Reproductive experience and opioid regulation of luteinizing hormone release in female rats

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The objective of the present study was to determine whether reproductive experience that produces shifts in opioid regulation of prolactin secretion and behavioural functions also alters opioid regulation of LH during the oestrous cycle or lactation. In Expt 1 the effect of naloxone administration (i.v.) on LH was compared between age-matched, nulliparous and primiparous, catheterized female rats on dioestrus II. In Expt 2, the effects of multiple reproductive experiences on opiate control of LH were investigated using cyclic, nulliparous and multiparous (three litters) rats. In both experiments, no differences in naloxone-stimulated LH release were found between groups even though multiple reproductive experiences resulted in the prolongation of oestrous cyclicity. In Expt 3, day 8 lactating primiparous rats were administered 2, 5, 10 or 25 mg naloxone kg⁻¹ i.v. The three lowest naloxone doses, but not the 25 mg kg⁻¹ dose, significantly increased LH concentrations. The possible effects of prior reproductive experience on opioid control of LH during lactation were then investigated. Naloxone at 0.5 mg kg⁻¹, but not at 2 mg kg⁻¹ or 10 mg kg⁻¹, stimulated a significantly greater rise in LH in multiparous (two litters) than in primiparous females. Overall, these data indicate that while modest differences were found in naloxone-induced LH responses between multiparous and primiparous animals during lactation, reproductive experience did not significantly alter opioid regulation of LH during subsequent oestrous cycles at the naloxone doses examined. Hence, the effects of reproductive experience on opioid regulation of LH are less pronounced than those previously found for opioid regulation of prolactin and behaviour.

Introduction

The endogenous opioid peptides (EOP) appear to influence reproductive function by tonic inhibition of hypothalamic GnRH secretion at all stages of the oestrous cycle (Gabriel *et al.*, 1983). When naloxone, a non-specific opioid receptor antagonist, is administered to rats, LH is no longer inhibited by the EOP resulting in an increase in GnRH release from the hypothalamus and a subsequent pituitary release of LH. However, marked age and sex differences in the LH response of rats to naloxone have been reported, suggesting a more complex influence of the EOP on GnRH secretion (Blank *et al.*, 1979; Cicero *et al.*, 1980; Chiba *et al.*, 1997).

In contrast to the inhibitory effects of opioids on LH, EOP stimulate the release of prolactin (Selmanoff and Gregerson, 1986). Opioids, such as β -endorphin, are thought to increase prolactin concentrations during lactation and possibly during the prepartum period (Dondi *et al.*, 1991). Recent studies have shown that reproductive experience alters the female's sensitivity to opioid regulation of prolactin secretion as well as maternal behaviour (Kinsley and

Bridges, 1988). When the effects of reproductive experience on maternal behaviour in rats was evaluated after central infusion of β -endorphin into the medial preoptic area (MPOA) (Mann and Bridges, 1992), an area of the brain that is responsible for the induction and maintenance of maternal behaviour (Numan, 1990), it was found that multiparous lactating rats were less sensitive than age-matched, primiparous lactating rats to the disruptive effects of β -endorphin administration on maternal behaviour. In addition, multiparous lactating rats had significantly lower concentrations of prolactin after suckling than did primiparous, lactating rats (Mann and Bridges, 1992). Thus, in general, multiparous rats appeared to be less affected by opioid administration than were age-matched, primiparous rats.

In the present study, the role of opioids in LH regulation was investigated using a number of experimental approaches. Unpublished findings from our laboratory indicate that the proestrous LH surge is dampened in cyclic primiparous rats compared with the surge in age-matched, nulliparous animals (R. S. Bridges and L. C. Vecellio, unpublished data). As opioids appear to inhibit GnRH, and thus LH, secretion in cyclic animals (Blank *et al.*, 1986; Weiner *et al.*, 1988), the aim was to delineate the possible

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involvement of opiates in LH release in reproductively experienced animals. In the first experiment the effects of the opiate antagonist naloxone on LH release was examined in cyclic, dioestrous II, nulliparous and primiparous female rats. On the basis of findings of earlier studies (Kinsley and Bridges, 1988; Mann and Bridges, 1992), we hypothesized that primiparous female rats would be less sensitive to the effects of naloxone than would nulliparous females. Thus, the rise in LH would be greater in nulliparous females. The possibility was also investigated that additional reproductive experience (more than two births) might alter the sensitivity of the hypothalamus to opioids in the regulation of LH by comparing the pattern of LH release on dioestrus II in age-matched cyclic, nulliparous and multiparous females that had raised three litters.

