) and ulcer formation (4–6). It is well known that non-steroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal lesions and that their action is correlated with a decrease of PG content in the stomach (7, 8). On the other hand, vagus nerve activity in the stomach is an important factor in gastric acid secretion and ulcer formation. However, the interaction between PGs and the activity of the vagus nerve in gastric acid secretion have not yet been clarified. In the present study, we investigated the effects of NSAIDs on gastric acid secretion and ulcer formation in rats induced both by various secretagogues and restraint and water-immersion stress (RWIS).

Materials and Methods

Animals: Male Wistar strain rats (180–230 g) were used. The animals were starved for 18 hr, but allowed free access to water.

Stress ulcer studies: Animals were placed in a stress cage and immersed in a water bath (23°C) for 6 hr as described by Takagi and Okabe (9). The test drugs were given orally just before the water immersion. The doses of test drugs used were the ED50 values of each drug for anticarrageenin edema (10): Aspirin (ASA, 180 mg/kg), indomethacin (IM, 5 mg/kg), flurbiprofen (FP, 0.2 mg/kg), ibuprofen (IP, 30 mg/kg), phenylbutazone (PBZ, 40 mg/kg) and flufenamic acid (FA, 20 mg/kg). At the end of RWIS, the animals were killed by a blow on the head, the stomachs were removed, inflated with 1% formalin solution and placed into 1% formalin solution for 10 min. The stomachs were then opened by cutting along the greater curvature and lesions in the glandular portion examined.
The ulcer index was calculated as the area of each lesion in the stomach using stereoscopic microscopy.

Assay of PGE₂ contents: The rat fundus strip superfusion technique was used to determine the PGE₂-like activity according to the method of Konturek et al. (11). Isolated gastric mucosal tissue was prepared for the generation of PGs as described by Vane (12). The mucosa was carefully stripped off from the antral and fundic regions in ice-cold 50 mM Tris-HCl buffer (pH 7.4). The mucosal tissues (about 300 mg) were chopped with scissors; blood and debris were washed out by shaking for 5 sec with 1 ml of ice-cold Tris-HCl buffer (pH 7.4) and centrifuging at 3000 rpm for 60 sec. After removing the supernatant, Tris-HCl buffer (pH 7.4) was added to residual tissue (0.5 ml buffer per 150 mg of the original), and the tissue was shaken for 60 sec at room temperature using a mixer at a steady speed and then centrifuged (3000 rpm) for 15 sec. The resulting preparation was left for 30 min at room temperature to destroy PG₁₂ for bioassay of PGE₂-like activity. A rat fundus strip was superfused in a cascade of Krebs solution, pregauged with 95% O₂ and 5% CO₂, containing each of the following pharmacological antagonists: phenoxybenzamine (1 μg/ml), atropine sulfate (0.1 μg/ml), cyproheptazine (1 mg/ml), diphenhydramine (0.1 μg/ml) and IM (1 μg/ml). The tone of the rat fundus strip was recorded with an isotonic transducer (TD-112S, Nihon Kohden) via an isotonic coupler and a coupler amplifier (EG-650H AA-600H, Nihon Kohden) connected to an ink-writing oscilloscope (WI-681G, Nihon Kohden).

Gastric acid secretion studies: These consisted of two experiments. Unanesthetized rats and rats anesthetized with urethane (1.25 g/kg, s.c.) were used. Both experiments were performed under the same conditions. The pylorus was ligated, and a cannula was inserted into the forestomach to collect the gastric juice. In the unanesthetized rats, gastric acid secretion was induced by RWIS. In the anesthetized rats, gastric acid secretion was induced by methacholine, gastrin, histamine, insulin and 2-D-G. When methacholine, gastrin and histamine were used as stimulators of acid secretion, truncal vagotomy was performed to exclude the influence of vagus nerve activity.

I.c.v. injection was carried out according to the technique of Okuyama and Aihara (13). Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and the head was held horizontally by a stereotaxic apparatus. The locus for insertion of a stainless guide cannula (outer diameter: 0.7 mm) was determined according to the stereotaxic atlas of Pellegrino and Cushman (14). The guide cannula was implanted into the left cerebral ventricle (A: 2.8, L: 1.5, H: +0.8). IM was dissolved in 10 μl of vehicle solution and injected at a rate of 10 μl/min through an intercannula made of stainless steel (outer diameter: 0.3 mm). Each animal was allowed one week to recover after surgery.

Gastric juice was collected in a test tube every 30 or 60 min for 6 hr by washing out with 10 ml of saline warmed at 37°C. The total acid output of each sample was determined by titration with 0.01 N NaOH using phenolphthalein as an indicator.

