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Action of epidermal growth factor on acid secretion by rat isolated parietal cells

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The site and mechanism of action of epidermal growth factor (EGF) on acid secretion by rat isolated parietal cells were investigated by using the intracellular accumulation of the weak base aminopyrine as an index of secretory activity. When parietal cells were stimulated with histamine (0.5 mM), the concentration of EGF required for half-maximal inhibition of acid secretion was 19 nM, with a maximally effective concentration of EGF producing 38% inhibition of secretory activity. EGF did not inhibit secretion stimulated by 0.1 mM-carbachol, or by 30 μM-, 56 μM-, 100 μM- or 1000 μM-dibutyryl cyclic AMP, low concentrations of which produced a secretory response comparable with that obtained with 0.5 mM-histamine. Addition of 0.1 mM-3-isobutyl-1-methylxanthine (IBMX) substantially increased aminopyrine accumulation in the presence of 0.5 mM-histamine. The inhibitory action of EGF on histamine-stimulated secretion was blocked by 0.1 mM-IBMX, even if low concentrations of histamine were used to generate aminopyrine accumulation ratios similar to those obtained with 0.5 mM-histamine alone. The cyclo-oxygenase inhibitor flurbiprofen (1–100 μM) and the cyclo-oxygenase and lipoxygenase inhibitor nor-dihydroguaiaretic acid (10–100 μM) did not affect the inhibitory action of EGF. The pattern of inhibition of secretion produced by the activator of Ca^{2+}-sensitive phospholipid-dependent protein kinase, 12-*O*-tetradecanoylphorbol 13-acetate, was markedly different from that produced by EGF. In conclusion, a major site of the action of EGF on acid secretion in the intact stomach is probably a decrease in the stimulatory effect of histamine by a mechanism which does not involve Ca^{2+}-sensitive phospholipid-dependent protein kinase or the production of prostaglandins, but which might involve enhancement of cyclic AMP phosphodiesterase activity.

INTRODUCTION

Epidermal growth factor (EGF) from mouse submaxillary glands is a single-chain polypeptide of 53 amino acid residues, which stimulates epithelial-cell proliferation (Dembinski *et al.*, 1982) and inhibits gastric acid secretion (Bower *et al.*, 1975; Konturek *et al.*, 1984; Finke *et al.*, 1985). EGF inhibits acid secretion induced *in vivo* by pentagastrin, histamine or cholinergic agonists, but has no effect on the serum gastrin concentration (Konturek *et al.*, 1984). Neither the site nor the mechanism of action of EGF on acid secretion is known. It is difficult to establish the primary site of action of inhibitors from experiments performed with intact tissue. For example, the histamine H_2-receptor antagonist cimetidine inhibits acid secretion induced not only by histamine but also by cholinergic agonists and gastrin *in vivo*, possibly because it prevents potentiating interactions between endogenous histamine and other secretagogues (Soll, 1982). An investigation of the site of action of EGF on secretory activity has therefore been undertaken in isolated parietal cells, where such interactions can be dissected out (Soll, 1982). In this system the accumulation of the weak base aminopyrine in acidic spaces within parietal cells provides an index of secretory activity (Soll, 1980*a*).

Activation of the Ca^{2+}-sensitive phospholipid-dependent protein kinase (protein kinase C) by TPA and 1-oleoyl-2-acetylglycerol inhibits histamine-stimulated gastric acid secretion (Pearce *et al.*, 1981; Anderson & Hanson, 1984, 1985; Shaw & Hanson, 1986). Since EGF activates protein kinase C in the A431 cell line (Sahai *et al.*, 1982), an interesting suggestion is that protein kinase C might be the intracellular mediator of the inhibitory action of EGF in parietal cells. This possibility has been investigated by comparing the characteristics of the inhibitory actions of EGF in parietal cells with the effects of an activator of protein kinase C. The possibility that eicosanoids might be involved in the action of EGF has also been investigated.

