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Phosphodiesterase isozymes involved in regulation of HCO_3^- secretion in isolated mouse duodenum *in vitro*

Msamune Hayashi, Kazutomo Kita, Yumi Ohashi, Eitaro Aihara, Koji Takeuchi *

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan

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ABSTRACT

We examined the effects of various isozyme-selective PDE inhibitors on HCO_3^- secretion in the mouse duodenum *in vitro* and investigated which type(s) of phosphodiesterase (PDE) isozymes are involved in the response to PGE_2 and NO. The duodenal mucosa of male DDY mice was stripped of the muscle layer and mounted on an Ussing chamber, and HCO_3^- secretion was measured at pH 7.0 by a pH-stat method using 2 mM HCl. Both PGE_2 and NOR-3 (NO donor) increased HCO_3^- secretion in the mouse duodenum *in vitro*, and the response to PGE_2 was inhibited by both EP3 and EP4 antagonists but not EP1 antagonist, while that to NOR-3 was inhibited by methylene blue. IBMX, a nonselective PDE inhibitor, significantly increased basal HCO_3^- secretion and potentiated the responses to both PGE_2 and NOR-3. Likewise, vinpocetine (PDE1 inhibitor) and cilostamide (PDE3 inhibitor) also increased the basal secretion at high doses and potentiated the HCO_3^- response to PGE_2 at doses that had no effect by themselves on the basal secretion. By contrast, the HCO_3^- stimulatory action of NOR-3 was significantly potentiated by vinpocetine but not cilostamide. Inhibitors of other PDE subtypes had no effect on the HCO_3^- secretion under basal or stimulated conditions. Both PDE1 and PDE3 mRNAs were expressed in the duodenal mucosa. These results suggested that PDE1 and PDE3 are involved in the regulation of duodenal HCO_3^- secretion and that the response to PGE_2 is associated with both PDE1 and PDE3, while the response to NO is mainly modulated by PDE1.

1. Introduction

Duodenal mucosal HCO_3^- secretion is a key process that helps prevent acid-peptic injury [1–3]. The mechanisms that govern mucosal HCO_3^- secretion involve neuro-humoral factors and luminal acid [1], yet both endogenous prostaglandins (PGs) and nitric oxide (NO) play a particularly important role in the local control of this secretion [4–6]. The stimulatory action of PGE_2 is known to be mediated by the activation of both EP3 and EP4 receptors and coupled intracellularly with Ca^{2+} and 3′,5′-cyclic-adenosine monophosphate (cAMP) [7–11]. It is also known that NO stimulates soluble guanylate cyclase and elevates the intracellular level of 3′,5′-cyclic-guanosine monophosphate (cGMP) [12]. We showed using the isolated bullfrog duodenum *in vitro* that NOR-3, a NO donor, increased the secretion of HCO_3^- via endogenous PGs in a cGMP-dependent manner [13], results later confirmed in the rat duodenum *in vivo* [6]. It is thus assumed that both PG/cAMP and NO/cGMP are involved in the local regulatory mechanism of HCO_3^- secretion in the duodenum.

These nucleotides are degraded into inactive metabolites due to hydrolysis by phosphodiesterase (PDE). At present, the PDE in mammalian tissues has been subdivided into 11 isozymes, each derived from separate gene families and having pharmacologically distinct roles in the body [14]. PDE1–PDE5 have been well characterized, and selective inhibitors of

* *Corresponding author*. Tel.: +81 75 595 4679; fax: +81 75 595 4774.
E-mail address: takeuchi@mb.kyoto-phu.ac.jp (K. Takeuchi).
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these isozymes are used for the treatment of heart disease, depression, asthma, inflammatory disease, and erectile dysfunction [15–17]. Since the secretion of HCO_3^- in the duodenum is intracellularly mediated by both cAMP and cGMP, it is possible that PDE affects the response by altering the levels of cyclic nucleotides. Indeed, Simson et al. [18] showed that theophylline, a nonselective PDE inhibitor, enhanced HCO_3^- secretion in the isolated bullfrog duodenum *in vitro*. We have reported a potentiation by isobutylmethylxanthine (IBMX) of PGE_2-induced HCO_3^- secretion in the rat duodenum *in vivo* [8,10]. It remains, however, unexplored which PDE isozyme(s) is involved in the regulation of duodenal HCO_3^- secretion.

In the present study, we examined the effects of subtype-selective inhibitors of PDE1–PDE5 on HCO_3^- responses to PGE_2 or NO in the isolated mouse duodenum *in vitro* and investigated which isozyme(s) of PDE is involved in the local regulation of duodenal HCO_3^- secretion.

