Cell Prolif. 1997, **30**, 219–235

Prolactin receptor gene expression in rat splenocytes and thymocytes during oestrous cycle, pregnancy and lactation

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(*Received* 11 *August* 1997; *accepted* 25 *August* 1997)

Abstract. Much evidence suggests that prolactin (PRL) has an immunoregulatory function. Part of this evidence is that the receptors for PRL are present on lymphocytes. Probably the effects of PRL on cells of the immune system depend on the level and specific forms of PRL-R present on the target cells. Therefore, PRL-R expression at both protein and mRNA levels was examined during oestrous cycle, pregnancy and lactation using Western blotting and PCR analysis. Antibody to the long form of PRL-R detected 84 and 42 kDa protein bands in the spleen but only 84 kDa band in the thymus. The expression pattern of these two protein bands was different in the spleen, suggesting that these two isoforms of PRL-R long form are differentially regulated by the hormones of oestrous cycle. In addition, depending on the tissue, the level of mRNA for the short and long forms of PRL-R showed a significant change at different stages of oestrous cycle. Moreover, 42 and 84 kDa PRL-R bands were detected in both spleen and thymus throughout the pregnancy and lactation; however, the expression pattern of 84 kDa protein band was different between tissues. This finding suggests that each tissue exhibits differential response to hormones which affect PRL-R content.

An anterior pituitary hormone PRL is primarily responsible for the development of mammary gland and the stimulation of milk protein genes. In addition, it has effect on reproduction, growth, metabolism, behaviour and immunoregulation. Immunoregulatory function of PRL has been supported by many studies. For example, decreased antibody production as well as cell mediated immunity have been observed in hypohysectomized rats (Nagy & –Berczi 1978). Administration of PRL or implantation of pituitary gland reconstituted these immune functions (Berczi *et al.* 1981, Nagy *et al.* 1983). In addition, PRL has been shown to regulate DNA synthesis *in vivo* in lymphoid tissue (Berczi *et al.* 1991) and to act directly on splenocytes and thymocytes *in vitro* (Mukherjee *et al.* 1990, Viselli, Stanek & Mukherjee 1991).

PRL-R expression has been shown in lymphoid cells. For example, binding studies with ^{125}I-labelled PRL provided evidence that human T- and B-cells have PRL-R (Russell 1985). More definitive studies based on the use of antibodies to PRL-R and flow cytometry confirmed these findings; and indicated that many subsets of rat splenocytes (Viselli & Mastro 1993), rat and murine lymphoid cells (Koh & Phillips 1993, Gagnerault *et al.* 1993)

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display PRL-R. By using *in-situ* hybridization and polymerase chain reaction, the long and the short forms of PRL-R have been detected in rat spleen and thymus (Ouhtit *et al.* 1993, Güneş *et al.* 1993, Touraine *et al.* 1994).

The short and long forms of PRL-R have been identified from normal rat liver and ovary (Shirota *et al.* 1990). These two receptors differ in the length and the sequence of the intracellular region. The short form (291 amino acids) is encoded by 1.8 kb (kilobase) transcript. However, the long form (591 amino acids) is encoded by three transcripts which are 2.5, 3, and 5.5 kb. Alternative splicing of a primary transcript appears to be responsible for these different mRNAs (Kelly, Ali & Rozakis 1993). An intermediate form of PRL-R has only been found in a rat lymphoma cell line, Nb2 (Shiu *et al.* 1983).

Hormonal expression and regulation of PRL-R have been the subject of several studies in many different tissues such as ovary, mammary gland, liver and brain (Jahn *et al.* 1991, Clarke & Linzer 1993, Clarke *et al.* 1993, Guillaumot & Cohen 1994, Sugiyama *et al.* 1994). However, less is known about PRL-R expression and regulation in lymphoid tissues. Because only lymphocytes from rats in certain hormonal states respond to PRL, it is possible that the effects of PRL on cells of immune system may depend on the level and specific forms of PRL-R on the target cells. Therefore, the aim of this study was to examine PRL-R expression during the oestrus cycle, pregnancy and lactation in rat splenocytes and thymocytes.

MATERIALS AND METHODS

Animals

Sprague–Dawley rats or pregnant rats (Harlan Laboratories, Indianapolis, IN) were housed in a room maintained at 20–22°C on a 14 h light, 10 h dark schedule with free access to food and water. Daily vaginal smears were taken to determine the stage of oestrus cycle. Only animals with a regular 4-day cycle were chosen for experiments. All rats were sacrificed between 11 and 12 am. The day when spermatozoa appeared in the vaginal smear was designated as day 0 of pregnancy.

Isolation of splenocytes and thymocytes

Rats were decapitated, the spleens and thymi removed, and placed in sterile saline. Cells were isolated as described previously (Güneş *et al.* 1993). Cell counts were performed with a haemacytometer and viability determined by trypan blue exclusion. Cell viability averaged 85% for splenocytes and 96% for thymocytes.

RNA isolation

Total cytoplasmic RNA was extracted from a range of 2×10^7 to 1×10^8 cells by the guanidinium thiocyanate method of Chomczynski & Sacchi (1987). Isolated RNA pellets were suspended in ribonuclease free water and quantified by spectrophotometry.

Primer design

Primers were designed and prepared (Oligo Synthesis by the Biotechnology Institute, The Pennsylvania State University, PA) in order to differentiate the mRNA of the long form of the PRL-R from the short form (Figure 1). The 5′ primer, TACATCGTTGAGCCCAGAG (352–370), was taken from a region preceding the transmembrane region. The sequences for both forms are homologous (Boutin *et al.* 1988). The sequences of the long and short forms diverge in their cytoplasmic regions. The short form 3′ primer, AGTCTGCAGCTT-CAGTAG (927–944), was taken from the sequences of the final 30 amino acids of the short

