Rat placental lactogen-I binds to the choroid plexus and hypothalamus of the pregnant rat

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ABSTRACT

Recent findings suggest that placental lactogen has a role in the regulation of hypothalamic function during pregnancy. To explore the mechanisms by which placental hormones may exert effects in the maternal central nervous system, we have examined the binding of rat placental lactogen-I (rPL-I) to brain slices from pregnant rats at mid- and late gestation. The binding of rPL-I to maternal rat brain was compared with that of human GH (hGH). Radiolabelled rPL-I bound specifically to ependymal cells of the choroid plexus in the lateral ventricles and in the roof of the third ventricle. The binding of $^{125}$I-labelled rPL-I was inhibited by unlabelled rPL-I, hGH or rat prolactin but not by rat GH, indicating that rPL-I and rat prolactin interact with a common binding site in maternal rat brain. Radiolabelled hGH bound to the choroid plexus and to ependymal cells lining the third ventricle in the region of the arcuate nucleus. In addition, hGH bound specifically to the ventromedial nuclei and to the medial preoptic area of the hypothalamus. The binding of radiolabelled hGH to all brain regions was inhibited by unlabelled rPL-I as well as hGH, indicating that rPL-I competes for lactogenic binding sites in the hypothalamus as well as the choroid plexus of the pregnant rat. These findings suggest potential mechanisms by which placental hormones may exert direct effects on the maternal central nervous system during pregnancy. The precise functions and roles of the PL-I binding sites in maternal choroid plexus and hypothalamus remain to be explored.

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INTRODUCTION

The hypothalamus plays a central role in the neuroendocrine and behavioural adaptations of the mother during pregnancy. Changes in hypothalamic hormone release produce striking alterations in maternal secretion of pituitary luteinizing hormone, follicle-stimulating hormone, growth hormone (GH) and prolactin (Yen & Jaffe, 1991), and hormonal effects on hypothalamic function appear to mediate the increases in maternal food intake during pregnancy and lactation and the induction of maternal behaviour in late gestation (Rosenblatt et al. 1985; Sanacora et al. 1990).

The factors that regulate maternal hypothalamic function during pregnancy are not clearly delineated but are thought to include the gonadal steroids, the pituitary and gastrointestinal hormones, the endorphins and other central neurotransmitters, and various olfactory, tactile and environmental cues. A role for the placental hormones in maternal behaviour and in the regulation of maternal hypothalamic-pituitary hormone secretion is suggested by three lines of evidence: first, the placental polypeptide hormone placental lactogen (PL) is detected in cerebrospinal fluid (CSF) in pregnant women at term and in pregnant rats in late gestation (Assies et al. 1978; Peake et al. 1983; Bridges & Lupini, 1991); secondly, the intraventricular administration of human PL (hPL) or of media containing rat PL-I (rPL-I) increases tyrosine hydroxylase activity in tuberoinfundibular dopaminergic neurones and suppresses circulating prolactin levels in the mid-gestational pregnant rat (Voogt, 1980; Demarest et al. 1983;
Voogt & DeGreef, 1989; Grattan & Averill, 1991; Arbogast et al. 1992); and thirdly; the infusion of hPL into the medial preoptic area induces maternal nesting and grooming behaviour in ovariectomized rats primed with oestrogen and progesterone and treated with bromocryptine to suppress endogenous prolactin secretion (Bridges & Freemark, 1991).

To explore the mechanisms by which placental hormones may exert effects on maternal hypothalamic or central nervous system function during pregnancy, we examined the binding of rPL-I to brain slices from pregnant rats at mid- and late gestation. The binding of rPL-I to maternal brain was compared with that of human GH (hGH), which interacts with somatotrophic as well as lactogenic binding sites (Maes et al. 1983).

MATERIALS AND METHODS

Hormones and materials

Recombinant rPL-I was purified by immunoaffinity chromatography from the conditioned media of Chinese hamster ovary cells transfected with rPL-I cDNA (Robertson et al. 1990, 1991). This preparation of glycosylated rPL-I has a mass of approximately 34 kDa and has potent lactogenic activity in the Nb2 lymphoma cell bioassay (Tanaka et al. 1980). Recombinant hGH was obtained from Genentech Corporation (South San Francisco, CA, U.S.A.). Rat PL-I and hGH were radiolabelled with $^{125}$I to specific activities of 35–80 $\mu$Ci/$\mu$g using chloramine T as a catalyst (Greenwood et al. 1963) and were purified by gel-exclusion chromatography. Highly purified rat prolactin (rPRL) and rat GH (rGH) were provided by the Hormone Distribution Program of the NIADDK. Bacitracin was purchased from Sigma Chemical Corporation (St Louis, MO, U.S.A.), while chromium potassium alumum (used for the preparation of chromalum slides) was obtained from ICN Biochemicals (Costa Mesa, CA, U.S.A.).