2. Materials and methods

2.1. Animals

Male DDY mice weighing 25–30 g (Charles River, Japan) were used in all experiments. Animals were kept in stainless steel cages with raised mesh bottoms, and deprived of food but allowed free access to tap water for 18 h before the experiments. All experimental procedures used were carried out in accordance with the Helsinki Declaration and have been approved by the Committee for Animal Experimentation established by Kyoto Pharmaceutical University.

2.2. Determination of HCO_3^- secretion

Under deep diethyl ether anesthesia, the mouse was killed and the abdomen opened by a midline incision. The proximal duodenum (1 cm distal from pylorus) was removed and immediately placed in ice-cold HCO_3^- Ringer's solution containing indomethacin (10^{-6} M) to suppress trauma-induced PG release. The duodenum was opened along the mesenteric attachment and striped from the muscular layers under a microscope (SZ-PT; Olympus). The tissues were, then, mounted between two halves of a lucite chamber, the exposed area being 12.5 mm^2, and bathed in unbuffered saline (mmol/L: Na^+, 154; Cl^-, 154) gassed with 100% O_2 on the mucosal side and HCO_3^- Ringer's solution (mmol/L: Na^+, 140; Cl^-, 120; K^+, 5.4; Mg^{2+}, 1.2; Ca^{2+}, 1.2; HPO_4^{2-}, 1.4; $H_2PO_4^-$, 2.4; HCO_3^-, 25; glucose 10; indomethacin 0.001) gassed with 95% O_2-5% CO_2 on the serosal side [19,20], and these solutions were warmed at 37 °C and continuously circulated by a gas-lift system. The osmolalities for both solution were approximately 308 mOsm/kg. The HCO_3^- secretion was measured by the pH-stat method (Comtite-980, Hiranuma industries, Ibaraki, Japan) using 2 mmol/L HCl as the titrant to keep the mucosal pH at 7.0. Measurements were made every 5 min starting at least 1 h after the mounting of the tissues. After the rate of secretion had stabilized for 45 min, the following agents were added to the serosal solution; PGE_2 (10^{-7} to 10^{-6} M), NOR-3 (NO donor; 10^{-4} to 10^{-3} M), IBMX (nonselective PDE inhibitor; 10^{-5} to 10^{-4} M), vinpocetine (PDE1 inhibitor; 10^{-6} to 10^{-5} M), EHNA (PDE2 inhibitor; 10^{-5} M), cilostamide (PDE3 inhibitor; 10^{-7} to 10^{-5} M), rolipram (PDE4 inhibitor; 10^{-5} M), and zaprinast (PDE5 inhibitor; 10^{-5} M). In some cases, ONO-8711 (EP1 antagonist; 10^{-5} M), AE5-599 (EP3 antagonist; 3×10^{-6} M) or AE3-208 (EP4 antagonist; 10^{-6} M) was added to the serosal side 10 min before PGE_2, while methylene blue (guanylate cyclase inhibitor; 10^{-4} M) or indomethacin (10^{-4} M) was added 30 min before NOR-3. In other cases, various PDE inhibitors were also added 30 min before the addition of PGE_2 or NOR-3. Furthermore, the effects of N^G-nitro-L-arginine methyl ester (L-NAME; nonselective NO synthase inhibitor) and indomethacin (nonselective cyclooxygenase inhibitor) on the responses to vinpocetine (10^{-5} M) and cilostamide (10^{-5} M) were also examined. L-NAME (10^{-3} M) or indomethacin (10^{-4} M) was added 30 min before the addition of these PDE inhibitors.

2.3. Preparation of drugs

Drugs used were PGE_2 (Funakoshi, Tokyo, Japan), NOR-3 [(±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamine](Dojindo, Tokyo, Japan), ONO-8711, AE3-208, AE5-599 (Ono Pharmaceutical Co., Osaka, Japan), isobutylmethylxanthine (IBMX), vinpocetine, cilostamide, rolipram, zaprinast, erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride (EHNA)(Aldrich, Milwaukee, WI), indomethacin, L-NAME (Sigma Chemicals, St. Louis, MO) and methylene blue (Nacalai tesque, Kyoto, Japan). All agents were dissolved in dimethyl sulfoxide (DMSO: Wako, Osaka, Japan) and diluted with distilled water to desired concentrations. All agents were prepared before use and added to the nutrient solution.

2.4. Statistical analysis

Data are expressed as the mean ± S.E. for 4–7 mice. Statistical analyses were performed with a one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparison test or, when appropriate, Student *t*-tests, and values of $P < 0.05$ were considered significant.

