Endocrine, *vol. 11, no. 1, 91–98, August 1999* 0969–711X/99/8:91–98/$12.00

Expression of Prolactin Receptor mRNA Is Increased in the Preoptic Area of Lactating Rats

Xiujun Pi and David R. Grattan

Department of Anatomy and Structural Biology, School of Medical Sciences and Neuroscience Research Centre, University of Otago, Dunedin, New Zealand

This study investigated expression of prolactin receptor (PRL-R) mRNA in the preoptic area in midlactating rats compared with diestrous rats. Tissues from specific nuclei were micropunched from 300-μm thick rat brain sections with 300- or 500-μm diameter needles. After total RNA was extracted, the two forms of PRL-R mRNA were evaluated by reverse transcriptase polymerase chain reaction and Southern hybridization. The results showed that levels of long-form PRL-R mRNA in the ventrolateral preoptic nucleus and lateroanterior nucleus in lactating rats were significantly higher ($p < 0.05$) than in diestrous rats. The ventromedial and medial preoptic nuclei in lactating rats also expressed moderately high levels of long-form mRNA when compared with ($p = 0.0547$) diestrous rats. The ventromedial and ventrolateral preoptic nuclei, and ventrolateral hypothalamic nucleus in lactating rats expressed significantly higher levels of short-form mRNA than in diestrous rats. The increased expression of both forms of PRL-R mRNA helps explain numerous effects of PRL on brain functions during lactation.

Key Words: Prolactin receptor; mRNA; preoptic area; hypothalamus; reverse transcriptase-polymerase chain reaction; Southern hybridization.

Introduction

Prolactin (PRL) acts on the brain to promote numerous physiological and behavioral changes *(1,2)*. The biological effects of PRL, a pituitary hormone, are presumably mediated by the PRL receptor (PRL-R), which is a member of the cytokine receptor superfamily *(3)*. PRL-Rs have been identified in the brain tissue of several vertebrate species including rats *(4)*, mice *(5)*, and humans *(6)*. Analysis of the cDNA sequence of PRL-R in normal rat tissues revealed that there are two isoforms of PRL-R protein and mRNA: the short form and long form, which are generated by alternative splicing of a single gene *(3)*.

Changes in expression of PRL-R mRNA in the brain during lactation have been observed in several studies. An earlier study using reverse transcriptase polymerase chain reaction (RT-PCR) showed that expression of the long-form PRL-R mRNA, but not the short form, was markedly increased in rat cerebrum during pregnancy and was maintained at high levels during lactation *(7)*. More specifically, the long-form PRL-R mRNA was shown to be significantly increased in the choroid plexus (ChP) of lactating rats, and markedly reduced by removal of pup contact *(8)*. However, these studies did not investigate the possible changes in expression of the two forms of PRL-R in individual hypothalamic nuclei.

In a recent study, we observed increased expression of PRL-R immunoreactivity in numerous preoptic and hypothalamic nuclei in lactating rats *(9)*. Consistent with these results, we detected increased expression of both forms of PRL-R in specific microdissected nuclei in the medial basal hypothalamus during lactation *(10)*. In the present study, we aimed to determine whether expression of PRL-R mRNA in different nuclei of the preoptic area is also increased during lactation by comparing expression levels of both forms of PRL-R mRNA in lactating rats with the same regions in diestrous rats.

Results

Microdissection

Tissue for evaluation of PRL-R mRNA was micropunched from individual nuclei in frozen brain sections. Figure 1 illustrates representative sections. The cortex (CTX) sample was punched from the rostral part of cingulate (Cg) CTX. The ChP sample was punched from the anterior part of the lateral ventricles. CgCTX, ventromedial preoptic nucleus (VMPO), medial preoptic nucleus

Received March 29, 1999; Revised May 12, 1999; Accepted June 1, 1999.
Author to whom all correspondence and reprint requests should be addressed: David Grattan, Department of Anatomy and Structural Biology, School of Medical Sciences, University of Otago, PO Box 913 , Dunedin, New Zealand. Email: david.grattan@stonebow.otago.ac.nz

