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PROSTAGLANDIN E RECEPTOR SUBTYPES INVOLVED IN STIMULATION OF GASTRODUODENAL BICARBONATE SECRETION IN RATS AND MICE

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We investigated prostaglandin E (EP) receptor subtypes responsible for the HCO_3^- stimulatory action of prostaglandin E_2 (PGE_2) in the gastroduodental mucosa, by examining the effects of various prostanoids with subtype specific EP receptor agonists in rats and those of PGE_2 in knockout mice lacking EP_1 or EP_3 receptors. In rats, gastric HCO_3^- secretion was stimulated by i.v. administration of PGE_2, 17-phenyl PGE_2 the selective EP_1 agonist as well as sulprostone the EP_1 and EP_3 agonist, but was not affected by other EP agonists such as butaprost the selective EP_2 agonist, ONO-NT-012 the selective EP_3 agonist or 11-deoxy PGE_1 the EP_3 and EP_4 agonist. In contrast, the HCO_3^- secretion in rat duodenums was stimulated by PGE_2, sulprostone, ONO-NT-012 as well as 11-deoxy PGE_1 but not affected by either 17-phenyl PGE_2 or butaprost. The HCO_3^- stimulatory effect of sulprostone in the stomach was significantly inhibited by ONO-AE-829, the selective EP_1 antagonist. On the other hand, PGE_2 applied topically to the mucosa for 10 min caused a dose-dependent increase of HCO_3^- secretion in both the stomach and duodenum of wild-type mice. The HCO_3^- stimulatory action of PGE_2 in the stomach was also observed dose-dependently in knockout mice lacking EP_3-receptors but was absent in EP_1-receptor knockout mice, while the stimulatory effect in the duodenum was observed in EP_1-receptor knockout mice, similar to wild-type animals, but not in knockout mice lacking EP_3-receptors. These results indicate that PGE_2 stimulates HCO_3^- secretion via different EP receptor subtypes in the stomach and duodenum; the former is mediated by EP_1-receptors, while the latter mediated by EP_3-receptors.

Key words: *EP receptor subtype, PGE_2, gastroduodenal HCO_3^- secretion rat, Ep receptor knockout mice.*

INTRODUCTION

The secretion of HCO_3^- in the surface epithelial cell is one of the main defense mechanism of the gastroduodenal mucosa against acid (1). Mucus adherent to the luminal surface of the mucosa provides a zone of low turbulence (unstirred layer), allowing the development of a gradient for HCO_3^- from the luminal side (2). Small amounts of HCO_3^- protect the mucosa against large amounts of acid by neutralizing H^+ ions that diffuse back into the mucus layer, and hence the HCO_3^- secretion is thought to play an important role in maintaining the integrity of the gastroduodenal mucosa (3, 4). Although the physiological regulation of HCO_3^- secretion involves prostaglandins (PGs) as well as neuro-humoral factors (3—7), it is thought that endogenous PGs are particularly important in the local control of this secretion. Indeed, PGE_2 and its analogues, whether applied luminally or vascularly, stimulate duodenal HCO_3^- secretion *in vivo* and *in vitro*, in a variety of species including man and in this way may contribute to protection of the mucosa against acid-induced injury (3, 4). On the other hand, recent studies showed that the receptors activated by PGE_2 are pharmacologically subdivided in 4 subtypes, $EP_1 \sim EP_4$ (8—10), but those mediating the HCO_3^- stimulatory action of PGE_2 remain to be much characterized.

The present study was designed to determine the EP receptor subtypes responsible for the HCO_3^- stimulatory action of PGE_2 in the gastroduodenal mucosa, by examining the effects of various subtype specific EP agonists on the HCO_3^- secretion in rats and by evaluating the HCO_3^- response to PGE_2 in knockout mice lacking EP_1- and EP_3-receptors.

MATERIALS AND METHODS

Animals

Male SD rats (200—220 g) or C57BL/6 mice (25—30 g) were used. Mice lacking the EP_1 or EP_3 receptors were generated as described previously (11, 12). In brief, the mouse genes encoding the EP_1 and EP_3 receptors were individually disrupted, and chimaeric mice were generated. These animals were then back crossed with C57BL/6 mice, and the resulting heterozygous litter mates [EP_1 (+/–) or EP_3 (+/–)] were bred to produce homozygous EP_1 (–/–) or EP_3 (–/–) mice. Distribution of the EP_1 and EP_3 receptor genes was verified by northern blot hybridization, which failed to detect messenger RNAs encoding the respective receptors in EP_1 (–/–) and EP_3 (–/–) mice. These knockout mice and rats were deprived of food but allowed free access to tap water for 18 hr before the experiments. All studies were performed under urethane anesthetized conditions (1.25 g/kg, IP).

Determination of HCO_3^- secretion

The HCO_3^- secretion was measured in both rats and mice under urethane anesthetized conditions, according to our previously published method (13). In brief, the stomach mounted on

