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Effect of bile on the intestinal bile-acid binding protein (I-BABP) expression. In vitro and in vivo studies

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Abstract Enterocytes actively transport bile acids from the ileal lumen to the portal blood. This physiological process greatly contributes to maintaining the bile acid homeostasis. However, little is known about the molecular mechanisms involved in this transport system. The effect of bile on gene expression of the intestinal bile-acid binding protein (I-BABP) expressed in the enterocytes was studied in vivo, using the by-pass method, and in vitro, using organ culture of ileum explants and Caco-2 cell line. The low cytosolic I-BABP concentration and I-BABP mRNA level found in diverted ileum was totally recovered when bile was added in the ileal lumen. Northern blot analysis of the ileal explants revealed a dose-dependent increase in the I-BABP mRNA in the presence of bile. In Caco-2 cells, the I-BABP transcript was dramatically increased in the presence of human bile while it was undectable in the control cultures. These data offer the first evidence that biliary components regulate the I-BABP gene expressed in the enterocytes.

Key words: Bile-acid binding protein; Caco-2 cell; Organ culture; By-pass; Gene regulation; Entero-hepatic circulation

1. Introduction

Bile acids (BA) are required for an efficient intestinal absorption of dietary lipids [1]. They undergo an entero-hepatic circulation which involves successive synthesis from cholesterol in the liver, secretion with bile into the lumen of the small intestine, uptake throughout the gut then re-circulation back to the liver via the portal blood [2]. This physiological process is essential to maintain the BA homeostasis. BA are absorbed by both diffusion across the jejunum and active sodium-dependent transport in the terminal ileum. Recently, the specific ileal uptake of BA has been the subject of in-depth research. It involves at least one 93–99 kDa integral membrane transporter [3,4] and a 14–15 kDa cellular binding protein termed ileal lipid binding protein (ILBP) or intestinal bile-acid binding protein (I-BABP). I-BABP has been identified and characterized in ileum from pig [5,6], rat [7–9], rabbit [10], mouse [11] and man [12]. Sequence analysis revealed that I-BABP belongs to a family of cytosolic hydrophobic ligand-binding proteins, the fatty acid-binding proteins (FABP). However, it has no binding ability as regards long-chain fatty acids [7]. In fact, several set of data suggest that I-BABP is involved in the intracellular transport of BA from the apical membrane towards the basolateral pole of the enterocyte. Indeed, not only can I-BABP bind conjugated BA [8,12–14], but it may also be associated with the BA transporter found in the brush border of ileal cells [10]. Surprisingly, the possible regulatory process affecting I-BABP expression remains unknown. In view of the hypothetic role of I-BABP in the BA intracellular trafficking, we have studied the effect of bile on the expression of I-BABP. Our data demonstrate that biliary components, probably the BA, induce I-BABP production by an increase in I-BABP mRNA.

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Abbreviations: I-BABP, intestinal bile-acid binding protein; ILBP, ileal-lipid binding protein; BA, bile acids; CRBPII, cellular retinol binding protein II.

2. Materials and methods

2.1. Animals

The regulations for the care and use of laboratory animals were observed. Sprague-Dawley male rats weighing 150–200 g were used in the bypass experiments. A partial ileal bypass was performed on sodium pentobarbital anaesthetized animals according to the following procedures. The ileum was cut 10 cm above the ileo-cecal valve, then the proximal portion of the ileum was anastomosed to the transverse colon while the terminal ileum was anastomosed to the skin (ileocutaneostomy). Three milliliters of saline (NaCl 0.9%), or bile, were infused 6 times a day for 3 days into the terminal ileum, from post-operative day 3 to day 5. The controls were sham-operated. The animals were killed by aortic puncture under ether anaesthesia and the serum was stored at −20°C until required for total BA determination. Male Swiss mice weighing 30–35 g were used in the organ culture of ileal explants. The entire intestine was removed, flushed with 0.9% NaCl at 4°C and a 9 cm segment of ileum was withdrawn 1 cm above the cecum. The mucosa was scraped off with a spatula at 4°C.

2.2. Cell culture

Caco-2 cells (passage 40–44) were cultured at 37°C in DMEM containing 10% fetal calf serum, 60 mg/ml Kanamycin, 1% non-essential amino acids and 4 mM L-glutamine. Five days after total confluency they were incubated for 12 h in the presence of 5% (v/v) human bile.

