Low-dose PGE₂ mimics the duodenal secretory response to luminal acid in mice

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Hirokawa, Masahiko, Osamu Furukawa, Paul H. Guth, Eli Engel, and Jonathan D. Kaunitz. Low-dose PGE₂ mimics the duodenal secretory response to luminal acid in mice. Am J Physiol Gastrointest Liver Physiol 286: G891–G898, 2004. First published February 5, 2004; 10.1152/ajpgi.00458.2003.—Luminal exposure to low-dose PGE₂, at least in terms of inhibitor profile, closely resembles secretory responses to one secretagogue, PGE₂, compared with high-dose PGE₂. High-dose PGE₂ substantially increases DBS by a mechanism that appears to be different than secretory response to physiological acid challenge. PGE₂ was chosen due to its universal acceptance as an important component of mucosal secretagogue response to luminal acid recently compounded by powerful evidence obtained from the study of transgenic mice (18). We hypothesized that the appropriate dose of exogenous PGE₂ would likely mimic the physiological mucosal response to luminal acid without producing additional pharmacological effects. We thus compared the effects of several known inhibitors on PGE₂ and acid-related DBS. We found that a low dose of PGE₂ closely mimicked the secretory effect of prior acid exposure and thus could be used in vitro to simulate the mucosal secretory response to luminal acid.

MATERIALS AND METHODS

Animals and Chemicals

Male C57BL/6 mice (Harlan Sprague Dawley, Hercules, CA) were maintained with free access to food and tap water up to 1 h before the experiment when food was removed. All studies were approved by the Animal Use Committee of the Greater Los Angeles Veterans Administration Healthcare System. DIDS, 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), methazolamide indomethacin, HEPES, and other chemicals were obtained from Sigma (St. Louis, MO). PGE₂ was obtained from Oxford Biochemical (Oxford, MD). HEPES-saline solution contained 135 mM NaCl and 20 mM HEPES at pH 7.0. For

BICARBONATE ION SPECIFICALLY protects the duodenal mucosa (4, 12). Because duodenal bicarbonate secretion (DBS) strongly correlates with mucosal injury susceptibility, mucosal bicarbonate secretion is the most commonly measured duodenal defensive factor (6). Due to the relative ease of this measurement, most are carried out in vivo, in which physiological acid concentrations (e.g., HCl 1–6.3 mM) can be applied to the luminal mucosal surface without producing gross or microscopic damage (13). Despite this relative ease of in vivo measurements, there are situations in which it would be desirable to study bicarbonate secretion in vitro. Examples include clinical studies, in which small mucosal biopsies can be used to measure the secretory response (14). Another example would be examining the role of basolateral membrane transporters, such as the basolateral sodium-bicarbonate cotransporter NBC1, which is not normally directly accessible in vivo (2, 11). One of the major drawbacks of the in vitro approach, however, is that the only accepted physiological stimulus for bicarbonate secretion, namely exposure of the luminal surface to 1–6.3 mM HCl, cannot be used due to the high probability of producing mucosal damage (20). Furthermore, the low pH used to stimulate secretion might alter the properties of inhibitors and other interventions designed to work optimally at neutral pH. Thus surrogate secretagogues, such as cyclic nucleotides and hormones have been used under the likely assumption that these compounds are involved in the secretory response to luminal acid. Indeed, variations in response of tissues to different secretagogues have been used to highlight possible differences between normal and disease-bearing tissues (14). Nevertheless, it is not known whether bicarbonate secretion to these surrogate stimuli is a physiological or a pharmacological phenomenon.

In response to this question, we have compared the mucosal secretory responses to one secretagogue, PGE₂, compared with the response to physiological acid challenge. PGE₂ was chosen due to its universal acceptance as an important component of the mucosal response to luminal acid recently compounded by powerful evidence obtained from the study of transgenic mice (18). We hypothesized that the appropriate dose of exogenous PGE₂ would likely mimic the physiological mucosal response to luminal acid without producing additional pharmacological effects. We thus compared the effects of several known inhibitors on PGE₂ and acid-related DBS. We found that a low dose of PGE₂ closely mimicked the secretory effect of prior acid exposure and thus could be used in vitro to simulate the mucosal secretory response to luminal acid.

