Characterization of multiple first exons in murine prolactin receptor gene and the effect of prolactin on their expression in the choroid plexus

Hidemi Tabata¹, Momoko Kobayashi¹, Junko H Ikeda¹, Nobuhiro Nakao¹, Toru R Saito² and Minoru Tanaka¹

¹Department of Animal Science and ²Behavioral Neuroscience Section, Graduate School of Veterinary Medicine, Nippon Veterinary and Animal Science University, Musashino, Tokyo 180-8602, Japan

(Correspondence should be addressed to M Tanaka; Email: mitanaka@nvlu.ac.jp)

Abstract

Prolactin (Prl) receptor (Prlr) gene is expressed in various brain regions, with the highest level present in the choroid plexus, a site for receptor-mediated PRL transport from the blood to cerebrospinal fluid. We investigated the regulatory mechanism of Prl gene expression by PRL in the murine choroid plexus. We first examined the organization of the alternative first exons in murine Prlr gene. In addition to the three known first exons, mE11, mE12, and mE13, two first exons, mE14 and mE15, were newly identified by cDNA cloning. Each first exon variant of Prlr mRNA exhibited tissue-specific or generic expression. In the choroid plexus of mice, the expression levels of mE12+, mE14+, and mE15-Prlr mRNAs were increased in the lactating mice compared with those in the diestrus mice. Furthermore, the expression level of mE14-Prlr mRNA was decreased in the PRL-deficient (Prl−/−) mice compared with the PRL-normal (Prl+/+) mice. In the ovariectomized Prl−/− mice, the expression level of mE14-Prlr mRNA was significantly increased by PRL administration but not by 17β-estradiol administration. The expression levels of the two last exon variants of Prlr mRNAs, encoding the long and short cytoplasmic regions of PrlR, were also increased in the lactating mice and decreased in the Prl−/− mice. These findings suggest that PRL stimulates the Prlr gene expression through the transcriptional activation of mE14 first exon, leading to increases in the long- and short-form variants of Prlr mRNA in the murine choroid plexus.

Journal of Molecular Endocrinology (2012) 48, 169–176

Introduction

Prolactin (PRL) exhibits many physiological functions, including a number of brain functions such as maternal behavior, stress tolerance, food intake, and sexual behavior (Bole-Feyset et al. 1998, Freeman et al. 2000). All these functions of PRL are mediated by PRL receptor (PRLR). PRLR mRNA expression has been detected in various brain regions in mammals, with the highest level detected in the choroid plexus (Brooks et al. 1992, Chiu et al. 1992, Di Carlo et al. 1992, Ouhtit et al. 1993, Chiu & Wise 1994, Nagano & Kelly 1994, Fujikawa et al. 1995, Brown et al. 2010). It is believed that PRL is transported from the blood to the cerebrospinal fluid (CSF) by a PRLR-mediated system in this brain region (Walsh et al. 1987). In addition, it has been reported that PRL enhances its own uptake in the choroid plexus (Mangurian et al. 1992). Supporting this function, a high level of PRLR expression in the choroid plexus has been observed during lactation when the plasma PRL level is also high (Muccioli & Di Carlo 1994, Escalada et al. 1996, Sugiyama et al. 1996, Pi & Grattan 1999, Augustine et al. 2003, Pi et al. 2003, Nogami et al. 2007, Anderson et al. 2008). Also, PRLR expression in this brain region increases during the stress response, accompanying an increase in the plasma PRL level (Fujikawa et al. 1995). Furthermore, both peripheral and central administration of PRL have been shown to increase the PRLR expression in the choroid plexus (Muccioli & Di Carlo 1994, Fujikawa et al. 2004). These findings suggest that PRL upregulates PRLR gene expression in the choroid plexus.