2.3. Organ culture of intestinal explants

2.5-month-old Swiss mice were fasted overnight and ileal explants were prepared and cultured as previously described [15]. In brief, a 9 cm ileal sample from 1 cm above the cecum was speedily removed and sliced into strips. The entire thickness of the intestine was retained except for the serosa which was stripped off. Explants were cultured in Hepes-buffered Dulbecco's modified Eagle medium (DMEM) containing 10% NCTC-135, 10% fetal calf serum, 0.1% fungizone and 0.1 mg/ml gentamycin (all from Gibco-BRL) at 37°C under an oxygenated atmosphere. The intestinal epithelium retained its morphological integrity in these conditions even after 24 h in culture [16]. After a 4 h preculture period, ileal explants were cultured in the presence of bile. At the end of the culture, the explants were quenched in liquid nitrogen and stored at −80°C until required for RNA extraction.

2.4. Molecular probes

Rat and human I-BABP cDNA [7,12] and human L-FABP cDNA were used. The L-FABP probe was amplified by PCR from a human ileum cDNA library [12] in the presence of the following primers: 5'-ATG AGT TTC TCC GGC AAG TAC-3' and 5'-AAT TCT CTT GCT GAT TCT CTT GAA-3'. β-actin cDNA was a gift from Dr A. Alonso (Institut Pasteur, Paris, France) [17]. These probes were la-

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beled with [α^{32}P]dCTP (3000 Ci/mmol; Amersham) using the random priming technique (Megaprime kit, Amersham). A 24-residue oligonucleotide specific for rat 18S rRNA [18] was used to ensure that equivalent amounts of RNAs were loaded and transferred. This oligonucleotide was 5′ end-labeled using T_4 polynucleotide kinase and [γ^{32}P]ATP (3000 Ci/mmol, Amersham).

2.5. Northern blot analysis

Total RNA were extracted according to Chomczynski and Sacchi [19]. They were denatured, electrophoresed on a 1% agarose gel and transferred to Gene Screen membrane (NEN), using 20-fold concentrated 150 mM NaCl and 15 mM trisodium citrate, pH 7 (NaCl/Cit). Filters were prehybridized for 4 h then hybridized for 16 h at 42°C according to previously published procedures [20]. They were washed twice in 2×NaCl/Cit at room temperature, twice in 2×NaCl/Cit with 1% SDS at 65°C for 30 min, and finally twice in 0.1×NaCl/Cit at room temperature. Autoradiograms were quantified with an automatic densitometric scanner (CS-9000, Shimadzu, Scientific Instruments).

2.6. Western blot analysis

Cellular homogenates were prepared in ice-cold 30 mM Tris HCl, 5 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol in a glass teflon potter. The homogenates were centrifuged for 20 min at 8000×*g*. The resulting supernatant was then centrifuged for 60 min at 105 000×*g*. The cytosolic protein concentration was measured by BCA (Pierce) and 1 μg denatured cytosolic proteins were separated by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) using 12% gels, in the presence of rainbow coloured protein molecular mass markers (Amersham). The separated proteins were electrophoretically transferred to a Hybond-ECL nitrocellulose membrane (Amersham) according to the manufacturer's protocol (BioRad). The membrane was blocked overnight at room temperature with 2% bovine serum albumin, then incubated for 1 h at 25°C with the rat I-BABP antiserum [21] diluted 1/1000. The filter was then incubated for 1 h at 25°C with anti-rabbit IgG-horseradish peroxidase conjugate (1/3000, by vol., Sigma), and the antigen–antibody complexes detected with ECL reagent.

2.7. Assays

The BA serum concentration was determined in the rat using a commercial kit (enzymatic determination, Sigma-Diagnostics). The determination of the BA composition of the human bile was performed by HPLC.

2.8. Statistical methods

Wherever possible, the results are expressed as mean ± S.E.M. The significance of differences between groups was determined by the Student's *t* test.

3. Results

3.1. Effect of a partial ileal bypass on I-BABP expression

To check whether or not bile may exert a physiological regulation on the I-BABP expression, a partial ileal bypass was carried out in rats. The efficiency of the ileal diversion was attested by the marked decrease in the serum total BA concentration found after surgery (3.1 ± 0.5 μmol/l in bypass saline group vs 19.4 ± 5.9 in sham-operated controls, $P < 0.001$). No significant difference in body mass gain or in protein, DNA and RNA contents of the ileum was found in bypass groups as compared to the sham-operated controls (Table 1). As shown in Fig. 1, the lack of bile circulation occurring in the terminal ileum in the bypass saline group leads to a decrease in the I-BABP mRNA level as compared with the sham-operated controls. This drop was totally avoided when the bile was directly infused in the terminal ileum (bypass bile group). This regulation appeared to be gene specific since the β-actin mRNA level was unaffected by the ileal diversion and bile supplementation (Fig. 1A). Similar modifications in the relative concentration of cytosolic I-BABP were also found by Western blot analysis (Fig. 2).

