Intracerebroventricular administration of the prolactin (PRL) receptor antagonist, S179D PRL, disrupts parturition in rats

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Abstract

The prolactin (PRL) receptor antagonist S179D PRL delays the onset of maternal behavior in steroid-primed nulliparous female rats. The present study investigated the role of the neural PRL system in the process of parturition. A preliminary study indicated that S179D PRL treatments administered by ALZET minipump to the lateral ventricle severely disrupted parturition. To examine the likely causes of this disruption, a group of timed-pregnant catheterized rats was continuously infused with S-179D PRL (0.001 and 0.1 ng/h) or vehicle control to the lateral ventricles for 3 days (gestation days 21–23), and serial blood samples were taken throughout this period. Effects of the treatments on parturition were recorded, and blood samples were assayed for PRL, progesterone, and oxytocin. Significantly fewer S179D PRL-treated rats successfully delivered by 1500 h on day 23 of gestation when compared with controls. The higher dose of S179D PRL also significantly suppressed the prepartum rise in PRL throughout the prepartum period, while the lower dose only affected plasma PRL during the first 24 h of treatment. No significant effects of the antagonist on plasma progesterone or oxytocin were detected. We conclude that disruption of parturition by S179D PRL is not caused by significant alterations in the plasma concentrations of progesterone or oxytocin. S179D PRL may indirectly act on parturition through the modulation of prepartum PRL. These findings suggest a previously unrecognized role for PRL in the regulation of parturition.

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Introduction

It has been established that successful mammalian parturition depends on the interactions of a number of hormones, including prolactin (PRL), progesterone, and oxytocin. Female mice with a null mutation of either the *PRL* gene (Lucas *et al.* 1998) or *PRL* receptor gene (Ormandy *et al.* 1997) are sterile, illustrating the importance of this hormone in general reproduction. PRL stimulates the production of progesterone by the corpus lutea early in pregnancy (Rothchild 1981), and is also involved in the prepartum decrease in progesterone secretion by initiating regression of the corpus luteum (Malven & Sawyer 1966). More recent reports identify PRL receptors on oxytocinergic neurons in the paraventricular nucleus (Kokay *et al.* 2006), and reveal that PRL stimulates oxytocin release in lactating rats (Parker *et al.* 1991). Based on these studies, alteration in the prepartum pattern in PRL secretion may interfere with

the normal progression of parturition at an ovarian or neural level directly, or through secondary actions on two other hormones, progesterone and oxytocin.

Modulation of plasma progesterone can have potent effects on parturition. Parturition may be dependent on PRL's role in promoting the prepartum decrease in progesterone secretion (Malven & Sawyer 1966), as the administration of progesterone prior to birth results in a 24-h delay of parturition, as well as a prolonged delivery (Antonijevic *et al.* 2000). These effects of progesterone may also be due to primary or secondary actions on oxytocin secretion since progesterone suppresses uterine oxytocin receptor expression (Larcher *et al.* 1995, Murata *et al.* 2000) and interferes with oxytocin binding in the myometrium (Grazzini *et al.* 1998). As a result, oxytocin is less effective at stimulating the uterus in the presence of high progesterone levels.

Oxytocin is another hormone that has been implicated in the control of parturition. During parturition, one-third

of oxytocin stores in the pituitary are depleted (Fuchs & Saito 1971), and if oxytocin function is impaired, parturition is slowed or ceases completely (Antonićević *et al.* 1995a, 2000). In mice and rats, treatment with an oxytocin antagonist slows parturition, while exogenous oxytocin treatment during impaired parturition can rescue normal parturition (Antonićević *et al.* 1995b, Douglas *et al.* 2002). PRL that binds to oxytocin neurons may play a role in this process by regulating central oxytocin activity (Kokay *et al.* 2006). Interestingly, genetically engineered mice with the oxytocin gene (Nishimori *et al.* 1996, Young *et al.* 1996) or receptor (Kimura 2002) disrupted are capable of delivering normally, suggesting that oxytocin is not absolutely necessary, and that compensatory mechanisms to regulate parturition may exist. One such mechanism may involve PRL.

In a preliminary study, we sought to determine whether the onset of parturition might be disrupted in pregnant rats centrally treated with the PRL-receptor antagonist, S179D PRL. This initial study indicated that continuous intracerebroventricular administration of S179D PRL in primiparous rats severely disrupts parturition by prolonging or completely stopping delivery. Since these effects were unexpected, we examined the most likely causes of this disruption in the present study. The present experiments, therefore, examined the effect of continuous intracerebroventricular administration of S179D PRL on parturition, and its possible mediation by endocrine factors. Specifically, we asked whether the actions of S179D PRL might alter the peripartum secretion of PRL, progesterone, and/or oxytocin. We hypothesized that S179D PRL would disrupt parturition, and significantly alter the prepartum profile of PRL, progesterone, and/or oxytocin.

