Endocrine, vol. 5, no. 1, 51–57, August 1996 1046-3976/5/1:51-57/$6.75

Mechanisms of the Effect of Icv IL-1β on Oxytocin Release in the Anesthetized, Lactating Rat

Brian C. Wilson,[1] Kathleen Fulop,[2] and Alastair J. S. Summerlee[2]

[1]Department of Medical Physiology, Faculty of Medicine, University of Calgary, Alberta, Canada ; [2]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Ontario, Canada

Interleukin-1β stimulates the release of many hypothalamic hormones, including oxytocin. Experiments were done to examine the effect of icv IL-1β on circulating oxytocin levels in rats throughout lactation, and to determine if alpha-adrenergic mechanisms and/or prostaglandins were involved as mediators. Blood samples were taken from urethane-anesthetized, nonlactating and lactating rats before and after icv treatment with either IL-1β or PBS-BSA; or icv treatment with IL-1β following pretreatment with either phentolamine or indomethacin. Plasma was assayed using a specific oxytocin radioimmunoassay. Interleukin-1β stimulated oxytocin release in all rats tested resulting in an approx 2- to 2.5-fold increase in plasma hormone levels. Throughout the first half of lactation, oxytocin responsiveness to IL-1β increased. On d 20 of lactation, the plasma oxytocin response to IL-1β was depressed. Adrenergic mechanisms may be involved in this differential effect on oxytocin release in lactation as phentolamine pretreatment attenuated IL-1β-induced oxytocin release in early lactation and reversed the depression in IL-1β-stimulation of oxytocin release observed in late lactation. It is possible that central IL-1β is involved in the weaning process: oxytocin responsiveness to IL-1β increases throughout the first half of lactation when oxytocin demands are high and is depressed in late lactation when weaning is occurring.

Key Words: α-Adrenergic, prostaglandins, weaning.

Introduction

Interleukin-1β (IL-1β) is a cytokine originally described as a mediator of acute inflammatory and immune responses (Dinarello, 1984). However, it is now known that IL-1β actions range from local autocrine and paracrine effects to widespread systemic and CNS effects. Through neuroendocrine actions in the hypothalamus, IL-1β is involved in the induction of fever (Blatteis, 1990; Rothwell, 1990; Kluger, 1991), slow-wave sleep (Krueger et al., 1984; Opp et al., 1988), and analgesia (Nakamura et al., 1988), as well as the suppression of food and water intake (Plata-Salamán et al., 1988; Plata-Salamán and ffrench-Mullen, 1992) and ovarian cycling (Rivier and Vale 1989, 1990; Kalra et al., 1990). Many of these IL-1β-induced changes result from the activation of the stress neuroendocrine axis: IL-1β stimulates corticotropin-releasing hormone (CRH) neurons in the paraventricular nuclei to produce and release CRH into the circulation and within the paraventricular nuclei (Berkenbosch et al., 1987; Sapolsky et al., 1987; Tsagarakis et al., 1989; Watanobe and Takebe, 1993). Central catecholamines and prostaglandins mediate this action of IL-1β on CRH release as antagonism of these systems attenuate or negate the efficacy of IL-1β in eliciting these responses (Weidenfeld et al., 1989; Katsuura et al., 1990). Preliminary evidence suggests that IL-1β is released in the rat hypothalamus during lactation in response to the suckling of young (Poterski, Wilson, and Summerlee, unpublished observations). Recent evidence suggests that centrally administered IL-1β stimulates oxytocin release into the circulation and enhances the release of oxytocin within the supraoptic nuclei of urethane-anesthetized, male rats (Landgaf et al., 1995). Therefore, it is possible that IL-1β mediates suckling-induced release of oxytocin in the lactating rat. Experiments were done to determine if icv IL-1β stimulated oxytocin release in both nonlactating and lactating urethane-anesthetized rats and to determine if alpha-adrenergic mechanisms and prostaglandins were involved as mediators.

Received January 15, 1996; Revised April 18, 1996; Accepted May 7, 1993.
Author to whom all correspondence and reprint requests should be addressed: Brian Wilson, Department of Medical Physiology, Faculty of Medicine, University of Calgary, 3330 Hospital Dr., NW, Calgary, Alberta, Canada T2N-4N1. E-mail: wilsonb@acs.ucalgary.ca

Results

Experiment 1: Effect of icv IL-1β on Oxytocin Release

Treatment with phosphate buffered saline-bovine serum albumin (PBS-BSA) had no significant effect on plasma concentrations of oxytocin in all rats tested. In contrast, IL-1β icv stimulated the release of oxytocin in nonpregnant, nonlactating rats and in rats throughout lactation. The

overall results are summarized in Figs. 1 and 2. In nonlactating rats, treatment with 250 pg IL-1β icv had no significant effect on oxytocin release. However, all three higher doses of IL-1β (500 pg, 1 ng, and 10 ng) caused a significant, dose-dependent increase in plasma oxytocin concentrations (Fig. 1A). Based on these data, a standard dose of 1 ng IL-1β in 1 μL PBS-BSA was used in the remainder of the experiments and the effect of this dose on oxytocin release was compared between nonlactating rats and rats at different stages of lactation.