EXPERIMENTAL

Animals

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

Reagents

EGF, purified from mouse submaxillary glands, was donated by ICI, Alderley Park, Cheshire, U.K., and Ro 20-1724 was provided by Dr. P. F. Sorter of Hoffmann–

Abbreviations used: EGF, epidermal growth factor; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; IBMX, 3-isobutyl-1-methylxanthine.

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La Roche, Nutley, NJ, U.S.A. Flurbiprofen was a gift from Boots, Pennyfoot Street, Nottingham, U.K. Nordihydroguaiaretic acid was purchased from Sigma. The sources of other reagents were as described previously (Anderson & Hanson, 1985; Shaw *et al.*, 1985).

Preparation and incubation of cells

Cells were prepared from the fundic portion of the stomach of fed rats by methods described by Shaw *et al.* (1985). Cell suspensions, containing about 20% parietal cells, were incubated in Eagle's minimal essential medium with Earle's salts containing bovine serum albumin (1 mg/ml), 20 mM-Hepes, [^{14}C]aminopyrine (0.1 μCi/ml; 0.9 μM) and [^{3}H]poly(ethylene glycol) (0.4 μCi/ml) for 30 min at 37 °C. The intracellular accumulation of aminopyrine, which only occurs in parietal cells (Sonnenberg *et al.*, 1979), was determined as described previously (Shaw *et al.*, 1985). Flurbiprofen, nordihydroguaiaretic acid, IBMX and Ro 20-1724 were dissolved in ethanol, and TPA in dimethyl sulphoxide, with the appropriate solvents [final concns. 0.1% (v/v) and 0.06% (v/v) respectively] being added to the control vials.

Analysis and presentation of results

Aminopyrine accumulation was expressed as the ratio of the concentration of aminopyrine in the cell to that in the incubation medium (Berglindh *et al.*, 1976). Although aminopyrine accumulation is an indirect index of acid secretion, results obtained with this technique correlate linearly with those employing stimulation of oxygen consumption (Chew *et al.*, 1980), which is a more direct measure of parietal-cell activity. Furthermore, aminopyrine accumulation is likely to be a linear measure of acid secretion, for otherwise data for the effect of histamine concentration on secretory activity would be unlikely to fit a classical dose–response model (see, e.g., histamine data in Table 2). The quantitative effect of secretagogues on accumulation of aminopyrine varied between batches of cells (see also Soll, 1980*a*), and it was therefore necessary to use statistical tests which surmounted this problem (Tables 1, 2 and 3), or to normalize data by expressing results as percentage inhibition of secretory activity (Fig. 1, Tables 3 and 4). Soll (1980*a*) found that the normalized pattern of dose–response relations was independent of a considerable variation in the absolute values for aminopyrine accumulation.

RESULTS AND DISCUSSION

Site of action of EGF

EGF inhibited aminopyrine accumulation stimulated by a near-maximally effective concentration of histamine (0.5 mM) (Table 1). The concentration of EGF required for half-maximal inhibition of secretory activity was 19±3 nM, and the maximum effect of EGF was to inhibit aminopyrine accumulation by 38% (Fig. 1). Increasing the concentration of histamine 10-fold to 5.0 mM had no significant effect on aminopyrine accumulation, or on the inhibitory action of 200 nM-EGF [inhibition: 0.5 mM-histamine, 35±3% (5); 5.0 mM-histamine, 36±2% (4)]. The absolute decrease in aminopyrine accumulation effected by 200 nM-EGF in a particular batch of cells was proportional to the extent of aminopyrine accumulation produced by histamine in that cell batch (Fig. 2). This relationship means that the percentage inhibition of secretion induced by 200 nM-EGF was independent of the absolute extent of histamine-stimulated aminopyrine accumulation. EGF had no effect on the basal extent of aminopyrine accumulation (Table 1). Furthermore, when parietal cells were stimulated by near-maximally effective concentrations of other secretagogues, namely 0.1 mM-carbachol and 1 mM-dibutyryl cyclic AMP (G. P. Shaw & P. J. Hanson, unpublished work), or by 0.5 mM-histamine coupled with 0.1 mM-IBMX (Table 2), no inhibitory effect of EGF was apparent (Table 1).