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acid perfusion, isotonic NaCl was adjusted to pH 4.5, 3.5, and 2.2 with HCl and adjusted to isotonicity (300 mosM) by reducing the NaCl concentration accordingly. Each solution was prewarmed to 37°C with temperature maintained by a heating pad during the experiment. PGE₂ was dissolved with absolute ethanol to make a concentrated stock solution. NPPB, methazolamide, and indomethacin were dissolved with DMSO, and DIDS was dissolved with distilled water to make a concentrated stock solution. PGE₂ was diluted with pH 7.0 saline to each concentration just before the stimulation.

**Measurement of Duodenal Loop Bicarbonate Secretion**

**Preparation of the duodenal loop.** Duodenal loops were prepared and perfused to measure duodenal HCO₃⁻ secretion modified from the similar system as described previously in rats (1, 17). In urethane-anesthetized mice (1.0 g/kg), the stomach and duodenum were exposed, and the forestomach wall was incised by using a miniature electrocautery. A polyethylene tube was inserted through the incision to the pyloric ring where it was secured with a nylon ligature. The distal duodenum was ligated proximal to the ligament of Treitz and was then incised through which another polyethylene tube was inserted and sutured into place. To prevent contamination of the perfusate with bile-pancreatic juice, the pancreaticobiliary duct was ligated just proximal to its insertion into the duodenal wall. The resultant closed proximal duodenal loop was perfused with prewarmed saline using a peristaltic pump at 0.2 ml/min.

**Bicarbonate secretion measurement by pH-stat.** Input (perfusate) and effluent of the duodenal loop were circulated through a reservoir and bubbled with 100% O₂. Perfusate pH was kept at pH 7.0 with a pH-Stat (models PHM290 and ABU901; Radiometer Analytical, Lyon, France). For pH-stat measurements, the amount of 10 mM HCl added to maintain constant pH of the perfusate was considered equivalent to duodenal HCO₃⁻ secretion. For duodenal HCO₃⁻ measurement, a 30-min stabilization with pH 7.0 saline (t = −35 to −5) was followed by baseline measurements with pH 7.0 saline (t = −5−25). To examine the acid-induced bicarbonate secretion, acid solution was perfused with a Harvard infusion pump at 0.2 ml/min for 10 min (t = 25–35). To examine PGE₂-induced bicarbonate secretion, PGE₂ (1–100 μg/ml) was perfused at 0.2 ml/min for 10 min (t = 25–35). The duodenal loop solution was returned to O₂ gas-bubbled pH 7.0 saline for 60 min after being gently flushed (t = 35–95). In some experiments, indomethacin (5 mg/kg) was administered subcutaneously 1 h before the experiment.

**CO₂ measurement.** To confirm that the titratable base secretion (acid disappearance) measured by the pH-stat technique reflected true HCO₃⁻ secretion (2), we also measured total dissolved CO₂ concentration ([CO₂]ₜ) in some but not all conditions. [CO₂]ₜ of the duodenal effluent was measured by a CO₂ electrode gas-sensing electrode (model 950200; Thermo Orion) connected to a pH meter [model PHM 62; Radiometer, Copenhagen, Denmark] (1). Duodenal loops were prepared and perfused with 20 mM HEPES containing saline (pH 7.0) at a rate 0.2 ml/min as described in Animals and Chemicals. Effluent was collected every 10 min; 0.2 ml of 1 M citrate buffer (pH 4.5) was then added to the sample solution (2 ml) to convert free HCO₃⁻ to CO₂, followed by measurement of electrode potential (mV) with the CO₂ electrode. [CO₂]ₜ was calculated according to a calibration curve using freshly prepared 0.1, 1, and 10 mM NaHCO₃ solutions as standards, which generate 0.1, 1, and 10 mM [CO₂]ₜ, respectively (1). For duodenal [CO₂]ₜ measurement, a 30-min stabilization with pH 7.0 saline (t = −30–0) was followed by baseline measurements with pH 7.0 saline (t = 0–30). To examine the acid-induced bicarbonate secretion, acid solution was perfused with a Harvard infusion pump at 0.2 ml/min for 10 min (t = 30–40). PGE₂ (1–100 μg/ml) was perfused at 0.2 ml/min for 10 min (t = 30–40). The duodenal loop solution was returned to pH 7.0 saline for 60 min after being gently flushed (t = 40–100).

