Intrinsic factor receptor during fetal development of the human intestine

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Intrinsic factor receptor activity was observed in mucosal homogenates from whole small intestine and colon of 10–19-week fetuses, whereas it was only detected in the distal part of the small intestine of a 25-week fetus. The receptor–intrinsic factor–cobalamin complex was eluted into the void-volume position when ileum mucosal extract was assayed for receptor activity by gel filtration after incubation with either fetal gastric extract or human gastric juice. The intrinsic-factor-binding capacity of intestinal mucosal extracts ranged from 2.6 to 30.5 fmol/mg and was correlated with the gestational age of six fetuses. The dissociation constant of the receptor for the intrinsic factor–cobalamin complex was estimated at 0.24–0.36 nM at pH 7.4. In conclusion, intrinsic-factor-receptor activity was detected in the whole intestine in 10–19-week fetuses, whereas it was only present in the distal ileum at the end of fetal development.

INTRODUCTION

In man, the assimilation of vitamin B12, also known as cobalamin (Cbl), involves two Cbl-binding proteins, haptocorrin which is secreted mainly in saliva and intrinsic factor (IF) which is secreted by parietal cells of the stomach (Guéant & Gräsbeck, 1990). In the duodenum, haptocorrin is degraded by pancreatic enzymes and Cbl binds to IF (Guéant *et al.*, 1990). The IF–Cbl complex is absorbed by an endocytic mechanism which involves a specific intrinsic factor receptor (IFR), located in the apical brush border of ileal enterocytes (Levine *et al.*, 1982; Guéant *et al.*, 1988). In adult mammals, IFR activity is not detected in the proximal small bowel or colon. In plasma, Cbl binds to either transcobalamin or haptocorrin. In the fetus, Cbl can be absorbed via cordal blood.

Only few data have been obtained concerning the synthesis and secretion of the Cbl-binding proteins in the digestive tract during fetal development. Sonneborn & Iwanik (1974) have shown the presence of both haptocorrin and IF in the stomachs of 16–20-week fetuses. The presence of IFR in fetal intestine has not been studied. In amniotic fluid, Cbl-binding capacity corresponds mainly to transcobalamin and haptocorrin (Stenman, 1974; Grossowicz *et al.*, 1983). The presence of IF has been demonstrated by gel filtration and isoelectrofocusing in the amniotic fluid after 13 weeks of gestation (Guéant *et al.*, 1989a; Hansen *et al.*, 1989).

In this study, we have determined the IFR activity of intestinal mucosal homogenates of fetuses aged from 10 to 25 weeks and the physicochemical properties of the two digestive Cbl binders, haptocorrin and IF, in fetal mucosal homogenates.

EXPERIMENTAL

Chemicals

Cyano[⁵⁷Co]cobalamin (8.15 MBq/μg, molecular mass: 1355 Da) and ³H₂O (185 MBq/ml) were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Phenyl-

Sephacrose 6 CL and Superose 6B were obtained from Pharmacia–LKB (Uppsala, Sweden). Other reagents were of analytical grade.

Preparation of stomach and intestinal mucosal homogenate

The small intestine and colon of aborted fetuses were collected at the Nursery Hospital of Nancy, kept at 4 °C and treated within 2 h. The intestine and colon were washed successively with 0.05 M-sodium acetate buffer, pH 4.5 containing 0.15 M-NaCl, 1.5 mM-NaN₃ and 0.4 mM-phenylmethanesulphonyl fluoride (PMSF) and twice with 0.02 M-Tris/HCl buffer, pH 7.4, containing 0.15 M-NaCl, 1.5 mM-NaN₃ and 0.4 mM-PMSF. The intestine and colon were then cut into segments and the mucosa was scraped off and homogenized in a Wheaton Potter mixer in the presence of 0.02 M-Tris/HCl buffer, pH 8.0, containing 0.15 M-NaCl, 1.5 mM-NaN₃, 0.4 mM-PMSF and 1 % (v/v) Triton X-100 (extracting buffer), incubated overnight at 4 °C and centrifuged at 100 000 g for 1 h at 4 °C. The supernatants were collected and corresponded to receptor extract. The final volume of each extract was adjusted to 2.5 ml with extracting buffer. Protein quantification of each extract was performed by the bicinchoninic acid procedure (Smith, 1985).

