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# ROLE OF PROSTAGLANDIN IN THE REGULATION OF GASTRIC $H^+$ - TRANSPORTING SYSTEM

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## ABSTRACT

**Prostaglandins and (PG) have been reported to be an important gastric acid suppressive factor. However, the mechanism underlying is yet to be clearly established. In vitro study with gastric microsomes in presence of both $PGE_2$ and $PGI_2$ shows a stimulation of gastric $H^+$ $K^+$ - ATPase activity below $1X10^{-6}$ M and $2.5X10^{-7}$ M concentrations repectively. However, with further increase in concentrations of both $PGE_2$ and $PGI_2$ , $H^+$, $K^+$ -ATPase activity shows an inhibition but $PGI_2$ completely obliterates the $K^+$ stimulated part of $H^+$, $K^+$ -ATPase activity at higher concentration. The $H^+$ ion transport study using chambered frog gastric mucosa shows that both $PGE_2$ and $PGI_2$ inhibit $H^+$ -ion transport at $5X10^{-6}$ M and $10X10^{-6}$M concentrations respectively but the effect of $PGI_2$ is reversible. These differential effects of $PGE_2$ and $PGI_2$ on microsomal $H^+$, $K^+$ -ATPase and on $H^+$ transport my be caused by the differential effects of these phospholipid mediators with the gastric mucosal cell membrane. This in vitro investigation shows the role of prostaglandin (s) as a physiological switch/regulator of gastric $H^+$ ion transport leading to the cessation of gastric acid secretion.**

KEY WORDS: Prostaglandin $E_2$, Prostaglandin $I_2$, Lipid mediators, $H^+$, $K^+$ -ATPase, $H^+$transport.

## INTRODUCTION

Prostaglandin(s) (PG) actively synthesised by the gastric mucosa of many species including human are shown to alter gastric function. Gastric mucosal protection and the role of prostaglandins are now well defined. Prostaglandin protects against gastric mucosal damage by various agents e.g. aspirin, indomethacin, ethanol etc. The postulated mechanism for the cytoprotective effect of prostaglandin, includes stimulation of (a) mucus production, (b) active gastric alkalistation and (c) active sodium and chloride ion transport (1-4). Apart from gastric cytoprotective activity, naturally occuring prostaglandins of the E-Series are potent inhibitors of gastric acid secretion in man. Soll and Whittle (5) reported an interaction of prostaglandins and intracellular C-AMP content and its regulation in gastric acid and protection. Sarosiek, et. al. (6) have shown that removal of associated lipids from partially purified gastric mucus increased its permeability to hydrogen ion in vitro. Ray and Nandi (7) have also demonstrated the role of membrane bound phospholipids in $H^+$, $K^+$ -ATPase activity. However, the exact role of prostaglandin(s), the lipid mediators in gastric acid regulation and their interaction with membrane bound phospholipid is not yet understood. The results of this investigation on the regulation of gastric $H^+$-transport in presence of prostaglandin(s) in vitro are communicated here.

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## MATERIALS AND METHODS

### Materials

Frogs (*Rana tigrina*) were purchased from local market. Fresh stomach for gastric microsomes preparation were collected from rabbits, weighing 1 - 1.2 kg. and reared on a balanced laboratory diet. β-mercapto-ethanol and n-butyl acetate were purchased from E.Merck, Germany; histamine hydrochloride, adenosine 5'-triphosphate-Na-Salt, tris (hydroxymethyl) aminomethane, pipes (1,4-piperazineethane sulphonic acid), EDTA (ethylenediamine tetra acetic acid), prostaglandin $E_2$ and prostaglandin $I_2$ - sodium salt were purchased from Sigma Chemicals (St.Louis, U.S.A.). All other chemicals were reagent grade.

## Methods

### Isolation of gastric microsomes (enriched with $H^+$, $K^+$ -ATPase)

Fresh rabbit stomachs were used for this purpose. The gastric microsomal membranes were harvested as described by Ray (8). Briefly, the fundic mucosa of rabbit was desquamated and scraped to collect the oxyntic cell enriched fractions. The mucosal cells were homogenised in 0.25 M sucrose containing 0.2 mM EDTA and 2 mM pipes buffer (pH 6.8) using Dounce homogeniser. The homogenate was centrifuged at 8000g for 5 min. Supernatent was layered over 13 ml of 37% (W/V) sucrose in 25 ml screw cap tubes and centrifuged at 100,000 g for 4 hr 30 mins in a A1-S41 angle rotor of Sorvall ultracentrifuge OTD-50B. The microsomal membrane band appeared at the interface of the clear soluble supernatant and 37% sucrose. The membrane bands were collected, diluted with the same buffer and centrifuged at 100,000g for 90 min. The pellet was resuspended in homogenising buffer at an appropriate protein concentration and used for the study. Protein was assayed by the method of Lowry et. al. (9). All procedures were carried out at 0-4°C.