In some cases, the anion channel inhibitor NPPB (0.2 mM), the anion transport inhibitor DIDS (0.5 or 1 mM), or the permeant carbonic anhydrase inhibitor methazolamide (0.1 or 1 mM) was added to the duodenal perfusate.

**Results**

**Effect of Methazolamide, DIDS, and NPPB on Acid-Induced DBS**

Initial experiments were designed to examine DBS in response to a prior pulse of acid in the mouse, with and without the presence of known transport inhibitors. Consistent with prior studies, DBS significantly increased after a 10-min exposure to pH 2.2 and 3.5 solutions (peak DBS: 0.053 ± 0.005 and 0.0044 ± 0.002 μmol·min⁻¹·cm⁻², respectively), but DBS did not increase after exposure to a pH 4.5 perfusate (peak DBS: 0.030 ± 0.001 μmol·min⁻¹·cm⁻²; Fig. 1). We then confirmed the effect of the anion transport inhibitor DIDS and the anion channel inhibitor NPPB on DBS. Both DIDS and NPPB inhibited acid-induced DBS (Fig. 2A), confirming the importance of DIDS- and NPPB-sensitive transport processes in DBS. We then tested the effect of the nonselective cyclooxygenase inhibitor indomethacin on DBS to examine the effects of endogenously generated prostaglandins on DBS. Pretreatment with indomethacin (5 mg/kg sc) inhibited acid-induced DBS increase as measured by back-titration (Fig. 2B). We then examined the effects of methazolamide and DIDS on.

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**Fig. 1. pH-dependent effect of acid on bicarbonate secretion by the pH-stat method.** Titratable alkalinity (bicarbonate secretion) was measured by the pH-stat method in duodenal loop perfusion experiments. Acid pH-dependently increased duodenal bicarbonate secretion (DBS) after acid exposure. All data are expressed as means ± SE from 6–8 mice. *P < 0.05 vs. pH 7.0 saline perfusion.
acid-induced DBS by measurement of $[\text{CO}_2]$t. DBS increased after pH 2.2 acid exposure and was abolished by DIDS and by methazolamide (Fig. 3).

**Effect of Methazolamide DIDS and NPPB on Basal DBS**

We then tested the effects of the inhibitors on basal (non-stimulated) DBS. NPPB (0.2 mM) had no effect on basal DBS. Methazolamide (1 mM) also did not alter DBS, whereas DIDS (0.5 mM) slightly reduced baseline DBS after prolonged perfusion (DIDS: 0.060 ± 0.005 μmol·min$^{-1}$·cm$^{-1}$; saline: 0.074 ± 0.005 μmol·min$^{-1}$·cm$^{-1}$; Fig. 4).

**Effect of Methazolamide, DIDS, and NPPB on PGE2-Induced DBS**

We then assessed the effects of PGE2 perfusion on DBS compared with acid perfusion. Figure 5 depicts the effects of three concentrations of PGE2 on DBS as measured by $[\text{CO}_2]$t. PGE2 dose-dependently increased peak DBS (PGE2: 100 μg/ml, 0.126 ± 0.008; 10 μg/ml, 0.101 ± 0.005; and 1 μg/ml, 0.093 ± 0.003 μmol·min$^{-1}$·cm$^{-1}$). By using similar conditions, we then tested the effects of methazolamide on PGE2-stimulated DBS. It is interesting that 0.1 mM methazolamide inhibited low-dose PGE2-induced DBS, but neither concentration of methazolamide inhibited high-dose PGE2-induced DBS (Fig. 6). Figure 7 depicts the effect of DIDS on PGE2-induced DBS by total CO2 measurement. DIDS (0.5 mM) inhibited low-dose PGE2-induced DBS, but DIDS (0.5, 1 mM) only partially attenuated high-dose PGE2-induced DBS. There was thus a difference between high-dose and low-dose PGE2-induced DBS, suggesting different secretory pathways. Figure 8 depicts the effect of three concentrations of PGE2 on DBS, as measured by back-titration. PGE2 dose-dependently increased
peak DBS (PGE$_2$: 100 μg/ml, 0.058 ± 0.004; 10 μg/ml, 0.052 ± 0.002; 1 μg/ml, 0.048 ± 0.003 μmol·min$^{-1}$·cm$^{-1}$) similar to but with a longer duration than PGE$_2$-induced increased [CO$_2$]$_I$. We then examined the effect of NPPB on PGE$_2$-induced DBS by back-titration. NPPB (0.2 mM) inhibited low-dose PGE$_2$-induced DBS, whereas NPPB did not abolish high-dose PGE$_2$-induced DBS (Fig. 9), suggesting that anion channels are involved in low-dose PGE$_2$-induced DBS. Pretreatment with indomethacin (5 mg/kg sc) did not inhibit low-dose as well as high-dose PGE$_2$-induced DBS increase as measured by [CO$_2$]$_I$ (Fig. 10).