Materials and Methods

Animals

Nulliparous female Sprague–Dawley rats (200–225 g) were purchased from Charles River Laboratories (Kingston, NY, USA). Females were housed in polypropylene cages (45×25×20 cm) in groups of two. Food and water were provided *ad libitum* in light (14 h light:10 h darkness cycle) and temperature (21–25 °C) controlled rooms. All animals used in this study were maintained in accordance with the guidelines of the National Research Council Guide for the Care and Use of Laboratory Animals (1996, National Academy of Science). The research protocol was approved by Tufts University Cummings School of Veterinary Medicine Institutional Animals Care and Use Committee.

Animals were mated by housing individual males with females. Mating was confirmed by the presence of sperm in the vaginal smear the following day (designated day 1

of pregnancy). Animals in our rat colony usually give birth during the light period on days 22 and 23 of pregnancy. Starting at 0800 h on day 22, the females were inspected hourly throughout the light period for signs of parturition. Animals were euthanized if they did not give birth by 1500 h on gestation day 23, if they showed signs of distress (dehydration, decreased activity, and abnormal postures), or if parturition was not completed within 4 h (in compliance with the Tufts Cummings School of Veterinary Medicine IACUC).

S179D PRL treatment

Thirty-six pregnant rats that received intraatrial catheters on day 20 of gestation were surgically implanted with ALZET osmotic minipumps (Durect Corp. Cupertino, CA, USA) between 0700 and 0900 h on gestation day 21. These pumps were connected to unilateral 28 gauge intracerebroventricular cannulae (Plastics One, Roanoke, VA, USA) directed at the right lateral ventricle via a 6 cm polyethylene catheter tube. The stereotaxic coordinates, based upon bregma, for placement of the cannulae were: AP=−0.8, ML=−1.5, DV=+3.5. Surgeries were conducted under isoflurane anesthesia. The osmotic pumps (which were incubated for 24 h prior to implantation to ensure immediate release of its contents upon implantation) contained either a high (0.10 ng/h), or low (0.001 ng/h) dose of S179D PRL (based on the initial pilot study), or saline vehicle. The infusions were continuous and pumps were left in the females until euthanasia at the conclusion of the experiment. The S179D PRL was produced as previously described (Chen *et al.* 1998), and only preparations with endotoxin levels below 0.004 EU/μg were used for *in vivo* work.

Blood sampling

Blood samples (500–600 μl) were collected at 4 h intervals from 0900 to 2100 h on days 21 and 22 of gestation, and on day 23 at 0900 h, 1300 h, and 1500 h from animals which had not given birth. Red blood cells were resuspended in physiological saline and returned to females between sampling.

Assays

Samples from 0900 and 1700 h time points were used for the progesterone and PRL assays, and samples taken at 1300, 2100 h, and day 23 1500 h were used for the oxytocin assay. It was necessary to divide the samples between the assays due to plasma volume requirements.

Plasma concentrations of PRL were measured using the NIDDK rat PRL kit that included RP NIDDK-rPRL-RP-3 and anti-rat PRL S-9 supplied by Dr A F Parlow through the National Hormone Pituitary Program, USA.

Plasma samples were assayed in duplicate. The assay sensitivity average was 0.5 ng/ml; inter- and intraassay coefficients of variation were 7.1 and 10.2% respectively. This assay showed no cross reactivity with S179D PRL.

The plasma progesterone levels were measured by RIA using a kit from Diagnostics Product Corporation (Los Angeles, CA, USA). The assay sensitivity was 0.02 ng/ml, intraassay variation was 7.6%, and all samples were run in a single assay.

Plasma oxytocin was measured by RIA. This assay has been described previously (Amico *et al.* 1981, 1985). Briefly, a starting volume of 300–400 µl plasma was extracted using the acetone–ether method. The following description is based on a starting plasma volume of 400 µl, and if the starting sample was less, all other volumes were adjusted accordingly. Plasma was mixed with three volumes of acetone and the supernatant was saved and washed with two volumes of anhydrous ether. After the ether phase was removed, the remaining extract was air-dried and reconstituted in 0.01 M potassium phosphate with added sodium chloride (8.7 g/l) BSA (1 mg/ml), and sodium azide (1 mg/ml). An antiserum to oxytocin (Pittsburgh antibody 2) specific for oxytocin and generated in rabbits was used. Final dilution of the antiserum in the assay was 1:125 000. The antiserum has <1% cross reactivity with arginine vasopressin or arginine vasotocin. Synthetic oxytocin was used to develop a standard curve. The standard curve was linear from 0.5 to 50 pg/ml, and the minimum detectable concentration was 0.5 pg/ml. Out of the 130 samples, 11 were beyond 50 pg/ml, and were reported as 50 pg/ml. For the assay, duplicate samples of 200 µl were incubated for 24 h at 4 °C with 50 µl diluted antiserum and 50 µl (2500 counts/min) ¹²⁵I-oxytocin (New England Nuclear, Boston, MA, USA), followed by additional incubation for 5 days. Antibody-bound oxytocin was separated, using γ-globulin and 25% polyethylene glycol. The sediments in each tube were counted in a gamma counter. The coefficients of variation were determined by assaying multiple replicates of charcoal-stripped plasma enriched with synthetic oxytocin at relevant concentrations. The intraassay coefficient of variation was 6.5% and the interassay coefficient of variation was 8%.

