Effects of Prostaglandin Biosynthesis Inhibitors and Ouabain on Duodenal Mucosa and HCO₃⁻ Secretion in Rats

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Abstract—A single injection (s.c.) of prostaglandin biosynthesis inhibitors such as indomethacin (5 mg/kg), aspirin (200 mg/kg) and quinacrine (100 mg/kg) or a Na⁺•K⁺ ATPase inhibitor such as ouabain (10 mg/kg) significantly reduced the adaptive increase of HCO₃⁻ output caused by acid in the duodenum of anesthetized rats. These agents had no effect on basal duodenal HCO₃⁻ secretion and histamine-stimulated gastric acid secretion. Either of these agents, when given alone, had no effect on the duodenal mucosa of conscious rats, but produced damage in the proximal duodenum within 8 hr when given together with histamine (40 mg/kg x 3, s.c., every 2.5 hr). A significant relationship was found between the degrees of inhibition of acid-induced HCO₃⁻ output and the severity of duodenal lesions induced by these drugs (r=0.8620, P<0.01). These results suggest that an impairment of the mechanisms related to acid-induced HCO₃⁻ secretion may be particularly relevant to the pathogenesis of duodenal lesions.

Recent studies have shown that HCO₃⁻ secretion from the surface epithelial cells may play a role in protection of the duodenal mucosa against acid (1, 2). Experimentally produced duodenal ulcer models using cysteamine, mepirizole or indomethacin plus histamine are, in fact, associated with an impairment of duodenal HCO₃⁻ secretion (3–5). Although the regulation of HCO₃⁻ secretion in the duodenum remains to be fully elucidated, this process is reportedly linked with the mucosal prostaglandins (PGs) and Na⁺•K⁺ ATPase activity (2, 6).

In the present study, we therefore investigated the effects of PG biosynthesis inhibitors such as indomethacin, aspirin and quinacrine and those of a Na⁺•K⁺ ATPase inhibitor such as ouabain on HCO₃⁻ secretion in the rat duodenum, and we examined whether these drugs induced duodenal lesions under acid hypersecretion caused by histamine.

Male Donryu rats (230–260 g) were deprived of food but allowed free access to tap water for 24 hr before the experiments.

Gastric acid and duodenal HCO₃⁻ secretion were measured in anesthetized rats with urethane (1.25 g/kg, i.p.; Nakarai) according to a previous paper (5). Briefly, the stomach or duodenal loop which was made between the pyloric ring and the area just proximal to the outlet of the common bile duct (1.7 cm) was perfused with saline (pH 7.4), and acid or HCO₃⁻ in the perfusion fluid was titrated to pH 7.4 using a pH-stat method (Hiranuma Comtite-7) and by adding 50 mM NaOH or 10 mM HCl. Approximately 1 hr after basal acid or HCO₃⁻ secretion had stabilized, indomethacin (5 mg/kg, Sigma), aspirin (200 mg/kg, Merck), quinacrine (100 mg/kg, Sigma) or ouabain (10 mg/kg, Sigma) was given s.c. in a volume of 0.5 ml per 100 g of body weight. The doses of indomethacin, aspirin and quinacrine have been shown to suppress PG biosynthesis by inhibiting cyclooxygenase or phospholipase A₂ activity (7, 8), and that of ouabain has been demonstrated to inhibit Na⁺•K⁺ ATPase activity (6). In the case of acid secretion, histamine (40 mg/kg, Nakarai), dissolved in 10% gelatin,
was given s.c. 30 min after administration of these drugs. To stimulate duodenal HCO₃⁻ secretion, the loop was perfused for 10 min with 10 mM HCl made isotonic with NaCl.

The effects of indomethacin, aspirin, quinacrine and ouabain on the duodenal mucosa were examined in conscious rats with or without histamine treatment. These drugs were given s.c. as a single injection, and half the number of those rats were given histamine (40 mg/kg) s.c. three times every 2.5 hr from 30 min after administration of these agents. The animals were killed 8 hr after treatment with those drugs, and the duodenum and stomach were examined for lesions under a dissecting microscope (×10).

**Fig. 1.** Effects of indomethacin, aspirin, quinacrine or ouabain on histamine-stimulated gastric acid secretion (A) and acid-induced duodenal HCO₃⁻ secretion (B) in anesthetized rats. Data are presented as the mean ± one S.E. of values read every 15 min. *Statistically significant difference from the controls, at P<0.05.
after fixation of the tissue with 2% formalin (5). The area (mm²) of macroscopically visible damage was measured and used as a lesion index. The person measuring the lesion did not know the treatment given to the animals.