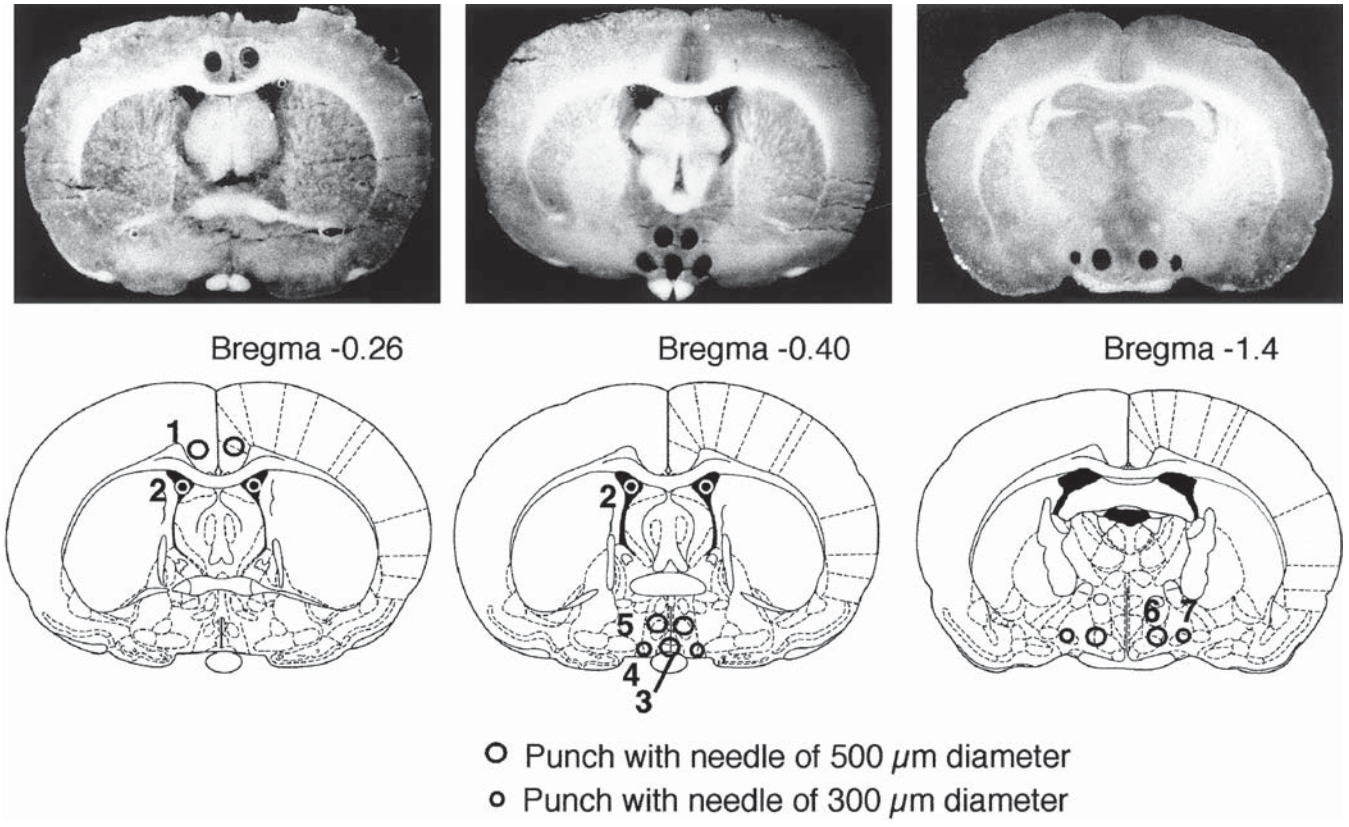

Fig. 1. Diagrams and representative sections showing locations of micropunches for the different brain regions. Rat brains were frozen on dry ice and then cut into 300-μm coronal sections in a cryostat at –9°C. Micropunches were collected from these frozen thick sections with 300-μm (small circles) or 500-μm diameter (large circles) autoclaved stainless steel needles. After microdissection, photographs were taken using a Nikon camera on a dissection microscope. Diagrams are taken from the Paxinos and Watson rat brain atlas *(11)*, which was used to identify anatomical structures and as a reference for abbreviations.

(MPO), and ventrolateral hypothalamic nucleus (VLH) were microdissected with 500-μm diameter needles. The smaller areas such as ChP, ventrolateral preoptic nucleus (VLPO), and lateroanterior hypothalamic nucleus (LA) were punched with 300-μm diameter needles. Table 1 shows the numbers of punches collected for each brain region. All diagrams and abbreviations are taken from the Paxinos and Watson rat brain atlas *(11)*.

RT-PCR Results

PCR amplification for the short and long forms of PRL-R mRNA identified either a single 330- or 420-bp band, respectively, which are consistent with predicted sizes (Fig. 2A). DNA bands from both forms of mRNA in the ChP were clearly visualized on agarose gels. To control for differences in RNA loading in the first strand synthesis, PCR amplification was performed using specific primers for β-actin gene, with the same RNA samples as used for PRL-R mRNA. RT-PCR products for β-actin mRNA showed a 395-bp band, which is consistent with the predicted size for this mRNA (Fig. 2A).

Southern Hybridization Results

As shown in Fig. 2B, DNA product from the long form of PRL-R mRNA could be detected on Southern blot films in most regions examined in both diestrous (n = 6) and lactating rats (n = 6). Results for the short form were less consistent. In four out of six pairs of rats (each pair consisting of one diestrous and one lactating rat processed in parallel), the short-form mRNA could be detected in most brain regions examined. However, in the other two pairs, the short-form mRNA was only detected in the ChP and not in any of the preoptic or hypothalamic regions. These two pairs were hence excluded from statistical analyses.

Comparison of mRNA Between Diestrous and Lactating Rats

Figure 3 illustrates the relative amounts of short and long forms of PRL-R mRNA in different brain regions of diestrous and lactating rats. All data are normalized against the amount of β-actin mRNA expressed in the same punch. In lactating rats, the ChP expressed the highest levels of both forms of PRL-R mRNA whereas the CgCTX contained the lowest of all regions examined. The expression of long-form PRL-R mRNA in the ChP, VLPO, and LA was significantly higher ($p < 0.05$) in lactating rats than in diestrous rats. The expression of long-form PRL-R mRNA in the VMPO (4.29 ± 1.90) and MPO (2.51 ± 0.54) in lactating rats tended to increase (p = 0.0547 for both areas,

Table 1
Microdissection of Discrete Brain Regions

Sample number	Brain region	Section coordinates relative to Bregma[a]	Number of punches per brain	Diameter of micropunch needle (μm)
1	CgCTX	–0.2, –0.5	4	500
2	ChP	–0.2, –0.5	4	300
3	VMPO[b]	–0.5	1	500
4	VLPO	–0.5	2	300
5	MPO	–0.5, –0.8	4	500
6	VLH	–1.4	2	500
7	LA	–1.4, –1.7	4	300

[a]Section coordinates represent the approximate position (relative to Bregma) of the rostral face of the section collected for microdissection. Where more than one coordinate is given, tissue was collected from more than one section.

