Pup contact induces the expression of long form prolactin receptor mRNA in the brain of female rats: effects of ovariectomy and hypophysectomy on receptor gene expression

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Abstract

Prolactin receptor (PRL-R) mRNA expression levels in the female rat brain (cerebrum) during pup contact stimulation were determined by the reverse transcription-PCR method. The high expression levels of long form PRL-R mRNA found in the brain of lactating rats were markedly reduced by removal of pups, and long form PRL-R mRNA levels were recovered by resumption of pup contact. Interestingly, pup contact stimuli of nulliparous virgin rats also markedly induced long form but not short form PRL-R mRNA expression in the brain in 1-3 days, together with the expression of maternal behaviour. In ovariectomized (OVX) or hypophysectomized (HYPOX) virgin rats, or in OVX plus HYPOX virgin rats, however, brain long form PRL-R mRNA was not significantly induced by pup contact stimuli for as long as 7 days, while maternal behaviour was fully expressed in these rats after 7 days of pup contact. The in situ hybridization experiments revealed that the long form PRL-R mRNA induced in virgin rats in contact with pups or in lactating rats was localized in the epithelial cells of the choroid plexus. No significant increase in mRNA was detected in other regions of the brain, such as the hypothalamus or cortex, in these maternal female rats. These results suggest that pup contact induces the expression of long form PRL-R mRNA in the choroid plexus of the brain in the presence of female sex steroid and pituitary hormones for the rapid expression of maternal behaviour. Our studies also suggested that maternal behaviour can be expressed in OVX plus HYPOX rats after exposure to pups for 7 days without any significant increase in brain PRL-R mRNA expression.


Introduction

In addition to its milk-producing actions in mammals and the pigeon, prolactin (PRL) is known to exert a wide variety of physiological functions in vertebrates (Nicoll 1974). Among these, maternal behaviour has been considered to be induced mainly by the action of PRL (Nicoll 1974, Meites 1988). In fact, maternal behaviour is induced in the rat by systemic administration of PRL in the presence of female sex steroid hormones (Bridges et al. 1984) or by bilateral direct infusions of PRL into the medial preoptic area (Bridges & Ronsheim 1990). Moreover, administration of bromocriptine, an inhibitor of PRL secretion, delays the rapid onset of maternal behaviour (Bridges & Ronsheim 1990). Histological investigations have identified several binding sites for PRL in the rat brain (Walsh et al. 1978, 1987, Barton et al. 1989, Muccioli et al. 1991, Mangurian et al. 1992), suggesting direct action of PRL on the brain. Recent studies on PRL receptor (PRL-R) gene expression in the rat brain have revealed the presence of two species of PRL-R, long and short forms, which are localized in the brain regions (Chiu et al. 1992). More recently, we have reported that a preferential expression of long form PRL-R mRNA is induced in the rat brain by the actions of PRL, growth hormone (GH), oestradiol or progesterone during the late gestation and lactation periods (Sugiyama et al. 1994).

In the present study, we have investigated mRNA expressions for long and short form PRL-Rs in the brain of female rats expressing maternal behaviour after exposure to pups. We report here that pup contact induces gene expression for the long form PRL-R in the brain and maternal behaviour expression, even in virgin rats in the presence of female sex steroids and pituitary hormones. We also present evidence that maternal behaviour can be induced in virgin rats by pup contact stimuli for a longer period in the absence of sex steroids, pituitary-derived hormones or a distinct increase in brain PRL-R gene expression.
Materials and Methods

Animals

Female Sprague–Dawley rats at 6 weeks of age were purchased from SLC, Inc. (Hamamatsu, Japan), and were kept in a temperature-controlled room. Lights were on during the day time (0700–1900 h), and the animals were fed on laboratory chow and tap water available ad libitum. The day on which spermatozoa were found in vaginal smears is referred to as day 0 of pregnancy. Ovariectomy was carried out on nulliparous rats at 8 weeks of age, and hypophysectomy was performed at 9 weeks of age. The animals were killed in the morning (0900–1100 h) and the brain (cerebrum) was quickly taken out, removing the pituitary gland and cerebellum. The brain was then frozen in liquid nitrogen before extraction of RNA.

