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Effects of Opioid Antagonism on Prolactin Secretion and c-Fos/TH Expression During Lactation in Rats

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Many studies have established that dopamine (DA) secreted by tuberoinfundibular dopaminergic (TIDA) neurons in the hypothalamus is the major inhibitory factor controlling prolactin (PRL) secretion from the anterior pituitary. Endogenous opioid peptides (EOPs), mainly the neuropeptide β-endorphin, facilitate PRL secretion by decreasing TIDA neuronal inhibitory tone in a number of physiological conditions, including pregnancy and lactation. We have previously demonstrated that there are many more c-Fos–expressing neurons than TIDA neurons in the arcuate nucleus, and treatment with naloxone (NAL), an opioid antagonist, activated these neurons in pregnant rats. Our previous data also suggest that the rostral region of the arcuate nucleus is more important than the caudal region in regulating the nocturnal PRL surge in pregnant rats. The aim of this study was to investigate the effects of NAL in regulating TIDA neuronal activity and therefore facilitating PRL secretion during lactation in rats. NAL was continuously infused (0.2 mg/10 µL/min iv) for 1 h before the separated pups returned, and then for 2 or 5 h after the separated pups were returned. Radioimmunoassay (RIA) was used to measure plasma PRL levels, and the immunocytochemical (ICC) staining of c-Fos was performed to detect changes in transcriptional activity of neurons in the hypothalamus. ICC of tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis, was performed to visualize TIDA neurons in the arcuate nucleus. The results showed that the peak of the PRL response to suckling was markedly delayed and dampened in NAL-treated rats ($p < 0.05$). The percentage of c-Fos positive TH neurons in the arcute nucleus increased in rats treated with NAL for 5 h after return of pups, but not in rats treated with NAL for 2 h.

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Introduction

Prolactin (PRL) has numerous effects on the brain, including regulation of appetite and feeding, regulation of oxytocin, and decreased stress response *(1)*. Release of dopamine (DA) from neurosecretory neurons is the primary hypothalamic inhibitor of PRL secretion from the pituitary *(2)*. Many studies reported that dopamine secreted from tuberoinfundibular dopaminergic (TIDA) neurons is the major inhibitory factor of PRL secretion. The arcuate nucleus is the location where the cell bodies of the TIDA neurons are located, with the nerve terminals in the median eminence *(3,4)*. Numerous studies have demonstrated that endogenous opioid peptides (EPOs), especially β-endorphin, are positive regulators of PRL secretion *(5–7)*. The receptors of endogenous opioid peptides (EOPs), including mu (μ), delta (δ), and kappa (κ), are found in the hypothalamus *(8–10)*. The μ-receptor is believed to be the predominant receptor of EOPs *(11)*. Administration of β-endorphin stimulates PRL secretion in many circumstances, including proestrus, stress, and lactation. Naloxone (NAL), a potent nonselective opioid receptor antagonist, lowers both basal and stimulated PRL levels.

The arcuate nucleus is a major source of both β-endorphin *(12)* and TIDA neurons *(13)*. Reports describe contacts between β-endorphin axon terminals and TIDA neurons in the arcuate nucleus *(14,15)*. In our previous studies, NAL treatment during the nocturnal surge in pregnant rats suppressed PRL secretion, whereas DOPA accumulation in the stalk median eminence of the hypothalamus was shown to increase significantly *(16,17)*. DOPA accumulation in the median eminence was used as an indicator of TIDA neuronal activity in the hypothalamus, and is a measure of all tyrosine hydroxylase (TH) neurons, which have terminals in that location. Although a measure of TH activity is a powerful tool in assessing neuronal activity, it does not present a complete picture. In order to look at subpopulations of TIDA neurons in the arcuate nucleus, dual labeling of c-Fos and TH was done to monitor the transcriptional activity of TIDA neurons in the lactating rat treated with NAL.