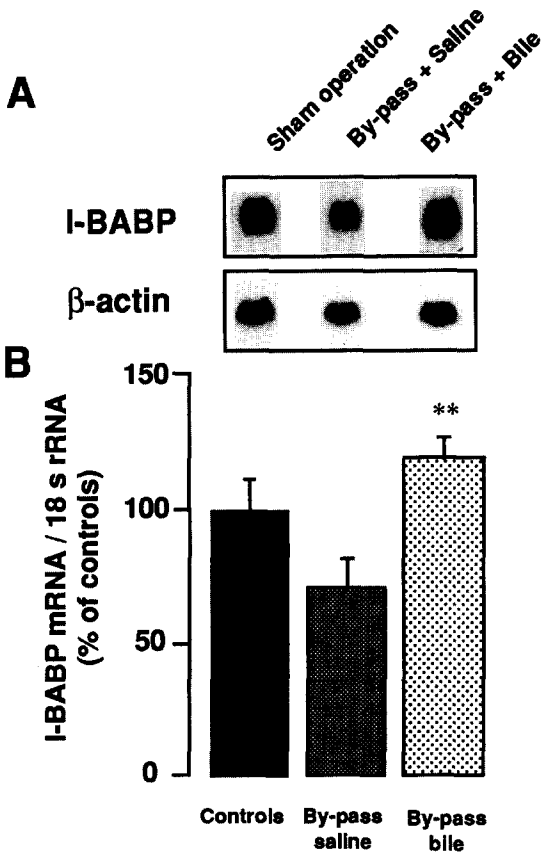

Fig. 1. The effect of chronic bile infusion on I-BABP mRNA levels in rats subjected to partial ileal bypass. The ileal diversion was performed as described in section 2. Three ml of saline (NaCl 0.9%) or bile were infused 6 times a day for 3 days into the terminal ileum of bypassed rats from post-operative day 3 to day 5. Controls were sham-operated. (A) Typical Northern blot analysis performed with total RNA extracted from the ileal mucosa (16 μg/lane). Filters were rehybridized with β-actin cDNA probe to check the specificity of the I-BABP data. (B) Bar graph representation of I-BABP data normalized to 18 S rRNA for differences in RNA loading. Mean ± S.E.M., $n = 4$. $^{**}P < 0.01$, bypass bile vs bypass saline; sham-operated controls, black column; bypassed rats plus saline, hatched column; bypassed rats plus bile dotted column.

Table 1
Effect of ileal diversion on the body mass gain and the ileal contents of protein, DNA and total RNA in bypassed rats chronically infused with saline (NaCl 0.9%) or bile

	Body mass gain (g)	Protein (μg/mg of ileal mucosa)	DNA (μg/mg of ileal mucosa)	Total RNA (μg/mg of ileal mucosa)
Controls	45.6 ± 2.5	100.3 ± 5.3	6.3 ± 0.4	6.3 ± 0.4
Bypass + saline	45.8 ± 1.7	87.9 ± 8.4	5.2 ± 0.4	5.2 ± 0.4
Bypass + bile	39.4 ± 1.6	98.6 ± 7.8	4.7 ± 0.2	4.7 ± 0.2

Controls were sham operated.

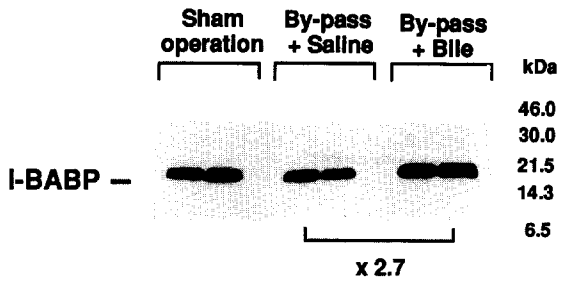

Fig. 2. The effect of chronic bile infusion on the relative cytosolic I-BABP abundance in rats subjected to partial ileal bypass. The ileal diversion was performed as described in section 2. Three milliters of saline (NaCl 0.9%) or bile were infused 6 times a day for 3 days into the terminal ileum of bypassed rats from post-operative day 3 to day 5. Controls were sham-operated. Western blot analysis was performed with 1 μg cytosolic proteins from ileal mucosa.