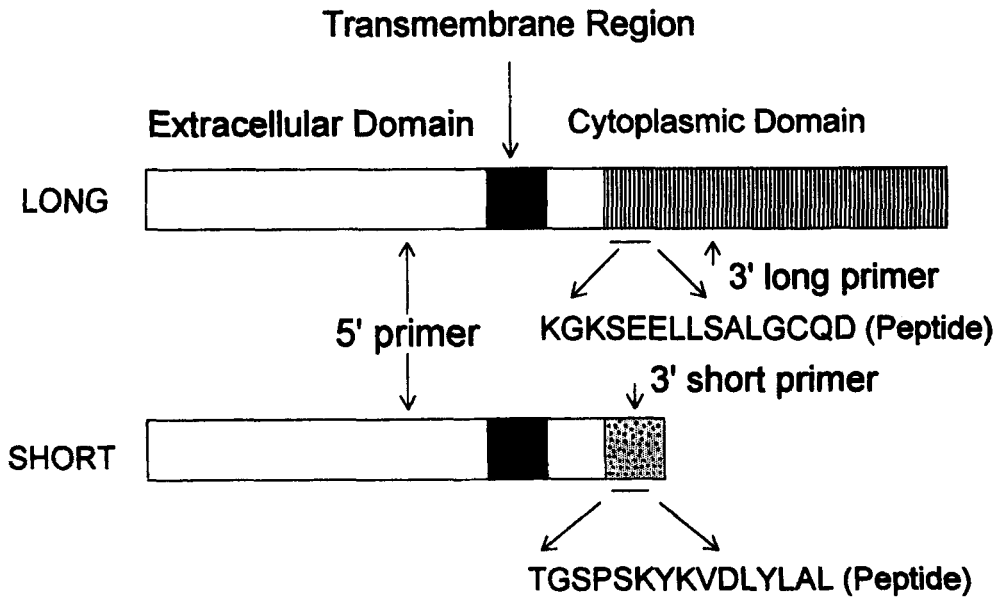


Figure 1. Peptide and primer design for antibody production and PCR of the long and short forms of PRL-R. 3′ primers were designed using non-homologous sequences in order to differentiate the long and short from mRNA. For antibody production, amino acid sequences were chosen from non-homologous sequences to distinguish the short and long forms.

form. The long form 3′ primer, CTGTCATAGCTTCCGTGA (1037–1054) (Shirota *et al.* 1990), is from sequences after the short form stops. The expected PCR products using these primers are 701 base pairs for the long form and 593 base pairs for the short form. Actin primers (5′ primer CACTGGCATTGTGATGGA; 3′ primer ACGGATGTCAACGTCACA) were chosen from the sequences of rat β-actin gene (Nudel *et al.* 1983) to produce 427 base pair PCR product.

Reverse transcription and PCR amplification (RT-PCR)

Total RNA was reverse transcribed to cDNA as described previously (Güneş *et al.* 1993). Briefly, 10 μg RNA was added to a reaction mixture of 0.75 μg random hexamers, 0.02 U of RNAsin, 75 μM of each dNTP, buffer (50 mM Tris HCl, pH 8.3; 75 mM KCl; 3 mM $MgCl_2$), 0.1 mM DTT and 200 U of M-MLV reverse transcriptase (Gibco BRL, Gaithersburg, MD). The cDNA was amplified using 2.5 U of Taq Polymerase (Perkin Elmer, Norwalk, CT) in a Thermocycler programmed with the following cycles: Cycle 1: 3 min at 94°C, 2 min at 50°C, 2 min at 72°C; Cycles 2 through to 38; 1 min at 94°C, 1 min at 50°C, 2 min at 72°C; and Cycle 40; 1 min at 94°C, 1 min at 50°C, 10 min at 72°C. A sample without cDNA served as negative control. After amplification, PCR products were kept at −20°C until used. A linear relationship between the log of RNA input (5, 10 and 20 μg) and the log of the chemiluminescent signal was observed previously (Güneş & Mastro 1996).

Southern blot analysis

The amplified cDNA was electrophoresed through a 1.5% agarose gel containing ethidium bromide and transferred to Immobilon-S membrane (Millipore, Beverly, MA). The membrane was hybridized with biotinylated cDNA probes for PRL-R or actin as described by

Güneş & Mastro (1996). The membrane was exposed to Polarplex Chemiluminescent reagents (Milipore, Beverely, MA) and XAR X-ray film for detection of the bands.

Quantification of PRL-R bands

In order to compare relative levels of the two forms of PRL-R, primers for actin, a housekeeping gene, were included in PCR reaction. The Southern blots were scanned by densitometry (Quick Scan Jr., Helena Laboratories, Beamont, TX). Quantitative analysis of mRNA for PRL-R was carried out by normalizing the densitometric signal for the short or long form with that of the actin. Also PRL-Rs detected with Western blotting were quantified in similar fashion.

Solubilization of lymphocyte proteins

Solubilization of membrane and cytoplasmic proteins of lymphocytes was carried out as described by Coligan *et al.* (1991). Briefly, cells were suspended at 2×10^7 cells in 5 ml ice-cold PBS and centrifuged for 5 min at 500 *g*, 4°C. The pellet was resuspended in 200 μl Triton X-100 lyses buffer (300 mM NaCl, 50 mM Tris-Cl pH 7.6, 0.5% Triton X-100) with protease inhibitors, 1 mM PMSF (phenylmethyl sulfonylfluoride), 10 μg/ml aprotinin, 10 μg/ml leupeptin and kept on ice for about 1 h. The samples were microcentrifuged for 15 min at 10000 *g* at 4°C. Supernatants were aliquoted at 50 μl and kept −20°C until used.

Western blot analysis

Solubilized membranes and cytosolic proteins were separated on 10% SDS-polyacrylamide gel electrophoresis (Laemmli 1970) and electrophoretically transferred (150 mA, 1 h, 0.9 mA/cm^2) to PVDF (polyvinylidene difluoride) membrane (Millipore, Beverely, MA) using a graphite plate electroblotting apparatus (Andersen 1984). Prestained molecular weight markers were electrophoresed and electroblotted as well. The membrane was incubated either with a monoclonal antibody U6 to PRL-R (a generous gift from Dr Paul Kelly, INSERM, Paris, France) or with a polyclonal antibody to the long form of PRL-R (Figure 1) produced and characterized by Güneş 1995; Güneş & Mastro (1996) or with an anti-actin mAb (Oncogene Science, Uniondale, NY) or with pre-immune rabbit serum as negative control. Protein bands were detected with an ECL Western blotting kit (Amersham, Arlington Heights, IL).