Animals and tissue preparation

Timed-pregnant Sprague-Dawley rats (70–85 days of age weighing 225–275 g purchased from Zivic Miller Corp., Allison Park, PA, U.S.A.) and age-matched non-pregnant female rats were housed under a constant schedule of 12 h light : 12 h darkness with free access to rat chow and water. On days 10, 11, 12 or 20 of gestation (presence of sperm in the vaginal canal signified day 0), rats were anaesthetized in mid-morning with carbon dioxide and then killed rapidly by decapitation. Three to four rats were studied at each gestational age. The brains and livers were frozen in crushed solid CO$_2$ and stored at $-70\degree$C prior to use. Coronal brain slices (20 $\mu$m) sectioned with a cryostat at $-20\degree$C were thaw-mounted onto gelatin/chromalum-coated slides. The brain slices were arranged into sets of four to five serial sections each and stored at $-70\degree$C in light-safe boxes containing dessicant capsules. Liver microsomal membranes (100 000 $\times$ g pellet) were prepared using methods described previously (Freemark et al. 1990) and stored at $-70\degree$C. The treatment of rats described in this study was approved by the Committee on Animal Care of the Duke University Medical Center.

Binding assays and autoradiography

The binding of rPL-I to maternal liver microsomes was examined using methods described previously by our laboratory (Freemark et al. 1990). Liver membranes suspended in binding buffer (25 mmol Tris/l, 10 mmol MgCl$_2$/l, 0-1% bovine serum albumin, pH 7.6) were incubated with radiolabelled rPL-I (100–150 000 c.p.m., approximately 0-1 nmol/l) in the presence or absence of varying concentrations of unlabelled rPL-I, rPRL or rGH. Following a 20-h incubation at 4 $\degree$C, the membranes were pelleted by centrifugation (1500 g), washed and counted by gamma scintillation spectrometry.

To examine the binding of rPL-I and hGH to rat brain, coronal brain slices were dried at room temperature using a fan. The slices were preincubated for 30 min in assay buffer (25 mmol Tris/l, 10 mmol CaCl$_2$/l, 0-1% bovine serum albumin, pH 7.4, containing 100 $\mu$g bacitracin/ml). The slices were then incubated in assay buffer containing radiolabelled rPL-I or hGH (0-75–1.0 $\times$ 10$^6$ c.p.m./ml, approximately 0-5–1 nmol/l) in the presence or absence of unlabelled hGH (400 nmol/l), rPRL (80–200 nmol/l), rGH (200–400 nmol/l) or rPL-I (15–30 nmol/l). Limitations of the supply of recombinant rPL-I precluded the use of higher concentrations of the unlabelled hormone in competitive binding studies. Following a 2-h incubation at 23 $\degree$C, the slices were washed twice for 5 min each in fresh assay buffer, dried four times in cold distilled water, and dried at room temperature. The sections were then exposed to photographic film (Beta-max Hyperfilm, Amersham Corp., Rockford, IL, U.S.A.) and exposed for 5–14 days at 4 $\degree$C. Standard $^{125}$I microsomes (d.p.m., Amersham Corp.) were coexposed with the tissue sections. The films were developed for 5 min with Kodak D-19 developer that had been prechilled to 14–15 $\degree$C, washed briefly with 10% acetic acid, and fixed with Kodak Rapid Fix (5 min).