Nonlactating Rats

The mean plasma oxytocin concentration in PBS-BSA-treated rats was 22.8 ± 1.8 pmol/L. Interleukin-1β icv significantly elevated plasma oxytocin levels by 10 min posttreatment, resulting in maximal levels (51.3 ± 1.8 pmol/L) by 60 min (Fig. 1B). Elevated levels were sustained beyond 120 min posttreatment.

Lactating Rats

Central injection of IL-1β caused a significant and sustained release of oxytocin on all four days of lactation tested although the pattern of release varied significantly between different days of lactation (Fig. 2). The most substantial release of oxytocin was observed on d 10 (approximately the midpoint of lactation) where IL-1β induced the fastest rate of oxytocin release (i.e., the highest peak in plasma oxytocin within the shortest period of time posttreatment) (Table 1). In contrast, the plasma oxytocin response to IL-1β was smaller and occurred less rapidly before and after this point in lactation. On d 26 of lactation, around the time of weaning, the plasma oxytocin response to icv IL-1β was not significantly different than that observed in nonlactating rats (Figs. 1 and 2).

Experiment 2: Mediators of the Central Actions of IL-1β

Nonlactating Rats

Pretreatment with phentolamine significantly augmented the peak release of oxytocin observed 30 min following icv IL-1β (Fig. 3), but had no significant effect on mean plasma oxytocin levels at any other time-point posttreatment. In contrast, indomethacin pretreatment completely negated the rise in plasma concentrations of oxytocin following icv IL-1β (Fig. 3).

Lactating Rats

Pretreatment of lactating (d 7 and d 20) rats with either phentolamine or indomethacin had different effects on IL-1β-induced release of oxytocin depending on the stage of lactation. On d 7 of lactation, both phentolamine and indomethacin pretreatments significantly attenuated the rise in mean plasma oxytocin levels following icv IL-1β (Fig. 3). On d 20 of lactation, indomethacin pretreatment had no effect while phentolamine pretreatment significantly augmented the IL-1β-induced rise in mean plasma oxytocin concentrations (Fig. 3).

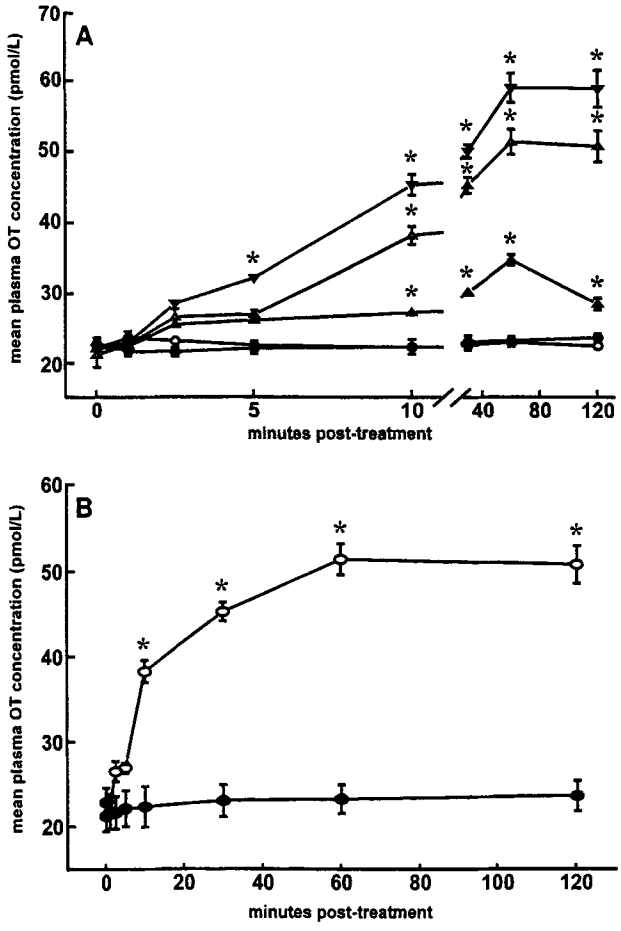

Fig. 1. (A) Mean plasma oxytocin concentrations before, and at timed intervals following treatment with four doses of IL-1beta in 1 μL PBS-BSA icv: 250 pg (filled circles); 500 pg (filled triangles); 1 ng (open triangles); 10 ng (inverted triangles), or 1 μL PBS-BSA icv (open circles) in nonpregnant, nonlactating rats. **(B)** Mean plasma oxytocin concentrations before, and at timed intervals following treatment with IL-1beta (1 ng/μL icv; open circles) or PBS-BSA (1 μL icv; filled circles) in nonpregnant, nonlactating rats. (Data is the same as presented in [A]). Data given as means ± SEM. *Significant difference when compared with PBS-BSA treated controls.

