Gastroduodenal HCO₃⁻ Secretion in Anesthetized Rats: Effects of 16,16-Dimethyl PGE₂, Topical Acid and Acetazolamide

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Abstract—Alkaline secretion was measured in the whole stomach and in the proximal duodenum (2 cm proximal to the outlet of the common bile duct) of anesthetized rats, under basal conditions and in response to topical acid and 16,16-dimethyl PGE₂ (16-dmPGE₂) given by various routes. Gastric alkaline secretion was unmasked by intraduodenal administration of omeprazole (30 mg/kg). Both the stomach and duodenum consistently secreted bicarbonate in amounts of 0.2–0.4 μEq/15 min and 1.5–2 μEq/15 min as a basal secretion, respectively. 16-dmPGE₂, either given subcutaneously (1–30 μg/kg), intravenously (3 μg/kg/hr) or by topical application for 30 min (0.3–10 μg/ml), dose (concentration)-dependently increased HCO₃⁻ secretion in both tissues, but this effect disappeared quickly after sacrifice with KCl (i.v.). Stimulation of HCO₃⁻ secretion was also caused by topical acid to the stomach (100 mM HCl for 10 min) or to the duodenum (10 mM HCl for 10 min), but was completely blocked by pretreatment with indomethacin (5 mg/kg, s.c.). Acetazolamide, given subcutaneously at 100 mg/kg, which gives over 80% inhibition of carbonic anhydrase activity in the gastroduodenal mucosa, had no effect on either basal or stimulated HCO₃⁻ secretion caused by 16-dmPGE₂ (10 μg/kg, s.c.). These results indicate that both endogenous and exogenous (16-dmPGE₂) prostaglandins stimulate alkaline secretion in the gastroduodenal mucosa of rats, and this mechanism is independent from the carbonic anhydrase activity of the tissue.

Previous studies have demonstrated that the gastroduodenal mucosa is capable of secreting HCO₃⁻ by a metabolism-dependent mechanism in various experimental animals including frogs, guinea pigs, cats and dogs (1–4). However, there are few studies in rats, although this species is most frequently used in experiments for screening antiulcer drugs or investigating the pathogenesis of peptic ulcers (5, 6). In most of the species, analogues of prostaglandins stimulate duodenal HCO₃⁻ secretion, but the effects of prostaglandins (PGE₂, 16-dmPGE₂) on gastric HCO₃⁻ secretion are not consistent, depending upon the route of administration, the experimental conditions, or the species of animals (2–4, 7–9). On the other hand, involvement of carbonic anhydrase in the mechanism of HCO₃⁻ secretion also remains controversial (1, 6, 10–12). Therefore, in the present study, we measured HCO₃⁻ secretion in the whole stomach and in the proximal duodenum of anesthetized rats, under basal conditions and in response to topical acid and 16-dmPGE₂ given by various routes, and the effect of acetazolamide on basal and stimulated HCO₃⁻ secretion was investigated.

Materials and Methods
Male Donryu rats (200–250 g), kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access to water for 24 hr prior to the experiments.

Determination of HCO₃⁻ secretion
Gastric HCO₃⁻ secretion: The abdomen of
rats anesthetized with urethane (1.25 g/kg, i.p.) (Nakarai) was incised, and the stomach and duodenum were exposed. An acute gastric fistula prepared by placing a polyethylene tube in the forestomach led to a three way tap. Another tube was passed into the stomach from the duodenum and was held in place by a ligature around the pylorus. The stomach was perfused at a flow rate of 0.7 ml/min with saline which was adjusted to pH 5.0, gassed with 100% O₂, heated at 37°C and kept in a reservoir (Fig. 1A and C). To unmask HCO₃⁻ secretion, acid secretion was completely inhibited by intraduodenal administration of omeprazole (30 mg/kg, Hassle). Since HCO₃⁻ was not detected in the lumen of the stomach at pH 7.4 even after acid secretion had ceased, gastric HCO₃⁻ was titrated at luminal pH 5.0 by using a pH-stat method (Hiranuma Comitte-7) and by adding 5 mM HCl to the reservoir.