[b]The punched tissue of this area may also contain the anteroventral periventricular nucleus.

Mann-Whitney *U*-test for nonparametric data) when compared with VMPO (1.34 ± 0.21) and MPO (1.32 ± 0.14) in diestrous rats. Levels of short form PRL-R mRNA in the ChP, VMPO, and VLPO in lactating rats were significantly higher than in diestrous rats ($p < 0.05$). The short-form of PRL-R mRNA was induced in the CgCTX (0.73 ± 0.42) in lactating rats, after being undetectable in diestrous rats.

Discussion

In our previous study *(10)*, we observed an increased expression of both forms of PRL-R mRNA in several medial basal hypothalamic nuclei in lactating rats when compared with diestrous rats. The present study extended these observations to evaluate the expression of the two forms of PRL-R mRNA in several preoptic nuclei in diestrous and lactating rats. For the first time, we detected both forms of PRL-R mRNA in the VLH and LA, regions identified to contain PRL-R immunoreactivity in lactating rats *(9)* but not in diestrous or ovariectomized plus estrogen-treated (OVX + E) rats *(12)*. The relative amounts of both forms of PRL-R mRNA in the VLPO of lactating rats were significantly higher than those of diestrous rats. In addition, the long-form mRNA was significantly increased in the LA during lactation and the short-form was significantly increased in the VMPO. The VMPO and MPO in lactating rats also tended to express higher levels of long-form PRL-R mRNA than in diestrous rats, although this difference was not significant. We have previously observed that PRL-R immunoreactivity is increased in several preoptic nuclei in lactating rats when compared with diestrous rats *(9)*. The increased expression of PRL-R mRNA observed in the present study is in agreement with those immunohistochemical data.

Increased expression of PRL-R mRNA in preoptic regions is also consistent with numerous effects of PRL in the brain that become apparent during lactation. There are extensive data suggesting that hyperprolactinemia inhibits the activity of gonadotropin-releasing hormone (GnRH) neurons, thereby causing infertility. Experimental hyperprolactinemia suppresses pulsatile secretion of luteinizing hormone (LH) in OVX rats *(13,14)*, reduces GnRH secretion into the portal blood *(15)*, and inhibits GnRH secretion from hypothalamic explants in vitro *(6)*. In normal cycling rats, ovine PRL treatment completely abolishes the preovulatory LH and estradiol surges, as well as ovulation *(17)*. Hence, the physiological hyperprolactinemia during lactation is likely to contribute to suppression of fertility of the lactating mother *(18,19)*. Whether the effects of PRL on GnRH are direct or indirect is not known. In the present study, expression of both forms of PRL-R mRNA was significantly increased in the VLPO and tended to increase in the MPO. These preoptic regions contain large numbers of GnRH-generating neurons in the rat *(20,21)*. Therefore, it is possible that increased expression of PRL-R in the VLPO and MPO during lactation might mediate inhibitory actions of PRL on GnRH release. Interestingly, in the GT1 cell line (a tumor cell line derived from GnRH neurons), PRL directly inhibits GnRH release in vitro *(22)*, suggesting that there may be PRL-R expressed on GnRH neurons. The VMPO region has been implicated in the control of the LH surge *(23)*. The increased expression of short-form PRL-R mRNA observed in the present study suggests that PRL may also contribute to inhibition of ovulation during lactation.

The MPO has also been suggested to be a major brain region controlling maternal behavior *(24)*, although the bed nucleus of the stria terminalis may also regulate this behavior *(25,26)*. Direct infusion of PRL or placental lactogen into the MPO of the rat stimulates maternal behavior in OVX females treated with steroid hormones *(27)*. In the present study, we observed a moderate (though not significant) increase in the amount of long-form PRL-R mRNA in the MPO of lactating rats. This finding was consistent with our previous study showing that PRL-R immunoreactivity was moderately increased in this region *(9)*. It seems likely

