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Prostaglandins Stimulate and Inhibit Acid Secretion in Amphibian Fundic Mucosa[1] (41449)

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Abstract. Stripped gastric fundic mucosae of *Rana catesbeiana* were mounted between two halves of a lucite chamber, and acid secretion was measured during continuous monitoring of transmucosal potential difference (*PD*), resistance (*R*), and short-circuit current (I_{sc}). At low concentrations ($<10^{-6}$ *M*), 16,16-dimethyl prostaglandin E_2 (16,16dmPGE$_2$) is inhibitory to the H^+ secretory mechanism, while at higher concentrations ($>10^{-6}$ *M*), 16,16dmPGE$_2$ stimulates H^+ secretion without significant change in electrical measurements. The stimulatory effect which is observed with titration at a luminal pH of either 4.8 or 7.4 shows tachyphylaxis, is abolished by metiamide (1×10^{-3} *M*) but not by atropine (1×10^{-6} *M*), and is prevented or reduced by pretreatment with compound 48/80 (1×10^{-4} g/ml), a substance which releases histamine from mast cells or by heparin which prevents release of histamine from mast cells. Similar stimulatory effects were observed with PGE_1, $PGF_{2\alpha}$, and PGI_2 at 1×10^{-5} *M*, with the magnitude of the effect being $PGE_1 \geq$ 16,16dmPGE$_2$ > $PGF_{2\alpha}$ > PGI_2. On the other hand, in tissues pretreated with compound 48/80, 16,16dmPGE$_2$ (10^{-9} to 10^{-5} *M*) caused inhibition of histamine-stimulated H^+ secretion only. These findings suggest that in addition to their inhibitory effects, prostaglandins (PGs) at higher concentrations stimulate H^+ secretion by releasing endogenous histamine from mast cells.

Prostaglandins (PGs) are widely distributed throughout the gastrointestinal tract and affect a variety of gastrointestinal functions (1). Several investigators have proposed a possible physiological role of PGs, especially the E compounds, in the negative feedback inhibition of gastric secretion (2, 3). Way and Durbin (4) showed that PGE_1 inhibits histamine-stimulated but not resting or cyclic AMP (cAMP)-stimulated secretion in amphibian mucosa, findings later confirmed *in vivo* in the rat (5). Recent studies have shown that the inhibitory effects of PGE_2 and its methyl analogs are likely brought about by suppression of the formation of cAMP (6, 7). However, high doses of PGI_2 stimulate adenyl cyclase in isolated parietal cells (6) and PGE_2 stimulates oxygen uptake in both isolated parietal and nonparietal cells (8). In a subsequent report, 16,16-dimethyl prostaglandin E_2 (16,16dmPGE$_2$) (1×10^{-8} *M*) was found to stimulate H^+ transport in rat gastric mucosa (9). Thus, there are conflicting data in the literature on the effects of PGs on H^+ secretion.

In the present study, we demonstrate that the effects of PGs on H^+ secretion are dose dependent in an *in vitro* system which has the advantage of eliminating the circulatory actions of PGs. Low doses inhibit acid secretion whereas high doses cause a tachyphylactic stimulation of H^+ secretion which is prevented by metiamide or compound 48/80, a specific histamine releaser from mast cells, but not by atropine.

Materials and Methods. Bullfrogs (*Rana catesbeiana*) were housed at 4° in 120 m*M* NaCl and tetracycline, 50 mg/liter. The frogs were pithed, stomachs isolated, and the fundic mucosa was stripped from the muscle layer by blunt dissection. The tissues were mounted between two halves of a lucite chamber. The exposed mucosal surface was 1.96 cm^2. Tissues were bathed in 120 m*M* NaCl gassed with 100% O_2 on

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the mucosal side and HCO_3^- Ringer's solution (mmole/liter: Na^+, 105; K^+, 5; Mg^{2+}, 1; Ca^{2+}, 2; Cl^-, 97; HCO_3^-, 18; PO_4^{2-}, 1; glucose, 10) gassed with 95% O_2–5% CO_2 on the serosal side, and these solutions were continuously circulated by a gas-lift system. H^+ secretion was measured by the pH-stat method (Radiometer, Copenhagen) using 50 m*M* NaOH as the titrant to keep the mucosal pH at 7.4 or 4.8. Measurements were made every 15 min starting at least one hour after mounting tissues.