3.2. *Evidence for a direct regulation of the I-BABP by biliary components*

In vitro studies were carried out in order to study the main characteristics of the regulation of I-BABP by bile. As shown in Fig. 3A, exposure of ileal explants to bile led to a concentration-dependent accumulation of I-BABP mRNA. A 2.3- and a 4.2-fold increase was found when 1% and 5% (v/v) of bile were added into the medium, respectively.

Northern blot analysis of RNA isolated from ileal explants in the culture exposed to 5% bile for 3 to 24 h demonstrated that I-BABP mRNA were induced in a time-dependent way. Indeed, an increase was first detected 6 h after the addition of bile into the medium. A maximum level was reached after 12 h and then it slowly decreased after 24 h (Fig. 3B). In contrast, no significant modification of I-BABP mRNA was found in control cultures (Fig. 3B).

Cell culture is a useful tool in studying the regulation of gene expression. Since Caco-2 cells and enterocytes reveal several common features [22], we have checked the effect of bile on the I-BABP mRNA abundance in this human cell line. Bile drained from a 66-year-old female having undergone a choledolithotomy and T-tube drainage was used on the 7th postoperative day. Its BA composition is recorded in Table 2. The I-BABP gene was apparently unexpressed in control cultures (Fig. 4). In contrast, I-BABP mRNA were highly expressed when 5% (v/v) human bile was added to the medium (Fig. 4). According to the in vivo results, this up-regulation was gene-specific since the L-FABP and β-actin mRNA levels were unchanged throughout the experiment (Fig. 4).

4. Discussion

To the best of our knowledge, this report shows for the first time that bile can induce I-BABP expression in the ileum. The lack of effect of the bile on both the L-FABP and β-actin mRNA levels demonstrates that this biliary action is gene-specific. Moreover, the fact that the addition of the bile led to an increase in the cytosolic I-BABP concentration strongly suggests the physiological relevance of our mRNA data. The biliary proteins are not involved in this regulation since a pretreatment of bile by heat or proteases does not prevent the induction of I-BABP (data not shown). Therefore, it is tempting to speculate that the main components of the bile, that is to say the BA, are physiological regulators of I-BABP expression. Preliminary experiments performed both in organ

