Determination of Bicarbonate Output Using pH Deflection in the Rat Duodenum: Influences of Prostaglandins and Cholinergic Agents

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Abstract—We set up a system to measure the luminal pH, potential difference (PD) and bicarbonate output in the anesthetized rat duodenum, and investigated these responses caused by prostaglandins (PGs) and cholinergic agents. When the proximal duodenum (1.7 cm) was perfused at a flow rate of 0.7 ml/min with saline adjusted to pH 4.5, the duodenal pH, PD and HCO₃⁻ output were 5.5 to 6.0, -4 to -6 mV and 1.2 to 1.6 µEq/10 min, respectively; they were markedly reduced by i.v. injection of saturated KCl. Both natural (PGE₁, PGE₂) and synthetic (PGE₂, PG₁₂) PGs, given either s.c. or i.v., significantly elevated all these parameters, while indomethacin (s.c.) decreased the pH as well as the PD. Small but significant increases of the pH were observed after i.v. administration of cholinergic agents (carbachol, bethanechol), a GABAergic agent (baclofen) and an analogue of thyrotropin releasing hormone (YM-14673), with a temporal elevation of the PD; the degree of net HCO₃⁻ output caused by these agents was 20–50% of the values obtained with PGE₂ (100 µg/kg, i.v.), and they were significantly reduced in the presence of atropine. These results suggest that (a) the system using pH deflections can be used to sensitively detect HCO₃⁻ output in the rat duodenum, and (b) duodenal acid neutralizing capacity may be regulated by central and peripheral cholinergic systems as well as endogenous PGs.

Materials and Methods

Male Sprague Dawley rats (300–350 g), kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access to tap water for 18 hr prior to the experiments.

Operative procedures: Animals were anesthetized with intraarterial administration of...
urethane (1.25 g/kg). Simultaneous measurement of luminal pH and PD in the proximal duodenum was performed according to previous papers (3, 11). Briefly, the abdomen was incised, and both the stomach and duodenum were exposed. An acute gastric fistula was prepared by means of a polyethylene tube in the forestomach through which the gastric contents were withdrawn to prevent accumulation of gastric juice in the stomach. A duodenal loop was made between the pyloric ring and the area just proximal to the outlet of the common bile duct (1.7 cm), excluding the influences of bile and pancreatic juice (Fig. 1). This loop was perfused at a flow rate of 0.7 ml/min with saline, which was gassed with 100% O₂, heated 37°C and kept in a reservoir. The pH of the saline was adjusted to 4.5 by adding 10 mM HCl. An exiting tube was connected to a glass electrode of the flow type (Horiba model 6901-25T), by which the pH of the duodenal perfusate was continuously measured. The duodenal PD was determined using two agar bridges: one positioned in the duodenal loop and the other in the abdominal cavity. Changes in both pH and PD were continuously monitored on a two channel recorder (Unicorder U-228).

Bicarbonate secretion was determined by introduction of an automatic titrator (Hiranuma Comtite-7) in the above perfusion system. Bicarbonate output was expressed as the amount of acid neutralized in the duodenal loop (acid neutralizing capacity), which was measured by back-titration of the duodenal perfusate to pH 4.5 using the pH-stat method and by adding 10 mM HCl.

Experimental protocols: At least 1 hr after the pH and PD had stabilized, the following drugs were given subcutaneously at the abdominal portion or intravenously into the tail vein: \textit{PGE}₁ (100 \( \mu \)g/kg, i.v.), \textit{PGE}₂ (300 \( \mu \)g/kg, s.c.; 100 \( \mu \)g/kg, i.v.), 16,16-dimethyl \textit{PGE}₂ (dm\textit{PGE}₂: 30 \( \mu \)g/kg, s.c.; 5 \( \mu \)g/kg, i.v.), orneprostil (a \textit{PGE}₁ derivative: 5 \( \mu \)g/kg, i.v.), TY-10957 (a \textit{PGI}₂ derivative: 40 \( \mu \)g/kg, i.v.), carbamylcholine chloride (4 \( \mu \)g/kg, i.v.), bethanechol chloride (100 \( \mu \)g/kg, i.v.), baclofen (PCPGABA: 1 mg/kg, i.v.) and YM-14673 (a derivative of thyrotropin releasing hormone: 1 mg/kg, i.v.). The doses of PGs were selected according to the previous papers (3, 4, 12) and those of the latter two

![Fig. 1. Schematic illustration of the perfusion system to simultaneously measure both the pH and PD in the proximal duodenum of anesthetized rats. Acid neutralizing capacity was determined using an automatic titrator introduced into the perfusion system.](image-url)
agents, selected to represent stimulation of acid secretion via the vagus nerves (13, 14). In some cases, indomethacin (5 mg/kg, s.c.) was given 1 hr before dmPGE2 treatment, and atropine (0.3 mg/kg, i.v.) was given 5 min before administration of carbachol and YM-14673. In a separate experiment, the pH and PD were monitored when the pH of the perfusate was lowered or elevated by adding dilute HCl or NaOH to the reservoir. At the end of the experiments, the animals were killed by injecting saturated KCl intravenously.