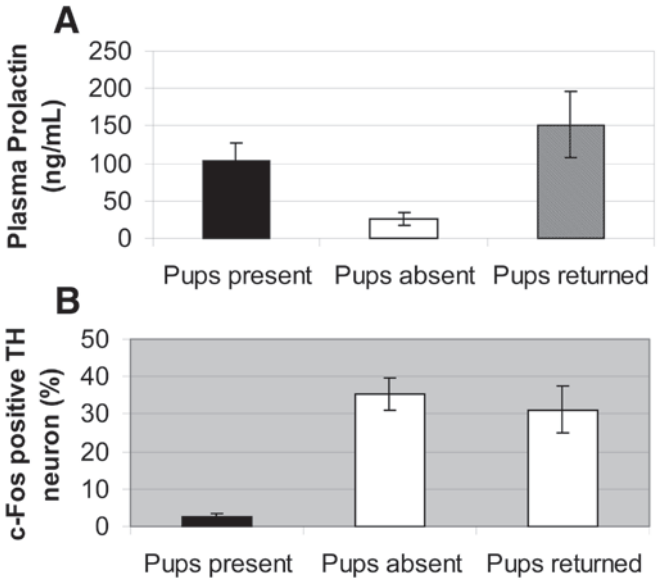

Fig. 1. Changes in plasma PRL levels and arcuate nucleus TH activity in dams with pups present, absent, or returned for 2 h. (**A**) Plasma PRL levels are significantly reduced when pups are absent ($p < 0.05$) but return of pups for 2 h increased PRL to levels seen in dams with pups continuously present. (**B**) The percentage of TH and c-Fos dual-labeled neurons in the absence of pups increased dramatically ($p < 0.05$) whereas return of pups for two hours did not change this response.

Results

Effect of Pup Suckling on Plasma PRL and TH Activity on d 9 of Lactation

When pups were left with the dam continuously, plasma PRL levels were about 100 ng/mL (Fig. 1A). Removal of pups for 17 h resulted in very low PRL levels. Return of pups for a 2 h suckling period resulted in an increase in PRL levels similar to dams in which pups were continuously present. TH neurons that show transcriptional activity as measured by c-Fos immunocytochemistry (ICC) are concentrated in the dorsal–medial part of the rostral arcuate nucleus. In these same dams, removal of pups resulted in a large increase in the percentage of TH neurons in the rostral arcuate nucleus that also expressed c-Fos (Fig. 1B). However, return of pups for 2 h of suckling did not reverse this non-suckling effect.

Effect of NAL Treatment on Plasma PRL Levels and TH Activity

In two groups of lactating rats, naloxone (NAL) or saline (SAL) was infused continuously into dams beginning at 10:00 h until 17:00 h on d 9 after pup delivery. Pups were returned to the dams after NAL infusion began. Blood was collected at 10:00 h and every 15 min beginning at 16:00 h. PRL levels in both groups of dams were low at 10:00 h. In SAL-treated rats (solid diamond), plasma PRL was elevated at 16:00 h and continued to be high during most blood sampling times