### $H^+$, $K^+$ -ATPase

This was assayed according to the method of Bandyopadhyay, et al. (10). Briefly, the incubation mixture contained, in a total volume of 1 ml, Tris-HCl buffer (pH-6.8) 50mM, Mg.acetate 5mM, membrane 10µg in the presence or absence of KCl 5mM. After preincubation for 10 min at 37°C, the reaction was started with 2mM ATP-Tris (pH-6.8) and incubated for 15 min. The reaction was stopped by adding 1 ml of 15% (W/V) TCA. $P_i$ was assayed by the procedure of Sanui(11).

### Transport studies with chambered frog gastric muscosa

These were done according to the method followed by Ray and Tague (12), where all experiments were carried out with gastric mucosa from frog, *Rana tigrina*. Briefly, the frogs were pitched and the stomachs were removed, opened along the lesser curvature. The mucosa was stripped from the submucosa (underlying external musclaris layers) and mounted over one end of a plastic tube (13 x 100 mm) with the mucosal surface facing out. The area of the mounted mucosa was 1.3 $cm^2$. The bathing solutions were bubbled continuously with 95% $O_2$, 5% $CO_2$ at room temperature. The solution in the nutrient side (N) contained the following (in mM): NaCl, 87; KCl, 4; $CaCl_2$,1; $KH_2PO_4$,1; $NaHCO_3$, 18; and glucose 11. The luminal (mucosal/secretory) side (S) contained 104 mM NaCl. Mucosal solutions were collected at 15 min intervals and placed in thoroughly washed plastic vials and pH of each sample was noted. $H^+$ -ion concentration in secretion was measured and expressed in terms of umole/hr. The unpaired student's test was used to compare secretory data obtained in the test condition with initial steady state value and the differences were regarded as significant when P was less than 0.05.

## RESULTS

### Effect of prostaglandin $E_2$ and prostaglandin $I_2$ on $H^+$, $K^+$ -ATPase activity

Fig.1 shows the effect of prostagladin(s) on $H^+$, $K^+$, -ATPase activity. With increase in concentration, $PGE_2$ stimulates $H^+$, $K^+$ -ATPase activity, reaches a maximum at 1 x $10^{-6}$M and thereafter the ATPase activity rapidly decreases

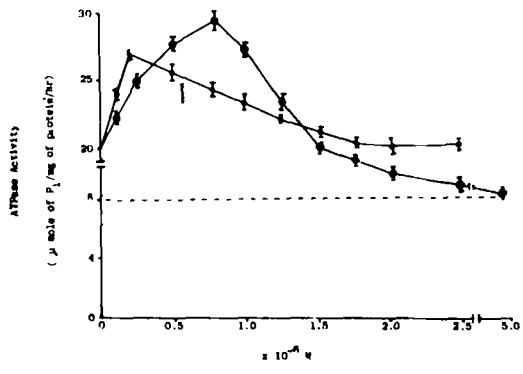



Figure : : Effects of prostaglandin(s) on the gastric microsomal ATPase activity. ATPase activity in presence of $Mg^{2+}$ alone (- - - -), $Mg^{2+}$ plus 5 mM K ( —— —— —— —— for $PGE_2$ and —— —— —— for $PGI_2$). Data are means ± SEM (n=7).

and reaches a plataue inhibiting only 15% of the $K^+$ stimulated part of $H^+$, $K^+$ -ATPase. Similarly $PGI_2$ stimulates the $H^+$, $K^+$ -ATPase activity but at much lower concentration and reaches a peak at 2.5 x $10^{-7}$M. Moreover, with further increase of $PGI_2$ concentration, the $K^+$ -stimulated part of the $H^+$, $K^+$ -ATPase is completely obliterated.

### Effect of $PGE_2$ and $PGI_2$ on gastric $H^+$-ion transport by chambered frog gastric mucosa

Different concentrations of $PGE_2$ and $PGI_2$ added to the nutrient solution of histamine stimulated chambered frog gastric mucosa inhibit $H^+$-ion transport in a dose dependent way. $PGE_2$ inhibits the $H^+$ -ion transport to the extent of 100% at 5 x $10^{-6}$M concentration and the effect is found to be irreversible (Fig 2). However, $PGI_2$ inhibits the $H^+$ -ion transport at a concentration of 10 x $10^{-6}$M but the effect is reversible in nature (Fig 3).