A final study examined the possible effects of reproductive experience on opioid regulation of LH secretion during lactation. Suckling both increases the concentrations of EOP (Selmanoff and Gregerson, 1986) and inhibits LH release (Smith and Neill, 1977). Therefore, a naloxone-LH dose-response curve was established in primiparous, lactating rats using four naloxone doses administered i.v. Afterwards the effects of naloxone administration during lactation in age-matched, primiparous and multiparous females were investigated to determine whether LH release was diminished in multiparous, lactating animals, reflecting a decreased sensitivity to opioid peptides.

Materials and Methods

Animals

Nulliparous female Sprague-Dawley [CrI:CD(SD)BR] virus-free rats (226–250 g) were purchased from Charles River Laboratories, Inc. (Kingston, NY) and housed upon their arrival in our animal facilities in 45 cm × 25 cm × 20 cm opaque polypropylene cages. Animals were maintained in temperature (21–25°C)- and light (14 h light:10 h dark cycle; lights on at 06:00 h)-controlled rooms and provided with food (Purina Rat Chow, Ralston-Purina Co., St Louis, MO) and water *ad libitum*. Female rats were initially housed two per cage for a minimum of one week at which time they were separated into experimental groups. Animals used in this study were maintained in accordance with the guidelines of the Division of Laboratory Animal Medicine at Tufts University School of Veterinary Medicine, which follow the guidelines for animal care prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council.

Experiment 1

Experiment 1 consisted of two parts. In the first part, seventy-five- to ninety-day-old nulliparous female rats were single-housed, and vaginal lavages were performed daily until these females demonstrated two consecutive 4 or 5 day oestrous cycles. On the day of vaginal oestrus, intra-atrial catheters were inserted via the right jugular vein under

Metofane (Mallinckrodt, Phillipsburg, NJ) anaesthesia. The following day catheters were flushed with heparinized saline (50 iu ml⁻¹). Blood samples (0.5 ml) were taken using heparinized syringes on the day of dioestrus II starting between 09:00 and 11:00 h. Samples were taken at -10, 0, 10, 20, 30, 45, 60 and 120 min. At the zero time point rats were injected via the intra-atrial catheters with one of the three treatments: vehicle (saline; *n* = 7), naloxone (1 mg kg⁻¹; *n* = 7) or naloxone (10 mg kg⁻¹; *n* = 7). Blood samples were centrifuged at 16000 g for 1 min and plasma samples were collected and stored at -20°C until assayed for LH by radioimmunoassay. Throughout each experiment when multiple samples (more than four) were collected, red blood cells from individual samples were resuspended in approximately 0.5 ml of physiological saline. The cell suspensions were returned to each subject after the subsequent blood sampling to maintain blood volume and haematocrit count. Cells from the final blood sample during each collection period were not returned to the experimental animals.

Rats in the second part of this experiment were separated into two groups. One group of nulliparous females was double-housed and remained unmated, while the other group was mated with males from our colony and housed individually before parturition. On the day after parturition the litters were culled to six pups and were raised to weaning on day 21 postpartum. After weaning the young, vaginal lavages were performed daily on primiparous rats, which were paired with the age-matched nulliparous females, until they demonstrated two consecutive 4 or 5 day oestrous cycles. As described previously, intra-atrial catheters were inserted on the day of vaginal oestrus and the following day catheters were rinsed with heparinized saline. Blood samples (0.5 ml) were collected on the day of dioestrus II starting between 09:00 and 11:00 h. Samples were taken at -10, 10, 20, 30 and 60 min. At the zero time point, rats were injected via the intra-atrial catheter with either vehicle (saline; primiparous *n* = 6, nulliparous *n* = 5) or naloxone (1 mg kg⁻¹; primiparous *n* = 12, nulliparous *n* = 9). Blood samples were processed as previously noted.

