Differential effects of sex steroid hormones on the expression of multiple first exons including a novel first exon of prolactin receptor gene in the rat liver

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Abstract

In addition to the known four alternative first exons E1₁, E1₂, E1₃ and E1₄ of the rat prolactin receptor (PRL-R) gene, a novel first exon, E1₅, was identified by cDNA cloning of the 5'-end region of PRL-R mRNA in the rat liver. Genomic fragments containing E1₅ and its 5'- or 3'-flanking regions were also cloned from rat kidney genomic DNA. A sequence search for E1₅ revealed that E1₅ is located 49 kb upstream of exon 2 of the PRL-R gene in rat chromosome 2q16. RT-PCR analysis revealed that E1₅ was preferentially expressed in the liver, brain and kidney. Expression profiles of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver of male and female rats at 5 days of age and those at 8 weeks of age were examined by RT-PCR. The levels of E1₂-PRL-R mRNA in the female rat increased remarkably in rats at 8 weeks of age compared with those at 5 days of age, and the levels of E1₅-PRL-R mRNA in the male rat decreased markedly at 8 weeks of age compared with those at 5 days of age. In the female rat, the levels of E1₂-PRL-R mRNA at 8 weeks of age decreased with ovariectomy performed at 4 weeks of age and recovered with the administration of β -oestradiol. On the contrary, the levels of E1₅-PRL-R mRNA increased with ovariectomy and decreased with the oestrogen treatment. In the male rat liver, the levels of E1₂-PRL-R mRNA at 8 weeks of age increased strikingly with castration performed at 4 weeks of age and became undetectable with the administration of testosterone. The levels of E1₅-PRL-R mRNA increased slightly with castration and were restored by testosterone treatment. Removal of gonadal tissues and sex steroid hormone treatment had no effect on the expression levels of E1₃-PRL-R mRNA in both female and male rat livers. These results indicated that the expression of the PRL-R gene in the liver is regulated by the differential effects of sex steroid hormones on the transcription of the multiple first exons including the novel one.

Journal of Molecular Endocrinology (2005) **34**, 667–673

Introduction

Prolactin (PRL) has many physiological functions in vertebrates (Doppler 1994, Freeman *et al.* 2000). The actions of PRL are mediated by the PRL receptor (PRL-R), which belongs to the type 1 cytokine receptor superfamily (Kelly *et al.* 1991). In mammals, the long and short forms of PRL-R differing in the length and sequences of their cytoplasmic domains are generated by alternative splicing of a primary transcript from a single PRL-R gene (Boutin *et al.* 1988, Davis & Linzer 1989, Shirota *et al.* 1990, Bignon *et al.* 1997, Schuler *et al.* 1997). The expression of the PRL-R gene is regulated by complex mechanisms. Multiple alternative first exons encoding 5'-untranslated regions have been identified in rat and human PRL-R genes, and the distinct expression patterns of these first exons are controlled by promoters located in the upstream regions of each first exon. In the rat PRL-R gene, three distinct first exons E1₁, E1₂ and

E1₃, together with their promoter regions PI, PII and PIII, have been identified (Hu *et al.* 1996, Moldrup *et al.* 1996). These three exons are located in the order of E1₃, E1₁, E1₂ from the distal side of exon 2 of the PRL-R gene (Hu *et al.* 1998a). E1₁ and E1₂ are predominantly expressed in gonadal tissues and liver respectively, while E1₃ is expressed in a wide range of tissues (Hu *et al.* 1996). The gonad-specific expression of E1₁ is dependent on the activation of its promoter by steroidogenic factor I (Hu *et al.* 1997), and hepatocyte nuclear factor 4 (HNF4) participates in the expression of E1₂ in the liver (Moldrup *et al.* 1996). The activation of the E1₃ promoter by the transcription factors C/EBP β and SP1 accounts for the generic expression of E1₃ (Hu *et al.* 1998b). We have recently found another first exon, E1₄, which is preferentially expressed in the brain, though its location in the rat PRL-R gene is not known (Tanaka *et al.* 2002). In the human PRL-R gene, six alternative first exons, hE1₃, hE1_{N1}, hE1_{N2}, hE1_{N3}, hE1_{N4} and

hE_{N5}, have been identified (Hu *et al.* 1999, 2002). hE₁₃ is considered to be the counterpart of rat E₁₃ because its promoter shows high sequence similarity to that of rat E₁₃ and shows high activity in various human tissues. The other five first exons show unique expression profiles in human tissues such as liver, ovary, testis and in a breast cancer cell line, T47D cells.