3. Results

3.1. Effects of PGE_2 or NOR-3 on duodenal HCO_3^- secretion

The isolated mouse duodenum consistently secreted HCO_3^- at rates of 0.4–0.7 μEq/h as basal secretion, in the absence or presence of 0.1% DMSO, a solvent for the agents used in the present study. Serosal addition of PGE_2 (10^{-7} to 10^{-6} M) caused a gradual increase of HCO_3^- secretion in a concentration-dependent manner, the ΔHCO_3^- output at 10^{-6} M being 0.26 ± 0.02 μEq/h (Fig. 1A and B). The HCO_3^- stimulatory effect of PGE_2 (10^{-6} M) was significantly attenuated by pretreatment of the tissue with either AE3-208 (EP4 antagonist: 10^{-6} M) or AE5-599 (EP3 antagonist: 3×10^{-6} M), and the ΔHCO_3^- output was reduced to 0.06 ± 0.02 μEq/h or 0.04 ± 0.01 μEq/h, respectively, both which are significantly lower than that obtained in the animals given vehicle in place of these antagonists. However, the EP1 antagonist ONO-8711 (10^{-5} M) had no effect on the HCO_3^- response to PGE_2.

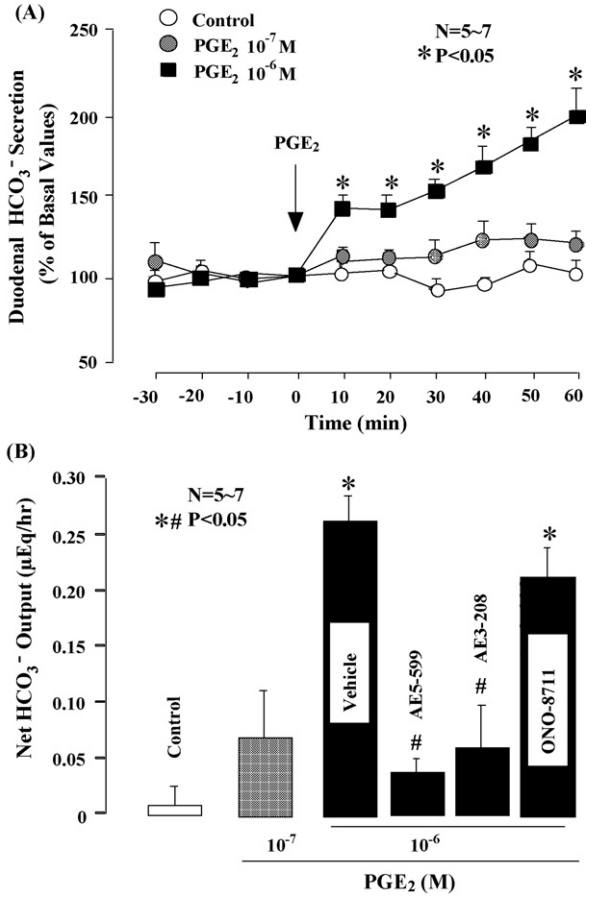

Fig. 1 – The stimulatory effect of PGE_2 on HCO_3^- secretion in the isolated mouse duodenum. PGE_2 (10^{-7} and 10^{-6} M) was added to the serosal solution. ONO-8711 (10^{-5} M), AE3-208 (10^{-6} M) or AE5-599 (3×10^{-6} M) was added to the serosal solution 30 min before PGE_2 (10^{-6} M). (A) Data are presented as a percentage of basal HCO_3^- secretion and represent the mean ± S.E. of values determined every 10 min from 5–7 mice. (B) Data show the total net HCO_3^- output for 1 h after the addition of PGE_2 and are presented as the mean ± S.E. for 5~7 mice. Significant difference at $P < 0.05$; * from control; # from vehicle.

