Stimulation by Prostaglandin E2 of Alkaline Secretion in the Rat Duodenum: Comparative Study with Hypertonic NaCl

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Abstract—Possible involvement of increased mucosal permeability in the stimulation by prostaglandin E2 (PGE2) of duodenal HCO3− secretion was investigated in rats. PGE2 (0.3, 1 mg/kg, s.c.) dose-dependently increased HCO3− secretion in the duodenum with a significant elevation of transmucosal potential difference (PD); the PD was increased from −4.5±0.3 mV to −10.0±1.5 mV (mucosa negative) at 1 mg/kg. These responses caused by PGE2 were abolished by sacrificing the animals with saturated KCl (i.v.). Although a significant increase of HCO3− output was observed after exposure of the mucosa to 1 M NaCl (0.5 ml), this response was accompanied by a significant reduction of PD and was not abolished after KCl injection. The mucosal permeability determined by Evans blue (1%, i.v.) was not affected by PGE2, while 1 M NaCl markedly elevated the amount of extravasated dye in both the luminal content and the mucosa. Stimulation of HCO3− output by PGE2 was significantly mitigated by ouabain (3 mg/kg, s.c.) or prior exposure of the mucosa to 1 M NaCl. These results suggest that stimulation by PGE2 of duodenal HCO3− secretion is not simply due to the increased mucosal permeability, but depends rather on both the Na/K ATPase activity and the intact perfusion of the organ. The HCO3− response as induced by 1 M NaCl may result from the increased permeability and is accompanied by a marked reduction of PD.

Duodenal HCO3− secretion is generally considered to depend on active, energy-requiring processes in the mucosa (1). Simson et al. (2) showed that prostaglandins (PGs) increased alkaline secretion via a PG-sensitive adenylate cyclase inducing sodium-coupled uptake of HCO3− at the basolateral membrane and conductive exit across the apical membrane, giving rise to electrogenic transcellular transport of HCO3−. However, a recent study by Heylings and Feldman (3) suggested that PGs may act by increasing the passive flux of HCO3− rather than by stimulating energy dependent HCO3− transport in the rat duodenum. Thus, the precise mechanism underlying the rise in HCO3− output by PGs remains unclear.

In the present study we have measured luminal alkalinization (pH), transmucosal PD and mucosal permeability in response to PGE2 in the rat duodenum and compared the changes with those induced by exposure of the mucosa to hypertonic NaCl. Since the stimulatory action of PGE2 on HCO3− secretion reportedly depends upon the Na/K ATPase activity at the serosal membrane (2), the effect of ouabain on the changes induced by PGE2 was also examined.

Materials and Methods
Male Sprague Dawley rats (Charles River, Shizuoka), weighing 250–300 g, were used. The animals were kept in individual cages with raised mesh bottoms to prevent coprophagy and deprived of food but allowed free access to tap water for 18 hr before the experiment. All studies were carried out using 4 to 8 rats per group, under anesthetized conditions induced by intraperitoneal administration of urethane (1.25 g/kg).
**Determination of duodenal HCO$_3^-$ secretion:** Bicarbonate secretion was determined in the proximal duodenum using a pH-stat method as previously described (4). Briefly, the abdomen was incised, and both the stomach and duodenum were exposed. A duodenal loop was made between the pyloric ring and the area just above the outlet of the common bile duct (1.7 cm). The loop was perfused at the flow rate of 1 ml/min with saline (154 mM NaCl) that was oxygenated with 100% O$_2$, heated at 37°C and kept in a reservoir. Alkaline secretion was titrated at pH 7.4 using the pH-stat method and by adding 10 mM HCl to the reservoir. Approximately 1 hr after basal HCO$_3^-$ output had stabilized, prostaglandin E$_2$ (PGE$_2$: 0.3, 1 mg/kg) was given subcutaneously (s.c.) or the duodenal mucosa was exposed for 10 min to 0.5 ml of hypertonic NaCl (0.5 M, 1.0 M), and the alkaline secretion was determined for 1.5 hr thereafter. In some cases, we examined the effect of saturated KCl injection on the increased HCO$_3^-$ responses caused by PGE$_2$ (1 mg/kg) or exposure of the mucosa to 1 M NaCl. The animals were given KCl intravenously (i.v.) as a single injection 1 hr after administration of PGE$_2$ or exposure to 1 M NaCl. In another experiment, the effects of ouabain pretreatment and prior exposure of the mucosa to 1 M NaCl on stimulation by PGE$_2$ (1 mg/kg) or exposure of the mucosa to 1 M NaCl were examined. Ouabain was given s.c. in a dose of 3 mg/kg, while 1 M NaCl was applied into the mucosa for 10 min. PGE$_2$ was given s.c. 30 min after these treatments. The dose of ouabain was selected to inhibit Na/K ATPase activity in the gastric mucosa in vivo (5).

