Inhibition of gastric acid secretion by epidermal growth factor

Effects on cyclic AMP and on prostaglandin production in rat isolated parietal cells

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Histamine (0.5 mM) stimulated the cyclic AMP content of cell suspensions containing > 80% parietal cells. Epidermal growth factor (EGF) inhibited this stimulatory effect of histamine, but had no effect on basal cyclic AMP content. The half-maximally effective concentration of EGF for inhibition of histamine-stimulated cyclic AMP was 3.9 nM. The equivalent measurement for the inhibition of histamine-stimulated aminopyrine accumulation was 3.0 nM. Aminopyrine accumulation was measured because it provides an index of the secretory activity of the cell. The cyclic AMP phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) prevented the inhibitory effect of EGF on cyclic AMP content. This effect of IBMX was not caused by its ability to raise cellular cyclic AMP content in the presence of histamine. Prevention by IBMX of the inhibitory action of EGF on histamine-stimulated aminopyrine accumulation had been shown previously [Shaw, Hatt, Anderson & Hanson (1987) *Biochem. J.* **244**, 699–704]. EGF stimulated prostaglandin E₂ (PGE₂) production in the cell fraction containing > 80% parietal cells, with the half-maximally effective concentration being 7.5 nM. EGF was ineffective in stimulating PGE₂ production if the cell fraction was depleted of parietal cells (12%), or if 0.5 mM-histamine was added to the enriched parietal-cell fraction. In conclusion, EGF may inhibit histamine-stimulated acid secretion by decreasing the cyclic AMP content of parietal cells. This effect could be mediated by an increase in cyclic AMP phosphodiesterase activity, but it is unlikely to involve an effect of EGF on parietal-cell prostaglandin production.

INTRODUCTION

Epidermal growth factor (EGF) is a polypeptide which was first isolated from the mouse submaxillary gland. In the rat, intraperitoneal or intragastric administration of EGF causes a trophic response in the gastroduodenal mucosa (Dembinski *et al.*, 1982). Intravenous or subcutaneous EGF inhibits gastric acid secretion (Bower *et al.*, 1975; Dembinski *et al.*, 1982; Gregory, 1985), but intragastric administration of EGF only inhibits acid secretion when very high doses are used (Konturek *et al.*, 1981). Thus EGF in the gastric lumen could be derived from saliva and might play a role in day-to-day maintenance of the gastric epithelium (Skinner *et al.*, 1984), but luminal EGF is unlikely to regulate gastric acid secretion. Inhibition of acid secretion by EGF might occur during acute damage to the epithelium, and the absence of acid might facilitate the process of repair (Gregory, 1985; Shaw *et al.*, 1987). In such circumstances EGF could be derived from platelets (Shaw *et al.*, 1987).

The inhibitory effect of EGF on acid secretion *in vitro* can be studied with gastric glands (Dembinski *et al.*, 1986) or isolated parietal cells (Shaw *et al.*, 1987), by using the accumulation of the weak base aminopyrine in acidic spaces within parietal cells as an index of secretory activity. Work with rat parietal cells suggested that EGF might act at a site close to the generation of hydrolysis of cyclic AMP (Shaw *et al.*, 1987), and this possibility is investigated here.

EGF seems a more potent inhibitor of acid secretion *in vivo* (Bower *et al.*, 1975) than *in vitro* (Shaw *et al.*, 1987).

Although prostaglandins were ruled out as being involved in the mechanism by which EGF inhibited aminopyrine accumulation in isolated cells (Shaw *et al.*, 1987), this could be because of their dilution in the incubation medium, which would not occur *in vivo*. Furthermore, EGF stimulated prostaglandin production by the perfused rat stomach (Chiba *et al.*, 1982), and the possibility of such an effect in isolated cells from the gastric mucosa was therefore investigated.

EXPERIMENTAL

Animals

Male Wistar rats (200–300 g body wt.) were obtained from Bantin 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 given by ICI, Alderley Park, Cheshire, U.K. Radioimmunoassay kits (¹²⁵I) for the measurement of cyclic AMP and PGE₂ were purchased from New England Nuclear. Insulin, cortisol, gentamicin and foetal-calf serum were from Sigma. The source of other chemicals has been described previously (Anderson & Hanson, 1985).

