The Inhibition of Gastric Acid Secretion by Epidermal Growth Factor

Epidermal growth factor (EGF) is a polypeptide isolated from the submaxillary glands of male mice which has the striking property of causing premature eye opening and incisor eruption when injected into new born mice[1]. This has been shown to be the result of increased keratinization of the epidermis[2]. The primary structure of EGF has been determined recently; it is composed of 53 amino-acid residues with 3 disulphide bonds although a fully active peptide of 51 residues has also been isolated[3].

EGF will stimulate cell proliferation in a variety of organ cultures[4]. It has similar effects to insulin in mouse mammary epithelial cells[5], and it will cause rapid regeneration of rabbit corneal epithelium[6]. The full range of actions has been reviewed recently[7]. It is not yet apparent whether this particular peptide fulfils any physiological role but it may possibly be involved in the control of growth of certain tissues. Relatively small amounts will stimulate DNA and RNA synthesis in cultured human fibroblasts[8] and a variety of mammalian tissues will bind epidermal growth factor at sites distinct from those occupied by other anabolic peptides such as insulin[9]. However, in addition to their effects on tissue growth, peptides such as insulin or gastrin also have a well defined 'short term' action and this report describes a direct effect of EGF upon gastric acid secretion which is unlikely to be the result of cell proliferation.

Materials and methods. Epidermal growth factor was isolated from the submaxillary glands of male and female mice according to the method of SAVAGE and COHEN[10] with minor modifications. The properties of the product were fully in accord with those published and in particular amino-acid analysis showed a complete lack of alanine, phenylalanine or lysine residues. The potency was confirmed by the action on new born mice (Alderley Park Strain). Subcutaneous injections of 5 µg peptide in 25 µl saline solution per day induced eye opening on day 10 compared to day 13 in controls.

The action of this product on gastric secretion was examined in both rats and dogs[11]. Rat testing was carried out in anaesthetized animals (Alderley Park Strain) with the stomach perfused and the acid content of the perfusate was determined by titration. Acid secretion was induced by s.c. injection of histamine (300 µg) at 1 h intervals and after 2 control responses, EGF was given by i.v. injection at the time of the next histamine injection (Figure 1). Alternatively, the action of EGF was examined in rats against a plateau of secretion evoked by repeated histamine (20 µg) injections at 5 min intervals[12]. In both cases near maximal inhibition was obtained with doses of 10–50 µg per kg.

The effect of the growth factor was also studied in Beagle dogs with a denervated gastric pouch, with denervated pouch and fistula in the gastric remnant, or with an innervated gastric pouch. The dogs were fasted overnight and then gastric secretion was stimulated by an i.v. infusion of either histamine, pentagastrin or methacholine to about 70% of the maximum output. When the secretion was steady, EGF was given as i.v. or s.c. injections at doses as low as 0.1 µg/kg (Figure 2). The duration of the inhibition was about 90 min but with the larger doses it took longer for the secretion to return to the original plateau level. However, using a sensitive radio-immunoassay quantitation process, it was found that the half time of disappearance of the peptide from blood was about 4 min[13].

Discussion. The original effect of EGF upon eye opening in new-born mice requires doses of approximately 200 µg/kg s.c. per day whereas doses of 10 µg/kg in rats and 0.5 µg/kg in dogs produces a profound effect upon gastric acid secretion. This effect appears to be specific for gastric secretion and doses of peptide up to 15 µg/kg s.c. in dogs, produce no untoward effects upon respiration, heart rate or temperature.

Although epidermal growth factor is found in large amounts in the salivary gland it also appears in other bodily fluids of the mouse including the saliva[14]. Inter-

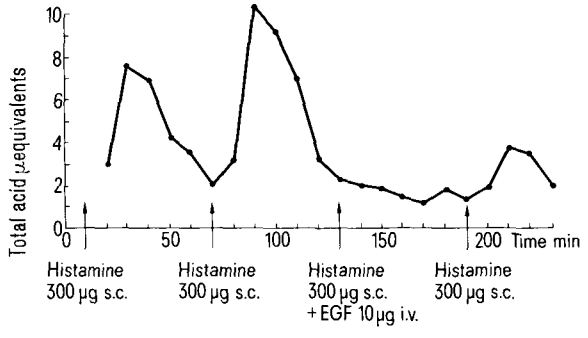

Fig. 1. Inhibition of histamine induced acid secretion in a perfused rat stomach preparation by epidermal growth factor.

