

Specific inhibition by prostaglandins E2 and I2 of histamine-stimulated [14C]aminopyrine accumulation and cyclic adenosine monophosphate generation by isolated canine parietal cells.

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Research Article

The effects of prostaglandins E2 and I2 on accumulation of [14C]aminopyrine and the generation of cyclic AMP by fractions of dispersed canine gastric mucosal cells, enriched in their content of parietal cells, have been studied. The parietal cell content of the fractions was enriched to between 43 and 70% using an elutriator rotor. The accumulation of [14C]aminopyrine was used as the index of parietal cell response to stimulation. Prostaglandin E2 (PGE2, 0.1 nM-0.1 mM) inhibited histamine stimulated aminopyrine uptake but did not block the response to carbachol, gastrin, or dibutyl cyclic AMP. PGE2 did, however, inhibit aminopyrine uptake stimulated by carbachol and gastrin when the response to these agents was potentiated by histamine. PGE2 (0.1 nM-0.1 mM) inhibited histamine-stimulated cyclic AMP production in a dose-dependent fashion with maximal inhibition at 1 microM PGE2. Prostacyclin also inhibited both histamine-stimulated aminopyrine accumulation and histamine-stimulated cyclic AMP production. In the absence of added histamine, PGE2 in concentrations above 1 microM and prostacyclin in concentrations above 10 microM stimulated cyclic AMP production, probably by acting on the nonparietal cells as shown in previous studies. These present data are consistent with the hypothesis that prostaglandins E2 and I2 inhibit the response of isolated parietal cells to histamine by specifically blocking histamine-stimulated cyclic AMP production.

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Specific Inhibition by Prostaglandins E₂ and I₂ of Histamine-stimulated [¹⁴C]Aminopyrine Accumulation and Cyclic Adenosine Monophosphate Generation by Isolated Canine Parietal Cells

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ABSTRACT The effects of prostaglandins E₂ and I₂ on accumulation of [¹⁴C]aminopyrine and the generation of cyclic AMP by fractions of dispersed canine gastric mucosal cells, enriched in their content of parietal cells, have been studied. The parietal cell content of the fractions was enriched to between 43 and 70% using an elutriator rotor. The accumulation of [¹⁴C]aminopyrine was used as the index of parietal cell response to stimulation. Prostaglandin E₂ (PGE₂, 0.1 nM–0.1 mM) inhibited histamine stimulated aminopyrine uptake but did not block the response to carbachol, gastrin, or dibutyl cyclic AMP. PGE₂ did, however, inhibit aminopyrine uptake stimulated by carbachol and gastrin when the response to these agents was potentiated by histamine. PGE₂ (0.1 nM–0.1 mM) inhibited histamine-stimulated cyclic AMP production in a dose-dependent fashion with maximal inhibition at 1 μM PGE₂.

Prostacyclin also inhibited both histamine-stimulated aminopyrine accumulation and histamine-stimulated cyclic AMP production. In the absence of added histamine, PGE₂ in concentrations above 1 μM and prostacyclin in concentrations above 10 μM stimulated cyclic AMP production, probably by acting on the nonparietal cells as shown in previous studies. These present data are consistent with the hypothesis that prostaglandins E₂ and I₂ inhibit the response of isolated parietal cells to histamine by specifically blocking histamine-stimulated cyclic AMP production.

INTRODUCTION

Several members of the prostaglandin family are synthesized by gastric mucosa (2, 3) and when given exogenously are capable of protecting gastric mucosa from ulcer and erosion formation (4–6). Prostaglandins are potent inhibitors of acid secretion, inhibiting stimulation by histamine, gastrin, cholinergic stimuli, and food (2, 4, 7–11). Prostacyclin (PGI₂),¹ a newly identified metabolite of the prostaglandin precursor arachidonic acid, is formed by gastric mucosa (3) and, like the E and A group prostaglandins, is also a potent inhibitor of gastric acid secretion (12–14). Prostaglandins, however, prevent ulcer formation by mechanisms in addition to inhibition of acid secretion, a property of prostaglandins that Robert (5) has termed “cytoprotection.” A few of the proposed mechanisms that may account for cytoprotection are increased mucosal blood flow (15), enhanced mucus production, bicarbonate secretion presumably by surface epithelial cells (16), and possibly alterations in sodium flux (cf. 5). The present study, however, concerns the mechanisms involved in the inhibition of parietal cell function by prostaglandins E₂ and I₂.

