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Effect of pertussis toxin on the inhibition of secretory activity by prostaglandin E_2, somatostatin, epidermal growth factor and 12-*O*-tetradecanoylphorbol 13-acetate in parietal cells from rat stomach

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Rat parietal cells were incubated for 2 h with pertussis toxin (100 ng/ml) which ADP-ribosylates and inactivates guanine nucleotide regulatory proteins (G proteins) of the 'G_i-like' family. The effect of this pretreatment on the action of inhibitors of parietal cell acid secretion was investigated by using the accumulation of the weak base aminopyrine as an index of secretory activity. The inhibitory actions of near maximally effective concentrations of prostaglandin E_2 (PGE_2), somatostatin and epidermal growth factor (EGF) on histamine-stimulated aminopyrine accumulation were reduced by 83%, 72% and 70%, respectively, by preincubation with pertussis toxin. By contrast, the inhibitory action of a near maximally effective concentration of 12-*O*-tetradecanoylphorbol 13-acetate on histamine-stimulated aminopyrine accumulation was reduced by only 12%. It is concluded that G-proteins are involved in the inhibitory actions of PGE_2, somatostatin and EGF on parietal cells. However, since the inhibitory actions of PGE_2 and EGF can be distinguished by the blockade of the action of EGF, but not that of PGE_2, by 3-isobutyl-1-methylxanthine, it is possible that PGE_2 and EGF either activate the same G-protein in different ways or work through different G-proteins.

Introduction

Three agents which act directly on rat parietal cells to inhibit secretory activity stimulated by histamine, and which may be of physiological importance, are prostaglandin E_2 (PGE_2), somatostatin and epidermal growth factor (EGF). PGE_2 appears to act by decreasing the cyclic AMP content of histamine-stimulated cells [1,2]. This effect is mediated by an inhibition of adenylate cyclase [3]. Somatostatin also inhibits secretory activity in isolated parietal cells [3,4] and inhibition of adenylate cyclase may contribute to this effect [3]. The site at which EGF inhibits histamine-stimulated acid secretion in rat parietal cells has yet to be established, but it seems to be close to the site of generation and hydrolysis of cyclic AMP [5].

Whereas the previous three inhibitors probably exert at least part of their effect by combining with receptors on the cell membrane, a fourth agent 12-*O*-tetradecanoylphorbol 13-acetate (TPA)

Abbreviations: PGE_2, prostaglandin E_2; EGF, epidermal growth factor; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; IBMX, 3-isobutyl-1-methylxanthine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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probably inhibits secretory activity by activation of the calcium-sensitive phospholipid-dependent protein kinase, protein kinase C [6]. Although TPA is acting as a pharmacological agent, the pathway it activates could be of physiological significance because muscarinic stimulation of the parietal cell generates diacylglycerol [7], the probable physiological activator of protein kinase C [8]. The pathway could therefore be involved in negative modulation of the histamine response by carbachol [6].

One route for receptor-mediated inhibition of adenylate cyclase appears to involve the guanine nucleotide regulatory protein G_i [9]. This action of the G_i protein can be prevented if the 41 kDa α subunit is ADP-ribosylated by *Bordetella* pertussis toxin [9]. Indeed, there appears to be a family of G proteins all of which are inactivated by pertussis toxin [10]. Our intention, with this work, was to establish whether proteins from this 'G_i family' might be involved in the inhibitory actions of PGE_2, somatostatin, EGF and TPA on parietal cells. The presence of a 41 kDa protein substrate for ADP-ribosylation by pertussis toxin has recently been demonstrated in parietal cell membranes [11].

Materials and Methods

Animals. Male Wistar rats (200–300 g body weight) were obtained from Bantin and Kingman, Hull, U.K., and were fed on Heygates' breeding diet, supplied by Pilsbury, Birmingham, U.K.