The presence of the multiple first exons allows the PRL-R gene to be regulated in different ways in individual tissues. We have previously demonstrated that the higher levels of PRL-R mRNA in the rat brain in late pregnancy and the following lactating period are dependent on the expression of E₁₄ (Sugiyama *et al.* 1994, Tanaka *et al.* 2002). It has also been shown that the expression levels of PRL-R mRNA in the different brain regions are regulated by oestrogen, which increases the utilization of E₁₂ and E₁₃ first exons (Pi *et al.* 2003). In the liver, the expression of the PRL-R gene is known to be regulated by sex steroid hormones; up-regulation by oestrogen and down-regulation by testosterone (Jolicœur *et al.* 1989, Sakaguchi *et al.* 1994). However, the molecular mechanisms of the effects of the sex steroid hormones on the expression of the PRL-R gene in the liver are not yet known.

In the present study, we have identified a novel first exon, E₁₅ of the rat PRL-R gene, and have examined the effects of sex steroid hormones on the expression of E₁₂, E₁₃ and E₁₅ first exons in the liver.

Materials and methods

Animals and hormone treatment

Sprague–Dawley rats were purchased from SLC (Sizuoka, Japan) and housed in a temperature-controlled room with 12 h light and 12 h darkness. Food and water were available *ad libitum*.

Castration or ovariectomy was performed on 4-week-old rats, and hormone treatment was started 2 weeks after surgery. Five micrograms of 17 β -oestradiol (Nacalai Tesque, Kyoto, Japan) in 0.1 ml olive oil, 1 mg testosterone propionate (Nacalai Tesque) in 0.1 ml olive oil, or 0.1 ml olive oil alone were injected subcutaneously into the back of the neck. The hormone injection was carried out daily for 2 weeks. The animals were killed by decapitation 24 h after the last injection and the liver was rapidly removed and frozen in liquid nitrogen until used. All procedures were performed in accordance with the NIH guidelines regarding the principles of animal care.

cDNA cloning for 5'-regions of PRL-R mRNAs in the rat brain

Total RNA was extracted from the liver with the ISOGEN kit (Nippon Gene, Toyama, Japan), and poly (A)⁺ RNA was isolated with oligotex-dT30 (TAKARA,

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GCAGGCTGTTACTACATGTGTTGTGACTGACACCACAACCAGCTAAAGGA 50
CACTTCTCTGTGAAGGTGAGCACTGCAGATGTTTTCACATGAGCCCTGA 100
AGGGAGCCTCTGATACATTGCCTATAGCAAGAAGAAGGGGCCAACCTGAA 150
GAAAAACATGCCATCTGCACCTTGCTTTCGTCTCTACTTGTCTCAACATCA 200
GCCTCCTGAAGGGACAGTCAACCCAGGAAACCTGAGATCCACAAATGT 250
CGCTCCTCTGACAAGGAAACATTCACTGCTGGTGGAAATCCTGGGACAGA 300
TGGAGGACTTCTACCAATTATTCACTGACTTACAGCAAGAAGGAGAGA 350
AAACCACCTACGAATGTCCAGACTACAAAACCAAGTGGCCCCAACTCCTGC 400
TTCTTTAGCAAGCAGTACACTTCCATCTGGAATATATATCATCACAGT 450
AAATGCCACGAACCAATGGGAAGCAGTT 479
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Figure 1 The nucleotide sequence of the E₁₅-PRL-R mRNA obtained by the oligo-capping method. The region corresponding to the E₁₅ first exon is underlined and the initiation codon ATG is indicated by bold letters.

Tokyo, Japan) according to the manufacturer's instructions. The 5'-end of the PRL-R cDNA was synthesized by the oligocapping method (Maruyama & Sugano 1994) as previously described (Tanaka *et al.* 2002), cloned into pGEM-T Easy vector (Promega, Palo Alto, CA, USA) and sequenced.