**Duodenal HCO₃⁻ secretion:** Under urethane anesthesia, the stomach and duodenum were exposed. A duodenal loop was made between the pyloric ring and the position just above the outlet of the common bile duct (2 cm), excluding the influences of bile and pancreatic juice. An acute fistula was prepared in the forestomach through which gastric contents were withdrawn to prevent an accumulation of gastric juice in the stomach (Fig. 1B and C). The duodenal loop was perfused at a flow rate of 0.7 ml/min with saline which was adjusted to pH 7.4, gassed with 100% O₂, heated at 37°C and kept in a reservoir. The titration of duodenal HCO₃⁻ secretion was performed at luminal pH 7.4 using the pH-stat method and by

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**Fig. 1.** Schematic illustration of the perfusion system and the order of connection of the loop for determination of HCO₃⁻ secretion in the whole stomach or in the proximal duodenum of an anesthetized rat.
adding 10 mM HCl to the reservoir.

The accuracy of HCO₃⁻ determination in the above system was examined by perfusing the stomach or the duodenum with various concentrations of NaHCO₃ (1–30 mEq/L) at a flow rate of 0.7 ml/min for 30 sec. The standard curve obtained was accurate and reliable within the range of amounts of HCO₃⁻ secretion (0.3–10 μmol) (Fig. 2). Back-diffusion of HCO₃⁻ through the gastric and duodenal mucosa did not occur in the present system when the alkaline secretion was measured before, during and after perfusion with the known amounts of HCO₃⁻ solution.

Experimental protocols

In the first study, basal HCO₃⁻ secretion was determined, and the effect of sacrifice with saturated KCl on it was examined. After stable basal secretion had been obtained for at least 1 hr, the animals were killed by a bolus injection of saturated KCl into the tail vein, and the secretion determined for 75 min thereafter. In the second study, the effect of 16-dmPGE₂ (Ono) on basal HCO₃⁻ secretion was examined. 16-dmPGE₂ was given either subcutaneously (1–30 μg/kg), by intravenous infusion into the tail vein (3 μg/kg/hr) or by topical application for 30 min through the three way tap (0.3–10 μg/ml), at least 1 hr after basal secretion had stabilized. Infusion of 16-dmPGE₂ was performed using a peristaltic pump (Harvard Apparatus, Model 931-D) at a rate of 1.2 ml/hr. In the case of topical application, the stomach or duodenum was exposed for 30 min to 2 ml or 0.3 ml of various concentrations of 16-dmPGE₂, respectively. 16-dmPGE₂ was first dissolved in absolute ethanol and diluted with saline. Control animals were given the vehicle alone. The HCO₃⁻ secretion was measured for 2 hr after treatment with 16-dmPGE₂. In another experiment, gastric or duodenal HCO₃⁻ secretion was stimulated with subcutaneous administration of 16-dmPGE₂ (10 μg/kg), and 90 min later, the animals were killed with saturated KCl (i.v.). In some rats, the effect of topical application of 16-dmPGE₂ (10 μg/ml) on transmucosal potential difference (PD) of the gastric and duodenal mucosa was examined using a method described in a previous paper (13). In the third study, the effect of topical acid on HCO₃⁻ secretion was examined. At least 1.5 hr after the basal HCO₃⁻ secretion had stabilized, the stomach or duodenum was perfused for 10 min with 100 mM HCl or 10 mM HCl solution made isotonic with NaCl, respectively. After exposure to acid, the mucosa was rinsed gently with saline, and HCO₃⁻ secretion was measured for 2 hr thereafter. In half of these animals, indomethacin (Merck), suspended in 1% carboxymethyl-cellulose solution (CMC), was given subcutaneously in a dose of 5 mg/kg, 1 hr before exposure to acid. In the last study, the effect of acetazolamide (Sigma) on basal and stimulated HCO₃⁻ secretion was examined. Acetazolamide, suspended in 1% CMC solution, was subcutaneously given in a dose...
of 100 mg/kg, 1 hr after the basal secretion had stabilized and 1 hr before subcutaneous administration of 16-dmPGE₂ (10 μg/kg). In different rats, carbonic anhydrase activity was determined in the corpus and antral mucosa of the stomach and in the proximal duodenal mucosa, excised at 1 and 3 hr after administration of acetazolamide, using the micromethod (14). In each study, HCO₃⁻ secretion was measured every 15 min, and the results were expressed as μEq/15 min. Carbonic anhydrase activity was expressed as Wilbur-Anderson (W-A) unit/mg protein.

