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Central prolactin infusions stimulate maternal behavior in steroid-treated, nulliparous female rats

(bromocriptine/estradiol/parenting/progesterone)

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ABSTRACT **A series of experiments were conducted to determine whether and under what conditions central prolactin (PRL) administration would stimulate the onset of maternal behavior in female rats and to identify possible neural sites of PRL action. In each experiment ovariectomized, nulliparous rats whose endogenous PRL levels were suppressed with bromocriptine were tested for maternal behavior toward foster young. In experiments 1, 2, and 4, females were also exposed to pregnancy-like levels of progesterone (days 1–11) followed by estradiol (days 11–17). In experiment 1 infusions (days 11–13) of four doses of ovine PRL (400 ng, 2 μg, 10 μg, or 50 μg, but not 80 ng) into the lateral ventricle resulted in a rapid onset of maternal behavior (behavioral testing, days 12–17). The stimulatory action of these doses of PRL appears to be central, since subcutaneous injections of 50 μg of ovine PRL failed to affect maternal responsiveness (experiment 2). Experiment 3 indicated that the stimulatory effect of intracerebroventricularly administered PRL is steroid dependent. Infusions of either 10 μg of ovine PRL or 10 μg of rat PRL failed to induce maternal behavior in nonsteroid-treated animals. In the final experiment (no. 4) bilateral infusions of 40 ng of ovine PRL into the medial preoptic area of steroid-treated rats resulted in a pronounced stimulation of maternal behavior. These findings demonstrate a central site of PRL action in the stimulation of maternal responsiveness and point to the medial preoptic area as a key neural site for PRL regulation of maternal behavior.**

The hormonal changes accompanying pregnancy and parturition play a critical role in preparing the female to respond maternally toward her newborn young (1–4). One hormone that recently was shown to have a key role in inducing maternal behavior is the pituitary hormone prolactin (PRL) (5, 6). PRL and other members of the PRL family—i.e., growth hormone and placental lactogens—are secreted in large amounts during pregnancy (7–9), making these molecules potential physiological regulators of maternal behavior. Eliminating or suppressing PRL secretion in steroid-treated, ovariectomized, nulliparous rats by either hypophysectomy or administration of bromocriptine, a dopamine agonist, blocks the rapid onset of maternal behavior (5, 6). These effects are prevented when PRL is given to hypophysectomized or bromocriptine-treated female rats.

Although PRL stimulates the onset of maternal behavior, little is known about PRL's site of action. The purpose of the present report was to investigate the possibility that PRL acts at the level of the central nervous system to stimulate maternal behavior. A central site of PRL action is feasible in light of a series of findings that indicate that circulating PRL can gain access to the brain through active transport across the blood–cerebrospinal fluid (CSF) barrier (10), that PRL, presumably of pituitary origin, is found in increased amounts within the CSF at times when circulating levels of PRL are elevated (11–13), and that receptors for PRL have been localized within the central nervous system (14–18).

In a series of four experiments, we first asked whether direct infusions of PRL into the ventricular system would stimulate the onset of maternal behavior in inexperienced female rats. We then asked whether the actions of PRL were central and under what conditions central PRL administration would stimulate maternal behavior. Finally, the behavioral effects of direct administration of PRL to the medial preoptic area (MPOA), an area known to be involved in the regulation of maternal behavior (19), were measured.

MATERIALS AND METHODS

Animals. Nulliparous female Sprague–Dawley rats [Crl: CD(SD)BR] weighing ≈200 g were purchased from Charles River Breeding Laboratories. Experimental females were individually housed in 45 × 25 × 20 cm opaque polypropylene test cages that contained ≈1.5 liters of medium-sized flake wooden shavings. Groups of lactating donor rats were also maintained to provide rat pups for behavioral testing. Animals were housed in light- (on 0500–1900 hr) and temperature-(21–24°C) controlled rooms and provided food (Purina Rat Chow) and water ad libitum.

Animals used in these experiments were maintained in accordance with the standards set forth by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (20).