In previous studies, histamine, gastrin, cholinergic agents, and cyclic AMP analogues were found to increase both oxygen consumption (17) and [¹⁴C]aminopyrine accumulation (18) by parietal cells isolated from canine fundic mucosa. Stimulation by histamine, but not by carbachol or gastrin, was found to be

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¹ *Abbreviations used in this paper:* AP, [¹⁴C]aminopyrine; APAR_b, [¹⁴C]aminopyrine accumulation ratio, with basal uptake subtracted; dbcAMP, dibutyl cyclic AMP; ID₅₀, the concentration of inhibitor producing 50% inhibition; IMX, 3-isobutyl, 1-methyl xanthine; PGE₂, prostaglandin E₂; PGI₂, prostaglandin I₂ (prostacyclin).

accompanied by increased parietal cell generation of cyclic AMP (19). Prostaglandin E₂ (PGE₂), which is known to stimulate cyclic AMP production by gastric mucosa (20, 21), was found to increase cyclic AMP production predominantly in nonparietal cells, with at most a very small effect on parietal cells (22). These observations with isolated mucosal cells did not resolve the question of the relation, if any, between the effects of PGE₂ on cyclic AMP production and inhibition of acid secretion by PGE₂. In the present study, the effects of PGE₂ on parietal cell aminopyrine accumulation and cyclic AMP production have been examined during stimulation by a variety of agents alone and in combinations. Because PGI₂ may be the most abundant prostaglandin produced by gastric mucosa, its effects on histamine-stimulated parietal cell activity and cyclic AMP formation have also been examined.

METHODS

Preparation of cells and determination of [¹⁴C]aminopyrine (AP) uptake. Cells were isolated from canine fundic mucosa that had been completely separated from submucosa and then sequentially treated with crude collagenase and EDTA. These methods and the methods for determining AP uptake have been described in detail previously (17, 18). The elutriator rotor, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif. (17, 22) was used to prepare fractions enriched in parietal cell content (43–70%); these fractions were used for all of the subsequent studies.

Stimulants and the prostaglandins were added simultaneously to the cell suspension, which was kept at 4°C until incubated with AP at 37°C for 20 min. In each cell preparation, the AP accumulation was determined on triplicate samples of one or two incubations for each treatment condition. AP accumulation was calculated as the ratio of the AP concentration in the parietal cell to that in the medium. After subtracting the basal accumulation ratio, each value was normalized by expressing it as the percentage of the accumulation ratio produced by a given concentration of the stimulant(s). The AP accumulation ratio to which each individual set of values was normalized is given in the appropriate figure legend.

Determination of cyclic AMP content. Cyclic AMP content was measured in cells that had been incubated for 15 min in 4 ml of the same Earles' balanced salt solution used for the AP studies (18), at which time the test agents were added simultaneously in a volume of <150 μ l. The phosphodiesterase inhibitor 3-isobutyl, 1-methyl xanthine (IMX) was added to all of the cyclic AMP experiments to retard degradation of cyclic AMP. The incubation was terminated by transferring 3.5 ml of the cell suspension to a chilled tube containing trichloroacetic acid (final concentration, 5%), which was then frozen at -20°C. Upon thawing, [³H]cyclic AMP marker was added to each tube for determination of recovery and the suspension was centrifuged for 15 min at 3,000 *g*. The supernate was extracted four times with 5 ml of water-saturated ether. The water phase was then dried under a stream of air in a water bath at 65°C and the residue resuspended in 1 ml of sodium acetate buffer (0.05 M, pH 6.2). Cyclic AMP content was then determined using the radioimmunoassay technique of Steiner et al. (23) as modified using acetylated cyclic AMP by Harper and Booker (24). The applications of these methods have been described elsewhere (22). In previous studies (22), <15% of

the total cyclic AMP content, either with IMX alone (0.1 mM) or stimulated by histamine (10 μ M) or PGE₂ (0.1 mM) plus IMX, was present in the supernate, and therefore the content of cyclic AMP in the present study has been determined on the entire cell suspension.

Cyclic AMP content was determined in triplicate for the one or two incubations for each treatment condition. The mean of these values was corrected for recovery and then expressed as the percentage of the response to a given concentration of stimulant. The amounts of cyclic AMP generated in picomoles per 10⁶ cells per 5 min incubation for these given response points to which other values are normalized, are stated in the figure legends.

Statistical analyses. The statistical significance of differences was evaluated using Student's *t* test, with *n* equal to the number of cell preparations. Standard errors are depicted in the figures and given after the \pm sign in the text and figure legends.