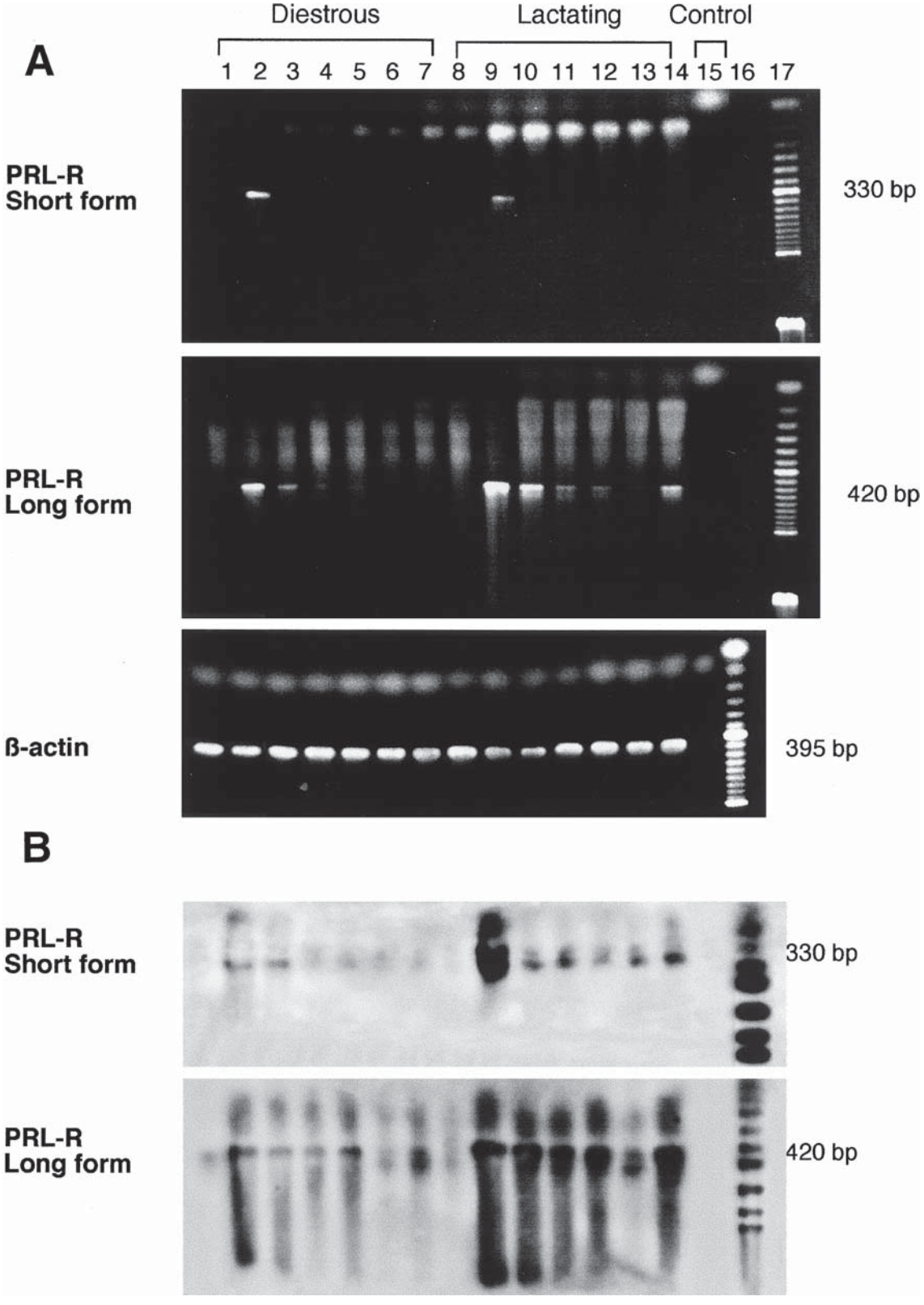

Fig. 2. (**A**) Ethidium bromide staining of RT-PCR products for the short **(upper panel)** and long forms **(middle panel)** of PRL-R mRNA, and β-actin mRNA **(lower panel)** from various preoptic nuclei of both diestrous (lanes 1–7) and lactating (lanes 8–14) rats. **(B)** Southern blot analysis of RT-PCR products generated with primers specific for the short **(upper panel)** and long forms **(lower panel)** of PRL-R mRNA in various preoptic nuclei of both diestrous (lanes 1–7) and lactating (lanes 8–14) rats. 1, 8 = CgCTX; 2, 9 = ChP; 3, 10 = VMPO; 4, 11 = VLPO; 5, 12 = MPO; 6, 13 = VLH; 7, 14 = LA; 15 = no RT controls; 16 = DIG mol wt marker; 17 = 50-bp DNA ladder with thicker band at 350 and 800 bp.

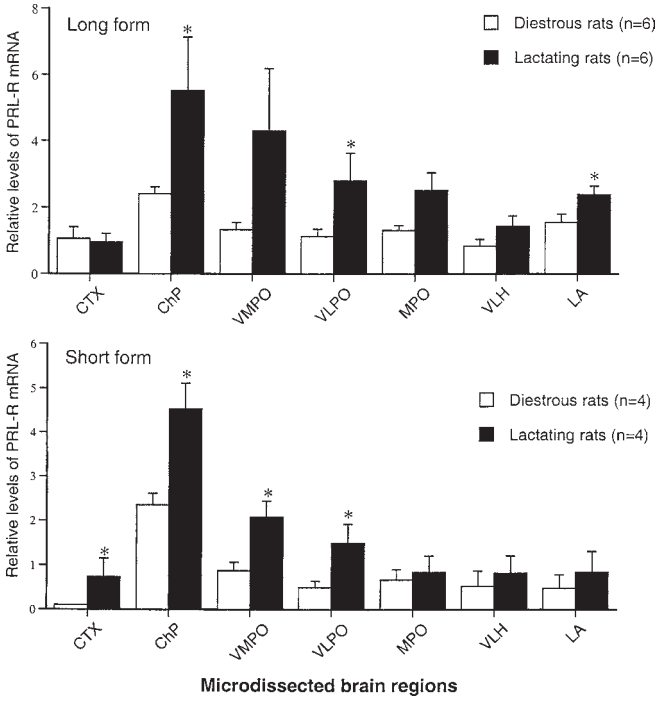

Fig. 3. Relative amounts for the long **(upper panel)** and short forms **(lower panel)** of PRL-R mRNA normalized to β-actin mRNA from different brain regions of diestrous (□) and lactating (■) rats. Data represent the mean ± SEM of four rats for the short form and six for the long form. The optical density was obtained from Southern blot films for PRL-R mRNA and from ethidium bromide-stained gels for β-actin mRNA. *, For both the short and long forms, $p < 0.05$ when compared with corresponding regions in diestrous rats.

