Increased expression of both short and long forms of prolactin receptor mRNA in hypothalamic nuclei of lactating rats

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ABSTRACT

This study investigated expression of prolactin receptor (PRL-R) mRNA in selected hypothalamic nuclei of lactating rats (days 7–10 post partum) compared with dioestrous rats. Rat brains were frozen with liquid nitrogen and cut into coronal sections of 300 µm. From these sections, tissues were micropunched from the parietal cortex (CTX), choroid plexus (ChP), and five hypothalamic regions: supraoptic (SO), paraventricular (Pa), arcuate (Arc) and ventromedial hypothalamic (VMH) nuclei, and median eminence (ME). Expression of both short and long forms of PRL-R mRNA were evaluated by reverse transcription-PCR and Southern hybridisation. The results showed that the relative amount of short form mRNA in the ChP of lactating rats was significantly higher than in dioestrous rats. The short form of PRL-R mRNA was undetectable in the SO, Pa,

VMH of dioestrous rats but was expressed at a significant level in lactating rats. Levels of long form mRNA in the ChP, SO, Pa and VMH in lactating rats were significantly increased compared with dioestrous rats. Moreover, the long form mRNA was induced in the CTX of lactating rats. In the Arc, levels of both forms of PRL-R mRNA tended to increase in lactating rats compared with dioestrous rats but changes were not statistically significant. Neither form of PRL-R mRNA was detectable in the ME in the two animal models. Increased expression of PRL-R mRNA in specific brain regions during lactation is consistent with the variety of PRL effects on the brain, and may help to explain profound physiological changes in the lactating mother.

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INTRODUCTION

Prolactin (PRL) exerts a wide range of effects on the brain to regulate many functions (Buntin 1993, Dutt *et al.* 1994, Bole-Feysot *et al.* 1998). Studies have demonstrated that circulating PRL can gain access to cerebral ventricles (Login & MacLeod 1977, Nicholson *et al.* 1980, Martensz & Herbert 1982), either through a receptor mediated mechanism (Walsh *et al.* 1987) or via circumventricular structures such as the *organum vasculosum* of the *lamina terminalis* that lack a blood–brain barrier. In addition, brain tissue itself may also produce PRL that could regulate brain functions, since both PRL immunoreactivity (Paut-Pagano *et al.* 1993) and PRL mRNA (Emanuele *et al.* 1992) have been identified in rat brain tissue including the hypothalamus.

The biological effects of PRL are presumably mediated by the PRL receptor (PRL-R) which is a member of the cytokine receptor superfamily (Kelly *et al.* 1993). Molecular analysis of the cDNA sequence for PRL-R has revealed that there are at least two isoforms of PRL-R protein: the short form and long form, which are generated by alternative splicing of a single gene (Kelly *et al.* 1993). Consistent with various actions of PRL on the brain, PRL-R protein has been identified in the brain and its distribution mapped in several hypothalamic nuclei of female rats by autoradiography in female rats (Crumeysrolle-Arias *et al.* 1993) and by immunohistochemistry in male (Roky *et al.* 1996) and female rats (Pi & Grattan 1998a). The corresponding PRL-R mRNA is also expressed in numerous hypothalamic nuclei of female rats (Chiu & Wise

1994, Bakowska & Morrell 1997, Pi & Grattan 1998b).

During lactation, the mother undergoes notable changes to certain neuroendocrine and behavioural functions. For example, lactating mothers exhibit markedly increased food intake (Rosso 1988), morphological changes in magnocellular hypothalamic nuclei (Hatton 1997) and increased secretion of oxytocin (Crowley & Armstrong 1992). These changes are accompanied by suckling-induced hyperprolactinaemia, and there is evidence that PRL may be involved in mediating some lactation-induced changes to hypothalamic function. For example, PRL has been shown to induce hyperphagia in virgin female rat (Sauve & Woodside 1996), and to increase oxytocin mRNA content in the hypothalamus (Ghosh & Sladek 1995).