Statistics

Data are presented as the mean±S.E.M. of values read every 15 min from 6 rats during each test period. Statistical analysis was performed using Student’s t-test for unpaired variables, and values of P<0.05 were regarded as significant.

Results

Basal HCO₃⁻ secretion: Both the whole stomach and the proximal duodenum of anesthetized rats consistently secreted alkaline in an amount of 0.2–0.4 μEq/15 min and 1.5–2 μEq/15 min of HCO₃⁻ as a basal secretion, respectively (Fig. 3). In the stomach, HCO₃⁻ secretion was detectable approximately 30 min after complete inhibition of acid secretion by intraduodenal administration of omeprazole. Basal HCO₃⁻ secretion in the stomach did not significantly change after the animals had been killed by a bolus injection of saturated KCl intravenously, while the duodenal HCO₃⁻ secretion decreased with time and reached about 40% of normal values at 60 min later.

Effect of 16-dmPGE₂ on HCO₃⁻ secretion: Subcutaneous administration of 16-dmPGE₂ (1–30 μg/kg) dose-dependently stimulated HCO₃⁻ secretion, which reached 3.8±0.5 μEq/15 min in the stomach and 4.5±0.4 μEq/15 min in the duodenum, in response to 30 μg/kg (Figs. 4 and 5). Gastric HCO₃⁻ secretion was significantly increased even at 1 μg/kg, while duodenal HCO₃⁻ secretion was significantly influenced at the dose of 3 μg/kg or greater. A similar increase of HCO₃⁻ secretion was observed in response to topical application of 16-dmPGE₂ (0.3–10 μg/ml) for 30 min into the stomach or the duodenal loop. In these cases, the maximal stimulation of HCO₃⁻ secretion was obtained in the first 15 min period after exposure to 16-dmPGE₂, and this was more apparent in the gastric HCO₃⁻ secretion (Figs. 4 and 5). At the concentration of 10 μg/ml of 16-dmPGE₂, HCO₃⁻ secretion reached over 3 μEq/15 min in both tissues. No significant changes in transmucosal PD values were observed even at the highest concentration of 16-dmPGE₂ in both the stomach and duodenum (data not shown). Intravenous infusion of 16-dmPGE₂ (3 μg/ kg/hr) also produced a significant and persistent increase of HCO₃⁻ secretion in both tissues (Fig. 6). The increase of HCO₃⁻ secretion by intravenously administered 16-dmPGE₂ appeared within 15 min and reached the plateau levels more rapidly as
compared to that by subcutaneous administration, and it was 6 or 2 times more than control values in the stomach or the duodenum, respectively. Stimulated \( \text{HCO}_3^- \) secretion caused by subcutaneous administration of 16-dmPGE\(_2\) (10 \( \mu \)g/kg) disappeared quickly when the animals were killed with saturated KCl (Fig. 7). After sacrifice, gastric \( \text{HCO}_3^- \) secretion markedly decreased in the first 15 min period, returned to the baseline level within 45 min, whereas duodenal \( \text{HCO}_3^- \) secretion gradually decreased and reached the level even lower than the baseline values within 45 min.