## DISCUSSION

Effect of prostaglandins on gastrointestinal secretion, blood flow and motility are quite well characterised (14,15). However the mechanisms underlying the cytoprotective actions and the regulation of acid secretion of this group of lipid mediators are yet to be clearly established. Our studies indicate that prostaglandin $E_2$ and prostaglandin $I_2$ regulate gastric acid secretion possibly interacting with the membrane bound phospholipids in vew of the report of Lichtengerger et.al. (16) that administration of $PGE_2$ may influence mucosal concentration of phospholipids. Both $PGE_2$ and $PGI_2$ at lower concentrations stimulate $H^+$, $K^+$ -ATPase activity. At higher concentrations they inhibit but at different rate and degree. This differential phenomenon of $PGE_2$ and $PGI_2$ might be due to their different permeability through the phospholipid bilayer of gastric microsomal, $H^+$, $K^+$ -ATPase vesicle. The activity and stability of the gastric $K^+$ stimulated ATPase depend on the unique orientation and nature of the microsomal phospholipid environment (17) and prostaglandins, the phospholipid mediators might have a significant role in gastric $H^+$, $K^+$ -ATPase activity. The differential permeability of prostaglandins and the role of phospholipid bilayer of plasma membrane are further confirmed by our $H^+$ -ion transport study. The inhibitory effect of $H^+$ -ion transport in presence of prostaglandin $I_2$ is found to be reversible on withdrawing from the medium whereas the effect of $PGE_2$ is irreversible. However, the immediate effect of $PGI_2$ is somewhat different from $PGE_2$. It is now postulated that addition of prostaglandins from nutrient side of gastric frog

Figure 2

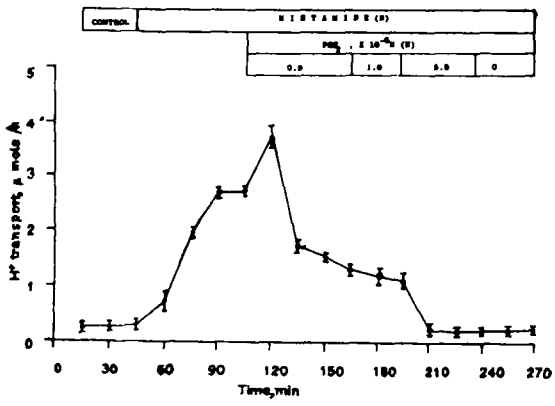
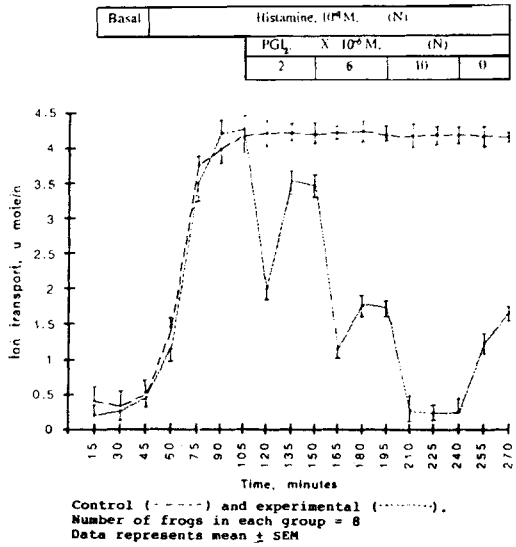



Figure 2 : Effects of incorporation of different concentration (/ml ) of $PGE_2$ in the nutrient solution on $H^+$ transport by histamine stimulated frog gastric mucosa in vitro. Values are means ± SEM (n=6)




Control (- - - - -) and experimental (·····).
Number of frogs in each group = 8
Data represents mean ± SEM

chamber or their treatment of $H^+$, $K^+$ -ATPase system alters the membrane resulting in either the loss of some of the essential membrane bound phospholipid or alteration of the orientation of the membrane, thus inhibiting $H^+$ -ion transport. The observations are consistent with the findings of Scheiman, et. al. (18). They have demonstrated that gastric cytoproective activity of $PGE_2$ may be caused by the release of surface active phospholipids by gastric mucus cells. The differential effect of $PGE_2$ and $PGI_2$ may also be due to their different structural property and the affinity towards the membrane bound phospholipids. Such extreme sensitivity of prostaglandins (2) suggests a precise and delicate regulatory mechanism for maintenance of controlled $H^+$ -ion uptake and also emphasizes the requirement of critical concentration of prostaglandin(s) for the regulation of gastric acid secretion.

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