Materials

AP (15.6 μ Ci/ μ mol) and the materials used in the assay of cyclic AMP were purchased from New England Nuclear, Boston, Mass. AP was stored in ethanol in a light-protected container at -20°C. Crude collagenase I was purchased from Sigma Chemical Co., St. Louis, Mo. PGI₂ was the gift of Dr. Brendan J. R. Whittle (Wellcome Research Laboratories, Beckenham, England) and PGE₂ was the gift of Dr. J. E. Pike and Dr. W. Bremer (Upjohn Co., Kalamazoo, Mich.). Other materials were obtained from sources listed in the indicated references.

PGI₂, as the sodium salt (25) was dissolved (10 mg/ml) in 1 M freshly prepared Tris buffer (pH 9.6) and stored for <2 h on ice; dilutions were made immediately before use in ice cold Earles' medium (pH 7.6). The highest Tris concentration added was 3.5 μ M (final) and at this concentration, parietal cell functional responses were not altered. PGE₂ was stored in ethanol (28 mg/ml, 80 mM) at -20°C, and diluted before use in Earles' medium. The highest concentration of ethanol delivered to a cell suspension was thus 0.13%. This concentration of ethanol did not alter either secretagogue stimulation of AP accumulation or the effects of PGE₂ or histamine on cyclic AMP production. However, ethanol at 1.3% not only impaired secretagogue stimulation of AP accumulation, but also blocked prostaglandin inhibition of histamine-stimulated cyclic AMP production.

RESULTS

AP accumulation

PGE₂ inhibition of histamine and IMX. PGE₂ inhibited histamine (10 μ M) stimulation of AP accumulation in a dose-dependent fashion, with 1 μ M PGE₂ causing 91% inhibition of the response (Fig. 1A). PGE₂ also inhibited the response to 1 μ M histamine with a 10- μ M IMX background (Fig. 1B) and the response to 100 μ M IMX as the sole stimulant (Fig. 1C). The concentration of PGE₂ producing 50% inhibition of the response to histamine and IMX in each of these instances was visually estimated at between 1 and 10 nM. PGE₂ inhibited the response of canine parietal cells to histamine concentrations from 1 to 100 μ M (Fig. 2).

Failure of PGE₂ to inhibit the response to dibutyryl

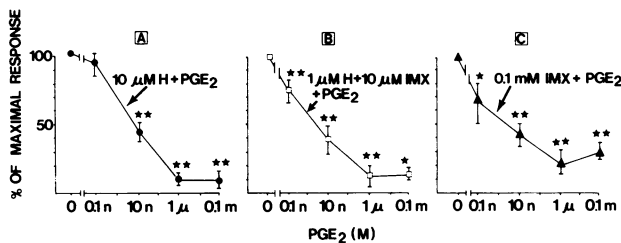


FIGURE 1 The effect of PGE₂ on histamine and IMX stimulation of AP accumulation. (A) PGE₂ inhibition of the response to 10 μ M histamine (H) was tested in nine preparations; the data were normalized to the response produced by 10 μ M histamine AP accumulation ratio with basal uptake subtracted ($APAR_b = 11.1 \pm 6.6$). (B) The effect of PGE₂ on the response to histamine (1 μ M) plus IMX (10 μ M) was tested in six preparations, with the data normalized to the response to this combination of agents in the absence of PGE₂ ($APAR_b = 32.9 \pm 10.1$). (C) The effect of PGE₂ on the response to 0.1 mM IMX was tested in five preparations; the data were normalized as a percentage of the response to this concentration of IMX alone ($APAR_b = 31.2 \pm 12.1$). ☆, $P < 0.05$; ☆☆, $P < 0.005$ vs. uninhibited response.

cyclic AMP (dbcAMP), carbachol, and gastrin. PGE₂ (1 μ M) failed to alter the dose-response relation for dbcAMP stimulation of AP accumulation (Fig. 3). In separate experiments PGE₂ in concentrations from 10 nM to 100 μ M, failed to inhibit the response to 0.33 mM dbcAMP ($n = 6$, $P > 0.2$, data not illustrated). PGE₂ (1 μ M) did not alter the dose-response relation for carbachol stimulation of AP accumulation (Fig. 4A), nor did PGE₂ between concentrations of 10 nM and 0.1 mM inhibit the response to 0.1 mM carbachol ($n = 6$, $P > 0.2$, data not illustrated). In addition, PGE₂ did not appear to inhibit the very small response to 0.1 μ M gastrin (Fig. 5A). PGE₂ did not significantly change basal AP accumulation; the accumulation ratio was 1.55 ± 0.38 for cells treated with 100 μ M PGE₂ compared with a value of 2.33 ± 0.62 ($n = 5$, $P > 0.2$) for control cells.

