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Tissue-specific regulation of the expression of rat intestinal bile acid-binding protein

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Abstract A lipid-binding protein identical to the rat intestinal bile acid-binding protein, termed I-15P, was expressed in steroid hormone-producing tissues such as ovary and adrenal gland, but not testis. In immature rats, I-15P was expressed in intestine but not in ovaries. The expression of I-15P in the ovaries of immature rats was induced to the level in immature rats by gonadotropin treatment. This suggests that the expression of I-15P in the ovaries is controlled by the ovarian cycle. The present results indicate that the expression of I-15P is developmentally and hormonally controlled in a tissue-specific manner.

Key words: Fatty acid-binding protein; Bile acid; Steroid hormone; Ovarian cycle; Gonadotropin

1. Introduction

Fatty acid-binding proteins (FABPs) are known to exhibit high affinity for long chain fatty acids [1,2]. FABPs have similar molecular masses (14–15 kDa), and form a family with other hydrophobic ligand-binding proteins such as cellular retinol-binding protein (CRBP), cellular retinoic acid-binding protein (CRABP), and myelin P2 protein (mP2) [3]. To date, at least fifteen members of the lipid-binding protein family have been isolated from various sources [4]. Although they are thought to be involved in the intracellular transport and/or metabolism of hydrophobic ligands which are responsible for cellular functions, their precise functions are not fully understood.

The intestinal 15 kDa protein (I-15P), a cytosolic protein with an apparent molecular mass of 15 kDa, was isolated originally from ileal mucosa along with intestine (I-) and liver (L-) FABPs [5]. In a previous study, although sequence analysis demonstrated that I-15P is a member of the FABP family, an in vitro binding assay revealed it has no binding ability as to long chain fatty acids [5]. More recently, we and another group have reported that the rat [6] or human homologue [7] of I-15P can bind to taurocholate, suggesting that I-15P may play a role in bile acid reabsorption in the ileum. However, the tissue distribution of I-15P, assessed by means of Northern blot and immunochemical analyses, revealed that I-15P was abundantly expressed not only in ileum but also in ovaries [8–10]. This tissue distribution implies that I-15P may also be involved in steroid metabolism, and raises the questions of the specific ligand and function of I-15P in extra-intestinal organs. Alternatively, it is possible that I-15P expressed in steroid hormone-producing tissues is a distinct protein homologous to the intestinal counterpart, which has a different ligand-binding specificity.

In the present study, to address these questions, we identified a cDNA for extra-intestinal I-15P, and examined whether or not the expression of I-15P is developmentally or hormonally controlled in a tissue-specific manner.

2. Materials and methods

2.1. Hormone treatment

Immature female Sprague Dawly rats (21 days old) were injected subcutaneously with 50 IU of pregnant mare serum gonadotropin (PMSG; UCB Bioproducts S.A.) or the vehicle (phosphate-buffered saline) alone, followed by the injection of 100 IU of human chorionic gonadotropin (hCG; Teikokuzoki) or the vehicle alone after 48 h. Two days after the injection of hCG, ovaries and intestines were removed from rats killed by means of ether inhalation. The organs were rapidly dissected out and quickly frozen in liquid nitrogen, and then stored at −80°C until use. Mature female rats (8 weeks old) were used for comparison with the immature rats.

2.2. Molecular cloning of ovarian I-15P

A rat ovary cDNA library (Clontech) was screened with a ^{32}P-labeled full length rat I-15P cDNA [8]. Positive clones were isolated and sequenced on both strands by the dideoxy termination method [11] with a 373A DNA Sequencer (Applied Biosystems).

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

As described previously [7,12], first-strand cDNAs were synthesized, using 1 μg of total cellular RNA prepared from ileum, ovaries, adrenal glands or testes of mature rats, according to the manufacturer's protocol (1st-Strand cDNA Synthesis Kit; Clontech). Aliquots (2 μl each) were used as templates for PCR amplification specific for rat I-15P. The primers used were synthesized on the basis of the nucleotide sequences of the N-terminal (residues 1–21) and C-terminal (residues 357–384) regions [8]. Amplification was performed in a 100 μl reaction mixture for 35 cycles; each cycle consisted of 1 min denaturation at 94°C, 2 min annealing at 60°C, and 3 min elongation at 72°C, followed by 5 min extension at 72°C. As a negative control, the reaction mixture contained no 1st-cDNA as a template. The PCR products were separated by 2% agarose gel electrophoresis, and then analyzed by Southern blotting using the internal probe. To further confirm the identity of the PCR products, the amplified DNA fragments prepared from agarose gel were ligated into the pGEM-T vector (Promega) and sequenced on both strands. Rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA was used as a control. The primers used were based on the nucleotide sequences of the upstream (residues 35–60) and downstream (residues 994–1017) regions of rat GAPDH cDNA [13]. Amplification of GAPDH cDNA for each tissue was performed as described above.