In mammals such as rats, mice, and humans, PRLR gene expression is regulated by the transcriptional activation of multiple alternative first exons encoding 5′-untranslated regions. In rat Prlr gene, five alternative first exons, E11, E12, E13, E14, and E15, have been identified previously (Hu et al. 1996, Moldrup et al. 1996, Tanaka et al. 2002, 2005). In rats, the E1 variant of Prlr mRNA is specifically expressed in the gonadal tissues (Hu et al. 1997) and the E12 variant is transcribed in the liver and kidney (Moldrup et al. 1996, Tanaka et al. 2005). In addition, the E13 variant is expressed in a wide range of tissues (Hu et al. 1998), whereas the E14 variant is preferentially expressed in the brain (Tanaka et al. 2002). Finally, the E15 variant is used in the brain, liver, and kidney (Tanaka et al. 2005). In murine Prl gene, three first exons homologous to rat
E11, E12, and E13 (referred to as mE11, mE12, and mE13 respectively) have been detected to date (Davis & Linzer 1989, Hu et al. 1997, 1998). In human PRLR gene, six alternative first exons, hE13, hE1N1, hE1N2, hE1N3, hE1N4, and hE1N5, have been identified (Hu et al. 1999, 2002).

In addition to the alternative first exons, mammalian PRLR genes also contain alternative last exons encoding the C-terminal regions of the long or short forms of PRLR proteins. In rats and humans, the two last exon variants of PRLR mRNA, one for the long form of protein and another for the short form, are present (Boutin et al. 1989, Shirota et al. 1990, Nagano & Kelly 1994, Trott et al. 2003). In murine Prlr mRNA, the four last exon variants, one long form (Prlr-S) and three short forms (Prlr-S1, -S2, and -S3), have been identified by cDNA cloning (Davis & Linzer 1989, Clarke & Linzer 1993, Moore & Oka 1993). However, the nucleotide sequence of PRLR-S3 contains one nucleotide deletion in codon 78, which results in the appearance of a stop codon after the following 19 amino acid residues. Therefore, PRLR-S3 encodes a truncated form of PRLR protein and is considered to be a transcript of a pseudogene (Davis & Linzer 1989). In rats and mice, the long-form PRLR is the predominant form in most tissues except for the liver where the short form is abundant (Davis & Linzer 1989, Shirota et al. 1990). Intracellular signaling of the long form is mainly mediated by the JAK2/STAT5 pathway (Bole-Feyt et al. 1998, Freeman et al. 2000). Although, short-form PRLR can activate the MAP kinase pathway (Das & Vonderhaar 1995), its intracellular signaling system is poorly understood.

To gain a better understanding of the molecular mechanisms of PRL function in the choroid plexus, it is essential to clarify the regulatory mechanisms involved in Prlr gene expression in the choroid plexus. In this study, we first demonstrated the presence of the five distinct first exons, including two newly identified first exons in murine Prlr gene, and then examined the tissue expression patterns of the individual first exon variants of Prlr mRNA. Subsequently, we used hyperprolactinemic (lactating) and PRL-deficient mice to examine the effects of PRL on the expression of the first exon variants together with the expression of the last exon variants of Prlr mRNA encoding long- or short-form PRLR in the choroid plexus.

**Materials and methods**

**Animals and hormone treatments**

C57BL/6j mice were purchased from Oriental Yeast (Tokyo, Japan). PRL-deficient (Prlr−/−) mice were generated as described previously (Horseman et al. 1997). The mice were housed under controlled temperature (22°C) and lighting conditions (12 h light:12 h darkness, lights were turned on at 0700 h). Food and water were given to the mice *ad libitum*. The presence of estrus cycle in the mice was determined by performing vaginal smears. Ovariectomy was performed on the mice at 5 weeks of age under pentobarbital anesthesia, and the hormone treatment was started on the mice after 2 weeks of their recovery. 17β-estradiol (E2; Nacalai Tesque, Kyoto, Japan) and human PRL (Shikibo Lifetech, Inc., Osaka, Japan) were individually dissolved in sesame oil and PBS respectively. E2 (1.25 mg/kg body weight) was injected s.c. into the back of the necks of the mice daily for 7 days, and PRL (2.5 mg/kg body weight) was s.c. injected twice daily (at 0900 and 1800 h) for 3 days. The effective doses and frequencies of E2 and PRL were determined by preliminary experiments. The animals were killed by decapitation 24 h after the last injection. All procedures were performed in accordance with the National Institutes for Health’s guidelines regarding the principles of animal care.