Cannulations. One week prior to the start of steroid treatment, female rats were fitted either with unilateral guide cannulas (22 gauge; Plastics One, Roanoke, VA) directed toward the right lateral ventricle [DeGroot coordinates: anterior-posterior (AP) = 5.6; lateral (L) = −1.6; horizontal (H) = 3.9] or with 22-gauge bilateral guides directed toward the MPOA (AP = 7.5; L = ±1.0; H = 4.0). Dummy cannulas were cut to extend 1 mm beyond the tip of the guide for the ventricular placements and to be flush with the guide cannulas for the MPOA placements. Infusion cannulas (28 gauge) were designed to extend 1 mm beyond the tip of the guide cannula for the ventricular placements (H = 2.9) and 5 mm beyond the tip of the bilateral guides (H = −1.0). All cannulations were performed under general anesthesia (chloropent or ketamine/xylazine). Females were also ovariectomized at the time of cannulation.

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Abbreviations: CSF, cerebrospinal fluid; i.c.v., intracerebroventricular(ly); MPOA, medial preoptic area; PRL, prolactin; oPRL, ovine PRL; rPRL, rat PRL; P, progesterone; E_2, estradiol; IR, immunoreactive.

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Steroid Hormone Regimen. The steroid regimen consisted of exposing rats sequentially to progesterone (P) and estradiol (E_2; Steraloids, Wilton, NH). Six to 8 days after ovariectomy on treatment day 1, rats were implanted s.c. with three 30-mm P-filled Silastic capsules (Dow Corning tubing no. 602-305; see ref. 6). These P capsules were removed between 0900 and 1000 hr on day 11, at which time each rat was given a single 2-mm E_2 implant. This hormone regimen was used because it results in a rapid stimulation of maternal behavior in nulliparous rats (6) and mimics changes in steroid concentrations found in pregnant rats (21). Rats were anesthesized with Metofane when implanting or removing hormone capsules.

Injections. All animals were injected s.c. twice daily at 0900 and 1700 hr from treatment day 11 through 17 (experiments 1, 2, and 4) or on days 1 to 7 (experiment 3) with bromocriptine (Sandoz Pharmaceutical) at a dose of 2 mg/kg. This dose of bromocriptine suppresses endogenous PRL secretion throughout the period of exogenous PRL treatment and behavioral testing (6).

Infusions. Ovine PRL (oPRL; lots oPRL-18, AFP 8277E and oPRL-19, AFP 9221A) and rat PRL (rPRL; lot rPRL-6, AFP 7545E) were obtained from the National Hormone and Pituitary Program and solubilized in 0.03 M $NaHCO_3$ in 0.15 M NaCl prior to infusions. Specified volumes were infused into the lateral ventricle over 52–58 sec and bilaterally into the MPOA over a 22-sec period with a Sage or Stoelting infusion pump fitted with 10- to 100-μl Hamilton syringes. Infusion cannulas remained in the guide cannulas for 30–40 sec after each infusion. In all intracerebroventricular (i.c.v.) studies, infusions were performed five times [from 1000 to 1100 hr and again between 1600 and 1700 hr one day before behavioral testing, twice on the first day of testing (0900–1000 hr and 1600–1700 hr), and a final time between 0900 and 1000 hr on the second test day].

Behavioral Testing. Animals were tested daily for maternal responsiveness beginning on treatment day 12 (or day 2; experiment 3) following previously described procedures (6). Animals were observed in their home cages for 1 hr—continuously for 15 min and then at 15-min intervals for the remainder of the hour. Behavioral testing lasted for 6 days or until a female displayed full maternal behavior on 2 consecutive test days, whichever first occurred. Females were scored as fully maternal if they retrieved all three test pups to the nest, grouped them in the nest, and crouched over them within the 60-min test session. The latency of the animal to exhibit a maternal response was based upon the test session in which the response was observed. An animal, for example, that responded on test day 1 was assigned a latency of 0 day.

Histology. At the completion of behavioral testing, rats from experiments 1, 3, and 4 were anesthetized with chloropent, the presence of E_2 capsules was validated for steroid-treated animals, and india ink was infused into the lateral ventricle or bilaterally into the MPOA. Rats were then perfused intracardially with physiological saline followed by 10% formalin, and brains were removed and stored in formalin prior to sectioning. The presence of ink in the ventricular system was considered evidence of a positive ventricular placement. MPOA cannula placements were determined independently by three investigators.

PRL Determinations. Plasma concentrations of PRL were measured by radioimmunoassay using the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) rPRL kit. The PRL assay kit includes the rPRL reference preparation (RP-3) and anti-rPRL (S-9). This assay has previously been validated in our laboratory (6).