Stomachs were cut and the insides scraped off. The scrapings were homogenized and prepared as above.

Radioisotope assay of the solubilized receptor of intrinsic factor

The method has previously been described by Guéant *et al.* (1991). Because of the low Cbl-binding capacity of fetal gastric extract, we used human gastric juice. Human gastric juice (20 μl corresponding to a Cbl-binding capacity for IF of 0.7 pmol) and 20 μl of CN[⁵⁷Co]Cbl in 1 ml of 0.02 M-Tris/HCl containing 0.15 M-NaCl were agitated for 20 min at room temperature. A portion (50 μl) of homogenate from each intestinal segment was incubated in the presence of either 10 μl of 0.1 M-CaCl₂/MgCl₂ (first series) or 10 μl of 1 M-EDTA (second series) with 70.4 fmol of the IF–CN[⁵⁷Co]Cbl solution for 1 h with shaking. The volume of each fraction was adjusted to 1 ml with 0.02 M-Tris/HCl buffer, pH 7.4, containing 0.15 M-NaCl. Then 200 μl of a

Abbreviations used: Cbl, cobalamin; IFR, intrinsic factor receptor; IF, intrinsic factor; PMSF, phenylmethanesulphonyl fluoride.

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suspension of Phenyl-Sepharose (1 vol. of decanted and washed Phenyl-Sepharose in 1 vol. of 0.01 M-Tris/HCl buffer, pH 7.4, containing 0.15 M-NaCl) and 300 μ l of 0.02 M-Tris/HCl buffer, pH 7.4, containing 3.33 M-NaCl and 0.5% (w/v) BSA were added successively to each tube. The tubes were agitated for 30 min at room temperature and then centrifuged at 1500 g for 5 min. The pellets were washed three times by repetitive addition of 3 ml of 0.02 M-Tris/HCl buffer, pH 7.4, containing 0.3 M-NaCl and 1 mM-CaCl₂ (first series) or 1 mM-EDTA (second series) and centrifuged at 4 °C. The radioactivity of the pellets was determined by γ -ray counting. The radioactivity corresponding to IFR-IF-CN[⁵⁷Co]Cbl was estimated from the difference in radioactivity between the pellets obtained in the first and second series. The dissociation constant of IF-Cbl for the receptor was determined by Scatchard (1949) plot analysis by using the same procedure with increasing amounts of IF-CN[⁵⁷Co]Cbl from human gastric juice. In this case, the small intestine of a 21-week fetus was cut into segments corresponding to the duodenum and ileum. These segments and the entire colon were washed and scraped as described above. The dissociation constant of the receptor to IF-Cbl complex was determined in each segment. The results were compared with those for mature ileum; a segment of ileum was obtained after surgical resection from a man presenting with a colonic carcinoma.

Gel filtration

Gel filtration of solubilized fetal IFR was performed through Superose 6B at a flow rate of 0.5 ml/min and was eluted with 0.02 M-Tris/HCl buffer, pH 7.4, containing 0.15 M-NaCl, 1 mM-CaCl₂ and 0.05% (v/v) Triton X-100. Human gastric juice (20 μ l corresponding to a Cbl-binding capacity for IF of 36.8 pmol/ml) was incubated successively with 90 pmol of cobinamide for 10 min and 0.7 pmol of CN[⁵⁷Co]Cbl for 20 min at room temperature. Then 1 ml of fetal ileal extract was added to the preparation for 1 h with mixing. The sample was finally injected on to the column. Fractions of 0.5 ml were collected for γ -ray counting. The same experiment was carried out with 100 μ l of fetal gastric extract (corresponding to a specific activity of 1.5 pmol/mg). Void volume and total volume were estimated with Dextran Blue and ³H₂O respectively.