**RNA isolation**

After the mice were killed, their tissues were rapidly removed and frozen in liquid nitrogen. The choroid plexus of each mouse was carefully removed from the forebrain. Total RNA was extracted from the tissues with TRIzol (Invitrogen). Poly(A)+ RNA was prepared from the total RNA of the forebrain with OligoTex Super-dT30 (Takara, Shiga, Japan) according to the manufacturer’s instructions.

**cDNA cloning of the first and last exon variants of Prlr mRNA**

The 5′-end of the Prlr cDNA was synthesized from the poly(A)+ RNA prepared from the forebrain by the oligo-capping method (Maruyama & Sugano 1994) using Cap Site cDNA dT kit (Nippon-capping, Toyama, Japan). Briefly, the cap structure of poly(A)+ RNA was removed with tobacco acid pyrophosphatase, and the decapped mRNA was recapped with an oligonucleotide RNA linker. Subsequently, the oligo-capped mRNA was reverse transcribed with oligo-dT primer, and the resultant cDNA was subjected to the first PCR using a combination of 1RDT linker primer supplied in the kit and murine PRLR-specific antisense primer. Then, the PCR products were subjected to nested PCR using a combination of 2RDT linker primer supplied in the kit and another murine PRLR-specific antisense primer. Prlr-S cDNA was amplified from the cDNA with a sense primer and an antisense primer derived from the reported Prlr-S3 cDNA sequence. The sequences of...
primers used are shown in Table 1. The amplified DNA was cloned into the pGEM-T Easy vector (Promega) and sequenced with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

**Real-time PCR analysis**

Total RNA (5 μg) was reverse transcribed at 50 °C for 60 min in 20 μl reaction mixture containing 200 units of Superscript III transcriptase (Invitrogen), 0.5 mM dNTPs, 10 mM dithiothreitol, 50 μM random primers, and 1× first-strand buffer supplied by the manufacturer. After the inactivation of the reverse transcriptase by heating at 70 °C for 15 min, the cDNA product was subjected to real-time PCR with the Real-time PCR system 7500 (Applied Biosystems, Tokyo, Japan). PCR was carried out with a thermal protocol consisting of 95 °C for 15 s and 60 °C for 35 s in 25 μl buffer containing 1× Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) and 0.2 μM each of the forward and reverse primers listed in Table 1. Quantitative measurement was performed by establishing a linear amplification of serial dilutions of the plasmid DNA (pGEM-T Easy vector) containing each cDNA fragment amplified by the PCR with the first- or last exon-specific primers.

**Statistical analysis**

All data were analyzed by one-way ANOVA and are expressed as mean ± S.D. The significance of the F values obtained was confirmed by Tukey’s post-hoc test. All analyses were performed using GraphPad Prism Software version 4 (GraphPad Software, San Diego, CA, USA).

### Results

**Identification and genomic organization of multiple first exons in murine Prlr gene**

Both the mE14- and the mE15-Prlr cDNAs were cloned from the brain by the oligo-capping method. The sequences of mE14- and mE15-first exons are shown in Table 1. mE14 and mE15 shared 69.4% and 91.8% sequence identities with rat E14 and E15 respectively. A computer-assisted sequence search revealed the positions of the five first exons in the Prlr gene (NT 039618) located in chromosome 15 of the mouse.

**Figure 1** Nucleotide sequences of mE14 and mE15 first exons and schematic representation of the organization of the five first exons of murine Prlr gene. (A) The sequences of the first exon portions of mE14- and mE15-Prlr cDNAs are shown. The sequence spanning exon 1 to exon 3 of murine Prlr gene was obtained from the Mouse Genome Database (NT 039618.3). (B) The diagrams show arrangement of the five first exons. The exons in gene are represented by vertical bars.