The transmucosal potential difference (*PD*) was measured between two agar bridges connected via two calomel electrodes to a voltmeter. Current was passed across the tissue between two other agar bridges which were connected via Ag–$AgCl_2$ electrodes to a 45-V battery. A microammeter measured current flow. The electrical resistance (*R*) was calculated from the changes in *PD* 0.5 sec after passage of a 100-μA current across the tissue. Correction was made for the fluid resistance (45 ohm·cm^2) which was calculated from the changes in *PD* after passing 100-μA current across the fluid-filled chamber with no tissue present. In the present study, all tissues were kept in the open-circuited state except for 60-sec periods at 15-min intervals when the short-circuit current (I_{sc}) was recorded.

PGE_1, 16,16dmPGE_2, and $PGF_{2\alpha}$ were dissolved in absolute ethanol and stored in a freezer, while PGI_2 was dissolved in 1 *M* Tris-(hydroxymethyl)aminoethane hydrochloride (Tris) buffer (pH 9.3) and was frozen as stock solution. The stock solution, which was kept for only 3 days, was diluted with 1.25% $NaHCO_3$ solution before use (10). PGs were instilled directly into the nutrient solution in an amount of 15 μl. Absolute ethanol or 1.25% $NaHCO_3$ solution was used as the control vehicle.

Data are presented as mean ± SE. The mean values of H^+ secretion, *R*, and I_{sc} were compared to values in paired control tissues using Student's *t* test for paired variates.

Results. A single instillation of 16,16-dmPGE_2 has a dose-dependent effect on H^+ secretion. Figure 1 shows the clear

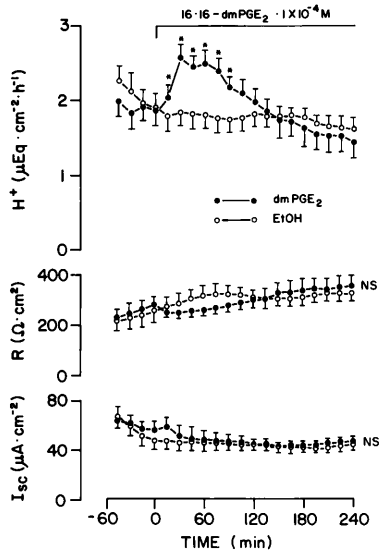

FIG. 1. Effect of 16,16dmPGE_2 on spontaneous H^+ secretion. Paired fundic mucosae were used and one-half was exposed to the 16,16dmPGE_2 while the other half was exposed to the ethanol vehicle as a control. Data are expressed as the mean ± SE from six paired tissues. In this figure and all subsequent figures, the percentage change in the experimental group was compared with that in the controls. Asterisks denote $P = 0.05$ or less.

stimulating effect of this compound on H^+ secretion at 1×10^{-4} *M*. The stimulation of H^+ secretion was accompanied by a small but consistent decrease in *R* and transient increase in I_{sc}, although neither of these changes reached statistical significance. The actual increase in H^+ secretion above baseline was 0.71 ± 0.19 μeq·cm^{-2}·hr^{-1} at a [16,16dmPGE_2] of 1×10^{-4} *M* and 0.55 ± 0.10 μeq·cm^{-2}·hr^{-1} at 1×10^{-5} *M*. As shown in Figure 2A, the stimulation of H^+ secretion at these higher doses was shorter in duration than that observed with 1×10^{-6} *M*, the effect of which was still evident at 4 hr. The inhibition of H^+ secretion by 16,16dmPGE_2 was also significant but small (0.3–0.4 μeq·cm^{-2}·hr^{-1}), possibly because of a simultaneous stimulatory effect on secretion, even at very low doses.

Figure 2B demonstrates that at 1×10^{-5} *M*, 16,16dmPGE_2 stimulates H^+ secretion at either luminal pH 7.4 or 4.8, and there was no statistical difference in the actual

stimulatory action between these two different luminal pH values.

Similar stimulatory effects were observed with PGE_1, $PGF_{2\alpha}$ at 1×10^{-5} *M* (Table I). Although a small increase in H^+ secretion was also obtained with PGI_2 at 1×10^{-5} *M*, this change was not statistically significant. These results suggest that the stimulatory action on H^+ secretion is a common property of PGs even though the magnitude of the effect was different, i.e., $PGE_1 \geq 16,16dmPGE_2 > PGF_{2\alpha} > PGI_2$.