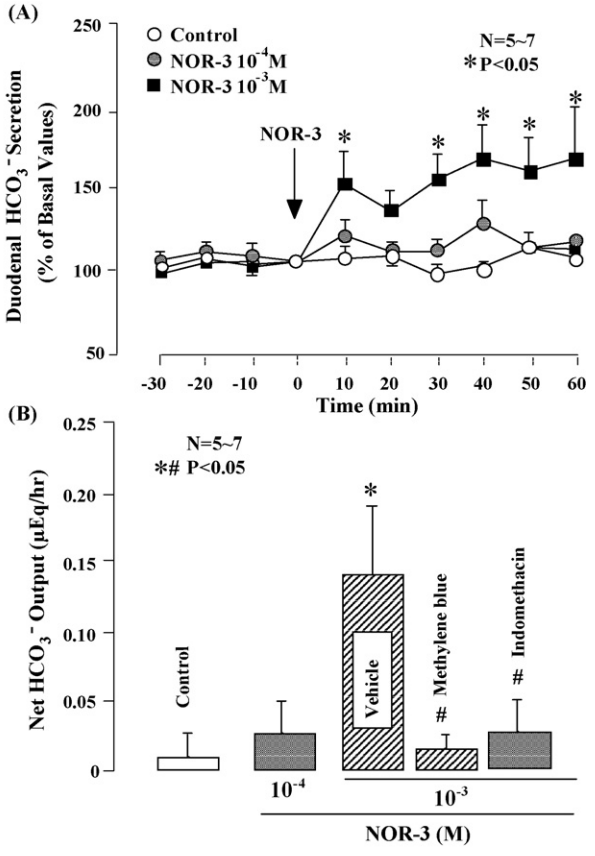

Fig. 2 – The stimulatory effect of NOR-3 on HCO_3^- secretion in the isolated mouse duodenum. NOR-3 (10^{-4} and 10^{-3} M) was added to the serosal solution. Methylene blue (10^{-4} M) or indomethacin (10^{-4} M) was added to the serosal solution 30 min before NOR-3 (10^{-3} M). (A) Data are presented as a percentage of basal HCO_3^- secretion and represent the mean ± S.E. of values determined every 10 min from 5–7 mice. (B) Data show the total net HCO_3^- output for 1 h after the addition of NOR-3 and are presented as the mean ± S.E. for 5–7 mice. Significant difference at $P < 0.05$; * from control; # from vehicle.

Likewise, the isolated mouse duodenum responded to the serosal addition of NOR-3 (10^{-4} or 10^{-3} M) with an increase of HCO_3^- secretion in a concentration- dependent manner, and the effect at 10^{-3} M reached a maximal level of about 180% of basal values, the ΔHCO_3^- output at 10^{-3} M being 0.14 ± 0.05 μEq/h (Fig. 2A and B). The response to NOR-3 (10^{-3} M) was totally attenuated by prior addition of methylene blue (10^{-4} M), the inhibitor of soluble guanylate cyclase, the inhibition being 99.6%. Furthermore, this response was also significantly mitigated by indomethacin, a cyclooxygenase inhibitor, the inibition being 81.2%.

3.2. *Effect of IBMX on basal and stimulated duodenal HCO_3^- secretion induced by PGE_2 or NOR-3*

A nonselective PDE inhibitor IBMX (10^{-5} to 10^{-4} M), added to the serosal solution, concentration-dependently increased the secretion of HCO_3^- in the isolated mouse duodenum, and the effect was significant at 10^{-4} M, the ΔHCO_3^- output being 0.20 ± 0.06 μEq/h (Fig. 3). IBMX at 3×10^{-5} M tended to increase the secretion under basal conditions, yet the effect was not statistically significant as compared to the control. When added at 3×10^{-5} M, together with PGE_2 (10^{-7} M) or NOR-3 (10^{-4} M), this nonselective PDE inhibitor significantly potentiated the response to either PGE_2 or NOR-3, the degree of increase being approximately 3.1 or 6.1 times over the corresponding control value, respectively. Neither PGE_2 (10^{-7} M) nor NOR-3 (10^{-4} M) by itself significantly stimulated HCO_3^- secretion in the isolated mouse duodenum.

3.3. *Effects of various PDE inhibitors on duodenal HCO_3^- secretion*

To determine which PDE isozyme is involved in the local regulation of duodenal HCO_3^- secretion, we examined the effects of various subtype-selective PDE inhibitors on the

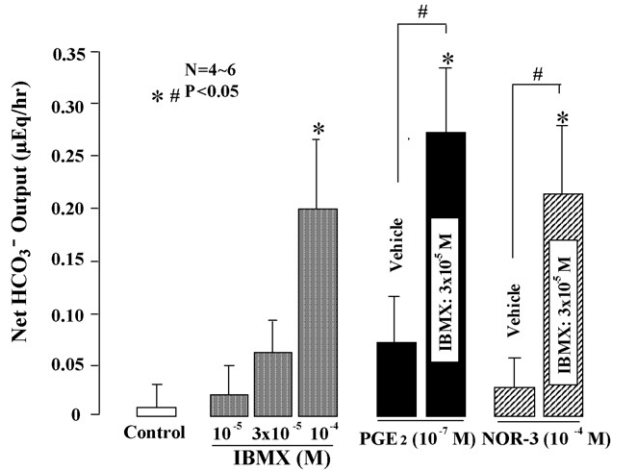

Fig. 3 – Effect of IBMX on the HCO_3^- stimulatory action of PGE_2 or NOR-3 in the isolated mouse duodenum. IBMX (10^{-5} to 10^{-4} M), PGE_2 (10^{-7} M) or NOR-3 (10^{-4} M) was added to the serosal solution. In some cases, IBMX (3×10^{-5} M) was added to the serosal solution 30 min before PGE_2 or NOR-3. Data show the total net HCO_3^- output for 1 h after the addition of IBMX, PGE_2 or NOR-3, and are presented as the mean ± S.E. for 4–6 mice. Significant difference at $P < 0.05$; * from DMSO; # from control.