Preparation, purification and incubation of parietal cells

Parietal cells were prepared from the fundic portion of the stomach of fed rats as described by Shaw *et al.* (1985). The crude cell preparation, which contained

Abbreviations used: EGF, epidermal growth factor; IBMX, 3-isobutyl-1-methylxanthine; PG, prostaglandin.

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about 20% parietal cells, was resuspended in Eagle's minimum essential medium containing 36 ml of iso-osmotic Percoll/100 ml, 12 mM-Hepes, 0.6 mg of bovine serum albumin/ml, 0.3 mM-dithiothreitol and 1.9 mM-EGTA, pH 7.35. This mixture was centrifuged for 13 min at 30000 g_{av} at 4 °C with a 20° angle rotor in a MSE Superspeed 50 centrifuge. The cell fraction removed from the top 20% of the gradient contained > 80% parietal cells (e.g. from ten experiments $\text{mean} \pm \text{s.e.m.} = 85 \pm 1\%$) and was used in all of the experiments described below unless stated otherwise. The other major band lower down the gradient at a density of 1.06 g/ml contained $12 \pm 1\%$ ($n = 5$) parietal cells and was used in one series of experiments.

Preincubation of the purified cell fractions for 2 h at 37 °C in Eagle's minimum essential medium containing 20 mM-Hepes, 1 mg of bovine serum albumin/ml, 8 μg of insulin/ml, 10 nM-cortisol, 50 μg of gentamicin/ml and 5% foetal-calf serum (Soll *et al.*, 1986) was used to make the purified cells responsive to histamine in the absence of a phosphodiesterase inhibitor.

Cell suspensions were incubated in polyethylene vials at $1.55 (\pm 0.12) \times 10^6$ cells/ml ($n = 13$) for the experiments involving measurement of cyclic AMP, and in silicone-treated glass vials at $1.6 (\pm 0.1) \times 10^6$ cells/ml ($n = 11$) for experiments involving assay of prostaglandin production. The incubation medium was always Eagle's minimum essential medium with Earle's salts plus bovine serum albumin (1 mg/ml) and 20 mM-Hepes, pH 7.35. When aminopyrine accumulation was measured, [^{14}C]aminopyrine (0.1 $\mu\text{Ci/ml}$; 0.9 μM) and [^3H]poly(ethylene glycol) (0.4 $\mu\text{Ci/ml}$) were also present. The aminopyrine accumulation ratio, which is the ratio of the concentration of aminopyrine in the cell to that in the incubation medium, was determined as described previously (Shaw *et al.*, 1985). For reasons which have been discussed previously (Shaw *et al.*, 1987), aminopyrine accumulation ratios are presented in normalized form.

Assay of cyclic AMP

Samples of the cell suspension were mixed with an equal volume of ice-cold ethanol and stored at -20 °C. Correction for cyclic AMP in the medium had no effect on the percentage inhibition of the histamine-stimulated cyclic AMP content by EGF, and routine measurements were therefore made on the cell suspension. This practice is also followed by other workers with parietal cells (Wollin *et al.*, 1979; Puurunen *et al.*, 1987). Furthermore, use of the cell suspension means that samples can be extracted immediately without the introduction of potential error from a centrifugation step to separate the cells from the medium.

Immediately before assay the samples were thawed, and centrifuged at 10000 g for 1 min, and the supernatant was evaporated to dryness in a vacuum oven at 55 °C. The residues were dissolved in buffer and assayed by using an acetylated radioimmunoassay procedure (New England Nuclear). The recovery of cyclic [^3H]AMP taken through this procedure was $85 \pm 1\%$ ($n = 6$), the lower limits of sensitivity of the assay were 0.10–0.25 pmol/ml, and the cross-reactivity with cyclic GMP, GMP, ATP, and ADP was less than 0.01%.