RESULTS

Haptocorrin and IF were found in stomach extracts from 18.5–25-week fetuses. Similar data were previously obtained by others in gel filtration (Sonneborn & Iwanik, 1974). We estimated

the Cbl-binding capacity respectively to be 0.75 pmol/mg of protein and 1.45 pmol/mg of protein by Sephacryl S200HR gel filtration (Schohn *et al.*, 1991). The two Cbl-binding proteins were eluted in the same positions as haptocorrin and IF of

Fig. 1. IFR activity of segments from the intestine and colon of 10–25-week fetuses

The radioisotopic assay of solubilized receptor was performed on each segment. The results are expressed as pmol of Cbl-IF bound to the receptor/mg of protein. Abbreviation: C, colon. (a) 10-week fetus; (b) 18.5-week fetus; (c) 19-week fetus; (d) 25-week fetus.

Fig. 2. Binding of increasing amounts of IF-Cbl to the solubilized receptor from the ileum, colon and jejunum of a 21-week fetus

(a) Saturation curve of the binding of IF-CN[⁵⁷Co]Cbl to IFR (from 50 μ l of fetal intestinal homogenate). The concentration of IFR-Cbl in the assay was calculated by measuring the difference between the concentration of IF-CN[⁵⁷Co]Cbl adsorbed to Phenyl-Sepharose in the presence of CaCl₂ (total binding) and that in the presence of 10 mM-EDTA (non-specific binding). (b) Scatchard plot of this experiment. The dissociation constant was estimated respectively to be 0.36, 0.33 and 0.24 nM in the duodenum (○), ileum (●) and colon extracts (■). Each point represents the mean of two determinations.

Table 1. Total IFR specific activity in fetal intestine as a function of age

Fetal age was estimated by crown-rump length as described by Keeling (1987). The receptor activity was calculated by radioisotopic assay as described in the Experimental section.

Fetal age (weeks)	Total activity (fmol/mg)		Crown-rump length (cm)
	Small intestine	Colon	
10	30.5	9.9	70
18.5	10.6	11.6	152
19	7.7	9.5	156
23	2.6	0.9	185
23	3.8	4.4	165
25	5.9	1.8	240

Fig. 3. Inhibition curve of the binding of IF- ^{57}Co Cbl (140 pmol/l) to the solubilized ileal receptor from a 21-week fetus (50 μl) in the presence of increasing concentrations (140, 280, 700, 1550 pmol/l) of unlabelled IF-Cbl (●)

No inhibition was obtained when IF- ^{57}Co Cbl was incubated with the same increasing concentrations of either Cbl alone (○) or saturated haptocorrin (□). Each point represents the mean of two measurements.

Fig. 4. Gel filtration of the solubilized receptor-IF-Cbl complex

CN ^{57}Co Cbl-IF complex was incubated with ileum homogenate from a 21-week fetus in the presence of CaCl_2 . Fetal gastric extract (●) and human gastric juice (○) were saturated with CN ^{57}Co Cbl and subsequently incubated with solubilized receptor. In both cases, the receptor-IF-Cbl complex was eluted into the void volume [arrows indicate the elution position of Dextran Blue (DB), IF-Cbl and free Cbl].

human gastric juice which were used as standard proteins (Schohn *et al.*, 1991). The molecular mass and mean isoelectric point of the fetal Cbl-binding protein were close to those observed by Marcoullis & Gräsbeck (1975) and Guéant *et al.* (1989a). Assimilation of Cbl requires the secretion of IF in the stomach and the presence of IFR in the intestinal mucosa.