Analysis of data

The binding of rPL-I to maternal liver microsomes was assessed in duplicate and was analysed using the
LIGAND program (Munson & Rodbard, 1980). The binding of rPL-I and hGH to brain slices was analysed using methods similar to those described by Walsh et al. (1990). After incubation with radiolabelled hormones and exposure to photographic film, the tissue sections were stained with cresyl violet (0.1%) and specific brain regions were identified using the atlases of Paxinos & Watson (1982) and Pellegrino & Cushman (1987). The corresponding autoradiographs were then analysed using the RAS 3000 Research Analysis System (Amersham/Loates Associates, Inc., Rockford, IL U.S.A.), a microdensitometry system utilizing digitized computer images. The input device of the computer was used to outline and measure the optical densities (O.D.) of specific brain regions in each autoradiograph including the cerebral cortex, choroid plexus, hippocampus, anterior commissure, optic chiasm, striatum, preoptic area and the medial basal hypothalamus. Certain regions, including the preoptic area and the medial basal hypothalamus, encompassed multiple nuclei. At least six O.D. readings from three different tissue sections were measured for each brain region outlined; the O.D. of the film background was subtracted from that of each brain region. The relative amount of radioactivity corresponding to a given O.D. was determined using $^{125}$I-microscales (Amersham Corp.) coexposed with the tissue sections. Total hormone binding equalled the amount of radioactivity in a region of the brain incubated with radioligand. Non-specific binding equalled the amount of radioactivity in the corresponding region in a comparable tissue slice incubated in the presence of excess unlabelled hormone. Specific hormone binding was calculated as the total binding minus the non-specific binding. Statistical differences between sample means were determined by analysis of variance.

RESULTS

Initial experiments examined the binding of rPL-I to liver membranes from pregnant rats at mid- and late-gestation. As shown in Text-fig. 1, radiolabelled rPL-I bound specifically to maternal liver (12.9–24.5% per 250 µg membrane protein). The binding of $^{125}$I-labelled rPL-I to liver membranes was inhibited in a dose-dependent fashion by unlabelled rPL-I ($K_d$ 0.9–2.4 nmol/l) or rPRL ($K_d$ 6.0 nmol/l). Human GH (2–100 nmol/l), which binds to both lactogenic and somatotrophic receptors (Maes et al. 1983), also inhibited $^{125}$I-labelled rPL-I binding, but rGH (100 nmol/l), which binds to somatotrophic but not to lactogenic receptors, was without effect. In
TABLE 1. Binding of rat placental lactogen-I (rPL-I) and human GH (hGH) to choroid plexus and hypothalamus of pregnant rats (days 11–12 of gestation). Coronal brain slices were incubated with radiolabelled rPL-I (0-5 nmol/l) or hGH (1 nmol/l) in the absence or presence of unlabelled hormones (rPL-I, 20 nmol/l; rat prolactin, 200 nmol/l; hGH, 400 nmol/l; or rat GH, 200 nmol/l).

After quantification of autoradiograph optical densities, the specific binding of radiolabelled hGH to choroid plexus was arbitrarily assigned a value of 1-0. The specific binding of rPL-I and hGH to various brain regions were quantified and assigned values relative to the specific binding of hGH to choroid plexus. Values are means ± s.e.m., n=4.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Radioligand</th>
<th>Relative specific binding</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rPL-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>0.89 ± 0.12</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>ETV</td>
<td>0.03 ± 0.01</td>
<td>&gt;0.1</td>
<td></td>
</tr>
<tr>
<td>VMN</td>
<td>0.01 ± 0.01</td>
<td>&gt;0.1</td>
<td></td>
</tr>
<tr>
<td>POA</td>
<td>0.06 ± 0.03</td>
<td>&gt;0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hGH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>1.00 ± 0.06</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>ETV</td>
<td>0.06 ± 0.01</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>VMN</td>
<td>0.04 ± 0.01</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>POA</td>
<td>0.21 ± 0.05</td>
<td>&lt;0.02</td>
<td></td>
</tr>
</tbody>
</table>

A P value <0.05 indicates that total binding of the radiolabelled hormone was significantly greater than non-specific binding (i.e. specific binding was significantly greater than 0).

CP, choroid plexus; ETV, ependymal lining of the third ventricle in the medial basal hypothalamus; VMN, ventromedial nuclei; POA, preoptic region.

parallel experiments, the same preparation of rat GH inhibited almost completely the binding of radiolabelled hGH to pregnant rabbit liver membranes. Scatchard plots of rPL-I binding were linear (Text-fig. 1), suggesting that rPL-I interacts with a single class of lactogenic sites in maternal rat liver.

Subsequent studies examined the binding of rPL-I to brain slices from pregnant rats. As shown in Pl. 1, 125I-labelled rPL-I bound intensely to ependymal cells of the choroid plexus in the lateral ventricles and in the roof of the third ventricle. The binding of radiolabelled rPL-I was abolished by coincubation of tissue slices with excess unlabelled rPL-I, hGH or rPRL, but not by excess unlabelled rGH (Pl. 1 and Tables 1 and 2). These findings indicate that rPL-I and other lactogenic hormones interact with a common binding site in maternal rat brain.