Reagents. Pertussis toxin was purchased from PHLS Centre for Applied Microbiology and Research, Porton Down, Wiltshire, U.K. EGF, PGE_2, somatostatin-14, TPA, 8-phenyltheophylline and N^6-phenylisopropyladenosine were obtained from Sigma, Poole, U.K. The sources of other reagents have been described previously [12].

IBMX and PGE_2 were dissolved in ethanol the concentration of which was 0.125% (v/v) in both control vials and those containing the agents. TPA was dissolved in dimethylsulphoxide, and the concentration of the solvent was 0.06% (v/v) in both control vials and those containing TPA. Pertussis toxin was dissolved in 50 mM Tris-chloride buffer (pH 8.0) containing 1 M NaCl. This vehicle was added to control incubations.

Isolation and incubation of cells. Cells were isolated from the fundic portion of the stomach of fed rats as described by Shaw et al. [12]. Cell suspensions, which contained about 20% parietal cells, were incubated for 2 h with or without 100 ng/ml pertussis toxin in Eagle's minimum essential medium with Earle's salts containing 1 mg/ml bovine serum albumin and 20 mM Hepes (pH 7.35). The cells were washed twice in the above medium without pertussis toxin and were then resuspended in the same medium and incubated for 30 min at 37 °C with the addition of [*dimethylamine*-^{14}C]aminopyrine (0.1 μCi/ml; 0.9 μM) and [1,2-^{3}H]poly(ethylene glycol) (0.4 μCi/ml) and, where appropriate, secretagogues and inhibitors. The aminopyrine accumulation ratio, which is the ratio of the concentration of aminopyrine inside the cell to that of the medium was determined as described in Ref. 12.

Expression of results. There was some variation between batches of cells in the aminopyrine accumulation ratio obtained in response to secretagogues. This has also been found by other workers with parietal cells [13]. The problem was overcome by always treating half the cells in a batch with pertussis toxin and using the remainder as a control. The effect of pertussis toxin could then be assessed by using a paired *t*-test. The inhibitory effect of agents on the aminopyrine accumulation ratio was expressed as a percentage of the aminopyrine accumulation ratio obtained in the absence of inhibitors. Basal aminopyrine accumulation ratios, which ranged from 1 to 3, were subtracted from measurements before calculation of the percent inhibition value.

Results

Effect of pertussis toxin on the aminopyrine accumulation ratio

Preliminary experiments established that the inhibitory action of PGE_2 on aminopyrine accumulation stimulated by 0.5 mM histamine and 0.1 mM IBMX was substantially blocked after 2 h of preincubation with 100 ng/ml pertussis toxin. Preincubation of cells with pertussis toxin (100 ng/ml) for 2 h was therefore adopted as the standard procedure. Measurements made in the absence of inhibitors demonstrated that pretreatment with

TABLE I

EFFECT OF PREINCUBATION WITH PERTUSSIS TOXIN ON THE AMINOPYRINE ACCUMULATION RATIO IN PARIETAL CELLS

Cells were preincubated with pertussis toxin (100 ng/ml) for 2 h, washed and then incubated with aminopyrine in the presence and absence of secretagogues for 30 min. The aminopyrine accumulation ratios are presented as means ± S.E. n is the number of batches of cells. * $P < 0.05$ for the comparison of results obtained with and without preincubation with pertussis toxin by a paired t-test.

Secretagogues	n	Aminopyrine accumulation ratio	
		control	pertussis toxin
None	13	1.5± 0.2	1.4± 0.2
Histamine (0.5 mM)	6	7.5± 1.7	17.9± 5.3 *
Histamine (0.5 mM) + IBMX (0.1 mM)	14	122 ±17	135 ±21

pertussis toxin had no effect on basal aminopyrine accumulation, or on that obtained in the presence of 0.5 mM histamine and 0.1 mM IBMX (Table I). However, the aminopyrine accumulation ratio obtained in response to 0.5 mM histamine alone was increased by preincubation with pertussis toxin (Table I).