ex-vivo chamber or the duodenal loop that was between the pylorus and the outlet of the common bile duct (1.5 cm in the rat and 0.8 cm in the mouse) was perfused at a flow rate of 1 ml/min in rats or 0.2 ml/min in mice, with saline that was gassed with 100% O_2, heated at 37° C and kept in a reservoir. The HCO_3^- secretion was titrated at pH 7.0 using a pH-stat method and by adding 5 mM HCL to the reservior. In the stomach preparation, acid secretion had been inhibited by pretreatment of the animals with omeprazole given i.p. in a dose of 60 mg/kg. After basal HCO_3^- secretion had well stabilized, the following prostanoids were administered i.v. as a single bolus injection in rats, and the HCO_3^- secretion was measured for 1 hr thereafter; PGE_2 ($EP_1/EP_2/EP_3/EP_4$ agonist: 0.1 ~ 3 mg/), sulprostone (EP_1/EP_3 agonist: 0.3 ~ 3 mg/kg), enprostil (EP_1/EP_3 agonist: 0.001 ~ 0.03 mg/kg), (misoprostol (EP_2/EP_3 agonist: 0.01 ~ 1 mg/kg), butaprost (EP_2 agonist: 1 ~ 10 mg/kg), 11-deoxy PGE_1 (EP_3/EP_4 agonist: 0.01 ~ 1 mg/kg), 17-phenyl-trinor PGE_2 (17-phenyl PGE_2: EP_1agonist: 0.3 ~ 3 mg/kg), and ONO-NT-012 (EP_3 agonist: 0.1 ~ 61 mg/kg). These prostanoids are considered to be EP receptor subtype specific agonists (13—15). In some animals, the effect of ONO-AE-829 (3 and 10 mg/kg), an EP_1 receptor antagonist, on gastroduodenal HCO_3^- secretion induced by PGE_2 was examined. ONO-AE-829 was administered s.c. 30 min before PGE_2. In mice, PGE_2 (0.01 ~ 0.3 mg/ml) was applied topically to the gastroduodenal mucosa for 10 min (16, 17).

Preparation of drugs

Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), prostaglandin E_2, butaprost, sulprostone, enprostil, misoprostol, ONO-NT-012, ONO-AE—829 (Ono Pharm. Co. Ltd.), 17-phenyl trinor-PGE_2, 11-deoxy PGE_1 (Nacalai tesque, Kyoto, Japan), and omeprazole (Hessle, Mondale, Sweden). Omeprazole was suspended in 0.5% carboxymethylcellulose solution. PGE_2 and other EP receptor ligands were first dissolved in absolute ethanol and then diluted with saline to a desired concentration. Each agent was prepared immediately before use and given in a volume of 0.5 ml per 100 g body weight in cases of i.p. and s.c. administration or in a volume of 0.1 ml per 100 g body weight in case of i.v. administration. In mice, PGE_2 was topically applied to the mucosa in a volume of 0.2 ml for 10 min. Control animals received saline in place of active agent.

Statistics

Data are presented as the means ± SE from 4 ~ 8 animals per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of $P < 0.05$ were regarded as significant.

RESULTS

Effect of Various EP Agonists on Gastroduodenal HCO_3^- Secretion in Rats

Under the present experimental conditions, the rat stomach secreted HCO_3^- secretion at a steady rate of 0.2 ~ 0.4 μEq/5 min in the animals given omeprazole (60 mg/kg, i.p.) to inhibit acid secretion. The HCO_3^- secretion in the

