Effect of epidermal growth factor on acid and pepsinogen secretion by rat isolated stomach cells stimulated with forskolin

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Epidermal growth factor (EGF) from mouse submaxillary glands is a polypeptide of M, 6045 which stimulates epithelial cell proliferation and which inhibits gastric acid secretion both in vivo [1] and in vitro [2, 3]. Inhibition of pepsinogen secretion by EGF has been observed in vivo [4], but this effect could be secondary to an inhibition of acid secretion. The mechanism by which EGF inhibits acid secretion induced by histamine may involve a decrease in the cyclic AMP content of the parietal cell [3].

The intention of this work was to investigate whether EGF could also inhibit acid secretion induced by forskolin which acts directly to activate adenylate cyclase. If such an effect were found then a comparison could be made with the effect of EGF on forskolin-stimulated pepsinogen secretion.

Stomach cells were isolated from the rat fundus by pronase digestion and intermittent calcium chelation [5]. The accumulation of the weak base aminopyrine was used as an index of acid secretion [5], and pepsinogen secretion was determined by measuring the ability of supernatant samples to hydrolyze acid-denatured haemoglobin [6].

Forskolin produced a dose-related increase in the aminopyrine accumulation ratio, which was 49 ± 6 (mean ± S.E.M. from 12 batches of cells) in the presence of 50 μM-forskolin. Responses to 1.6, 5 and 16 μM-forskolin were 13, 33 and 58% of that obtained with 50 μM-forskolin. EGF (200 nm) only inhibited aminopyrine accumulation stimulated by 1.6 and 5 μM-forskolin. Similar results were obtained in the presence of 10 μM-cimetidine, which was added to block the effects of any endogenous histamine, should it be present. The inhibitory action of EGF against stimulation induced by low concentrations of forskolin was prevented by the presence of 3-isobutyl-1-methylxanthine (0.1 mm) and by preincubation of the cells for 2 h with pertussis toxin (100 ng/ml). Forskolin also produced a dose-related stimulation of pepsinogen secretion, but its action was not inhibited by 200 nm-EGF, whatever the level of the secretory response. EGF (200 nm) did not affect either basal aminopyrine accumulation or basal pepsinogen secretion.

An enriched fraction containing greater than 80% of parietal cells was somatotyped and was used for assay of adenylate cyclase [7]. Stimulation of adenylate cyclase activity by 5 μM-forskolin was 81 ± 17 (n = 4) pmol cyclic AMP formed/10 min per 106 cells. This stimulation was unaffected by the presence of 200 nm-EGF.

The mechanism by which EGF inhibits acid secretion induced by histamine [3] and by forskolin may be similar, because, in both cases, the action of EGF was prevented by preincubation with pertussis toxin or by the presence of 3-isobutyl-1-methylxanthine. The lack of effect of EGF on forskolin-stimulated adenylate cyclase activity, and the action of 3-isobutyl-1-methylxanthine as a phosphodiesterase inhibitor, suggest that EGF may activate a cyclic AMP phosphodiesterase enzyme in the parietal cell, and that EGF may only activate one phosphodiesterase with a low Kₘ for cyclic AMP. At high concentrations of cyclic AMP, the contribution of this phosphodiesterase to the rate of cyclic AMP breakdown, and therefore any effect of EGF, would be minimal. There was no evidence for a similar action of EGF against forskolin-stimulated pepsinogen secretion by chief cells.

Abbreviation used: EGF, epidermal growth factor.


Expression of recombinant horseradish peroxidase C in Escherichia coli

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Peroxidases form a broad class of haem-containing enzymes that utilize H₂O₂ and act on a wide range of substrates. Although several different genes encoding plant peroxidase have been isolated and sequenced [1], as far as we are aware none have been successfully expressed in heterologous systems such as Escherichia coli or Saccharomyces cerevisiae. With a view to performing site-directed mutagenesis on horseradish (Armoracia rusticana) peroxidase C (HRP C; EC 1.11.1.7) in order to understand further its mechanism of action [see e.g. [2]], we have now synthesized a synthetic gene encoding the enzyme and have achieved expression in E. coli.

Abbreviation used: HRP C, horseradish peroxidase C.

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The gene was chemically synthesized on the basis of the protein sequence determined by Welinder [3]. It is 956 nucleotides in length and contains multiple restriction endonuclease sites (Fig. 1A).

To regulate expression, the synthetic gene was cloned into a plasmid vector containing a tac promoter and transfected into E. coli HW110. When induced with isopropylthiogalactoside, insoluble and inactive HRP C protein was produced. Solubilization under denaturing conditions, subsequent refolding and purification yielded enzymically active HRP C. Fig. 1(B) shows the activity of native enzyme purified by f.p.l.c. from horseradish root (Sigma), the insoluble enzyme as prepared from induced E. coli and the refolded active recombinant enzyme after fractionation on f.p.l.c.

These initial observations suggest that sufficient quantities of recombinant HRP C can be obtained for kinetic and physical studies on the enzyme. Attempts to optimize the refolding reaction are in progress.

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Analysis of conformation of mutant proteins by studying in vitro translation products

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Existing methods for monitoring the effects on protein conformation and stability of specific mutations in the DNA sequence involve expressing the protein in vivo and subsequent purification and renaturation. We are developing methods which will enable the identification of mutations, which lead to changes in protein conformation or stability, without the need to express the protein in vivo. The genes of interest are cloned into transcription/translation vectors and transcribed in vitro. The resulting transcript is translated in a rabbit reticulocyte lysate supplemented with dog pancreas microsomes. This system has been optimized for translocation and processing of the newly synthesized protein and for co-translational disulphide bond formation. The radio-labelled translation product can then be characterized by electrophoretic techniques after various treatments to assess its folding, proteinase susceptibility or enzymic function; hence the effects of specific mutations on protein conformation, stability or function can be evaluated. Two examples of the application of these techniques are described here.

Abbreviation used: SDS/PAGE, SDS/polyacrylamide-gel electrophoresis.

Prochymosin is a zymogen of the aspartic proteinase chymosin produced in the calf stomach and exploited in cheese processing [1]. When the cDNA for prochymosin is expressed in Escherichia coli, insoluble inclusion bodies are formed which contain incorrectly folded prochymosin [2]. Activatable prochymosin can be recovered from these inclusion bodies by solubilization and renaturation. This reversible denaturation contrasts with the irreversible denaturation of the active enzyme chymosin. Thus it appears that the pro-sequence is important in the folding of prochymosin. When in vitro transcribed RNA coding for pre-prochymosin is translated in vitro in the absence of microsomes a single translation product is observed (Fig. 1, lane 4). When microsomes are included in the translation system, a polypeptide with a slightly faster electrophoretic mobility is synthesized indicating processing of the signal peptide (lane 2, cf. lane 4). When proteinase K is added post-translationally, the product translated in the absence of microsomes is completely degraded (lane 8), whereas the product translated in the presence of microsomes is protected from proteolysis (lane 6). Thus the newly synthesized protein is translocated to the vesicle interior and is protected from exogenous proteinase. When the microsomes are solubilized with detergent before proteolysis, the translation product is only partially degraded and a large fragment remains (lane 7). This proteinase-resistant peptide was also observed with authen-