Assay of PGE₂

Cells were removed by rapid centrifugation (10000 g

for 30 s), and assays were performed on the incubation medium. Only the medium was assayed, because Schepp *et al.* (1986) showed that PGE₂ produced during a 30 min incubation was not retained inside the cells, but was released into the medium. Such samples were immediately acidified to pH 3 with 1.0 M-HCl and were extracted twice with 3 vol. of ethyl acetate. The extracts were evaporated to dryness under a stream of N₂ and stored at -20 °C. The recovery of [^3H]PGE₂ taken through this procedure was $89 \pm 3\%$ ($n = 4$). Samples were reconstituted in buffer and assayed with a [^{125}I]PGE₂ assay kit (New England Nuclear). The lower limits of sensitivity of the assay were 0.25–0.5 pg/100 μl . The cross-reactivity with PGE₁ was 3.7% and with 6-oxoPGF_{1 α} , PGB₂ and PGF_{2 α} was less than 0.01%. The inter-assay and intra-assay coefficients of variation were 9.3 and 3.9% respectively.

RESULTS

Effect of incubation time on the aminopyrine accumulation ratio and the cyclic AMP content of the cell suspension

The aminopyrine accumulation ratio reached a plateau after 30 min incubation with 0.5 mM-histamine. The presence of 200 nM-EGF in cell suspensions incubated with 0.5 mM-histamine significantly decreased the aminopyrine accumulation ratio after 30 and 60 min of incubation (Fig. 1). The cyclic AMP content of the cell suspension reached a maximum after 5 min incubation with 0.5 mM-histamine and then remained essentially unchanged for a further 55 min. The presence of 200 nM-EGF decreased histamine-stimulated cyclic AMP after both 5 and 30 min of incubation (Fig. 1). There was no significant effect of incubation time on basal cyclic AMP content, or on the basal aminopyrine accumulation ratio between 5 and 60 min after the start of incubation.

Effects of EGF concentration on cyclic AMP content and aminopyrine accumulation ratio

EGF produced a dose-related inhibition of the cyclic AMP content of cells incubated with 0.5 mM-histamine for 30 min (Table 1). The cyclic AMP content was significantly decreased by 20 nM- and 200 nM-EGF; 200 nM-EGF had no effect on the basal cyclic AMP content.

Comparison of the above results with the effects of EGF concentration on aminopyrine accumulation under similar conditions was effected by measuring cyclic AMP content and aminopyrine accumulation simultaneously on the same batch of cells. Basal values for aminopyrine accumulation and cyclic AMP were subtracted, and then the inhibitory effect of EGF was calculated as a percentage of the uninhibited value (Fig. 2).

The half-maximally effective concentration of EGF for inhibition of aminopyrine accumulation was 3.0 nM, and that required for half-maximal inhibition of cyclic AMP content was 3.9 nM. No significant difference was detectable between the inhibition of cyclic AMP content and aminopyrine accumulation at either 20 nM- or 200 nM-EGF (paired t test) (Fig. 2). There was a significant correlation ($P < 0.05$) between the stimulation of the cyclic AMP content above basal by 0.5 mM-histamine (x) and the stimulation of the aminopyrine accumulation ratio above basal (y): $y = 6.49 + 27.9x$, $r = 0.59$, 13 cell batches.

Fig. 1. Effect of incubation time on the aminopyrine accumulation ratio, and the cyclic AMP content of the cell suspension in control cells and in those incubated with 0.5 mM-histamine in the presence and the absence of 200 nM-EGF

Results from three or four batches of cells have been normalized by expressing them as a percentage of the value obtained at 30 min in the presence of 0.5 mM-histamine. Results are expressed as means $\pm$ S.E.M.: $\triangle$, no addition; $\square$, 0.5 mM-histamine; $\blacksquare$, 0.5 mM-histamine plus 200 nM-EGF. Values used for normalization were: aminopyrine accumulation ratio, 17.3 ± 5.1 (4); cyclic AMP, 2.1 ± 0.4 (4) pmol/ 10^6 cells. $*P < 0.05$, $**P < 0.01$ for a significant effect of EGF by a paired *t* test on histamine-stimulated values.

