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Presence and characteristics of epidermal growth factor receptors in human fetal small intestine and colon

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In the present study, we demonstrate for the first time the presence of important concentrations of EGF binding sites in isolated epithelial cells of both human fetal small intestine and colon as early as 12 weeks gestation. The pattern of EGF binding in the small intestine between 12 and 17 weeks show that binding was significantly higher (2.5-fold) in younger fetuses than in older fetuses. Moreover, the fetal colon exhibited a much higher binding capacity (1.5–2.5 times) than corresponding intestinal cells for all age groups studied. Analysis of Scatchard representations reveal that the concentration of high- and low-affinity binding sites in colonic epithelial cells are twice the values observed in corresponding intestinal cells. The present data raise interesting possibilities as to the role of this growth factor in human fetal gut development.

Small intestine; Colon; EGF receptor; (Human fetal, Isolated cell)

1. INTRODUCTION

Epidermal growth factor (EGF), a potent 53-amino acid single-chain polypeptide, has fast become one of the better characterized growth factors and has been identified in a number of biological fluids and tissues [1-4]. In man, recent immunohistochemical localization of EGF in several fetal tissues such as submandibular gland, kidney and developing gut seems to indicate that this growth factor may already exert possible exocrine or paracrine functions early in human development [5, 6]. Moreover, significant concentrations of EGF have been detected in amniotic fluid (0.25–4.3 ng/ml) as early as 16 weeks gestation and may derive from endogenous sources [6–8]. This peptide has already been shown to influence both fetal and neonatal gastrointestinal tissues in rodents [9–11] and may therefore also play a role in fetal development of the human GI tract. However, any possible effect of EGF on these tissues requires the presence of specific EGF receptors on their cell surface. In a previous report, we were able to isolate, in a complete and selective manner, epithelial cells of both fetal intestine and colon, regardless of their stage of development [12]. Using these pure cell preparations, we have established the presence of specific EGF receptors in both small intestine and colon of 12–17-week-old fetuses.

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2. MATERIALS AND METHODS

2.1. *Tissue specimens and cell preparations*

Small intestine and/or colon from over 31 fetuses ranging from 12 to 17 weeks in age (post-fertilization) were obtained after legal abortion. Epithelial cell preparations were obtained by manual shaking of everted segments of small intestine or colon in a 1.5 nM EDTA–0.25 M NaCl solution at 4°C as described in [12]. Aliquots of the resulting cell suspensions were put aside for protein determination by the method of Lowry et al. [13].

2.2. *EGF iodination*

Receptor-grade mouse EGF (Collaborative Research,

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Waltham, MA) was iodinated by the chloramine-T method and separated on an AG-1 ion-exchange resin column as described [14, 15]. Specific activity ranged between 350 and 390 μCi/μg.

2.3. *Binding measurements*

Cell suspensions (0.06–1.0 mg/ml) were incubated with 2×10^{-11} M ^{125}I-EGF (approx. 50000 cpm) in Krebs' Ringer phosphate (KRP) buffer + 2% bovine serum albumin. Non-specific binding was determined by adding 10^{-6} M native EGF. Incubation was carried out in duplicate samples for 45 min at 22°C, unless stated otherwise. Cell-bound labelled EGF was then separated by rapid centrifugation (15000 × g in 1 min) and counted in a Beckman autogamma scintillation spectrometer. Specific binding is obtained by subtracting non-specific binding from the total radioactivity bound.

For Scatchard analyses, cells were incubated with 2×10^{-11} M ^{125}I-EGF and increasing concentrations of unlabelled EGF (10^{-11}–10^{-7} M). The non-linear Scatchard plots were analyzed according to the two-site model [14] by an iterative process for non-linear regression. Equilibrium parameters of the non-linear function (K_1, K_2, n_1 and n_2) were estimated by the least squares method using a pseudo-Gauss-Newton algorithm. The predicted values were calculated as described [15, 16].