Endogenous adenosine inhibits histamine-stimulated aminopyrine accumulation by suspensions of canine parietal cells by acting at A_1 receptors [14]. Since pertussis toxin is known to interfere with the action of A_1 receptors [15], endogenous adenosine might complicate the interpretation of the results in the present study with rat parietal cells. For example, prevention of the inhibitory effect of endogenous adenosine might be the cause of the enhancements of histamine-stimulated aminopyrine accumulation by preincubation with pertussis toxin. However, the specific adenosine A receptor antagonist, 8-phenyltheophylline (20 μM), did not block the enhancement of the histamine-stimulated aminopyrine accumulation ratio by pertussis toxin pretreatment (histamine-stimulate aminopyrine accumulation ratios (means ± S.E., with the number of determination in parentheses, using a single batch of cells): adenosine antagonist absent, control 5.3 ± 0.4 (3); pertussis toxin-treated, 11.0 ± 0.3 (4); 20 μM 8-phenyltheophylline present, control 4.3 ± 0.3 (4); pertussis toxin-treated, 11.4 ± 0.3 (4) ($P < 0.001$ for effect of pertussis toxin in both cases)). Furthermore, the adenosine receptor agonist N^6-phenylisopropyladenosine (10 μM) had no effect on the aminopyrine accumulation ratio in cells stimulated with 0.5 mM histamine or histamine plus the phosphodiesterase inhibitor Ro 20-1724 (data not shown).

Effect of preincubation with pertussis toxin on the inhibitory action of PGE_2, somatostatin and TPA

Preincubation with pertussis toxin significantly reduced the inhibitory action of 10^{-8}, 10^{-7} and 10^{-6} M PGE_2 on aminopyrine accumulation (Fig. 1). Indeed, in cells preincubated with pertussis toxin, the dose-related inhibitory effect of PGE_2 was abolished (F ratio for the effect of PGE_2 concentration by Anovar = 2.36, which does not reach significance).

The inhibitory effect of somatostatin on aminopyrine accumulation stimulated with 0.5 mM histamine and 0.1 mM IBMX (Fig 2) was less than that obtained with PGE_2. Nevertheless, the results obtained are not substantially different from those of Chew [4] or Schepp et al. [16], who both included dithiothreitol in their incubation medium.

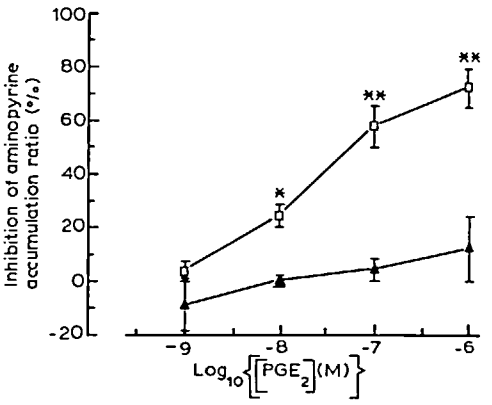

Fig. 1. Effect of preincubation with pertussis toxin on the inhibitory action of PGE_2 on aminopyrine accumulation stimulated by 0.5 mM histamine and 0.1 mM IBMX. Results are from four batches of cells, are expressed as means ± S.E., and the effect of preincubation with pertussis toxin has been assessed by a paired t-test. ** $P < 0.01$; * $P < 0.05$. □, preincubated without pertussis toxin; ▲, preincubated with pertussis toxin (100 ng/ml) for 2 h. Stimulation of aminopyrine accumulation above basal by histamine in the absence of PGE_2 was 154±34 with pertussis toxin absent and 180±44 with it present during preincubation.