Given that PRL can up-regulate its own binding sites in rat hypothalamus (Muccioli & Di Carlo 1994) and increase PRL-R mRNA in the brain of ovariectomised rats (Sugiyama *et al.* 1994), we hypothesised that PRL-R expression in the hypothalamus would be likely to increase in order for PRL to induce these physiological changes during lactation. Consistent with this suggestion, we have recently reported that there are significant increases in PRL-R immunoreactivity in the hypothalamus during lactation, particularly in the supraoptic (SO), paraventricular (Pa), arcuate (Arc) and ventromedial hypothalamic (VMH) nuclei (Pi & Grattan 1999). The increased expression of PRL-R immunoreactivity suggests that levels of the two forms of PRL-R mRNA in different brain regions may also be increased during lactation. Therefore, the aim of the present study was to evaluate the expression of both forms of PRL-R mRNA in selected hypothalamic nuclei of lactating rats, and to compare this expression with that in dioestrous rats. We focused on several hypothalamic nuclei in which we have previously identified PRL-R protein during lactation (Pi & Grattan 1999).

MATERIALS AND METHODS

Animal preparation

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. Animals were group-housed in plastic cages under conditions of controlled temperature 22 °C and lighting (lights on from 0600 to 1800 h). They were given free access to food and water. The stages of the oestrous cycle were monitored by daily cytological examination of

vaginal smears. Dioestrous rats were selected after the animals had exhibited at least two 4-day oestrous cycles. A group of pro-oestrous 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 day 18 of pregnancy. On day 2 post partum (parturition day=day 0), the litters were culled to 12 pups per dam.

On each experimental day, tissue was collected from one lactating (days 7–10 post partum) and one dioestrous rat, and then processed in parallel. All animals were killed between 0900 and 1200 h. 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 dioestrous rats were examined. All protocols for animal experimentation were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals.

Microdissection

Coronal sections of 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 (Palkovits & Brownstein 1988), tissues were carefully micro-punched from the choroid plexus (ChP), S1BF parietal cortex (CTX), four specific hypothalamic nuclei and the median eminence (ME) with blunted stainless-steel needles of 300 or 500 µm in diameter, depending on the size of the target nucleus (Table 1 and Fig. 1). Microdissected tissue of each brain region from each individual animal was treated as a single data point.

Extraction of total ribonucleic acid (RNA)

Total cellular RNA was extracted from various microdissected brain tissues using the RNeasy Mini Kit (Qiagen Inc., Santa Clarita, CA, USA). Briefly, brain tissues were placed in 300 µl lysis buffer containing 4 M guanidinium salt and β-mercaptoethanol, and homogenised by a sonicator using a cup-horn attachment. The tissue homogenate was then mixed with 300 µl 70% ethanol, loaded onto the RNeasy spin column and centrifuged for 0.5 min at 8000 *g* (~10 000 r.p.m.). The supernatant was washed with a buffer containing 70% ethanol and centrifuged two times. Total RNA was finally collected with 30 µl DEPC-treated water and stored at −70 °C.

TABLE 1. Microdissection of discrete brain regions

Brain regions	Section coordinates relative to Bregma*	Number of punches per brain	Diameter of micropunch needle (μm)
1 S1BF cerebral cortex (CTX)	-2.3, -2.6, -2.9	8	500
2 Choroid plexus (ChP)	-0.6, -0.9, -1.2	6	500
3 Supraoptic nucleus (SO)	-0.9, -1.2, -1.5	6	500
4 Paraventricular hypothalamic nucleus (Pa)	-1.5, -1.8	4	500
5 Arcuate nucleus (Arc)	-2.6, -2.9, -3.2	3†	500
6 Ventromedial hypothalamic nuclei (VMH)	-2.6, -2.9, -3.2	6	500
7 Median eminence (ME)	-2.6, -2.9, -3.2	3†	500

*The rat brain atlas by Paxinos & Watson (1997) was used as a guide for the microdissection. Section coordinates represent the approximate position (relative to Bregma) of the rostral face of each section collected for microdissection. †Arc was removed by a single punch centred on the midline, after the removal of the ME. ME was dissected with single punch to include only the medial parts of this area.

FIGURE 1. Diagrams and representative sections showing locations of micropunches for the different brain regions. See Table 1 for definition of different numbered brain regions. Rat brains were frozen on dry ice and then cut into coronal sections of 300 μm in a cryostat at -9 °C. Micropunches were collected from these frozen thick sections with autoclaved stainless steel needles of 300 μm- or 500 μm-diameter. After microdissection, photographs were taken of thawed tissue using a dissecting microscope. Diagrams are taken from the Paxinos & Watson rat brain atlas (1997), which was used to identify anatomical structures and as a reference for abbreviations .

Reverse transcription (RT)

The first strand cDNA synthesis reaction was catalysed by Superscript II RNase H⁻ reverse transcriptase (RT, GibcoBRL, Life Technologies, Gaithersburg, MD, USA). In all cases, 10 μl total RNA (about 50–200 ng) were reverse transcribed.