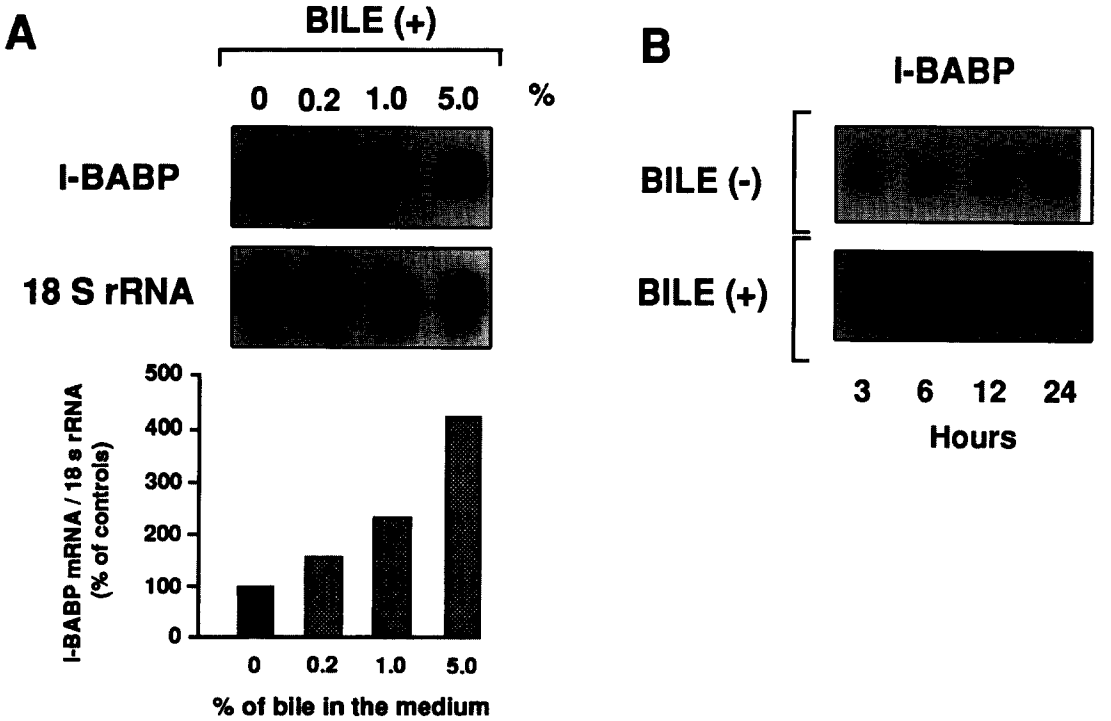

Fig. 3. (A) The effect of bile concentration on I-BABP mRNA in organ culture of ileal explants. Explants were cultured for 24 h in the presence of various concentrations of bile. Control cultures were performed without bile. The data were normalized to 18 S rRNA for differences in RNA loading. (B) Time course of bile action on I-BABP mRNA in organ culture of ileal explants. Explants were cultured in presence of 5% (v/v) bile alone for 24, 12, 6 or 3 h before they were harvested. Control cultures were performed without bile. RNA were extracted and analyzed as described in section 2.

Table 2
Bile acids composition of the human bile used in the Caco-2 study

Bile-acids	mM
Glycoursodeoxycholic acid	nd
Tazuroursodeoxycholic acid	nd
Ursodeoxycholic acid	nd
Glycocholic acid	11.5
Taurocholic acid	1.7
Cholic acid	0.1
Glycochenodeoxycholic acid	4.5
Taurochenodeoxycholic acid	0.6
Chenodeoxycholic acid	nd
Glycodeoxycholic acid	nd
Taurodeoxycholic acid	nd
Deoxycholic acid	nd
Glycolithocholic acid	nd
Taurolithocholic acid	nd
Lithocholic acid	nd

The bile from a 66-year-old female with a cholelithisis who had undergone surgery (choledocholithotomy followed by T-drainage) was collected on the 7th postoperative day (nd = not detected).

culture and in Caco-2 cells suggest that this hypothesis is right (H. Fudji and T. Kanda, personal data).

The regulation of a binding protein by its specific ligands has already been observed in other members of the FABP family. For instance, long-chain fatty acids trigger the transcription of the adipocyte-lipid binding protein (ALBP) [23] and 9-cis retinoic acid induces the cellular retinol binding protein II (CRBPII) in the small intestine [24]. Moreover, the regulation by BA of the cholesterol 7α-hydroxylase [25,26] and the sterol 27-hydroxylase genes [26], the rate-limiting enzymes involved in the conversion of cholesterol to BA in the liver, has recently been demonstrated. In this hypothesis, glycocholic acid, glycochenodexoxycholic acid and taurocholic acid, the main BA found in the human bile used in the Caco-2 experiment (Table 2), are potential candidates. Finally, the rapid increase of the I-BABP mRNA level found in the organ culture and the switch-on of the I-BABP gene occurring in Caco-2 cells after bile addition strongly suggest a transcriptional control of the I-BABP gene by biliary components.

In conclusion, the data reported here demonstrate that the I-BABP gene can quickly respond to changes in the luminal environment. They also show that the Caco-2 cells make up a useful tool for determining the factors affecting I-BABP expression and the molecular mechanisms of its regulation.

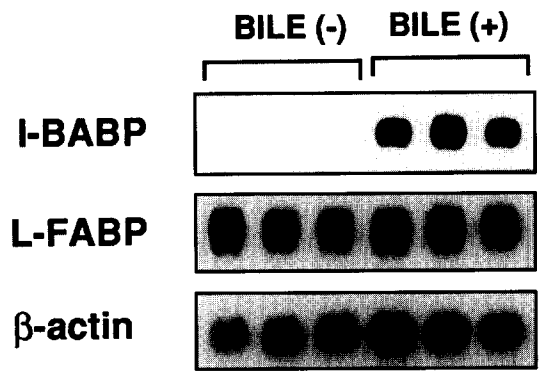

Fig. 4. The effect of bile on I-BABP, L-FABP and β-actin mRNA levels in the Caco-2 cells. The cells were cultured for 12 h in medium containing 5% (v/v) human bile. Control cultures were performed without bile. Total RNA (20 μg/lane) were extracted and analyzed as described in section 2.

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