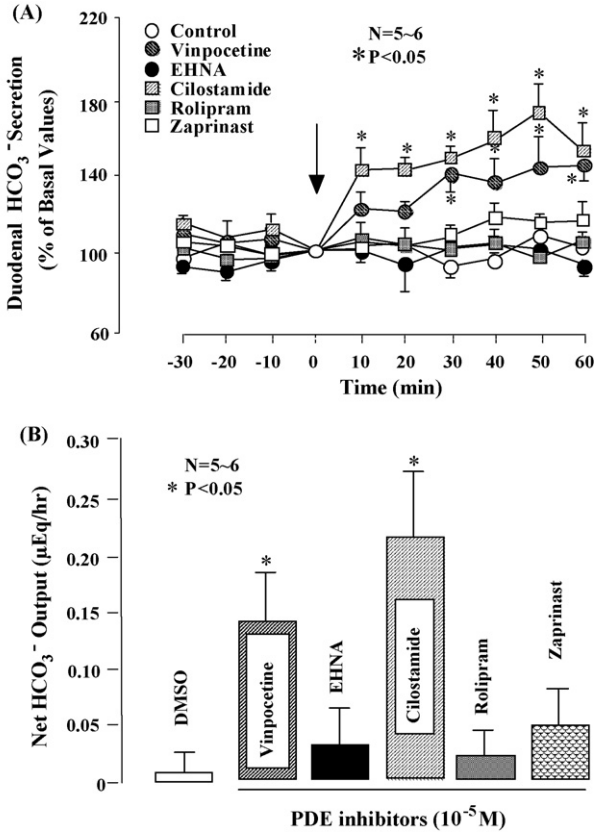

Fig. 4 – Effect of various subtype-selective PDE inhibitors on HCO_3^- secretion in the isolated mouse duodenum. Vinpocetine (PDE1 inhibitor), EHNA (PDE2 inhibitor), cilostamide (PDE3 inhibitor), rolipram (PDE4 inhibitor), or zaprinast (PDE5 inhibitor) was added to the serosal solution at the concentration of 10^{-5} M. (A) Data are presented as a percentage of basal HCO_3^- secretion and represent the mean ± S.E. of values determined every 10 min from 5–6 mice. (B) Data show the total net HCO_3^- output for 1 h after the addition of each agent and are presented as the mean ± S.E. for 5–6 mice. * Significant difference from control, at $P < 0.05$.

rate of basal HCO_3^- secretion in the isolated mouse duodenum.

Among the subtype-selective PDE inhibitors tested, both the selective PDE1 inhibitor vinpocetine and the selective PDE3 inhibitor cilostamide significantly increased the basal rate of HCO_3^- secretion at 10^{-5} M, the ΔHCO_3^- output being 0.14 ± 0.04 μEq/h and 0.02 ± 0.05 μEq/h, respectively (Fig. 4A and B). However, neither EHNA (the selective PDE2 inhibitor), rolipram (the selective PDE4 inhibitor), nor zaprinast (the selective PDE5 inhibitor) had a significant effect on the HCO_3^- secretion, even at a concentration of 10^{-5} M.

To further investigate the involvement of endogenous PGs and NO in these responses, we examined the effects of indomethacin and L-NAME on the HCO_3^- stimulatory action of these PDE inhibitors at 10^{-5} M. Both vinpocetine (10^{-6} and 10^{-5} M) and cilostamide (10^{-7} to 10^{-5} M) increased the rate of HCO_3^- secretion in a concentration- related manner (Fig. 5). The response induced by vinpocetine was significantly abrogated by the co-treatment with indomethacin (10^{-5} M) or L-NAME (10^{-3} M), the inhibition being almost complete, 95.1% or 96.0%, respectively. On the other hand, the increase of HCO_3^- secretion in response to cilostamide was all but totally attenuated by indomethacin but not L-NAME, the inhibition being 82.6% and 17.4%, respectively. The HCO_3^- output caused by cilostamide (10^{-5} M) in the presence of L-NAME was 0.22 ± 0.07 μEq/h, which was not significantly different from that (0.18 ± 0.03 μEq/h) obtained in the absence of L-NAME.