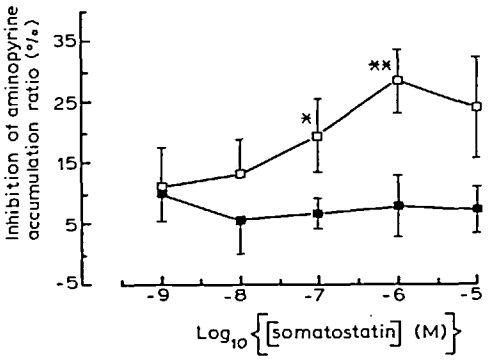

Fig. 2. Effect of preincubation with pertussis toxin on the inhibitory action of somatostatin on aminopyrine accumulation stimulated by 0.5 mM histamine and 0.1 mM IBMX. Results are from five batches of cells and have been compared and presented as in Fig. 1. ** $P < 0.01$; * $P < 0.05$. □, preincubated without pertussis toxin; ■, preincubated with pertussis toxin (100 ng/ml) for 2 h. Stimulation of aminopyrine accumulation by histamine above basal in the absence of somatostatin was 70±26 with pertussis toxin absent and 77±26 with it present during preincubation.

No effect of 0.5 mM dithiothreitol on the inhibitory action of somatostatin was detectable in the present experiments, and it was therefore omitted. Preincubation with pertussis toxin reduced the inhibitory effect of 10^{-7} or 10^{-6} M somatostatin.

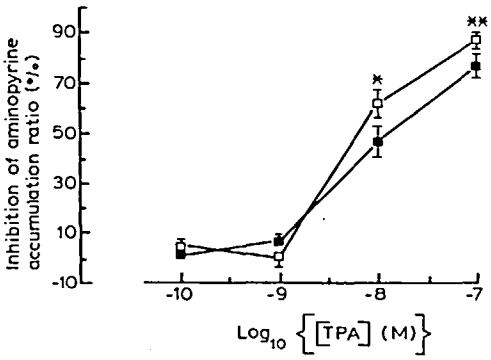

Fig. 3. Effect of preincubation with pertussis toxin on the inhibitory action of TPA on aminopyrine accumulation stimulated by 0.5 mM histamine and 0.1 mM IBMX. Results are from five batches of cells and have been compared and presented as in Fig. 1. ** $P < 0.01$; * $P < 0.05$. □, preincubated without pertussis toxin; ■, preincubated with pertussis toxin (100 ng/ml) for 2 h. Stimulation of aminopyrine accumulation above basal by histamine in the absence of TPA was 145±20 with pertussis toxin absent and 153±27 with it present during preincubation.

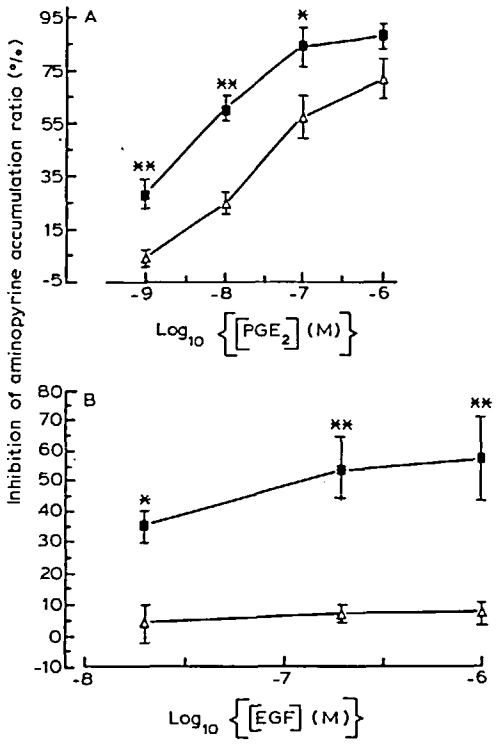

Fig. 4. Effect of 0.1 mM IBMX on the inhibitory action of PGE_2 (A), and EGF (B) on aminopyrine accumulation stimulated by 0.5 mM histamine. ■, IBMX absent; △, IBMX present. (A) Results from four separate batches of cells in each case are expressed as means±S.E. and have been compared by an unpaired *t*-test. ** $P < 0.01$; * $P < 0.05$. Stimulation of aminopyrine accumulation ratios above basal in the absence of PGE_2 was 154±34 and 4.6±2.0 in the presence and absence of IBMX, respectively. (B) Results are from four or five separate batches of cells in each case and have been expressed and compared as in A. Stimulation of aminopyrine accumulation ratios above basal in the absence of EGF was 123±18 and 4.6±0.99 in the presence and absence of IBMX, respectively.