---

**Table 1** PCR primers

<table>
<thead>
<tr>
<th>Application</th>
<th>cDNA</th>
<th>Forward (5′–3′)</th>
<th>Accession no.</th>
<th>Reverse (5′–3′)</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA cloning</td>
<td>5′-variants</td>
<td>gatgctagctgcaagtcagtc</td>
<td>1RDTa</td>
<td>ggtagtgccaacattaccac</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>5′-variants</td>
<td>caggtcagctgcaagtcagtc</td>
<td>2RDTa</td>
<td>ccagggctctgcgttcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>mPRLR-S4</td>
<td>tagggaaactgagaagagc</td>
<td>M22957</td>
<td>tcaaaaactacgcttcagtc</td>
<td>M22957</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>mE12-PRLR</td>
<td>acctgctgcatctggtgtcagtc</td>
<td>M22959</td>
<td>ggaactgctgcatctggtgtcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>mE13-PRLR</td>
<td>attttacacgctggtgtcagtc</td>
<td>BC005555</td>
<td>cagggaactgctgcatctggtgtcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>mE14-PRLR</td>
<td>caggtcagctgcaagtcctgcag</td>
<td>L13593</td>
<td>ccagggctctgcgttcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>Total PRLR</td>
<td>tcgcatgcatctggtgtcagtc</td>
<td>L13593</td>
<td>ccagggctctgcgttcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>mPRLR-L</td>
<td>atccacccgctgcaagtcctgcag</td>
<td>L13593</td>
<td>ccagggctctgcgttcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>mPRLR-S1</td>
<td>gcaggtctgctcttgagaggtta</td>
<td>L13593</td>
<td>ccagggctctgcgttcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>mPRLR-S2</td>
<td>atccacccgctgcaagtcctgcag</td>
<td>L13593</td>
<td>ccagggctctgcgttcagtc</td>
<td>L13593</td>
</tr>
<tr>
<td></td>
<td>mGAPDH</td>
<td>tgtcagactgctctgcagctgcag</td>
<td>NM_008084</td>
<td>ccagggctctgcgttcagtc</td>
<td>NM_008084</td>
</tr>
</tbody>
</table>

aPrimers supplied with the Cap Site cDNA kit.

bSequences in the Fig. 1A.

cSequences in the Fig. 1B.
Expression levels of the first exon variants of Prlr mRNAs in the choroid plexus of diestrus, lactating, and PRL-deficient mice

As the expression of *mE1* and *mE1*-*Prlr* mRNAs were not observed in the choroid plexus at day 3 of lactation mice as well as the diestrus mice by RT-PCR analysis (data not shown), the expression levels of *mE1*, *mE1*-*Prlr* mRNAs in the choroid plexus were examined in the diestrus, lactating, and PRL−/− mice (Fig. 3). In the wild-type (*Prl+/+*), mice, the levels of *mE1*, *mE1*-*Prlr* mRNAs were significantly increased at day 3 of lactation compared with those at the diestrus state, with the most prominent difference occurring in *mE1*-*Prlr* mRNA. Reflecting these results, the level of total *Prlr* mRNA was markedly increased at the lactation state. The *mE1*-*Prlr* mRNA level was significantly lower in the diestrus PRL−/− mice than in the diestrus *Prl+/+* mice, whereas no significant difference was observed in the levels of *mE1*-*Prlr* and *mE1*-*Prlr* mRNAs. In addition, the total *Prlr* mRNA level was significantly lower in the diestrus PRL−/− mice than in the diestrus *Prl+/+* mice.