3.4. *Effect of various subtype-selective PDE inhibitors on duodenal HCO_3^- secretion in response to PGE_2 or NOR-3*

It was found in the present study that the nonselective PDE inhibitor IBMX significantly potentiated the HCO_3^- stimulatory action of PGE_2 or NOR-3 in the mouse duodenum. In addition, we found that both vinpocetine and cilostamide significantly stimulated the secretion, depending on endogenous PGs and/or NO, suggesting the involvement of PDE1 and PDE3 in the regulation of duodenal HCO_3^- secretion. To confirm these points, we examined the effects of various PDE inhibitors on the HCO_3^- stimulatory action of PGE_2 or NOR-3 in the isolated mouse duodenum.

PGE_2, added serosally at 10^{-7} M, slightly increased the rate of secretion, but the effect was not statistically significant as compared with the control given vehicle. When various subtype-selective PDE inhibitors were added at the dose of 10^{-5} to 10^{-7} M, together with PGE_2 (10^{-7} M), it was found that both vinpocetine and cilostamide markedly enhanced the PGE_2-induced response, the HCO_3^- output being 0.21 ± 0.04 μEq/h and 0.33 ± 0.08 μEq/h, respectively (Fig. 6). However, other PDE inhibitors such as EHNA, rolipram, and

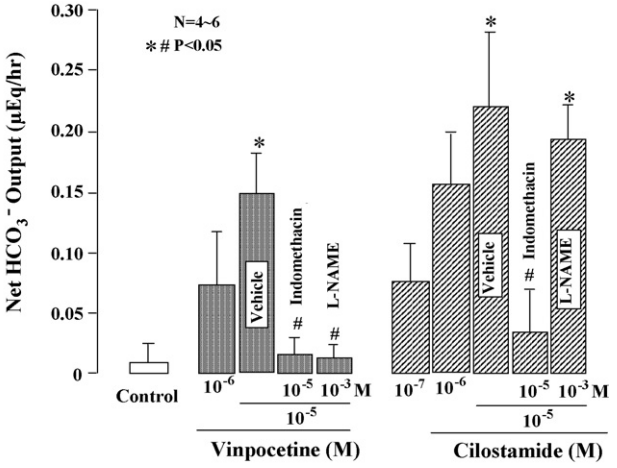

Fig. 5 – Effects of indomethacin and L-NAME on the HCO_3^- response to vinpocetine or cilostamide in the isolated mouse duodenum. Vinpocetine (10^{-6} to 10^{-5} M) or cilostamide (10^{-7} to 10^{-5} M) was added to the serosal solution. Indomethacin (10^{-5} M) or L-NAME (10^{-3} M) was added to the serosal solution 30 min before vinpocetine (10^{-5} M) or cilostamide (10^{-5} M). Data show the total net HCO_3^- output for 1 h after the addition of vinpocetine or cilostamide and are presented as the mean ± S.E. for 4–6 mice. Significant difference at $P < 0.05$; * from DMSO; # from control.

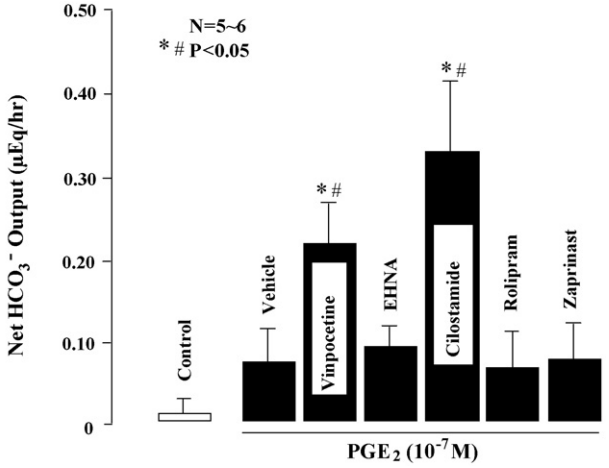

Fig. 6 – Effect of various subtype-selective PDE inhibitors on the HCO_3^- stimulatory action of PGE_2 in the isolated mouse duodenum. PGE_2 (10^{-7} M) was added to the serosal solution. Vinpocetine (10^{-6} M), EHNA (10^{-5} M), cilostamide (10^{-7} M), rolipram (10^{-5} M), or zaprinast (10^{-5} M) was added to the serosal solution 30 min before PGE_2. Data show the total net HCO_3^- output for 1 h after the addition of PGE_2 and are presented as the mean ± S.E. for 5–6 mice. Significant difference at $P < 0.05$; * from control; # from vehicle.

zaprinast did not affect the HCO_3^- response to PGE_2, and the HCO_3^- output was equivalent to that observed in the tissues treated with PGE_2 alone.