Cloning of the E₁₅ exon-containing region of the rat PRL-R gene

Genomic DNA was prepared from a rat testis by the SDS-proteinase K method, and 5'-flanking regions of E₁₅ were cloned with an LA-PCR *in vitro* cloning kit (TAKARA) according to the manufacturer's instruction. Briefly, the genomic DNA was digested with EcoRI restriction enzyme, and ligated with EcoRI cassette. The 5'-flanking region of E₁₅ was then amplified by PCR with cassette primer C1 supplied by the manufacturer and primer 1 (5'-CTGGTTGTGTTGTGTCAGTCACAA-3') corresponding to the antisense sequence of nucleotides 21–42 of E₁₅ cDNA (Fig. 1). The second PCR amplification was performed with cassette primer C2 supplied by the manufacturer and primer 2 (5'-TCAGTCACAACACATGTAGTAAC-3') corresponding to the antisense sequence of nucleotides 8–30 of E₁₅ cDNA. For the cloning of the 3'-flanking region of E₁₅, the inverse PCR method was employed (Ochman *et al.* 1990). The genomic DNA was digested with SacI restriction enzyme and self-ligated. The circularized DNA was subjected to the PCR with primer 3 (5'-AATGCTGGCTCCAGCTAGGTCTTT-3') corresponding to nucleotides –146 to –122 and primer 4 (5'-TGCCTGTGTGAATAGCCTACTGGCT-3') corresponding to the antisense sequence of nucleotides –1115 to –1091 of the 5'-flanking region of E₁₅ (Fig. 2). The amplified DNA fragments were cloned into pGEM-T Easy vector and sequenced.

RT-PCR analysis

Total RNA (10 μ g) from rat tissues was reverse transcribed using oligo-dT primer (TAKARA) and the

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gaattcgattagttagctctgtccgtgaaaggacagtataattccatctgtctcacgcccaactgctcctctgggactggtagcatctgtgtcctctgatt -1449
tctgctcttttagaaaaatcaataccacatcatattgtcaacctttcaagagctcaaggagagtgcaggagggaattgctccaggaggagctccag -1349

      CCAAT
ttttgatgaaagtctgatccaaataacacttctctagcagaaagtaccatattgtcaggtcattccaaataacctgtgacaagataccagtagactgtccta -1249
ccgattgtgtagtagtgactcttaaaacagttacttagtaaccaagtgttgcctgggatacaggcagaggagcatgttcatgagacacagggaacttaac -1149
aaggagagaggtggcttcttaaatcccatcttataagccagtaggctattcacacaggcagaccacaccctctaggcctctcttcttcttgggacatga -1049
gaaccttgacccctcatcttgaagacatcttatccaccatcctcttatatttgagaagtataattttctgagggttaagggtggagccagcaatcttc -949

      GAS
tcaaagggttgagcttacaattatgtagttgacattctcaaacacttttttaatcagagaggaatacagtaaaattcattcatggaatgatcctgtatccc -849
tattcatttaagtgggaaaagttagacataataacataacataacataacataacataacataacataacattctcaaagtagtcaggagaaag -749

      GATA
aatttaaaagtatgttctagagttcagatagattctctgatttagtgataagtttgagttctatggatttgggatacacaaactatgattggtgtttggac -649
gtctagaagttaagatattatagctatgagagaatggtagagtgagatggagcttatgaagtaatgatattcagatatagaaatttttcatatcaca -549
atagtaattggagtcagtgcttcttccactgaagtcactgacaagcttcttctgtgattggaaggaaggtcatcagacattatgaaaagggtgttagaaa -449
tgcatgtgtagaaaattttcttatgcaagattcactgagctttttgtgaccatatacccatccatagaaaaatatgattaaagtgtctaaatccacaat -349
aactcaagcttggagccttggaataatagcttttagaaaaaaaatcacctggcagagctccatgtttcctttctcattggatttttggaaataggattag -249
ggtaaacagtaatttttaattcaccacaaaaaaacccctatttcttacaacactatctgtgtgcaagtttggtttttccacacggatccaggctgcca -149
agaaatgctggctccagctaggtccttctgttagccaagtcaggggatggtcagggcattcatccagggtgacctgagccaccccaactcaaaaaaccaa -39

gaggagtttgctcggtagagcaaggggctcactgactgcaggctgttactacatgtgtgtgactgacaccacaaccaggtatgtcatgagaaaacctt 62

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Figure 2 Nucleotide sequence of E1₅ and its 5'-flanking region. The sequence of E1₅ is shown in capitals. Transcription factor elements CCAAT, GATA and GAS are overlined.

