DEMONSTRATION OF SPECIFIC RECEPTORS FOR EGF–UROGASTRONE IN ISOLATED RAT INTESTINAL EPITHELIAL CELLS

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1. Introduction

Epidermal growth factor (EGF), a polypeptide first isolated from submaxillary glands [1,2], was found identical to urogastrone, a polypeptide prepared from urine [3,4]. Both substances inhibit gastric acid secretion, [4,5] stimulate the growth of various cells in culture [6–14], and are powerful mitogens causing epithelial proliferation and keratinisation of squamous epithelial cells in vivo [7]. EGF, like a polypeptide hormone, acts on its target-cells via specific membrane receptors [8,9,14–19]. The facts that the normal epithelial cells of the intestine are in rapid proliferation [20], and that EGF–urogastrone is present in the small intestine [21,22] induced us to test the first step of action of this peptide in isolated intestinal epithelial cells. Here we show the presence of high affinity binding sites specific for EGF–urogastrone in isolated rat intestinal epithelial cells. The existence of such receptors is a strong argument for the action of EGF–urogastrone at the level of normal intestinal epithelial cell.

2. Materials and methods

Mouse EGF was a kind gift of Novo Res. Inst. (Copenhagen) and was prepared as in [23]. Monocomponent pork insulin was purchased from Novo, fatty acid free bovine serum albumin was from Sigma (MO). Carrier-free $Na^{125}I$ (IMS-300) was from the Radiochemical Centre (Amersham). All chemicals were of reagent grade.

Rat intestinal epithelial cells from the jejuno-ileum of female Sprague-Dawely rats (150–200 g), fed ad libitum, were isolated by hand shaking in a dispersing solution containing EDTA (2.5 mM) as in [24]; content, 0.24 mg cell protein/10^6 cells [24]. EGF was iodinated by the chloramin T method and purified on Sephadex G-50 as for insulin [25]. The specific activity was ~160 μCi/μg, i.e., 0.4 atoms iodine/molecule as a mean. Binding studies were performed in Krebs Ringer phosphate buffer (pH 7.4) containing 1% bovine serum albumin, [^{125}I]EGF (~$6 \cdot 10^{-11}$ M), and when indicated, increasing concentrations of unlabelled EGF. After incubation, the cell-bound radioactivity was separated by a rapid centrifugation as for insulin binding [26] and counted in a gamma spectrometer. Values are reported as specific binding: this is obtained by subtracting from the total the non-specific binding, i.e., the amount of [^{125}I]EGF that is not displaced by 10^{-7} M unlabelled EGF. For dissociation studies, cell-bound radioactivity was separated by filtration on glass fiber filters as for insulin [27]. The integrity of EGF in the incubation medium was tested by its ability to bind to rat liver plasma membranes as for insulin [28]. Except for fig.4, the results are expressed as means of duplicate or triplicate determinations. Each individual value differed by $\leqslant$10%.

3. Results and discussion

Fig.1(left) shows the time course of association of [^{125}I]EGF to rat intestinal epithelial cells at 20 and 30°C. The binding is time- and temperature-dependent, the equilibrium being achieved in 30 min at 20°C and 15 min at 30°C. Non-specific binding accounted for 20–25% and 40–45% of total binding