**Measurement of mucosal permeability:** Since alkaline responses caused by PGE$_2$ and 1 M NaCl might be due to an increased mucosal permeability of the duodenum, we used a dye method to evaluate the effects of PGE$_2$, 1 M NaCl and KCl on the mucosal and vascular permeability, according to a previous paper (7). The duodenal loop was made as described previously, and it was perfused at the flow rate of 1 ml/min with saline. The duodenal perfusate was collected into tubes every 10 min. The animals were injected with 1 ml of Evans blue (1% w/w) i.v.; and 30 min later, they were killed by bleeding from the abdominal aorta. The duodenum was opened along the mesenteric attachment, and the mucosa was scraped off using two glass slides, weighed, and put into a tube containing 2 ml of distilled water. The extraction of dye from both the duodenal perfusate and mucosa was performed according to the modified method described by Katayama et al (8). The absorbances of the samples were measured at 620 nm in a Hitachi spectrophotometer (Model 200-100). The amounts of dye recovered from the duodenal perfusate and mucosa were expressed as μg/ml of duodenal contents and μg/100 mg of wet tissue weight, respectively. Thirty minutes before Evans blue injection, PGE$_2$ (1 mg/kg) was given s.c., while 1 M NaCl (0.5 ml) was topically applied into the duodenal loop for 10 min. Saturated KCl was given i.v. 5 min after Evans blue injection.

**Determination of transmucosal potential difference and pH in the duodenum:** The duodenal loop was made in the proximal part of the duodenum as described previously, and it was perfused at the flow rate of 1 ml/min with saline (pH 5.8). The duodenal PD was determined using a PD meter (Yokokawa, Type 2575) through two agar bridges, one positioned in the lumen of the duodenum and the other in the abdominal cavity (6). The pH of the duodenal perfusate was measured using a pH glass electrode of the flow type (Horiba, Model 6901-25T), and continuously monitored on a two channel recorder (Hitachi, Model 056), simultaneously with the duodenal PD. Approximately 1 hr after the basal PD and pH had stabilized, PGE$_2$ (1 mg/kg) was given s.c. or the loop was exposed to 0.5 ml of 1 M NaCl for 10 min. In the latter, the measurement of pH was interrupted for 10 min while the mucosa was exposed to 1 M NaCl. In some experiments, the effects of indomethacin (5 mg/kg), ouabain (3 mg/kg) and 1 M NaCl were examined on the PD and pH responses caused by PGE$_2$ (1 mg/kg). Both indomethacin and ouabain were given s.c., while 1 M NaCl (0.5 ml) was topically applied into the duodenal loop for 10 min. PGE$_2$ was given s.c. 30 min after these treatments. At the end of each experiment, the animals were killed by i.v. injection of saturated KCl.
Preparation of drugs: Drugs used were urethane (Tokyo Kasei, Tokyo), prostaglandin E₂ (Ono, Osaka), indomethacin, ouabain (Sigma Chemicals, St. Louis, MO) and Evans blue (Merck, Darmstadt, West Germany). Prostaglandin E₂ was first dissolved in absolute ethanol and diluted with saline to the desired concentrations. Indomethacin was suspended in saline with a drop of Tween 80 (Nacalai Tesque, Kyoto). Other drugs were dissolved in saline. Each agent was prepared immediately before use, and was given s.c. in a volume of 0.5 ml per 100 g of body weight or i.v. in a volume of 1 ml per rat.

Statistics: Data are presented as the mean±S.E. from 4 to 8 rats per group. Statistical analysis was performed using a two-tailed Dunnett's multiple comparison test (9), and values of P<0.05 were regarded as significant.