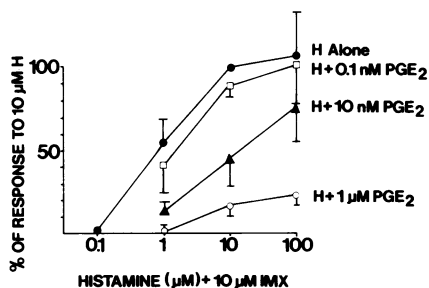


FIGURE 2 The effect of PGE₂ on the dose response for stimulation of AP uptake by histamine (H) in the presence of 10 μ M IMX. The dose-response relationship for histamine-stimulated AP accumulation was tested without PGE₂ and with three concentrations of PGE₂. The data from four preparations were normalized to the response produced by 10 μ M IMX ($APAR_b = 60.5 \pm 32.0$).

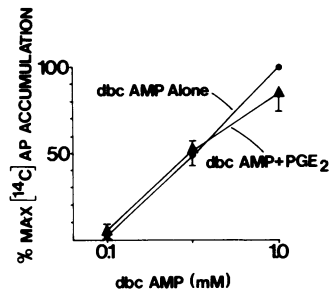


FIGURE 3 The effect of PGE₂ on dbcAMP stimulation of AP accumulation. Data for the effect of PGE₂ on the dose response for dbcAMP stimulation of AP uptake were expressed as a percentage of the response to 1 mM dbcAMP ($APAR_b = 71.1 \pm 21.4$). Four preparations were tested.

Effects of PGE₂ on AP accumulation during secretagogue interactions. When the AP accumulation in response to carbachol was potentiated by interaction with histamine, then PGE₂ did inhibit this combined response; at the highest concentration of PGE₂ (0.1 μ M), the residual effect was similar to that found with carbachol alone (Fig. 4B). Similarly, when gastrin action was potentiated by interaction with histamine, PGE₂ also inhibited the response to this combination, and at the highest concentration of PGE₂ (0.1 μ M), the residual response was equivalent to that found with gastrin alone (Fig. 5C). In contrast, when the response to gastrin was potentiated by interaction with dbcAMP, not only did PGE₂ fail to inhibit stimulation by this combination, but the highest concentration of PGE₂ tested (0.1 mM) significantly enhanced the response ($P < 0.05$, Fig. 5B). The ID₅₀ for PGE₂ inhibition of the

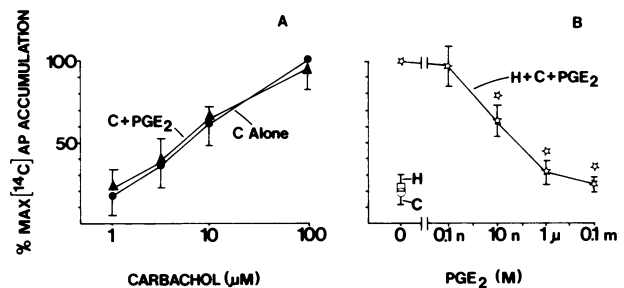


FIGURE 4 The effect of PGE₂ on carbachol stimulation of AP accumulation. (A) The effect of PGE₂ on the dose response for carbachol (C) stimulation of AP accumulation was tested in four preparations. The data are expressed as a percentage of the response to this concentration of carbachol alone ($APAR_b = 43.1 \pm 18.5$). (B) The effect of PGE₂ upon the response to the combination of 1 μ M carbachol plus 10 μ M histamine (H) was expressed as a percentage of the response to this histamine-carbachol combination alone. The data are from four preparations ($APAR_b = 24.6 \pm 5.6$) with the statistical significance of the differences from the uninhibited response indicated by ☆ ($P < 0.005$). The response to the combination of histamine plus carbachol was greater than the sum of the individual responses ($P < 0.05$), which are also depicted in the figure.