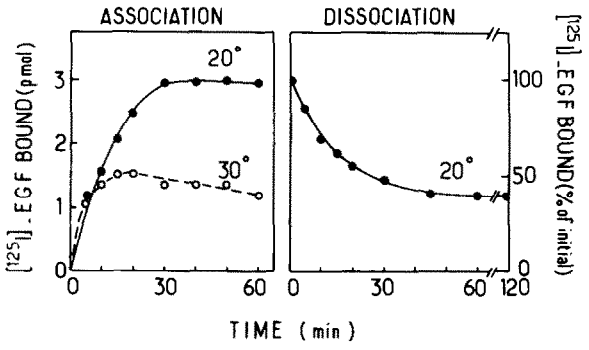

Fig.1. Left: time curves of [^{125}I]EGF specific binding to rat intestinal epithelial cells at 30°C (○) and 20°C (●). Cells (1 mg cell protein/ml) were incubated with $8.4 \cdot 10^{-11}$ M [^{125}I]EGF for the times indicated on the abscissa. Right: dissociation of bound [^{125}I]EGF to rat intestinal epithelial cells at 20°C, initiated by dilution (×20) of the incubation medium. Cells (0.66 mg cell protein/ml) were incubated for 30 min at 20°C, and the specific binding determined; this corresponds to 100% on the ordinate (time 0 of dissociation). After dilution, the specific binding was determined by filtration at the times indicated on the abscissa.

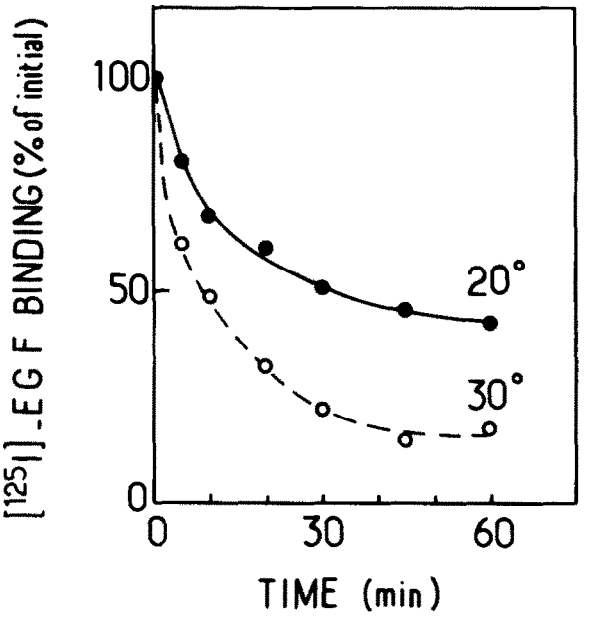

Fig.2. Time curves of inactivation of binding sites. Cells (1 mg cell protein/ml) were preincubated without EGF for the times indicated on the abscissa at 20°C (●) and 30°C (○). Then $8 \cdot 10^{-11}$ M [^{125}I]EGF was added, the incubation continued for 30 min at 20°C and the specific binding determined.

at 20 and 30°C, respectively. Binding is higher at 20°C and all subsequent studies were performed at this temperature. Fig.1(right) shows the dissociation, at 20°C, of bound [^{125}I]EGF to the cells by dilution of the medium of incubation (×20). 50% of bound [^{125}I]EGF is dissociated in 25 min, and ~40% remained associated after 60 min. No further dissociation is obtained after 120 min. [^{125}I]EGF is not degraded in the medium neither at 20 nor at 30°C after 1 h incubation with intestinal epithelial cells (0.76 mg/ml). Similarly, no degradation of the peptide could be detected after 30 min at 20°C even in the presence of 2 mg cell protein/ml (not shown). This absence of degradation of the peptide was found also in the presence of rat liver and placental membranes [15]. On the contrary, as shown in fig.2, the binding sites are rapidly inactivated by preincubation of the cells at 20 or 30°C before adding the ^{125}I-labelled peptide. The inactivation concerns 50% of the sites after 35 and 10 min at 20 and 30°C, respectively (fig.2). The stronger inactivation of binding sites at 30°C as compared to 20°C can explain at least in part the fact that binding is lower at 30 than at 20°C (fig.1, left). The binding of [^{125}I]EGF to rat intestinal epithelial cells is proportional to [cell protein] up to 2 mg/ml (fig.3). Fig.4(left) shows the competition