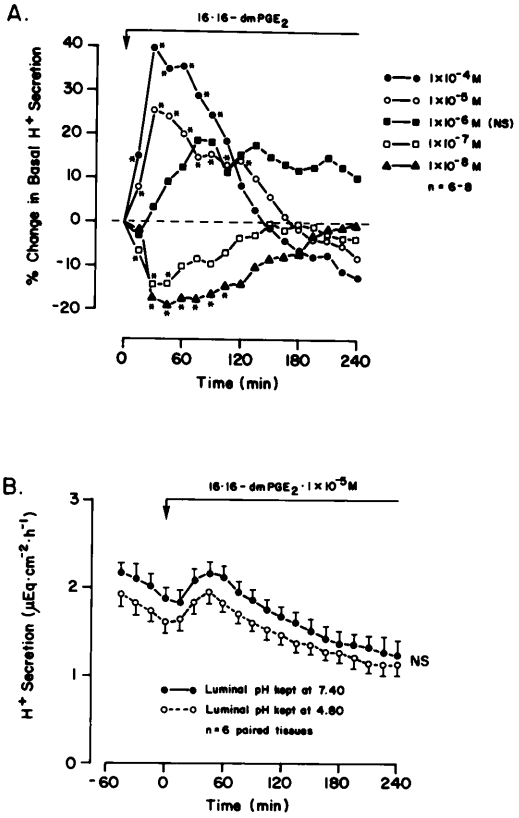

FIG. 2. (A) Effect of various doses of $16,16dmPGE_2$ on spontaneous H^+ secretion. Paired fundic mucosae were used and one-half was exposed to the $16,16dmPGE_2$ while the other half was exposed to the ethanol vehicle as a control. The values plotted in (A) were derived by subtracting the percentage changes from baseline value in the control tissues from the percentage change from baseline values in the paired tissue treated with $16,16dmPGE_2$. Six to eight paired tissues for each concentration of $16,16dmPGE_2$ were used. (B) Effects of $16,16dmPGE_2$ (1×10^{-5} *M*) on spontaneous H^+ secretion at luminal pH of 7.40 and 4.80. Experiments were done in paired tissues. In half of the tissues, luminal pH was titrated to pH 7.40 and in the other half of the tissues pH 4.80 was used as endpoint. Data are expressed as the mean ± SE from six paired tissues.

Figure 3 demonstrates that the stimulatory effect of the high concentration of $16,16dmPGE_2$ (1×10^{-5} *M*) exhibits tachyphylaxis, since the second and third doses had no effect on H^+ secretion while the tissue retained its ability to respond to histamine (1×10^{-4} *M*). Atropine (1×10^{-6} *M*) did not alter the stimulatory effect of the PGs on H^+ secretion, whereas metiamide (1×10^{-3} *M*), an H_2 receptor antagonist completely abolished it (not shown). The lower dose of atropine was chosen because this dose has been shown to be active in amphibian gastric mucosa at this concentration. Figure 3B shows that $16,16dmPGE_2$ failed to stimulate H^+ secretion in tissues pretreated with compound 48/80 to release histamine from mast cells in the tissue. The *PD* and *R* were unaffected by the compound 48/80. It is unlikely that compound 48/80 had irreversible injurious effects on the mucosa because the addition of exogenous histamine stimulated H^+ secretion to a similar degree in both treated and control tissues.

Heparin is known to prevent the release of histamine from mast cells. In five tissues, heparin, 133 units/ml in the nutrient solution, had no effect on H^+ secretion, *PD*, *R*, or I_{sc}. In two sets of paired fundic tissue, heparin, 133 units/ml, prevented the initial increment in H^+ secretion induced by compound 48/80, but the subsequent decline in secretion was unaffected (Fig. 3B). In two experiments with paired tissues, pretreatment with heparin, 133 units/ml in the nutrient solution, completely prevented the increment in H^+ secretion caused by $16,16dmPGE_2$ (1×10^{-5} *M*) in the control of one pair and halved the stimulatory effect in the other pair.