Statistical Analysis. Hormone data were analyzed by using an analysis of variance. Behavioral data were analyzed with the Kruskal–Wallis test for multiple group comparisons, the Mann–Whitney U test for comparisons between groups, and the Fisher test for exact probability (22). Behavioral latencies are expressed in terms of cumulative percentage of animals responding over days and as medians.

Experiment 1: I.c.v. Infusions of oPRL and the Induction of Maternal Behavior in Steroid-Treated, Nulliparous Rats. The objective of these studies was to determine which doses of oPRL when given i.c.v. might stimulate the onset of maternal behavior. In the initial i.c.v. studies the effects of two doses of oPRL on the induction of maternal behavior were examined. On day 1, one week after cannulations, females were started on the steroid hormone regimen. Between 1000 and 1100 hr on day 11, immediately after bromocriptine injections and changing Silastic implants, rats were infused i.c.v. with either oPRL or vehicle. One set of experimental rats (n = 10) was administered 50 μg of oPRL in 3.8 μl, whereas a second set of females (n = 11) was given 10 μg of oPRL in 0.8 μl. Separate control groups (n = 11 per group) were given 3.8 μl and 0.8 μl of vehicle, respectively.

In the final i.c.v. dose–response study, the effects of three lower doses of oPRL on maternal behavior were measured. Starting 1 week after cannulations, all females were treated with steroid hormones as previously described. After bromocriptine injections and capsule changing on treatment day 11, four groups of rats (n = 8–11) were infused i.c.v. with oPRL at a dose of either 2 μg, 400 ng, or 80 ng or with vehicle (2 μl).

Behavioral testing for all i.c.v.-infused rats began 30–60 min after the central infusions on day 12 and was repeated on days 13–17, starting 30–60 min after i.c.v. infusions (day 13) or bromocriptine injections (days 14–17).

Experiment 2: Systemic oPRL Administration and the Induction of Maternal Behavior in Steroid-Treated, Nulliparous Rats. Experiment 2 investigated the possibility that the behavioral effects of oPRL in experiment 1 were due to leakage of oPRL into the periphery with a subsequent action outside of the brain. Specifically, we asked whether peripheral administration of the highest dose of oPRL (50 μg) given i.c.v. would stimulate the onset of maternal behavior in steroid-treated females. Adult nulliparous rats were ovariectomized and treated with the sequential steroid hormone regimen of P followed by E_2. After bromocriptine administration and Silastic capsule surgery on day 11, rats (n = 12 per group) were injected s.c. with either 50 μg of oPRL in 0.2 ml of vehicle [$Na(CO_3)_2$/NaCl, pH = 7.8–8.0] or vehicle alone. Injections were repeated between 1600 and 1700 hr on day 11, twice again on day 12 (0900–1000 hr and 1600–1700 hr), and a final time on day 13 (0900–1000 hr).

Behavioral testing began 30–60 min after the oPRL/vehicle injections on day 12 and was conducted once daily for 6 days. At the completion of testing on day 17, pups were removed, and trunk blood samples were collected 4–5 hr later between 1600 and 1700 hr. Plasma samples were assayed for rPRL content to validate the suppressive action of bromocriptine on the E_2-induced diurnal PRL surge (6).

Experiment 3: I.c.v. Infusions of oPRL and rPRL and Maternal Behavior in Nonsteroid-Treated Rats. The objective of experiment 3 was to determine whether i.c.v. PRL administration would stimulate maternal behavior in the absence of steroidal treatment. Seventeen days after cannulations and gonadectomies at 0900 hr (treatment day 1), rats were injected with bromocriptine and 1 hr later were infused i.c.v. with 10 μg of rPRL, 10 μg of oPRL, or vehicle (2 μl). Infusions were repeated as previously noted. Behavioral testing began 30–60 min after the central infusions on day 2 and was repeated on days 3–7 starting 30–60 min after i.c.v. infusions (day 3) and bromocriptine injections (days 4–7).