Effect of EGF in the presence of IBMX

Shaw *et al.* (1987) showed that 200 nM-EGF did not decrease the aminopyrine accumulation ratio in cells stimulated with a range of concentrations of histamine in the presence of 0.1 mM-IBMX. A similar lack of effect of EGF was obtained when the cyclic AMP content of the cell suspensions stimulated with histamine and 0.1 mM-IBMX was determined (Table 2). IBMX is a cyclic AMP phosphodiesterase inhibitor and therefore increases cyclic AMP. An increased cyclic AMP content was not responsible for this lack of effect of EGF, because cyclic AMP contents of 2.1 ± 0.4 (4) (Fig. 1) and 1.15 ± 0.27 (4)

Table 1. Effect of EGF on the cyclic AMP content of a suspension of rat parietal cells incubated in the presence and the absence of 0.5 mM-histamine

Results from four batches of cells are expressed as means $\pm$ S.E.M.: $**P < 0.01$, $*P < 0.05$ by Dunnett's test (Zar, 1984) for the effect of EGF on the cyclic AMP content of suspensions stimulated with 0.5 mM-histamine. There was no effect of 200 nM-EGF on the cyclic AMP content of cell suspensions incubated without secretagogue (paired *t* test).

Secretagogue	Concn. of EGF (nM)	Cyclic AMP content (pmol/ 10^6 cells)
0.5 mM-Histamine	0	1.15 ± 0.27
	0.2	1.13 ± 0.26
	2	1.09 ± 0.24
	20	$0.94 \pm 0.20^{**}$
	200	$0.96 \pm 0.23^*$
None	0	0.83 ± 0.19
	200	0.80 ± 0.17

Fig. 2. Effect of the concentration of EGF on the inhibition of aminopyrine accumulation and of the cyclic AMP content of the cell suspension

The inhibitory effect of EGF is expressed as a percentage of the stimulatory effect of 0.5 mM-histamine above basal. Results are means $\pm$ S.E.M. from four batches of cells. $\square$, Aminopyrine accumulation ratio; $\blacksquare$, cyclic AMP content. The basal and stimulated values for cyclic AMP content are shown in Table 1. The basal aminopyrine accumulation ratio was 2.51 ± 0.90 , and stimulation above basal by 0.5 mM-histamine was 9.0 ± 0.76 . The lines have been drawn by using the computer program FIT (Barlow, 1983).

(Table 1) were obtained with 0.5 mM-histamine alone when EGF was effective, and these were higher than some cyclic AMP contents obtained in the presence of low histamine and 0.1 mM-IBMX (Table 2). The inhibitory action of 10 nM-PGE₂ on cyclic AMP content was not prevented by IBMX.

Effect of EGF on PGE₂ production

Results obtained for PGE₂ production were variable, but this variation seemed to reside in the background

Table 2. Action of 200 nM-EGF and 10 nM-PGE₂ on cyclic AMP in cell suspensions incubated with different concentrations of histamine in the presence of constant 0.1 mM-IBMX

Results are presented as means $\pm$ S.E.M.: ** $P < 0.01$ for the effect of the agent, by paired t test. Basal cyclic AMP contents were as follows: control, 0.77 ± 0.09 (6); 200 nM-EGF, 0.91 ± 0.19 (6); 10 nM-PGE₂, 0.72 ± 0.13 (4). There was no significant effect of either EGF or PGE₂ on basal values.