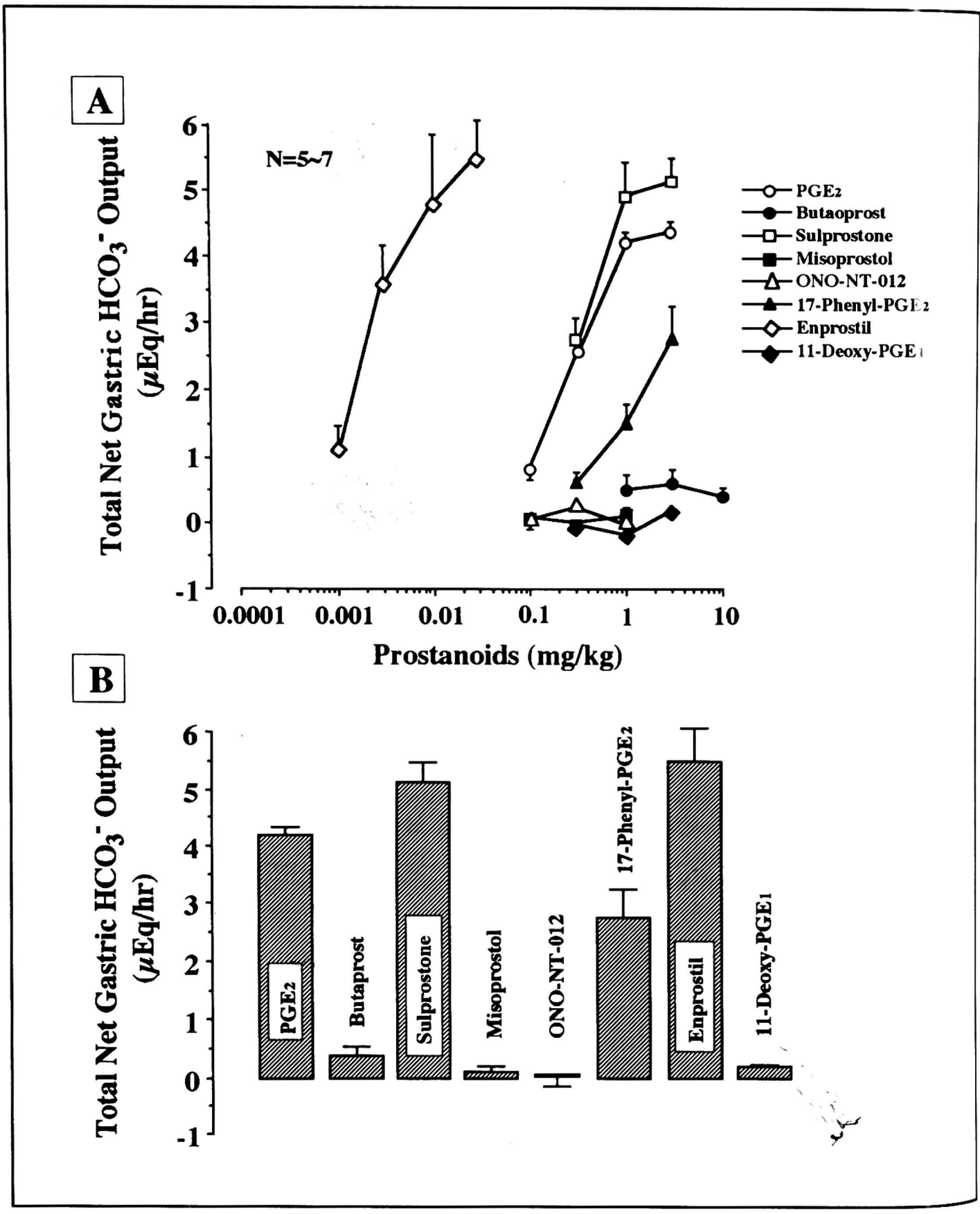


Fig. 1. Effects of various EP agonists on gastric HCO_3^- secretion in anesthetized rats. EP agonists were administered i.v. as a single injection after the secretion had well stabilized. The animals were pretreated with omeprazole (60 mg/kg, i.p.) to inhibit acid secretion completely. ○: PGE_2 (0.1 ~ 3 mg/kg); ●: butaprost (1 ~ 10 mg/kg); □: sulprostone (0.3 ~ 3 mg/kg); ◇: enprostil (0.001 ~ 0.03 mg/kg); ◆: 11-deoxy PGE_1 (0.001 ~ 0.03 mg/kg); ■: misoprostol (0.1 ~ 1 mg/kg); △: ONO-NT-012 (0.1 ~ 1 mg/kg); ▲: 17-phenyl PGE_2 (0.3 ~ 3 mg/kg). Data are presented as the means ± SE of total net HCO_3^- output obtained for 1 hr from 5 ~ 7 rats. In Figure B, values indicate total net HCO_3^- output obtained for 1 hr after administration of the agents and are presented as the means ± SE from 5 ~ 7 rats.

stomach was increased dose-dependently when the animals were administered with PGE_2 i.v. in doses of 0.1 ~ 3 mg/kg; the total net HCO_3^- at 3 mg/kg was 4.9±0.4 μEq/hr (*Fig. 1A*). Likewise, both sulprostone (0.3 ~ 3 mg/kg) and enprostil (0.001 ~ 0.03 mg/kg) also increased gastric HCO_3^- secretion in a dose-dependent manner, and the total net HCO_3^- output obtained by these agents at the highest doses used was 5.0±0.4 μEq/hr and 5.5±0.7 μEq/hr, respectively, which were approximately equivalent to that induced by PGE_2 at 3 mg/kg (*Fig. 1B*). Although neither misoprostol, butaprost, 11-deoxy PGE_1 nor ONO-NT012 exhibited any stimulatory effect on gastric HCO_3^- secretion at any doses used in this study, 17-phenyl PGE_2 (0.3 ~ 3 mg/kg) dose-dependently stimulated HCO_3^- secretion in the stomach, similar to PGE_2, the total net HCO_3^- output being 2.8±0.4 μEq/hr at a dose of 3 mg/kg.

On the other hand, the rat duodenum also spontaneously secreted HCO_3^- at a steady rate of 0.2 ~ 0.4 μEq/5 min during a 90-min test period. Intravenous administration of PGE_2 (0.1 ~ 3 mg/kg) as a single bolus injection produced a dose-dependent increase of HCO_3^- secretion in the duodenum (*Fig. 2A*). After administration of PGE_2 at 1 mg/kg, the duodenal HCO_3^- secretion was increased from 0.25±0.30 μEq/5 min to the maximal values of 0.78±0.12 μEq/5 min, about 3 times as great as basal levels, and remained elevated for at least 60 min. The HCO_3^- output also increased dose-dependently in response to i.v. administration of other prostanoids such as sulprostone (0.3 ~ 3 mg/kg), and ONO-NT-012 (0.1 ~ 1 mg/kg); the total net HCO_3^- output at the highest doses used was 4.1±0.3 μEq/hr, 6.9±0.6 μEq/hr, 4.2±0.3 μEq/hr, 7.9±0.3 μEq/hr and 4.7±1.2 μEq/hr, respectively (*Fig. 2B*). However, both butaprost (1 ~ 10 mg/kg) and 17-phenyl PGE_2 (0.3 ~ 3 mg/kg) did not cause either an increase or a decrease in duodenal HCO_3^- secretion at any doses used in this study.

To further clarify which EP receptor subtype is involved in the stimulation of HCO_3^- secretion, we examined the effect of ONO-AE-829 (EP_1 antagonist) on the HCO_3^- response induced by PGE_2. Intravenous administration of PGE_2 (0.3 mg/kg) increased gastric HCO_3^- secretion over control levels, from 0.40±0.06 μEq/5 min to the maximal values of 0.98±0.07 μEq/5 min; the total net HCO_3^- output was 4.9±0.5 μEq/hr. ONO-AE-829 (3 and 10 mg/kg) administered s.c. dose-dependently antagonized the HCO_3^- stimulatory action of PGE_2 in the stomach, and a significant effect was observed at 10 mg/kg, the inhibition being 80%. In contrast, ONO-AE-829 at 10 mg/kg, however, did not have any effect on the HCO_3^- response to PGE_2 in the duodenum; the total net HCO_3^- output was 4.0±0.3 μEq/hr and 4.3±0.3 μEq/hr, respectively, the absence or presence of ONO-AE-829.