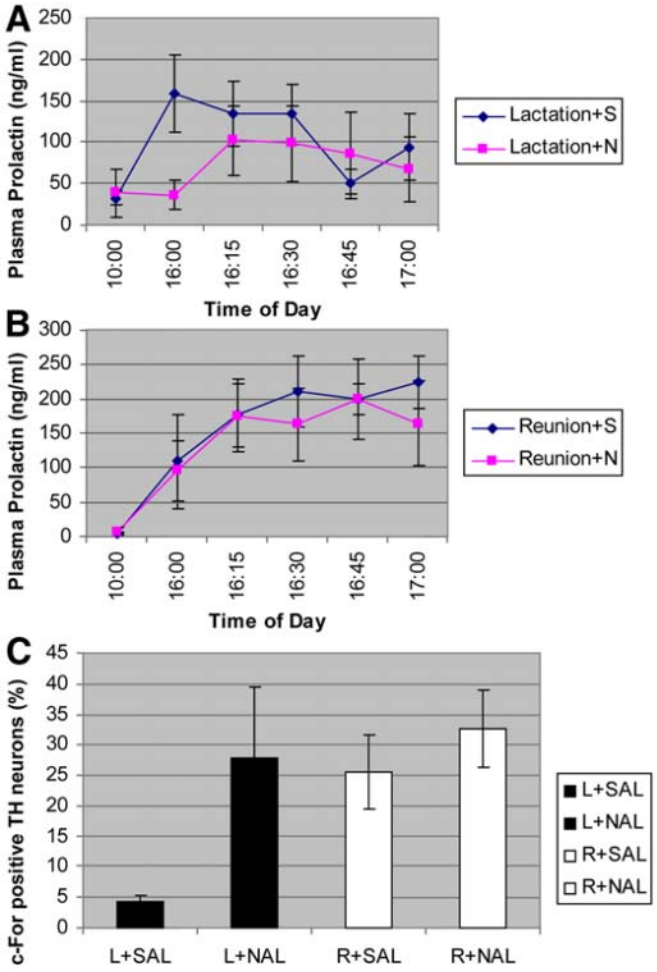

Fig. 2. Changes in plasma PRL levels (**A,B**) and arcuate nucleus TH activity (**C**) in dams which received infusion of NAL or SAL for 7 h, begun at 10:00 h. (**A**) In the SAL-treated group (solid diamonds), plasma PRL levels were high at 16:00 h, and then gradually returned to baseline. In the NAL-treated group (solid squares), PRL levels were low at 16:00 h ($p < 0.05$) followed by a slight increase after 15 min, that remained stable up to 1 h. (**B**) In the SAL-treated group (solid diamonds), return of pups for 2 h beginning at 15:00 h significantly increased PRL levels compared to levels at 10:00 h. NAL treatment (solid squares) did not alter the response to suckling. (**C**) The percentage of c-Fos positive TH neurons were increased significantly ($p < 0.05$) in continuously suckled dams treated with NAL (L+NAL). Return of pups (R+NAL or SAL) for 2 h did not reduce c-Fos activity in TH neurons in SAL-treated controls, and NAL treatment had no effect.

(Fig. 2A). In NAL-treated rats, PRL levels were low at 16:00 h and somewhat higher over the next 60 min. This may reflect a partial escape from the inhibitory effects of NAL. As seen in Fig. 2C, these dams that had pups present beginning at 10:00 h showed increased c-Fos activity in the TH neurons of the rostral arcuate nucleus when NAL was infused (L+NAL) compared to dams that received SAL (L+SAL).

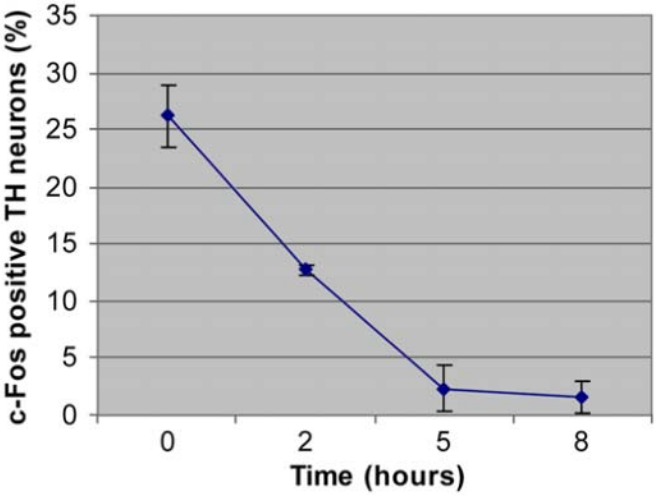

Fig. 3. Changes in percentage of c-Fos positive TH neurons in the arcuate nucleus of dams following increased periods of suckling. Two hours of suckling reduced TH activity by approx 50% compared to no suckling, whereas 5 and 8 h of suckling reduced TH activity by 90%.

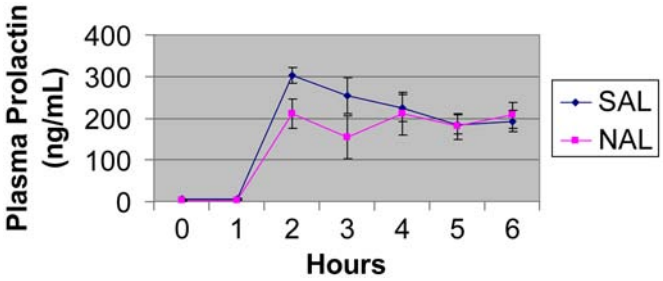

Fig. 4. Changes in plasma PRL levels in dams infused with NAL (solid squares) or SAL (solid diamonds) for 6 h. Pups were returned to dams 1 h after NAL or SAL infusion began (0 h) and blood samples were taken every hour for 5 h. PRL levels were significantly lower at 1 h after return of pups in NAL-treated dams ($p < 0.05$) compared to controls, but also significantly higher than levels before pups were returned ($p < 0.05$).