that PRL-R in the MPO participates in the steroid hormone–dependent onset and maintenance of maternal behavior induced by lactogenic hormones. Whether increased expression of PRL-R during lactation is initiated during pregnancy or during lactation could not be determined in the present study. Levels of long-form PRL-R mRNA in the MPO have been demonstrated to be significantly higher in d 21 than d pregnant rats *(28)*, suggesting that an increase in the levels of PRL-R mRNA in the MPO may be initiated during pregnancy and then maintained at elevated levels during lactation.

The present study also detected expression of both forms of PRL-R mRNA in the LA and VLH. Levels of the long form in the LA in lactating rats were significantly higher than in diestrous rats. These exciting new data provide strong support for our previous results showing that PRL-R immunoreactivity could be detected in these regions in lactating rats *(9)*. These observations suggest that these regions are specially regulated by PRL during lactation, but the functional significance is not yet clear. The LA contains specific binding sites for somatostatin receptor in spontaneously hypertensive rats *(29)*. Interestingly, the LA also receives efferent projection from estrogen receptor–containing neurons that are located in the VMH. These neurons are implicated in the regulation of female sexual behavior by steroid hormones in the guinea pig *(30)*. The VLH was recently identified for the first time by using the chemical marker NADPH-diaphorase *(11)*, but its function is not known.

The presence or absence of PRL-R mRNA in CTX has been an area of controversy in the literature. An earlier study reported undectectable PRL-R mRNA in the CTX of cycling rats *(31)* whereas we *(32)* and others *(33)* were able to detect low levels in OVX + E rats or cycling rats. In the present study, we observed that the short form of PRL-R mRNA in the CgCTX was induced during lactation. In a previous study *(10)*, we reported that the long form of PRL-R mRNA was induced in the S1BF CTX (a part of parietal CTX) during lactation. We have also reported limited expression of PRL-R immunoreactivity in the parietal cortex during lactation *(10)*. More work is required to determine how widespread PRL-R expression is in the CTX during lactation and to determine whether the two forms are differentially regulated in different parts of the CTX. Nevertheless, observations of increased expression of PRL-R mRNA and protein in the CTX during lactation is suggestive of additional, as yet unsuspected roles of PRL in the brain.

Combining data in the present study with our previous work *(10)*, we find that it is apparent that there are relatively widespread increases in both forms of PRL-R expression in hypothalamic and preoptic regions during lactation. Based on our work examining expression of PRL-R protein by immunohistochemistry *(9)*, it seems likely that the PRL-R mRNA in these regions will be associated with neurons, rather than glial cells, although PRL has been reported to have mitogenic effects on glial cells, in culture *(34)*. Whether both forms are expressed on the same neuronal cell types is unknown at present. The functional significance of the fact that both forms of PRL-R mRNA are increased in specific brain regions during lactation remains to be investigated. Analysis of cDNA transcripts for the short, long, and Nb2 intermediate forms of PRL-R has demonstrated that each form has a high affinity for PRL. However, only the long and intermediate forms transduce hormonal signal, as assessed by their capacity to stimulate the transcription of milk protein gene promoters and interferon regulatory factor 1 via the Jak/Stat pathway *(35,36)*. Recent data suggest that the short form may mediate the mitogenic activity of PRL through a mitogen-activated protein kinase pathway *(37)*. Such a function, however, is unlikely to be relevant in postmitotic neuronal cells, but could be important in glia *(34)*. The increased expression of both forms of PRL-R mRNA in some brain regions supports the idea that both the short and long form may mediate different functions of PRL during lactation.

In our previous *(10)* and present studies, we have consistently observed that both the short and long forms of PRL-R mRNA in the ChP were significantly higher in lac-

tating rats than in diestrous rats. Similar data were also obtained for PRL-R immunoreactivity in the ChP of diestrous and lactating rats *(8,9)*. Clearly, these data support an important role for PRL-R in the ChP during lactation. It has been suggested that PRL-R in this region may be involved in the transport of systemic PRL into the cerebral ventricles *(38)*. Therefore, increased expression of PRL-R may be required to augment the transport of PRL into the brain during condition as of elevated PRL secretion such as lactation. Both forms of PRL-R mRNA were elevated during lactation, and it remains to be elucidated which form is involved in the transport functions.

In conclusion, the present study described expression of both forms of PRL-R mRNA in the CTX, ChP, and five preoptic nuclei—VMPO, VLPO, MPO, VLH, and LA—in both diestrous and lactating rats. Expression of the long-form mRNA was significantly increased in the ChP, VLPO and LA during lactation. Expression of the short form was significantly increased in the ChP, CgCTX, VMPO, and VLPO. The increased expression of both forms of PRL-R mRNA in these brain regions in lactating rats suggests that PRL plays several roles in the brain, as well as stimulates mammary glands during lactation.