Drugs: The drugs used were ASA (Nihon Kayaku), IM (Sumitomo Kagaku), FP (Kaken-yaku Kogyo), IP (Hokkou Kagaku), PBZ (Sigma), FA (Sankyo), PGE₂ (Sigma), phenoxybenzamine (Wako Junyaku), cyproheptazine (Banyu), atropine sulfate (Wako Junyaku), diphenhydramine hydrochloride (Wako Junyaku), methacholine chloride (Nakarai Kagaku), amogastrin (Nihon Kayaku), histamine dihydrochloride (Wako Junyaku), insulin (Torii Yakuhin), 2-D-G (Nakarai Kagaku), urethane (Wako Junyaku) and pento-barbital-Na (nembutal, Dainippon). Water-insoluble drugs were suspended with 0.4% sodium carboxy methylcellulose (CMC) in saline, and water-soluble drugs were dissolved in saline solution. These drugs were administered at a dose of 0.2 ml/100 g body weight. When IM was administered by i.c.v., it was dissolved in 0.1 M Tris-HCl buffer (pH 7.9).

Statistical analysis: The significance of differences between values was examined by Student's t-test.
Results

Effect of NSAIDs on stress-induced ulceration: As shown in Fig. 1, all NSAIDs significantly increased the ulcer index induced by RWIS. The effects of ASA, IM, FP and IP were more marked than those of PBZ and FA. Subcutaneous injection of IM also increased the ulcer formation induced by RWIS dose-dependently within a dose range of 1.0 to 10.0 mg/kg. Ten mg/kg IM increased the ulcer index about 8 times compared with the control value (3.61±0.68 mm² (Fig. 2, right side). However, i.c.v. injection of IM (10 μg) did not increase the ulcer formation induced by RWIS (Fig. 3).

Effect of IM on mucosal PGE₂-like activity: The PGE₂-like activities in the antrum...
and fundus of control rats were 1.61±0.18, and 1.95±0.24 ng/30 mg tissue weight, respectively. The effect of IM on PGE$_2$-like activity is shown on the left side of Fig. 2. IM decreased gastric mucosal PGE$_2$-like activity dose-dependently within a dose range of 1.0 to 10.0 mg/kg.

**Effect of IM on gastric acid output:** In non-vagotomized rats, the gastric acid output of rats given RWIS increased time-dependently, reaching a peak at 60 min after water-immersion and then decreasing gradually (Fig. 4). At this point, the value of the gastric acid output was 22.9±5.4 μEq/30 min, and this output was enhanced by pretreatment with IM (10 mg/kg, s.c.). On the other hand, the gastric acid output of vagotomized rats given RWIS was not changed by pretreatment with IM (Fig. 5). Methacholine-stimulated gastric acid output was not enhanced by IM pretreatment except for a small increase with 0.03 mg/kg methacholine and 2.5 hr after the administration of 0.3 mg/kg methacholine (Fig. 6). Gastrin-stimulated gastric acid output was not influenced by IM pretreatment except at the highest dose of gastrin (1.0 mg/kg) (Fig. 7). Histamine-stimulated gastric acid output was not influenced by IM pretreatment except at the lowest dose of histamine (Fig. 8). The gastric acid outputs induced by insulin (Fig. 9) and 2-D-G (Fig. 10) were significantly potentiated by pretreatment with IM (Fig. 12). At a dose of 0.3 U/kg, however, insulin did not stimulate gastric acid output, and under this condition, IM did not show any significant effects on insulin induced gastric acid output (Fig. 9). Pretreatment with an i.c.v. injection of IM (10 μg) did not affect the gastric acid secretion which was induced by 2-D-G (Fig. 11).

![Fig. 3. Effect of IM (10 μg, i.c.v.) on ulcer formation induced by RWIS in rats. Each value represents the mean±S.E.](image)

![Fig. 4. Effect of IM (10 mg/kg, s.c.) on gastric acid output induced by RWIS in unanesthetized acute gastric fistula rats. Each value represents the mean±S.E. *P<0.05, **P<0.01 and ***P<0.001, compared with the control values. #P<0.05, ##P<0.01 and ###P<0.001, compared with the values obtained before the water-immersion.](image)
Vagotomy

Fig. 5. Effect of IM (10 mg/kg, s.c.) on gastric acid output induced by RWIS in unanesthetized vagotomized acute gastric fistula rats. Each value represents the mean±S.E.