Data are presented as the mean±S.E. from 6 to 8 rats per group. The means were compared using the unpaired Student's t-test, and values of P<0.05 were regarded as significant.

Under anesthetized conditions, control animals secreted acid at a rate of 3-7 μEq/15 min. Acid secretion was markedly stimulated by histamine, reached the maximal values of 23-25 μEq/15 min within 75 min and remained elevated for about 2 hr (Fig. 1A). Indomethacin, quinacrine or ouabain did not significantly affect both basal and histamine-stimulated acid secretion. Aspirin also had no effect on basal acid secretion, but significantly potentiated the stimulated acid secretion caused by histamine. On the other hand, the proximal duodenum secreted HCO₃⁻ at a rate of 1.5-2 μEq/15 min as a basal secretion. The HCO₃⁻ secretion was significantly increased after exposure of the mucosa for 10 min to 10 mM HCl, and it reached the levels of approximately 2-2.5 times greater than basal values (Fig. 1B). Either of the above agents had no effect on basal HCO₃⁻ secretion, but significantly inhibited the increased HCO₃⁻ output in response to acid.

Neither indomethacin, aspirin, quinacrine nor ouabain induced any damage in the duodenal and gastric mucosa of conscious rats after a single s.c. administration (not shown). Repeated injections of histamine also had no effect on the mucosa of these tissues. However, the combined treatment with histamine and either of the above drugs produced lesions in the proximal duodenum within 8 hr (Fig. 2). Although the combined treatment with aspirin plus histamine caused multiple lesions in the stomach (12.2±3.4 mm²) as

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Fig. 2. Effects of indomethacin, aspirin, quinacrine or ouabain on duodenal and gastric mucosa of conscious rats given histamine. The rats were given one of the above agents s.c. as a single injection and subsequently injected with histamine (40 mg/kg), s.c., three times every 2.5 hr from 30 min after administration of the above agents, and they were killed 2.5 hr after the final injection of histamine (8 hr after administration of the above agents). Data are presented as the mean±one S.E. from 8 rats per group.
well as in the duodenum, such gastric lesions were less apparent in other cases. When the lesion index in the duodenum was plotted against % inhibition of the acid-induced \( \text{HCO}_3^- \) output caused by these agents, there was a significant relationship between these two factors, the correlation coefficient being 0.8620 (P<0.01).

An increase of \( \text{HCO}_3^- \) secretion by luminal acid was first reported in amphibian duodenums by Heylings et al. (9), and it was later confirmed in other species including rats (5, 10). Since the increased \( \text{HCO}_3^- \) output caused by acid is known to be mediated by endogenous PGs (9), the inhibition of this response by indomethacin, aspirin or quinacrine may be accounted for by suppression of PG biosynthesis in the mucosa. These results are consistent with our previous findings that these drugs significantly decreased the luminal pH and acid-neutralizing capacity in the rat duodenum perfused with acid solution (11). On the other hand, the ouabain effect on adaptive \( \text{HCO}_3^- \) secretion may be associated with the inhibitory action on \( \text{Na}^+\text{-K}^+ \) ATPase activity, since Simson et al. (6) reported that this agent inhibited both \( \text{HCO}_3^- \) output and \( \text{Na}^+ \) flux across the mucosa in amphibian duodenums in vitro.

These PG biosynthesis inhibitors and ouabain produced damage in the duodenum in the presence of sufficient acid. Since these drugs significantly decreased the luminal pH and acid-neutralizing capacity in the rat duodenum perfused with acid solution (11). On the other hand, the ouabain effect on adaptive \( \text{HCO}_3^- \) secretion may be associated with the inhibitory action on \( \text{Na}^+\text{-K}^+ \) ATPase activity, since Simson et al. (6) reported that this agent inhibited both \( \text{HCO}_3^- \) output and \( \text{Na}^+ \) flux across the mucosa in amphibian duodenums in vitro.

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In agreement with the findings of Bugat et al. (12), the combined treatment with aspirin plus histamine produced damage in the stomach as well as in the duodenum. They showed in cats that parenteral administration of aspirin produced lesions in the gastric mucosa if the stomach was bathed in sufficient acid. The mechanism underlying such lesions seems to involve PG deficiency in the gastric mucosa. However, since other PG biosynthesis inhibitors such as indomethacin and quinacrine induced few lesions in the stomach, other factors may contribute to the formation of gastric lesions in response to aspirin plus histamine.

Taken together, the present study suggests that a defect in the mechanisms related to adaptive \( \text{HCO}_3^- \) secretion may be particularly relevant to the pathogenesis of duodenal ulcers.

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