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Presence and binding characteristics of calcitriol receptors in human fetal gut

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In the present study, we show for the first time the presence of calcitriol-specific binding sites in hypertonic extracts of cells isolated from human fetal small intestine and colon from 13–21 weeks of gestation. Woolf plot analysis of the binding characteristics revealed the presence of a single class of high affinity receptors. The presence of specific receptors for calcitriol in fetal intestine and colon opens interesting possibilities as to the role of this hormone in human gut development.

Vitamin D; Calcitriol receptor; Small intestine; Colon; (Human fetus)

1. INTRODUCTION

Besides its well-recognized role in mineral homeostasis [1,2], calcitriol, the active form of vitamin D, has been involved in cellular differentiation and proliferation processes [3,4]. Epithelial cells of the small intestine mucosa are in continuous renewal and differentiation, and are target cells for calcitriol [5]. Between 9 and 20 weeks of gestation, human gut acquires many of the morphological and functional characteristics found in adult. During this period, high proliferative activities followed by differentiation are recorded in all segments of the gut [6–8]. To further our understanding of the ontogenesis of fetal human gastrointestinal tract, the present study was undertaken to verify the presence and distribution of calcitriol receptors in the small and large intestine of 13–21-week-old fetuses.

2. MATERIALS AND METHODS

2.1. *Tissue specimens and cell preparation*

Small intestine and/or colon from 17 fetuses ranging from 13 to 21 weeks were obtained after legal abortion. Epithelial cells were obtained by manual shaking of everted segments of small intestine or colon in a 1.5 mM EDTA/0.25 M NaCl solution at 4°C as already described [8,9]. Cells were centrifuged at 500 × *g* × 5 min and frozen at −80°C until used.

2.2. *Binding measurement*

Frozen cells were thawed and resuspended in 1–2 ml of hypertonic buffer (0.3 M KCl, 10 mM Tris-HCl, 15 mM EDTA) adjusted at pH 7.4 and containing aprotinin (Trasylol, Boeringher-Mannheim Canada, Montreal) as protease inhibitor and dithiotreitol (Sigma Chemicals, St. Louis, MI) as antioxidant. Cells were disrupted by sonication (4 bursts of 2 s each at an amplitude of 8 μm) at 4°C and centrifuged at 220,000 × *g* × 30 min. The supernatants were assayed for the presence of calcitriol receptors by incubating 200 μl of cytosol extract in presence of 500 pM 1α,25-dihydroxy[26,27-methyl-^{3}H]cholecalciferol ([^{3}H]calcitriol, 176 Ci/mmol; Amersham, Oakville, Canada) with and without a 100-fold excess of unlabeled hormone (kind gift of Dr Milan Uskokovic, Hoffman-Laroche, Nutley, NJ). The steroid-receptor complex was adsorbed on hydroxyapatite [10]. In some samples, saturation analysis of the receptors was performed in presence of increasing concentrations of tritiated calcitriol (40–500 pM). Dissociation constants (K_d) and maximum binding capacity (B_{max}) were estimated by the Woolf plot analysis [11]. An aliquot of each hypertonic supernatant was kept for the measurement of DNA [12].

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3. RESULTS

3.1. *Comparative development pattern of calcitriol binding*

Specific binding pattern of calcitriol to the hypertonic extracts of epithelial cells is shown in table 1. All proximal small intestine samples tested, as early as 13 weeks of gestation, exhibited calcitriol binding activity. No particular developmental pattern could be elicited with the samples collected. In several fetuses, specific binding was measured in all intestinal segments including the colon. The specific binding had a tendency to be lower in the distal portion of small intestine and in the proximal and distal parts of colon.