**DISCUSSION**

We demonstrated that acid-stimulated DBS in C57BL/6 mice is inhibited as it is in rats by NPPB, DIDS, and metha-

Fig. 4. Effects of methazolamide, DIDS, and NPPB on basal bicarbonate secretion by the CO$_2$-sensitive electrode method. Methazolamide (1 mM) and NPPB (0.2 mM) did not alter basal DBS. DIDS (0.5 mM) slightly reduced basal DBS. All data are expressed as means ± SE from 6–8 mice. *P < 0.05 vs. pH 7.0 saline perfusion.

Fig. 5. Effect of PGE$_2$ on bicarbonate secretion by the CO$_2$-sensitive electrode method. PGE$_2$ dose-dependently increased DBS after PGE$_2$ exposure for 10 min (1–100 μg/ml) by total CO$_2$ measurement. All data are expressed as means ± SE from 6–8 mice.

Fig. 6. Effects of methazolamide on high-dose and low-dose PGE$_2$-induced bicarbonate secretion by the CO$_2$-sensitive electrode method. A: methazolamide (0.1 mM) inhibited low-dose PGE$_2$ (1 μg/ml)-induced DBS. B: methazolamide (0.1 mM, 1 mM) did not alter high-dose PGE$_2$ (100 μg/ml)-induced DBS. All data are expressed as means ± SE from 6 mice. *P < 0.05 vs. PGE$_2$ perfusion group.
zolamide. The same compounds inhibited DBS in response to 1 μg/ml-perfused PGE2 but not in response to 100 μg/ml-perfused PGE2. On the basis of these data, we conclude that 1 μg/ml PGE2 mimics the physiological response to duodenal acid perfusion.

The duodenal bicarbonate secretory response to luminal acid perfusion is thought to be the primary means by which duodenal injury due to luminal acid is prevented (6). Luminal acid is thought to be the principal endogenous stimulus of DBS. Although the entire secretory mechanism consisting of afferent acid sensors, neural circuits, cellular transduction, and plasma membrane ion transporters is only beginning to be fully understood, the broad pathways that regulate DBS are well characterized. The main classes of secretory signals consist of cAMP, cGMP, and prostaglandin-related signals. The necessity of studying acid-induced DBS in an in vivo system has somewhat limited detailed knowledge of the cell biology of acid-related secretion, although several recent studies have indicated that this response involves prostaglandin generation in a step that is likely close to the final secretory pathway, in that cyclooxygenase inhibition suppresses DBS in response to acid and to a variety of stimuli (10, 16, 18). Moreover, mice lacking the gene for the prostaglandin EP3 receptor have defective DBS in response to duodenal acid perfusion (18). Furthermore, luminal acid perfusion releases mucosal prostaglandins (16). We therefore chose PGE2 as a logical secretagogue to mimic the physiological duodenal secretory acid response.