Concn. of histamine (μ M)	No. of cell batches	Agent	Cyclic AMP content (pmol/ 10^6 cells)	
			Control	Agent
0.5	4	200 nM-EGF	1.06 ± 0.18	1.03 ± 0.19
1	4	200 nM-EGF	1.38 ± 0.23	1.35 ± 0.23
3	5	200 nM-EGF	1.62 ± 0.04	1.51 ± 0.13
20	5	200 nM-EGF	1.82 ± 0.09	1.79 ± 0.13
500	5	200 nM-EGF	3.03 ± 0.40	2.94 ± 0.32
500	4	10 nM-PGE ₂	3.27 ± 0.41	$1.70 \pm 0.24^{**}$

Fig. 3. Concentration-dependence of the stimulation of PGE₂ production by EGF

Results are from six batches of cells and are presented as means $\pm$ S.E.M. of the stimulation of PGE₂ production above that in the absence of EGF, which was 6.7 ± 2.00 . The line has been drawn by using the computer program FIT (Barlow, 1983).

and not in the stimulatory effect of EGF. Thus 200 nM-EGF stimulated PGE₂ production (pg/30 min per 10^6 cells) by 5.4 ± 1.4 when basal PGE₂ production was 6.7 ± 2.0 (Fig. 3), and by a similar amount, 6.9 ± 1.6 , when basal production was 20.1 ± 1.9 (Table 3). This variation in basal PGE₂ could not be ascribed to inter-assay variation (see the Experimental section). The half-maximally effective concentration of EGF for stimulation of PGE₂ production was 7.5 nM. In the presence of 200 nM-EGF, the concentration of PGE₂ in the medium was 84 ± 13 pM (11).

PGE₂ production in a cell fraction isolated from a region close to the bottom of the Percoll gradient, and which was depleted of parietal cells, was lower than that in the fraction enriched with parietal cells (Table 3). A similar result was found by Postius *et al.* (1985). EGF did

Table 3. Effect of the proportion of parietal cells and the presence of histamine on the stimulation of PGE₂ production by 200 nM-EGF

Results for five batches of cells are expressed as means $\pm$ S.E.M. Basal PGE₂ production was significantly higher in the cell fraction enriched with parietal cells ($P < 0.01$) than in the fraction depleted of parietal cells. ** $P < 0.025$ for a significant stimulation of PGE₂ production above basal by 200 nM-EGF. Data were analysed by a paired t test.

Proportion of parietal cells (%)	Histamine concn. (mM)	PGE ₂ production (pg/30 min per 10^6 cells)	
		Basal	Stimulation by 200 nM-EGF above basal
11.5 ± 0.7	0	8.3 ± 1.3	0.30 ± 0.37
82.0 ± 1.6	0	20.1 ± 1.9	$6.9 \pm 1.6^{**}$
82.0 ± 1.6	0.5	20.2 ± 1.2	0.89 ± 2.4

not stimulate PGE₂ production in the fraction depleted of parietal cells (Table 3). Addition of 0.5 mM-histamine to the fraction enriched with parietal cells did not alter PGE₂ production, but abolished the stimulatory effect of EGF.

DISCUSSION

The stimulation of cyclic AMP content by histamine alone was small. The reason for this low response is unclear. Although compartmentalization of cyclic AMP may occur (see below), it is a somewhat unlikely explanation of this low response of total cyclic AMP to histamine, for the changes in aminopyrine accumulation were also small (see legends of Figs. 1 and 2) by comparison with results obtained in cells stimulated near-maximally with 0.5 mM-histamine and 0.1 mM-IBMX [mean stimulation above basal in six batches of cells ($\pm$ S.E.M.), 196 ± 44].

Since the histamine-induced change in total cyclic AMP was not at variance with the secretory response, it is not necessary to invoke compartmentalization of cyclic AMP to explain the small overall response. Although the cell fraction used in the majority of this work contained predominantly parietal cells, it was not pure. Histamine stimulation of cyclic AMP content is probably restricted to the parietal cells, because the magnitude of the histamine stimulation correlates with the proportion of parietal cells (Sonnenberg *et al.*, 1978; Wollin *et al.*, 1979). Since EGF inhibits histamine-stimulated cyclic AMP content, it is likely that this effect also resides in the parietal cell. An important point which requires examination is whether the decrease in cyclic AMP induced by EGF causes the decrease in the aminopyrine accumulation ratio which is used as an index of acid secretion.