The inability of EGF to inhibit secretion induced by a muscarinic cholinergic agonist contrasts with results obtained *in vivo* (Bower *et al.*, 1975; Konturek *et al.*, 1984). This contradiction could be explained by an action of EGF on the potentiating effect of endogenous histamine on secretion induced by the cholinergic agonist *in vivo* (Soll, 1982). Furthermore, acetylcholine can effect a release of histamine from specialized cells in the gastric mucosa (Nylander *et al.*, 1986), and an alternative possibility is that EGF might prevent the

Table 1. Effect of EGF on acid secretion by rat isolated parietal cells incubated under various conditions

Secretory activity, estimated from the intracellular accumulation of aminopyrine (see the Experimental section), is presented as means ±S.E.M., with the numbers of cell batches in parentheses. Significant effects of EGF, determined by a paired *t* test, are denoted: $^{**}P < 0.01$, $^{***}P < 0.001$.

Incubation conditions	Aminopyrine accumulation ratio					
	Control	20 nM-EGF	Control	200 nM-EGF	Control	1 μM-EGF
No addition	2.3±0.5 (4)	2.1±0.5 (4)	3.7±0.6 (4)	3.9±0.8 (4)	2.1±0.4 (4)	2.0±0.4 (4)
Histamine (0.5 mM)	5.4±0.8 (5)	4.3±0.6 (5)**	9.7±1.0 (5)	6.3±0.6 (5)**	5.2±1.0 (4)	3.5±1.0 (4)***
Carbachol (0.1 mM)	4.2±0.9 (4)	4.2±1.5 (4)	10.3±1.1 (4)	10.5±1.7 (4)	4.6±0.8 (5)	4.2±1.0 (5)
Histamine (0.5 mM) +IBMX (0.1 mM)	114±23 (5)	115±30 (5)	146±21 (11)	136±21 (11)	105±28 (4)	99±27 (4)
Dibutyryl cyclic AMP (1 mM)	147±31 (5)	144±30 (5)	201±38 (7)	201±41 (7)	126±31 (4)	121±33 (4)

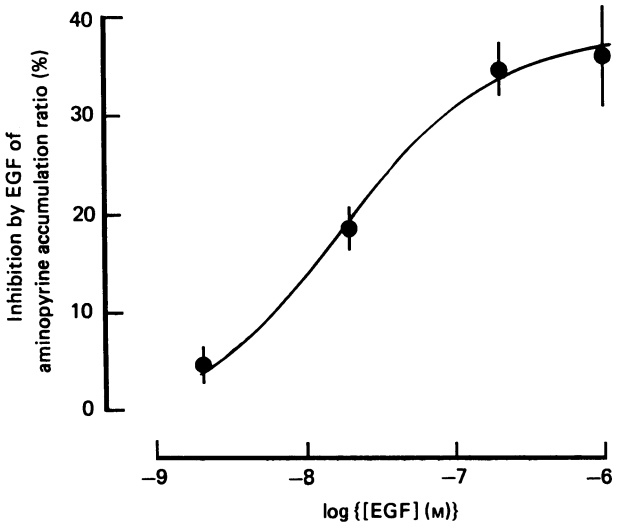

Fig. 1. Effect of the concentration of EGF on the inhibition of aminopyrine accumulation in parietal cells stimulated by 0.5 mM-histamine

Results, for the percentage inhibition of secretion effected by EGF, from four or five batches of cells, are presented as means ± S.E.M. The half-maximally effective concentration of EGF was 19 ± 3 nM, and the maximum effect of EGF was 38 ± 1% inhibition. These constants, and the line drawn, were calculated by using the computer program FIT (Barlow, 1983).