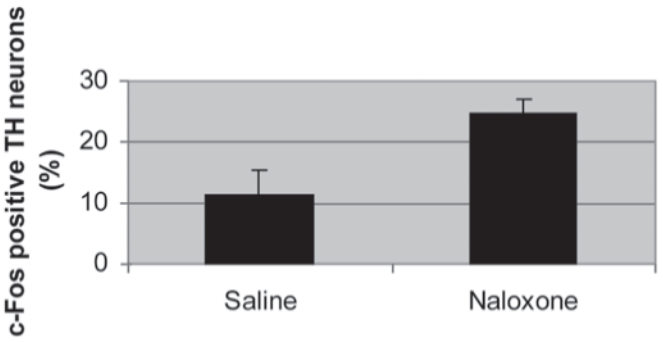

Fig. 5. Changes in percentage of c-Fos positive TH neurons in arcuate nucleus of suckled dams infused with SAL or NAL for 6 h. NAL infusion significantly increased the percentage of c-Fos positive TH neurons ($p < 0.05$). The c-Fos positive TH neurons comprises about 10% of the total TH neurons in the SAL-treated group, and about 25% of the total TH neurons in the NAL-treated group ($p < 0.05$).

Return of pups to the dams for 2 h beginning at 15:00 h resulted in plasma PRL levels that were increased compared to levels when pups were absent (10:00 h). However, NAL infusion beginning at 10:00 h did not alter the PRL response to 2 h of suckling (Fig. 2B). Furthermore, NAL infusion had no effect on the percentage of dual labeled TH and c-Fos neurons present (Fig. 2C).

TH Neuronal Activity Changes in Response to Length of Time Pups Suckled

The results of the above experiment in which 2 h of suckling did not reduce TH activity prompted us to do a time course in which pups were returned to the dams for various lengths of suckling time. As seen in Fig. 3, 5 and 8 h of continuous suckling reduced TH activity levels by 90% whereas the reduction after 2 h was much less (about 50%).

TH Neuronal Activity and PRL Levels in Dams Treated with NAL for 6 h and Suckled for 5 h

Plasma PRL levels increased in dams in which pups were returned for 5 h compared to pre-suckling levels (Fig. 4). Treatment with NAL partially lowered the PRL response initially ($p < 0.05$), but after 2 h of suckling, the levels were the same and remained so during the 3 additional hours. Measurement of c-Fos activity in the rostral arcuate nucleus revealed that NAL significantly elevated TH activity detected in neurons compared to SAL-treated controls (Fig. 5).

Discussion

In this study, we examined the role of endorphins in regulating TIDA neuronal activity and PRL secretion during lactation in rats. Naloxone (NAL), an opioid receptor antagonist that acts primarily on the μ receptor, was used to gain insight into the hypothalamic response to suckling in regulating PRL release.

The regulation of release of PRL is a complex process involving various nonhypothalamic and hypothalamic factors *(18,19)*. EPOs are positive regulators of PRL secretion *(5–7)*. β-Endorphin is the most potential stimulator of PRL secretion among EOPs. There are three major opioid receptors present within the hypothalamus: μ-, κ-, and δ-receptors, and the μ-receptor is generally recognized to be the predominant one for PRL control *(10,15)*. Naloxone is a broad spectrum opioid antagonist with a fourfold higher affinity for μ-receptors than κ-receptors and a 20-fold higher affinity for μ-receptors than δ-receptors *(9)*. The route and dosage of NAL treatment selected for our study was previously shown to effectively block the nocturnal PRL surge *(16)*.