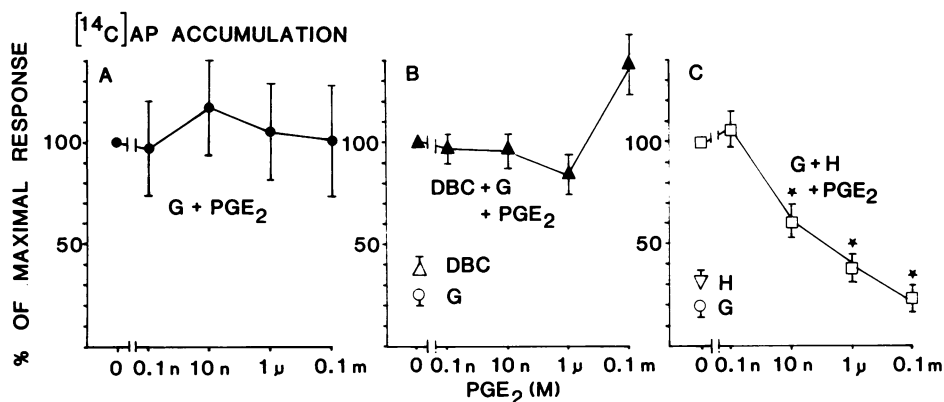


FIGURE 5 The effect of PGE₂ on gastrin (G) stimulation of AP accumulation. (A) The effect of PGE₂ upon the response to 0.1 μM gastrin was tested in six preparations of cells, with the data expressed as a percentage of the response to this concentration of gastrin alone (APAR₀ = 2.0 ± 0.5). (B) The effect of PGE₂ upon the response to 0.1 mM dbcAMP plus 0.1 mM gastrin was tested in six preparations. The data were normalized to the response produced by this dbcAMP-gastrin combination alone (APAR₀ = 11.2 ± 4.6). The response to dbcAMP plus gastrin was significantly increased by adding 0.1 mM PGE₂ ($P < 0.05$). The responses to the combination of dbcAMP and gastrin produced a response that was greater than the sum of the individual responses ($P < 0.05$), which are also shown. (C) The data for PGE₂ inhibition of the response to 10 μM histamine (H) plus 0.1 μM gastrin were normalized to the response produced by this histamine-gastrin combination alone (APAR₀ = 11.2 ± 1.3, $n = 4$). Statistical significance of the difference from the uninhibited response is indicated by ★ ($P < 0.005$). The histamine plus gastrin combination produced a response that was greater than the sum of the individual responses ($P < 0.05$), which are also shown.

histamine-potentiated responses to carbachol and gastrin was visually estimated at 10 nM.

Effect of PGI₂ on AP accumulation. Incubation of parietal cells with PGI₂ produced a dose-related inhibition of histamine-stimulated AP accumulation (Fig. 6A).

Cyclic AMP Production

PGE₂ inhibition of histamine-stimulated cyclic AMP production. PGE₂ in concentrations between 0.1 nM and 1 μM inhibited stimulation of cyclic AMP production by 10 μM histamine plus 0.1 mM IMX (Fig. 7). Maximal inhibition was found with 1 μM PGE₂ and at 0.1 mM less inhibition occurred (Fig. 7). This lessened inhibition occurred in a concentration range that corresponded to the region in which PGE₂ acting alone stimulated cyclic AMP production (Fig. 7). In the presence of 100 μM IMX, PGE₂ inhibited cyclic AMP production in response to histamine concentrations from 1 to 100 μM (Fig. 8).

PGI₂ inhibition of histamine-stimulated cyclic AMP production. Treatment of parietal cells with PGI₂, in concentrations similar to those that inhibited histamine-stimulated AP accumulation, also produced a dose-related fall in histamine-elevated cyclic AMP levels (Fig. 6B). In the absence of histamine, PGI₂ produced elevation of cyclic AMP levels but these effects were only found at concentrations of 10 μM and higher (Fig. 6B).

DISCUSSION

In the present study, PGE₂ inhibited histamine-stimulated AP accumulation by isolated canine fundic mucosal cells. In contrast, PGE₂ failed to inhibit stimulation of AP uptake by carbachol, or the cyclic AMP analogue dbcAMP. However, when carbachol stimulation of AP accumulation was enhanced by potentiating interaction with histamine, then PGE₂ did inhibit the combined response. PGE₂ did not appear to block the very small effects of gastrin on AP accumulation; PGE₂ also failed to decrease the response to gastrin when potentiated by dbcAMP, providing further indication that prostaglandins do not directly inhibit gastrin effects on the parietal cell. In contrast, when gastrin action was potentiated by histamine, then PGE₂ caused marked inhibition. Thus, among the stimulants tested, PGE₂ appeared to block specifically the effects of histamine on the accumulation of AP. The present studies were done on fractions enriched to a parietal cell content of ~50%. It is likely that the effects of PGE₂ on histamine-stimulated AP accumulation and cyclic AMP production are exerted directly on the parietal cells in these fractions. This conclusion is based upon previous cell separation studies in which histamine stimulation of AP accumulation (18) and cyclic AMP production (22) correlated closely with the parietal cell content of the fractions examined.