Experiment 4: Bilateral Infusions of oPRL into the MPOA and Maternal Behavior of Steroid-Treated Rats. The aim of this experiment was to determine whether PRL infusions directly into the MPOA, an important neural region in the regulation of maternal behavior, would stimulate the onset of maternal care. One week after bilateral MPOA cannulations,

rats were started on the sequential steroid treatment of P plus E_2. At 0800 hr on day 11 of treatment, all rats were injected with bromocriptine and had their P capsules removed and E_2 capsules inserted s.c. Then, between 1000 and 1100 hr females were infused bilaterally with either oPRL at a dose of 40 ng per MPOA side or vehicle (0.4 μl). Infusions were repeated on day 11, twice on day 12, and a fifth time on day 13 at the times previously noted. This dose of oPRL was selected, since 80-ng infusions of oPRL i.c.v. failed to stimulate maternal behavior in experiment 1. Thus, a stimulation of maternal care after infusions of 80 ng of oPRL into the MPOA could not be interpreted to be the result of leakage into the ventricular system with an action elsewhere within the brain. Behavioral testing began 30–60 min after the 1000 hr infusions on day 12 and was repeated daily for 6 days.

RESULTS

Experiment 1: I.c.v. Infusions of oPRL and the Induction of Maternal Behavior in Steroid-Treated, Nulliparous Rats. In the initial set of studies, infusions of both 50 μg and 10 μg of oPRL stimulated a rapid onset of maternal behavior when compared with controls. As shown in Fig. 1, significantly more rats displayed full maternal behavior on test day 3 and 4 in the 50-μg oPRL group and on test days 3–6 in the 10-μg oPRL group versus their respective control group (P values < 0.05). Median latencies to display maternal behavior for the 50-μg and 10-μg oPRL treatment groups were 2.9 and 1.6 days, respectively, whereas the control values were 5.0 and 5.7 days. These data suggest a central role for PRL in the regulation of maternal behavior in the female rat.

The effects of smaller amounts of oPRL (80 ng–2 μg) on maternal behavior are shown in Fig. 2. Whereas both the 2-μg and 400-ng doses of oPRL stimulated the onset of full maternal behavior when infused into the lateral ventricle, the lowest dose of oPRL (80 ng) failed to stimulate the onset of maternal behavior. Median latencies to display maternal behavior for the oPRL-treated rats were 2.5 and 2.3 days for the 2-μg and 400-ng oPRL groups, respectively, and 5.6 days for both the 80-ng oPRL and vehicle groups. These studies demonstrate that i.c.v. administration of oPRL at doses ranging from 400 ng to 50 μg stimulate a rapid onset of maternal behavior.

Experiment 2: Systemic oPRL Administration and the Induction of Maternal Behavior in Steroid-Treated, Nulliparous Rats. Multiple s.c. injections of 50 μg of oPRL failed to stimulate the onset of maternal behavior in ovariectomized, steroid-treated, nulliparous rats whose endogenous PRL levels were suppressed with bromocriptine. The average

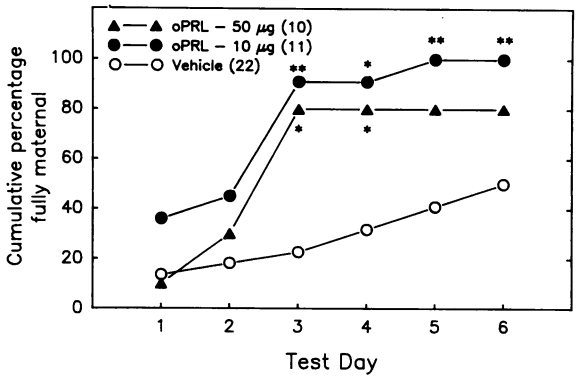

FIG. 1. Maternal behavior after i.c.v. infusions of 50 μg and 10 μg of oPRL into steroid-primed rats. oPRL groups were compared with their separate control groups (n = 11 per group). Since the two control groups did not differ from one another, they are shown here as a single group. *, $P < 0.05$; **, $P < 0.01$ versus controls. The numbers in parentheses here and in Figs. 2–4 are the number of rats per group.