RESULTS

Prolactin receptor expression during the rat oestrus cycle

Lysates of splenocytes from rats at pro-oestrus and di-oestrus were subjected to Western blot analysis to determine how different hormonal states affected PRL-R expression at protein level. As it was shown in our previous study (Güneş & Mastro 1996), the polyclonal antibodies produced against the long form of the PRL-R recognized two protein bands with molecular mass of 42 kDa and 84 kDa in the rat splenocytes (Figure 2a). The expression of relative amount of 42 kDa and 84kDa bands exhibited different pattern during oestrous cycle (Figure 2b). For example, the level of 42 kDa band was at its highest level during pro-oestrus but decreased by 1.6-fold during oestrus and twofold during di-oestrus. However, the level of the 84 kDa band was at its lowest during pro-oestrus and increased by twofold and sixfold during oestrus and di-oestrus, respectively. The amount of 42 kDa band displayed no significant change during various stages of oestrous cycle (Table 1). However, the level of 84 kDa

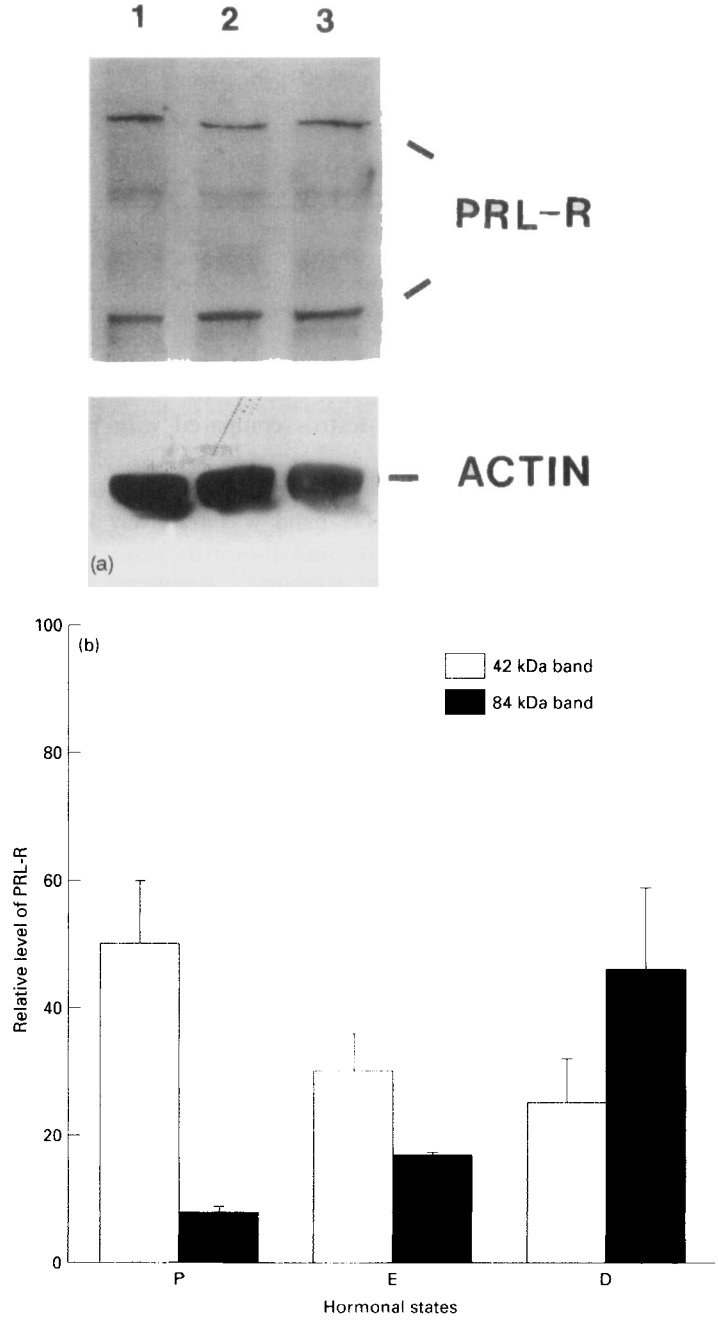


Figure 2. Western blot analysis of PRL-R expression in splenocytes during the rat oestrus cycle. Splenocytes 2×10^7 from rats at pro-oestrus (1), oestrus (2), and di-oestrus (3) stages of the oestrus cycle were lysed in 200 μl lysis buffer. Cell lysate 20 μl from each group was subjected to 10% SDS-PAGE, transfered onto PVDF membrane. Protein bands were detected with antibody to the long form of PRL-R (1/1000) and actin (2 μg/ml) antibodies using the ECL method. (a) Western blot analysis. (b) Normalization of the level of PRL-R to that of actin. P, pro-oestrus; E, oestrus; D, di-oestrus. Data are the average $\pm$SEM of four rats.

Table 1. Relative levels of 42 kDa and 84 kDa bands detected with antibody to the long form of PRL-R during oestrus cycle

Oestrus cycle	84 kDa in spleen	42 kDa in spleen	84 kDa in thymus
Pro-oestrus	8 (±0.8)	50 (±10)	50 (±11)
Oestrus	17 (±3)[a]	30 (±6)	170 (±41)[a]
Di-oestrus	46 (±13)[a,b]	25 (±7)	100 (±12)

PRL-R levels, detected with Western blotting, during at various stages of the oestrus cycle. Data are the mean ±SEM of four rats. Statistical analysis was done using a one-way analysis of variance with Tukeys post hoc test to detect the differences between different stages of the oestrous cycle. The significant differences ($P<0.05$) between groups were shown by different letters. [a]Different from pro-oestrus. [b]Different from oestrus.

band changed significantly in oestrus and di-oestrus compared with pro-oestrus; and in oestrus compared with di-oestrus.

In thymocytes, only the 84 kDa band was detected with antibodies to the long form of PRL-R (Figure 3a). The amount of the 84 kDa band was relatively low at pro-oestrus; however, it increased three- and twofold at oestrus and di-oestrus, respectively. These results indicated that unlike in the spleen 84 kDa band exhibited a significant change only during oestrus compared to pro-oestrus and di-oestrus in the thymus (Table 1). Moreover, the level of the 84 kDa band peaked during di-oestrus in the spleen but at oestrus in the thymus.

In addition to the protein levels, mRNA levels of PRL-R short and long forms were examined during various stages of oestrous cycle. PCR analysis of total RNA from splenocytes (Figure 4a) indicated that the relative level of the long form mRNA was higher than that of the short form mRNA (Figure 4b) at all the stages of oestrous cycle. The level of the long form mRNA was the highest during pro-oestrus. A dramatic decrease (1.8-fold and 1.5-fold) was observed during oestrus and di-oestrus as compared to that in pro-oestrus (Table 2). On the other hand, the amount of mRNA for the short form did not significantly change at different stages of oestrous cycle.