Experiment 2

Rats in Expt 2 were separated into two age-matched groups. One group remained nulliparous, while rats in the other group (multiparous, three litters) were mated to males from our colony at three separate times. Litters were culled to six pups the day after each parturition and raised to weaning on day 21 postpartum. After weaning the third litter at approximately eight months of age, vaginal lavages were taken daily from multiparous females together with the age-matched, nulliparous females until they demonstrated two consecutive 4 or 5 day oestrous cycles. On the day of vaginal oestrus, intra-atrial catheters were inserted as previously described. The following day, catheters were flushed with heparinized saline (50 iu ml⁻¹). Blood samples (0.6–0.7 ml) were collected on the day of dioestrus II starting between 09:00 and 10:30 h at the following time points: -10, 10, 20, 30, and 60 min. At the zero time point, rats were injected i.v. with naloxone (1 mg kg⁻¹; multiparous *n* = 17, nulliparous *n* = 6).

Catheters were kept patent on non-sampling days by flushing with heparinized saline. Blood samples were again collected on the day of dioestrus II in the subsequent oestrous cycle starting between 09:00 and 10:30 h. The sampling time points were the same as in the prior cycle; however, the rats were injected i.v. at the zero time point with 0.5 mg naloxone kg⁻¹ (multiparous $n = 16$, nulliparous $n = 4$). The dosage of naloxone was reduced in the subsequent oestrous cycle to address the possibility that the 1 mg kg⁻¹ dose may have produced a maximum response in both the nulliparous and multiparous rats. Blood samples were processed and assayed for LH as previously described.

Experiment 3

Rats in part A of Expt 3 were mated to males from our colony and randomly assigned to one of four groups. The day after parturition the litters were culled to six pups. On day 6 of lactation intra-atrial catheters were inserted via the right jugular vein into these primiparous rats. On day 7 catheters were flushed with heparinized saline (50 iu ml⁻¹).

Between 08:00 and 09:00 h on day 8 of lactation, pups were removed and sampling of these primiparous rats began between 12:00 and 13:00 h, 4 h after pup removal. Blood samples were taken at -10, 0, 10, 20, 30, and 60 min. At the zero time point, separate groups of rats were injected i.v. with either 2 ($n = 8$), 5 ($n = 5$), 10 ($n = 8$), or 25 ($n = 5$) mg naloxone kg⁻¹. Blood samples were processed and assayed for LH determinations.

Two groups of rats were used in part B of Expt 3. One group initially was unmated, while the other was mated to males from our colony. The day after parturition litters were culled to six pups and subsequently raised to day 21 postpartum. Seven to ten days after weaning, these primiparous rats along with the initially unmated group were mated to males in our colony to produce multiparous and primiparous females, respectively. Again, on the day after parturition, litters were culled to six pups. As in the first part of this experiment, females received intra-atrial catheters on day 6 of lactation. On day 7, catheters were flushed with heparinized saline. Between 08:00 and 09:00 h on day 8 of lactation, pups were removed and sampling began between 12:00 and 13:00 h, 4 h after pup removal. Blood samples were taken at 0, 10, 20 and 30 min based on results of the first part of the experiment. At the zero time point, rats were injected i.v. with 2 mg kg⁻¹ (multiparous $n = 19$, primiparous $n = 11$) naloxone. On day 9, catheters were again flushed with heparinized saline. Between 08:00 and 09:00 h on day 10 of lactation, pups were removed and sampling began between 12:00 and 13:00 h. Blood samples were taken at the same time points, but rats were separated into two groups and administered either 0.5 (multiparous $n = 7$, primiparous $n = 5$) or 10 (multiparous $n = 10$, primiparous $n = 6$) mg naloxone kg⁻¹ i.v. at the zero time point. The dosage of naloxone was both decreased and increased to establish whether the initial 2 mg kg⁻¹ i.v. dose was producing a maximal response in both the multiparous and primiparous rats. Blood samples were processed as previously described and plasma assayed for LH content.