Materials and Methods

Preparation of Animals

Adult Sprague-Dawley female rats (200–250 g) were purchased from the Animal Breeding Station of the Department of Laboratory Animal Sciences, University of Otago. All protocols for animal experimentation were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Animals were group housed in plastic cages under conditions of controlled temperature (22 ± 1°C) and lighting (lights on from 6:00 AM to 6:00 PM). They were given free access to food and water. The stages of the estrous cycle were monitored by daily cytological examination of vaginal smears. Diestrous rats were selected after the animals had exhibited at least two 4-d estrous cycles. A group of proestrous animals was paired with adult male rats, and successful mating was assessed by the presence of sperm in the vaginal lavage on the following day. Rats were housed individually from d 18 of pregnancy. On d 2 postpartum (parturition day = d 0), the litters were culled to 12 pups per dam, and lactating animals were used between d 7 and 10 postpartum.

All animals were sacrificed between 9:00 AM and 12:00 noon. On each experimental day, tissue was collected from one lactating and one diestrous rat, and then processed in parallel. The rat brains were removed and snap-frozen using isopentane cooled in liquid nitrogen. Tissue from one animal was counted as one sample and a total of six lactating and six diestrous rats was examined.

Microdissection

Coronal sections (300 μm) were cut from frozen rat brains at –9°C in a cryostat and thaw-mounted onto glass slides. Sections were then placed on a precooled microdissection plateau. By using Palkovits' microdissection technique *(39)*, tissues were carefully micropunched from the ChP, CgCTX, and five specific preoptic or anterior hypothalamic nuclei with blunted 300- or 500-μm diameter stainless-steel needles, depending on the size of the target nucleus (Table 1, and Fig. 1). To prevent possible tissue contamination, each brain area was microdissected with a different autoclaved needle of the appropriate size. Microdissected tissue of each brain region from one individual animal was treated as a single data point.

Extraction of Total RNA

Total cellular RNA was extracted from various microdissected brain tissues using the RNeasy Mini Kit (Qiagen, Santa Clarita, CA). Briefly, brain tissues were placed in 300 μL of lysis buffer containing 4 *M* guanidinium salt and β-mercaptoethanol, and homogenized by a sonicator using a cup-horn attachment. The tissue homogenate was then mixed with 300 μL of 70% ethanol, loaded onto the RNeasy spin column, and centrifuged for 0.5 min at 8000*g*. The supernatant was washed with a buffer containing 70% ethanol and centrifuged two times. Total RNA was finally collected with 30 μL of DEPC-treated water. Usually, the RNA was immediately processed through the RT procedure (*see* next section).

Reverse Transcription

The first strand cDNA synthesis reaction was catalyzed by AMV reverse transcriptase (Promega, Madison, WI). In all cases, 10 μL of total RNA (about 50–200 ng) was reverse transcribed. The final RT mixture contained 5 m*M* $MgCl_2$, 10 m*M* Tris-HCl (pH 9.0), 50 m*M* KCl, 0.1% Triton X-100, 1 m*M* dNTP, 1 U of Ribonuclease inhibitor, 15 U of RT, and 0.5 μg of Oligo(dT)15 Primer. After incubating at 42°C for 60 min, the RT mixture was heated to 99°C for 5 min followed by a 5-min incubation at 0°C. Two control experiments were carried out: (1) A blank control was prepared using all reagents except the RNA sample, for which an equivalent volume of water was substituted; and (2) two controls were prepared using RNA samples from the ChP or MPO, in which RT was omitted. These controls then underwent identical PCR procedures to experimental samples.

Oligonucleotide Primers

The oligonucleotides used for RT-PCR were designed according to the published cDNA sequence of the rat PRL-R *(40,41)*. Table 2 lists the sequence of the primers used in the present study. Primers 1 and 2 were used to detect the short form of PRL-R mRNA and primers 1 and 3 were used to detect the long form in the RT-PCR studies. Primer 4

Table 2
Oligonucleotides Used for RT-PCR and Southern Hybridization

Primer	Sequence	Position (nucleotide) on PRL-R cDNA	Usage
1	5'-ATA-CTG-GAG-TAG-ATG-GAG-CCA-GGA-GAG-TTC-3'	624–653	RT-PCR
2	5'-TCC-TAT-TTG-AGT-CTG-CAG-CTT-CAG-TAG-TCA-3'	924–953	RT-PCR
3	5'-CTT-CCG-TGA-CCA-GAG-TCA-CTG-TCG-GGA-TCT-3'	1014–1043	RT-PCR
4	5'-CAA-AGC-CAC-TGC-CCA-GAC-3'	742–759	Southern hybridization
5	5'-TGA-ACC-CTA-AGG-CCA-ACC-GTG-3'	—	RT-PCR (β-actin)
6	5'-GCT-CAT-AGC-TCT-TCT-CCA-GGG-3'	—	RT-PCR (β-actin)

was common to both forms of PRL-R cDNA and was used as a probe for Southern hybridization. Primers 5 and 6 were used to amplify cDNA from β-actin mRNA [32]. The predicted DNA sizes for the short- and long-form PRL-R mRNA and β-actin mRNA were 330, 395, and 420 bp, respectively.