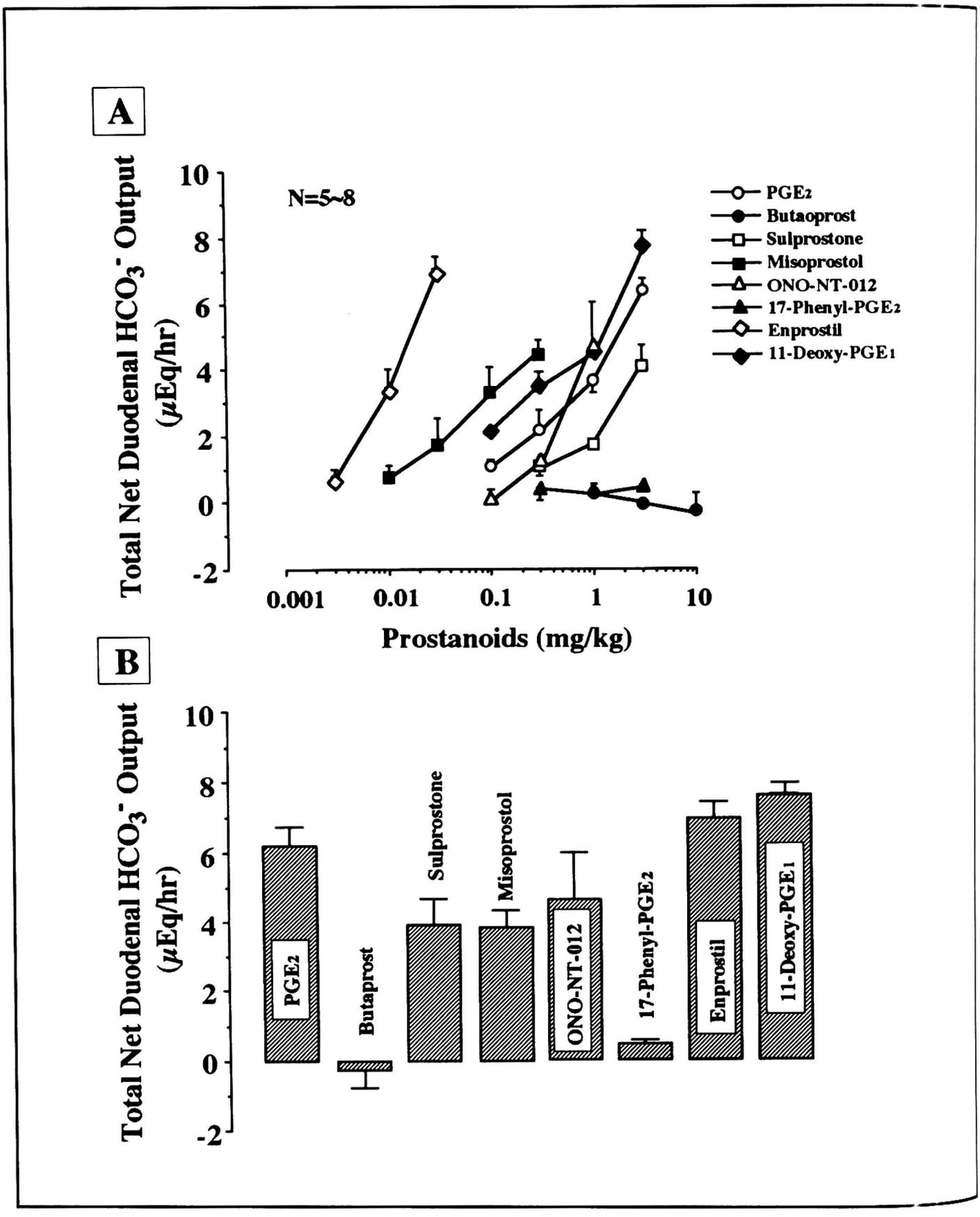


Fig. 2. Effects of various EP agonists on duodenal HCO_3^- secretion in anesthetized rats. EP agonists were administered i.v. as a single injection after the secretion had well stabilized. ○: PGE_2 (0.1 ~ 3 mg/kg); ●: butaprost (1 ~ 10 mg/kg); □: sulprostone (0.3 ~ 3 mg/kg); ◇: enprostil (0.003 ~ 0.03 mg/kg); ◆: 11-deoxy PGE_1 (0.001 ~ 0.03 mg/kg); ■: misoprostol (0.1 ~ 1 mg/kg); △: ONO-NT-012 (0.1 ~ 1 mg/kg); ▲: 17-phenyl PGE_2 (0.3 ~ 3 mg/kg). Data presented as the means ± SE of total net HCO_3^- output obtained for 1 hr from 5 ~ 8 rats. In Figure B, values indicate total net HCO_3^- output obtained for 1 hr after administration of the agents and are presented as the means ± SE from 5 ~ 8 rats.