resulting cDNA was subjected to 25 cycles (E1₂-, E1₃- and E1₅-PRL-R mRNAs) and 17 cycles (glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) of PCR (94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min). Primer 5 (5'-AAGAACAACCTTGCCTGGCAAAGA GG-3', nucleotide positions -289 to -266 of E1₂ (Hu *et al.* 1996) and primer 6 (5'-TTCCTGCTCTGTCT CACTCGCTCC-3', nucleotide positions -261 to -238 of E1₃ (Hu *et al.* 1996)) and primer 7 (5'-GCAGGCTGT TACTACATGTGTTG-3', nucleotide positions 1-23 of E1₅ cDNA in Fig. 1) were used as sense primers for the respective PRL-R mRNAs. Primer 8 (5'-GAAGAAGG GGCCAACCTGAAGAAAAAC-3', nucleotide positions 131-157 of E1₅ cDNA in Fig. 1) was used as the sense primer for the analysis of total PRL-R mRNA. Primer 9 (5'-AAGTGGTTCCTGCTGTTG-3', nucleotide positions 456-479 of E1₅ cDNA in Fig. 1) was used as the antisense primer for all of the PRL-R mRNAs. A sense primer (5'-TGAAGGTCGGTGTGA ACGGATTT-3') and an antisense primer (5'-CACA GTCTTCTGAGTGGCAGTGA-3') were used for the analysis of rat GAPDH mRNA. The PCR products were separated by electrophoresis on a 1.5% agarose gel and transferred to a nylon membrane. The membrane was subjected to Southern blot analysis with the AlkPhos direct labelling and detection system (Amersham Bioscience, Tokyo, Japan) according to the manufacturer's instruction. A cDNA fragment derived from a common region of E1₅-PRL-R cDNA (nucleotides 131-479 in Fig. 1) was used as a probe. The fluorescent signal from the probe was detected on an X-ray film and the density of bands was measured by using the NIH image analysis system.

Statistical analysis

The data were analyzed for statistical significance using the Macintosh super ANOVA program and are

expressed as the means $\pm$ S.D. The significance of the differences between the values was analyzed using Scheffé's post-hoc test, and $P < 0.05$ was considered significant.

Results

Identification of a novel first exon, E1₅, in the rat PRL-R gene by cDNA and genomic DNA cloning

Sequence analysis of the oligo-capping PRL-R cDNA clones obtained from the rat liver revealed the presence of a cDNA clone containing a unique sequence of 42 bp at its 5'-end. The unique sequence in the cDNA is followed by the full-length exon 2 sequence, hence the unique sequence is considered to be derived from a novel first exon and is referred to as E1₅ (Fig. 1). The 5'- and 3'-flanking regions of E1₅ were cloned by PCR-based methods and sequenced (Fig. 2). A computer-assisted search revealed that E1₅ was localized between E1₂ and exon 2 in the draft sequence of the PRL-R gene located in chromosome 2q16 (Fig. 3). No canonical TATA box or GC box was observed but consensus sequences for transcription factors such as CCAAT, GATA and γ -interferon-activated sequence (GAS) were present in the 5'-flanking region. The splicing donor site sequence, gt, was observed immediately downstream of E1₅.

Tissue distribution of E1₅-PRL-R mRNA

Tissue distribution of E1₅-PRL-R mRNA was examined by RT-PCR. As shown in Fig. 4, a 349 bp fragment derived from E1₅-PRL-R mRNA was detected in the liver, brain and kidney, but not in the other tissues examined.

Figure 3 Schematic representation of the position of the E1₅ first exon in the rat PRL-R gene. The positions of the four first exons and exon 2 in the rat genome draft sequence (NW 047622.1; National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD, USA) are shown by thick vertical lines. E1₅ genomic clones obtained by PCR are indicated at the corresponding positions.

Expression profiles of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver during sexual maturation

Expression levels of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver of male and female rats at 5 days of age and those at 8 weeks of age were examined by RT-PCR (Fig. 5). Levels of E1₂-PRL-R mRNA were faintly detected in the male rat liver at 5 days of age but were not detected at 8 weeks of age. In the female rat liver, the levels of E1₂-PRL-R mRNA were slightly higher than those in the male rat liver at 5 days of age, and increased remarkably at 8 weeks of age. The levels of E1₅-PRL-R mRNA did not differ with sex at 5 days of age, and decreased at 8 weeks of age with the male liver showing the higher extent. The levels of E1₃-PRL-R mRNA did not change with sex and age.