After being denatured together with 0.5 μg (1 μl) oligo (dT)_{12–18}, RNA samples were incubated with 200 units (1 μl) Superscript II RT and the following reagents: 1) 2 μl 10 × PCR buffer (375 mM KCl, 250 mM Tris-HCl, pH 8.3); 2) 2 μl 25 mM MgCl₂; 3) 1 μl 10 mM deoxy-NTPs mixture; and 4) 2 μl 0.1 M dithiothreitol. Two control experiments were

TABLE 2. Oligonucleotides used for RT-PCR and Southern hybridisation

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

carried out routinely: 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 and Arc, 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 (Boutin *et al.* 1988, Shirota *et al.* 1990). The sequence of the primers used in the present study are listed in Table 2. Primers 1 and 2 were used to detect the short form of PRL-R mRNA while primers 1 and 3 were used to detect the long form in the RT-PCR studies. Primer 4 was common to both forms of PRL-R cDNA and used as a probe for Southern hybridisation. Primers 5 and 6 were used to amplify cDNA from β-actin mRNA (Roelen *et al.* 1994). The predicted DNA sizes for the short and long form of PRL-R mRNA, and for β-actin mRNA were 330, 395 and 420 base pairs respectively.

Polymerase chain reaction (PCR)

For each microdissected brain region, three separate PCRs were set up using primer pairs to detect the short and long form of PRL-R mRNA and β-actin mRNA respectively. PCR was carried out with PCR SuperMix (GibcoBRL, Life Technologies), which contained (per ml): 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM MgCl₂, 220 μM dNTPs, 22 U recombinant Taq DNA polymerase. The reaction mixture contained 45 μl PCR SuperMix, 1 μl primers at the concentration of 20 μM, and 4 μl RT product solution. PCR incubations were conducted in a programmable

thermal controller (MJ Research, Watertown, MA, USA). During each cycle, the samples were denatured at 96 °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. A total of 33 cycles were performed for PCR tubes containing β-actin template whereas 40 cycles were used for each form of PRL-R cDNA. These PCR procedures were selected based on previous control experiments to ensure that detection of PCR products was completed within the linear part of the amplification curves for each cDNA (Pi & Grattan 1998b).

The RT-PCR products were separated in a 1.5% (w/v) agarose gel LE (Boehringer Mannheim, Indianapolis, IN, USA). A 50 bp DNA Ladder (GibcoBRL, LifeTechnologies) was used as standard size markers. Gels were stained for 20 min in 50 mM TRIS base, 50 mM boric acid, 1 mM disodium EDTA buffer containing 0.5 μg/ml ethidium bromide (EthBr), destained for 20 min, and examined on a UV transilluminator. DNA bands were photographed with a polaroid camera.

Southern hybridisation

Southern filter hybridisation was carried out to validate PCR products from the PRL-R mRNA and to increase sensitivity of detection. The digoxigenin (DIG) labelled PRL-R probe was made by labelling primer 4 according to the DIG oligonucleotide 3'-end labelling Kit (Boehringer Mannheim). Gels were first denatured in a solution containing 0.5 M NaOH and 1.5 M NaCl, and neutralised 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 20× SSC buffer. Membranes were baked at 120 °C for 30 min, prehybridised in DIG Easy Hyb buffer (Boehringer Mannheim) at 48 °C for 2 h, and hybridised in DIG Easy Hyb containing 3 pmol/ml

DIG-labelled PRL-R probe at 48 °C for 6 h. Membranes were then washed twice in $2 \times$ SSC (5 min each) and washed twice in $0.5 \times$ SSC (15 min each) at room temperature.

Hybridisation 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-Ar) from 15 min to 1 h.

Data analysis

Exposed films and polaroid images of EthBr-stained gels were scanned at 600 dpi to generate high resolution TIFF files, and DNA bands were analysed using NIH Image 1.61 software for Macintosh. The density of the bands was measured in pixels. For comparison of expression of mRNA in dioestrous and lactating rats, the optical density for PRL-R mRNA in Southern blots was normalised against that for β -actin in EthBr-stained gels. These ratios were then analysed by Mann-Whitney U test for non-parametric data. In the dioestrous group, no data were available for the short form mRNA in the SO, Pa, Arc, VMH and for the long form in the CTX. 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 less than 0.05.