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Table 1

Distribution of calcitriol-specific binding in individual fetal small intestine and colon specimens

Weeks gestation	Small intestine			Colon	
	Proximal	Median	Distal	Proximal	Distal
13	0.336				
16	0.048				
16	0.150				
17	0.117				
17	0.084	0.091	0.077		
17	0.111	0.090	0.078		
17	0.099				
18	0.462	0.494	0.356		
19	0.227				
19	0.031	0.033	n.d.	0.017	
19	0.080	0.069	0.007	0.088	
19	0.034	0.034			n.d.
19	0.067			0.055	0.040
19	0.458				
20	0.377				
21	0.331	0.097	0.088	0.096	0.053
21	0.380	0.389	0.270		

Specific binding was measured as described in section 2 in the presence of 50 pM [^{3}H]calcitriol with or without 100-fold concentration of unlabeled hormone. Results are expressed as fmol calcitriol bound per μg DNA. n.d. = not detectable

3.2. *Characteristics of calcitriol binding*

Woolf plot of bound [^{3}H]calcitriol displacement by increasing concentration of radioinert hormone were performed on hypertonic extracts of epithelial cells of the proximal small intestine. An example is given in fig.1. A single class of high affinity binding sites was exhibited. The individual dissociation constants (K_d) and maximal binding (B_{max}) are listed in table 2. Values were of the same order of magnitude whether they were derived from samples obtained at 18 or 21 weeks of gestation. They range from 189 to 323 pM and from 0.571 to 0.770 fmol/μg DNA, respectively.

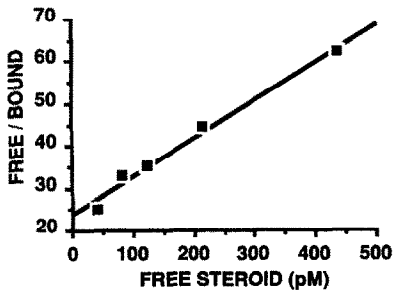

Fig.1. Woolf plot of calcitriol-specific binding by hypertonic cytosolic extracts of cells isolated from proximal small intestine. The extracts, prepared as described in section 2, were incubated for 16 h at 4°C with increasing concentrations of [^{3}H]calcitriol in the presence of a 100-fold molar excess of radioinert hormone. Bound sterol-receptor complex was adsorbed on hydroxyapatite. The line fits a simple linear regression ($r = 0.99$).

Table 2

Binding characteristics of proximal small intestine calcitriol receptors*

Weeks gestation	K_d (pM)	B_{max} (fmol/μg DNA)
18	189 ± 41	0.620 ± 0.13
19	323 ± 17	0.770 ± 0.05
20	252 ± 57	0.571 ± 0.13
21	265 ± 26	0.603 ± 0.06

*The K_d and B_{max} values were calculated from the Woolf plot analysis [9]. The values are the mean ± SE ($n = 3$)

4. DISCUSSION

We have established the presence of specific receptors for calcitriol in epithelial cells from both intestine and colon of human fetuses. Data concerning such receptors in human gut is scarce and in fact, nonexistent for fetal tissues. The observed binding affinities and capacities for calcitriol in the epithelial cells from the proximal small intestine are similar to those reported for human adult jejunum [14] illustrating that the intestinal cells acquire this function very early during development. The binding affinities reported here are, however, slightly lower than those reported earlier for human cultured amniocytes [13]. The presence of specific calcitriol binding sites in the normal fetal colon is particularly interesting since such sites were also found to be present in the human colonic cell line HT-29 [15]. However, our data differ markedly from those published for developing rodents. Indeed in both rat [16] and rabbit [17], the ontogeny of calcitriol receptors is initiated only during postnatal development. Therefore this report warrants caution in extrapolating, to human, data observed in rodents. The physiological role of these receptors is not fully understood. The vitamin D-dependent CaBP are best known molecular markers of vitamin D action in target cells. Recently, 9K-CaBP gene expression was elicited as early as 14 weeks of gestation in human duodenum with no significant change in 9K-CaBP mRNA levels from 14 to 32 weeks of gestation [18]. In rodents, sucrase, a marker enzyme for intestinal cell maturation, is elicited at the time of weaning [19]. The onset of this activity is concomitant to the appearance of calcitriol receptors [16]. Interestingly sucrase appears early in human fetal development when calcitriol receptors, as we show in this report, are also present.

Based on these observations and on the fact that calcitriol can promote cell differentiation, the presence of specific receptors in human fetal small intestine and colon opens interesting possibilities as to the role of calcitriol in human gut development and in the ontogeny of digestive enzymes.

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