Preincubation with pertussis toxin reduced the inhibitory effect of TPA against aminopyrine accumulation stimulated by 0.5 mM histamine and 0.1 mM IBMX by 25% at 10^{-8} M TPA and by 12% at 10^{-7} M TPA (Fig. 3). However, by contrast with the action of pertussis toxin on the inhibitory effects of PGE_2, there was still a significant inhibitory effect of TPA concentration on the aminopyrine accumulation ratio ($P < 0.01$, by Anovar).

Effect of IBMX on the inhibitory actions of PGE_2 and EGF

0.1 mM IBMX caused a rightward shift in the dose-response curve for the effect of PGE_2 on histamine-stimulated aminopyrine accumulation (Fig. 4A). However, the effect of IBMX on the inhibitory action of EGF was different. This is illustrated by examining the effect of IBMX on the action of near maximally effective concentrations of EGF (Fig. 4B). IBMX totally abolished the inhibitory action of EGF and this effect could not be overcome by raising the concentration of EGF.

Effect of preincubation with pertussis toxin on the inhibitory action of EGF

For reasons mentioned above, these experiments were performed with cells stimulated with histamine alone. Preincubation with pertussis toxin significantly reduced the inhibitory effects of 20 and 200 nM EGF (Fig. 5). It was pertinent (see discussion) to examine whether the extent of the stimulation of the aminopyrine accumulation ratio by histamine (x) affected the percent inhibition of this measurement by EGF (y). Examination of

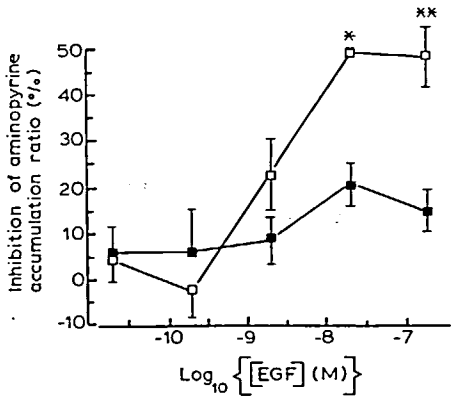

Fig. 5. Effect of preincubation with pertussis toxin on the inhibitory action of EGF on the stimulation of aminopyrine accumulation by 0.5 mM histamine. Results are from four batches of cells and have been compared and presented as in Fig. 1. ** $P < 0.01$; * $P < 0.05$. □, preincubated without pertussis toxin; ■, preincubated with pertussis toxin (100 ng/ml) for 2 h. Stimulation of aminopyrine accumulation above basal by histamine in the absence of EGF was 9.7 ± 3.3 with pertussis toxin absent and 26.8 ± 8.1 with it present during preincubation ($P < 0.025$ for the effect of pertussis toxin using a paired t-test).