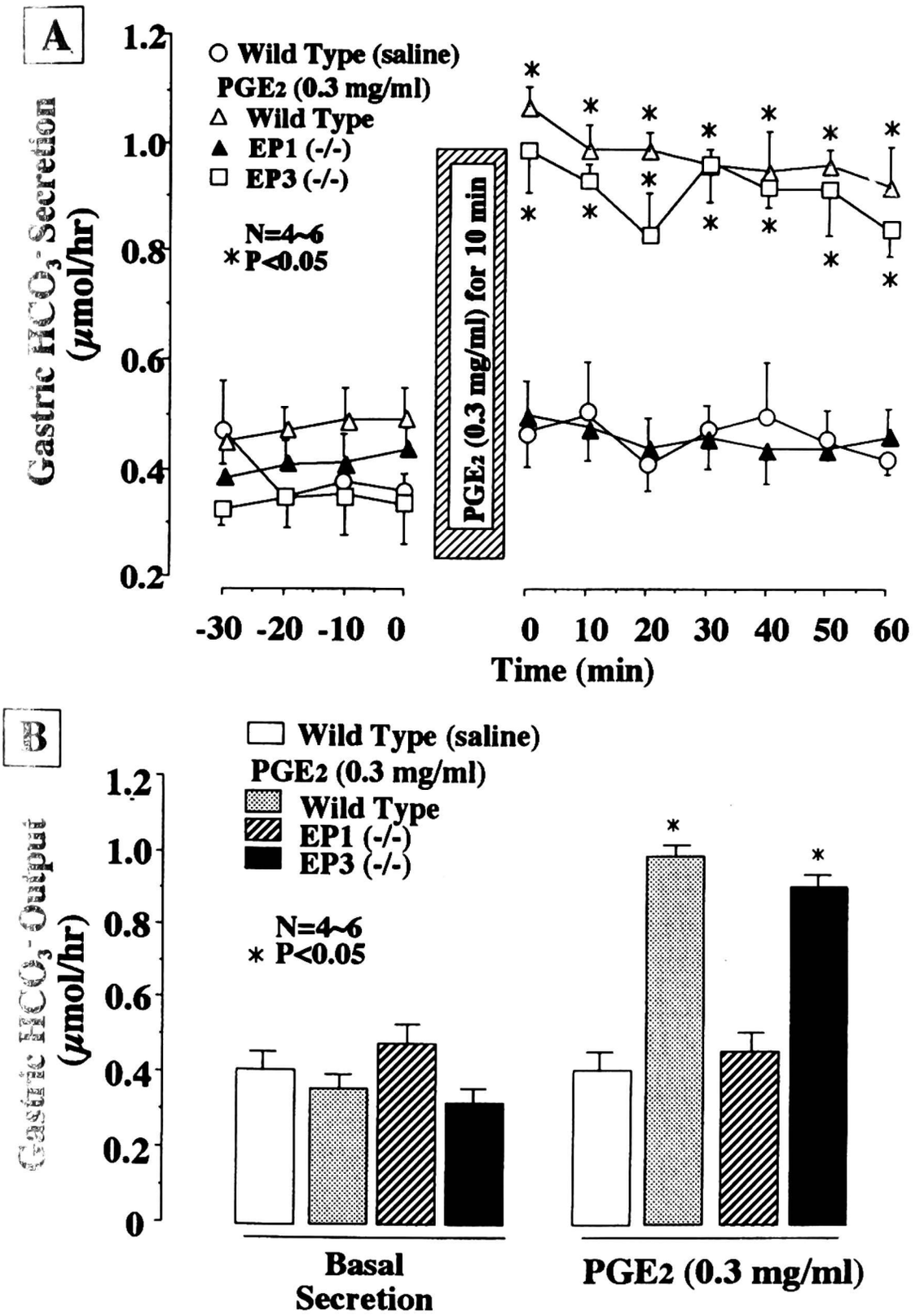


Fig. 3. Gastric HCO_3^- secretory responses induced by PGE_2 in wild type- and EP receptor knockout mice. The animals were pretreated with omeprazole (60 mg/kg, i.p.) to inhibit acid secretion completely. PGE_2 (0.1 mg/ml) was applied luminally to the stomach for 10 min. ○: wild type (saline); △: wild-type (PGE_2); ▲ EP_1 (–/–); □; EP_3 (–/–). Data are presented as the means ± SE of values determined every 10 min from 4 ~ 6 mice. * Statistically significant difference from values observed immediately before PGE_2 treatment, at $P < 0.05$. In Figure B, values indicate total HCO_3^- output obtained for 1 hr before and after PGE_2 treatment and are presented as the means ± SE from 4 ~ 6 mice per group. * Statistically significant difference from values in before, at $P < 0.05$.

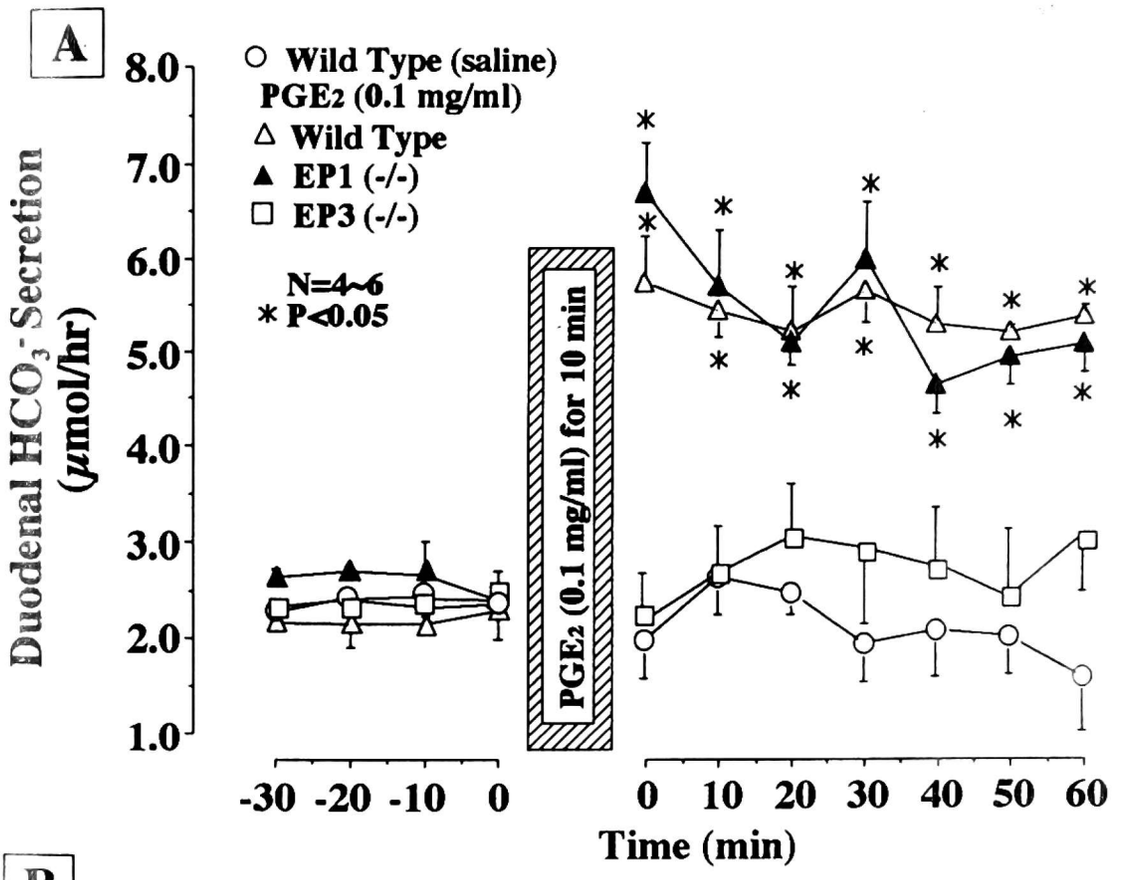
HCO_3^--Secretory Response to PGE_2 in Knockout Mice