Results

Effects of PGE₂ and 1 M NaCl on HCO₃⁻ output and PD responses in the duodenum

HCO₃⁻ output: The duodenum of anesthetized rats secreted alkali at the rate of about 1 μEq/10 min and remained unaltered when saline (0.15 M NaCl) was given s.c. or applied intraluminally for 10 min. PGE₂ (0.3, 1 mg/kg, s.c.) increased duodenal HCO₃⁻ secretion significantly in a dose-related manner; HCO₃⁻ output elevated to the levels of 2.3 and 4.9 times greater than the control at 0.3 and 1 mg/kg, respectively, and reached to the maximal values of 5.0±0.9 μEq/10 min at the latter dose (Fig. 1). Duodenal HCO₃⁻ output was also significantly increased in a concentration-dependent manner when the mucosa was exposed for 10 min to hypertonic NaCl. Application of 1 M NaCl into the duodenum raised HCO₃⁻ output from 1.2±0.2 μEq/10 min to the maximal values of 2.6±0.3 μEq/10 min within 30 min after the exposure, followed by a gradual decrease (Fig. 2).

To compare the increased HCO₃⁻ responses caused by PGE₂ and 1 M NaCl, HCO₃⁻ secretion was measured before and after i.v. injection of saturated KCl. As shown in Fig. 3, HCO₃⁻ secretion was significantly increased to about 4 and 2.5 times greater than the basal levels in response to PGE₂ (1 mg/kg, s.c.) and intraluminal application of 1 M NaCl, respectivey. When the perfusion of the organ was terminated by sacrificing the animals with KCl injection, the increased HCO₃⁻ responses induced by PGE₂ were reduced to or below the basal values within 30 min. On the contrary, KCl injection failed to produce a marked reduction in the HCO₃⁻ output caused by 1 M NaCl, and the HCO₃⁻ output was significantly greater than the basal values, even at 1 hr after KCl treatment.

PD response: The duodenal mucosa gener-
ated a stable PD of -4 to -6 mV (mucosa negative) under the present conditions. PGE2 (1 mg/kg, s.c.) significantly raised the duodenal PD: the values were increased from -5.1 ± 0.3 mV to -8.2 ± 0.5 mV within 10 min, and they remained elevated for about 1 hr (Figs. 4 and 5). In contrast, the PD was significantly decreased in response to intra-duodenal application of 1 M NaCl for 10 min. While the PD was increased during exposure to 1 M NaCl, it decreased immediately after removal of the solution from the duodenum and remained lowered for about 1 hr; the PD was decreased from -5.0 ± 0.3 mV to the minimal values of -2.6 ± 0.2 mV. In either case, KCl injection abolished the generation of PD with a sharp decline of the PD to zero.

Effects of PGE2 and 1 M NaCl on vascular permeability of the duodenal mucosa

Under normal conditions, the duodenal mucosal permeability was minimum: the amount of extravasated dye in the mucosa was 0.9 ± 0.2 μg/100 mg tissue, and there was no dye detectable in the luminal solution (Fig. 6). PGE2 (1 mg/kg, s.c.) did not affect the permeability of the duodenal mucosa, and these values were not significantly different from those obtained in the control animals. On the other hand, the exposure of the duodenal mucosa to 1 M NaCl for 10 min markedly enhanced the permeability response, and the extravasated amount of dye in the mucosa reached to 5.3 ± 1.2 μg/100 mg tissue, which
was about 5 times greater than the control values. Exposure of the mucosa to 1 M NaCl also increased the leakage of dye into the luminal contents, the amount of dye being 0.38±0.18 μg/ml. Sacrifice of the animals with KCl 5 min after dye injection completely abolished the extravasated leakage of dye into the mucosa and the lumen.