In tissues pretreated with compound 48/80, $16,16dmPGE_2$ (10^{-10} to 10^{-5} *M*) caused a significant inhibition of H^+ secretion stimulated by histamine (1×10^{-5} *M*) as shown in Fig. 4. The inhibitory action appeared at 1×10^{-9} *M* $16,16dmPGE_2$ and reached a maximum level at 1×10^{-6}

TABLE I. EFFECTS OF PROSTAGLANDINS (1×10^{-5} *M*) ON SPONTANEOUS ACID SECRETION IN *IN VITRO* AMPHIBIAN STOMACH

	Initial rate of acid secretion (μeq·cm^{-2}·h^{-1})	ΔH^+ after application of PGs (1×10^{-5} *M*)				
		15 min	30 min	60 min	90 min	120 min
Control	2.24 ± 0.23	0.25 ± 0.03**	0.55 ± 0.09**	0.52 ± 0.05**	0.46 ± 0.05**	0.39 ± 0.09**
PGE_1	2.31 ± 0.23					
Control	2.11 ± 0.22	0.20 ± 0.04**	0.60 ± 0.11**	0.47 ± 0.12**	0.38 ± 0.11*	0.35 ± 0.08**
16,16dmPGE_2	1.97 ± 0.28					
Control	2.24 ± 0.15	0.06 ± 0.02*	0.15 ± 0.02**	0.20 ± 0.01**	0.27 ± 0.05**	0.28 ± 0.06**
$PGF_{2\alpha}$	2.18 ± 0.15					
Control	1.79 ± 0.14	0.36 ± 0.18	0.27 ± 0.19	0.27 ± 0.24	0.23 ± 0.24	0.15 ± 0.29
PGI_2	1.80 ± 0.25					

Note. All values are expressed as the mean ± SE from six (6) paired tissues. ΔH^+ is the difference in H^+ secretion between prostaglandin-treated group and control group. The H^+ secretion in the prostaglandin and control group is the value derived by subtracting the baseline from the value at 15–20 min.

* $P < 0.05$.

** $P < 0.01$. Those values which do not have an asterisk (PGI_2) were not statistically significantly different.

M, with ED_{50} being approximately 1×10^{-8} *M*. No stimulation was observed even at 1×10^{-5} *M*.

Discussion. A stimulatory effect of PGs on gastric H^+ secretion in amphibian gastric mucosa has not previously been described to our knowledge. While an unexpected stimulation of H^+ secretion by *in vitro* frog mucosa has been observed in response to arachidonic acid, a precursor of PGs (11), it is unlikely that this effect is mediated by PGs because it is not prevented by aspirin, an inhibitor of PGs cyclooxygenase.

Since some investigators have suggested that some PGs (16,16dmPGE_2) disrupt the gastric mucosal barrier and increased back-diffusion of luminal H^+ (12), we chose to titrate the luminal solution at pH 7.4 to avoid possible errors in titration of secreted acid caused by back-diffusion of luminal H^+. Although it has also been suggested that differential gassing of the luminal and nutrient solutions will overestimate H^+ secretion if titration is at 7.4 (13), the stimulatory action of PGs was still clearly evident and was of the same magnitude at a luminal pH of 4.8.

The stimulatory action of H^+ secretion seems to be a common property of PGs, because similar effects were obtained with other PG analogs. A variety of substrates including fatty acids, glucose, and metabolites of the citric acid cycle have been shown to increase H^+ secretion in frog stomach (14, 15). Since tachyphylaxis was observed in response to the PGs and because most of the substrates are effective only at concentrations of 10^{-2} *M* or higher, we feel that the stimulatory effect of the PGs acts by a mechanism different from that induced by substrates. The observed tachyphylaxis suggests that an intermediate substance, probably limited in amount, is released by the PGs. The finding that metiamide completely abolished the stimulatory action is consistent with the proposal that the stimulatory effect of the PGs might be mediated by histamine. Many substances which stimulate gastric H^+ secretion such as Ba^{2+}, tetragastrin, or reserpine have been reported to release endogenous histamine (16, 17). On the basis of our current findings we believe that PGs can be added to this list. In the frog gastric mucosa, compound 48/80 has been shown by others to decrease H^+ secretion dramatically after an initial, transient stimulation (18). These observations, together with our own, indicating a failure of the PGs to elicit an increase in H^+ secretion after treatment