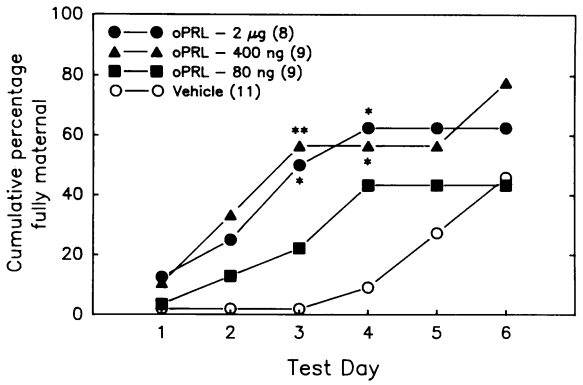

FIG. 2. Maternal behavior after i.c.v. infusions of 80 ng to 2 μg of oPRL into steroid-primed rats. *, $P < 0.05$; **, $P < 0.01$ versus controls.

latency of PRL-treated rats to display full maternal behavior was 3.5 days, whereas that of controls was 3.0 days. Plasma rat PRL levels in these groups were equally suppressed by bromocriptine. PRL levels measured in oPRL- and vehicle-injected rats on treatment day 17 were 5.6 ± 1.4 and 5.1 ± 0.8 ng/ml (mean ± SEM), respectively. That maternal behavior is not stimulated by systemic oPRL injections indicates that the rapid onset of maternal behavior induced by equal and lower i.c.v. doses of oPRL in experiment 1 resulted from PRL's central action.

Experiment 3: I.c.v. Infusions of oPRL and rPRL and Maternal Behavior in Nonsteroid-Treated Rats. In the absence of steroid exposure, i.c.v. administration of either oPRL or rPRL at doses of 10 μg per infusion failed to stimulate the onset of maternal behavior (Fig. 3). Only one oPRL-treated and one rPRL-treated rat became maternal over 6 test days. Hence, the median latencies to display maternal behavior were 5.9–6.0 days. These findings indicate that the actions of centrally administered PRL are dependent upon the presence of either P or E_2 or both.

Experiment 4: Bilateral Infusions of oPRL into the MPOA and Maternal Behavior in Steroid-Treated Rats. Direct infusions of 40 ng of oPRL bilaterally (80 ng total) into the region of the MPOA resulted in a highly significant stimulation of maternal behavior ($P < 0.001$; Fig. 4). The median latencies to respond maternally were 1.3 days for the oPRL group and 5.6 days for vehicle controls. Significantly more rats treated with oPRL responded maternally from test day 2 through 6 (P values < 0.025 to < 0.005), with all PRL-treated females becoming maternal within the 6 test days. Histological examination revealed that the tips of the infusion cannulas were located either within the MPOA or in close proximity to this region (see Fig. 5). Thus, direct infusions of oPRL into the

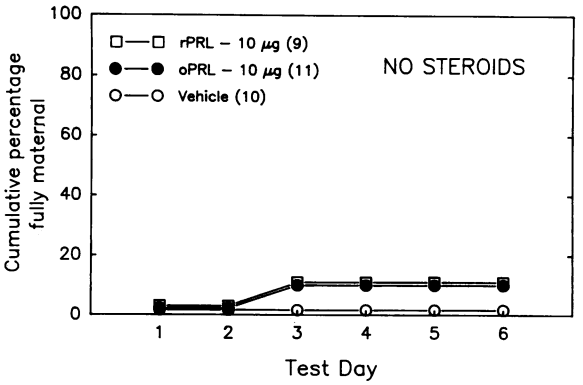

FIG. 3. Maternal behavior in nonsteroid-treated rats after i.c.v. infusions of 10 μg of oPRL or rPRL.

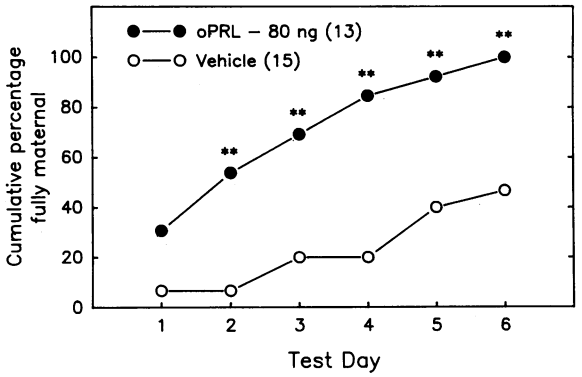

FIG. 4. Maternal behavior after bilateral infusions of 40 ng of oPRL into the MPOA region of steroid-primed rats. **, $P < 0.01$ versus controls.

MPOA, in amounts that fail to affect maternal behavior when infused into the ventricular system, result in a profound stimulation of the onset of maternal behavior.