PGE₂ in concentrations of 0.1 nM and 100 μM

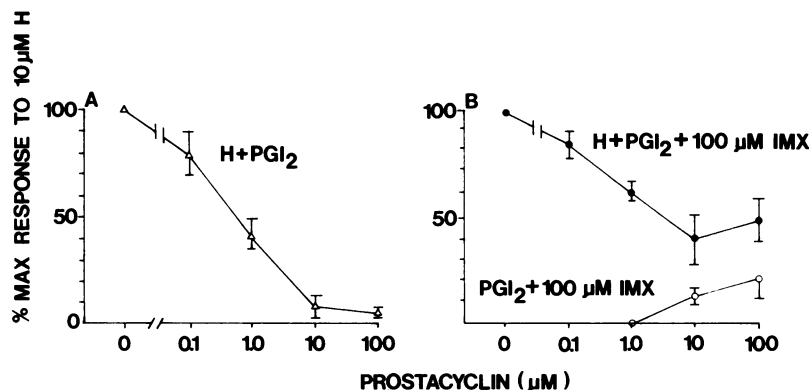


FIGURE 6 PGI₂ inhibition of histamine-stimulated parietal cell function. (A) The effects of PGI₂ on AP accumulation stimulated by 10 μ M histamine (H) were tested. Data are normalized as the percentage of the response to 10 μ M histamine and are from four preparations of cells in which histamine (10 μ M) produced an APAR₀ of 8.1 ± 2.5 . (B) The effect of PGI₂ on cyclic AMP production stimulated by 10 μ M histamine plus 100 μ M IMX was tested. Data are normalized as the percentage of the response over 100 μ M IMX produced by 10 μ M histamine plus 100 μ M IMX. Data are from four preparations of cells in which cyclic AMP formation in the presence of 100 μ M IMX was 3.7 ± 0.6 pmol/ 10^6 cells and in which addition of histamine (10 μ M) produced additional cyclic AMP formation of 11.7 ± 1.1 pmol/ 10^6 cells.

inhibited histamine stimulation of cyclic AMP production by isolated parietal cells in a dose-dependent fashion. Thus PGE₂ appeared to inhibit histamine activation of adenylate cyclase and this effect could be

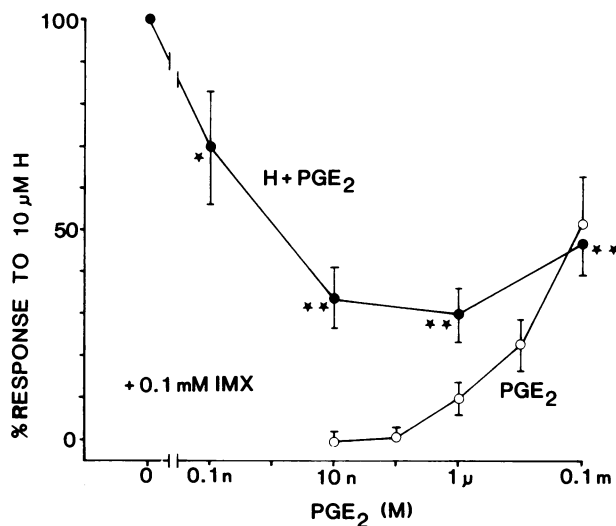


FIGURE 7 The effect of PGE₂ on histamine-stimulated cyclic AMP production. PGE₂ effects on cyclic AMP production stimulated by 10 μ M histamine were tested in five preparations of enriched parietal cells as were the effects of PGE₂ alone. IMX (0.1 mM) was present in all incubations. The data were normalized to the response above the 0.1 mM IMX background (2.7 ± 0.5 pmol cyclic AMP/ 10^6 cells) produced by 10 μ M histamine (13.5 ± 3.0 pmol cyclic AMP/ 10^6 cells). ★, $P < 0.05$; ★★, $P < 0.005$ vs. histamine. Stimulation of cyclic AMP by PGE₂ plus IMX (0.1 mM) was significantly greater than the response to IMX alone at concentrations of PGE₂ > 1 μ M ($P < 0.01$).

the mechanism by which PGE₂ inhibits AP uptake. The hypothesis would account for the failure of PGE₂ to directly inhibit carbachol and gastrin stimulation of AP uptake because previous findings showed that the action of these latter two agents are dependent upon the presence of extracellular calcium (26) rather than upon enhanced cyclic AMP generation (19).