Measurement of maternal behaviour

The test for maternal responsiveness was carried out as described previously (Bridges et al. 1984) beginning between 0930 and 1100 h. The behavioural responses of the animal toward three pups (3 to 7 days old), which were placed in the three quadrants (one pup per quadrant) away from the nest site, were measured. The test animals were observed for pup retrieval, grouping and crouching over the young in a nursing position, as well as for pup-killing responses. Animals were observed for the presence or absence of these behaviours for 1 h; continuously for 15 min, then at 15-min intervals for about 5 min three times. On test days 2–11, the pups used in the previous day’s tests were removed from the cages. Approximately 30 min later, a new set of pups was introduced into each test cage, thereby commencing another 1-h test for maternal behaviour. Tests were continued for 10 days or

Figure 1 Effects of lactation and its interruption on PRL-R gene expression in the brain of female rats. (A) The scheme for the experiments. Four dams were used for each experimental group divided as follows: group 1, dams lactating for 8 days; group 2, dams lactating for 3 days and the pups removed for 5 days; group 3, dams lactating for 3 days, the pups were removed for 5 days and then the dams lactated again for 5 days. Ten litters (0–3 days of age) were put into the cages of the dams to be tested. Total RNA samples were obtained from the brains of four dams on the days indicated, and cDNA fragments amplified by RT-PCR were hybridized with the radiolabelled oligonucleotide probe. (B) Autoradiograms of RT-PCR analysis of PRL-R mRNAs. (C) The densitometrically determined PRL-R mRNA levels. Data are expressed as values relative to those for group 2 (defined as 1.0). Values indicate means ± S.E.M. of the rats. *P<0.01 compared with group 2 (Student’s t-test).
As the results recovered of rat eightfold expression RT-PCR method, mined The Determination of mRNA levels

The expression levels of PRL-R mRNAs were determined by the reverse transcription-PCR (RT-PCR) method, and then the cDNA fragments amplified by RT-PCR were hybridized with the radiolabelled oligonucleotide probe as described previously (Sugiyama et al. 1994).

Results

Effects of lactation and its interruption on PRL-R gene expression in the rat brain

As shown in Fig. 1, the high level expression (about eightfold) of the long form PRL-R mRNA in the lactating rat brain (group 1) was remarkably reduced by the removal of the pups from the cage (group 2) and completely recovered by resumption of pup contact (group 3). Prompt recovery of the long form PRL-R mRNA expression in the brain was observed within 1 day after resumption of pup contact (data not shown). On the other hand, the mRNA level for short form PRL-R remained minimal in all groups of rats.

Effects of pup contact stimuli on behaviour and brain PRL-R mRNA expressions in nulliparous, ovariectomized (OVX), hypophysectomized (HYPOX), or OVX plus HYPOX rats

Pup contact stimulation induced full maternal behaviour in nulliparous virgin rats in approximately 1-3 days (Fig. 2). On the other hand, in OVX, HYPOX, or OVX plus HYPOX rats, full maternal behaviour was induced by longer contact periods, i.e. 7-0, 6-9 or 7-6 days respectively (Fig. 2). In the brain of non-operated nulliparous rats which expressed full maternal behaviour after exposure to young, long form PRL-R mRNA but not short form receptor mRNA was remarkably increased compared with that in dioestrous control rats (Fig. 3). However, in OVX, HYPOX, or OVX plus HYPOX rats expressing full maternal behaviour, brain long form mRNA was not significantly induced (Fig. 3). The mRNA expression level of short form PRL-R, decreasing in the brain of OVX or HYPOX rats, was not affected by pup contact stimuli either.

Figure 2 Effects of ovariectomy, hypophysectomy, or ovariectomy plus hypophysectomy on the induction time of full maternal behaviour. One week after hypophysectomy of intact or OVX rats, each animal was housed in an individual cage, where ten pups (0-3 days of age) were also placed. Four rats were used for each OVX, HYPOX and OVX plus HYPOX group. Four intact nulliparous rats were also housed in individual cages, where ten litters were also placed. Responses are expressed as means ± S.E.M. of the time needed for the onset of full maternal behaviour. *P<0.01 compared with nulliparous rats with pup contact (Student's t-test).
Discussion

Analysis of the dynamic induction of PRL-R mRNA species in the rat brain during pup contact stimuli and expression of maternal behaviour was made possible by the sensitive RT-PCR method. In a previous work (Sugiyama et al. 1994), we reported that the expression of long form PRL-R mRNA is highly induced in the brain of female rats during the late gestation and lactation periods. The present study showed that the high expression of long form PRL-R mRNA in the brain during the nursing period was dramatically abolished by interruption of pup contact and lactation. The brain gene expression of PRL-R that was abolished was rapidly recovered by resumption of pup contact and lactation. Moreover, pup contact stimulation of nulliparous virgin rats also induced long form PRL-R mRNA expression in the brain concomitantly with the exhibition of maternal behaviour in about 1-3 days. These findings indicate that pup contact stimuli induce the gene expression of long form PRL-R mRNA in the brain, implying a direct action of PRL on the brain of nursing female rats.