NAL administration decreases both basal *(20)* and stimulated PRL *(7,21)* levels. In this experiment, infusion of NAL delayed and dampened plasma PRL levels in response

to suckling (Fig. 2A). This delay and dampening of PRL levels is consistent with previous reports on the nocturnal surge of PRL during pregnancy *(16,17)*. Whether the NAL was insufficient to totally block the μ receptors, or whether there were other hypothalamic stimulatory factors or PRL-releasing factors able to activate the PRL surge is not discernible by this experiment. The PRL response to suckling also has been shown to be dampened but not totally blocked by NAL *(22)*. In the latter report, NAL was shown to increase both TH enzyme activity and TH mRNA in the arcuate nucleus during lactation, suggesting the EOPs play a key role in increasing the PRL response to suckling.

The arcuate nucleus is a major source of both β-endorphin and TIDA neurons, and it can be divided into rostral and caudal regions. Our previous data suggest that the rostral region of the arcuate nucleus is more important than the caudal region in regulating the nocturnal PRL surge in pregnant rats *(17)*. TH gene expression during lactation is significantly suppressed in TIDA neurons in the rostral, but not in the caudal, arcuate nucleus *(23)*. In another report, suckling-induced c-Fos expression (measure of neuronal activity) was measured in specific subgroups of hypothalamic POMC neurons located in the rostral part of the arcuate nucleus *(24)*.

Because TH neuronal numbers have not been shown to change with NAL treatment *(16)*, it is unlikely that changes in TH numbers are part of the mechanism involved in EOPs' regulation of PRL in response to suckling. Other possibilities include changes in dopamine synthesis and release, TH activity, or modulation of TH mRNA synthesis *(25–27)*. In the present study, the actual percentage of TH neurons that were active was determined by counting the number of colabeled c-Fos positive TH neurons. Separation of pups resulted in a large increase in the percentage of c-Fos-positive TH neurons in the rostral arcuate nucleus in the dams (Fig. 1B). However, return of pups for 2 h of suckling surprisingly did not reverse this nonsuckling effect. When NAL was infused for 2 h, dams with pups continuously present showed increased c-Fos activity in the TH neurons of the rostral arcuate nucleus compared to dams that received SAL (Fig. 2C). However, NAL infusion for 2 h had no effect on the percentage of c-Fos positive TH neurons when pups were returned after 17 h separation (Fig. 2C). This result prompted a time course experiment. As shown in Fig. 3, pups were returned to the dams for 0, 2, 5, and 8 h of suckling, and the percentages of c-Fos-positive TH neurons in the rostral arcute nucleus at these different time points were determined. Results indicate that at 5 and 8 h of suckling, TH activity levels decreased significantly to levels found in continuously suckled rats, while the reduction at 2 h was less. This led us to treat dams with NAL for 6 h, in which suckling by pups occurred during the final 5 h. NAL treatment significantly increased the number of TH neurons that were c-Fos positive compared to SAL-treated controls (Fig. 5). Why it took more than 2 h of NAL exposure to increase TIDA neuronal activity is not evident. Even though NAL turnover is very rapid, given that NAL administration was continuous should provide adequate antagonism to the opiate receptors. It is possible that NAL acts as a partial opiate agonist to inhibit the TIDA neurons initially, but eventually as an antagonist.

Our previously report indicated that NAL treatment given to pregnant rats increased c-Fos expression in the arcuate nucleus *(17)*. These neurons were subsequently determined to be TH positive. The present study yielded similar results in lactating dams. TIDA neurons in the arcuate nucleus are at the center of PRL regulation. It is reasonable to investigate changes in the TIDA neuronal activity after NAL treatment as it has been postulated that β-endorphin neurons function by inhibiting TIDA neurons. Our results demonstrate that NAL treatment for 5 h resulted in an increase in the percentage of c-Fos-positive TH neurons in the rostral arcuate nucleus in dams, indicating that NAL treatment during lactation is associated with an increased TIDA neuronal activity in the arcuate nucleus.

In conclusion, the results from this study reveal that EOPs, especially β-endorphin, play a critical stimulatory role in PRL secretion in lactation rats. The mechanism of the regulation of the PRL secretion is, in part, by inhibiting either directly or indirectly TIDA neuronal activity in the rostral region of the arcuate nucleus.