Discussion

There are two main areas of discovery derived from these studies: Icv IL-1β stimulated oxytocin release and the magnitude of release shifted through the course of lactation and there appeared to be different roles for alpha-adrenergic mechanisms and prostaglandins in mediating this action of IL-1β. Although the overall profile for oxytocin release following icv IL-1β was similar in lactating rats compared with nonlactators, the latency, rate of increase, and magnitude of the hormone responses following icv IL-1β differed between rats at separate stages of lactation. During the first half of lactation (d 7 and 10), the peak in plasma oxytocin concentrations observed following IL-1β treatment was not different than that observed in nonpregnant, nonlactating rats, except that the latency to the rise in plasma oxytocin following icv IL-1β became progressively shorter. At d 20

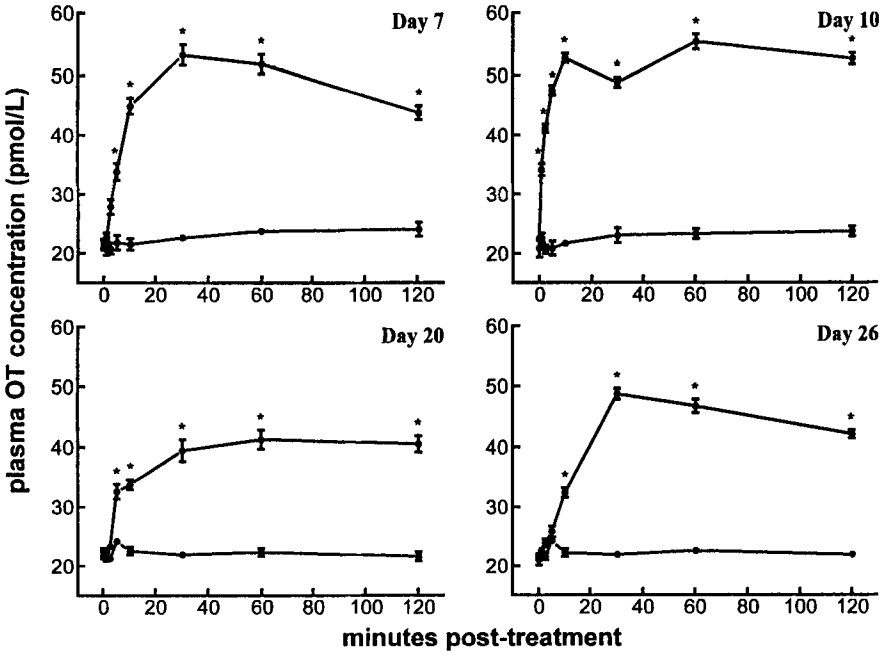

Fig. 2. Mean plasma oxytocin concentrations before, and at timed intervals following treatment with IL-1beta (1 ng/μL icv; open circles) or PBS-BSA (1 μL icv; filled circles) in rats at d 7, d 10, d 20, and d 26 of lactation. Data given as means ± SEM. *Significant difference when compared with PBS-BSA treated controls.

Table 1
Changing Effect of icv IL-1β on Oxytocin Release Throughout Lactation in Anesthetized, Lactating Rats

	Non-lactating	Lactating d 7	d 10	d 20	d 26
Time to significant elevation in plasma oxytocin levels (min)	10	5	1	5	10
Time to peak plasma oxytocin levels (min)	60	30	10	30	30
Estimate of the peak increase in mean plasma OT levels (pmol/L) (peak levels–levels induced by PBS-BSA)	28.1	30.6	31.1	17.6	26.9
[a]Approximate rate of increase (pmol/L/min)	0.6	1.2	3.5	0.7	1.3

[a]The rate of increase of plasma oxytocin levels was estimated by dividing peak increases in mean plasma hormone levels by the time that levels were significantly elevated.

of lactation, the plasma oxytocin response to icv IL-1β was attenuated when compared with nonlactating controls and rats in early lactation: there was a significant depression in peak hormone levels and the latency to the rise in plasma hormone levels following treatment was longer. By d 26 of lactation, oxytocin release in response to IL-1β was indistinguishable from the response observed in nonpregnant, nonlactating controls.

A limitation of this study is that specificity of this IL-1β effect was not demonstrated. Repetition of these experiments using the IL-1 receptor antagonist or soluble forms of the IL-1 receptors to block IL-1β-induced oxytocin release would strengthen these findings. However, the data presented in this paper are consistent with previously published reports that IL-1β stimulates oxytocin release under other experimental situations. Christensen et al. (1990) showed that IL-1β released oxytocin from isolated, perfused neurohypophyses obtained from male rats. Interleukin-1β also raised plasma levels of oxytocin when it was administered peripherally to male rats (Naito et al., 1991; Landgraf et al., 1995). Recently, Landgraf et al. (1995) demonstrated that icv IL-1β stimulates release of oxytocin within the supraoptic nuclei of urethane-anesthetized, male rats. In their study, icv infusion of IL-1β (200 ng/5 μL) resulted in a 1.7-fold increase in plasma oxytocin in 30-min dialysates obtained during a 90-min post-treatment period (Landgraf et al., 1995). The studies described within the current paper are the first indication that icv IL-1β affects oxytocin release in intact female nonlactating and lactating rats. In nonlactating rats, plasma oxytocin levels peaked at 60 min following icv IL-1β representing a 2.3-fold increase above controls. The higher peak in oxytocin release observed in female rats compared with males might suggest that oxytocin release is more sensitive to IL-1β modulation in female vs male rats. The different responsiveness may also result from the different treatment modalities used in this study and those of Landgraf et al. (1995). In the current study, rats received a single icv treatment of 1 ng/μL IL-1β, whereas Landgraf et al. (1995) infused a 40-fold higher dose of IL-1β in a 5 μL volume over 30 s.