Effects of sex steroid hormone treatments on the levels of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver

The effects of sex steroid hormone treatments on the levels of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver

Figure 4 Tissue distribution of E1₅-PRL-R mRNA. Ten micrograms each of total RNA in the tissues of female and male (testis) rats at 8 weeks of age were reverse transcribed, and the E1₅-PRL-R cDNA was amplified by 25 cycles of PCR. The 349 bp of amplified cDNA fragment was hybridized with the AlkPhos-labelled PRL-R cDNA probe and the fluorescent signal from the probe was detected on an X-ray film.

Figure 5 Expression profiles of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver of male and female rats at 5 days of age and 8 weeks of age. (A) Autoradiograms of RT-PCR Southern blot analysis of E1₂-, E1₃- and E1₅-PRL-R mRNAs and GAPDH mRNA with four rats in each group. (B) Densities of the hybridization signals were measured and expressed as the values relative to the value of each PRL-R mRNA of female rats at 8 weeks of age. Each value represents the mean $\pm$ S.D. * P < 0.05, ** P < 0.01, *** P < 0.001, ND, not detected.

were investigated (Figs 6 and 7). Two weeks after castration or ovariectomy of 4-week-old rats, 17 β -oestradiol or testosterone propionate was subcutaneously administered daily for 2 weeks, and the levels of the PRL-R mRNAs were examined by RT-PCR 1 day after the final administration. In the female rat liver, the levels

Figure 6 Effect of oestrogen (E2) treatment on the expression levels of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver of female rats. (A) Autoradiograms of RT-PCR Southern blot analysis of E1₂-, E1₃-, E1₅- and total PRL-R mRNAs and GAPDH mRNA with four rats in each group. (B) Densities of the hybridization signals were measured and expressed as values relative to the value of each PRL-R mRNA of the sham-operated group. Each value represents the mean $\pm$ S.D. * P < 0.05, ** P < 0.01 vs the sham-treated group. OvX, ovariectomized.

of E1₂-PRL-R mRNA decreased significantly with ovariectomy and recovered with oestrogen treatment. On the other hand, the levels of E1₅-PRL-R mRNA increased slightly with surgery, and decreased to the levels of sham-operated rats with oestrogen administration. The levels of E1₃- and total PRL-R mRNAs were not affected by ovariectomy and hormone treatment. In the male rat liver, the levels of E1₂-PRL-R mRNA increased strikingly with castration and decreased to the levels of sham-operated animals with the administration of testosterone. The levels of E1₅-PRL-R mRNA slightly increased with castration and were restored by testosterone treatment, while castration and testosterone treatment had no effect on the levels of E1₃-PRL-R mRNAs. The profile of the total PRL-R mRNA was similar to that of E1₂-PRL-R mRNA.

Discussion

In addition to the known four first exons, E1₁, E1₂, E1₃ and E1₄, a novel first exon E1₅ was identified in the rat

Figure 7 Effect of testosterone (Te) treatment on the expression levels of E1₂-, E1₃- and E1₅-PRL-R mRNAs in the liver of male rats. (A) Autoradiograms of RT-PCR Southern blot analysis of E1₂-, E1₃-, E1₅- and total PRL-R mRNAs and GAPDH mRNA with four rats in each group. (B) Densities of the hybridization signals were measured and expressed as values relative to the value of each PRL-R mRNA of the sham-operated group. Each value represents the mean $\pm$ S.D. ** P < 0.01, *** P < 0.001 vs the sham-treated group. Cast, castrated.

PRL-R gene by cDNA and genomic DNA clonings, and found to be localized in the most proximal position to exon 2. E1₅ was preferentially expressed in the liver, brain and kidney. Such an expression profile differs from those of any other known first exons of rat and human PRL-R genes. Mouse counterparts of rat E1₁, E1₂, E1₃ and E1₅ are found in the 5'-flanking region of exon 2 of mouse PRL-R gene contained in a bacterial artificial chromosome clone derived from mouse chromosome 15. Although mouse E1₁ and E1₃ have been shown to be expressed in a mouse Leydig tumour cell line (Hu *et al.* 1997), expression profiles of each first exon in mouse tissues are not known. In the rat liver, E1₂ and E1₃ are also expressed with E1₂ as the major form (Hu *et al.* 1996). It has been shown that the expression of E1₂ in the liver depends on the action of HNF4 on the

promoter (Hu *et al.* 1998b) and that the expression of $E1_3$ in a wide range of tissues including the liver is operated by the activation of the promoter with the generic transcription factors, C/EBP β and SP1 (Moldrup *et al.* 1996). Within the 1.5 kb 5'-flanking region of $E1_5$, there is no consensus element for these transcription factors participating in the activation of $E1_2$ or $E1_3$. CCAAT, GATA and GAS elements are present, but no canonical TATA box or GC box exists in the 5'-region. The promoter regions directing the preferential expression in the liver, brain and kidney remain to be investigated.