Expression levels of the first exon variants of Prlr mRNAs in murine tissues

The expression levels of *Prlr* mRNAs containing each of the first exons in the tissues of the diestrus mice were examined by real-time PCR (Fig. 2). The expression of *mE1*-*Prlr* mRNA was not observed in any tissues under the experimental conditions we used. However, *mE1*-*Prlr* mRNA was expressed in both the liver and the kidney, with higher levels present in the liver. The *mE1*-*Prlr* mRNA expression was observed in all the tissues examined, with markedly high levels in the liver and choroid plexus and moderately high levels in the adrenal gland. The *mE1*-*Prlr* mRNA was expressed in the choroid plexus and forebrain, with remarkably high levels present in the choroid plexus. Finally, *mE1*-*Prlr* mRNA expression was observed in the choroid plexus, forebrain, liver, and kidney, with the most abundant levels apparent in the choroid plexus. The expression levels of total *Prlr* mRNA were markedly high in the liver and choroid plexus, moderately high in the kidney, and relatively low in the other tissues; this reflects the expression levels of each of the first exon in the respective tissues.

Figure 2 Tissue distributions of the first exon variants of murine *Prlr* mRNA. Expression levels of (A) *mE1*-, (B) *mE1*-, (C) *mE1*-, (D) *mE1*-, and (E) total *Prlr* mRNAs in the liver (Li), kidney (Ki), spleen (Sp), adrenal gland (Ad), ovary (Ov), forebrain (FB), and choroid plexus (CP) in diestrus mice were determined by real-time PCR. Values are expressed as relative to the value of *Gapdh* mRNA, and they represent mean ± S.D. (n=4). Data for each mRNA variant are shown in the tissues where each mRNA was detected.

Figure 3 Expression levels of the first exon variants of *Prlr* mRNA in the choroid plexus of diestrus, lactating, and PRL-deficient mice. The expression levels of (A) *mE1*-, (B) *mE1*-, (C) *mE1*-, and (D) total *Prlr* mRNAs in diestrus (Di) and day 3 lactating (Lac) *Prl+/+* mice, and diestrus PRL−/− and PRL−/− mice were determined by real-time PCR. Values are expressed as relative to the value of *Gapdh* mRNA, and they represent mean ± S.D. (n=4). Values with different letters are significantly different (P<0.05).

C57BL/6 mouse strain. Figure 1B shows a schematic representation of the organization of these first exons together with exons 2 and 3 in *Prlr* gene. *mE1* was located downstream of exon 2, accounting for the lack of the exon 2 sequence in the *mE1*-cDNA (data not shown).
Effects of PRL and E$_2$ on expression levels of the first exon variants of Prlr mRNA in the choroid plexus

The effects of PRL and E$_2$ on the expression levels of mE$_{1\tau}$, mE$_{1\tau}$, and mE$_{1\tau}$Prlr mRNAs in the choroid plexus were examined in the sham-operated or ovariectomized Prl$^{+/−}$ and Prl$^{−/−}$ mice (Fig. 4). Neither the ovariectomy nor the administration of PRL or E$_2$ had a significant effect on the expression levels of mE$_{1\tau}$ or mE$_{1\tau}$Prlr mRNAs in the Prl$^{+/−}$ or Prl$^{−/−}$ mice. However, in the Prl$^{+/−}$ mice, the expression level of mE$_{1\tau}$Prlr mRNA was markedly decreased by the ovariectomy and was recovered by the E$_2$ administration. In the Prl$^{−/−}$ mice, neither the ovariectomy nor the E$_2$ administration had an effect on the expression level of mE$_{1\tau}$Prlr mRNA, although the PRL administration significantly increased the expression level. No additive effect was observed by the simultaneous administration of PRL and E$_2$.