Under urethane anesthesia, the mouse stomach or duodenum spontaneously secreted HCO_3^- at a steady rate of 0.3 ~ 0.5 μEq/hr or 1.9 ~ 2.4 μEq/hr, respectively, during a 90 min test period. Gastric HCO_3^- secretion was increased in response to luminal exposure to PGE_2 (0.3 mg/ml) in wild-type mice, from 0.45±0.05 μEq/hr to a maximal value of 1.10±0.03 μEq/hr; the total HCO_3^- output (1.02±0.06 μEq/hr) was about two fold greater than basal values (0.38±0.02 μEq/hr) (*Fig. 3A, 3B*). This process was, however, almost absent in EP_1-receptor knockout mice, and the total HCO_3^- output remained in the same ranges (0.4 ~ 0.5 μEq/hr) before and after the mucosal exposure to PGE_2. On the other hand, the perfusion of the duodenum with PGE_2 (0.1 mg/ml) also caused a significant increase of the HCO_3^- secretion in wild-type mice; the secretion increased from 2.0±0.2 μEq/hr to a maximal value of 5.9±0.5 μEq/hr, the total HCO_3^- output being 5.5±0.2 μEq/hr (*Fig. 4A, 4B*). The HCO_3^- stimulatory effect of PGE_2 was similarly observed in EP_1 -but not EP_3-receptor knockout mice. In the latter animals lacking EP_3 receptors, PGE_2 failed to stimulate HCO_3^- secretion in the duodenum, the total HCO_3^- output being 2.1±0.5 μEq/hr which is not significantly different from basal values (2.6±0.7 μEq/hr).

The mucosal perfusion of PGE_2 (0.01 ~ 0.3 mg/ml) caused a dose-dependent increase of HCO_3^- secretion in both the stomach and duodenum of wild-type mice, although the dose required to stimulate HCO_3^- secretion was higher in the stomach as compared to the duodenum (*Fig. 5A, 5B*). Likewise, a dose-dependent HCO_3^- response to PGE_2 was observed in the stomach of EP_3-receptor knockout mice or in the duodenum of EP_1-receptor knockout mice, and the degrees of HCO_3^- response to PGE_2 in such animals were similar to those observed in wild-type mice.

DISCUSSION

PG_s, either generated endogenously or administered exogenously, act on multiple receptors (9). In the previous study using subtype-specific various EP_1 agonist in rats, we demonstrated that different EP receptors are involved in the HCO_3^- response to PGE_2 in the gastroduodenal mucosa; EP_1-receptors in the stomach and EP_3-receptors in the duodenum (13). In the present study, we further demonstrated using EP-receptor knockout mice that the HCO_3^- response in the stomach and duodenum was totally absent in the animals with EP_1-or EP_3-receptor gene disruption, respectively, confirming the findings in rats obtained with subtype-specific various EP agonists.



