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

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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 *Prlr* 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 mE1₃⁻, 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*^{+/+} and *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.

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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-Feysot *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 E1₁ 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 *Prlr* 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, Shiota *et al.* 1990, Nagano & Kelly 1994, Trott *et al.* 2003). In murine *Prlr* mRNA, the four last exon variants, one long form (*Prlr-L*) 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, Shiota *et al.* 1990). Intracellular signaling of the long form is mainly mediated by the JAK2/STAT5 pathway (Bole-Feysot *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 (*Prl*^{-/-}) 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 Gene, 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-S4* 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

Table 1 PCR primers

Application	cDNA	Forward (5'–3')	Accession no.	Reverse (5'–3')	Accession no.
cDNA cloning	5'-variants	gatgctagctgcgagtgcaagtc	1RDT ^a	ggtaggtggcaaccattttacc	L13593
	5'-variants	cgagtcgaagtcgacgaagtgc	2RDT ^a	ccgaggaggctctggttcaac	L13593
Real-time PCR	<i>mPRLR-S4</i>	tagggaaacgtagaagagca	M22957	tcagaaaaccacactgcag	M22957
	<i>mE1₂-PRLR</i>	actgtctgctctttcagaagtct	M22959	ggacaagcagcatgtaagca	L13593
	<i>mE1₃-PRLR</i>	attttacacggggctcagg	BC005555	caggggaacgacatttggtg	L13593
	<i>mE1₄-PRLR</i>	agagaggcaccctccacag	– ^b	caggggaacgacatttggtg	L13593
	<i>mE1₅-PRLR</i>	caggctgtgactccatgtgt	– ^c	caggggaacgacatttggtg	L13593
	Total <i>PRLR</i>	tgccatctgcacttgcttac	L13593	caggggaacgacatttggtg	L13593
	<i>mPRLR-L</i>	atcattcaccggccgttctctc	L13593	ccagcaagtctccacagtca	L13593
	<i>mPRLR-S1</i>	gcagtggtcttgaagggtta	L13593	ccaggggaagtcaactggaga	M22958
	<i>mPRLR-S2</i>	atcattcaccggccgttctctc	L13593	ggctgtggtcgaagtgggtta	M22959
	<i>mPRLR-S4</i>	atcattcaccggccgttctctc	L13593	gtattgtctggagagccagt	AB643813
	<i>mGAPDH</i>	tgtcagcaatgcactctgca	NM_008084	ccgttcagctctgggatgac	NM_008084

^aPrimers supplied with the Cap Site cDNA kit.^bSequences in the Fig. 1A.^cSequences in the Fig. 1B.

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 Fig. 1A. *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 *Prlr* gene (NT 039618) located in chromosome 15 of the

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.

Figure 2 Tissue distributions of the first exon variants of murine *Prhr* mRNA. Expression levels of (A) *mE12-Prhr*, (B) *mE13-Prhr*, (C) *mE14-Prhr*, (D) *mE15-Prhr*, and (E) total *Prhr* 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 $\pm$ s.d. ($n=4$). Data for each mRNA variant are shown in the tissues where each mRNA was detected.

C57BL/6 mouse strain. Figure 1B shows a schematic representation of the organization of these first exons together with exons 2 and 3 in *Prhr* gene. *mE14* was located downstream of exon 2, accounting for the lack of the exon 2 sequence in the *mE14*-cDNA (data not shown).

Expression levels of the first exon variants of *Prhr* mRNAs in murine tissues

The expression levels of *Prhr* 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 *mE12-Prhr* mRNA was not observed in any tissues under the experimental conditions we used. However, *mE13-Prhr* mRNA was expressed in both the liver and the kidney, with higher levels present in the liver. The *mE15-Prhr* 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 *mE14-Prhr* mRNA was expressed in the choroid plexus and forebrain, with remarkably high levels present in the choroid plexus. Finally, *mE15-Prhr* 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 *Prhr* 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.

Expression levels of the first exon variants of *Prhr* mRNAs in the choroid plexus of diestrus, lactating, and PRL-deficient mice

As the expression of *mE12-Prhr* and *mE13-Prhr* 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 *mE15-Prhr*, *mE14-Prhr*, and *mE15-Prhr* mRNAs in the choroid plexus were examined in the diestrus, lactating, and *Prhr*^{-/-} mice (Fig. 3). In the wild-type (*Prhr*^{+/+}) mice, the levels of *mE15-Prhr*, *mE14-Prhr*, and *mE15-Prhr* mRNAs were significantly increased at day 3 of lactation compared with those at the diestrus state, with the most prominent difference occurring in *mE14-Prhr* mRNA. Reflecting these results, the level of total *Prhr* mRNA was markedly increased at the lactation state. The *mE14-Prhr* mRNA level was significantly lower in the diestrus *Prhr*^{-/-} mice than in the diestrus *Prhr*^{+/+} mice, whereas no significant difference was observed in the levels of *mE15-Prhr* and *mE15-Prhr* mRNAs. In addition, the total *Prhr* mRNA level was significantly lower in the diestrus *Prhr*^{-/-} mice than in the diestrus *Prhr*^{+/+} mice.

Figure 3 Expression levels of the first exon variants of *Prhr* mRNA in the choroid plexus of diestrus, lactating, and PRL-deficient mice. The expression levels of (A) *mE13-Prhr*, (B) *mE14-Prhr*, (C) *mE15-Prhr*, and (D) total *Prhr* mRNAs in diestrus (Di) and day 3 lactating (Lac) *Prhr*^{+/+} mice, and diestrus *Prhr*^{+/+} and *Prhr*^{-/-} mice were determined by real-time PCR. Values are expressed as relative to the value of *Gapdh* mRNA, and they represent mean $\pm$ s.d. ($n=4$). Values with different letters are significantly different ($P<0.05$).