DISCUSSION

The results of the present studies demonstrate that PRL can act centrally to stimulate the onset of maternal behavior in female rats. I.c.v. infusions of oPRL at doses that have no behavioral effects when given systemically stimulated a rapid onset of maternal care in steroid-treated, nulliparous females. Moreover, infusions of oPRL directly into the region of the MPOA at a dose that does not affect maternal behavior when infused into the ventricles resulted in a pronounced stimulation of maternal care toward foster young. These data suggest that PRL acting at the level of the central nervous system has an important role in stimulating the onset of maternal behavior under physiological conditions.

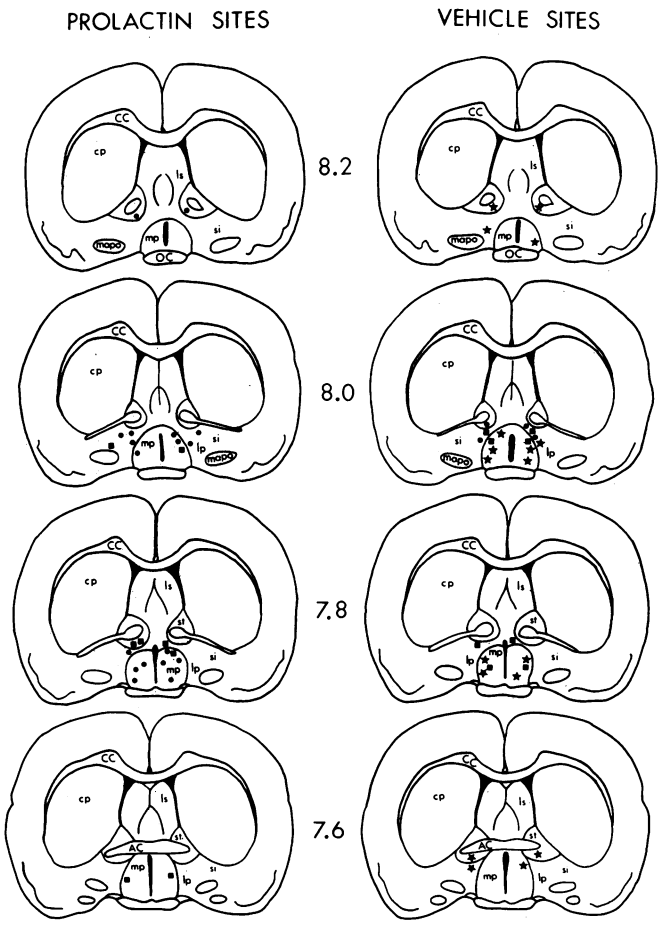

FIG. 5. Histological drawings showing oPRL or vehicle infusion sites in the MPOA region (DeGroot atlas; 8.2–7.6). Symbols (shown bilaterally) depict latencies to display full maternal behavior for individual rats: ●, 0–2 days; ■, 3–5 days; ★, >5 days. AC, anterior commissure; CC, corpus callosum; cp, caudateoputamen; lp, lateral preoptic area; ls, lateral septal area; mapo, magnocellular preoptic nucleus; mp, medial preoptic area; OC, optic chiasm; si, substantia innominata; st, bed nucleus of the stria terminalis.

The MPOA appears to be one site of PRL's behavioral action within the brain. Earlier research has demonstrated that the MPOA has a critical role in the expression of maternal behavior in female rats (19). Electrolytic lesions and deafferentation of the lateral connections of the MPOA, as well as destruction of cell bodies within the MPOA with the neurotoxin *N*-methylaspartic acid, result in severe deficits in maternal care in lactating rats (23–25). Comparative studies indicate that PRL may act in the region of the MPOA to regulate maternal behavior. PRL-like receptors have been identified in this region in birds (17), in the hypothalamus of rabbits and rats (14, 16), and most recently in the MPOA of female rats (18).