Specific binding of rPL-I to choroid plexus was detected in non-pregnant as well as pregnant rats. However, the specific binding of 125I-labelled rPL-I to choroid plexus of mid (days 10–12) and late (day 20) gestational pregnant rats exceeded the binding of the hormone to choroid plexus of age-matched non-pregnant rats by 133% and 78% respectively (Table 3). There was no detectable specific binding of radiolabelled rPL-I to cerebral cortex, hippocampus, anterior commissure, optic chiasm, preoptic area or medial basal hypothalamus.

TABLE 2. Per cent displacement of radiolabelled rat placental lactogen-I (rPL-I) and human GH (hGH) by various unlabelled hormones. Coronal brain slices were incubated with radiolabelled rPL-I (0-5 nmol/l) or hGH (1 nmol/l) in the absence or presence of unlabelled hormones [rPL-I, 20 nmol/l; rat prolactin (rPRL), 200 nmol/l; hGH, 400 nmol/l; or rat GH (rGH), 200 nmol/l]. The per cent displacement of each radioligand was calculated by subtracting the amount of specific binding of the radioligand in the presence of each unlabelled hormone from the specific binding of the radioligand in the presence of unlabelled rPL-I or hGH. Values are means ± s.e.m., n=4.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Radioligand</th>
<th>Unlabelled hormone</th>
<th>% Displacement</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rPL-I</td>
<td>rPRL</td>
<td>89 ± 10.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>hGH</td>
<td>rPRL</td>
<td>87.9 ± 9.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>hGH</td>
<td>rPL-I</td>
<td>45 ± 5.2</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td></td>
<td>hGH</td>
<td>rGH</td>
<td>75.5 ± 9.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>rPRL</td>
<td>rPL-I</td>
<td>94.5 ± 9.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>rGH</td>
<td>rPL-I</td>
<td>6.8 ± 4.7</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td></td>
<td>hGH</td>
<td>rPRL</td>
<td>64.5 ± 7.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>hGH</td>
<td>rPL-I</td>
<td>83.4 ± 8.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>hGH</td>
<td>rPL-I</td>
<td>73.2 ± 10.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>rPRL</td>
<td>rPL-I</td>
<td>80.5 ± 9.8</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>rGH</td>
<td>rPL-I</td>
<td>20.8 ± 8.4</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

A P value <0.05 indicates that the competing hormone caused a statistically significant reduction in specific binding of the radioligand. CP, choroid plexus; ETV, ependymal lining of the third ventricle; VMN, ventromedial nuclei; POA, medial preoptic area.
As biological examined DISCUSSION choroid thalamus the choroid area binding labelled third means ± rPL-I choroid densities, presence lactogen Pregnant 125I-labelled The shown plexus, fivefold in ventromedial ventricle in 20) indicated that rPL-I choroid plexus of pregnant rats was arbitrarily assigned a value of I-0. The specific binding of rPL-I to choroid plexus of pregnant rats at mid (days 11-12) and late (day 20) gestation were quantified and assigned values relative to the specific binding of rPL-I to choroid plexus of non-pregnant rats. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>Relative specific binding</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>3</td>
<td>1.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>4</td>
<td>2.3 ± 0.3</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>(days 11-12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>3</td>
<td>1.8 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(day 20)</td>
<td></td>
<td></td>
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</tbody>
</table>

A P value <0.05 indicates that the specific binding of rPL-I to choroid plexus of pregnant rats was significantly greater than the specific binding of rPL-I to choroid plexus of non-pregnant female rats.

The binding of 125I-labelled hGH to pregnant rat brain was compared with that of 125I-labelled rPL-I. As shown in Pl. 2, radiolabelled hGH bound to choroid plexus as well as to ependymal cells lining the third ventricle in the region of the arcuate nucleus; 125I-labelled hGH also bound with low intensity to the ventromedial nuclei in the medial basal hypothalamus (Pl. 2). In each case the binding of 125I-labelled hGH was inhibited by unlabelled hGH or rPL-I (Tables 1 and 2). There was also specific binding of radiolabelled hGH to the medial preoptic area (Pl. 3 and Tables 1 and 2). The binding of radiolabelled hGH to the preoptic area, which was approximately fivefold lower than its binding to choroid plexus, was inhibited by unlabelled rPL-I as well as hGH; in contrast, rGH had little or no effect (Table 2). These findings indicate that rPL-I binds to lactogenic sites in maternal hypothalamus as well as choroid plexus.