Polymerase Chain Reaction

For each microdissected brain region, three separate PCR reactions were set up using primer pairs to detect the short and long forms of PRL-R mRNA, and β-actin mRNA, respectively. PCR was carried out with PCR Master Mix (Advanced Biotechnologies, Surrey, UK). The reaction mixture contained 45 μL of PCR Master Mix, 1 μL of primers at the concentration of 20 μM, and 4 μL of RT product solution. Each PCR reaction contained 1.25 U of *Taq* DNA polymerase, 75 m*M* Tris-HCl (pH 8.8), 20 m*M* $(NH4)_2SO_4$, 2.5 m*M* $MgCl_2$, 0.2 m*M* dNTPs, and 0.01% Tween-20. PCR incubations were conducted in a programmable thermal controller (MJ Research, Watertown, MA). During each cycle, the samples were denatured at 94°C for 30 s, annealed at 55°C for 30 s, and extended at 72°C for 90 s. The final extension was at 72°C for 10 min. Thirty-three cycles were performed for PCR tubes containing β-actin template, and 40 cycles were used for each form of PRL-R cDNA. These PCR profiles were determined by the amplification for both forms of PRL-R mRNA and β-actin to ensure that PCR was performed within the linear part of the amplification curves *(32)*.

The RT-PCR products were separated in a 1.5% (w/v) agarose gel LE (Boehringer Mannheim, Auckland, NZ). A 50-bp DNA Ladder (Gibco-BRL, Life Technologies, Auckland, NZ) was used as a standard size marker. Gels were stained for 20 min in TBE buffer containing 0.5 μg/mL of ethidium bromide, destained for 20 min, and examined on an ultraviolet transilluminator. DNA bands were photographed with a Polaroid camera.

Southern Hybridization

Southern filter hybridization was carried out to validate PCR products from the PRL-R mRNA and to increase sensitivity of detection. The digoxigenin (DIG)-labeled PRL-R probe was made by labeling primer 4 according to the DIG oligonucleotide 3'-end labeling kit (Boehringer Mannheim). Gels were first denatured in a solution containing 0.5 *M* NaOH and 1.5 *M* NaCl, and neutralized in a solution containing 0.5 *M* Tris-HCl (pH 8.0) and 1.5 *M* NaCl. DNA bands were then capillary-transferred onto positively charged nylon membrane (Boehringer Mannheim) with 20X SSC (3 *M* NaCl, 0.3 *M* sodium citrate pH 7.0) buffer. Membranes were baked at 120°C for 30 min, prehybridized in DIG Easy Hyb buffer (Boehringer Mannheim) at 48°C for 2 h, and hybridized in DIG Easy Hyb containing 3 pmol/mL of DIG-labeled PRL-R probe at 48°C for 6 h. Membranes were then washed twice in 2X SSC in 0.1% SDS (5 min each) and washed twice in 0.5X SSC in 0.1% SDS (15 min each) at room temperature.

Hybridization was detected using a DIG luminescent detection kit (Boehringer Mannheim). Membranes were incubated in blocking solution containing anti-DIG-alkaline phosphatase (1:10,000 diluted) at room temperature for 30 min, followed by incubation in detection buffer containing CSPD® (1:100 diluted) at room temperature for 8 h. Finally membranes were exposed to normal X-ray film (Kodak, X-OMAT-A*R*, Radiographic Supplies, Christchurch, NZ) from 15 min to 1 h.

Data Analysis

Exposed films and images of stained gels were scanned to generate high-resolution TIFF files. DNA bands were analyzed using NIH Image 1.61 software for Macintosh. The density of the bands was measured in pixels. For comparison of expression of mRNA in diestrous and lactating rats, the optical density for PRL-R mRNA in Southern blots was normalized against that for β-actin in ethidium bromide–stained gels. These ratios were then analyzed by the Mann-Whitney *U*-test for nonparametric data. In the diestrous group, no data were available for the short-form mRNA in the CgCTX. In these cases, an arbitrary ratio value of 0.1 was designated in order to allow statistical comparison. Differences were considered to be statistically significant if the p value was <0.05.

Acknowledgments

This research was supported in part by a grant from the Health Research Council of New Zealand and by University of Otago Bequest Funds.