Likewise, NOR-3 added serosally at 10^{-4} M, which in itself did not significantly increase the secretion of HCO_3^- in the isolated duodenum, markedly stimulated the L-NAMEcretion in the presence of vinpocetine but not other PDE inhibitors including cilostamide (Fig. 7). The HCO_3^- output induced by NOR-3 in the presence of vinpocetine was 0.22 ± 0.07 μEq/h, which was significantly greater than that observed in the vehicle-treated group.

4. Discussion

It has been well established that the secretion of HCO_3^- from the duodenal surface epithelial cells is regulated by both humoral and neuronal factors, including endogenous PGs, NO, and sensory neurons [5,6,21], and intracellularly mediated by cAMP and cGMP as well as Ca^{2+} [1,10,13,22]. These nucleotides are degraded into inactive metabolites by the catalytic action of PDE. It remains, however, unexplored which PDE isozyme(s) is involved in the regulation of duodenal HCO_3^- secretion. The present study showed for the first time that both PDE1 and PDE3 are involved in the local regulation of duodenal HCO_3^- secretion and that the response to PGE_2 is associated with PDE1 and PDE3 while the response to NO is mainly modulated by PDE1.

We previously reported that PGE_2 or NOR-3 (a NO donor) stimulated HCO_3^- secretion in the rat duodenum and these responses are intracellularly mediated by cAMP and cGMP, respectively [6,8,13]. Several studies have confirmed the involvement of these nucleotides in the HCO_3^- response in the duodenum; cf., both pituitary adenylate cyclase activating polypeptide and vasoactive intestinal polypeptide stimulated

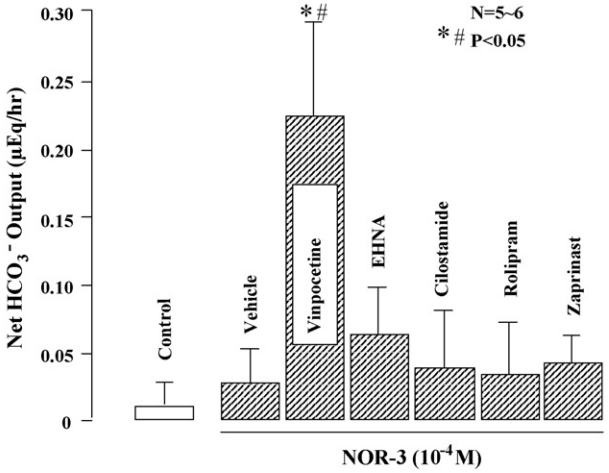

Fig. 7 – Effect of various subtype-selective PDE inhibitors on the HCO_3^- stimulatory action of NOR-3 in the isolated mouse duodenum. NOR-3 (10^{-4} M) was added to the serosal solution. Vinpocetine (10^{-6} M), EHNA (10^{-5} M), cilostamide (10^{-7} M), rolipram (10^{-5} M), or zaprinast (10^{-5} M) was added to the serosal solution 30 min before NOR-3. Data show the total net HCO_3^- output for 1 h after the addition of NOR-3 and are presented as the mean ± S.E. for 5–6 mice. Significant difference at $P < 0.05$; * from control; # from vehicle.

HCO_3^- secretion by increasing intracellular cAMP production [9], while guanylin increased the response by activating soluble guanylate cyclase [1,22]. Furthermore, exogenous dbcAMP and dbcGMP stimulated HCO_3^- secretion in the isolated amphibian duodenum *in vitro* [13]. Thus, there is no doubt that both cAMP and cGMP play a central role in the local regulation of duodenal HCO_3^- secretion.

It was confirmed in the present study that PGE_2 increased the secretion of HCO_3^- in the isolated mouse duodenum. Consistent with previous findings in the rat duodenum *in vivo*, this PGE_2 action is thought to be mediated by the activation of both EP3 and EP4 receptors, in as much as the response was significantly inhibited by both AE5-599 and AE3-208 [10,23]. Likewise, NOR-3 stimulated HCO_3^- secretion in this preparation, and this effect was completely attenuated by methylene blue, a guanylate cyclase inhibitor, suggesting that the stimulatory effect of NOR-3 on duodenal HCO_3^- secretion is mediated by cGMP. Consistent with the observations in amphibian duodenums *in vitro* [13] and rat duodenums *in vivo* [24], we observed that the response to NOR-3 in the mouse duodenum *in vitro* was also significantly mitigated by indomethacin, confirming the involvement of endogenous PGs in the stimulatory action of NOR-3.