DISCUSSION

Rat PL-I has been cloned and expressed only recently (Robertson et al. 1990, 1991), and the binding of the hormone to tissues of the pregnant rat has not been examined previously. Studies in other species, however, have suggested that the placental lactogens exert biological actions in the mother primarily or exclusively through binding to peripheral tissues such as liver, mammary gland and ovary (Soares et al. 1991). The results of the current studies indicate that rPL-I binds specifically to sites in the brain of the pregnant rat, suggesting that the PLs might also exert direct biological actions in the maternal central nervous system.

Our initial studies demonstrated that rPL-I binds with high affinity to liver membranes from pregnant rats at mid- and late gestation. These observations concur with previous studies demonstrating binding of mouse PL-I to liver membranes from pregnant mice (Harigaya et al. 1988). The binding of radiolabelled rPL-I to maternal rat liver was inhibited by unlabelled rPL-I or rPRL not but by rGH, suggesting that rPL-I and rPRL interact with a common hepatic binding site.

Because recent findings suggested a role for the placenta in the regulation of maternal hypothalamic function, we examined the binding of rPL-I to brain slices from pregnant rats. We showed that rPL-I binds specifically to ependymal cells of the maternal choroid plexus. As in the liver, the binding of radiolabelled rPL-I to choroid plexus was inhibited by unlabelled rPRL or hGH but not by rGH, suggesting that rPL-I and rPRL interact with a common choroid plexus binding site. These findings are consistent with previous studies demonstrating specific binding of prolactin to human choroid plexus and to the choroid plexus of non-pregnant rats, rabbits, horses, sheep, pigs and ring doves (Walsh et al. 1978, 1984, 1990; Posner et al. 1983; Muccioli et al. 1988, 1989; Lai et al. 1992). Specific binding of radiolabelled rPL-I to choroid plexus was highest at mid-gestation, when rPL-I concentrations in maternal rat plasma reach a peak and maternal pituitary prolactin secretion is suppressed (Robertson & Friesen, 1981; Robertson et al. 1982, 1990; Voogt et al. 1982). This observation suggests that rPL-I might bind to the choroid plexus of the pregnant rat in vivo. The functional significance of rPL-I binding to choroid plexus in pregnancy is unclear. RPL-I might regulate choroid plexus function in the pregnant rat; alternatively, the lactogenic binding sites in choroid plexus may serve to degrade PL or may transport PL from the systemic circulation into the CSF, as they may transport prolactin (Walsh et al. 1987; Mangurian et al. 1992). The human PL detected in the CSF of pregnant women at term (Assies et al. 1978; Peake et al. 1983) and the rPL-II activity detected in the CSF of pregnant rats in late gestation (Bridges & Lupini, 1991) presumably derive from the systemic circulation because PL is expressed only in the placenta during pregnancy.

In addition to binding to maternal choroid plexus, rPL-I competed with radiolabelled hGH for binding to ependymal cells lining the third ventricle. Conceivably, rPL-I or other lactogenic hormones taken up by ventricular ependymal cells might gain
access to contiguous regions of the hypothalamus such as the arcuate nucleus and thereby regulate tuberoinfundibular hypothalamic function during pregnancy. A role for the PLs in the regulation of tuberoinfundibular function is suggested by three lines of evidence: first, the rise in maternal rPL-I concentrations at mid-gestation coincides with a sustained increase in tuberoinfundibular dopaminergic activity and a consequent fall in pituitary prolactin secretion (Voogt et al. 1982; Tonkowicz & Voogt, 1983; Arbogast & Voogt, 1991); secondly, hysterectomy of pregnant rats on days 11 or 12 of gestation triggers the resumption of pituitary prolactin surges (Voogt, 1980); and thirdly, the intraventricular administration of hPL or of culture media containing rPL-I stimulates tyrosine hydroxylase activity in tuberoinfundibular neurones and suppresses circulating prolactin levels (Voogt, 1980; Demarest et al. 1983; Voogt & DeGreeff, 1989). The effect of rPL-I on prolactin secretion in vivo may be exerted at the level of the hypothalamus because purified rPL-I has no effect on prolactin secretion in isolated rat pituitary cells (Tomogane et al. 1993).