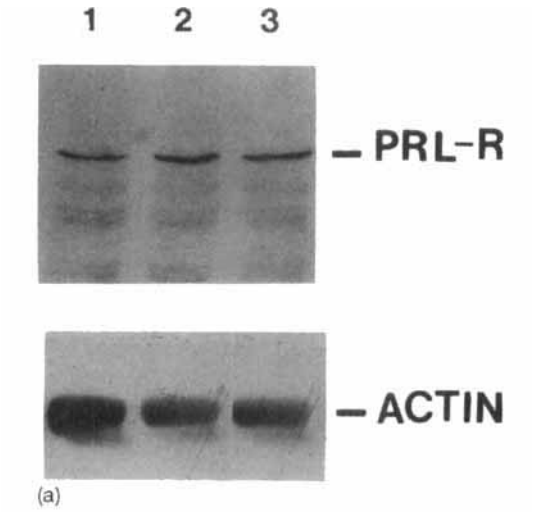
In contrast to splenocytes, thymocytes did not show a significant change in the amount of the long form mRNA during the oestrous cycle (Table 2). Moreover, unlike in splenocytes, the relative levels of short form mRNA declined significantly during oestrus in comparison with pro-oestrus and di-oestrus in thymocytes.

Prolactin receptor expression during pregnancy and lactation

Western blot analysis with antibodies to the long form PRL-R indicated the presence of both 84 kDa and 42 kDa bands in the spleen throughout the pregnancy and lactation (Figure 5a). Densitometric analysis of Western blots revealed that the relative amount of 42 kDa band was 51 at the beginning of pregnancy (Figure 5b). Then, it decreased twofold during mid-pregnancy (day 10) and increased 1.5-fold toward the end of pregnancy (day 20). It rose to the highest level during lactation day 8 and day 14. Toward the end of lactation (day 22), the level of 42 kDa band decreased sharply (2.2-fold).

The relative amount of the 84 kDa band remained low during day 1 and 10 of pregnancy (Figure 5b). However, it started to increase at day 20 and reached to the highest level during lactation day 8. There was a rapid decrease at day 14 of lactation. At the end of lactation, it further decreased to the level, almost the same level as at day 20 of pregnancy.

The 84 and 42 kDa bands showed similar patterns during pregnancy and lactation (Figure 5b). For example, the levels of both protein bands were low at day 10 of pregnancy, reached



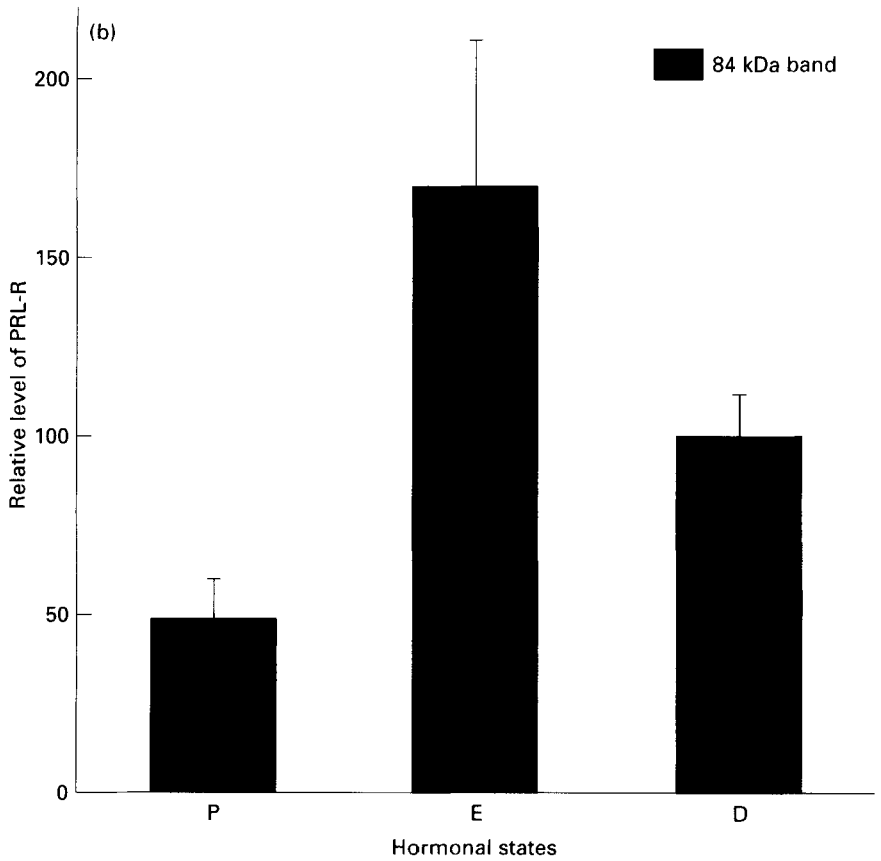
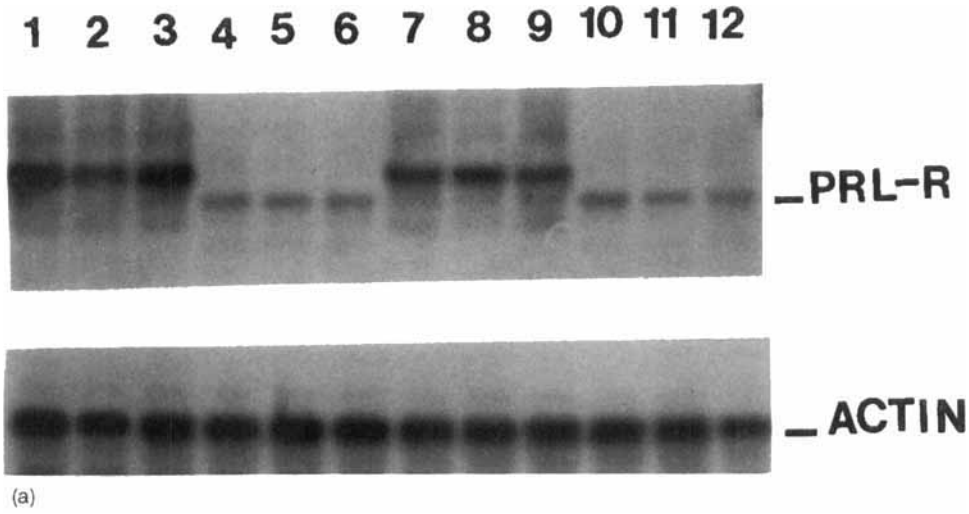


Figure 3. Western blot analysis of PRL-R expression in thymocytes during the rat oestrus cycle. Samples are exactly as described in the legend to Figure 1 except that thymocytes were used. (a) Western blot. (b) Normalization of the level of PRL-R to that of actin. P, pro-oestrus; E, oestrus; D, di-oestrus. Data are the average ±SEM of four rats.