The mechanism by which HCO₃⁻ is secreted by the duodenum remains controversial. There is consensus that HCO₃⁻ is taken into the cell by the basolateral NBC1 and as CO₂, and that the main secretory pathways are CFTR and an anion exchanger on the enterocyte apical membrane. Furthermore, basal and carbonic anhydrase-inhibitable secretion is mostly electroneutral, whereas cAMP-stimulated secretion is mostly electrogenic, and is thought to result from upregulation of NBC1 function (3, 11). Because measurement of short-circuit current in vivo is not possible, we would prefer not to speculate on the mechanism of secretion elicited by low and high-dose PGE2. The secretory response to PGE2 and acid perfusion is generally slow to develop and is prolonged. One interesting observation is that although acid-induced secretion as measured either by [CO₂], or back-titration is prolonged, secretion in response to PGE2 develops and falls more quickly when measured by [CO₂], compared with back-titration. This suggests that the long recovery to baseline rates of secretion reflects probable non-HCO₃⁻ alkaline secretion.

Fig. 7. Effects of DIDS on high-dose and low-dose PGE2-induced bicarbonate secretion by the CO₂-sensitive electrode method. A: DIDS (0.5 mM) inhibited low-dose PGE2-induced DBS. B: DIDS (0.5 mM, 1 mM) partially attenuated high-dose PGE2-induced DBS. All data are expressed as means ± SE from 6 mice. *P < 0.05 vs. PGE2 perfusion group.

Fig. 8. Effect of PGE2 on bicarbonate secretion by the pH-stat method. PGE2 dose-dependently increased DBS after PGE2 exposure for 10 min (1–100 μg/ml) by pH-stat back-titration. All data are expressed as means ± SE from 6–8 mice.
Since the discovery of the intestinal prosecretory effects of the E-type prostaglandins in the 1970s, and the development of stable prostaglandin analogs such as 16–16-dimethyl PGE$_2$, these compounds have been the preferred secretagogues for duodenal bicarbonate. Despite the extensive use of prostaglandins to augment DBS in vivo, there have been few dose-response studies of its action. In rabbit duodenum, Granstam et al. (8) used 5–80 μM (1.9–30 μg/ml), with a rough ED$_{50}$ of 20 μM (8 μg/ml). In the rat, Isenberg et al. (10) used doses of 10$^{-7}$ – 4 × 10$^{-4}$ M, finding an ED$_{50}$ at ~50 μM (19 μg/ml) and ED$_{50}$ at 200 μM [76 μg/ml]. Other rough ED$_{50}$ values published include 0.1 μg/ml (0.3 μM) in cats (19) and 10 μg/ml (26 μM) in rats (17). A dose of 10 μM (3.8 μg/ml) in rats also is commonly used (21). Thus the dose employed by us (1–100 μg/ml or 0.38–38 μM) ranges from ~2 to 200% of the consensus mammalian ED$_{50}$ for this compound of ~20 μM.

Secretagogues used for augmentation of HCO$_3^-$ secretion in vitro include cAMP, prostaglandins, vasoactive intestinal peptide, carbachol, and cGMP agonists such as heat-stable *Escherichia coli* toxin (STa) with the most commonly used being cAMP analogs (3, 15a, 14, 22). Although all of these compounds can produce a robust secretory response, few studies have addressed the comparability of this response to the phys-

![Fig. 9.](http://ajpgi.physiology.org/) Effects of NPPB on high-dose and low-dose PGE$_2$-induced bicarbonate secretion by the pH-stat method. A: NPPB (0.2 mM) inhibited low-dose PGE$_2$ (1 μg/ml)-induced DBS. B: NPPB did not alter high-dose PGE$_2$ (100 μg/ml)-induced DBS. All data are expressed as means ± SE from 6–8 mice. *P < 0.05 vs. PGE$_2$ perfusion group.