**Effect of topical acid on \( \text{HCO}_3^- \) secretion:** As observed in many other species, \( \text{HCO}_3^- \) secretion in the gastroduodenal mucosa of rats was also stimulated by exposure to acid. Perfusion of the duodenal loop for 10 min with 10 mM HCl solution increased \( \text{HCO}_3^- \) secretion to 2–2.5 times greater than the basal values (Fig. 8). Exposure of the stomach for 10 min to 10 mM HCl solution did not affect \( \text{HCO}_3^- \) secretion (not shown), but acidification with 100 mM HCl solution caused a 6-fold increase in \( \text{HCO}_3^- \) secretion.
Fig. 5. Effect of 16-dmPGE$_2$ given subcutaneously (upper) or by topical application (lower) on HCO$_3^-$ secretion in the proximal duodenum. In topical application, the duodenal loop was exposed for 30 min to 0.3 ml of various concentrations of 16-dmPGE$_2$ (1–10 μg/ml). *Significantly different from controls, at P < 0.05.

Increase in HCO$_3^-$ secretion caused by topical acid was rather transient and returned to the baseline values within 2 hr. Pretreatment of rats with indomethacin (5 mg/kg, s.c.) all but completely blocked the increased response of HCO$_3^-$ secretion caused by topical acid in both tissues. Indomethacin alone tended to decrease duodenal but not gastric HCO$_3^-$ secretion.

Effect of acetazolamide on basal and stimulated HCO$_3^-$ secretion: Subcutaneous administration of acetazolamide (100 mg/kg) had no effect on basal HCO$_3^-$ secretion in both the stomach and the proximal duodenum (Fig. 9). This agent also had no influence on the stimulatory effect of 16-dmPGE$_2$ on HCO$_3^-$ secretion. In response to subcutaneous administration of 16-dmPGE$_2$ (10 μg/kg), HCO$_3^-$ secretion increased in the same magnitude in both the stomach and duodenum, regardless of whether or not the animals had been pretreated with acetazol-
amid. Carbonic anhydrase activity in the corpus and antral mucosa of the stomach and in the proximal duodenal mucosa was all but completely inhibited for at least 3 hr after this agent had been given in a dose of 100 mg/kg (Fig. 10).

**Discussion**

Several prostaglandins of the E and F series prevent gastric lesions induced by noxious agents including necrotizing substances in rats (15, 16). This protective ability, termed "cytoprotection", is completely independent from their gastric acid inhibitory activity, but seems to be related to their stimulatory action on mucus and HCO₃⁻ secretion (16, 17). However, only a few studies have been reported on rat HCO₃⁻ secretion. Kivilaakso and Flemström (5) showed that the rat duodenum has an ability to increase HCO₃⁻ secretion and the mucus pH gradient in response to PGE₂. A stimulatory effect of 16-dmPGE₂ on gastric HCO₃⁻ secretion was first reported by Reischtein and Cohen (6) using the chambered rat oxyntic mucosa. Although many studies have demonstrated the stimulatory effect of prostaglandins on gastro-duodenal HCO₃⁻ secretion in other species, these effects on gastric HCO₃⁻ secretion...
differed from species to species (2–4, 8, 9, 18). Even in the same species results were controversial, depending upon the route of administration, the preparation of the stomach, or the group of investigators (7, 8, 19, 20). Thus, it is important to establish a method for determination of gastric HCO$_3^-$ secretion in the rat and to examine prostaglandin effects on it.