RESULTS

Microdissection

It was important to establish that tissue samples were micropunched from within the specific brain regions of interest. Hence, microdissection was validated by examining thawed sections under a dissecting microscope (thawing of sections allowed clear differentiation of grey and white matter). Photographs were taken after brain sections were thawed (Fig. 1). The CTX sample was punched from the rostral part of S1BF CTX, which is part of the parietal CTX. The ChP tissue was punched from the anterior region of the lateral ventricles. Most of hypothalamic nuclei were microdissected with needles of 500 μ m diameter whereas the smaller areas (ChP and SO) were punched with needles of 300 μ m diameter. The number of punches collected for each brain region is described in Table 1. All diagrams and abbreviations were

based on the Paxinos & Watson rat brain atlas (1997).

RT-PCR results

The conditions used for RT-PCR have been characterised previously (Pi & Grattan 1998b). PCR amplification of cDNA with primers specific for the long form of PRL-R identified a single 420 bp band, which is consistent with the predicted size of the mRNA for the long form of PRL-R. PCR amplification of cDNA from the same punched tissue with primers specific for the short form of PRL-R identified a single 330 bp product, which agrees with the predicted size of the short form of PRL-R mRNA. Under the conditions used, cDNA for both forms of PRL-R mRNA were clearly detectable in EthBr-stained gels from samples of ChP from both dioestrous and lactating rats. Much lower levels of cDNA were observed in the microdissected brain regions, however, with some regions showing faint bands, and others being undetectable (Fig. 2A, top and middle panels). Hence, it was necessary to use the more sensitive Southern hybridisation to detect and quantify the cDNA products from PRL-R mRNA in different brain regions. To control for differences in amount of tissue in each dissection and possible differences in RNA loading in the first strand synthesis, PCR amplification was performed using specific primers for β -actin, from the same cDNA samples as used for PRL-R mRNA. RT-PCR products for β -actin mRNA showed a single 395 bp band which is consistent with the predicted size for this mRNA. The DNA bands from β -actin mRNA were easily visualised on EthBr-stained agarose gels in all brain regions examined (Fig. 2A, bottom panel).

Southern hybridisation results

A representative image is shown in Fig. 2. In the dioestrous rat, DNA product from the short form of PRL-R mRNA was only detected in the ChP and Arc while the long form was visualised in the ChP, SO, Pa, Arc and VMH. In the lactating rat, the short form mRNA could be detected in the ChP, SO, Pa, Arc, VMH but not in the S1BF CTX and ME. DNA bands amplified from the long form of PRL-R mRNA were detected in all examined regions of lactating rats except the ME.

Comparison of mRNA between dioestrous and lactating rats

The normalised amount of short and long form PRL-R mRNA in different brain regions of

FIGURE 2. (A) Representative gels showing EthBr staining of RT-PCR products for the short form, long form PRL-R mRNA, and β -actin mRNA from various hypothalamic nuclei of a dioestrous (lanes 1–7) and a lactating (lanes 8–14) rat. Lanes 1 and 8=S1BF CTX; 2 and 9=ChP; 3 and 10=SO; 4 and 11=Pa; 5 and 12=Arc; 6 and 13=VMH; 7 and 14=ME; 15 and 16=no RT controls; 17=DIG molecular weight marker in top and middle panel; 50 bp DNA ladder in bottom panel, with thicker band at 350 and 800 bp; 18=50 bp DNA ladder in top and middle panel. (B) Representative Southern blot film of RT-PCR products from the gels presented in part A. The RT-PCR products were capillary-transferred onto a nylon membrane and then hybridised with DIG-labelled PRL-R probe common for both forms of PRL-R.

FIGURE 3. Relative amounts of mRNA for the short form (upper panel) and long form (lower panel) of PRL-R mRNA normalised to β -actin mRNA from different brain regions of dioestrous (open bars) and lactating rats (solid bars). Data represent the mean $\pm$ S.E.M. of four pairs (each consisting of a dioestrous and a lactating rat) of rats for the short form and six for the long form. Two pairs of rats were excluded from the short form analysis because the short form was only detected in the ChP in these two animals due to poor recovery of mRNA. For both the short and long form, $*P < 0.05$ when compared with corresponding regions in dioestrous rats.