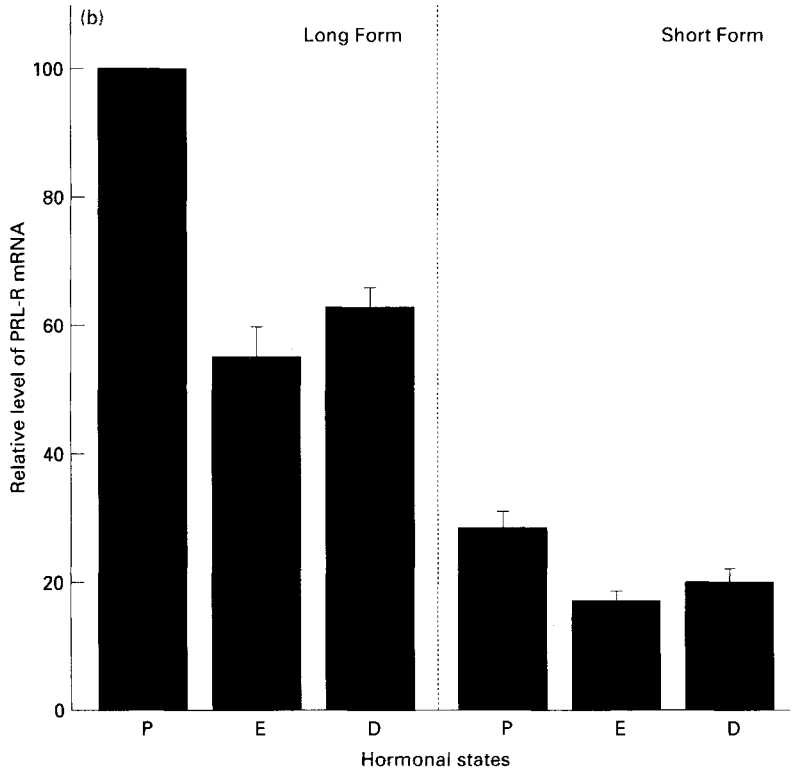


Figure 4. PRL-R mRNA expression in splenocytes during the rat oestrus cycle. Total RNA 10 μg from rats at pro-oestrus (P), oestrus (E), and di-oestrus (D) was reverse transcribed to cDNA. Ten per cent of each cDNA reaction was used in 40 cycles of PCR amplification. After separation on an agarose gel, PCR products were transferred to an immobilon membrane and hybridized with biotinylated probes. (a) Southern blot analysis of PCR products. Lanes 1, 2, 3 are P, E, D for the long form; lanes 4, 5, 6 are P, E, D for the short form; lanes 7, 8, 9, 10, 11, 12 are the repeat of the experiment in the same order as above. (b) Normalization of PRL-R level with the level of actin. Data are the average $\pm$SEM of three rats.

Table 2. Relative levels of PRL-R short and long form mRNA during the oestrous cycle in splenocytes and thymocytes

Oestrus cycle	Long form in spleen	Short form in spleen	Long form in thymus	Short form in thymus
Pro-oestrus	100 (±0)	28 (±2.5)	70 (±13)	34 (±1)
Oestrus	55 (±5)[a]	17 (±1.5)	50 (±0)	27 (±0)[a,b]
Di-oestrus	65 (±3)[a]	20 (±2.0)	40 (±3)	34 (±3)

PRL-R levels, detected with PCR, during at various stages of oestrus cycle. Data are the mean ±SEM of three rats. Statistical analysis was carried out as described in the legend to Table 1. The significant differences ($P<0.05$) between groups were shown by different letters. [a]Different from pro-oestrus. [b]Different from di-oestrus.

a maximum during the early stage of lactation (day 8) and decreased continuously as lactation progressed (Figure 6a).

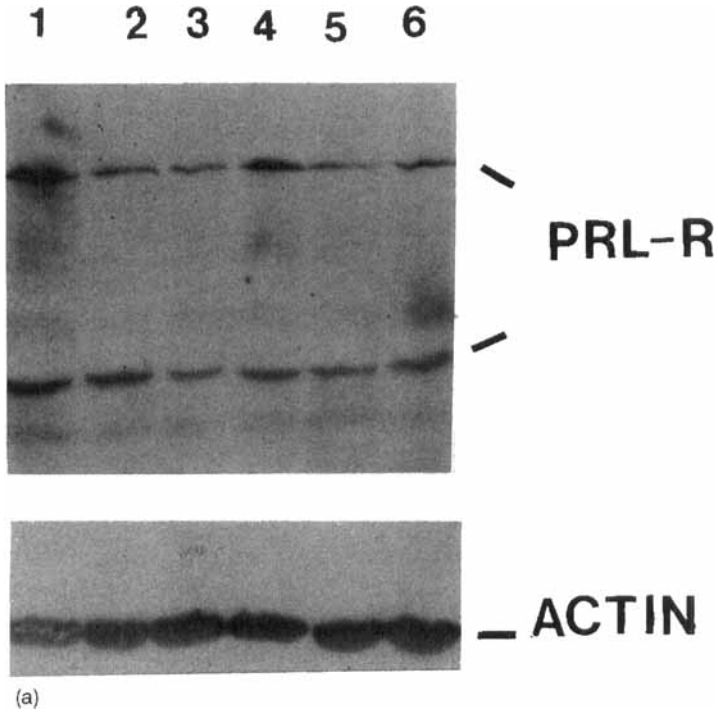
In contrast to spleen, only the 84 kDa band was detected in the thymus during pregnancy and lactation (Figure 7a). The level of 84 kDa band gradually decreased with progression of pregnancy. After parturition, the level increased markedly 10-fold, ninefold and 17-fold during days 8, 14 and 22 of lactation, respectively (Figure 7b). This expression pattern of PRL-R in the thymus differs from that of PRL-R in the spleen (Figure 6a & b) in that at the end of lactation the level of 84 kDa band increased twofold in the thymus, but it decreased in the spleen.

In addition, PRL-R expression was examined using a monoclonal antibody U6, known to recognize the short form (Okamura *et al.* 1989). We noted that proteins (38–40 kDa) detected using U6 were not recognized by antibodies to the long form of PRL-R. Therefore, all the Western blots were stripped and relabelled with U6. The pattern of the expression of PRL-Rs detected with U6 and the antibodies to the long form of PRL-R was similar in both tissues (data not shown).