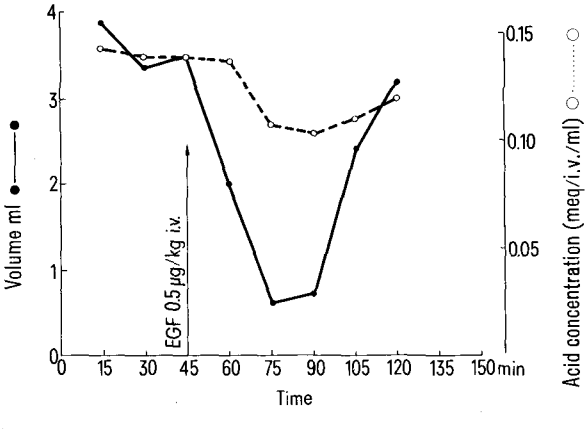

Fig. 2. The effect of a single i.v. injection of epidermal growth factor in a Heidenhain pouch dog upon volume and acid concentration – secretion induced by an infusion of histamine at 30 µg/kg/h.

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estingly there is an association between human saliva and the inhibition of acid secretion. Thus in 1949 it was found that the injection of human saliva into animals would inhibit gastric acid secretion[15] and it was found later that the sublingual gland provided the greatest amount of inhibitory material[16]. The extract called sialogastrone caused the inhibition of secretion in pylorus ligated rats by over 50% at doses of 30 mg/kg. Further purification gave material with antisecretory activity at 50 μg/kg in rats but the product behaved as a compound with a molecular weight of about 150,000[17]. At the same time inhibitory material (MW 50,000) was also obtained from mouse submaxillary glands with an activity of 25 μg/kg but these workers found none of the sexual dimorphism associated with epidermal growth factor[18]. This sex difference is quite striking; the male mouse contains ca. 1000 ng/mg wet tissue and the female only ca. 70 ng/ml[19], but treatment of the female with testosterone causes a dramatic rise in the levels of EGF[14]. Smaller amounts were also observed in rat salivary glands[1], but inhibition of acid secretion can be effected in rats, albeit at higher doses than are necessary in the dog. A recent publication described the identification of immunoreactive EGF in the plasma of pregnant human females but not in non-pregnant females or males[20] and levels up to 6 ng/ml were reported during early pregnancy. However, if the doses administered to dogs ~ 0.5 μg/kg i.v., were calculated as blood levels then the maximum briefly attainable would be about 6 ng/ml and this represents near maximal inhibition of gastric acid secretion. If similar doses applied to the human then a persistent blood level of 6 ng/ml would presumably be associated with greatly reduced acid secretion unless, of course, sensitivity was reduced during pregnancy. This may relate to the known low incidence of peptic ulceration during pregnancy.

Among the known properties of EGF in causing cell proliferation is the stimulation of ornithine decarboxylase[21]. More recently it was reported that histidine decarboxylase was also stimulated by EGF in skin and other tissues although gastric mucosa was not amongst those studied[22]. Nevertheless a number of other inhibitors of gastric secretion are also associated with increased histidine decarboxylase activity in the gastric mucosa even though histamine itself is a stimulant of acid secretion[23]! Possibly EGF exerts a similar effect upon gastric mucosa.

The doses of EGF required to affect gastric secretion in dogs are in the same region as those for the hormone gastrin[24], and are low compared to those required for the other observable effects, so that it is tempting to speculate that it is a truly physiological effect. Indeed it may well be that comparable peptides from different species are involved in the control of gastric acid secretion.

Résumé. Le facteur de croissance épidermique (EGF) a été isolé à partir des glandes sous-maxillaires des souris. On a trouvé que ce facteur est un inhibiteur de la sécrétion gastrique. Chez les chiens des doses aussi faibles que 0,1 μg/kg font baisser la sécrétion gastrique provoquée par divers stimulants.

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Modification by Calcium Dobesilate of Histamine Effects on Capillary Ultrastructure

As is well known, the existence of close contacts between cells is the most peculiar feature of epithelial structures. In the vascular endothelium, and particularly at the blood capillary level, intercellular bonds through interdigitation create a selective barrier between the vascular compartment and the interstitial fluid[1]. The stability of these intercellular contacts depends on the chemical composition of the cell membrane, and more precisely of the cell coat, as has been shown in several experimental studies[2]. On the other hand, endothelial cells, and bonds between them, constitue the major structural base of capillary permeability and capillary resistence. Several endogenous chemicals, and among them particularly histamine, are capable of disturbing capillary function, leading to an increase in capillary permeability and a fall in capillary resistence. Histamine produced and stored in mast cells is released in response to a local injury[3], following an antigen-antibody reaction, or even through the effects of physiological or pharmacological substances. In fact, a role for histamine has been postulated in connection with the estrogen-induced increase in capillary permeability at the endometrial level[4].

Since the classical studies of MAJNO and PALADE[5], it is generally accepted that histamine effects on capillary permeability mainly result from an action of histamine on postcapillary venules. The increase in capillary permeability would be the result of separation on endothelial cells at their boundaries, due to the contraction and shrinkage of these cells with formation of stomata[6]. It must be stressed, however, that the evidence in favour of a true contraction of the endothelial cell under the effects of histamine is only weak, and consists mainly in the similitude with the effects of histamine on smooth muscle.

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