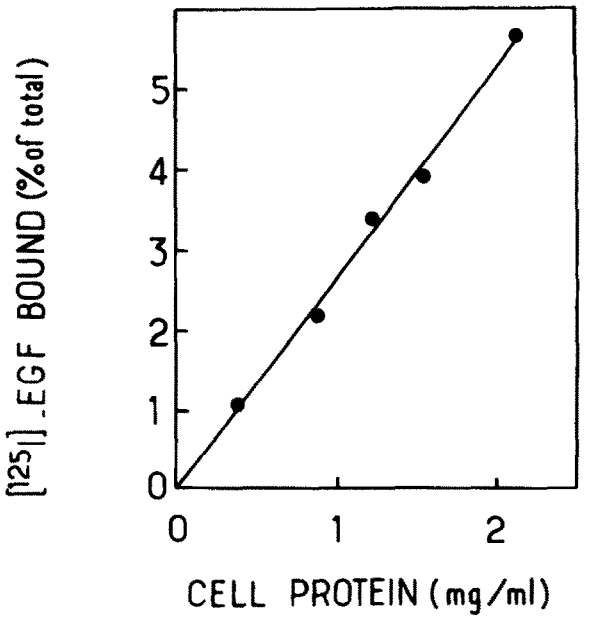

Fig.3. Specific binding of [^{125}I]EGF as a function of [cell protein]. Cells (0.4–2.14 mg cell protein/ml) were incubated at the different concentrations indicated on the abscissa with $8.4 \cdot 10^{-11}$ M [^{125}I]EGF for 30 min at 20°C.

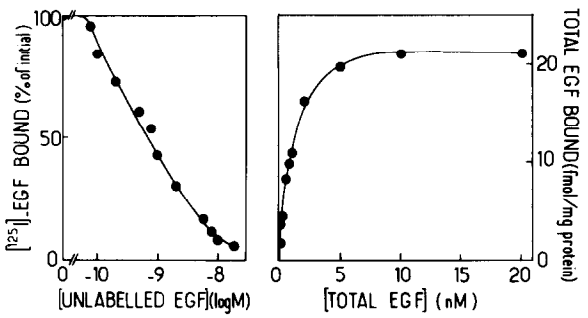

Fig.4. Concentration dependence of EGF binding. Left: inhibition of binding of [^{125}I]EGF by unlabelled EGF. Right: total binding as function of total EGF concentration. Cells (0.5–1.5 mg cell protein/ml) were incubated for 30 min at 20°C with [^{125}I]EGF ($6-8 \cdot 10^{-11}$ M) in the absence (left: 100% on the ordinate) and presence of increasing concentration of unlabelled EGF ($8 \cdot 10^{-11}$ to $2 \cdot 10^{-8}$ M). Each value is the mean of 3–6 separate expt. performed in duplicate.

between [^{125}I]EGF and unlabelled EGF. Unlabelled EGF inhibits competitively tracer binding in the range of $8 \cdot 10^{-11}$ to $2 \cdot 10^{-8}$ M. Half maximum inhibition is obtained with $7 \cdot 10^{-10}$ M unlabelled peptide. The saturation of binding sites is attained with 10^{-8} M EGF (fig.4, right). As calculated from the curve, the total number of binding sites is $1.26 \cdot 10^{10}$/mg cell protein, i.e., 3000 sites/cell. This amount of EGF bound at saturation is comparable with the amount bound to human fibroblasts [8], a target-cell for EGF [6,10]. As calculated from fig.4(right) the apparent $K_d = 10^{-9}$ M, as found for rat liver plasma membranes [15] and human fibroblasts [6]. Insulin, at 10^{-6} M did not compete with [^{125}I]EGF for the binding site, as found in other tissues [15]. These results show the presence of specific EGF receptors in intestinal epithelial cells. The characteristics of these EGF receptors are compatible with the physiological involvements of EGF–urogastrone in the control of the function of intestinal epithelium: in regard to the powerful mitogenic action of EGF [1], further studies should investigate its eventual effects on the control of growth of the rapidly proliferating intestinal epithelium, and its interaction with the insulin [27] and VIP [24] receptors described in these cells.

Acknowledgements

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