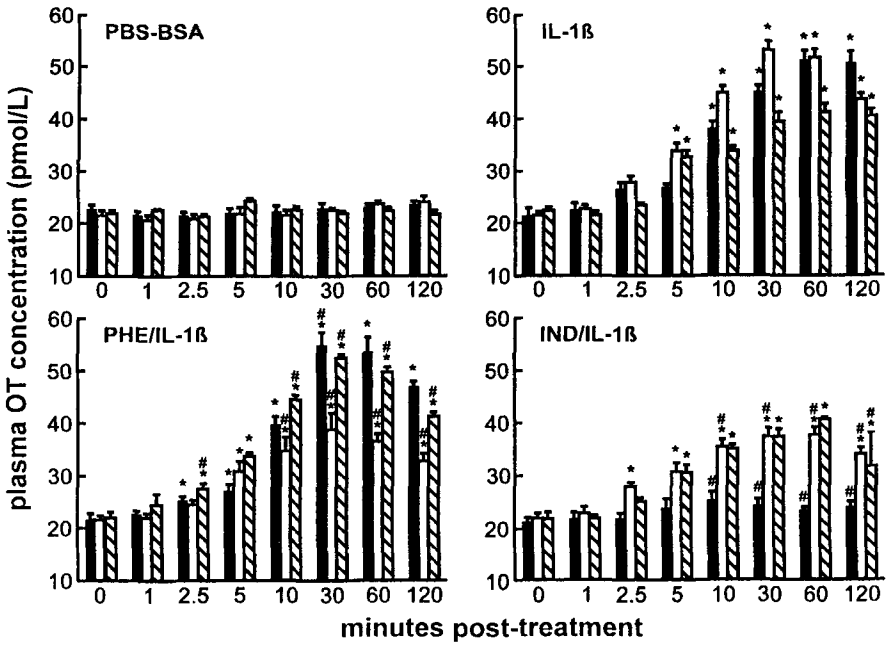

Fig. 3. Mean plasma oxytocin concentrations before, and at timed intervals following treatment with either phentolamine (PHE; 1.7 μg/μL icv), or indomethacin (IND; 1 μg/μL icv) followed by IL-1beta (1 ng/μL icv) in anesthetized, nonlactating rats (filled bars) and rats at d 7 (open bars) and 20 (hatched bars) of lactation. Data given as means ± SEM. *Significant difference when compared with PBS-BSA treated controls.# Significant difference when compared with rats treated with IL-1beta alone.

Throughout the first half of lactation (d 7 and 10) there was an upregulation of the responsiveness of the oxytocin system to IL-1β stimulation. In contrast, in late lactation (d 20), the plasma oxytocin response to central IL-1β was attenuated. Based on the change in oxytocin responsiveness to IL-1β stimulation in early vs late lactation, it is possible that IL-1β, through the decrease in oxytocin releasing ability, is a component of the weaning process. Weaning is a complex process involving many humoral changes, including a decrease in the availability of oxytocin and a decrease in mammary responsiveness to oxytocin (Tucker, 1994). The suckling stimulus would presumably become less important as young rats began to find their own food and rely less on milk from the dam for nourishment. Support for this concept comes from the work of Taya and Sasamoto (1991), who have shown that the role that suckling stimulation plays in suppressing gonadotropin release is less important in later stages of lactation as ovarian factors begin to exert the main inhibitory influence on luteinizing hormone and follicle-stimulating hormone release.

It has been shown that oxytocin is an important promoter of maternal nursing behavior. Intraventricular injections of 400 ng of oxytocin induced pup retrieval, licking, nest-building, and crouching behaviors in ovariectomized, estrogen-replaced, virgin rats (Pedersen and Prange, Jr., 1979; Pedersen et al., 1982; Fahrbach et al., 1984). However, oxytocin only appears to be important in establishing nursing behaviors of the dam immediately postpartum as icv injections of an oxytocin antagonist does not affect maternal behavior in rats that have begun to nurse their litters (Fahrbach et al., 1985). Clearly, other factors contribute to maternal behavior in established lactators. However, as lactation progresses and young rats become more experienced, they are able to find and attach to nipples themselves, without help from the dam (Stern and Johnson, 1990). Therefore, a decrease in the oxytocin response to icv IL-1β in later stages of lactation might also contribute to a cessation in maternal behavior that promotes suckling by the young.