Effects of PRL and E₂ on expression levels of the first exon variants of *Prlr* mRNA in the choroid plexus

The effects of PRL and E₂ on the expression levels of *mE1₃*, *mE1₄*, and *mE1₅-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 E₂ had a significant effect on the expression levels of *mE1₃* or *mE1₅-Prlr*

Figure 4 Effects of PRL and estrogen treatment on the expression levels of the first exon variants of *Prlr* mRNAs in the choroid plexus of *Prl*^{+/-} and *Prl*^{-/-} mice. Expression levels of (A) *mE1₃*, (B) *mE1₄*, and (C) *mE1₅-Prlr* mRNAs in the choroid plexus of sham-operated (Sham) mice, ovariectomized (OvX) mice, estrogen-treated ovariectomized (OvX + E₂) mice, PRL-treated ovariectomized (OvX + PRL) mice, and estrogen- and PRL-treated ovariectomized (OvX + E₂ + PRL) mice are presented. Values represent mean ± s.d. (n=4). Values with different letters are significantly different (P < 0.05).

mRNAs in the *Prl*^{+/-} or *Prl*^{-/-} mice. However, in the *Prl*^{+/-} mice, the expression level of *mE1₄-Prlr* mRNA was markedly decreased by the ovariectomy and was recovered by the E₂ administration. In the *Prl*^{-/-} mice, neither the ovariectomy nor the E₂ administration had an effect on the expression level of *mE1₄-Prlr* mRNA, although the PRL administration significantly increased the expression level. No additive effect was observed by the simultaneous administration of PRL and E₂.

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. AB643813), showing 93% overall sequence identity with the rat short-form PRLR. The nucleotide sequence of *Prlr-S4* was similar to that of *Prlr-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 the murine choroid plexus, *mE1₃*, *mE1₄*, and *mE1₅* *Prhr* mRNAs were expressed in a similar manner as in the rat choroid plexus. It has been previously demonstrated that the expression level of *Prhr* 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 *Prhr* gene expression in the choroid plexus is upregulated by PRL. Our present study showed that the expression levels of *mE1₃*, *mE1₄*, and *mE1₅* *Prhr* 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 *mE1₄* *Prhr* mRNA. Furthermore, the expression level of *mE1₄* *Prhr* mRNA but not *mE1₃* or *mE1₅* *Prhr* mRNAs were decreased in the *Pr^l^{-/-}* mice compared with the corresponding levels in the *Pr^l^{+/-}* mice. These results indicate that PRL upregulates the expression of *mE1₄* *Prhr* mRNA in the murine choroid plexus and that the increased expression of *mE1₃* and *mE1₅* *Prhr* mRNAs at lactation may depend on factors other than PRL. It has been previously shown that estrogen upregulates *Prhr* 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 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 $\pm$ s.d. ($n=4$). Values with different letters are significantly different ($P<0.05$).

involving the ovariectomized mice showed that estrogen upregulates the expression of *mE1₄Prhr* mRNAs in the *Prhr*^{+/−} mice but not in the *Prhr*^{−/−} mice. These findings indicate that the upregulating effect of estrogen on the expression of murine *Prhr* 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 *Prhr* 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. *Prhr-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 *Prhr-S4* cDNA was completely identical with that of the corresponding regions of *Prhr* 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 (Ouhit *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 compared with the levels in the PRL-normal (i.e. *Prhr*^{+/+} and *Prhr*^{+/−}) mice. These expression patterns are similar to those of *mE1₄Prhr* mRNA, indicating that the expression of *Prhr-L* and *Prhr-S4* mRNAs in the choroid plexus largely depend on the transcriptional activation of *mE1₄* first exon by PRL.

It is well known that the JAK2/STAT5 pathway is a major intracellular signaling pathway of the long-form PRLR. In this pathway, binding of PRL to the long-form receptor activates the kinase JAK2 and subsequently the transcription factor STAT5 by phosphorylation. In the murine choroid plexus, the amount of phosphorylated STAT5 is increased by the administration of PRL (Brown *et al.* 2010). Collectively, these findings suggest that the JAK2/STAT5 pathway is activated by PRL action on the long-form PRLR, and the activated STAT5 stimulates the *Prhr* gene expression by transcriptional activation of *mE1₄* in the murine choroid plexus. In addition, they also suggest that the increased long and/or short forms of PRLR facilitate the uptake of blood PRL into the CSF by the receptor-mediated transport system. However, the molecular mechanisms of transcriptional activation of *mE1₄* first exon and the PRLR-mediated transport system of PRL in the choroid plexus remain to be elucidated.

In conclusion, our present study demonstrated the presence of *mE1₄*, and *mE1₅* first exons in addition to the known *mE1₁*, *mE1₂*, and *mE1₃* first exons in murine

Prhr gene. Our experiments also revealed the tissue expression pattern of each first exon variant of *Prhr* mRNA. The expression level of *mE1₄Prhr* mRNA in the choroid plexus was markedly increased in the lactating mice and was significantly decreased in the PRL-deficient mice. PRL administration, but not estrogen, to the ovariectomized PRL-deficient mice significantly recovered the expression level of *mE1₄Prhr* mRNA. The expression levels of long- and short-form *Prhr* mRNAs were closely related to those of *mE1₄Prhr*. 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.

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