Identification of the murine ortholog for rat short-form Prlr mRNA and genomic organization of the multiple last exons in murine Prlr gene

A cDNA that encodes the mouse ortholog for the rat short-form PRLR was cloned from the choroid plexus of a C57BL/6 mouse (Fig. 5A). The obtained cDNA, which is referred to as Prlr-S4, encoded a protein consisting of 310 amino acids (accession no. AB645381), showing 93% overall sequence identity with the rat short-form PRLR. The nucleotide sequence of Prlr-S4 was similar to that of Pbr-S3 cDNA cloned from a Swiss Webster mouse (Davis & Linzer 1989), but there are considerable mismatches including the deletion of a single nucleotide at codon 78, which is the cause for the truncated amino acid sequence of PRLR-S3. Four alternative last exons encoding the 3′-end sequences of Prlr-L,-S1,-S2, or -S4 mRNAs were found in Prlr gene of the C57BL/6 mouse (NT 039618). The arrangement of the alternative last exons is shown in Fig. 5B.

Expression levels of the last exon variants of Prlr mRNAs in the choroid plexus of diestrus, lactating, and PRL-deficient mice

The expression levels of the last exon variants of Prlr mRNAs in the choroid plexus were examined by real-time PCR (Fig. 6). These results revealed that Prlr-L mRNA was abundantly expressed, but among the three short-form variants, only Prlr-S4 mRNA was detected at a measurable level. In the Prl$^{+/−}$ mice, the expression level of PRLR-L mRNA was markedly higher in the lactation state than in the diestrus state. The expression levels of Prlr-S4 mRNA in the lactating mice were also significantly higher than those in the diestrus mice. The expression levels of Prlr-L and Prlr-S4 mRNAs in the diestrus Prl$^{+/−}$ mice were similar to those in the diestrus Prl$^{+/+}$ mice and significantly decreased in the diestrus Prl$^{−/−}$ mice.

Discussion

In addition to the three known first exons, two other distinct first exons were identified in murine Prlr gene. These five first exons are similar to the rat counterparts.
in terms of both their sequence and positional arrangement in Prlr gene (Hu et al. 1996, Kobayashi et al. 2007). The tissue expression patterns of each of the first exon variants of murine Prlr mRNA were also comparable to those of the rat counterparts, except for the silent expression of mE11-Prlr mRNA in all the tissues analyzed. In rats, E11 has been shown to be a gonad-specific first exon activated by steroidogenic factor 1 (SF-1 (NR5A1); Hu et al. 1997). Although, mE1-Prlr mRNA has been detected in a murine Leydig tumor cell line, the expression level was very low due to the absence of the functional SF-1 binding element in the promoter region (Hu et al. 1997, 1998). In our present study, mE1ɔ-PRLR was the only Prlr mRNA detected in the ovary, suggesting that the Prlr gene expression in the mouse ovary depends on the transcription of mE1ɔ.

In the murine choroid plexus, mE1σ, mE1τ, and mE1ω Prlr mRNAs were expressed in a similar manner as in the rat choroid plexus. It has been previously demonstrated that the expression level of Prlr mRNA in the rat choroid plexus increases to the highest level during lactation (Augustine et al. 2003, Anderson et al. 2008) and is accompanied by a high serum PRL level (Escalada et al. 1996, Augustine et al. 2003). These findings suggest that Prlr gene expression in the choroid plexus is upregulated by PRL. Our present study showed that the expression levels of mE1σ, mE1τ, and mE1ω-Prlr mRNAs in the murine choroid plexus were more increased in the lactating mice than in the diestrus mice, with a particularly large increase in the levels of mE1σ-Prlr mRNA. Furthermore, the expression level of mE1σ-Prlr mRNA but not mE1σ or mE1ω-Prlr mRNAs were decreased in the Prl+/− mice compared with the corresponding levels in the Prl+/+ mice. These results indicate that PRL upregulates the expression of mE1σ-Prlr mRNA in the murine choroid plexus at lactation may depend on factors other than PRL. It has been previously shown that estrogen upregulates Prlr gene expression in the choroid plexus (Pi et al. 2003) as well as Prl gene expression in the pituitary gland (Shull & Gorski 1990, Kansra et al. 2005). Our experiments in this study

---

Figure 5 Nucleotide sequence of Prlr-S4 cDNA that encodes short-form PRLR. (A) The deduced amino acid sequence is shown under the cDNA sequence. The transmembrane domain is denoted with a thick underline. The unique region encoded by an alternative last exon is boxed. (B) The diagrams show arrangement of the four last exons. The exons in the gene are represented by vertical bars.