3. RESULTS

3.1. *Kinetics and specificity of EGF binding*

As shown in fig.1, ^{125}I-EGF binding to isolated epithelial cells was found to be time and temperature-dependent (left panel). At 37°C, maximal binding was achieved quickly (20 min), but decreased sharply soon afterwards. At 22°C, binding increased gradually with time to reach a plateau after 45 min of incubation. This steady-state binding was readily reversible by subsequent dilution and/or addition of non-radioactive EGF in the incubation medium (right panel). After 90 min post-incubation at 22°C, over 80% of bound labelled EGF was readily dissociated from its receptor after addition of cold EGF (half-time: 20 min), thus indicating that very little internalization of the ligand-receptor complex had occurred. Therefore, all subsequent binding experiments were performed at 22°C for 45 min. Specificity of EGF binding was also tested by addition, in excess concentrations, of various hormones or growth factors into the incubation medium. As seen in table 1, no cross-competition was observed between ^{125}I-EGF and all tested steroid or peptidic hormones. Only insulin-growth-factor II (10^{-6} M) competed slightly for EGF binding sites.

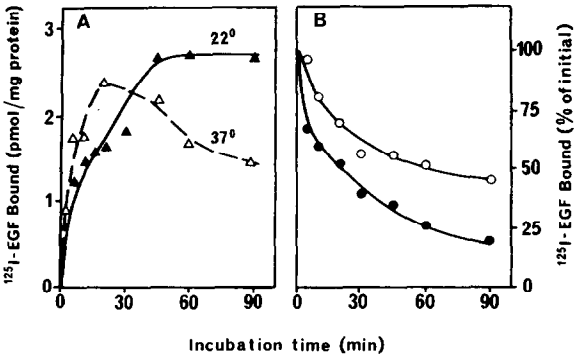

Fig.1. Association and dissociation kinetics of ^{125}I-EGF binding to isolated intestinal epithelial cells. In panel A, cells (0.4–0.7 mg cell protein/ml) were incubated with 2×10^{-11} M ^{125}I-EGF at 22°C (▲) or 37°C (△) for the time intervals indicated. In panel B, after 45 min incubation at 22°C, dissociation was initiated by dilution (20-fold) of the incubation medium in the presence (●) or absence (○) 10^{-7} M unlabelled EGF. The remaining radioactivity bound is plotted as % of initial binding vs time. Each point represents the mean of two separate experiments, in duplicate determinations.

3.2. *Comparative development pattern of EGF binding*

Specific binding patterns of ^{125}I-EGF to isolated intestinal and colonic epithelial cells as a function of gestational age is shown in fig.2. In the small intestine, binding was highest between 12 and 14 weeks of age (2.24–2.86 pmol/mg cell protein) and decreased steadily afterwards (1.13 pmol/mg protein at 17 weeks). In percentage terms, specific binding in younger fetuses represented more than

TABLE 1

Specificity of ^{125}I-EGF binding

Addition	Concentration (M)	Specific binding		
		Expt 1	Expt 2	Expt 3
None		2132	2001	1721
EGF	10^{-6}	579	590	433
Insulin	10^{-6}	2188	2076	1899
ACTH	10^{-6}	2307	ND	1864
Thyroxine	10^{-5}	2169	2149	1538
Hydrocorticosone	10^{-6}	2029	1803	1478
IGF-I	10^{-6}	2041	1863	1483
IGF-II	10^{-6}	1517	1763	1206
Dexamethasone	10^{-5}	ND	1923	ND

The hormones or analogues indicated were added simultaneously with 2×10^{-11} M labelled EGF to isolated intestinal epithelial cells for 45 min at 22°C. Cells were obtained from fetuses aged 14–16 weeks. Values for each experiment are the mean of duplicate determinations and are expressed in cpm. ND, not determined