Measurements made after aminopyrine accumulation had reached a plateau, in cells stimulated under conditions where there was moderate increase in cyclic AMP, demonstrate a correlation between cyclic AMP content and the aminopyrine accumulation ratio (the present work; Soll & Wollin, 1979; Batzri & Dyer, 1981). However, the rise in cyclic AMP content to a plateau after stimulation precedes in time the rise in aminopyrine

accumulation (Fig. 1; cf. Wollin *et al.*, 1979; Soll, 1980). Accumulation of aminopyrine is caused by the sequestration of acid in spaces (secretory canaliculi) inside the parietal cell, and it is probably only after a steady state has been achieved that this measurement can be used as a reliable index of secretory activity. Thus an effect of EGF on cyclic AMP content of parietal cells after 5 min of incubation, but no effect on aminopyrine accumulation (Fig. 1), cannot be used to disprove a link between the effects of EGF on cyclic AMP and aminopyrine accumulation. The absence of a significant decrease in cyclic AMP by EGF after 1 h incubation may reflect the onset of down-regulation of the EGF receptor, but aminopyrine accumulation, which reflects the previous history of the cells, remains depressed by EGF.

Although the half-maximally effective concentrations of EGF for inhibition of aminopyrine accumulation and cyclic AMP content are close, the dose-response curves for the effect of EGF concentration on these two measures of secretory activity appear to be of different shape. This difference seems to derive from a greater percentage inhibition of cyclic AMP than of aminopyrine accumulation by 20 nM- and 200 nM-EGF, although the significance of the difference is not proved by paired *t* tests. The potential difference in the shape of the dose-response curves to EGF could be explained by EGF-induced changes in the concentration of cyclic AMP in the vicinity of type I cyclic AMP-dependent protein kinase (Chew, 1985), being less than those for total cyclic AMP.

In conclusion, the evidence that the effect of EGF on secretory activity is caused by a decline in cyclic AMP content is the following. (i) Cyclic AMP is thought to be the second messenger for histamine (Soll & Berglin, 1987), and cyclic AMP content correlates with aminopyrine accumulation provided that the cells are not over-stimulated. A fall in cyclic AMP content should therefore produce a fall in secretory activity. (ii) The half-maximally effective concentrations of EGF for inhibition of cyclic AMP content and aminopyrine accumulation are close (Fig. 2). (iii) The effects of EGF on cyclic AMP content and aminopyrine accumulation are both blocked by IBMX.

The parietal cells seem the most likely site of the stimulation of prostaglandin production by EGF. Firstly, an effect of EGF was not detectable in the cell fraction depleted of parietal cells. Secondly, the half-maximal concentration of EGF required for stimulation of prostaglandin production was close to that required for depression of cyclic AMP content. Thirdly, the effect of EGF was blocked by 0.5 mM-histamine, and histamine receptors are known to be present on parietal cells. The inhibitory effect of histamine on the stimulation of PGE₂ production by EGF could have been mediated by elevation of cyclic AMP content, which has been shown in several cell types to inhibit prostaglandin production (Hassid, 1982).

In cells incubated with 200 nM-EGF the concentration of PGE₂ was 84 pM, whereas the half-maximally effective concentration of PGE₂ for inhibition of aminopyrine accumulation in rat parietal cells is 24 nM (M. M. Atwell & P. J. Hanson, unpublished work). Therefore essentially no effect of prostaglandins in the medium on aminopyrine accumulation would be detectable in the isolated-cell preparation (Shaw *et al.*, 1987). The prevention of the effect of EGF on prostaglandin production by histamine

also explains why no involvement of prostaglandins in the action of EGF was found in the isolated-cell preparation. In intact tissue prostaglandins released into the extracellular space will not be diluted, and could be at a sufficient concentration to inhibit secretion. However, since histamine will be present in actively secreting tissue, it is difficult, taking the present results into account, to see how EGF could exert an inhibitory effect via prostaglandin production from parietal cells. An alternative possibility is that EGF might cause prostacyclin production from endothelial cells *in vivo* (Vane *et al.*, 1987), and that the prostacyclin might exert an inhibitory effect on parietal cells (Soll & Whittle, 1981).