Another factor contributing to the process of weaning may be IL-1β-induced release of vasopressin. In a similar study, we have shown that icv IL-1β stimulates vasopressin release in anesthetized, female rats and in rats at d 20 and 26 of lactation (Wilson et al., 1996). On d 7 and 10 of lactation, the vasopressin response to IL-1β is attenuated and this may be permissive for oxytocin delivery to mammary tissue. The magnitude of IL-1β-induced vasopressin release increases throughout middle to later stages of lactation and in these stages, high plasma vasopressin levels may interfere with oxytocin delivery to the mammary gland by restricting blood flow to the gland (Nakamo, 1974; Liard, 1985).

The possible role of alpha-adrenergic and prostaglandin mechanisms as mediators of IL-1β stimulation of oxytocin release was also examined. Pretreatment with phentolamine and indomethacin affected IL-1β-stimulated release of oxytocin differently throughout lactation. In summary, alpha-adrenergic agonists appear to mediate the stimulatory effect of icv IL-1β on oxytocin release in d 7 lactators, whereas adrenergic systems appear to cause the depression in IL-1β-induced release of oxytocin in d 20 lactating rats.

In nonlactators, alpha-adrenergic mechanisms are not involved. In contrast, prostaglandins appear to play a prominent role in mediating IL-1β-induced oxytocin release in nonlactating rats. In lactating animals, the stimulatory influence of prostaglandins was more prominent in early (d 7) vs late (d 20) stages of lactation. Based on the different effects of both classes of mediator throughout lactation, the mechanism(s) behind IL-1β-stimulated release of oxytocin appears complex. Suckling increases noradrenaline turnover in the paraventricular nuclei (Crowley et al., 1987) and central injection of noradrenaline or adrenergic agonists results in an increase in intramammary pressure (Clarke et al., 1979). Furthermore, administration of alpha-adrenergic receptor antagonists and noradrenaline synthesis inhibitors blocks the milk-ejection reflex (Moos and Richard, 1975; Tribollet et al., 1978). The stimulatory effect of adrenergic agonists on oxytocin release is mediated by alpha-1 adrenoreceptors (Clarke et al., 1979) that are found in the supraoptic and paraventricular nuclei (Wilcox et al., 1990; Pieribone et al., 1994). In addition, there is noradrenergic innervation of magnocellular oxytocin neurons (McNeill and Sladek, 1980; Alonso and Assenmacher, 1984; Hornby and Piekut, 1987; Ginsberg et al., 1994). Phentolamine pretreatment affected IL-1β-induced oxytocin release differently in early (d 7) vs late (d 20) lactation. In rats at d 7 of lactation, blockade of both alpha-1- and alpha-2 adrenoreceptors resulted in a significant reduction in IL-1β-stimulated oxytocin release. In d 20 lactating rats, phentolamine pretreatment had the reverse effect and appeared to reverse the depression in IL-1β-induced oxytocin release. This difference in effect could be a result of the expression of different subclasses of alpha-1 or alpha-2 adrenoreceptors on oxytocin neurons in early vs later stages of lactation. Alternatively, the difference in effect may result from the structural modifications that occur in the supraoptic and paraventricular nuclei as lactation progresses. In times of prolonged stimulation of the oxytocin system, such as parturition and lactation, glial processes and neuropil that normally divide oxytocin perikarya, withdraw permitting direct soma-soma and soma-dendritic juxtapositions (Hatton, 1988; Theodosis et al., 1988). In addition, the number of double synapses on oxytocin neurons increases. This may increase the responsiveness of oxytocin neurons to catecholaminergic modulation. As lactation progresses, there is a progressive reversal in these changes as suckling stimulation wanes (Hatton, 1988; Theodosis et al., 1988), and it is possible that responsiveness to catecholaminergic stimulation also decreases as double-synapses disappear.

Prostaglandins are known to mediate the febrile response to iv and icv IL-1 (Morimoto et al., 1987) and the effect of iv IL-1 on adrenocorticotropic hormone release (Katsuura et al., 1988; Rivier and Vale, 1991). It has been shown that centrally administered prostaglandins stimulate the release of both oxytocin and vasopressin (Hashimoto et al., 1989), and data obtained from this study is consistent with this. Indomethacin pretreatment completely negated IL-1β-stimulation of oxytocin release in nonlactating animals and caused a significant reduction in stimulated oxytocin release in d 7 lactators. However, in d 20 lactating rats, indomethacin pretreatment had no effect on the plasma oxytocin response to icv IL-1β. The central site of prostaglandin mediation of the effect of IL-1β on oxytocin release could be the organum vasculosum of the lamina terminalis (OVLT), one the circumventricular organs where circulating peptides gain access to brain parenchyma (Bartanusz and Jezova, 1992). The OVLT has been implicated as a site that mediates the febrile response to iv IL-1β and it has been suggested that prostaglandins are involved in this mechanism (Blatteis et al., 1983; Stitt and Shimada, 1989). This may represent a mechanism that is acting independently of noradrenergic systems in modulating oxytocin release in the lactating rat since noradrenergic projections that innervate the supraoptic and paraventricular nuclei come from brain stem centers and do not involve circumventricular structures (Wakerley et al., 1994).