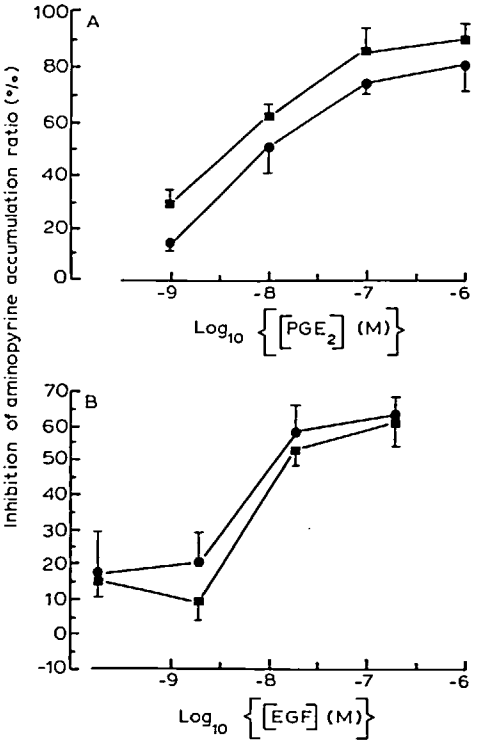

Fig. 6. Effect of a constant concentration of EGF (1 nM) on the inhibitory action of PGE_2 on histamine-stimulated aminopyrine accumulation (A), and of a constant concentration of PGE_2 (2 nM) on the inhibitory action of EGF on histamine-stimulated aminopyrine accumulation (B). ■, no second inhibitor at a constant concentration; ●, constant concentration of second inhibitor present. (A) Results from four batches of cells are expressed as means ± S.E. The stimulation of the aminopyrine accumulation ratios above basal in the absence of PGE_2 was 4.56 ± 2.04 and 3.71 ± 2.00 in the absence (■) and presence (●) of 1 nM EGF, respectively (the effect of 1 nM EGF was significant, $P < 0.01$, using a paired t-test). There was no difference between the inhibitory effect of PGE_2 in the presence and absence of EGF (paired t-test). (B) Results are from five batches of cells and are expressed as means ± S.E. The stimulation of the aminopyrine accumulation ratios above basal in the absence of EGF was 3.3 ± 0.84 and 2.0 ± 0.44 in the absence (■) and presence (●) of PGE_2, respectively (there was a significant effect of PGE_2 ($P < 0.05$) using a paired t-test). There was no difference in the inhibitory effects of EGF in the presence and absence of PGE_2 (paired t-test).

results for 20 and 200 nM EGF from 9 and 10 separate experiments, respectively, by linear regression analysis showed no such relationship (20 nM EGF, $y = 55 - 0.71x$; 200 nM EGF, $y = 55 + 0.03x$, slope not different from zero in both cases).

Potential interactions between PGE_2 and EGF

The ability of IBMX to block the action of EGF, but not that of PGE_2 suggested that these two inhibitors might employ different mechanisms of action. The possibility that there might be a synergistic interaction between the two compounds was investigated by examining the inhibitory effect of PGE_2 in the presence and absence of a fixed concentration of EGF (Fig. 6A), and the action of EGF in the presence and absence of a fixed concentration of PGE_2 (Fig. 6B). There was no significant effect of EGF on the action of PGE_2, or of PGE_2 on the action of EGF against aminopyrine accumulation in cells stimulated with 0.5 mM histamine.

Discussion

The accumulation of aminopyrine only occurs in parietal cells [17] and, therefore, the above experiments could be performed with an impure cell preparation containing other stomach cells. The determinant of aminopyrine accumulation is the sequestration of acid in spaces inside the parietal cells. Aminopyrine accumulation does not, therefore, measure acid secretion directly, but rather acts as an index of this process [13]. Nevertheless, results obtained using aminopyrine accumulation correlate linearly with those employing more direct measures of secretory activity such as oxygen consumption [18].

There is no evidence that treatment of cells with pertussis toxin caused any non-specific interference with cell function. Thus, neither the basal aminopyrine accumulation ratio, nor that stimulated by 0.5 mM histamine and 0.1 mM IBMX, was affected by preincubation with pertussis toxin. The stimulation of the response to histamine alone by pertussis toxin pretreatment could have been the consequence of the removal of an inhibitory tone. This inhibitory tone was not due to the presence of adenosine in the medium, because the addition of the adenosine A receptor antagonist, 8-Phenyltheophylline, did not prevent the effect of pertussis toxin. Indeed, rat parietal cells may not possess A_1 adenosine receptors because N^6-phenylisopropyladenosine does not inhibit aminopyrine accumulation induced by histamine (this work and Ref. 19). A stimulatory effect of pertussis toxin on the response to agonists has been observed in other cell types [20,21]. No effect was observed in parietal cells stimulated with 0.5 mM histamine and 0.1 M IBMX probably because these cells were already maximally stimulated [16].