Radioimmunoassay

LH was measured by radioimmunoassay using the National Hormone Pituitary Program LH kit. This assay has previously been validated in our laboratory (Rubin *et al.*, 1985). The LH antibody was NIDDK-anti-rLH-S-11. The reference preparation was NIDDK-rLH-RP-3. Iodinated (I125) LH was obtained from Hazelton Washington Inc. (Vienna, VA). The assay sensitivity ranged from 10 to 20 pg per tube and the interassay and intra-assay coefficients of variation were 8.9% and 4.9%, respectively.

Statistical analyses

Statistical analyses of the LH data were performed using an analysis of variance (ANOVA). Multiple comparisons were performed using the least significant difference test. Nonparametric data were analysed using the Fisher's exact probability test. Since in Expt 3 baseline LH concentrations were significantly different between the primiparous and multiparous groups, difference scores were calculated. This computation included subtraction of baseline LH values at each time point before performing an ANOVA.

Results

Experiment 1

The aim of the first part of this experiment was to establish a dose of the opiate antagonist naloxone that would effectively stimulate LH release in nulliparous rats on dioestrus II. There was a significant main effect of group ($F_{2,126} = 5.67$, $P < 0.05$) and time ($F_{7,126} = 2.61$, $P < 0.01$). In addition, there was a significant interaction between group and time ($F_{14,126} = 2.47$, $P < 0.01$). Naloxone treatment at 1 mg kg⁻¹ i.v. resulted in high LH values which differed significantly from vehicle at 10, 20, 45 and 60 min. Plasma LH concentrations increased from about 0.4 ng ml⁻¹ to 0.8 ng ml⁻¹ at 10 min. LH concentrations after the 10 mg kg⁻¹ dose of naloxone differed significantly at 10, 45 and 60 min, rising from 0.5 ng ml⁻¹ to 0.8 ng ml⁻¹ at 10 and 20 min. LH values in vehicle-treated animals decreased between 30 and 45 min ($P < 0.05$) and returned to baseline concentrations thereafter. The group differences present at the 45 min and possibly the 60 min collection points may have been the result of low concentrations of LH in the vehicle-treated controls. LH concentrations in naloxone-treated animals at these times remained fairly constant or tended to decline slightly.

The goal of the second part of Expt 1 was to compare the LH responses to naloxone on dioestrus II between age-matched, nulliparous and primiparous females (Fig. 1). With the 1 mg kg⁻¹ dose, significant main effects of drug ($F_{1,28} = 23.83$, $P < 0.001$) and time ($F_{4,112} = 10.73$, $P < 0.001$) were found. Neither the two-way interaction between group and drug ($F_{1,28} = 0.37$, $P > 0.05$) nor group and time ($F_{4,112} = 0.68$, $P > 0.05$) were significant. However, the two-way interaction between time and drug was significant ($F_{4,112} = 8.08$, $P < 0.001$). There were no significant differences between

Fig. 1. Comparison of plasma concentrations of LH after administration of naloxone (1 mg kg^{-1} i.v.) or vehicle (saline) to nulliparous and primiparous female rats on dioestrus II. (●) primiparous/naloxone ($n = 12$); (○) nulliparous/naloxone ($n = 9$); (▲) primiparous/vehicle ($n = 6$); (△) nulliparous/vehicle ($n = 5$). There were no significant differences between the nulliparous and primiparous females. Naloxone treatment resulted in significant increases in LH in the nulliparous and primiparous animals within 10 min of naloxone administration. * $P < 0.05$ compared with baseline values.

nulliparous and primiparous groups after either vehicle or naloxone treatments (Fig. 1), although the groups administered naloxone had significantly higher LH concentrations than did vehicle-treated animals after naloxone administration ($P < 0.05$ – 0.001).

Experiment 2

The aim of this experiment was to compare circulating LH concentrations collected on dioestrus II between age-matched nulliparous and multiparous (three litters) female rats after naloxone (1 mg kg^{-1}) treatment. As shown in Fig. 2a, there was a significant main effect of time ($F_{4,84} = 17.29$, $P < 0.0001$). LH concentrations were significantly higher at the 10 min time point compared with the baseline ($P < 0.05$) and decreased throughout the remaining time points. There were no significant differences between nulliparous and multiparous rats at any time point ($F_{4,84} = 0.18$, $P > 0.05$).