Since pituitary PRL appears essential for the rapid onset of maternal behavior in steroid-primed virgin rats, our present results demonstrating a central site of action for PRL suggest that circulating PRL reaches the CSF, the brain, and the MPOA in particular. Evidence supporting this view exists. First, circulating PRL appears capable of entering the brain at the level of the CSF–brain barrier. Walsh *et al.* (10) documented a receptor-mediated transport mechanism for carrying PRL into the CSF from blood. Systemically administered ^{125}I-labeled oPRL and other lactogenic hormones entered the CSF of female rats, a process inhibited by concurrent administration of excess unlabeled oPRL. Similar studies in female macaques also demonstrated that PRL can gain access to the CSF (26). Next, actual measurements of PRL in CSF indicate that under certain physiological conditions elevated circulating levels of PRL are associated with increased concentrations of immunoreactive (IR) PRL in CSF. Specifically, suckling is associated with concurrent increases in blood and CSF PRL levels in the mother rats (13). CSF PRL concentrations are also higher at times of the reproductive cycle when females exhibit elevations in circulating PRL (11, 12). Moreover, in male rats administration of haloperidol, a dopamine receptor antagonist that elevates peripheral PRL concentrations, increases CSF IR PRL concentrations (27). Interestingly, basal levels of IR PRL in the CSF of these male rats prior to haloperidol treatment appear to be of neural origin, independent of the pituitary gland (27, 28). Together, these data indicate that PRL, like other molecules (29), can gain access to CSF by means of transport across the CSF–brain barrier. Once within the CSF, PRL in principle has access to numerous neural regions.

Physiological studies demonstrate that during the course of pregnancy rats are exposed to high circulating titers of PRL as well as other lactogenic hormones—i.e., growth hormone and placental lactogens (8, 9). Interestingly, CSF concentrations of immunoreactive PRL are undetectable just prior to parturition (13). One possible explanation to account for the lack of PRL in the CSF at this time is that the high levels of rat placental lactogen II prepartum (9) compete with PRL for the lactogenic receptors in the choroid plexus and hence for transport into the CSF and brain. If indeed a number of lactogenic hormones are transported into the CSF during pregnancy, then it would suggest that maternal behavior may normally be induced by a set of lactogenic molecules, one of which is PRL.

Whereas the results of this report point to an important action of PRL in the region of the MPOA, as this time we have not established whether this behavioral effect is limited to

this neural region. Further work is needed to examine other neural sites not involved in the regulation of maternal behavior—i.e., the anterior hypothalamus—and to delineate the chemical specificity of PRL's action.

The stimulation of maternal behavior induced by central PRL administration appears to be steroid dependent. In experiment 3, infusions of either 10 μg of oPRL or rPRL in nonsteroid-treated rats failed to stimulate maternal care. This same dose of oPRL when given together with P and E_2 in experiment 1 stimulated maternal behavior. The actions of these steroids could be multiple and their biochemical actions at this point are not clearly understood within the context of maternal behavior. One set of studies suggests that increased concentrations of estrogen (30, 31) and estrogen receptors (32, 33) in the MPOA prepartum may help stimulate the onset of maternal behavior. E_2 and P may stimulate synthesis of PRL receptors, receptors that can then bind endogenous PRL and PRL-like molecules and alter their access to the CSF at the level of the choroid plexus (10) or within the region of the MPOA. It is also possible that PRL is the agent responsible for the increase in estrogen receptors (34), which then may allow estrogen to directly stimulate maternal behavior.

In addition to systemic PRL reaching the brain, it is also noteworthy that IR PRL, including messenger RNA for PRL, is found in neurons in the brain (28, 35–40), including the preoptic area, and that the brain PRL system, which is responsive to estrogen (41), appears to be distinct from the pituitary PRL system. One intriguing possibility is that this brain PRL-like system may be involved in the regulation of maternal behavior at times when peripherally produced lactogens are no longer required for the stimulation of maternal responsiveness. It is well recognized that once maternal behavior becomes established in female rats the behavior is maintained independent of pituitary regulation (42, 43). Perhaps the brain/PRL system regulates maternal care at this time. It is possible that long-term alterations in anatomical and biochemical processes, similar to those that have been identified in the synaptic connectivity in the supraoptic nucleus of female rats (44), occur within the MPOA as a consequence of pregnancy and lactation. Some form of prolonged up-regulation of the brain/PRL system as a consequence of prior parity, for example, might free maternal behavior from peripheral endocrine control and be involved in the retention or remembering of maternal behavior in nonlactating parous animals (45). It would be of interest to know whether in humans and other primates, species in which hormones appear to play a less critical role in inducing parental behavior, similar brain/hormonal systems become functional during development independent of pregnancy and lactation, which might modulate the expression of maternal care.

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