In addition to binding to choroid plexus and to third ventricular ependymal cells, rPL-I competed with radiolabelled hGH for binding to lactogenic sites in the medial preoptic area. This observation concurs with previous findings demonstrating specific binding of lactogenic hormones to the preoptic area in the rabbit (Walsh et al. 1990) and ring dove (Fechner & Buntin, 1989). The amount of specific binding of hGH to the medial preoptic area in the pregnant rat was fivefold lower than its binding to choroid plexus, a finding that may be explained by the low levels of prolactin receptor mRNA in the rat hypothalamus (Chiu et al. 1992). The relative paucity of lactogenic binding sites in the rat hypothalamus may explain the failure to detect specific binding of radiolabelled rPL-I to the ventromedial nuclei or to the medial preoptic area. However, the available data cannot exclude the possibility that the lactogenic binding sites in rat hypothalamus differ subtly in structure or affinity from the lactogenic sites in rat choroid plexus.

The preoptic area plays important roles in reproductive function, sexual behaviour and the induction of maternal behaviour in late gestation (Numan, 1985; Rosenblatt et al. 1985; Merchenthaler et al. 1990). The existence of lactogenic binding sites in the preoptic region of the pregnant rat suggests a mechanism by which the pregnancy lactogens and/or prolactin might play roles in the regulation of reproductive function and maternal behaviour in pregnancy. This hypothesis is supported by recent studies demonstrating that the infusion of prolactin or hPL into the medial preoptic area induces maternal caretaking and nesting behaviour in non-pregnant rats (Bridges et al. 1985, 1990; Bridges & Ronheim, 1990; Bridges & Freemark, 1991). The mechanisms by which placental hormones might gain access to the medial preoptic area during pregnancy are unclear. One possible mechanism might involve neuronal uptake of blood-borne PL at the organum vasculosum lamina terminalis, a preoptic circumventricular organ in which axons terminate in the spaces surrounding fenestrated capillaries lacking a functional blood-brain barrier (Gross & Weindl, 1987; Pardridge, 1991).

The results of these and other studies suggest that placental hormones may bind to and exert direct effects on structures in the maternal central nervous system during pregnancy. Future studies exploring the regulation of PL concentrations in maternal CSF and the biological actions of PLs in maternal choroid plexus and hypothalamus may clarify the roles of the PLs in maternal central nervous system function.

ACKNOWLEDGEMENTS

The authors thank Dr R. Schwartz and her colleagues for permitting us to use the RAS 3000 densitometer. The studies were supported by grants HD 24192 and HD 00901 from the National Institute for Child Development.

REFERENCES


DESCRIPTION OF PLATES

Plate 1.
Characterization of rat placental lactogen-I (rPL-I) binding sites in maternal rat brain. Typical autoradiographs of serial coronal slices of the brain of a pregnant rat (11 days of gestation), incubated with radiolabelled rPL-I (10⁶ c.p.m./ml, approximately 0.7 nmol/l) in the absence (top) or presence of unlabelled rPL-I (20 nmol/l), human GH (hGH; 400 nmol/l), rat prolactin (rPRL; 100 nmol/l) or rat GH (rGH; 400 nmol/l). Similar results were obtained in experiments using slices of brain from pregnant rats at days 10, 12 and 20 of gestation. cp, choroid plexus.

Plate 2.
Binding of hGH to choroid plexus and medial basal hypothalamus. Typical autoradiographs of serial coronal slices (at the level of the diencephalon) of the brain of a pregnant rat (12 days of gestation), incubated with ¹²⁵I-labelled hGH (10⁶ c.p.m./ml, 1 nmol/l) in the absence (top) or presence of unlabelled hGH (400 nmol/l) or rPL-I (30 nmol/l). Similar results were obtained in three separate experiments. e, ependymal cells lining the third ventricle; v, ventromedial nuclei; cp, choroid plexus.

Plate 3.
Lactogenic binding sites in the medial preoptic area. Typical autoradiographs of serial coronal slices (at the level of the anterior commissure) from the brain of a pregnant rat (12 days of gestation), incubated with ¹²⁵I-labelled hGH (10⁶ c.p.m./ml, 1 nmol/l) in the absence (top) or presence of unlabelled hGH (400 nmol/l), rPL-I (20 nmol/l) or rGH (250 nmol/l). Similar results were obtained in four separate experiments. poa, medial preoptic area; oc, optic chiasm; cp, choroid plexus.