Major and Scholes (27) have also demonstrated in parietal cells isolated from canine mucosa that prostaglandins inhibit histamine-stimulated cyclic AMP production. PGE₂ caused about 80% inhibition of the histamine effect with the ID₅₀ of ~ 10 nM; similar to findings in present study.

PGI₂ was also capable of inhibiting both histamine-stimulated AP accumulation and histamine-stimulated cyclic AMP production by parietal cells. The true

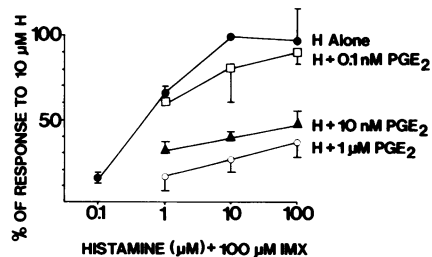


FIGURE 8 The effect of PGE₂ on the dose response for stimulation of cyclic AMP production by histamine. The effect of three concentrations of PGE₂ on histamine stimulation of cyclic AMP production was tested in three preparations. All incubations were in the presence of 0.1 mM IMX. The data were normalized to the response produced by 10 μ M histamine (15.5 ± 3.9 pmol cyclic AMP/ 10^6 cells) after subtracting the response to IMX alone (2.7 ± 0.3 pmol cyclic AMP/ 10^6 cells).

potency of PGI_2 is difficult to assess because of the very rapid breakdown to the far less active product 6-oxo-PGF $_{1\alpha}$ (25). Thus estimates of potency in a given system depend upon the mode of administration and the propensity for decomposition. Probably as a result of rapid decomposition in the present system, PGI_2 was found to be much less potent in relation to PGE_2 than had been found when antisecretory activity was tested in the dog (13) and the rat (12) using continuous intravenous infusion to compensate for rapid turnover. The present studies thus allow one to conclude that PGI_2 inhibits histamine-stimulated parietal cell function and histamine-stimulated cyclic AMP production, but the comparative potency of PGI_2 and PGE_2 will require further elucidation.

Prostaglandin actions on adenylate cyclase in gastric mucosa are complex, in that prostaglandins can both stimulate and inhibit cyclic AMP production. The present study and the work of Major and Scholes (27) indicate that the inhibitory effect of prostaglandins on histamine-activation of adenylate cyclase occur at low concentrations of prostaglandins and may represent the physiologic mechanism by which prostaglandins inhibit acid secretion. On the other hand, it appears unlikely that the stimulatory effects of prostaglandins on adenylate cyclase are relevant to parietal cell function. In the present study, both PGE_2 and PGI_2 , acting in the absence of histamine, stimulated cyclic AMP production. However, in accord with the findings in intact mucosa (20, 21, 28), stimulation of cyclic AMP production was only found at concentrations of the prostaglandins $>1 \mu\text{M}$ and such high concentrations are unlikely to occur in vivo even under extreme conditions (29). Furthermore, PGE_2 appears to stimulate cyclic AMP production primarily in mucosal cells other than parietal cells themselves. Data supporting this latter point come from cell separation studies (22) in which an inverse correlation was found between PGE_2 -stimulated cyclic AMP production and the parietal cell content of the separated cell fractions. If high concentrations of PGE_2 also stimulated cyclic AMP production in parietal cells, one might expect these same high concentrations to stimulate AP accumulation, for the reason that parietal cell function does appear to respond to cyclic AMP (18, 19). However, even high concentrations of prostaglandins have at most a minor stimulatory effect on parietal cell adenylate cyclase (22), which is not accompanied by a detectable increase in AP accumulation. As an aside, it is of course possible that certain of the complex actions of prostaglandins on gastric mucosa are mediated by activation of adenylate cyclase in a specific cell type that constitutes only a small fraction of all the mucosal cells, but that the present techniques are not sufficiently sensitive to detect such changes.