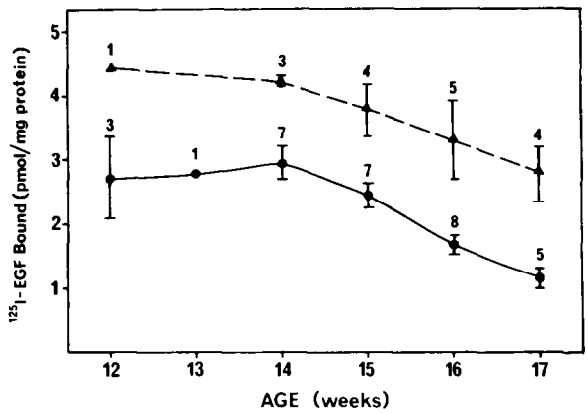

Fig.2. Developmental pattern of specific ^{125}I-EGF binding in isolated intestinal (●—●) and colonic (▲---▲) epithelial cells of human fetuses aged between 12 and 17 weeks. Cells were incubated at 22°C for 45 min. The number of experiments is indicated above each point.

16.5% of labelled EGF/mg cell protein whereas 17-week-old fetuses bound less than 6% of labelled EGF per mg cell protein. When incubated under the same optimal conditions, isolated colonic epithelial cells demonstrated the same pattern of high-binding capacity between 12 and 14 weeks and gradual diminished binding thereafter (fig.2). However, compared to the small intestine, specific binding in the colon was substantially higher (1.4–2.4-times higher) in all age groups studied. At 12 weeks, colonic cells bound 4.43 pmol labelled EGF (23.4%/mg protein) while at 17 weeks, cells bound 2.75 pmol labelled EGF (14.5%/mg protein).

3.3. *Characteristics of EGF binding*

Scatchard plots of bound ^{125}I-EGF displacement by increasing concentrations of native EGF were performed in both intestinal and colonic epithelial cells for which an example is given in fig.3. Both cell populations exhibited two classes of binding sites for low- and high-affinity binding. At equilibrium, the association constants K_1 (high-affinity) and K_2 (low-affinity) for EGF binding remained relatively similar between intestinal and colonic epithelia. In intestinal cells, K_1 and K_2 values were 1.90 ± 0.45 and 0.033 ± 0.016 × 10^9 M^{-1}, respectively (mean ± SE, n = 5), while in colonic cells, mean values for the constants K_1 and K_2 were 1.78 ± 0.83 and 0.014 ± 0.005 × 10^9