Materials and Methods

Animals

Nonpregnant, nonlactating (hereafter referred to as nonlactating) and lactating female Sprague-Dawley rats (280–330 g) used in these studies were housed in the Central Animal Facilities of the University of Guelph. They were maintained on a 14-h light/10-h dark photoperiod (lights on at 06:00) and received food and water *ad libitum*. For lactating rats, litters were standardized to contain 10 pups on d 1 of lactation. Following experimentation, rats were euthanized with an overdose of somnotol (sodium pentobarbital; MTC Pharmaceuticals, Canada Packers, Cambridge, ON; 100 mg/kg iv). All procedures were carried out according to guidelines set by the Canadian Council for Animal Care and approved by the Animal Care Committee of the University of Guelph.

Cannulations

All rats were anesthetized with urethane (ethyl carbamate; Sigma, St. Louis, MO; 1.25 g/kg ip) and rompun (xylazine; BAYVET, Chemagro, Etobicoke, ON; 1 mg/kg im). Polyethylene cannulae were surgically inserted into the following blood vessels: a) the left saphenous vein (ID 0.28 mm; OD 0.61 mm), for iv administration of drugs; the left femoral artery (ID 0.58 mm; OD 0.96 mm), connected to a calibrated Grass polygraph (Grass model 7E) via a pressure transducer (Viggo-Spectramed model P23XL, Viggo-Spectramed, Oxnard, CA) to monitor arterial blood pressure; the right common carotid artery (ID 0.58 mm; 0.96 mm), for blood sampling; and the right external jugular vein (ID 0.63 mm; OD 1.4 mm), connected to an infusion pump for simultaneous replacement of physiological saline (0.9%) during blood sampling.

Drugs

Human recombinant interleukin-1β (IL-1β) was supplied by the Biological Resources Branch of the Biological Response Modifiers Program, Division of Cancer Treatment, US National Cancer Institute. Interleukin samples were determined by the supplier to be free of *Escherichia coli* and endotoxin contamination (*Limulus sp.* amoebocyte lysate assay). Four dosages of IL-1β (250 pg, 500 pg, 1 ng, and 10 ng) were dissolved in 1 μL phosphate-buffered saline in 0.01% bovine serum albumin (PBS-BSA). These were used initially to determine the dose-response effects of the cytokine on oxytocin release in Experiment 1. Based on these data, a standard dose of 1 ng IL-1β dissolved in 1 μL PBS-BSA was chosen to use in the rest of the experiments described in this paper. Alpha-adrenergic receptors were antagonized by pretreating animals with phentolamine (Rogitine; CIBA-GEIGY Pharmaceuticals Division, Mississauga, ON; 1.7 μg in 1 μL PBS icv). Prostaglandin synthesis was blocked by pretreating rats with indomethacin (Indocid PDA; Merck Sharp and Dohme Canada, Kirkland, QC; 1 μg in 1 μL PBS icv).

Experiment 1: Effect of icv IL-1β on Oxytocin Release

Nonlactating Rats

EXPERIMENTAL PROTOCOL. Anesthetized, nonlactating rats ($n = 25$) were placed in a stereotaxic frame (Narishige SR6, Narishige, Tokyo, Japan), and a 10 μL Hamilton microsyringe was placed with the tip of the needle in the left lateral cerebral ventricle (coordinates: 0.9 mm caudal to bregma; 2.5 mm lateral to midline; 3.0 mm ventral to the cortical surface). Microsyringe placement was verified after each experiment by injecting 1 μL india ink icv and looking for the distribution of ink throughout the ventricular system. Blood samples (1 mL) were collected over 1 min periods through the carotid cannula before, and 1, 2.5, 5, 10, 30, 60, and 120 min after icv injection of IL-1β (250 pg, 500 pg, 1 ng or 10 ng in 1 μL PBS-BSA; experimental; $n = 5$/group) or PBS-BSA (1 μL; control; $n = 5$). Physiological saline (0.9%) was infused (1 mL/min) concurrently through the jugular cannula to offset any hypovolemic effects of sampling. To further limit cardiovascular effects of blood withdrawal, blood pressure was monitored during sampling and the rate of sampling controlled to maintain a stable pressure recording on the polygraph. After collection, blood samples were centrifuged (10,000*g* for 5 min) and plasma was frozen at –25°C until assay.

Lactating Rats

Rats at d 7 ($n = 8$), d 10 ($n = 10$), d 20 ($n = 12$), and d 26 ($n = 12$) of lactation were used in this study. One-half of the rats in each lactational state were assigned to either experimental or control groups. Based on the dose-response results, we chose to use a standard dose of IL-1β (1 ng dissolved in 1 μL PBS-BSA) for the remainder of the experiments to compare results obtained at the different points of lactation. Rats were anesthetized and prepared as described for nonlactating rats. Blood samples were collected following icv IL-1β and plasma was stored until assay.