dioestrous and lactating rats is illustrated in Fig. 3. The results can be summarised as follows: 1) the relative amount of short form of PRL-R mRNA in the ChP of lactating rats was significantly higher than that in dioestrous rats ($P < 0.05$). In addition, the short form mRNA was induced in the SO, Pa, and VMH in the lactating rat; 2) compared with dioestrous rats, the levels of long form mRNA in lactating rats were significantly amplified in four brain regions including the ChP, SO, Pa and VMH ($P < 0.05$); 3) moderate levels of two forms of mRNA were detected in the Arc in both dioestrous (short = 0.9 ± 0.3 ; long = 1.6 ± 0.5) and lactating rats (short = 1.8 ± 0.4 ; 3.0 ± 1.3). Despite the tendency for increased expression in lactating rats, the differences in the Arc between animal models were not statistically significant; 4) both forms of mRNA

were undetectable in S1BF CTX of dioestrous rats whereas a low level of long form was detected in the S1BF CTX of lactating rats, indicating that expression of the long form was induced in the S1BF CTX during lactation; and 5) neither of two forms of PRL-R mRNA were detected in the ME in either dioestrous or lactating rats.

DISCUSSION

It is well known that hyperprolactinaemia has numerous effects on the brain (Moore 1987, Dutt *et al.* 1994). While the mechanisms of these effects are unknown, certain discrete hypothalamic nuclei have been implicated. Clearly, the understanding of changes in PRL-R expression in specific nuclei

during lactation is essential to elucidate mechanism(s) of actions of hyperprolactinaemia on the brain. The present study combined the sensitivity of the RT-PCR approach with the relatively precise neuroanatomical localisation provided by microdissection techniques. With this approach, the expression of both short and long forms of PRL-R mRNA in specific brain regions in dioestrous rats and lactating rats has been described for the first time. We observed that expression levels of both short and long forms of PRL-R mRNA were significantly increased in the ChP, SO, Pa, and VMH of lactating rats when compared with dioestrous rats. The long form of mRNA in the S1BF CTX appeared to be induced in the lactating rat. Both forms of mRNA were detected in the Arc of dioestrous rats and lactating rats, but no significant differences between the two animal models were observed. These results are consistent with our previous data showing that PRL-R immunoreactivity was increased in these brain regions in lactating rats (Pi & Grattan 1999).

There have been a few previous studies showing that levels of PRL-R mRNA in the brain change during pregnancy and lactation. The long form, but not the short form, of PRL-R mRNA was reported to be induced in the rat brain during pregnancy and lactation (Sugiyama *et al.* 1994) or by pup contact (Sugiyama *et al.* 1996). These studies only investigated mRNA changes in the whole rat cerebrum and, hence, provided no anatomical resolution as to the location of the described changes. In contrast to lack of effect on short form mRNA observed in these studies (Sugiyama *et al.* 1994, 1996), the present study showed that the short form mRNA was also induced in some brain areas. It is likely that the earlier studies using whole cerebrum may have missed some region specific increase in the short form mRNA. More recently, expression of the long form PRL-R mRNA during pregnancy was studied by *in situ* hybridisation, and data showed increased expression between days 2 and 21 of pregnancy in the medial preoptic nucleus, with no change in the VMH (Bakowska & Morrell 1997). However, the short form of mRNA was not investigated in that study. To date, changes in two forms of PRL-R mRNA in discrete hypothalamic nuclei during lactation have not been reported.

Because of exponential nature of PCR amplification, quantitation of mRNA by RT-PCR can be unreliable. We have used Southern hybridisation to confirm the identity of the PCR product, but the relative amount of DNA described do not represent true levels of cellular mRNA. Nevertheless, the techniques provide a qualitative comparison of

relative amounts of mRNA within different brain regions in the two experimental models.

Clearly, levels of expression of PRL-R mRNA are significantly increased in the rat brain during pregnancy (Sugiyama *et al.* 1994, Bakowska & Morrell 1997) and during lactation (Sugiyama *et al.* 1996 and the present data). The mechanisms which mediate the increased expression of PRL-R mRNA are currently unclear. In the ChP, up-regulation of the long form of PRL-R mRNA may be mediated by high levels of PRL, since a concomitant increase in serum PRL level and long form mRNA level was observed in male rats by pup-contact (Sakaguchi *et al.* 1996). Whether the long form is also regulated by PRL or placental lactogen in discrete hypothalamic nuclei is not known. Oestrogen, progesterone and growth hormone have also been suggested to be involved in induction of the long form mRNA during pregnancy, given that these hormones markedly increase mRNA levels in the whole cerebrum of ovariectomised and hypophysectomised nulliparous rats (Sugiyama *et al.* 1994).