Figure 6 Expression levels of the last exon variants of Prlr mRNA in the choroid plexus of diestrus, lactating, and PRL-deficient mice. The expression levels of (A) Prlr-L and (B) Prlr-S4 mRNAs in diestrus (Di), day 3 lactating (Lac) Prl+/+, Prl+/−, and Prl−/− mice were determined by real-time PCR. Values are expressed as relative to the value of Gapdh mRNA, and they represent mean ± S.D. (n=4). Values with different letters are significantly different (P<0.05).
involving the ovariecctomized mice showed that estrogen upregulates the expression of mE14-Prlr mRNAs in the Prl\(^+/\) mice but not in the Prl\(^{+/−}\) mice. These findings indicate that the upregulating effect of estrogen on the expression of murine Prlr gene is mediated through the transcriptional activation of mE14 by PRL, whose production is stimulated by estrogen.

The expression of the long- and short-form Prlr mRNAs has been observed previously in the rat choroid plexus. Similarly, PRLR-L, the long-form variant, and PRLR-S4, the murine ortholog of the rat short-form PRLR, were expressed in the mouse choroid plexus. Prlr-S3 cDNA cloned from the liver of a Swiss Webster strain showed high sequence similarity with the rat short-form PRLR, but it encoded a truncated form of PRLR consisting of 97 amino acids and, therefore, is considered as a product of a pseudogene (Davis & Linzer 1989). However, the sequence of Prlr-S4 cDNA was completely identical with that of the corresponding regions of Prlr gene of the C57BL/6 strain, and no pseudogene sequence was found in this mouse strain. In the rat choroid plexus, the expression levels of both the mRNAs increase during lactation (Ouhtit et al. 1993, Bakowska & Morrell 1997, Augustine et al. 2003, Nogami et al. 2007). In this study, the expression levels of the PRLR-L and PRLR-S4 variants were significantly increased in the lactating mice compared with those in the diestrus mice and were decreased in the PRL-deficient mice. PRL administration, but not estrogen, to the ovariecctomized PRL-deficient mice significantly recovered the expression level of mE14-Prlr mRNA. The expression levels of long- and short-form Prlr mRNAs were closely related to those of mE14-Prlr. These data suggest that PRL upregulates transcription of mE14 first exon mainly through the long-form PRLR signaling pathway in the murine choroid plexus.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

This work was supported in part by the Japan Pet Care Association.

**References**


Das R & Vonderhaar BK 1995 Transduction of prolactin's (PRL) growth signal through both long and short forms of the PRL receptor. Molecular Endocrinology 9 1750–1759. (doi:10.1210/me.9.12.1750)

Davis JA & Linzer DI 1989 Expression of multiple forms of the prolactin receptor in mouse liver. Molecular Endocrinology 3 674–680. (doi:10.1210/mend-3-4-674)


Muccioli G & Di Carlo R 1994 Modulation of prolactin receptors in the rat hypothalamus in response to changes in serum concentration of endogenous prolactin or to ovine prolactin administration. Brain Research 663 244–250. (doi:10.1016/0006-8993(94)91269-6)


Pi XJ & Grattan DR 1999 Increased expression of both short and long forms of prolactin receptor mRNA in hypothalamic nuclei of lactating rats. Journal of Molecular Endocrinology 23 13–22. (doi:10.1677/jme.0.0230013)


Walsh RJ, Slaby FJ & Posner BI 1987 A receptor-mediated mechanism for the transport of prolactin from blood to cerebrospinal fluid. Endocrinology 120 1846–1850. (doi:10.1210/end-120-3-1846)

Received in final form 5 January 2012
Accepted 31 January 2012
Made available online as an Accepted Preprint 31 January 2012