Experiment 2: Mediators of the Central Actions of IL-1β

Experiment 2a

Noradrenergic mediation of IL-1-induced oxytocin release was tested in three groups of rats: group 1 were nonlactating rats ($n = 6$); group 2 were d 7 lactators ($n = 6$), and group 3 were d 20 lactators ($n = 5$). In each group, the effect of icv IL-1β on oxytocin release was tested in rats pretreated with an alpha-adrenergic antagonist phentolamine (1.7 μg/μL icv) or with PBS-BSA.

Experiment 2b

Prostaglandinergic mediation of IL-1β-induced oxytocin release was tested in three separate groups of rats: group 1 were nonlactating rats ($n = 6$); group 2 were d 7 lactators ($n = 6$), and group 3 were d 20 lactators ($n = 5$). In each group, the effect of icv IL-1β on oxytocin release was tested in rats pretreated with indomethacin, a cyclooxygenase inhibitor (1 μg/μL icv) or with PBS-BSA.

EXPERIMENTAL PROTOCOL. Following an initial blood sampling period, rats were treated with either phentolamine, indomethacin or PBS-BSA alone, left for 5 min, then treated with 1 ng IL-1β icv. Blood samples were taken at 1, 2.5, 5, 10, 30, 60, and 120 min after IL-1β treatment and blood volume was replaced with physiological saline (0.9%) during each sampling period as described in Experiment 1. Blood samples were centrifuged and plasma was collected and frozen until assayed for oxytocin.

Oxytocin Radioimmunoassay

Plasma concentrations of oxytocin were measured by specific radioimmunoassay (Fourth International Standard for oxytocin 76/578) on unextracted plasma. Rabbit antiporcine antiserum (85/F2) was prepared by S. Birkett, Dept. of Anatomy, University of Bristol, Bristol, UK). The lower limit of detection was 5.2 pmol/L and the inter- and intrassay coefficients of variation were 7.1 and 3.7%, respectively, at 10 pmol/L. Cross-reactivity was 12.5% for mesotocin, 0.03% with lysine vasopressin, and <0.02% for arginine vasopressin, the neurophysins and a variety of other hypothalamic peptides.

Statistical Analysis

Mean plasma concentrations for oxytocin were calculated for each of the sampling time-points. Multivariate analysis of variance (ANOVA) was used to compare trends in the profile of hormone release at different times after IL-1β treatment and at different stages of lactation. Comparisons between mean hormone levels at individual time-points were made using Bonferroni's method for pairwise comparison of means. Means were considered significantly different at $p < 0.05$.

Acknowledgments

The authors would like to thank S. Birkett (University of Bristol) for the supplies of oxytocin antisera and David Jones (University of Bristol) for carrying out the oxytocin radioimmunoassays. We also want to thank the staff of the Animal Care Facility at the University of Guelph for their expert care of the experimental animals. Funding for these studies was obtained from NSERC Canada and OMAFRA.

References

Alonso, G. and Assenmacher, I. (1984). *Neurosci. Lett.* **49,** 45–50.

Bartanusz, V. and Jezova, D. (1992). *Endocr. Regul.* **26,** 3–9.

Berkenbosch, F., van Oers, J., del Rey, A., Tilders, F., and Besedovsky, H. (1987). *Science* **238,** 524–526.

Blatteis, C. M. (1990). *Yale J. Biol. Med.* **63,** 133–146.

Blatteis, C. M., Bealer, S. L., Hunter, W. S., Llanos, Q. J., Ahokas, R. A., and Masburn, Jr., T. A. (1983). *Brain Res. Bull.* **11,** 519–526.

Christensen, J. D., Hansen, E. W., and Fjalland, B. (1990). *Pharmacol. Toxicol.* **67,** 81–83.

Clarke, G., Lincoln, D. W., and Merrick, L. D. (1979). *J. Endocrinol.* **83,** 409–420.

Crowley, W. R., Shyr, S.-W., Kacsoh, B., and Grosvenor, C. E. (1987). *Endocrinology* **121,** 14–20.

Dinarello, C. A. (1984). *Rev. Infect. Dis.* **6,** 51–95.

Fahrbach, S. E., Morrell, J. I., and Pfaff, D. W. (1984). *Hormone Behav.* **18,** 267–286.

Fahrbach, S. E., Morrell, J. I., and Pfaff, D. W. (1985). *Neuroendocrinology* **40,** 526–532.

Ginsberg, S. D., Hof, P. R., Young, W. G., and Morrison, J. H. (1994). *J. Comp. Neurol.* **341,** 476–491.

Hashimoto, H., Noto, T., and Nakajima, T. (1989). *Prostaglandins Leukot. Essent. Fatty Acids* **36,** 9–14.

Hatton, G. I. (1988). In: *Stimulus-Secretion Coupling in Neuroendocrine Systems.* Ganten, D. and Pfaff, D. (eds). Springer-Verlag: New York. pp. 1–27.

Hornby, P. J. and Piekut, D. T. (1987). *Cell Tissue Res.* **248,** 239–246.

Kalra, P. S., Sahu, A., and Kalra, S. P. (1990). *Endocrinology* **126,** 2145–2152.

Katsuura, G., Gottschall, P. E., Dahl, R. R., and Arimura, A. (1988). *Endocrinology* **122,** 1773–1779.