The functional significance of induction of both forms mRNA for PRL-R in the various hypothalamic nuclei (SO, Pa and VMH) needs to be elucidated further, such as by experiments using gene or protein knockout techniques. Nonetheless, some suggestions can be made. It is suggested that the VMH regulates feeding behaviour (Li *et al.* 1995). Since experimental hyperprolactinaemia produces an increase in food intake in female rats (Sauve & Woodside 1996), PRL-R in the VMH may be involved in the increased feeding behaviour specific to lactating rats. This assumption is supported by the observation that PRL-R mRNA in the VMH was not changed during pregnancy (Bakowska & Morrell 1997) but was augmented during lactation (present data), and the observation that PRL-R protein was not detected in the ovariectomised plus oestrogen-treated rat (Pi & Grattan 1998a) or dioestrous rat (Pi & Grattan 1999) but induced during lactation (Pi & Grattan 1999).

Within the Pa and SO are neuronal cell bodies of magnocellular neurons which synthesise either vasopressin or oxytocin (Leng *et al.* 1992). Both oxytocin and vasopressin mRNA in the SO and Pa were markedly increased during lactation (Van Tol *et al.* 1988), and this region also exhibits marked structural plasticity during lactation, particularly in oxytocin neurons and their associated glial cells (Hatton 1997). We have observed that PRL-R immunoreactivity tended to be concentrated in the dorsal part of the SO and ventral and lateral portions of the Pa (Pi & Grattan 1998a, 1999), a distribution that is more closely correlated with that of oxytocin-containing neurons than vasopressin

neurons (Mejia *et al.* 1997). Hence, PRL-R in the SO and Pa may be involved in regulation of oxytocin secretion during pregnancy and/or lactation.

In the present study, both forms of PRL-R mRNA were detected in the Arc of both dioestrous and lactating rats. PRL-R in this area are likely to be involved in negative feedback regulation of tuberoinfundibular dopamine (TIDA) neuronal activity (Lerant & Freeman 1998). It is interesting to note that while levels of PRL-R mRNA and protein tended to increase in the Arc during lactation, PRL effects on TIDA neurons are not increased but rather are suppressed during the last 24 h of pregnancy (Grattan & Averill 1995, Fliestra & Voogt 1997) and during lactation in the rat (Demarest *et al.* 1983, Arbogast & Voogt 1996). This phenomenon permits the lactating mother to maintain high levels of serum PRL, but the mechanism is currently unknown. It seems clear that the reduced responsiveness of TIDA neurons to PRL is not caused by decreased expression of PRL-R in the Arc, based on our present and previous data (Pi & Grattan 1999).

Both forms of PRL-R mRNA were detected in the ME of estrogen-treated ovariectomised rats in our previous study (Pi & Grattan 1998b). These data were unexpected, because there are no neuronal cell bodies in this region. In order to ensure that there was no possible contamination from adjacent nuclei, such as the ventral Arc, we modified our microdissection method in the present study. Rather than collecting both the medial and lateral portions of the ME (Kobayashi *et al.* 1978) as we had done previously (Pi & Grattan 1998b), we only dissected the medial portion of the ME in the present study. The absence of detectable mRNA in the ME in the present study suggests that there may have been minor contamination of the ME dissection with tissue from the ventral Arc in our previous study (Pi & Grattan 1998b), or that mRNA expression is limited to lateral aspects of the ME.

The implication of induction of PRL-R mRNA in the cerebral CTX remains to be investigated. S1BF CTX is part of the parietal CTX, which is responsible for primary somatosensory function. Lactating mothers display parental behaviour, aggressive actions towards foreign adults, and become less fearful than usual during lactation. The parietal CTX, and prefrontal CTX are activated in the lactating rat in terms of Fos immunoreactivity (Fleming & Korsmit 1996) but show reduced responses to N-methyl-D,L-aspartic acid (Abbud *et al.* 1993). It would be interesting to determine whether induction of the long form of PRL-R in this area is associated with these changes in the lactating mother.

In conclusion, the present study showed significant increases in levels of PRL-R mRNA in the ChP and three hypothalamic nuclei during lactation. The data suggest that expression of PRL-R gene undergoes significant alteration during pregnancy and/or during lactation with region-specific increases in receptor mRNA expression. The change in expression of PRL-R during lactation may help to explain hyperprolactinaemia-induced physiological changes in the lactating mothers.

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