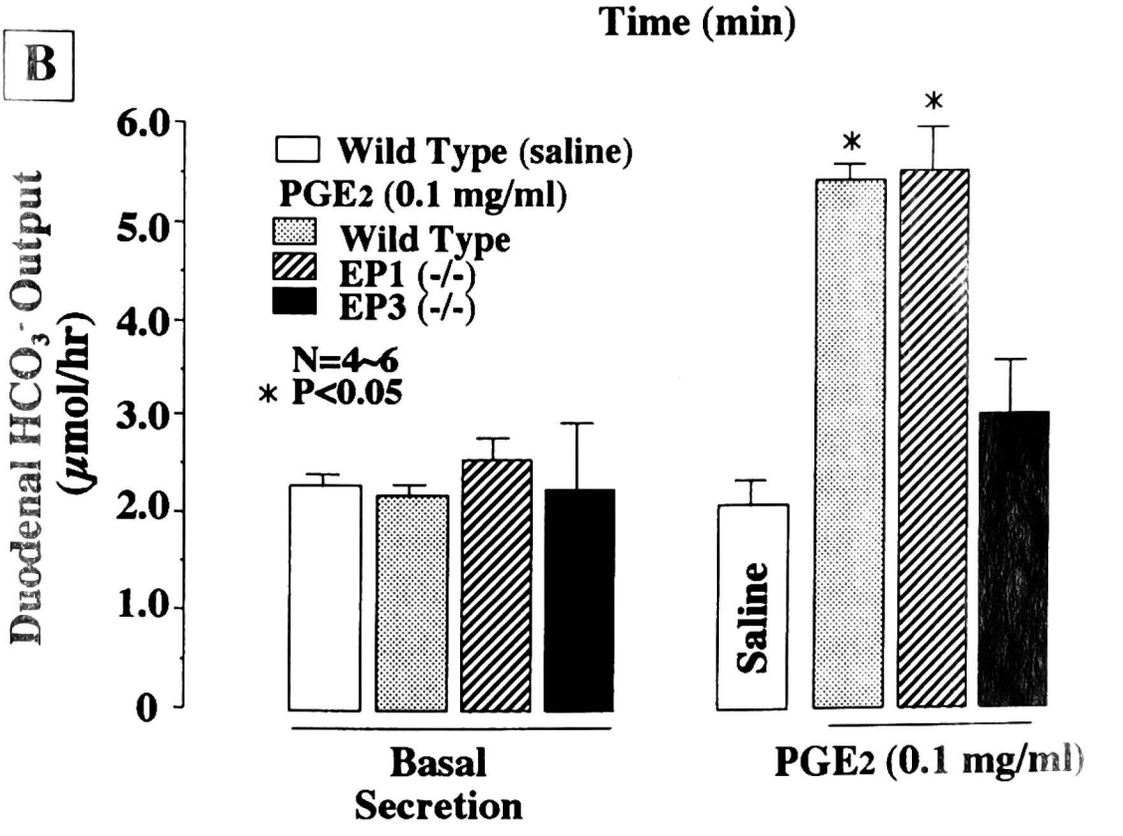


Fig. 4. Duodenal HCO_3^- secretory responses induced by PGE_2 in wild type- and EP receptor knockout mice. PGE_2 (0.1 mg/ml) was applied luminally to the duodenal loop for 10 min. ○; wild type (saline); △: wild-type (PGE_2); ▲: EP_1 (–/–); □; EP_3 (–/–). Data are presented as the means ± SE of values determined every 10 min from 4 ~ 6 mice. * Statistically significant difference from values observed immediately before PGE_2 treatment, at $P < 0.05$. In Figure B, values indicate total HCO_3^- output obtained for 1 hr before and after PGE_2 treatment and are presented as the means ± SE from 4 ~ 6 mice per group. * Statistically significant difference from values in before, at $P < 0.05$.

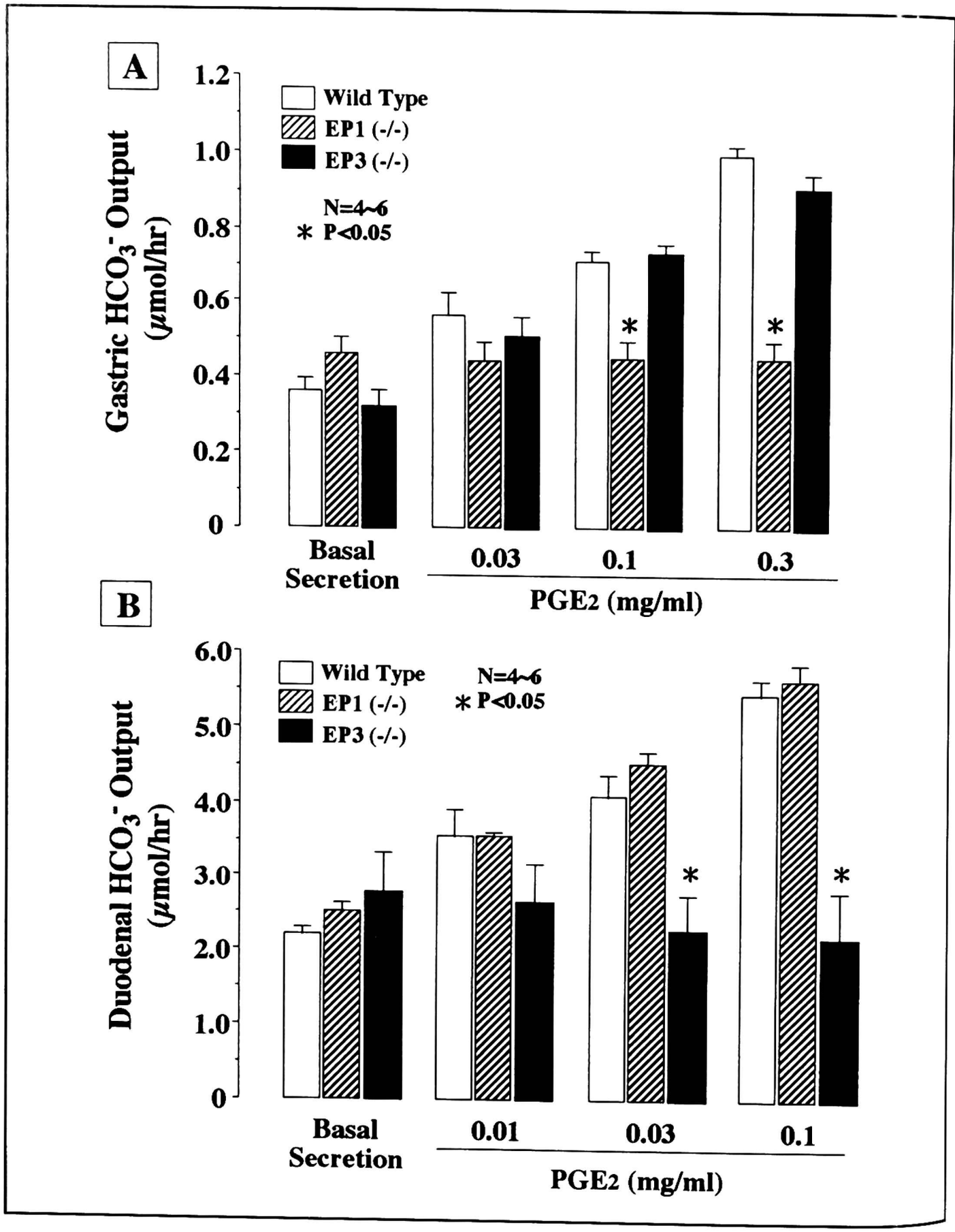


Fig. 5. Dose-response effects of PGE_2 on gastric (A) and duodenal (B) HCO_3^- secretion in wile-type and EP receptor knockout mice. PGE_2 (0.03 ~ 0.3 mg/ml) was applied luminally to the stomach or the duodenal loop for 10 min. In case of determination of gastric HCO_3^- secretion, the animals were pretreated with omeprazole (60 mg/kg, i.p.) to inhibit acid secretion completely. Values indicate total HCO_3^- output obtained for 1 hr after PGE treatment and are presented as the means ± SE from 4 ~ 6 mice per group. * Statistically significant difference from basal secretion, at $P < 0.05$.