References

1. Buntin, J. D. (1993). *Am. Zool.* **33,** 229–243.
2. Dutt, A., Kaplitt, M. G., Kow, L. M., and Pfaff, D. W. (1994). *Neuroendocrinology* **59,** 413–419.
3. Kelly, P. A., Ali, S., Rozakis, M., Goujon, L., Nagano, M., Pellegrini, I., Gould, D., Djiane, J., Edery, M., Finidori, J., and Postel-Vinay, M. C. (1993). *Recent Prog. Horm. Res.* **48,** 123–164.
4. Walsh, R. J., Posner, B. I., Kopriwa, B. M., and Brawer, J. R. (1978). *Science* **201,** 1041–1043.
5. Davis, J. A. and Linzer, D. I. (1989). *Mol. Endocrinol.* **3,** 674–680.
6. Lai, Z., Roos, P., Olsson, Y., Larsson, C., and Nyberg, F. (1992). *Neuroendocrinology* **56,** 225–233.
7. Sugiyama, T., Minoura, H., Kawabe, N., Tanaka, M., and Nakashima, K. (1994). *J. Endocrinol.* **141,** 325–333.
8. Sugiyama, T., Minoura, H., Toyoda, N., Sakaguchi, K., Tanaka, M., Sudo, S., and Nakashima, K. (1996). *J. Endocrinol.* **149,** 335–340.
9. Pi, X. J. and Grattan, D. R. (1998). *J. Neuroendocrinol.*, in press.
10. Pi, X. J. and Grattan, D. R. (1998). *J. Mol. Endocrinol.*, **23,** 13–22.
11. Paxinos, G. and Watson, C. (eds.). (1997). *The rat brain in stereotaxic coordinates*, 3rd ed. Academic: San Diego.
12. Pi, X. J. and Grattan, D. R. (1998). *J. Comp. Neurol.* **394,** 462–474.
13. Cohen-Becker, I. R., Selmanoff, M., and Wise, P. M. (1986). *Neuroendocrinology* **42,** 328–333.
14. Park, S. K., Grattan, D. R., and Selmanoff, M. (1993). *J. Reprod. Fertil.* **99,** 209–217.
15. Sarkar, D. K. and Yen, S. S. (1985). *Endocrinology* **116,** 2080–2084.
16. Koike, K., Miyake, A., Aono, T., Sakumoto, T., Ohmichi, M., Yamaguchi, M., and Tanizawa, O. (1991). *Horm. Res.* **35,** 5–12.
17. Wise, P. M. (1986). *Endocrinology* **118,** 1237–1245.
18. Smith, M. S. (1978). *Biol. Reprod.* **19,** 77–83.
19. Rogers, I. S. (1997). *Early Hum. Dev.* **49,** S185–S190.
20. Malik, K. F., Silverman, A. J., and Morrell, J. I. (1991). *Anat. Rec.* **231,** 457–466.
21. Sagrillo, C. A., Grattan, D. R., McCarthy, M. M., and Selmanoff, M. (1996). *Behav. Genetics* **26,** 241–277.
22. Milenkovic, L., D'Angelo, G., Kelly, P. A., and Weiner, R. I. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 1244–1247.
23. Gu, G. B. and Simerly, R. B. (1997). *J. Comp. Neurol.* **384,** 142–164.
24. Numan, M., Rosenblatt, J. S., and Komisaruk, B. R. (1977). *J. Comp. Physiol. Psychol.* **91,** 146–164.
25. Lin, S. H., Miyata, S., Matsunaga, W., Kawarabayashi, T., Nakashima, T., and Kiyohara, T. (1998). *Brain Res.* **787,** 226–236.
26. Numan, M., Numan, M. J., Marzella, S. R., and Palumbo, A. (1998). *Brain Res.* **792,** 348–352.
27. Bridges, R. S., Robertson, M. C., Shiu, R. P., Sturgis, J. D., Henriquez, B. M., and Mann, P. E. (1997). *Endocrinology* **138,** 756–763.
28. Bakowska, J. C. and Morrell, J. I. (1997). *J. Comp. Neurol.* **386,** 161–177.
29. Krantic, S., Martel, J. C., and Quirion, R. (1991). *Peptides* **12,** 81–87.
30. Ricciardi, K. H., Turcotte, J. C., De Vries, G. J., and Blaustein, J. D. (1996). *J. Neuroendocrinol.* **8,** 673–685.
31. Chiu, S., Koos, R. D., and Wise, P. M. (1992). *Endocrinology* **130,** 1747–1749.
32. Pi, X. J. and Grattan, D. R. (1998). *Brain Res. Mol. Brain Res.* **59,** 1–12.
33. Shamgochian, M. D., Avakian, C., Truong, N. H., Stone, S., Tang, K. T., and DeVito, W. J. (1995). *Neuroreport* **6,** 2537–2541.
34. DeVito, W. J., Okulicz, W. C., Stone, S., and Avakian, C. (1992). *Endocrinology* **130,** 2549–2556.
35. Lesueur, L., Edery, M., Ali, S., Paly, J., Kelly, P. A., and Djiane, J. (1991). *Proc. Natl. Acad. Sci. USA* **88,** 824–828.
36. O'Neal, K. D. and Yu-Lee, L. Y. (1994). *J. Biol. Chem.* **269,** 26076–26082.
37. Das, R. and Vonderhaar, B. K. (1995). *Mol. Endocrinol.* **9,** 1750–1759.
38. Mangurian, L. P., Walsh, R. J., and Posner, B. I. (1992). *Endocrinology* **131,** 698–702.
39. Palkovits, M. and Brownstein, M. J. (eds.). (1988). *Maps and guide to microdissection of the rat brain*. Elsevier: New York.
40. Boutin, J. M., Jolicoeur, C., Okamura, H., Gagnon, J., Edery, M., Shirota, M., Banville, D., Dusanter-Fourt, I., Djiane, J., and Kelly, P. A. (1988). *Cell* **53,** 69–77.
41. Shirota, M., Banville, D., Ali, S., Jolicoeur, C., Boutin, J. M., Edery, M., Djiane, J., and Kelly, P. A. (1990). *Mol. Endocrinol.* **4,** 1136–1143.
42. Roelen, B. A., Lin, H. Y., Knezevic, V., Freund, E., and Mummery, C. L. (1994). *Dev. Biol.* **166,** 716–728.