Preparation of drugs: Drugs used were PGE1, PGE2, 16,16-dimethyl PGE2 (Funa-koshi), ornoprostil, TY-10957 (Toa-Eiyo), carbamylcholine chloride, bethanechol chloride, indomethacin (Sigma), YM-14673 (Yamanouchi), atropine (Merck) and baclofen (kindly supplied from Dr. Y. Goto of Tokushima Bunri Univ.). Indomethacin was suspended in saline with a drop of Tween 80 (Nacalai Tesque). PGE1, PGE2 and dmPGE2 were first dissolved in absolute ethanol and diluted with saline to the desired concentrations, while other agents were dissolved in saline; the final concentration of ethanol in the drug solution was less than 1%. Each agent was prepared immediately before use and given subcutaneously in a volume of 0.5 ml per 100 g of body weight or intravenously in a volume of 0.1 ml per 100 g of body weight.

Statistics: Data are presented as the mean±S.E. from 4 to 7 rats per group. Values are compared by a two-tailed Dunnett's multiple comparison test (15) and are considered to be statistically significant if P<0.05.

Results

“Bench” testing of the system: When the duodenal loop was perfused with the saline solution of pH 4.5, the pH of the fluid emerging from the duodenal loop was around 5.5 and remained in this range during the test period, suggesting a significant neutralization with HCO3- secretion in the loop. Duodenal PD was maintained at values of −4 to −6 mV (mucosa negative) under the above conditions. The PD remained unaltered when the duodenal pH was lowered to 4.0 or elevated to 7.0 by changing the pH of the duodenal perfusate, suggesting that the luminal pH change by itself did not affect the degree of PD generation (Fig. 2A). In response to subcutaneously administered PGE2 (300 µg/kg, s.c.), the PD was increased promptly, followed by a gradual return to the basal levels within 30 min, while the pH was progressively elevated, reached the maximal values about 30 min later and persisted for over 1 hr (Fig. 2B). Subcutaneous administration of indomethacin (5 mg/kg) reduced the pH with slight depression of the PD, but the subsequent dose of dmPGE2 (30 µg/kg, s.c.) produced a marked elevation of the pH as well as the PD in the presence of indomethacin (Fig. 2C). When the animal was killed by injecting saturated KCl intravenously, the pH was decreased to about 4.7, and the generation of PD was completely abolished.

Effects of prostaglandins on duodenal pH, PD and acid neutralizing capacity: Duodenal pH and PD were significantly increased by intravenous administration of various types of PGs (Fig. 3, A and C). PGE1 (100 µg/kg) as well as PGE2 (100 µg/kg) produced a marked
Fig. 3. Representative figures showing the effects of various types of PGs on the pH and PD in the rat duodenum. A: PGE1 (100 μg/kg, i.v.); B: ornoprostil (5 μg/kg, i.v.); C: PGE2 (100 μg/kg, i.v.); D: dmPGE2 (5 μg/kg, i.v.); E: TY-10957 (40 μg/kg, i.v.). Each agent was given intravenously after basal pH and PD had stabilized.

elevation of the pH and PD, and the degrees of the changes were similar in both cases; in the latter, the pH increased from 5.8±0.1 to the maximal value of 6.7±0.1, and the PD rose from −4.6±0.2 mV to the values of −8.3±1.1 mV. Although the pH remained significantly elevated for 30 min after administration of PGE2, a significant rise in the PD was observed only for 20 min (Fig. 4). The acid neutralizing capacity was significantly increased by PGE2 from 1.3±0.2 μEq/10 min to 2.7±0.3 μEq/10 min and persisted for about 50 min; the net increase of HCO3− output was 7.2±1.0 μEq/rat. The increased pH and PD responses were similarly induced by dmPGE2 (5 μg/kg) and TY-10957, a PGI2 derivative (40 μg/kg), and the degree of elevation caused by these PG derivatives was almost comparable to those produced by PGE2 (100 μg/kg). Although ornoprostil, a PGE1 derivative (5 μg/kg), affected both the pH and PD, this effect was less marked as compared with those of the other PG derivatives (Fig. 3, B, D and E).