First, we confirmed that PGE_2 stimulates HCO_3^- secretion in the stomach as well as in the duodenum of rats, in agreement with other observations (1, 3, 7, 13). The present study also confirmed that this effect on HCO_3^- secretion is mediated by different EP receptor subtypes in the stomach and duodenum. Duodenal HCO_3^- secretion is stimulated by either sulprostone (EP_1/EP_3 agonist), enprostil, 11-deoxy PGE_1 (EP_3/EP_4 agonist) or misoprostol (EP_2/EP_3 agonist). However, neither 17-phenyl PGE_2 (EP_1 agonist) nor butaprost (EP_2 agonist) was effective in stimulating this secretion, while ONO-NT-012 (EP_3 agonist) dose-dependently increased HCO_3^- secretion in the duodenum. In contrast, gastric HCO_3^- secretion was stimulated by 17-phenyl PGE_2, sulprostone and enprostil, whereas neither butaprost, ONO-NT-012, misoprostol nor 11-deoxy PGE_1 effectively altered HCO_3^- secretion in the stomach. These results strongly suggest that PGE-stimulated HCO_3^- secretion in the duodenum is brought about by activation of EP_3 receptors, while that in the stomach occurs via EP_1 receptors. The latter is supported by the fact that ONO-AE-829, an EP_1 antagonist, significantly attenuated the HCO_3^- stimulatory action of PGE_2 in the stomach but not in the duodenum. Hassen et al (18) reported that gastric mucus secretion is stimulated by PGs through EP_4 receptors. However, 11-deoxy PGE_1, a potent EP_4 agonist, did not show any effect on gastric HCO_3^-, suggesting the different mechanisms involved in mucus and HCO_3^- secretions in the stomach.

Of most important is the finding that the HCO_3^- stimulatory action of PGE_2 in the stomach is absent in EP_1-receptor knockout mice, while that in the duodenum is not observed in knockout mice lacking EP_3 receptors. These results confirmed the finding in rats and strongly indicate that PGE_2 stimulates HCO_3^- secretion in the stomach *via* EP_1-receptors and in the duodenum via EP_3-receptors. It should be noted that the effective dose of PGE_2 in stimulating HCO_3^- secretion was different between the stomach and duodenum; the ED_{50} was much lower in the latter. This phenomenon remains to be explained but might be related with a low rate of acid secretion in the stomach was about 1/5 of that in the duodenum. Since the surface area of the tissue used or the route of PGE_2 administration was different in the experiments for rats and mice, it is difficult to compare the rate of HCO_3^- secretion between these two species. Yet, considering only the surface of the tissue, it was revealed that the rate of basal HCO_3^- secretion in the duodenum was almost same in both rats and mice, about $5 \sim 6$ $\mu Eq/cm^2/hr$.

Morimoto *et al* (19) recently examined the cellular distribution of the mRNAs for the EP receptors in the mouse gastrointestinal tract by *in situ* hybridization. They showed that strong signals for EP_1 transcripts was observed in the smooth muscle cells in the muscularis mucosa throughout the tract, while moderate EP_3 mRNA expression was detected in the epithelial cells and also in the neurons of the myenteric glanglia throughout the gastrointes-

tinal tract. These results are not necessarily compatible with the present finding that HCO_3^- secretion, one of the epithelial functions, is mediated by EP_1 receptors in the stomach and by EP_3 receptors in the duodenum. On the other hand, the EP receptor subtypes are coupled with different signal transduction systems; activation of EP_1 receptors are coupled with Gq protein, resulting in an increase of intracellular Ca^{2+}, while the activation of EP_3 receptors are coupled with Gi protein, leading to an inhibition of andenylate cyclase activity (9, 20). We previously reported that the HCO_3^- response to PGE_2 in the stomach was mitigated by verapamil the Ca^{2+} antagonist but was not affected by isobutylmethyl xanthine the inhibitor of phosphodiesterase, suggesting a mediator role of Ca^{2+} in this response. On the other hand, duodenal HCO_3^- secretion results from an elevation of intracellular 3′5′-cyclic adenosine monophosphate levels (21, 22) and can be elicited also by receptor-independent adenylate cyclase activator like forskolin (23). Since duodenal HCO_3^- secretion is stimulated by activation of EP_3 receptors, there seems to be some controversy in the signal transduction coupled with EP_3 receptors. However, a recent study showed the existence of four isoforms in the EP_3 receptors (24). EP_{3A} is linked to activation of Gi protein, while that of EP_{3B} and EP_{3C} is coupled with activation of Gs protein, resulting in stimulation of adenylate cyclase activity.

The present results taken together indicate that the presence of EP_1- or EP_3-receptors is essential for maintaining the HCO_3^- secretion in the stomach or the duodenum, respectively. Further studies should certainly be needed regarding signal transduction of the HCO_3^- secretion mediated by these different EP receptors as well as the cellular distribution of EP receptor subtypes.

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