Statistical analysis

Parturition data were analyzed with χ^2 analyses. PRL, progesterone, and oxytocin data were analyzed using a two-way ANOVA to test for significant differences between treatments, over time, and for interactions between treatment and time. In addition, the PRL data were ln transformed to normalize the variance, and the first three time points were analyzed with a two-way ANOVA for treatment and time. Statistical significance was denoted as $P < 0.05$.

Results

Parturition

By 1500 h on day 23, significantly fewer S179D PRL-treated females had given birth when compared with controls. At that time, zero out of eight of the low S179D PRL dose treated and 1 out of 7 of the high-dose-treated animals completed parturition, when compared with 8 out of 12 of the controls ($P < 0.05$ versus controls, χ^2 , Fig. 1). Pregnancies were confirmed in euthanized animals that failed to give birth. One other high-dose-treated animal started parturition, but only delivered 11 out of 16 pups before being euthanized due to distress. Including the one high-dose animal that successfully completed parturition, only 2 out of the 15 S179D PRL-treated animals initiated parturition. The fact that one-third of the control animals did not complete parturition by 1500 h on day 23 may be an artifact of the experimental protocol. To avoid subjecting the dams to unnecessary distress (in accordance with IACUC requirements), 1500 h on day 23 was chosen as the termination point for parturition, and any dam that had not initiated parturition at this point was killed. None of the pregnant control animals appeared to be in distress at the time of euthanasia.

PRL

The higher dose S179D PRL treatment significantly suppressed plasma PRL concentrations ($P < 0.01$, see Fig. 2). In contrast, PRL concentrations in the low-dose-treated animals were similar to those of the controls over days 21–23 of gestation. In both the controls and the low S179D PRL groups, PRL gradually increased between days 21 and 22. However, when the plasma PRL data are ln transformed to normalize the variances, and only the first three sampling times are analyzed (24 h of treatment), there are significant treatment effects from both doses ($P < 0.01$). No interactions between time and treatment were found.

Figure 1 Percentage of females that did not complete parturition by 1500 on gestation day 23 following treatment with 0.001 or 0.1 ng/h of the prolactin antagonist S179D PRL, or saline control. * $P < 0.05$ when compared with controls.

Figure 2 Plasma concentrations (means $\pm$ s.e.m.) of prolactin on gestation days 21–23 following treatment with 0.001 or 0.1 ng/h of the prolactin antagonist S179D PRL, or saline control. Both doses of the prolactin antagonist S179D PRL significantly decreased plasma prolactin over the first three sampling times, but only the 0.1 ng/h dose significantly decreased prolactin throughout the prepartum period. * $P < 0.01$ as compared with control and 0.001 ng/ml dose.

Progesterone

As shown in Fig. 3, there was a significant effect of time on plasma progesterone ($P < 0.01$), with progesterone progressively decreasing from an average of 46 ng/ml at 0900 h on day 21 to 15 ng/ml at 0900 on day 23. However, there was no effect of S179D PRL treatment on plasma progesterone concentrations, and no interaction between time and treatment.

Oxytocin

There was a significant effect of time on plasma oxytocin, with a large increase at 1500 h on day 23 ($P < 0.01$, Fig. 4). Treatment with S179D PRL failed to affect oxytocin concentrations, and there were no interactions between time and treatment.

Discussion

Both low and high doses of S179D PRL had a pronounced effect on parturition, with significantly

Figure 3 Plasma concentrations (means $\pm$ s.e.m.) of progesterone on gestation days 21–23 following treatment with 0.001 or 0.1 ng/h of the prolactin antagonist S179D PRL, or saline control. #Represents a significant effect of time ($P < 0.01$).