Effects of cholinergic drugs on duodenal pH and acid neutralizing capacity: The pH of the duodenal perfusate was significantly increased by intravenously administered carbacbol (4 μg/kg) from 5.5±0.2 to 6.0±0.1, with a concomitant increase of the PD (−4.2±0.2 mV to −7.9±0.3 mV) and the increased pH responses persisted for about 30 min. Bethanecol (100 μg/kg) produced similar elevations of the pH and PD, the degree of these changes being equivalent to those induced by carbacbol (Fig. 5). This agent at lower doses (10, 30 μg/kg) had no effect on the pH (not shown). The net increase of acid neutralizing capacity (HCO3− output) was 1.7±0.5 μEq for carbacbol and 1.9±0.6 μEq for bethanecol, the values respectively being 23.6%

Fig. 4. The effects of PGE2 (100 μg/kg, i.v.) on the PD, pH and acid neutralizing capacity (HCO3− output) in the rat duodenum. Data are presented as the mean±S.E. of values determined every 10 min from 4 rats per group. *Statistically significant difference from basal values (means of 4 points observed before PGE2 treatment), at P<0.05.
Fig. 5. Representative figures showing the effects of PGE$_2$ (100 µg/kg, i.v.), PCPGABA (1 mg/kg, i.v.) and bethanechol (100 µg/kg, i.v.) on the pH and PD in the rat duodenum. Note that the pH responses caused by PCPGABA and bethanechol were much less than that by PGE$_2$.

![Graph showing pH and PD responses](image)

Fig. 6. The net increase of HCO$_3^-$ output produced by intravenously administered carbachol (4 µg/kg), bethanechol (100 µg/kg), YM-14673 (1 mg/kg) and PCPGABA (1 mg/kg) in the rat duodenum and the effect of atropine (0.3 mg/kg) on the responses caused by carbachol and YM-14673. Data are expressed as a percentage (%) of the values produced by PGE$_2$ (100 µg/kg, i.v.) and represent the mean±S.E. from 5–7 rats per group. The net increase of HCO$_3^-$ output caused by PGE$_2$ was 7.2±1.1 µEq/rat. *Statistically significant difference from the corresponding groups at P<0.05.

and 26.4% of the net increase of HCO$_3^-$ output (7.2±1.0 µEq) caused by 100 µg/kg of PGE$_2$ (Fig. 6). Both YM-14673 (1 mg/kg) and PCPGABA (1 mg/kg) significantly increased the duodenal pH and PD; in the case of YM-14673, the pH was increased from 5.6±0.2 to 6.4±0.2 and remained elevated for 40 min. The net increase of HCO$_3^-$ output caused by YM-14673 and PCPGABA was 3.8±0.9 µEq and 1.8±0.3 µEq, respectively, the values being respectively 52.8% and 25.0% of that induced by PGE$_2$ (100 µg/kg). Both pH and PD responses caused by carbachol (4 µg/kg) and YM-14673 (1 mg/kg) were apparently blocked by prior administration of atropine (0.3 mg/kg, i.v.) (Fig. 7, A and B). The net increase of HCO$_3^-$ output caused by these agents was also significantly inhibited by atropine, the inhibition being 92.4% and 86.7% in the cases of carbachol and YM-14673, respectively (Fig. 6).

**Discussion**

The duodenal preparations described herein respond to a variety of PGs and their derivatives by a significant rise in the luminal pH (acid neutralizing capacity) and may allow us to investigate the regulatory mechanism of duodenal HCO$_3^-$ secretion. Since this system represents the net changes of HCO$_3^-$ output by pH deflections in the duodenal perfusate, it is capable of detecting a feeble stimulatory effect on HCO$_3^-$ secretion caused by cholinomimetics.
In most of the studies, HCO$_3^-$ secretion was measured at pH 7.4 using a pH-stat method and by titrating the duodenal perfusate with diluted HCl (1, 3, 7-10). In the present study, the duodenal loop was perfused with acidic solution (pH 4.5) and the HCO$_3^-$ output was monitored using pH deflections and determined by back-titration of the perfusate to pH 4.5. Since sacrificing the animals with KCl (i.v.) abolished the generation of PD and reduced the duodenal pH to near 4.5, the pH deflection observed in this system may represent HCO$_3^-$ changes without much modification by H$^+$ back-diffusion due to the pH gradient across the mucosa. Under these conditions, HCO$_3^-$ secretion might be slightly stimulated over the basal levels, as it has been shown that alkaline secretion increases in response to the mucosal acidification, partly mediated with endogenous PGs (6). This is supported by the finding that the duodenal pH was slightly reduced after indomethacin treatment. However, a previous study showed that the acidification of the mucosa with 1 mM HCl (pH 3.0) did not produce a significant alteration in the rate of duodenal alkaline secretion when HCO$_3^-$ was determined by direct titration at pH 7.4 (16). In the latter study, the mucosa was exposed to 1 mM HCl for only 10 min, while in the present study, the mucosa was acidified throughout the test period with less concentrated HCl. These different conditions may partly account for the different mucosal responses to acidification observed in these two studies. The basal HCO$_3^-$ output obtained in this system was 1–1.5 μEq/10 min, which is slightly higher than that observed by direct titration at pH 7.4 (0.8–1.2 μEq/10 min) (3, 16). It may be assumed that the prolonged acidification of the mucosa with a slightly acidic solution itself does not actively stimulate the basal HCO$_3^-$ output, but modifies (potentiate) the mucosal alkaline responses to secretagogues. In a preliminary study, we found that the pH deflection was the maximum between pH 4.0 and 7.0 when the titration curve was made by addition of NaHCO$_3$ to acid solution (pH 3.0); the pH increased linearly against the amount of NaHCO$_3$ added to the system. Thus, this system using pH deflection may be a sensitive method for continuously detecting changes in duodenal HCO$_3^-$ secretion.