IFR was present in intestinal homogenates from 10–25-week fetuses (Table 1 and Fig. 1). It was distributed along the whole intestine in 10–23-week fetuses but only in the ileum in the 25-week fetus (Fig. 1). Specific IFR activity of the small-intestinal extract ranged from 2.6 to 30.5 fmol/mg (Table 1), the maximal specific activity being observed in the extract from the youngest

fetuses. In the colon, the highest activity was observed in 10–19-week fetuses and was of the order of 10 fmol/mg (Table 1). A negative correlation was found between IFR specific activity of the whole intestine ($r = -0.972$, $P < 0.001$) and gestational age. A similar correlation was observed when only the IFR specific activity of the small intestine was considered ($r = -0.94$, $P < 0.005$). The correlation found for the IFR specific activity of the colon was less significant ($r = -0.74$, $P < 0.1$).

The dissociation constant of the receptor to IF-Cbl complex was estimated respectively to be 0.36, 0.33 and 0.24 nM in duodenum, ileum and colon extracts of a 21-week fetus (Fig. 2). By comparison, the dissociation constant was estimated at 0.5 nM for adult human ileum extract. Using the radioisotopic assay, we showed that the binding of CN ^{57}Co Cbl-IF from human gastric juice to fetal IFR was inhibited in the presence of increasing amounts of unlabelled saturated IF but not in the presence of either free CN ^{57}Co Cbl or saturated haptocorrin (Fig. 3). The receptor-IF-Cbl complex was eluted into the void volume when the ileal mucosal extract from a 21-week fetus was assayed for IFR activity by gel filtration with either fetal gastric extract or human gastric juice (Fig. 4). The receptor-IF-Cbl peak was not observed when the experiment was repeated in the presence of EDTA instead of CaCl_2 (not shown).

DISCUSSION

The present study is the first to demonstrate the presence of IFR activity in fetal intestine. As shown in Fig. 1, the IFR activity was found in the small intestine and colon of 10–23-week fetuses, whereas it was mainly limited to the distal portion of the small intestine of a 25-week fetus, with only very low activity in the colonic extract. It has been shown in our laboratory that IFR is present in HT29 cells (Guéant *et al.*, 1992), and Dix *et al.* (1990) and Ramanujam *et al.* (1991) have observed binding of IF-Cbl complexes in CaCo2 cells. These cell lines are derived from colon carcinoma and have the same brush-border enzyme activities as fetal intestinal cells (Rousset, 1986). The results obtained with HT29 and CaCo2 cells suggested therefore that IFR could also be present in fetal colon. The present data confirmed this hypothesis since IFR was present in colonic mucosal homogenates of 10–21-week fetuses. In addition, IFR was detected in the proximal small intestine of these fetuses. IFR specific activity decreased between 10 and 25 weeks of fetal age in the small intestine and correlated negatively with gestational age (Table 1). The present data suggest that IFR gene expression is repressed in the colon and duodenojejunum part of the small intestine during fetal development. Such an observation has previously been reported for the sucrase-isomaltase activity from intestinal mucosa, which decreased in the colon after 30 weeks of gestational age (Triadou and Zweibaum, 1985).

The IFR of fetal mucosal extract is eluted in the same position on gel filtration as the solubilized IFR of pig mucosal extract (Kouvonen & Gräsbeck, 1979; Guéant *et al.*, 1989b). The affinity of the IFR from ileal extract for the IF-Cbl complex is similar to that of solubilized ileal IFR from an adult patient. It is also close to the affinity of solubilized pig receptor for the IF-Cbl complex (Hooper *et al.*, 1973; Guéant *et al.*, 1991). The IFR also binds fetal gastric IF, demonstrating that absorption of IF-Cbl complexes may occur *in utero*, via the fetal intestinal lumen. Endocytosis of receptor-IF-Cbl complex can therefore occur in the digestive tract during fetal development, since the presence of IF has been reported in amniotic fluid and fetal gastric mucosa (Sonneborn & Iwanik, 1974; Guéant *et al.*, 1989a).

In conclusion, the different proteins involved in Cbl assimilation are present in the human fetus after 10 weeks of gestation. IFR seems to be a marker of intestinal maturation during fetal

development. Further studies are needed to compare IFR activity with brush-border enzyme markers at different stages of fetal development.

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