Effects of 1 M NaCl and ouabain on duodenal \( \text{HCO}_3^- \) output caused by PGE2

PGE2 (1 mg/kg, s.c.) increased \( \text{HCO}_3^- \) secretion from 1.0±0.1 μEq/10 min to 3.9±0.9 μEq/10 min within 1 hr, which remained elevated for 1 hr thereafter (Fig. 7). Prior exposure of the duodenal mucosa to 1 M NaCl significantly raised the rate of \( \text{HCO}_3^- \) secretion but completely abolished the increased \( \text{HCO}_3^- \) responses caused by PGE2; the \( \text{HCO}_3^- \) output obtained by 1 M NaCl remained unaltered before and after administration of PGE2. Pretreatment of the animals with ouabain (3 mg/kg, s.c.) also produced a slight but significant inhibition against the stimulation by PGE2 of duodenal \( \text{HCO}_3^- \) secretion. The total amount of \( \text{HCO}_3^- \) output caused by PGE2 was 49.3±4.3 μEq/2 hr and 36.1±5.2 μEq/2 hr, respectively, in the control and ouabain-treated groups.

Effects of PGE2 on duodenal pH and PD under various conditions

To further characterize the stimulatory action of PGE2 on duodenal \( \text{HCO}_3^- \) secretion, we investigated the effect of PGE2 on duodenal pH and PD under various conditions. When the duodenal loop was perfused with saline (pH 5.8), the pH of the duodenal perfusate was about 7.0, and PD was −4 to −6 mV (Fig. 8A). Under these conditions, PGE2 (1 mg/kg, s.c.) immediately elevated the PD, followed by a gradual increase of pH, and these responses persisted for about 1.5 hr. Prior administration of indomethacin (5 mg/kg, s.c.) did not significantly affect these responses caused by PGE2 (Fig. 8B). However, the duodenal PD was markedly reduced in response to ouabain (3 mg/kg, s.c.) with a slight decrease of the pH; the PD was reduced from −4.3±0.5 to 0.6±0.4 mV. Subsequent administration of PGE2 elevated both PD and pH in these animals, but the degrees of these responses were less marked when compared to those in the control animals (Fig. 8D). Similar responses of PD and pH were observed after PGE2 injection in the duodenum exposed previously to 1 M NaCl. On the other hand, perfusion of the duodenal loop with 22 mM NaHCO3 raised the pH of the perfusate to about 8.3 with no effect on the PD. Under these conditions, PGE2 produced a significant elevation of the PD with very
little increase of the pH, but these responses were also less marked as compared to those observed in normal rats (not shown).

Discussion

The present study confirmed a marked stimulation by PGE$_2$ of duodenal HCO$_3^-$ secretion and further showed that the process is not accompanied by an increased mucosal permeability but depends on the Na/K ATPase activity and the intact perfusion of the organ. Although HCO$_3^-$ output was significantly increased after exposure of the mucosa to hypertonic NaCl, this response was accompanied by a lowering of the PD and an increase of mucosal permeability, suggesting a phenomenon due to an increase of passive diffusion of HCO$_3^-$ from the mucosa into the luminal solution.

We previously demonstrated that stable duodenal HCO$_3^-$ secretion in vivo occurred at luminal pH 7.4, responded to a low luminal pH or exogenous PGs, and reduced by about 50% after sacrifice of the animals with KCl injection, suggesting that 50% of the basal HCO$_3^-$ output may be due to passive movement from the blood to the luminal fluid (4). Sacrifice by KCl injection does not substantially negate the active component of HCO$_3^-$ only, because this treatment causes stasis of perfusion in the organ, results in insufficient supply of HCO$_3^-$ to the epithelial cells, and may affect the passive component of HCO$_3^-$ output. Yet, sacrifice of the animals with KCl abolished the leakage of Evans blue into the mucosa and the luminal solution, suggesting no change in the mucosal permeability after death. Cessation of the organ perfusion with KCl might possibly separate the active component of HCO$_3^-$ secretion from the passive diffusion of this ion.

In agreement with other studies (3, 4), the luminal alkalinization in the rat duodenum responded to natural PGE$_2$ by a dose-dependent rise in HCO$_3^-$ output with concomitant elevation of the transmucosal PD. HCO$_3^-$ output was similarly increased after exposure of the duodenal mucosa to hypertonic NaCl for 10 min, but this case was accompanied by a significant reduction of the PD. Furthermore, the increased HCO$_3^-$ responses caused by PGE$_2$ and hypertonic NaCl were in contrast to the dependence of the intact perfusion of the organ; after KCl injection the alkaline response induced by PGE$_2$ was quickly abolished, while the increased HCO$_3^-$ output caused by hypertonic NaCl remained relatively unchanged even 1 hr later. These results indicate that a different mechanism could account for the increased alkaline responses induced by PGE$_2$ and hypertonic NaCl. The dependence of PGE$_2$ action on the intact organ perfusion is also supported by our previous findings (10) that the reduction of mucosal blood flow by hemorrhagic shock significantly impaired the increased alkaline response caused by PGE$_2$ as well as basal HCO$_3^-$ output.