![Fig. 10.](http://ajpgi.physiology.org/) Effects of indomethacin on high-dose and low-dose PGE$_2$-induced bicarbonate secretion by the CO$_2$-sensitive electrode method. A: pretreatment with indomethacin (5 mg/kg sc) did not inhibit low-dose PGE$_2$-induced DBS. B: indomethacin also did not inhibit high-dose PGE$_2$-induced DBS. All data are expressed as means ± SE from 6 mice.
iological effect of a prepulse of luminal acid measured in vivo. Although the use of inhibitors of limited specificity could be considered somewhat crude, their effects are strikingly dependent on the mode of HCO₃⁻ stimulation, uncovering similarities and differences with acid-induced secretion in vivo. The same is true in the case of dysfunction of a key element of the secretory pathway, the apical membrane CFTR. For example, in the presence of a dysfunctional, mutant CFTR, or in CFTR knockout mice, basal HCO₃⁻ secretion is reduced, and the response to acid is abolished (9). In an in vitro system using tissue obtained from cystic fibrosis patients carrying the ΔF508 mutation, this pattern of response was observed with cAMP but not with SfTa or with carbachol, suggesting that the cAMP response more closely resembled the acid response (14). These studies confirm that the presumed secretory mechanism is dependent on the stimulus provided.

To help further establish specificity, the inhibitors NPPB, DIDS, and methazolamide were used, due to their well-known inhibition of DBS. NPPB, an anion channel inhibitor, is believed to inhibit the CFTR anion channel, which is functionally closely tied to bicarbonate secretion. The inhibitory mechanism of DIDS, a relatively nonspecific stilbene anion transport protein inhibitor, on bicarbonate secretion is less agreed on but may reflect inhibition of either the apical membrane anion exchanger, whose identity is at present controversial (15a), and/or the basolateral membrane sodium-bicarbonate cotransporter NBC1 (3, 7, 11). In vivo, DIDS and NPPB abolish the HCO₃⁻ secretory response to luminal acid (2). Similar to our results obtained in vivo, luminal applied DIDS in vitro in rabbit duodenum abolished the HCO₃⁻ secretory response to 100 μM (38 μg/ml) PGE₂ but not to cAMP (22), and basolaterally applied DIDS abolished the secretory response to 10 μg/ml PGE₂ in bullfrog duodenum (7). Other groups have also confirmed that the secretory response to cAMP measured in vitro is not inhibited by DIDS alone (3, 15a). This lack of DIDS sensitivity to cAMP-induced secretion is hence unlike the secretory response to acid observed in vivo. This observation supports the hypothesis that the response to PGE₂ in vitro and in vivo, which is DIDS inhibitable, more closely resembles the physiological acid-induced secretory response than does the response in vitro to cAMP. Methazolamide, or the related compound acetazolamide, inhibitors of carbonic anhydrase, also inhibits basal and stimulated bicarbonate secretion, presumably by inhibiting hydration of intracellular CO₂. Although methazolamide given subcutaneously did not inhibit secretion stimulated by 10 μg/ml PGE₂ in rats (17), when used in vitro it inhibited electroneutral secretion in mouse duodenum (3) and basal but not cAMP-stimulated secretion in rabbit duodenum (11). Indomethacin, the nonselective cyclooxygenase inhibitor, strongly inhibited acid-induced DBS but did not affect PGE₂-induced secretion in rat duodenum in vivo in our study or as observed by other groups (10, 19) consistent with its mechanism of inhibiting endogenous prostanoid production but not the effects of exogenous prostanoid administration. By using an array of inhibitors, each with an effect on a different component of the bicarbonate secretory pathway, we created a pattern that identified the secretory pathway augmented by luminal acid exposure. More detailed studies have indicated that the DIDS-resistant, cAMP-elicited pathway occurs by a different mechanism than basal secretion and may reflect secretion under conditions such as inflammation and not the more common response to luminal acid (15a).

A limitation of this study is that the intended in vitro preparation may differ in sensitivity from the in vivo preparations used. Although it is not possible to use concentrated acid with in vitro preparations due to the aforementioned high likelihood of damaging the mucosa (20) and hence make direct comparisons, the limited published data (22) from which an assessment can be made suggests that comparable PGE₂ concentrations can be used in vivo.

In summary, we found that a dose of prostaglandin lower than is usually used in vitro augmented DBS measured in vivo with similar magnitude and inhibitor profile as did a prepulse of luminal acid. In in vitro studies of DBS, low doses of prostaglandin can be used as a surrogate for luminal acid exposure to help better understand the apical and basolateral mechanisms involved with bicarbonate secretion. With more understanding of the mechanism of duodenal HCO₃⁻ secretion, other secretagogues such as melatonin (15) might also be used in vitro to simulate the duodenal secretory acid response.

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