Whittle and Kauffman (21) reported the effect of topical application of stable analogues of PG12 on HCO$_3^-$ secretion in the whole stomach of the rat. They used a bolus injection of cimetidine (60 mg/kg, i.p.) and intravenous infusion of PGE$_2$ (1 mg/kg/hr) to inhibit acid secretion and titrated HCO$_3^-$ at luminal pH 5.0. In the present study, gastric HCO$_3^-$ secretion was unmasked by a bolus injection of omeprazole (H+-K+ATPase inhibitor) into the duodenum. The dose (30 mg/kg) used was shown previously to completely inhibit acid secretion for more than 7 hr in the rat (22) and seemed to afford sufficient conditions for the present experiments. As demonstrated in other species (1–4), both the stomach and the proximal
duodenum of rats spontaneously secreted \( \text{HCO}_3^- \) in amounts of 0.2–0.4 \( \mu \text{Eq}/15 \text{ min} \) and 1.5–2 \( \mu \text{Eq}/15 \text{ min} \), respectively. The amount of \( \text{HCO}_3^- \) in the stomach and duodenum was roughly estimated as 5% and 30% of that of basal acid secretion in the same species under anesthetized conditions (23). However, since gastric \( \text{HCO}_3^- \) secretion did not decrease after sacrificing the animals with saturated KCl (i.v.), it appears to be formed by passive diffusion of \( \text{HCO}_3^- \). In contrast, duodenal \( \text{HCO}_3^- \) secretion was due to both active (60%) and passive (40%) \( \text{HCO}_3^- \) transport, as shown in the amphibian duodenum (10). This is also supported by the fact that inhibition of endogenous prostaglandin synthesis with indomethacin reduced duodenal but not gastric \( \text{HCO}_3^- \) output in the rat. Since \( \text{HCO}_3^- \) in the stomach was not detected at luminal pH 7.4 under omeprazole inhibition of acid secretion, gastric \( \text{HCO}_3^- \) secretion was titrated at pH...
5.0, according to the previous papers reported by others using dogs and rats (4, 21). Therefore, alkalinization in the stomach under basal conditions may be accounted for by a diffusion of $\text{HCO}_3^-$ and/or acid back-diffusion due to the pH gradient.

Konturek et al. (4) demonstrated that PGE$_2$ or 16-dmPGE$_2$, given either by topical application or intravenous infusion, dose-dependently increased $\text{HCO}_3^-$ output in the dog duodenal pouch. Smeaton et al. (9) showed large increases of duodenal $\text{HCO}_3^-$ output in response to topical application of 16-dmPGE$_2$ in cats, but failed to confirm its stimulatory action when given intravenously (1 µg/kg/hr). We found in the rat duodenum that $\text{HCO}_3^-$ secretion increased dose-dependently and significantly in response to 16-dmPGE$_2$ given subcutaneously (1–30 µg/kg) or topically (1–10 µg/ml) or by intravenous infusion (3 µg/kg/hr). The maximal increase of $\text{HCO}_3^-$ output in the rat duodenum was about 2–2.5 times greater than the basal values. This was consistent with findings in the dog (4), but far less than in the cat (9). Recently, Tabata et al. (24)

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**Fig. 9.** Effect of acetazolamide (100 mg/kg) on basal and 16-dmPGE$_2$-stimulated $\text{HCO}_3^-$ secretion in the stomach (upper) and in the proximal duodenum (lower). Acetazolamide was given subcutaneously 1 hr before administration of 16-dmPGE$_2$ (10 µg/kg).
reported that intravenous infusion of 16-dmPGE$_2$ (10 $\mu$g/kg/hr) had no effect on HCO$_3^-$ secretion in the rat duodenum. They used conscious rats and measured HCO$_3^-$ secretion indirectly, whereas we used anesthetized rats and measured HCO$_3^-$ output by direct titration using a pH-stat method. However, Isenberg et al. (25) reported that duodenal HCO$_3^-$ secretion was stimulated by endogenous and exogenous (PGE$_2$) prostaglandins in chronic unanesthetized rats. Therefore, the above difference appears not to be ascribed to anesthesia, but may be due to acute surgical trauma in conscious rats.