When 0.5 mg naloxone kg^{-1} i.v. was administered on dioestrus II of the subsequent oestrous cycle, LH concentrations in the nulliparous and multiparous female rats once again differed significantly over time ($F_{4,72} = 19.53$, $P < 0.0001$), but the groups did not differ significantly from each other ($F_{4,72} = 1.38$, $P > 0.05$; Fig. 2b). As with the 1 mg kg^{-1} i.v. dose, the administration of 0.5 mg naloxone kg^{-1} i.v. resulted in a maximal response at 10 min ($P < 0.05$) followed by a gradual decline over the time period.

The advancing age of these rats combined with the lack of reproductive experience in the nulliparous group had a significant effect on oestrous cyclicity. More nulliparous females (9 of 15) displayed irregular oestrous cycles or were acyclic (Fisher's $P < 0.0001$) at 8 months of age than were the

Fig. 2. Comparison of plasma concentrations of LH in nulliparous (○) and multiparous (●) rats on dioestrus II after administration of naloxone at 1 mg kg^{-1} i.v. (a) ($n = 6$ and 17 , respectively), and on the subsequent oestrous cycle at a dose of 0.5 mg kg^{-1} i.v. (b) ($n = 4$ and 16 , respectively). No significant differences were found between the nulliparous and multiparous females, although naloxone treatments resulted in significant increases in LH versus baseline samples (* $P < 0.05$).

multiparous females (0 of 23). This difference exemplifies the effect parity exerts on maintaining reproductive cyclicity.

Experiment 3

In the first part of this experiment plasma LH concentrations were measured in primiparous females after i.v. administration of four different doses of naloxone (Fig. 3) on day 8 of lactation. There was a significant main effect of time ($F_{5,110} = 7.95$, $P < 0.0001$); LH concentrations were significantly higher at the 10 min time point compared with the baseline after the 2, 5 and 10 mg kg^{-1} i.v. doses of naloxone ($P < 0.05$). The 25 mg kg^{-1} i.v. dose did not significantly increase LH ($P > 0.05$). LH concentrations were significantly lower at 10 min after treatment with 25 mg naloxone kg^{-1} . The LH response to this naloxone dose was significantly lower than that of the 2 and 5 mg kg^{-1} doses.

In the second part of Expt 3, an examination of plasma LH concentrations collected on day 8 of lactation after 2.0 mg naloxone kg^{-1} revealed that plasma LH concentrations differed over time in both primiparous and multiparous rats

Fig. 3. Comparison of plasma concentrations of LH after administration of 2 (○), 5 (●), 10 (▽), and 25 (▼) mg naloxone kg⁻¹, i.v. in primiparous lactating rats. Plasma concentrations of LH during lactation increased significantly compared with baseline at the 10 min time point for the 2, 5, and 10 mg kg⁻¹ doses only. **P* < 0.05 at the 10 min time point for 25 mg kg⁻¹ compared with 2, 5 and 10 mg kg⁻¹ doses, and at the 20 min time point compared with the 2 and 5 mg kg⁻¹ doses.

($F_{3,84} = 27.17$, $P < 0.0001$). Basal LH concentrations averaged about 0.17 ± 0.03 ng ml⁻¹ and increased to about 0.35 ± 0.05 ng ml⁻¹ at the 10 min time point in both groups. The responses of these groups did not differ significantly from each other ($F_{3,84} = 0.11$, $P > 0.05$). The stimulatory effect of naloxone on LH secretion was maximal at 10 min and then diminished over time in both groups at approximately the same rate ($P < 0.05$).