Fig. 6. Effect of IM (10 mg/kg, s.c.) on gastric acid output induced by methacholine (0.03, 0.1 and 0.3 mg/kg, s.c.) in anesthetized acute gastric fistula rats. Each value represents the mean±S.E. *P<0.05, **P<0.01 and ***P<0.001, compared with the control values.

Fig. 7. Effect of IM (10 mg/kg, s.c.) on gastric acid output induced by gastrin (0.1, 0.3 and 1.0 mg/kg, s.c.) in anesthetized acute gastric fistula rats. Each value represents the mean±S.E. *P<0.05, compared with the control values.
Fig. 8. Effect of IM (10 mg/kg, s.c.) on gastric acid output induced by histamine (0.3, 1.0 and 3.0 mg/kg, s.c.) in anesthetized acute gastric fistula rats. Each value represents the mean±S.E. *P<0.05, compared with the control values.

Fig. 9. Effect of IM (10 mg/kg, s.c.) on gastric acid output induced by insulin (0.3, 1.0 and 3.0 U/kg, s.c.) in anesthetized acute gastric fistula rats. Each value represents the mean±S.E. *P<0.05, compared with the control value.

Fig. 10. Effect of IM (10 mg/kg, s.c.) on gastric acid output induced by 2-D-G (50, 100 and 200 mg/kg, i.p.) in anesthetized acute gastric fistula rats. Each value represents the mean±S.E. *P<0.05, **P<0.01 and ***P<0.001, compared with the control values.
Discussion

The severity of ulceration induced by RWIS was markedly increased by the administration of NSAIDs. The increase in severity of ulceration was in parallel with the reduction of PGE₂-like activity in the gastric mucosa. The gastric acid output induced by the peripheral gastric secretagogues, meth-acholine, gastrin and histamine, were little influenced by IM (Fig. 12). It is well known that both insulin and 2-D-G are activators of the vagus nerve (15–17). The gastric acid secretions induced by insulin and 2-D-G were markedly increased by IM. However, these effects were not observed in vago-tomized rats. In this respect, the significance of IM-mediated increases of gastric acid secretion induced by methacholine (0.03 mg/kg), gastrin (1 mg/kg) and histamine (0.3 mg/kg) are now not clear. However, when the total amounts of acid output were calculated, the increase of acid output induced by IM was not so large compared with its effect on the action of the acid secretagogues which stimulate vagus nerve activity (Fig. 12). I.c.v. injection of IM did not have any effect on either gastric acid secretion or ulcer formation. These findings suggest that concerning its potentiation of gastric acid output, the main site of action of IM is not the central nervous system. Recently, Maeda-Hagiwara and Watanabe (18) indicated that insulin and 2-D-G enhanced the severity of ulceration caused by IM (40 mg/kg, i.p.). In this study, we showed the selectivity of the actions of NSAIDs on gastric acid output. The gastric

![Graph](image-url)

Fig. 11. Effect of IM (10 μg/, i.c.v.) on gastric acid output induced by 2-D-G (200 mg/kg, i.p.) in anesthetized acute gastric fistula rats. Each value represents the mean±S.E.

![Graph](image-url)

Fig. 12. Effect of IM (10 mg/kg, s.c.) on gastric acid output for 6 hr induced by several secretagogues. Each value represents the mean±S.E. *P<0.05, **P<0.01 and ***P<0.001, compared with the control values.
acid secretion induced by the peripheral secretagogues was not significantly influenced by the NSAIDs, but vagal-mediated gastric acid secretion was markedly increased by pretreatment of NSAIDs. It is well known that PGs have potent inhibitory action on gastric acid secretion. PGs inhibit the gastric acid output induced by all secretagogues (peripheral and central-mediated secretagogues). The mechanism, however, is not yet clear. In this study, we observed the selective action of NSAIDs that potentiate the gastric acid output induced by vagus nerve-stimulating secretagogues more than that induced by peripheral site-stimulating secretagogues. Recently, Goto (19) reported that a PGE₁ derivative inhibited the gastric acid output induced by stimulation of the vagus nerve by β-(P-chlorophenyl)-γ-amino-butyric acid rather than the acid output caused by peripheral site-stimulating secretagogues such as histamine or bethanechol, and that this effect of the PGE₁ derivative disappeared upon vagotomy. From these results, he suggested that PGs play a role in inhibiting gastric acid secretion by altering the function of the central nervous system. In the present study, we did not observe any enhancing effect of IM when it was administered by i.c.v. on gastric acid secretion induced by 2-D-G, but we did observe an enhancing effect when IM was administered s.c.