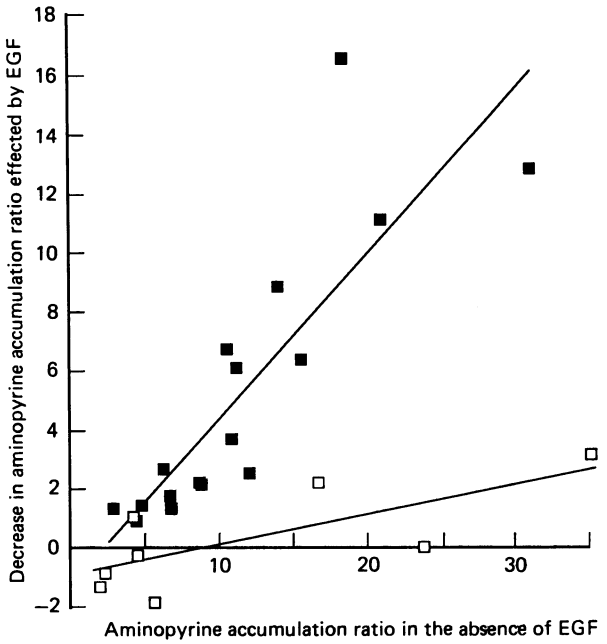

Fig. 2. Relationship between the absolute decrease in aminopyrine accumulation effected by 200 nM-EGF (y), and the extent of histamine-stimulated aminopyrine accumulation (x), for different batches of cells

□, Values obtained in the the presence of IBMX (0.1 mM) and 1–30 μM-histamine. Equation of the regression line: $y = -0.97 + 0.11x$; correlation coefficient = 0.74, $P < 0.05$. ■, Values obtained in the presence of 0.5 mM-histamine alone. Equation of the regression line: $y = -1.23 + 0.56x$; correlation coefficient = 0.85, $P < 0.01$. Analysis of covariance (Snedecor & Cochran, 1967) demonstrated significant difference ($P < 0.001$) between the slopes of the two lines.

carbachol-induced release of histamine and/or the subsequent action of histamine on the parietal cell. In the rat isolated-parietal-cell preparation the action of carbachol was not mainly the consequence of a release of histamine, because the H_2-receptor antagonist cimetidine had a very small effect on carbachol-stimulated secretion (Anderson & Hanson, 1984). Also, little endogenous histamine is present in this preparation, because the phosphodiesterase inhibitor IBMX does not increase basal secretion (G. P. Shaw & P. J. Hanson, unpublished work). Interactions between carbachol and histamine, which could be associated with an effect of EGF on secretion in the presence of carbachol *in vivo*, were therefore not seen in the present isolated-cell preparation.

Lack of effect of EGF on secretion stimulated by dibutyryl cyclic AMP

EGF (200 nM) was ineffective at inhibiting secretion induced by dibutyryl cyclic AMP whether the extent of aminopyrine accumulation was much higher than or within the range of that produced by 0.5 mM-histamine alone (Table 2 and Fig. 2). The action of histamine on

Table 2. Effect of 200 nM-EGF on secretory activity stimulated by a range of concentrations of dibutyryl cyclic AMP and by different concentrations of histamine in the presence of constant 0.1 mM-IBMX

The presentation and statistical analysis of the results for aminopyrine accumulation ratios is as in Table 1. EGF had no effect at any concentration of either secretagogue. n = number of batches of cells.