2.4. Western blot analysis

Five grams of each organ (ovaries and intestines) was homogenized

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in 15 ml of 50 mM Tris-HCl, pH 7.5, containing 150 mM KCl and 0.1 mM dithiothreitol. The homogenates were centrifuged at 20,000 × *g* for 20 min. The resulting supernatants were centrifuged at 105,000 × *g* for 60 min to yield the cytosolic fraction. The cytosolic proteins (10 μg) were fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then electroblotted onto nitrocellulose membranes. I-15P was detected using anti-rat I-15P rabbit serum (diluted 1:500). As described previously [14], rat heart-type (H)-FABP was detected using a specific polyclonal antibody raised against the purified H-FABP. After incubation with the first antibodies at 37°C for 1 h, the nitrocellulose membranes were incubated with peroxidase-conjugated anti-rabbit IgG (diluted 1:400). Peroxidase labeling was detected as chemiluminescence using the ECL reagent (Amersham). The signals were visualized by exposure to Fuji RX film.

2.5. Northern blot analysis

Organs (ovaries and intestines) from control or hormone-treated rats were rapidly dissected out and quickly frozen in liquid nitrogen. Poly $(A)^+$ RNA was isolated by the method previously reported [8] and then fractionated by electrophoresis in a 1% agarose gel containing formaldehyde. After transfer to a NYTRAN nylon membrane (Schleicher and Schuell), hybridization was performed with ^{32}P-labeled I-15P cDNA or H-FABP cDNA. As described previously [15], H-FABP cDNA, as a probe, was synthesized by RT-PCR using the primers corresponding to the C-terminal and N-terminal regions on the basis of the H-FABP cDNA sequence. Hybridization was performed at 55°C in 5× SSPE (0.9 M sodium chloride/50 mM sodium phosphate/5 mM EDTA, pH 7.0), 0.1% SDS, 100 μg/ml denatured sonicated salmon sperm DNA and 5× Denhard't solution. The membrane was washed in 1× SSC (0.15 M sodium chloride/0.015 M sodium citrate, pH 7.0) at 65°C, followed by visualization. After removing the I-15P probe, the same membrane was reprobed with the H-FABP cDNA.

3. Results and discussion

In a previous study, Northern blot analysis of rat I-15P under a moderately stringent conditions revealed a single transcript only in ileum and ovary [8], however, the RT-PCR in the present study demonstrated expression in other steroid hormone-producing tissues such as adrenal gland, but not in testis (Fig. 1). Therefore, it is possible that a potent lipid-binding protein homologous or identical to I-15P is present in these tissues, but its precise primary structure and specific ligand are not known. To establish the nucleotide identity between intestinal and extra-intestinal I-15P, we performed molecular cloning of extra-intestinal I-15P cDNA. Screening of the 2×10^5 clones from the rat ovary cDNA library with the full length rat I-15P cDNA probe yielded several positive clones. The cDNA inserts obtained from these positive clones were isolated. One of these clones contained a 507 bp nucleotide with a single open reading frame encoding 384 bp, as well as 5′ (35 bp) and 3′ (88 bp) untranslated regions (data not shown). It was demonstrated that the primary nucleotide sequence of the open reading frame of the ovarian counterpart was identical to that of I-15P in intestine. In addition, RT-PCR of adrenal gland revealed that the protein expressed in this tissue was also identical to I-15P, but the level was much lower than in ileum or ovary (data not shown). Interestingly, although the reason for the lack of expression of I-15P in testis is unknown, it is possible that I-15P specifically plays a role in ovary but not in testis.