DISCUSSION

In this study, we investigated the PRL-R expression during different hormonal states. Antibodies to the long form of PRL-R revealed two protein bands at 42 and 84 kDa in the spleen (probably two different isoforms of the long form of the PRL-R) but only 84 kDa in the thymus as it was shown previously (Güneş & Mastro 1996). The level of 84 kDa band increased during oestrus and di-oestrus in both tissues; whereas it decreased during pro-oestrus. Based on previous studies, plasma concentration of LH, FSH, PRL, progesterone and oestradiol peaks during the pro-oestrus stage of the rat oestrus cycle (Butcher, Collins & Fuge 1974, Freeman 1988). Therefore, it seems that elevation of all these hormone levels at pro-oestrus results in a decrease in expression of 84 kDa PRL-R in both spleen and thymus.

In contrast to the 84 kDa protein, the 42 kDa protein reached to its maximum during pro-oestrus and decreased at oestrus and di-oestrus. These results indicate that these two isoforms of PRL-R long form are differentially regulated in the spleen by the hormones of oestrus cycle. This finding is consistent with the results of Guillaumot & Cohen (1994) who found, by using the binding of ^{125}I-labelled ovine PRL, four different molecular forms of PRL-R, 80, 50, 40 and 16 kDa which varied according to the physiological stages in the mammary gland. For example, the 40 kDa form was present during all the stages of the



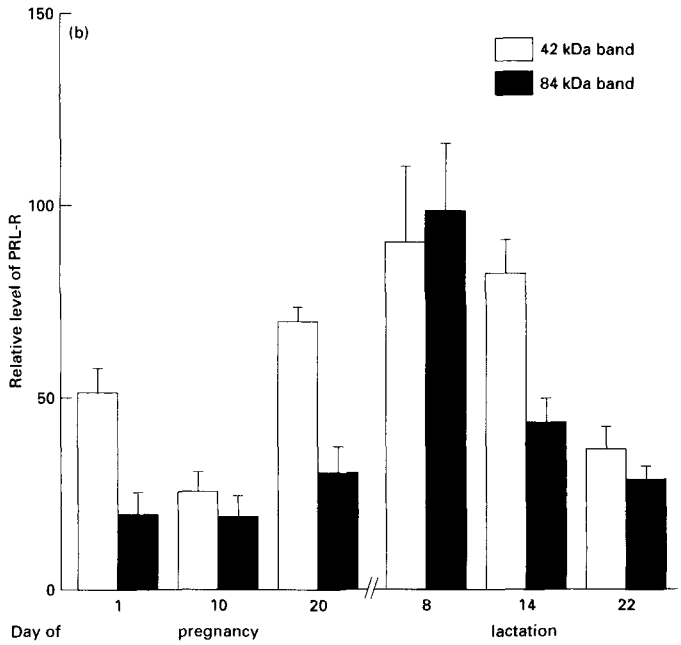


Figure 5. Western blot analysis of PRL-R expression in splenocytes during pregnancy and lactation. Splenocytes 2×10^7 from 1 day (1), 10 days (2), and 20 days (3) pregnant rats; rats lactating for 8 days (4), 14 days (5), and 22 days (6) were lysed in 200 μl lysis buffer. Twenty microlitres of cell lysate from each group was subjected to Western blotting as described in the legend to Figure 1. (a) Western blot analysis. (b) Normalization of the level of PRL-R to that of actin. Data are the average $\pm$SEM of three rats.

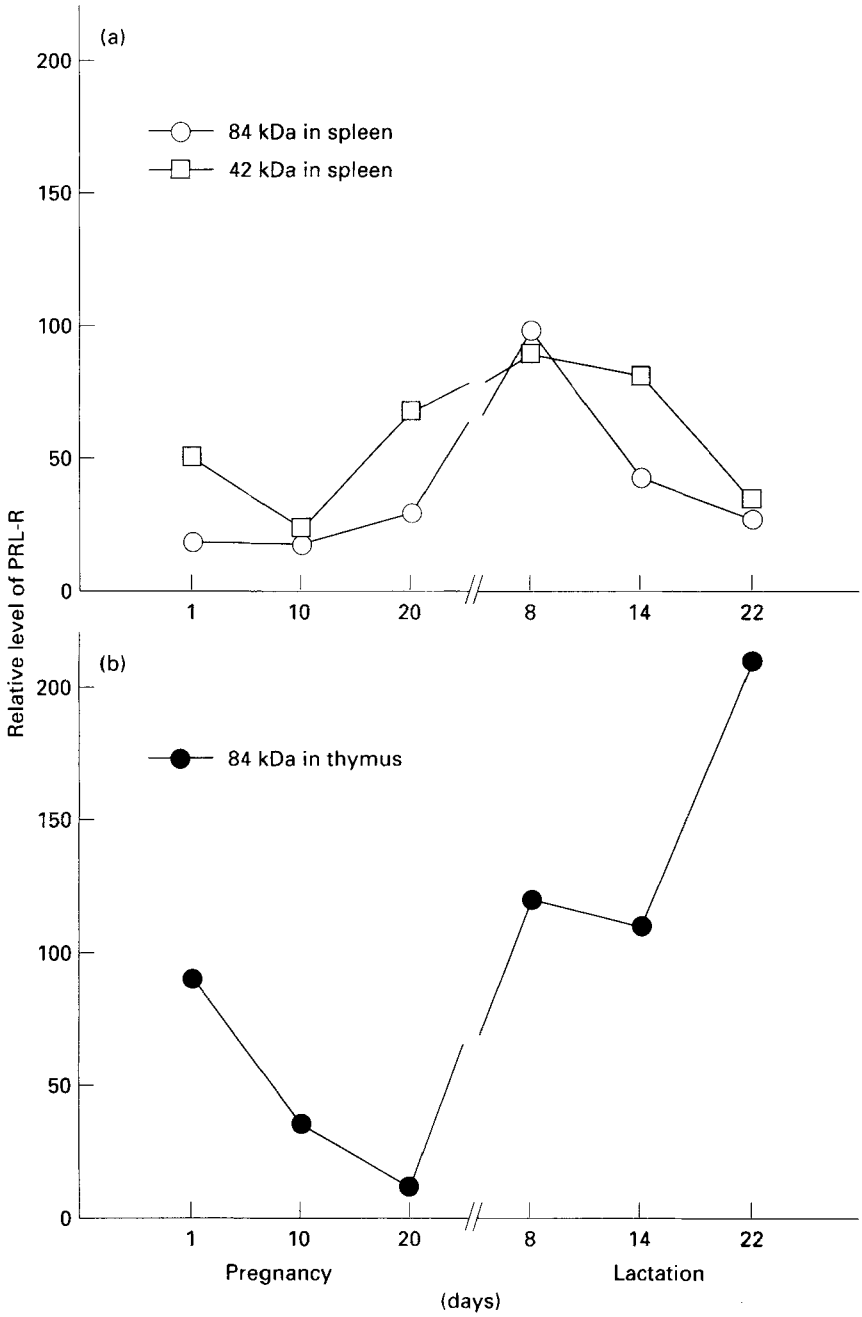
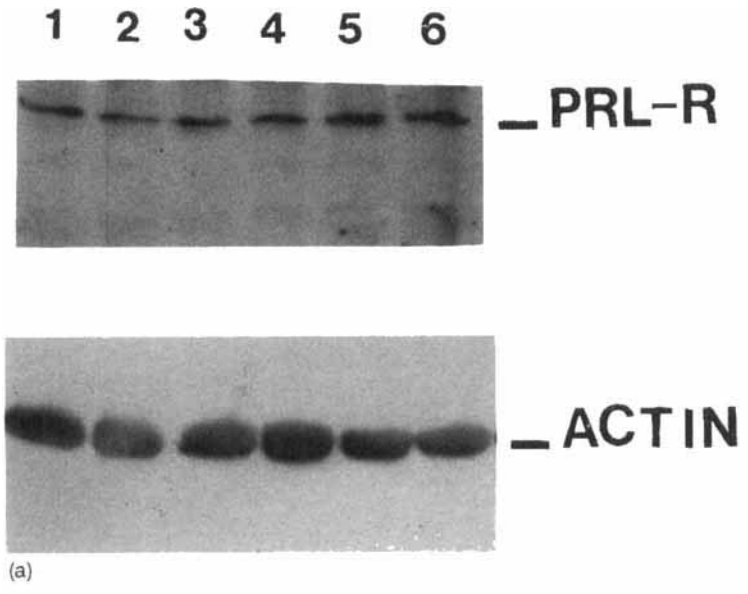