On day 10 of lactation females received either 10 mg kg⁻¹ or 0.5 mg kg⁻¹ naloxone i.v. The 10 mg naloxone kg⁻¹ resulted in increases in LH concentrations for both primiparous and multiparous rats which differed over time ($F_{3,42} = 16.1$, $P < 0.0001$). Baseline LH concentrations in primiparous rats averaged about 0.18 ± 0.05 ng ml⁻¹ and increased to about 0.34 ± 0.08 ng ml⁻¹ at the 10 min time point, while basal LH concentrations in the multiparous rats averaged 0.17 ± 0.03 ng ml⁻¹ and increased to 0.27 ± 0.04 ng ml⁻¹ at 10 min. The LH responses of these rats did not differ from each other ($F_{3,42} = 0.85$, $P > 0.05$). The overall trend, therefore, was a maximal LH response to naloxone administration at 10 min ($P < 0.05$) followed by an equivalent and gradual decline over the duration of sampling. In contrast, 0.5 mg naloxone kg⁻¹ i.v. (Fig. 4) resulted in LH concentrations that differed over time ($F_{3,30} = 9.54$, $P < 0.001$) and were significantly different between the primiparous and multiparous female rats at all time points, including the baseline time point ($F_{1,30} = 17.1$, $P < 0.01$). Difference scores were calculated (see statistical methods) and compared to determine whether the difference at all other time points was due to the significant difference in baseline. The statistical difference found between the primiparous and multiparous females after naloxone treatment was not due to differences in baseline LH concentrations. At the 10 min sampling time, LH concentrations in multiparous females had increased

Fig. 4. Comparison of plasma concentrations of LH after administration of naloxone (0.5 mg kg⁻¹ i.v.) to primiparous (○; $n = 5$) and multiparous (●; $n = 7$) females on day 10 of lactation. Multiparous females had significantly higher plasma concentrations of LH after administration of naloxone (0.5 mg kg⁻¹ i.v.) than did primiparous females. **P* < 0.05 compared with primiparous rats.

0.175 ng ml⁻¹ from baseline concentrations, while LH concentrations only increased by 0.075 ng ml⁻¹ in primiparous mothers ($P < 0.05$). Comparable differences persisted at 20 and 30 min ($P < 0.05$). Thus, the overall trend was increases in LH in response to naloxone, increases which were significantly larger in multiparous females and which declined gradually over time.

Discussion

The overall hypothesis in this study was that with increases in reproductive experience, that is, pregnancy and lactation, rats would become less sensitive to the effects of EOP and thus less sensitive to the effects of the nonspecific opioid antagonist, naloxone. In the first part of Expt 1 the effects of two naloxone doses (1 mg kg⁻¹ and 10 mg kg⁻¹ i.v.) on LH secretion were measured in nulliparous rats. Naloxone at 1 mg kg⁻¹ i.v. which stimulated an increase in LH release within 10 min was then used to compare the LH response of age-matched, cyclic nulliparous and primiparous female rats. No significant difference in the LH response to naloxone was found between these groups, indicating that opioid regulation of LH may not be altered by prior reproductive experience. One explanation for these findings is that this dose of naloxone may have been too high to detect differences in opioid sensitivity between these groups, for example it may have inhibited the action of the EOP equally in nulliparous and primiparous cyclic female rats. Future studies will be necessary to examine adequately the effect lower doses of naloxone might have in detecting whether there are differences in opioid sensitivity in rats as a function of different numbers of births. Alternatively, the difference of a single parity between nulliparous and primiparous females

may not be great enough to influence the EOP system sufficiently to produce a significant difference in LH release, in contrast to the differential response seen with regard to prolactin release and maternal behaviour (Mann and Bridges, 1992). It is also possible that changes in opioid sensitivity produced by prior reproductive experience are not uniformly evident across all stages of the oestrous cycle. Although we did not detect changes in opioid regulation of LH on dioestrus II, an examination of LH responsiveness on other days of the oestrous cycle, for example prooestrus, or under a different hormonal background, that is oestrogen dominance, might allow for detection of differences.

Increasing the amount of prior reproductive experience from one to three litters in the second experiment also did not significantly affect the LH responses of the nonlactating, multiparous (three births) female rats to naloxone at a dose of either 1 mg kg⁻¹ or 0.5 mg kg⁻¹ i.v. on dioestrus II of the oestrous cycle. One complication of this study was that animals were 8 to 9 months of age at the time of sampling. By this age many of the age-matched, nulliparous female rats had entered a state of persistent vaginal oestrus prior to sampling. Hence, the actual number of cyclic, nulliparous animals used was low. It is possible that differences between groups might have been found had the sample sizes been larger. The phenomenon of decreased cyclicity with age in nulliparous animals confirms the earlier results of Matt *et al.* (1987). In rats, multiparous animals continue to exhibit normal oestrous cycles into middle age, whereas few nulliparous rats display 4 or 5 day oestrous cycles (Matt *et al.*, 1987).