Next, to investigate the function of I-15P in steroid hormone-producing tissues, we examined whether or not the ovarian I-15P is developmentally or hormonally controlled. We first examined the expression of I-15P in the intestine or ovaries of immature rats. As shown in Fig. 2, lanes 1 and 3, Western blot analysis revealed that I-15P was expressed in the intestine of the immature female rats but not in ovaries. On the other hand, another member of the FABP family, H-FABP, which is local-

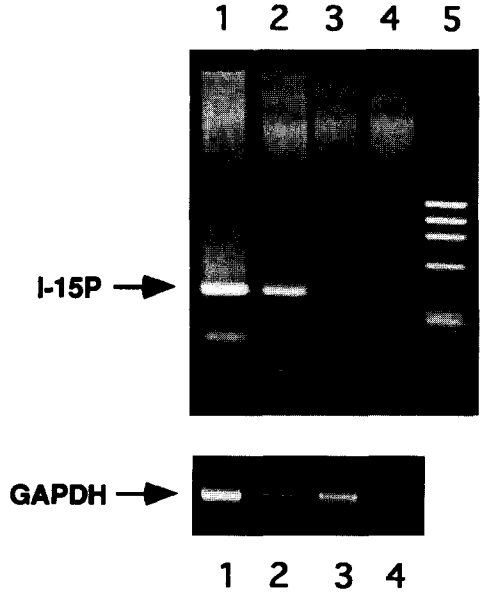

Fig. 1. RT-PCR of mRNA from steroid hormone-producing tissues. First-strand cDNAs of rat ileum, ovary, adrenal gland and testis were synthesized, and used as templates for PCR specific for rat I-15P or GAPDH. Upper panels, ethidium bromide staining of the amplified products of I-15P. The arrow indicates a 384 bp fragment. Lower panels, ethidium bromide staining of the amplified products of GAPDH. The arrow indicates a 983 bp fragment. Lane 1, ileum; lane 2, ovary; lane 3, adrenal gland; lane 4, testis; lane 5, ϕX174/HaeIII.

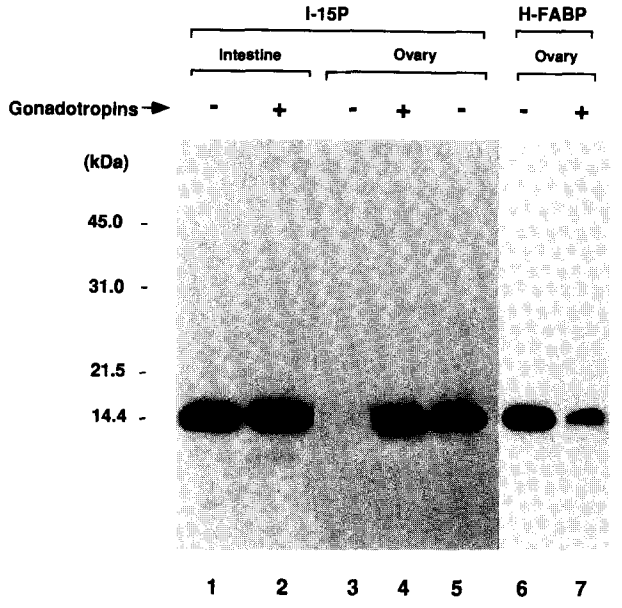

Fig. 2. Western blot analysis of ovarian or intestinal I-15P and ovarian H-FABP after treatment of immature rats with gonadotropins. Immunoblot analysis of ovarian or intestine cytosolic I-15P, and ovarian cytosolic H-FABP (10 μg each) in immature rats with anti-rat I-15P (lanes 1–5) or H-FABP antiserum (lanes 6 and 7) was performed. Samples were fractionated and subjected to Western blot analysis as described in section 2. Lane 1, untreated intestine; lane 2, treated intestine; lanes 3 and 6, untreated ovary; lanes 4 and 7, treated ovary; lane 5, untreated mature rat ovary.