Secretagogue...	Dibutyryl cyclic AMP				Histamine with constant 0.1 mM-IBMX			
	Concn. of dibutyryl cyclic AMP (μM)	n	Aminopyrine accumulation ratio		Concn. of histamine (μM)	n	Aminopyrine accumulation ratio	
			Control	200 nM-EGF			Control	200 nM-EGF
					0	3	2.3 ± 0.4	2.6 ± 0.4
	30	4	5.5 ± 1.7	5.0 ± 0.8	1	5	3.9 ± 0.7	4.0 ± 0.9
	56	6	49.3 ± 15.3	46.3 ± 16.8	10	3	44.3 ± 14.0	45.7 ± 16.0
	100	5	139 ± 47.7	125 ± 42.5	30	5	102 ± 18.2	116 ± 27.8
	1000	7	201 ± 38	202 ± 41	500	5	172 ± 37.1	159 ± 38.1

acid secretion is mediated by an elevation of the intracellular cyclic AMP concentration (Major & Scholes, 1978; Soll & Wollin, 1979). Since EGF did not inhibit secretion induced by dibutyryl cyclic AMP, its action against histamine-stimulated secretion was probably associated with an effect on either the generation or the hydrolysis of cyclic AMP.

Effect of IBMX and Ro 20-1724 on the action of EGF

The combination of IBMX with histamine produced a much greater aminopyrine accumulation than that obtained with histamine alone (Table 1). This effect of IBMX is also found in dog parietal cells (Soll, 1980*a*), and it is probably related to an enhanced elevation of intracellular cyclic AMP resulting from an inhibition of cyclic AMP phosphodiesterase activity by IBMX. Different extents of aminopyrine accumulation, in the presence of a constant concentration of 0.1 mM-IBMX, were produced by changing the concentration of histamine. Under such conditions 200 nM-EGF did not inhibit secretory activity, despite the aminopyrine accumulation ratio at low histamine concentrations being within the range of that obtained with 0.5 mM-histamine alone (Table 2). A similar lack of effect of EGF in the presence of IBMX was also seen when results from individual batches of cells were compared by plotting the decrease in aminopyrine accumulation effected by EGF against the uninhibited aminopyrine accumulation ratio (Fig. 2). In the presence of IBMX (□, Fig. 2), there was little inhibitory effect of EGF at extents of aminopyrine accumulation where EGF was obviously effective against histamine alone (■, Fig. 2). It is concluded that IBMX blocks the inhibitory action of EGF against histamine-stimulated secretion.

The phosphodiesterase inhibitor Ro 20-1724 is structurally dissimilar to IBMX and does not act as an adenosine-receptor antagonist (Gerber *et al.*, 1984). Aminopyrine accumulation in the presence of 0.5 mM-histamine plus Ro 20-1724 (0.1 mM) was 105±16 (6). Inhibition by EGF of aminopyrine accumulation in the presence of histamine (0.5 mM) plus Ro 20-1724 (0.1 mM) [11±6% (6)] was smaller ($P < 0.01$) than that obtained when EGF was added to histamine alone [39±4 (23)].

The most straightforward interpretation of these results is that EGF was inhibiting histamine-induced acid secretion by stimulating cyclic AMP phosphodiesterase activity. However, IBMX and Ro 20-1724 have other effects (Wells & Kramer, 1981), and the site at which they block EGF action thus cannot be stated with certainty.

Effect of flurbiprofen on the action of EGF

EGF stimulates prostaglandin release from the isolated perfused rat stomach (Chiba *et al.*, 1982), and prostaglandins, like EGF, inhibit histamine-stimulated aminopyrine accumulation, but not secretion induced by carbachol or dibutyryl cyclic AMP (Soll, 1980*b*). The arachidonic acid required for the formation of prostaglandins by cyclo-oxygenase could result from an EGF-induced phosphorylation of lipocortin (Pepinsky & Sinclair, 1986) effecting a de-inhibition of phospholipase A_2 (Hirata, 1981). It was therefore of interest to determine whether the inhibition of cyclo-oxygenase activity could prevent the inhibitory action of EGF. Flurbiprofen, one of the most potent and selective cyclo-oxygenase inhibitors (MacAdams *et al.*, 1984), was chosen for these experiments.