Since PDE inactivates both cAMP and cGMP by converting them into 5′-AMP and 5′-GMP, respectively, the physiological responses mediated by these nucleotides are expected to be augmented by inhibitors of PDE. PDE is genetically subdivided into 11 izosymes, five of which, PDE1–PDE5, have been well characterized pharmacologically [14]. PDE1 is activated by Ca^{2+}/calmodulin and PDE2 by cGMP, yet both of them catalyze the conversion of cAMP and cGMP into inactive metabolites [15]. By contrast, both PDE3 and PDE4 selectively bind to cAMP as the substrate, while PDE5 catalyzes cGMP's conversion to 5′GMP [16,17,25]. In general, the fundamental properties of PDE isozymes are well preserved among species [14]. In a preliminary study, we examined the gene expression of PDE isozymes, PDE1–PDE5, in the mouse duodenum by RT-PCR and confirmed that all of them were clearly expressed in this tissue, although it remains unknown which cell type expressed each PDE isozyme (data not shown).

We previously showed that the secretion of HCO_3^- in response to PGE_2 and PACAP in the rat duodenum was enhanced in the presence of IBMX, a nonselective PDE inhibitor [9]. In the present study, IBMX significantly increased the response induced by not only PGE_2 but also NOR-3, confirming the involvement of both cAMP and cGMP in the mechanism of these responses. We further found that the response to PGE_2 was enhanced by vinpocetine, the PDE1 inhibitor, and cilostamide, the PDE3 inhibitor, while the response to NOR-3 was potentiated only by vinpocetine. Other PDE inhibitors, including those of PDE2, PDE4, and PDE5, had no effect on the response induced by PGE_2 or NOR-3. These results suggest that PDE1 and PDE3 contribute to the regulatory mechanism of HCO_3^- secretion in the mouse duodenum; they are both involved in the response to PGE_2/cAMP, while only PDE1 is involved in the response to NO/cGMP.

It should be noted in the present study that vinpocetine and cilostamide increased by themselves the rate of basal HCO_3^- secretion in the isolated mouse duodenum. A similar effect was certainly obtained with IBMX, the nonselective PDE inhibitor, but not by the other subtype-selective PDE inhibitors even at a dose of 10^{-5} M. Furthermore, the response to the PDE1 inhibitor vinpocetine was attenuated by both indomethacin and L-NAME, while the response to the PDE3 inhibitor cilostamide was mitigated only by indomethacin. These results suggest that the stimulatory effect of the PDE1 inhibitor on HCO_3^- secretion is mediated by endogenous PGs and NO, while that of the PDE3 inhibitor is mediated only by PGs. We previously reported that the HCO_3^- stimulatory action of NOR-3 as well as dbcGMP was attenuated by pretreatment with indomethacin, suggesting the involvement of endogenous PGs in the stimulatory action of NO/cGMP [6,13,25]. In the present study, however, we observed that the PDE3 inhibitor cilostamide did not affect HCO_3^- secretion in response to NOR-3. PDE3 binds to cAMP and catalyzes the hydrolysis of this nucleotide to produce 5'AMP. If the HCO_3^- stimulatory action of NO/cGMP is partly mediated by PG/cAMP, the response to NOR-3 would be expected to be enhanced by not only vinpocetine (PDE1 inhibitor) but cilostamide (PDE3 inhibitor) as well. PDE3 is often referred to as the cGMP-inhibited PDE [26]. Indeed, some biological effects of endogenous cGMP may be mediated by inhibition of PDE3, which results in an increase of cAMP and activation of cAMP-dependent protein kinase [27,28]. If this occurs also in the mouse duodenum, then it would be expected that PDE3 is inhibited by NOR-3, and therefore the stimulatory action of NOR-3 was not enhanced by cilostamide, the PDE3 inhibitor. Further study is certainly needed to verify this point.

It has been shown that cystic fibrosis transmembrane conductance regulator (CFTR) has a major role in the regulation of duodenal HCO_3^- secretion [29]. Furthermore, O'Grady et al. [30] showed that both PDE1 and PDE3 are involved in the activation of CFTR in T_{84} cells and human colonic epithelial cells. Thus, these PDE isozymes may be expressed in the epithelial cell of mouse duodenums. On the other hand, it is known that PDE2 is expressed in the entire body, especially in brain and adrenal gland, while PDE4 is mainly expressed in inflammatory cells and PDE5 in smooth muscle [14]. These findings together with the present results strongly suggest that neither PDE2, PDE4, nor PDE5 is involved in the local regulatory mechanism of duodenal HCO_3^- secretion.

In conclusion, these results suggest that both PDE1 and PDE3 are involved in the regulation of duodenal HCO_3^- secretion and that the response to PGE2 is associated with both PDE1 and PDE3 while the response to NO is mainly modulated by PDE1.

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