Katsuura, G., Arimura, A., Koves, K., and Gottschall, P. E. (1990). *Am. J. Physiol.* **258,** E163–E171.

Kluger, M. J. (1991). *Physiol. Rev.* **71,** 93–127.

Krueger, J. M., Walter, J., Dinarello, C. A., Wolff, S. M., and Chedid, L. (1984). *Am. J. Physiol.* **246,** R994–R999.

Landgraf, R., Neumann, I., Holsboer, F., and Pittman, Q. J. (1995). *Eur. J. Neurosci.* **7,** 592–598.

Liard, J. F. (1985). In: *VASOPRESSIN.* Schrier, R. W. (ed.). Raven: New York. pp. 59–67.

McNeill, T. H. and Sladek, J. R. (1980). *J. Comp. Neurol.* **193,** 1023–1033.

Moos, F. and Richard, P. (1975). *J. Physiol. (Paris)* **70,** 307–314.

Morimoto, A., Murakami, N., Nakamori, T., and Watanabe, T. (1987). *J. Physiol. (Lond.)* **383,** 629–637.

Naito, Y., Fukata, J., Shindo, K., Ebisui, O., Murakami, N., Tominaga, T., Nakai, Y., Mori, K., Kasting, N. W., and Imura, H. (1991). *Biochem. Biophys. Res. Commun.* **174,** 1189–1195.

Nakamo, J. (1974). In: *Handbook of Physiology Endocrinology* IV. The pituitary gland and its neuroendocrine control. Knobil, E. and Sawyer, W. H. (eds.). American Physiological Society: Washington DC. pp. 395–442.

Nakamura, H., Nakanishi, K., Kita, A., and Kadokawa, T. (1988). *Eur. J. Pharmacol.* **149,** 49–54.

Opp, M. R., Obál Jr., F., and Krueger, J. M. (1988). *Am. J. Physiol.* **255,** R914–R922.

Pedersen, C. A. and Prange, Jr., A. J. (1979). *Proc. Natl. Acad. Sci. USA* **76,** 6661–6665.

Pedersen, C. A., Ascher, J. A., Monroe, Y. L., and Prange, Jr., A. J. (1982). *Science* **216,** 648,649.

Pieribone, V. A., Nicholas, A. P., Dagerlind, A., and Hokfelt, T. (1994). *J. Neurosci.* **14,** 4252–4268.

Plata-Salamán, C. R., Oomura, Y., and Kai, Y. (1988). *Brain Res.* **448,** 106–114.

Plata-Salamán, C. R. and ffrench-Mullen, J. M. H. (1992). *Physiol. Behav.* **51,** 1277–1279.

Rivier, C. and Vale, W. (1989). *Endocrinology* **124,** 2105–2109.

Rivier, C. and Vale, W. (1990). *Endocrinology* **127,** 849–856.

Rivier, C. and Vale, W. (1991). *Endocrinology* **129,** 384–388.

Rothwell, N. J. (1990). *Eur. Cyt. Net.* **1,** 211–213.

Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P., and Vale, W. (1987). *Science* **238,** 522–524.

Stern, J. M. and Johnson, S. K. (1990). *Physiol. Behav.* **47,** 993–1011.

Stitt, J. T. and Shimada, S. G. (1989). *J. Appl. Physiol.* **67,** 1734–1739.

Taya, K. and Sasamoto, S. (1991). *J. Endocrinol.* **129,** 119–130.

Theodosis, D. T., Poulain, D. A., Montagnese, C., and Vincent, J. D. (1988). In: *Neurosecretion:* cellular aspects of the production and release of neuropeptides. Pickering, B. T., Wakerley, J. B., and Summerlee, A. J. S. (eds.). Plenum: New York. pp. 157–165.

Tribollet, E., Clarke, G., Dreifuss, J. J., and Lincoln, D. W. (1978). *Brain Res.* **142,** 69–84.

Tsagarakis, S., Gillies, G., Rees, L. H., Besser, M., and Grossman, A. (1989). *Neuroendocrinology* **49,** 98–101.

Tucker, H. A. (1994). In: *The Physiology of Reproduction,* vol. 2. Knobil, E. and Neill, J. D. (eds.). Raven: New York. pp. 1065–1098.

Wakerley, J. B., Clarke, G., and Summerlee, A. J. S (1994). In: *The Physiology of Reproduction,* vol. 2. Knobil, E. and Neill, J. D. (eds.). Raven: New York. pp. 1131–1177.

Watanobe, H. and Takebe, K. (1993). *Neuroendocrinology* **57,** 593–599.

Weidenfeld, J., Abramsky, O., and Ovadia, H. (1989). *Neuropharmacology* **28,** 1411–1414.

Wilcox, B. J., Raskind, M. A., Ko, G. N., Baskin, D. G., Pascualy, M., and Dorsa, D. M. (1990). *Neuroendocrinology* **51,** 315–319.

Wilson, B. C., Fulop, K., and Summerlee, A. J. S. (1996). *J. Neuroendocrinol.* (in press).