The inhibition of the action of EGF by IBMX suggests that one site of action of EGF may be a cyclic AMP phosphodiesterase. Establishment of the target upon which EGF acts to decrease cyclic AMP in parietal cells will require measurement of the effects of preincubation with EGF on adenylate cyclase and cyclic AMP phosphodiesterase activities assayed subsequently in broken-cell preparations.

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REFERENCES

- Anderson, N. G. & Hanson, P. J. (1985) *Biochem. J.* **232**, 609–611
- Barlow, R. B. (1983) *Biodata Handling with Microcomputers*, pp. 126–133, Elsevier, Amsterdam
- Batzri, S. & Dyer, J. (1981) *Biochim. Biophys. Acta* **675**, 416–426
- Bower, J. M., Camble, R., Gregory, H., Gerring, E. L. & Willshire, I. R. (1975) *Experientia* **31**, 825–826
- Chew, C. S. (1985) *J. Biol. Chem.* **260**, 7540–7550
- Chiba, T., Hirata, Y., Taminato, T., Kadowaki, S., Matsukura, S. & Fujita, T. (1982) *Biochem. Biophys. Res. Commun.* **105**, 370–374
- Dembinski, A., Gregory, H., Konturek, S. J. & Polanski, M. (1982) *J. Physiol. (London)* **325**, 35–42
- Dembinski, A., Drozdowicz, D., Gregory, H., Konturek, S. J. & Warzecha, Z. (1986) *J. Physiol. (London)* **378**, 347–357
- Gregory, H. (1985) *J. Cell. Sci. Suppl.* **3**, 11–17
- Hassid, A. (1982) *Am. J. Physiol.* **243**, C205–C211
- Konturek, S. K., Brzozowski, T., Piastucki, I., Dembinski, A., Radecki, T., Dembinska-Kiec, A., Zmuda, A. & Gregory, H. (1981) *Gut* **22**, 927–932
- Postius, S., Ruoff, H.-J. & Szeleyni, I. (1985) *Br. J. Pharmacol.* **84**, 871–877
- Puurunen, J., Lohse, M. J. & Schwabe, U. (1987) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **336**, 471–477
- Schepp, W., Steffen, B., Schusdzarra, V. & Classen, M. (1986) *J. Clin. Endocrinol. Metab.* **63**, 886–891
- Shaw, G. P., Anderson, N. G. & Hanson, P. J. (1985) *Biochem. J.* **227**, 223–229
- Shaw, G. P., Hatt, J. F., Anderson, N. G. & Hanson, P. J. (1987) *Biochem. J.* **244**, 699–704
- Skinner, K. A., Soper, B. D. & Tepperman, B. L. (1984) *J. Physiol. (London)* **351**, 1–12

- Soll, A. H. (1980) *Am. J. Physiol.* **238**, G366–G375
- Soll, A. H. & Berglindh, T. (1987) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., ed.) 2nd edn., pp. 883–909, Raven Press, New York
- Soll, A. H. & Whittle, B. J. R. (1981) *Prostaglandins* **21**, 353–365
- Soll, A. H. & Wollin, A. (1979) *Am. J. Physiol.* **237**, E444–E450
- Soll, A. H., Chen, M. C. Y., Amirian, D. A., Toomey, M. & Alvarez, R. (1986) *Am. J. Med.* **81**, suppl. 2A, 5–11
- Sonnenberg, A., Hunziker, W., Koelz, H. R., Fischer, J. A. & Blum, A. L. (1978) *Acta Physiol. Scand. Spec. Suppl.* pp. 307–317
- Vane, J. R., Gryglewski, R. J. & Botting, R. M. (1987) *Trends Pharmacol. Sci.* **8**, 491–496
- Wollin, A., Soll, A. H. & Samloff, I. M. (1979) *Am. J. Physiol.* **237**, E437–E443
- Zar, J. H. (1984) *Biostatistical Analysis*, pp. 194–195, Prentice-Hall, Englewood Cliffs, NJ

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