Similar to duodenal HCO$_3^-$ secretion, 16-dmPGE$_2$ given by various routes increased gastric HCO$_3^-$ secretion in the rat, and the maximal HCO$_3^-$ output obtained was 5–6 times greater than the basal values, regardless of the route of administration. Since transmucosal PD was not significantly changed even after exposure to the highest concentration (10 $\mu$g/ml) of 16-dmPGE$_2$, the increase in mucosal permeability due to mucosal damage can be excluded from the mechanism. We cannot separate HCO$_3^-$ secretion from antral and oxyntic mucosa in the present study. However, since it has been shown that HCO$_3^-$ secretion from the Heidenhain pouch and antral pouch of dogs responded similarly to PGE$_2$ and 16-dmPGE$_2$ (4), the same might be assumed for the rat stomach. In an earlier study, Garner and Heylings (7) reported that 16-dmPGE$_2$ and PGF$_{2\alpha}$ stimulated HCO$_3^-$ secretion in vitro amphibian antral and fundic mucosa, and they proposed that this effect may be responsible for cytoprotection. Schiessel et al. (8), however, have been unable to confirm these findings in the same species. Miller et al. (19) found that 16-dmPGE$_2$ increased HCO$_3^-$ secretion in fundic mucosa of the canine chambered stomach. Later, Kuo et al. (20) from the same laboratory reported that HCO$_3^-$ secretion in isolated fundic mucosa of the dog stomach was not stimulated by 16-dmPGE$_2$, suggesting that an intact blood circulation is required for this action to occur. These results taken together suggest that the whole stomach preparation of the rat is a useful model to examine the effect of 16-dmPGE$_2$ or probably other prostaglandins on gastric HCO$_3^-$ secretion.
Flemstrom (1) first proposed that the main source of luminal alkalinization is endogenous HCO$_3^-$, because nutrient HCO$_3^-$ was found not to modify this process in an in vitro preparation of amphibian fundic and duodenal mucosa. However, Silen and colleagues (10, 11, 26) found in the same species that HCO$_3^-$ secretion was not influenced by acetazolamide, but markedly reduced by removal of nutrient HCO$_3^-$. We also found that acetazolamide at the dose of 100 mg/kg which showed over 80% inhibition of carbonic anhydrase activity in the gastroduodenal mucosa of rats, failed to affect HCO$_3^-$ secretion under basal conditions and in response to 16-dmPGE$_2$. In fact, the protective effects of 16-dmPGE$_2$ on gastric and duodenal lesions in rats were not affected by pretreatment with acetazolamide (12). Studies in other tissues such as canine submaxillary gland and hamster ileum suggest that extracellular fluid is a major source of the secreted HCO$_3^-$ (27, 28). Even in the pancreas, where cellular metabolism is sufficient to supply CO$_2$ for maximal rates of secretion, studies with labeled HCO$_3^-$ have shown that extracellular fluid is an important source of the secretion of HCO$_3^-$ (29). Thus, in a variety of tissues that secrete HCO$_3^-$, some portion of the luminal HCO$_3^-$ seems to originate in the extracellular fluid regardless of the rate of cellular metabolism. These results suggest that carbonic anhydrase does not significantly contribute to the process of HCO$_3^-$ secretion in either the stomach or duodenum of rats.

We confirmed in the rat that topical acid to the gastroduodenal mucosa stimulates HCO$_3^-$ secretion, in agreement with the findings in other species (30, 31). The degree of HCO$_3^-$ output caused by topical acid was roughly equivalent to the maximal HCO$_3^-$ output induced by 16-dmPGE$_2$, and this effect was completely blocked by the prior administration of indomethacin. Although the concentration of acid required to elicit the increased response of HCO$_3^-$ secretion differed in the stomach and duodenum, this is what we would expect based on the amount of acid normally present in these two tissues.

In summary, we have shown in the rat that both endogenous and exogenous (16-dmPGE$_2$) prostaglandins stimulate HCO$_3^-$ secretion in the whole stomach as well as in the proximal duodenum. We have also demonstrated that HCO$_3^-$ secretion in the rat is independent from carbonic anhydrase activity in the gastroduodenal mucosa. Our method in the rat to measure gastric and duodenal HCO$_3^-$ secretion will be useful for studying the mechanisms of HCO$_3^-$ secretion and for screening cytoprotective drugs.

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