The finding that pretreatment with pertussis toxin prevents the inhibitory action of PGE_2 is in agreement with the preliminary study of Rosenfeld [22] who employed a single concentration of PGE_2 (300 nM). It has already been established that PGE_2 inhibits adenylate cyclase in parietal cells [3], and it is not unreasonable to assume that G_i protein may be involved in the inhibition of adenylate cyclase by PGE_2. Similarly, somatostatin inhibits adenylate cyclase in parietal cells [3], and its inhibitory effect on aminopyrine accumulation has been shown here to be blocked by preincubation with pertussis toxin. Thus, as in other cells [23], it is possible that somatostatin acts via G_i protein to inhibit parietal cell adenylate cyclase and thereby counters the effects of histamine stimulation.

A small effect of preincubation with pertussis toxin was found on the inhibitory effect of TPA on histamine-stimulated aminopyrine accumulation. Brown and Chew [11] could find no such effect of pertussis toxin pretreatment in rabbit gastric glands. This lack of agreement could be a consequence of differences in experimental protocol, for Brown and Chew [11] preincubated their glands with 100 nM TPA for 30 min before a 45-min challenge with histamine. In the present experiments, histamine and TPA were added simultaneously and incubation was for 30 min. Recent work (Hatt, J.F. and Hanson, P.J., in preparation) suggests that TPA decreases the cyclic AMP content of histamine-stimulated parietal cells, but also inhibits at a second site downstream in the secretory pathway from adenylate cyclase. It is possible that pretreatment with pertussis toxin might interfere with the action of TPA on cyclic AMP content, but that, since the action of TPA at the second site was unaffected, no major effect on the inhibition of aminopyrine accumulation was evident.

Aminopyrine accumulation obtained in the presence of histamine in cells pretreated with pertussis toxin was higher than in control cells treated with histamine alone (see legend to Fig. 5). However, it is unlikely that the elevation of the

aminopyrine accumulation ratio by pertussis toxin was responsible for its prevention of the inhibitory effect of EGF. Thus, there was no evidence that the inhibitory effect of EGF was related to the extent of histamine-stimulated aminopyrine accumulation. A G-protein may therefore be involved in the action of EGF in parietal cells. In other cells, some actions of EGF are blocked by pertussis toxin. Thus, the action of EGF on inositol trisphosphate formation and elevation of intracellular calcium is blocked by preincubation of hepatocytes with pertussis toxin [24], but pertussis toxin does not seem to block the mitogenic effect of EGF on fibroblasts [25].

IBMX blocks the action of EGF (Ref. 5 and above). This inhibitory effect of IBMX on the action of EGF occurs over a wide range of secretory activity which encompasses that found in cells stimulated with histamine alone [5]. Thus, the ability of IBMX to enhance the secretory response to histamine does not seem to be the reason for its prevention of the inhibitory action of EGF [5]. By contrast, IBMX does not block the inhibitory effect of PGE_2 [2,6]. Thus, although the action of both EGF and PGE_2 is blocked by pertussis toxin, the two agents appear to act differently. There are two potential explanations. Firstly, both agents might act on G_i protein but they might activate it in different ways, thereby leading to different effects. Alternatively, G_i is not the only pertussis toxin substrate [10], and another member of the 'G_i family' might mediate the action of EGF. If both EGF and PGE_2 acted on the same G-protein is seemed possible that there might be a synergistic interaction between the two inhibitors. However, no such interaction was detectable.

In conclusion, it seems likely that G-proteins sensitive to pertussis toxin play a major role in the mechanisms by which PGE_2, somatostatin and EGF inhibit secretory activity stimulated by histamine in parietal cells.

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