It is well known that PRL-R is abundantly present in the female rat liver and that sex steroid hormones strongly affect the expression levels of PRL-R in the liver (Norstedt & Mode 1982, Yasui *et al.* 1999). We have previously shown that the PRL-R gene expression increases and decreases during sexual maturation in female and male rat liver respectively (Sakaguchi *et al.* 1994). The mRNA levels in the female liver decreased with ovariectomy and were restored by the administration of oestrogen. On the other hand, castration of male rats resulted in the induction of the PRL-R gene expression, and testosterone completely blocked the induction of the gene expression. Our present study revealed that expression of the three PRL-R mRNA species containing $E1_2$, $E1_3$ or $E1_5$ first exons were distinctly regulated by sex steroid hormones in the liver. In the female, the levels of $E1_2$ -PRL-R mRNA markedly increased during sexual maturation while, in the male, the mRNA levels were very low irrespective of sexual maturation. $E1_5$ -PRL-R mRNA levels in both sexes decreased during sexual maturation with male rats showing the higher extent. No notable change was observed in the levels of $E1_3$ -PRL-R mRNA with sex or age. In female rat liver, oestrogen treatment after ovariectomy resulted in the up-regulation of $E1_2$ -PRL-R mRNA expression and the down-regulation of $E1_5$ -PRL-R mRNA expression. In male rat liver, testosterone treatment following castration showed a strong and moderate suppression of the expression of $E1_2$ - and $E1_5$ -PRL-R mRNAs respectively. The expression of $E1_3$ -PRL-R mRNA was not affected by treatment with sex steroid hormones in either sex. The expression profiles of the total PRL-R mRNA in both sexes were represented by the profile of $E1_2$ -PRL-R mRNA, consistent with the reported finding that $E1_2$ -PRL-R mRNA is the most abundant PRL-R mRNA species in the liver (Hu *et al.* 1996). The presence of two potential half sites of the oestrogen-responsive element in the promoter region of $E1_2$ (Hu *et al.* 1996) suggested the possibility of a direct effect of oestrogen on $E1_2$ expression. The molecular mechanisms of the inducible effect of oestrogen together with the suppressive effect of androgen on $E1_2$ expression in the liver remain to be elucidated.

In addition to hepatocytes, the liver contains several cell types such as Kupffer cells, endothelial cells and hepatic stellate cells with minor populations. It has been shown that PRL-R is largely expressed in hepatocytes in the rat liver with higher levels in the female than in the male and that the PRL-R levels increase and decrease in both sexes with oestrogen and androgen treatments respectively (Smirnova *et al.* 1994). More recently, PRL-R gene expression has been observed in Kupffer cells as well as in hepatocytes in the male rat liver (Yokoyama *et al.* 2003). Although the profiles of the first exon usage in each cell types in the liver are not known, our present findings suggest that the $E1_2$ first exon is largely responsible for the stimulative and depressive effects of sex steroid hormones on the levels of PRL-R mRNA in hepatocytes. Moreover, it can be speculated that $E1_3$ and $E1_5$ are used in the minor hepatic cells to show the differential effects on their expression by sex steroid hormones.

In conclusion, the expression of the PRL-R gene in the liver is regulated by sex steroid hormones with different effects on the transcription of $E1_2$, $E1_3$ and the newly identified $E1_5$ first exons. Oestrogen moderately increases the overall PRL-R mRNA level in the female liver by stimulating the expression of $E1_2$ mRNA, while the overall PRL-R mRNA level in the male liver is strongly depressed by testosterone due to the suppression of $E1_2$ and $E1_5$ mRNA expression. These findings contribute to the understanding of the regulatory mechanisms of PRL-R gene expression by sex steroid hormones in the liver.

Acknowledgements

This work was supported in part by the grants-in-aid for Scientific Research 15380203 from the Ministry of Education, Science, Sports and Culture of Japan. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 3 December 2004

Accepted 8 March 2005

Made available online as an Accepted Preprint 21 March 2005