The possibility was also investigated that another physiological state, that is, lactation, may provide an alternative model to assess the effects of reproductive experience on opioid regulation of LH. Earlier studies in rats established that throughout most of early to mid-lactation plasma LH concentrations are suppressed (Smith and Neill, 1977), primarily as a consequence of the suckling stimuli provided by the pups (McCann *et al.*, 1967). Hence, in the present study, basal LH concentrations prior to naloxone treatments were quite low. In the third experiment, on day 8 of lactation age-matched, primiparous and multiparous (second litter) females had comparable LH responses to 2 mg naloxone kg⁻¹. In contrast, on day 10 of lactation, at 0.5 mg naloxone kg⁻¹, a significant difference emerged between primiparous and multiparous animals at every time point, including baseline concentrations. Subsequent analysis using difference scores likewise revealed that at the 10 min sampling time point multiparous females displayed a greater LH response to naloxone than did primiparous dams. The reason for the difference in baseline LH values between the 0.5 mg kg⁻¹ treatment groups is unknown. However, it may in part reflect the limited sample size in animals given this dose of naloxone.

The finding that day 10 multiparous females are more sensitive to low doses of naloxone than are primiparous dams is in opposition to our initial hypothesis that reproductive experience renders females less sensitive to opioid regulation. Although this difference appears to be limited to a single low dose of naloxone, one possible explanation of this finding is that the dose-response curve to

the opiate antagonist is bell-shaped and shifts to the left in multiparous animals so that at lower doses naloxone stimulates LH secretion more readily, while at high naloxone doses LH is more likely to be inhibited. Shifts in opiate responsiveness and concentrations of opiate receptors in the medial preoptic area have been demonstrated in reproductively experienced lactating rats (Bridges and Hammer, 1992; Mann and Bridges, 1992). In fact, the concentrations of μ -opiate receptors is greater in day 12 multiparous lactating rats than in age-matched, primiparous controls (Bridges and Hammer, 1992). Might similar changes in μ -opiate receptor or other opiate receptor numbers or occupancy account for this shift in naloxone-induced LH secretion? Further studies are needed to clarify the relationships between opiate antagonist dose, reproductive experience, and neural receptor densities.

The lack of an LH response in primiparous lactating rats administered 25 mg naloxone kg⁻¹ in Expt 3 was unexpected, since the three lower doses of naloxone stimulated increases in plasma LH. This lack of LH stimulation to this high dose of naloxone may be a reflection of the superpharmacological treatment. This 25 mg kg⁻¹ dose of naloxone is higher than those previously used to access opiate regulation of LH secretion, and thus may functionally shut down opioid regulation of LH. It would be of interest to examine the central actions of this high naloxone dose to see how it affects opiate receptors and endogenous opioid synthesis. Finally, while we used plasma LH release as our dependent variable in these studies, it may also be worthwhile to examine pituitary LH stores and message in reproductively experienced and naive females to determine whether the processing of pituitary LH and not just its release may be affected by prior pregnancies and lactations. It is possible that differences in pituitary LH synthesis and storage occur that may not be reflected in plasma LH concentrations.

In summary, our results indicate that prior reproductive experience prolongs normal oestrous cyclicity and appears to increase the magnitude of the LH response of lactating, multiparous females to low doses of opiate antagonists. During lactation, a change in opioid regulation of LH in response to maximal doses of naloxone was not evident during the oestrous cycle of reproductively experienced animals at the doses of naloxone examined. Further studies, however, are needed to determine how a female's past reproductive experience may affect her subsequent neurochemical state, since it would influence, for example, the nature and dose of drugs that should be administered. Moreover, shifts in neurochemical sensitivity to substances that regulate GnRH and LH after prior pregnancies and lactations would be expected to affect reproductive ageing, influencing the duration of fertility in adulthood.

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