Figure 6. Comparison of PRL-R levels detected with Western blotting during pregnancy and lactation in spleen and thymus.



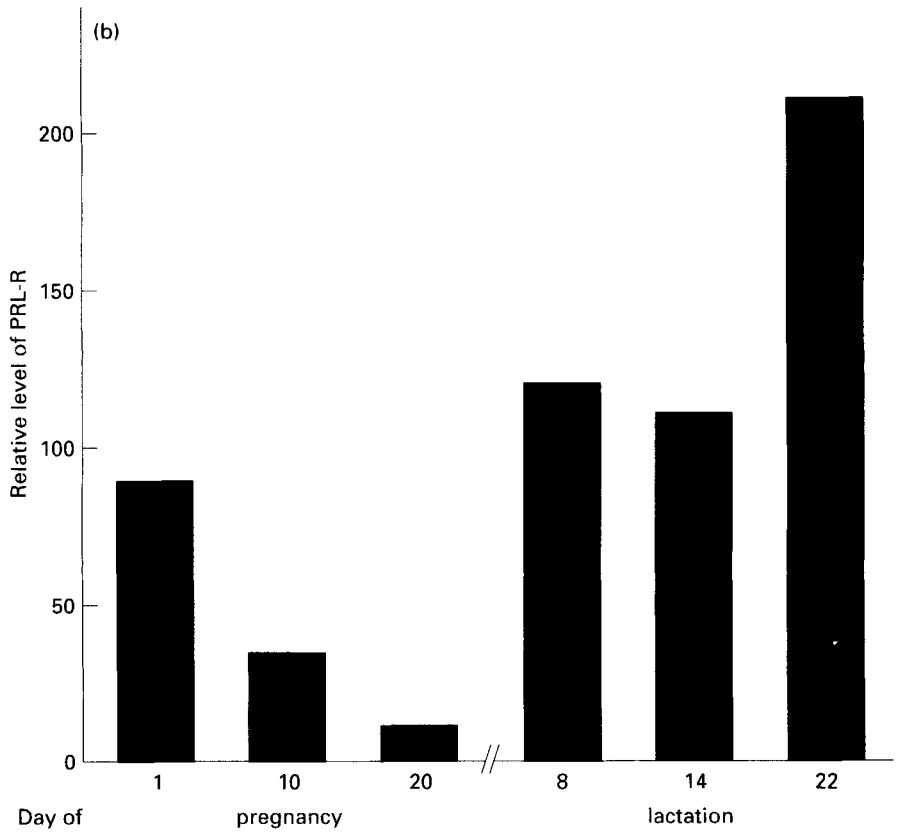


Figure 7. Western blot analysis of PRL-R expression in thymocytes during pregnancy and lactation. Samples are exactly as described in the legend to Figure 5 except that thymocytes were used. (a) Western blotting. (b) Normalization of the level of PRL-R to that of actin. Experiment was repeated twice with similar results.

oestrus cycle; however, the 50 kDa form was observed only during pro-oestrus; and 16 kDa form was present only during lactation.

We found that the level of 84 kDa PRL-R in thymus reached its maximum level at oestrus, a result consistent with the previous studies (Guillaumot *et al.* 1984) that PRL-R levels rose to their maximum level in the mammary gland during the day of oestrus. Likewise, the amount of 84 kDa PRL-R in the spleen was also relatively high during oestrus and di-oestrus as compared to pro-oestrus in this study. Previous reports indicated that steroid hormones as well as PRL alter the cellular concentration of PRL-R (Posner 1976, Barkey *et al.* 1981, Ormandy & Sutherland 1993). At this point, we cannot determine which of these hormones caused the change in PRL-R protein in spleen and thymus during different stages of oestrus cycle. In fact, all hormones, oestradiol, LH, FSH, PRL and progesterone, increase during the day of pro-oestrus (Butcher *et al.* 1974, Freeman 1988) and probably each hormone alone or in synergy with others may be involved in the alteration of PRL-R level during the oestrus cycle.

Differential regulation of the long forms of PRL-R levels during various stages of the oestrus cycle may be one of the possibilities for the *in vitro* finding of a mitogenic effect of PRL on splenocytes from rats at dioestrus and from ovariectomized rats. However, this needs to be confirmed in further studies. It is also possible that the level of short form of the PRL-R may have an important function for PRL-responsiveness of splenocytes because both the short and long forms of PRL-R have been shown to signal cells to grow in the presence of PRL (Banerjee & Vonderhaar 1994).