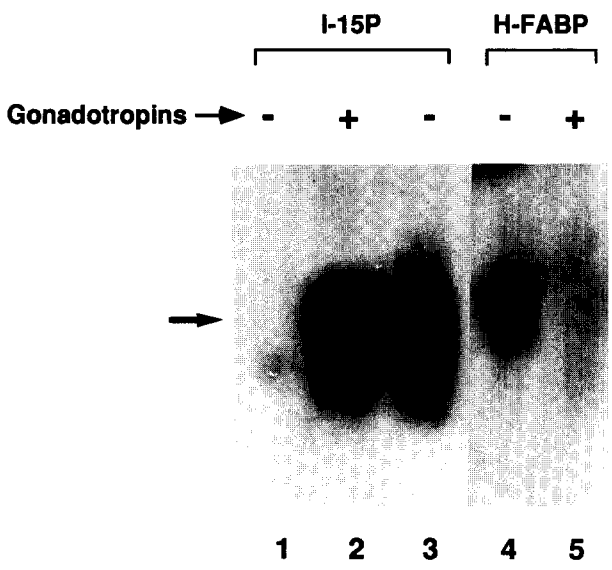

Fig. 3. Northern blot analysis of ovarian I-15P and H-FABP mRNA after treatment of immature rats with gonadotropins. Two μg of ovarian poly $(A)^+$ RNA isolated from untreated or gonadotropin-treated rats was electrophoresed and then transferred to a nylon membrane. The membrane was probed with the full length rat I-15P cDNA (lanes 1–3) or H-FABP cDNA (lanes 4 and 5). Lanes 1 and 4, untreated immature rat; lanes 2 and 5, treated immature rat; lane 3, untreated mature rat. The arrow indicates I-15P or H-FABP transcript.

ized in the interstitial cells of rat ovaries [16], was found to be expressed even in immature rats (Fig. 2, lane 6). In our immunochemical study, H-FABP was observed in rat ovaries on postnatal day 3 [17]. These findings were confirmed at the RNA level, as assessed by Northern blot analysis (Fig. 3, lane 1). The ovarian I-15P mRNA was not detected in immature rats, whereas H-FABP mRNA was significantly detected under the same conditions (Fig. 3, lane 4). Thus, the expression of rat I-15P is developmentally controlled in a tissue-specific manner.

To gain further insight into the mechanism by which the ovarian I-15P gene is developmentally controlled, we examined the effect of gonadotropin treatment of immature rats on the expression of ovarian I-15P. Western blot analysis revealed that no immunoreactive signal was observed for immature female rats (Fig. 2, lane 3), whereas a strong signal equivalent to that in mature rats (Fig. 2, lane 5) was detected in the cytosol derived from gonadotropin-treated immature rats (Fig. 2, lane 4). This finding suggests that the expression of ovarian I-15P is hormonally and developmentally controlled.

To determine whether or not the regulation of the expression of ovarian I-15P is correlated with that of the mRNA level, Northern blot analysis of total RNA from control and gonadotropin-treated immature rats was performed (Fig. 3). The expression of ovarian I-15P mRNA was also found to be induced by the hormone treatment (Fig. 3, lane 2), suggesting that the ovarian I-15P gene expression might be controlled by the ovarian cycle at the transcriptional level. In fact, the expression of I-15P in mature rats was shown to exclusively occur in corpus luteum cells of ovaries [9,10]. On the other hand, the expression of H-FABP mRNA was not induced but markedly suppressed by the gonadotropin treatment (Fig. 3, lane 5). Thus, there is a remarkable difference in the regulation of the gene expression between I-15P and H-FABP during the ovarian development. Further study is required to elucidate the regulatory mechanism of the tissue-specific gene expression of I-15P. We next examined if the I-15P expression in the rat intestine is affected by gonadotropin treatment. As shown in Fig. 2, lanes 1 and 2, no significant change in the protein level was observed in intestine, suggesting that the expression of I-15P in the intestine is independent of the ovarian cycle.

More recently, it was shown that I-15P isolated from rat or human ileum has the ability to bind to taurocholate, suggesting that taurocholate may be a specific ligand for I-15P in the ileum and that I-15P may be responsible for reabsorption of bile acids in this tissue [6, 7]. However, these findings raised a question regarding the function of I-15P in extra-intestinal tissues, considering its tissue distribution. As steroid hormones such as progesterone are synthesized from cholesterol and exhibit structural similarities to bile acids, they might be candidates for the ligands of I-15P in steroid hormone-producing tissues. Further study is required to clarify these issues.

Overall, we conclude that the expression of I-15P is regulated in a tissue-specific manner, and that the function of I-15P in extra-intestinal tissues might be different from that in the intestine.

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