Flurbiprofen at 1 or 10 μM had no effect on histamine-stimulated aminopyrine accumulation or on its inhibition by 200 nM-EGF (Table 3); 100 μM-flurbiprofen inhibited histamine-stimulated aminopyrine accumulation, but an inhibitory effect of EGF was still apparent (Table 3). Two other cyclo-oxygenase inhibitors, 10 μM-mefenamic acid and 100 μM-ibuprofen, were also unable to block the inhibitory action of EGF (G. P. Shaw & P. J. Hanson, unpublished work). Also, whereas IBMX blocked the inhibitory action of EGF, it does not prevent the inhibition of histamine-stimulated secretion by prostaglandin E_2 (Soll, 1980*b*). Furthermore, the combined cyclo-oxygenase/lipoxygenase inhibitor nordihydroguaiaretic acid did not prevent the inhibitory action of EGF (Table 3). This result supports the lack of involvement of prostanoids in EGF action, and also

Table 3. Lack of effect of flurbiprofen and nordihydroguaiaretic acid (NDGA) on the inhibition of histamine-stimulated aminopyrine accumulation by EGF

Results are presented as means±S.E.M. The significance of effects of EGF (200 nM) on histamine (0.5 mM)-stimulated aminopyrine accumulation in the presence and absence of the cyclo-oxygenase inhibitors were determined by using analysis of variance, followed by a Newman–Keuls multiple-comparison test. This analysis also showed that both 100 μM-flurbiprofen and 100 μM-NDGA decreased histamine-stimulated aminopyrine accumulation by comparison with the effect of histamine alone ($P < 0.01$). A paired *t* test was used to examine the effect of agents on the percentage inhibition of histamine-stimulated secretion induced by EGF. *$P < 0.05$; ***$P < 0.01$.

Cyclo-oxygenase inhibitor	Concn. of agent when added (μM)	No. of cell batches	Aminopyrine accumulation ratio				Inhibitory effect of EGF on histamine-stimulated secretion (% inhibition)	
			Histamine	Histamine +EGF	Histamine +agent	Histamine +agent +EGF	No agent	+Agent
Flurbiprofen	1	4	11.1±0.8	6.1±0.4***	9.9±1.0	6.3±1.0***	43±6	38±5
Flurbiprofen	10	4	12.0±1.8	6.7±0.8***	13.5±2.4	8.2±1.3***	43±4	37±4
Flurbiprofen	100	4	10.3±1.2	6.4±0.7***	6.6±1.1	4.0±0.6*	38±2	38±5
NDGA	10	3	11.6±1.0	6.4±0.1***	12.0±1.5	6.9±0.6***	44±4	42±2
NDGA	100	4	11.3±0.1	6.3±0.1***	6.8±0.9	3.5±0.5***	43±3	44±2

suggests that products of lipoxygenase activity do not mediate the inhibitory action of EGF. In conclusion, stimulation of prostaglandin production by EGF does not therefore seem to form part of the anti-secretory action of EGF. Konturek *et al.* (1981*a*) concluded that prostaglandins were not involved in the 'cytoprotective' effect of EGF, which occurs at a dose of EGF below that needed to inhibit acid secretion.