In addition to the protein level of the PRL-R, the mRNA levels for the short and the long forms of the PRL-R were examined during the rat oestrus cycle. The mRNA for the long form of PRL-R was present in greater quantities than that of the short form of PRL-R at all the times during the oestrus cycle (Table 2). These results are consistent with those of Sugiyama *et al.* (1994) who showed that the level of the long form of the PRL-R mRNA was greater than that of the short form in rat brain during oestrus cycle; and by Clarke, Arey & Linzer (1993) who found the same results in rat ovary.

In this present study, the maximum level was observed during pro-oestrus for the long form of PRL-R mRNA, then the level dropped significantly during oestrus and stayed at the similar level during di-oestrus. Similarly, the short form mRNA was at the highest level during pro-oestrus but it did not alter significantly during oestrus and di-oestrus (Figure 4). The change of the long form of PRL-R mRNA levels may be due to alterations in the rate of PRL-R gene transcription or PRL-R mRNA stability as a function of hormonal environment.

High levels of expression of PRL-R long form mRNA during pro-oestrus had also been observed in the studies of others. For example, Sugiyama *et al.* (1994) found that the long form of mRNA in rat brain increased twofold during pro-oestrus, and stayed almost at the same level during oestrus in comparison with di-oestrus. Similarly, the long form of PRL-R mRNA increased during pro-oestrus and oestrus in rat liver (Nagao & Kelly 1992). In contrast to these results, we found that the long form mRNA levels in the spleen decreased almost twofold during oestrus in the present study. The differences between the results are most likely due to the different tissues used in these studies. The results also show that the same hormones exert different effects on different tissues. This significant increase of PRL-R long form mRNA during pro-oestrus at the time during which there is a surge in serum PRL and oestradiol levels (Freeman 1988) implies that oestradiol and PRL are involved in the induction of the long form mRNA as well as the short form of PRL–R mRNA in the spleen.

In the thymus, the amount of PRL-R long form mRNA did not significantly change during the oestrus cycle. However, the short form of PRL-R mRNA decreased during oestrus but remained at the highest level during pro-oestrus and di-oestrus (Table 2). These results indicated that hormonal regulation of PRL-R mRNA expression is tissue specific. As in the spleen, the long form of PRL-R mRNA was at higher quantity than the short form mRNA during every stage of the oestrus cycle in thymus.

When the protein level of PRL-R in the spleen (Fig. 2) was compared to the level of PRL-R mRNA (Fig. 4), it was observed that the level of long form of mRNA paralleled the level of 42 kDa band during the oestrus cycle. However, the 84 kDa band exhibited an opposite pattern to the long form mRNA. These findings indicate that PRL-R expression is regulated at both transcriptional and translational level during different hormonal states. In other words, both 84 kDa and 42 kDa PRL-R are present in the spleen, and an increase in the expression of either one or the other depends upon the hormonal environment. Indeed, previous investigators reported that post-translational regulation of PRL-R protein was mediated through steroid hormones. For instance, PRL-R protein half-life increased in response to progestin (Murphy *et al.* 1993). Also oestradiol results in an increase in PRL-R protein without further increase in PRL-R mRNA level in liver (Jolicoeur, Boutin & Okamura 1989).

Comparison of PRL-R protein with PRL-R mRNA in the thymus (Tables 1 & 2) showed that the 84 kDa protein increased significantly during oestrus and di-oestrus but the mRNA level for the long form was almost the same at all the stages of the oestrus cycle. This result suggests that PRL-R expression in thymus is regulated at translational level during different hormonal states.

In addition to the oestrus cycle, PRL-R expression was examined during pregnancy and lactation. In the spleen, the levels of the 42 kDa and 84 kDa proteins were relatively low during early stages of pregnancy but increased at the end of pregnancy (day 20) and early lactation, then decreased gradually as lactation progressed (Fig. 5). In the thymus, the 84 kDa PRL-R was relatively high during first day of pregnancy and it decreased sharply throughout the pregnancy. It increased dramatically during lactation and continued to rise at the end of lactation (Fig. 7). This pattern of PRL-R expression in thymus is similar to those reported in mammary gland (Jahn *et al.* 1991). Although the expression pattern of PRL-R in spleen looks similar to that in the liver (Jahn *et al.* 1991), they do not parallel exactly. For example, PRL-R levels increased during the end of pregnancy in both tissues. However, PRL-R level dropped to the lower level throughout the lactation in liver but in the spleen it remained high at early stage of lactation and decreased gradually toward the end of lactation. These results show that effects of hormones on PRL-R expression are tissue specific.

In addition, U6 monoclonal antibodies to the PRL-R detected 38 kDa and 40 kDa proteins during pregnancy and lactation in both tissues. The pattern of expression of these protein bands was similar to that of the long form of the PRL-R in the spleen, i.e. the level was high at the beginning of pregnancy and lactation and decreased with progression of these hormonal states.

A rise in PRL-R expression during end of pregnancy and early stages of lactation in spleen and thymus is in agreement with the fact that the fall in progesterone levels induces PRL secretion (Deis, Corrizo & John 1989) as well as PRL-R expression (Jahn *et al.* 1991). Indeed, Morishige, Pepe & Rothchild (1973) reported that serum concentration of progesterone increase at the early stages of pregnancy in the rat, reaches a maximum around day 16; and decreases to undetectable level by day 21 of pregnancy. Furthermore, a rapid increase in the concentrations of PRL and oestradiol several days before delivery and after

birth (Morishige *et al.* 1973, Neill 1974, Saunders *et al.* 1976) supports the idea that PRL secretion during end of pregnancy and the beginning of lactation induces and upregulates expression of its own receptor in the spleen and the thymus.

In conclusion, the expression levels of 42 kDa and 84 kDa protein bands detected with antibody to the long form of PRL-R were differentially regulated in the spleen throughout the oestrus cycle. In other words, an increase in the expression level of either one or the other depends on the stage of the oestrus cycle. Moreover, the level of the long form mRNA in the spleen and that of the short form mRNA in the thymus showed significant change at different stages of oestrus cycle, indicating that hormonal regulation of PRL-R mRNA expression is tissue specific. Furthermore, the expression pattern of 84 kDa PRL-R band was different in both spleen and thymus throughout pregnancy and lactation. However, a consistent finding was that the level of PRL-R expression increased rapidly at the beginning of lactation. As a result, PRL-R expression at both mRNA and protein levels showed a change during different hormonal stages and the effect of hormones on PRL-R expression was specific to each tissue.

ACKNOWLEDGEMENTS

We thank Dr Paul Kelly for his generous gifts of plasmids containing cDNA for prolactin receptor short and long form. This work was supported in part by NIH grants CA-24385 and CA-23248 to AMM.

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