It has been reported that maternal behaviour is dependent on hormones, since PRL, as well as oestradiol and progesterone, can induce full maternal behaviour in the rat (Bridges et al. 1984, 1985, Bridges & Millard 1988). The site on which PRL acts to stimulate the onset of maternal behaviour is considered to be the central nervous system in recent anatomical, physiological and behavioural investigations. Initially, abundant PRL-binding sites were found on the epithelial cells of the choroid plexus in the rat brain (Walsh et al. 1978, 1987), apparently mediating the transport of circulating PRL into the ventricular system. In situ hybridization studies on the brain showed that PRL-R mRNA expression was increased mainly in the choroid plexus epithelial cells by pup contact stimulation (T Sugiyama, K Sakaguchi & K Nakashina, unpublished results). This result supports the role of PRL-R in the choroid plexus in the transport of PRL from the blood to the brain. Similar mRNA induction for long form PRL-R at the choroid plexus has been observed in rats during tolerance of restraint stress (Fujikawa et al. 1995). We consider that the PRL transported into the brain may act on such regions as the hypothalamus, substantia nigra or medial preoptic area. It has recently been demonstrated by the binding assay method that PRL-R exists in the hypothalamus, substantia nigra or striatum of the rat brain (Muccioli et al. 1991). Muccioli & Di Carlo (1994) have demonstrated that endogenous or ovine PRL administration induce specific PRL binding in the hypothalamus of female rats. Moreover, mRNA expression for PRL-R has been demonstrated in these tissue cells in the brain (Ouhit et al. 1993). These reports are consistent with our suggestions.

In the previous study, we reported marked induction of long form PRL-R mRNA expression during late gestation and lactation in the rat brain (Sugiyama et al. 1994). Brain mRNA expression for long form PRL-R but not for the short form receptor began to increase on day 7 of pregnancy, and a dramatic elevation in the amount of mRNA was observed on day 14 of pregnancy, a high level being maintained during the following gestation and lactation periods. We also showed that the brain mRNA expression of the long form PRL-R could be induced directly by PRL, GH or progesterone. From these results,
we suggested that sex steroid hormones or PRL are important regulatory factors for PRL-R gene expression in the brain of rats expressing maternal behaviour during pregnancy and lactation. In the post-partum period, however, the levels of serum oestradiol and progesterone are very low, so that PRL and pup contact stimuli may be critical factors for PRL-R gene expression in the brain during these periods.

On the other hand, we have shown in the present study that PRL-R mRNA was not induced significantly in the brain of OVX, HYPOX, or OVX plus HYPOX rats which started to exhibit full maternal behaviour after about 1 week of pup contact stimuli. It has also been shown previously that maternal behaviour can be maintained and retained independently of hormonal regulation by steroids or pituitary hormones (Rosenblatt 1967). Rosenblatt (1967) demonstrated that maternal behaviour is seen in OVX or HYPOX rats. In fact, in our present study, full maternal behaviour eventually appeared in OVX or HYPOX, or OVX plus HYPOX rats after about 7 days of pup contact. Recent studies have shown that PRL mRNA is expressed at a very low level in the hypothalamus or other regions of the rat brain (Wilson et al. 1992) and that the extrapituitary PRL mRNA expression is stimulated by vasoactive intestinal peptide injected i.c.v. (Bredow et al. 1994). It may be possible that PRL expressed at low levels in the hypothalamus or other regions of the brain is responsible for the expression of maternal behaviour in OVX and HYPOX rats. However, this prediction is still open to investigation.

Neural control of maternal behaviour has also been investigated by many laboratories and it is suggested that pup contact stimuli may act on a particular region of the brain via olfactory bulbs stimulated by odour and on other sensory signal-receiving areas by visual, auditory and tactile stimuli. In the present study, the intact nulliparous rats became maternal in a relatively short time after pup contact. We consider that this may have resulted from the housing conditions under which the virgin rats were kept. Their cages were placed in the same room as that in which the mother rats delivered and nursed the pups. Preliminary stimuli by smell or sound of the pups might have promoted the onset of maternal behaviour in these virgin rats. It has recently been suggested that maternal behaviour is caused by stimulation of the medial preoptic area (MPOA) and its neural connections (Numan 1988). It has been shown previously that oestradiol acts on the MPOA to facilitate expression of maternal behaviour (Numan et al. 1977). It is therefore possible that there are multiple pathways to induce maternal behaviour in the rat. Fleming & Rosenblatt (1974) also predicted that these olfactory-limbic-preoptic pathways mediate maternal behaviour in rats, and that hormones such as oestrogen and PRL may act on a number of different limbic and hypothalamic sites to accomplish the expression of maternal behaviour.

We conclude that circulating PRL in the presence of sex steroids may induce long form PRL-R expression in the choroid plexus which enhances the transport of PRL from the blood to the brain. The transported PRL may act on the hypothalamus or other regions of the brain to induce a rapid onset of maternal behaviour. The neural stimulations may also interact with the PRL-induced maternal behaviour signalling pathway to enhance expression of the behaviour. In OVX or HYPOX rats, neural stimulations lasting for a longer time and/or low levels of brain-derived endogenous PRL may be involved in the onset and maintenance of maternal behaviour.

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