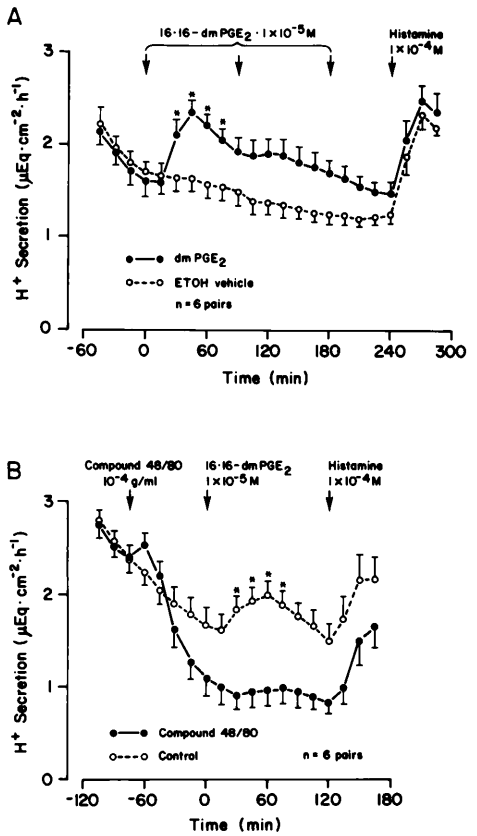

FIG. 3. (A) Effect of single and repeated application of 16,16dmPGE$_2$ (1×10^{-5} *M*) on acid secretion. Paired fundic mucosae from the same frog were used. At times indicated by the arrows, each half of the tissues was exposed to 16,16dmPGE$_2$ or its ethanol vehicle alone. At the end of the experiment, histamine (1×10^{-4} *M*) was placed in the nutrient solution of both groups. Data are expressed as the mean ± SE from six paired tissues. (B) Effect of compound 48/80 (1×10^{-4} g/ml) on stimulatory action of 16,16dmPGE$_2$ (1×10^{-5} *M*) in acid secretion. Experiments were performed in paired tissues. Half of the tissues were pretreated with compound 48/80 75 min before application of 16,16dmPGE$_2$, and the other half of the tissues received saline as the control vehicle before application of 16,16dmPGE$_2$. At the end of the experiment, histamine (1×10^{-4} *M*) was given to both groups. Data are expressed as the mean ± SE from six paired tissues.

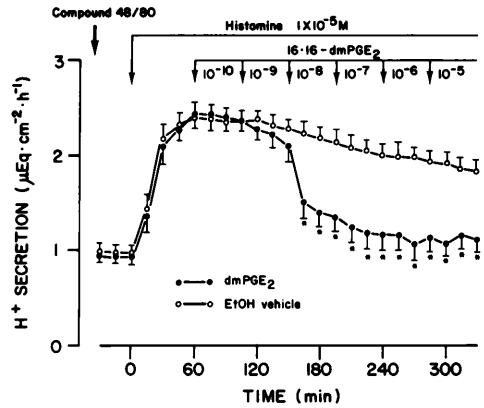

FIG. 4. Effect of 16,16dmPGE$_2$ in augmented doses (10^{-10} to 10^{-5} *M*) on histamine-stimulated H^+ secretion in compound 48/80 pretreated tissues. Paired fundic mucosae were pretreated with compound 48/80 (1×10^{-4} g/ml) 60 min before application of histamine (1×10^{-5} *M*). One-half of the tissues was given 16,16dmPGE$_2$ in augmented doses every 45 min, while the other half received the ethanol as control vehicle. Data are expressed as the mean ± SE from six paired tissues. * denotes statistical significance at $P < 0.05$.

with compound 48/80, heparin, or metiamide, are consistent with the proposal that PGs in higher doses release endogenous histamine from mast cells.

Although it has been shown that tissue damage caused by aspirin or taurocholic acid results in histamine release together with a marked decrease in *PD* and *R* (19, 20), it is unlikely that the release of histamine caused by PGs is due simply to tissue damage because there was no significant change either in *PD* or *R* in our studies. In other tissues such as mammalian lung and unfractionated rat peritoneal mast cells, pharmacological agents which increase total cAMP inhibit mediator (histamine) release, whereas those which cause a fall in cAMP enhance release (21–23). However, some agents which increase cAMP in highly purified rat peritoneal mast cell preparations, including PGs, have been reported to have varying effects on mediator release, including inhibition (22, 23), no effect (24, 25), or enhancement (26). These variable effects are possibly the result of differences in concentrations of PGs used in the reported studies, especially since opposite effects of PGs have been observed with different concentrations of PGs in platelets (27) and red blood cells (28). It is also possible that peritoneal mast cells respond differently to PGs than do those in the gastric mucosa, although most studies with peritoneal mast cells used PGs at a concentration of 1×10^{-6} *M* or less.

Our current results clearly demonstrate a dual effect of PGs on gastric acid secretion in the frog. It is likely that the stimulatory effect is mediated by the release of endogenous histamine. Under conditions in which PGs are produced in high concentrations, e.g., after injury of surface cells, the interaction of PGs and histamine would play an important role in the local response to such injury.

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