Comparison of the inhibitory effects of TPA and EGF

Neither EGF (Table 1) nor 32 nM-TPA (Anderson & Hanson, 1984) affected the basal extent of aminopyrine accumulation by parietal cells. However, there were marked differences between the inhibitory actions of EGF and TPA, and these are illustrated by the data presented in Table 4. The concentration-dependence of the inhibitory action of TPA on histamine-stimulated aminopyrine accumulation has been described previously (Anderson & Hanson, 1985), and, as an example of the action of TPA, results obtained at a concentration of 32 nM-TPA are presented. This concentration is approx. 10 times the half-maximally effective concentration of TPA required for inhibition of histamine-stimulated secretion (Anderson & Hanson, 1985), and therefore the effects of TPA will be near-maximal. At 32 nM, TPA also produces near-maximal inhibition of aminopyrine accumulation induced by 1 mM-dibutyryl cyclic AMP (J. F. Hatt & P. J. Hanson, unpublished work). These results on the effect of TPA are compared with those obtained with 200 nM-EGF, which in turn exerts a near-maximal inhibition of histamine-stimulated aminopyrine accumulation (Fig. 1). Thus near-maximally effective concentrations of TPA and EGF both inhibited histamine-stimulated aminopyrine accumulation, but TPA was the more powerful inhibitor (Table 4). This difference might be accounted for by TPA being a better activator of protein kinase C than is EGF. However, whereas TPA produced inhibition of aminopyrine accumulation stimulated by 0.5 mM-histamine plus 0.1 mM-IBMX, or by dibutyryl cyclic AMP (Table 3; Anderson & Hanson, 1984, 1985), 200 nM-EGF had no effect on cells stimulated under such conditions. Furthermore, this inability of EGF to inhibit secretion induced by histamine in the presence of IBMX, or by dibutyryl cyclic AMP, was apparent over a range of concentrations of EGF (Table 1), and over a range of cellular secretory activities (Table 2). In conclusion, there are substantial differences in the anti-secretory actions of TPA and EGF, and the proposal that protein kinase C might mediate the inhibitory action of EGF now seems untenable.

Table 4. Illustration of the different effects of TPA and EGF on secretory activity in rat isolated parietal cells

The effect of agents on secretory activity was assessed from the decrease in intracellular aminopyrine accumulation (see the Experimental section), expressed as a percentage of the aminopyrine accumulation in the absence of TPA or EGF. The results are presented as means ± S.E.M., with the numbers of cell batches in parentheses. The concentration of EGF was 200 nM, and that of TPA was 32 nM. A significant difference between the results for EGF and TPA with the same secretagogue (Student's t test) is indicated by: $^{**}P < 0.01$; $^{***}P < 0.001$.

	Inhibition of aminopyrine accumulation ratio (%)	
Secretagogues	EGF	TPA
Histamine (0.5 mM)	41.8 ± 4.6 (4)	77.1 ± 4.4 (4)**
Histamine (0.5 mM) + IBMX (0.1 mM)	6.7 ± 2.4 (11)	76.4 ± 3.5 (10)***
Dibutyryl cyclic AMP (1 mM)	2.7 ± 2.9 (7)	48.5 ± 9.8 (6)***

General discussion and conclusions

The concentration of EGF in mouse plasma [0.16 nM (Carpenter & Cohen, 1979)] is substantially lower than the concentration of EGF (19 nM, Fig. 1) required to exert half-maximal inhibition of histamine-stimulated secretion by rat parietal cells. EGF is present in saliva, but does not inhibit acid secretion when added to the lumen of the rat stomach (Konturek *et al.*, 1981*b*). EGF therefore might function as a paracrine regulator of acid secretion. In man EGF has not been detected in the gastric mucosa (Elder *et al.*, 1978), and in human blood virtually all of the EGF appears to be in platelets (Oka & Orth, 1983). In our view, EGF may be released from activated platelets during acute injury to the gastric epithelium, and its inhibitory effect on acid secretion may prevent the accumulation of acid under the protective cap of mucus and fibrin (Ito & Lacy, 1985), which forms over the damaged area. Accumulation of acid would probably inhibit the process of re-epithelialization which occurs under this protective cap.

A major site for the inhibitory action of EGF on acid secretion in the intact stomach may be the antagonism of histamine-stimulated secretion at a location close to the generation or hydrolysis of cyclic AMP. EGF probably acted directly on the parietal cells rather than by releasing an inhibitor, such as a prostaglandin or somatostatin, from another cell type. Reasons for a lack of involvement of prostaglandins in the action of EGF have been presented above, and an involvement of somatostatin is also unlikely, for, by contrast with EGF, somatostatin inhibits histamine-induced secretion in the presence of IBMX (Schepp *et al.*, 1983). Protein kinase C does not seem to mediate the inhibitory action of EGF. The proposal that EGF increases cyclic AMP phosphodiesterase activity and thereby inhibits histamine-stimulated acid secretion deserves further investigation.

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