Taking these things together, it can be considered that at least some of the actions of PGs or NSAIDs regulate gastric acid output induced by stimulation of the vagus nerve even though this is due to alteration of the activity of the central nervous system which is in turn related to the activity of the vagus nerve. Furthermore, it is suggested that as far as their enhancing effect on gastric acid output is concerned, NSAIDs do not have a site of action in the central nervous system, at least at the area near to the ventricle, but acts in the peripheral nervous system, probably the presynaptic site of gastric vagus nerve.

References

1 Robert, A., Nezamis, J.E. and Phillips, J.P.: Inhibition of gastric secretion by prostaglandins. Am. J. Dig. Dis. 12, 1073–1076 (1967)

2 Dajani, E.Z., Driskill, D.R., Bianchi, R.G. and Collins, P.W.: Comparative gastric antisecretory and antiulcer effects of prostaglandin E₂ and its methyl ester in animals. Prostaglandins 10, 205–215 (1975)

3 Boughton-Smith, N.K. and Whittle, B.J.R.: The gastric antisecretory actions of prostaglandin E₂ and stable prostacyclin analogues against different secretagogues in perfused whole-stomachs of rat or mouse in vitro. Br. J. Pharmacol. 72, 291–298 (1981)

4 Kauffman, G.L., Jr. and Grossman, M.I.: Prostaglandin and cimetidine inhibit the formation of ulcers produced by parenteral salicylates. Gastroenterology 75, 1099–1102 (1978)

5 Tepperman, B.L., Miller, T.A. and Johnson, L.R.: Effect of 16, 16-dimethyl prostaglandin E₂ on ethanol-induced damage to canine oxyntic mucosa. Gastroenterology 75, 1061–1065 (1978)

6 Tabata, K. and Okabe, S.: Effects of 16, 16-dimethyl prostaglandin E₂ methyl ester on aspirin- and indomethacin-induced gastrointestinal lesions in dogs. Dig. Dis. Sci. 25, 439–448 (1980)

7 Main, I.M.H. and Whittle, B.J.R.: Investigation of the vasodilatory and antisecretory role of prostaglandins in the rat gastric mucosa by use of non-steroidal anti-inflammatory drugs. Br. J. Pharmacol. 53, 217–224 (1975)

8 Whittle, B.J.R., Higgs, G.A., Eakins, K.E., Moncada, S. and Vane, J.R.: Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. Nature 284, 271–273 (1980)

9 Takagi, K. and Okabe, S.: The effects of drugs on the production and recovery processes of the stress ulcer. Japan. J. Pharmacol. 18, 9–18 (1968)

10 Otomo, S., Higuchi, S., Tanaka, M. and Ohzeki, M.: Studies of antiinflammatory drugs (I). Anticarrageenin edema and gastrointestinal lesions. The 100th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, Abstract p. 341 (1980)

11 Konturek, S.J., Piastucki, I., Brzowski, T., Radecki, T., Dembinska-Kiec, A., Zmuda, A. and Gryglewski, R.: Role of prostaglandins in the formation of aspirin-induced gastric ulcers. Gastroenterology 80, 4–9 (1981)

12 Vane, J.R.: The use of isolated organs for detecting active substances in the circulating blood. Br. J. Pharmacol. 23, 360–373 (1964)

13 Okuyama, S. and Aihara, H.: The mode of action of analgesic drugs in adjuvant arthritic rats as
an experimental model of chronic inflammatory pain: possible central analgesic action of acidic nonsteroidal antiinflammatory drugs. Japan. J. Pharmacol. 35, 95–103 (1984)

14 Pellegrino, L.J. and Cushman, A.J.: A Stereotaxic Atlas of the Rat Brain. Appleton-Century-Crofts, New York (1967)

15 Hirschowith, B.I. and Sachs, G.: Vagal gastric secretory stimulation by 2-deoxy-D-glucose. Am. J. Physiol. 209, 452–460 (1965)

16 Eisenberg, M.M., Emas, G.S. and Grossman, M.I.: Comparison of the effect of 2-deoxy-D-glucose and insulin on gastric acid secretion in dogs. Surgery 60, 111–117 (1966)

17 Hirano, T. and Nijima, A.: Effects of 2-deoxy-D-glucose, glucose and insulin on effenter activity in gastric vagus nerve. Experientia 36, 1197–1198 (1980)

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

19 Goto, Y.: Inhibitory effect of a prostaglandin E1 analog on rat gastric acid secretion—Possible action on central mechanisms. The Japanese Pharmacological Society, Kinki Area Regional Meeting 65, Kanazawa, Abstract p. 79 (1984)