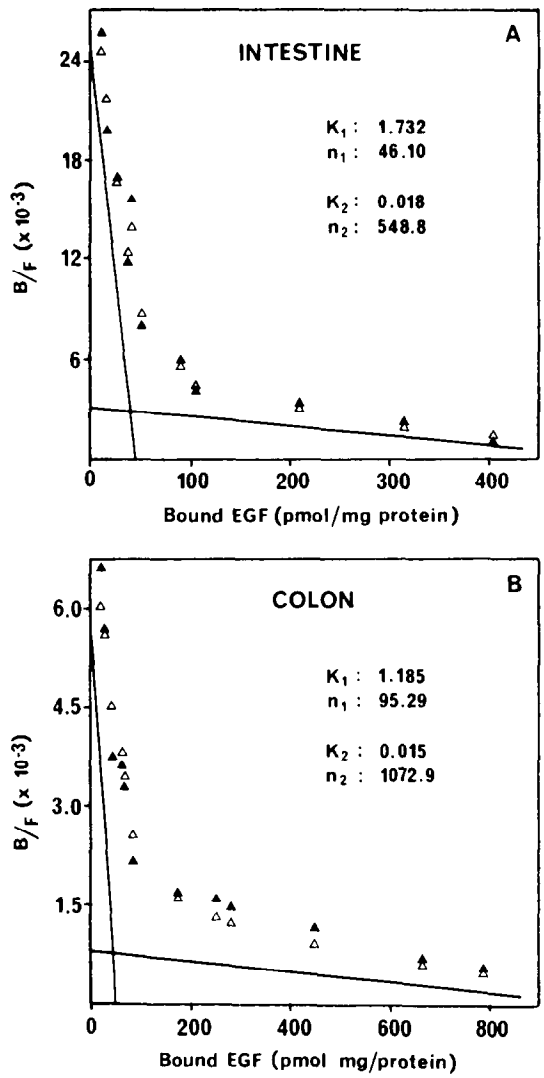

Fig.3. Representative Scatchard plots of bound ^{125}I-EGF displacement by unlabelled EGF in isolated intestinal (top panel) and colonic (bottom panel) epithelial cells. Bound vs free (B/F) EGF is plotted against bound (B) EGF. (▲) Experimental values, (△) predicted values. Corresponding equilibrium parameters for high (K_1, n_1)- and low (K_2, n_2)-affinity binding sites are given for each plot. Affinity constants (K_1, K_2) are expressed as 10^9 M^{-1} while concentration of binding sites (n_1, n_2) are expressed as pmol per mg cell protein.

M^{-1}, respectively (n = 3). On the other hand, the concentrations of EGF binding sites (n_1, n_2) were higher in colonic epithelial cells (126 ± 79 and 1394 ± 302 pmol/mg cell protein) compared to those found in intestinal epithelial cells (61 ± 10 and 487 ± 252 pmol/mg cell protein).

4. DISCUSSION

The present study establishes the presence of specific EGF receptors in both intestinal and colonic epithelial cells of the human fetus. The characteristics of these receptors in terms of binding kinetics, ligand specificity and binding affinities (K_d = 10^{-10}–10^{-9} M) are typical of those found in rodent intestinal epithelial cells [14, 17]. Unfortunately, very little data exist concerning EGF receptors in human gut tissues, especially during fetal life. The only developmental profiles of EGF receptors that are available are limited to postnatal development in rodents in which receptor ontogeny has been characterized in both small intestinal [15, 18] and colonic [16] epithelium. In the human fetus, of particular interest is the binding patterns each of these two tissues reflect during development and, in particular, the timeframes in which they occur. Between 8 and 12 weeks of fetal life, the small intestine undergoes extensive morphogenesis with the onset of villous and crypt formation and subsequent transformation of the epithelium from pseudostratified columnar to simple columnar [19]. Labelling indices within this epithelium are also at their highest value between 8 and 10 weeks gestation (approx. 26–30%) and decrease markedly during the next 4–6 weeks [20]. Interestingly, it is during this critical period of morphogenesis and differentiation that the intestinal epithelium exhibits its highest EGF binding capabilities (fig.2). Moreover, Poulsen and co-workers [6] have recently demonstrated the presence of immunoreactive EGF in intestinal Paneth cells of the 20-week-old fetus. Paneth cells normally appear at approximately 11–12 weeks gestation [20]. If these Paneth cells are indeed able to synthesize an active form of EGF, then the appearance of endogenous sources of this growth factor coincides well with the high number of EGF receptors of both gut tissues at this early age. The pattern of EGF binding in the fetal human colon is also noteworthy since colonic epithelial cells exhibited even higher EGF binding than their intestinal counterparts. A similar observation was also noted in an earlier study involving EGF receptors during postnatal development of mouse colon [16]. This higher binding in colonic epithelial cells can be attributed partly to a higher concentration of EGF receptors on their cell surface. Since the onset of villus formation and epithelial differentiation in the developing colon occurs 3–4 weeks after initiation of morphogenesis in the small intestine, there may exist a relation between the fact that levels of EGF binding in the colon at 17 weeks coincide with peak levels observed in the 12–14-week-old intestine (fig.2). The presence of specific EGF receptors in human fetal small intestine and colon certainly lend further support to an important role of EGF in human gut development. Indeed, EGF, along with other hormonal supplements, has recently been shown to enhance differentiation of human fetal intestine in organ culture [21] as well as in serially passaged human fetal normal colonic epithelial cells [22]. The use of human colonic carcinoma lines may also provide additional information since several of these moderately well-differentiated cell lines exhibit increased expression of EGF receptors [23].

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