A number of mechanisms could account for the rise in alkaline secretion from the duodenal mucosa, including the direct stimulation of HCO$_3^-$ output by enhancing either cellular transport or mucosal permeability (3, 11–13). In fact, passive diffusion of HCO$_3^-$ via shunt pathways contributes substantially to luminal alkalinization by this tissue in vitro (13). Evans blue used in the present study generally
binds to serum protein and is employed as a marker of vascular permeability. The appearance of this dye in the luminal fluid may reflect an increased mucosal permeability due to endothelial and/or epithelial injury (14). As expected from the decreased PD responses, hypertonic NaCl markedly elevated the recovery of this dye in the luminal fluid, probably by inducing epithelial injury. In fact, the mucosa exposed to 1 M NaCl appears to lose normal cellular activity of the epithelium, since PGE2 failed to stimulate HCO3− secretion in the duodenum preexposed to 1 M NaCl. On the other hand, PGE2 did not affect the mucosal permeability as determined from the dye experiment. These results are consistent with the findings by Wilkes et al. (12) who showed that PGE2 produced no changes in mucosal permeability of the duodenum. However, since the HCO3− ion can permeate very small channels and since Evans blue is a macromolecule which would not appear through such small channels, the results presented with Evans blue may not totally prove that passive permeability of HCO3− ions is unaltered by PGE2.

Simson et al. (12, 15, 16) postulated that duodenal HCO3− secretion is an electrogenic process that depends upon cellular metabolism through the energy-requiring Na/K exchange pump at the nutrient membrane. In contrast, a recent study by Heylings and Feldman (3) suggested that PGE2 may act by increasing the passive flux of HCO3− rather than by stimulating energy-dependent HCO3− transport. They demonstrated that PGE2 significantly increased HCO3− secretion into a HCO3−-free luminal solution but had no effect on HCO3− secretion into the luminal solution containing 22 mM HCO3−. We previously reported that both ouabain (10 mg/kg, s.c.) and digitoxin (10 mg/kg) significantly blocked the acid-induced HCO3− secretion in the rat duodenum without much effect on basal HCO3− output (17, 18). The present study further showed that ouabain significantly lessened the magnitude of PD and HCO3− responses induced by PGE2. Since this dose of ouabain did not affect basal rates of HCO3− secretion, it is unlikely that such effects may be due to nonspecific intoxication of this drug in other organs such as the cardiovascular system. These results are consistent with the findings by Simson et al. (16) who demonstrated that ouabain significantly reduced HCO3− secretion using an in vitro amphibian duodenum. In the present study, we also confirmed that the rise in luminal pH after PGE2 treatment was minimal when the duodenum was perfused with saline containing 22 mM HCO3−. However, since the pH of the luminal perfusate containing 22 mM HCO3− was about 8.5 and as NaHCO3 can be absorbed in the cell due to the gradient, the rise in HCO3− output caused by PGE2 may be masked by the luminal loss of this ion. If PGE2 enhanced luminal alkalization simply by increasing the passive diffusion, then this effect should remain unaltered even after KCl injection, similar to that induced by hypertonic NaCl. Thus, it may be assumed that stimulation of HCO3− secretion caused by PGE2 is not simply due to passive diffusion of this ion into the luminal solution, but may depend on the cellular mechanism related to Na/K ATPase activity. Certainly, further studies including determination of ionic fluxes in vitro are needed to characterize the role of Na/K ATPase activity in the cellular mechanisms of HCO3− secretion.

In conclusion, the present study, together with the previous findings (10), suggests that stimulation by PGE2 of duodenal HCO3− secretion is not simply due to the increased mucosal permeability, but depends rather on the intact perfusion of the organ and a cellular mechanism such as one involving the Na/K ATPase activity.

References


4 Takeuchi, K., Tanaka, H., Furukawa, O. and