Figure 4 Plasma concentrations (means $\pm$ s.e.m.) of oxytocin on gestation days 21–23 following treatment with 0.001 or 0.1 ng/h of the prolactin antagonist S179D PRL, or saline control. #Represents a significant effect of time ($P < 0.01$).

fewer females delivering by 1500 h on day 23 of gestation. While both doses suppressed plasma PRL concentrations over the first 24 h of treatment, only the high dose significantly altered PRL throughout the prepartum period. There were no significant treatment effects on either progesterone or oxytocin. It appears, therefore, that centrally infused S179D PRL may indirectly act on parturition through the modulation of prepartum PRL.

Although S179D PRL treatment significantly lowered PRL following both doses over the first 24 h of treatment, it is unclear whether the effects of the antagonist on plasma PRL are the proximate cause of the parturition impairment. While S179D PRL treatment only affected plasma PRL throughout the prepartum period at the high dose, both the low and high doses were very effective at disrupting normal parturition. If the antagonist's action on plasma PRL was the causative factor, it would be expected to affect plasma concentrations to a similar degree at both doses. However, since the low dose did have a transient effect on plasma PRL over the first 24 h of treatment, it is possible that S179D PRL is exerting effects on PRL which activate an undetermined primary mechanism for the disruption of parturition.

Similar effects of S179D PRL on plasma PRL have been reported previously. S179D PRL reduces endogenous PRL release when administered peripherally to male animals (Johnson *et al.* 2003). Female animals are somewhat more resistant to this effect (Johnson *et al.* 2003), but central rather than peripheral administration may well have resulted in greater sensitivity in the current experimental animals. Although for the sake of simplicity S179D PRL has been referred to throughout as a PRL receptor antagonist, it achieves this activity both by blockade of signaling from PRL (Schroeder *et al.* 2003) and by the generation of an alternate signal (Wu *et al.* 2003). S179D PRL is a molecular mimic of phosphorylated PRL (Chen *et al.* 1998) which both directly and indirectly inhibits the release of PRL from the pituitary (Walker 2006). It has recently been suggested that it be more aptly referred to as

a selective PRL receptor modulator (Walker 2006). We propose that the effects of S179D PRL on plasma PRL in the current study are due to an ultra short loop feedback mechanism at the pituitary that has previously been established *in vitro* (Ho *et al.* 1989), or via hypothalamic feedback on arcuate dopaminergic neurons (Kokay & Grattan 2005).

All the dams exhibited the typical preterm decrease in plasma progesterone, and this decline was unaffected by central S179D PRL. Although these data do not explain the effects on parturition, they support the hypothesis that the disruptive actions of this antagonist on parturition and PRL were not mediated by its effects on ovarian progesterone secretion. Whereas the typical prepartum surge in PRL can be inhibited by progesterone (Grattan & Averill 1990), the present data indicate no significant effects of the antagonist on progesterone during the time when the antagonist is depressing PRL.

Due to observations during the initial pilot studies on S179D PRL, we hypothesized that oxytocin may be involved in the mechanism of the antagonist's effects on parturition. While some animals would not begin parturition at all, other dams would fail to complete parturition after delivering a few pups. Evidence supporting the role of oxytocin in the early stages of parturition (Russell *et al.* 2003), the presence of PRL receptors on PVN oxytocinergic neurons (Kokay *et al.* 2006), and the stimulation of oxytocin by PRL (Parker *et al.* 1991), led to the hypothesis that the antagonist was blocking the amplification of oxytocin release by PRL, thereby interfering with the stimulation of uterine muscle contraction. However, no significant changes in the concentrations of oxytocin were observed. Nevertheless, the possibility of some kind of relationship or effect between central PRL or S179D PRL and oxytocin cannot be excluded.

It is interesting to note the trend of higher plasma oxytocin in the antagonist-treated animals on day 23 at 1500 h. We postulate that the treated animals may be compensating for the adverse effects of the antagonist on parturition by increasing oxytocin output. Another possibility is that vaginal distention is triggering oxytocin release in these animals. Because the experiment had to be terminated at this time due to IACUC recommendations, it is unknown whether this would have been sufficient to result in normal parturition, although the preliminary investigation of this phenomenon would suggest otherwise. While significant differences in plasma oxytocin were not recorded, it is possible that S179D PRL was exerting significant effects on peripheral oxytocin receptors. However, when S179D PRL was peripherally delivered throughout pregnancy at a rate that resulted in 50 ng/ml in the circulation, there was no statistically significant effect on the timing of parturition (Yang *et al.* 2001). It seems unlikely, therefore, that the much lower peripheral concentrations that might result from intraventricular delivery of 0.1 ng/h would have a greater effect.

In conclusion, this study documented the pronounced disruption of parturition by the central administration of S179D PRL. The results exclude modulation of progesterone or total oxytocin secretion, and suggest that the transient effects on PRL secretion may mediate the effects of S179D PRL on parturition.

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