The ability of prostaglandins to both stimulate and

inhibit cyclic AMP production in a given tissue has been described previously. Butcher and Baird (30) reported that PGE_1 inhibition of epinephrine-stimulated lipolysis in fat tissue was paralleled by inhibition of epinephrine-stimulated cyclic AMP production. These effects of PGE_1 were found in a low concentration range ($\text{ID}_{50} = 1 \text{ nM}$). However, at concentrations $>1 \mu\text{M}$, PGE_1 also increased cyclic AMP production on its own. Although the inhibition of epinephrine action was also found in isolated fat cells, prostaglandin directly stimulated cyclic AMP production only in intact fat pads and not in the isolated adipocytes, indicating that this effect was probably on another cell type. The parallel of these findings to prostaglandin action on histamine-stimulated parietal cell function is striking. There are other examples in the literature of inhibition of hormone-stimulated cyclic AMP function by prostaglandins (31, 32). However, in certain cell types, such as platelets (33, 34) and heart tissue (35), prostaglandins in concentrations in the nanomolar range may also stimulate adenylate cyclase, whereas in other cell types, this action is only found at 1,000-fold higher concentrations (36, 37). Thus, depending on the cell type and the concentration tested, the effect of prostaglandins on adenylate cyclase may be stimulatory or inhibitory.

The mechanism of inhibition of acid secretion by prostaglandin has been perplexing because in vivo its effects are not specific in that stimulation by histamine, pentagastrin, vagal pathways, and food are all inhibited (2, 4, 7–11). There are, however, in vitro studies that indicate that prostaglandin action may be specific for histamine. While studying frog mucosa, Way and Durbin (38) found that PGE_1 inhibited histamine-stimulated acid secretion but failed to inhibit the response to exogenous cyclic AMP. They therefore proposed that prostaglandin inhibition was specific for histamine, blocking activation of adenylate cyclase. Similarly, Main and Whittle (39) found in rat gastric mucosa that PGE_1 and PGE_2 inhibited the response to histamine but not the small stimulation by dbcAMP alone. Furthermore, Glick (40) demonstrated that PGE_1 inhibited histamine- but not carbachol-stimulated chloride transport by polarized isolated parietal cells. The view that prostaglandins inhibit histamine-stimulated cyclic AMP production was not widely accepted both because the role of cyclic AMP as a mediator of histamine action on the parietal cell was highly disputed (41), and because prostaglandins were shown to stimulate, not inhibit adenylate cyclase in gastric mucosa (20, 21). However, this latter effect of prostaglandin occurs only with micromolar concentrations of prostaglandins both in intact mucosa and with isolated cells. The present findings with lower concentrations of prostaglandins and using isolated parietal cells confirmed the earlier hypothesis (38) that prostaglandins

inhibit both histamine-stimulated parietal cell function and cyclic AMP generation. These present data, therefore, also support the view that histamine action on the parietal cell is closely linked to stimulation of cyclic AMP generation. Prostaglandin inhibition of histamine-stimulated adenylate cyclase may be masked in intact mucosa because histamine-stimulated cyclic AMP generation in parietal cells may be only a small portion of total cyclic AMP content, whereas prostaglandins cause prominent stimulation of adenylate cyclase primarily in nonparietal cells. The use of lower prostaglandin concentrations may be helpful by reducing direct stimulation of adenylate cyclase. Furthermore, because PGE₂ inhibition of histamine action is surmountable in the intact parietal cell,² these effects may not be clearly evident against maximal histamine-stimulated activity.

In vivo, both histamine H₂ receptor antagonists and prostaglandins inhibit all forms of acid secretion. However, in the isolated parietal cell, both of these inhibitors are specific against histamine, failing to block the action of cholinergic agents or gastrin. This apparent discrepancy between the in vivo and in vitro observations may however be explained by assuming that the parietal cell in the basal state in vivo is exposed to endogenous histamine in amounts sufficient to support potentiating interactions between stimulants. By isolating the parietal cell, this endogenous histamine background is largely removed, thereby allowing potentiation to be demonstrated when stimulants are added back in combination. When the actions of gastrin or carbachol are potentiated by interaction with histamine, then both prostaglandins and H₂-blockers (42) display an apparent nonspecificity, reminiscent of that found in vivo and presumably resulting from specific inhibition of the histamine component of these interactions.

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² The present data does not clearly show that PGE₂ inhibition of histamine action is surmountable at higher concentrations of histamine. However in parietal cells prepared in a similar manner from rabbit fundic mucosa, PGE₂ (1.0 μM) inhibition of histamine-stimulated AP accumulation was fully surmounted by concentrations of histamine between 10 and 100 μM (unpublished observations). These data indicate that the effects of prostaglandins need to be examined over the full dose response curves for both histamine and the prostaglandins. Furthermore, the differences found between canine and rabbit parietal cells indicate that the quantitative aspects of prostaglandin action may vary among the species.

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