The present study not only confirmed the potent stimulatory effects of natural PGs (PGE$_1$ and PGE$_2$) on duodenal HCO$_3^-$ secretion (5, 6) but those of their derivatives (dmPGE$_2$, TY-10957) as well (3, 12). The net increase of HCO$_3^-$ output caused by PGE$_2$ (100 μg/kg, i.v.) was about 8 μEq/rat, which was equivalent to that obtained by 300 μg/kg of this agent given s.c. (17). The duration of stimulation after i.v. treatment (50 min) was much shorter when compared to s.c. administration (1.5 hr). However, ornoprostil, a PGE$_1$ derivative, produced a minimal increase of pH in the duodenal perfusate at the dose used (5 μg/kg, i.v.). We previously observed that this derivative of PGE$_1$ at 10 to 100 μg/kg (s.c.) did not significantly affect
HCO₃⁻ secretion in the stomach and the duodenum (12). The present results support the previous observations, but it remains a question why this derivative of PGE₁ did not affect the alkaline secretory responses.

Flemstrom (1) first demonstrated that carbachol stimulates alkaline secretion in the bullfrog antrum. Cholinergic involvement in the process of alkaline secretion is supported by recent studies showing that vagal stimulation increased alkaline secretion from the duodenal mucosa (8, 9). However, the effects of cholinergic drugs on alkaline secretion still remain controversial. Smedfors and Johansson (10) reported that neither carbachol nor bethanechol significantly affected duodenal HCO₃⁻ secretion in the rat. In the present study, both carbachol and bethanechol significantly increased the duodenal pH and acid neutralizing capacity, and those effects were blocked by atropine, although the degrees of increase caused by these agents were only about 20% of that induced by PGE₂. Such feeble stimulation may account for the controversial effect of these cholinergic agents on duodenal alkaline secretion. Since the luminal acidification enhanced PG generation in the mucosa (6, 18), such cholinergic effects might be potentiated by endogenous PGs in the duodenum perfused with a slightly acidic solution. At present, it remains undetermined whether or not the alkaline responses induced by these agents are affected by indomethacin pretreatment.

Involvement of a neural pathway in the regulation of alkaline secretion is also supported by the finding that both PCPGABA and YM-14673 (an analogue of TRH) significantly increased HCO₃⁻ output in the duodenum, the degrees of stimulation being 20% and 50%, respectively, of that induced by PGE₂. These two agents stimulate acid secretion by acting on the central nervous system and through the vagus nerves (13, 14). It may be assumed that these agents also stimulate duodenal HCO₃⁻ secretion via the cholinergic systems, since atropine significantly inhibited the increase of duodenal pH and HCO₃⁻ output caused by YM-14673, similar to the case of carbachol. These results suggest that both central and peripheral neuronal systems are involved in the regulation of duodenal HCO₃⁻ secretion. Further studies are currently under way in our laboratory to characterize the alkaline response induced by these agents.

Simson et al. (19, 20) reported that duodenal HCO₃⁻ secretion is an electrogenic process, probably depending upon Na⁺/K⁺ ATPase activity at the serosal membrane. However, Heylings and Feldman (21) showed that the increased PD response caused by PGE₂ were observed only temporarily after administration and not during changes in the HCO₃⁻ output. We also found a similar discrepancy in the duration of the increased PD and pH responses caused by PGs and other drugs. These results support their suggestion that the rise in PD seen after PGE₂ in the rat duodenum is not necessarily due to an increase in electrogenic transcellular HCO₃⁻ transport but may reflect the electrogenic secretion of other ions. Further investigations on the association between PD and HCO₃⁻ responses are warranted.

In conclusion, our apparatus has the advantage of not only giving a continuous display of pH but also providing a direct visualization of the acid neutralizing capacity in the duodenum. This system responds well to cholinergic agents as well as various PGs, and it may be useful for evaluating drugs which affect duodenal alkaline secretion.

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