Materials and Methods

Animals

Adult female Sprague-Dawley rats (Sasco Co., Omaha, NE) weighing 180-200 g were housed in a temperature (24 ± 2°C) and light (06:00–18:00 h) controlled room. Animals were provided with food and water *ad libitum*. Each female was paired with one male for the purpose of mating. After the pups were born, a litter of eight pups were kept with the mother and the others were removed. On the 7th day after delivery, the right jugular vein of the dam was cannulated under anesthesia. The cannula was exteriorized through the back of the neck and extended outside of the cage. Patency of the cannula was maintained by flushing with 20 IU heparin/mL saline solution. The dam was returned to the cage after the operation, and allowed to stay with her pups. On the next day, the pups were separated from the dam overnight.

Sample Preparation

On the 9th day after delivery, the jugular cannula was connected to a peristaltic pump. Naloxone (Sigma Chemical Co., St. Louis, MO) (0.2 mg/10 μL/min) or saline was infused for 3 or 6 h. One hour after the infusion began, the pups were returned to the cage. Blood samples were collected (0.3 mL/sample) just before the infusion began and thereafter at every hour. After the last sample was taken, rats were injected with an over-dose of sodium phenobarbital (1 mL/kg body weight) through the cannula. Immediately following injection, rats were perfused transcardially

with 150 mL potassium phosphate, pH 7.6 (KPBS solution) followed by 200 mL 4% paraformaldehyde (Polysciences Inc., Warrington, PA) in KPBS. Fixed brains were removed and then postfixed in the same fixative overnight. Brains were immersed into 25% sucrose in KPBS solution until they sank to the bottom of the container. Sucrose-saturated brains were snap frozen in the HistoFreeze TM-2000 (Fischer Scientific, Pittsburgh, PA) for 10–15 s, wrapped with foil, and kept in a −70°C freezer. Sectioning was done with a cryostat (Reichert-Jung 1800, Germany) at 20 µm. Sections were mounted on slides, stored at −70°C until the ICC was performed.

RIA

Plasma PRL concentrations were determined by RIA methods with materials supplied by the NIDDK and expressed in terms of the rat PRL RP-3 standard. The sensitivities of the PRL RIAs (defined as the amount of unlabeled PRL that caused a 10% displacement of iodinated PRL from antibody) ranged from 0.05 to 0.1 ng/tube. Since 50 µL of serum were routinely used in the assay, the sensitivity was 1–2 ng/mL serum. The interassay coefficient of variation was 10%, and the intraassary coefficient of variation was 5%.

ICC

c-Fos antibodies were obtained from Santa Cruz Biotech (Santa Cruz, CA) These antibodies were raised in rabbits against a highly conserved domain of p62 Fos of human origin (amino acid 128–152). This region of Fos is identical in the human, mouse, rat, and chicken. According to the data provided by the company, the specificity of the c-Fos antibody is broad, recognizing c-Fos, FosB, Fra-1, and Fra-2. Every third section from the anterior commissure (Bregma-0.26 mm) to the end of the arcuate nucleus (Bregma-4.52 mm) was analyzed, according to the rat brain atlas.

Brain sections were washed with KPBS and treated with 0.6% hydrogen peroxide in methanol (Fischer Scientific) to quench endogenous peroxidase activity, and with 10% normal goat serum in KPBS to reduce nonspecific binding. The sections were then incubated with the c-Fos antibody diluted at 1:100 in KPBS containing 2% normal goat serum and 0.01% thimerosal for 24 h at room temperature. Brain sections were incubated consecutively with biotinylated goat anti-rabbit immunoglobulin (1:100, vectastain Elite Kit; Vector Lab., Burlingame, CA) in KPBS with 0.4% Triton X-100 for 1 h, followed by KPBS with 0.4% Triton X-100 for 1 h at room temperature. This was followed by incubation with avidin DH-biotinylated horseradish peroxidase-H complex (45 µL of each A and B reagents /10 mL KPBS with 0.4% Triton X-100, Elite Kit) for another hour at room temperature. The sections were rinsed in 0.175 *M* sodium acetate buffer (pH 6.5) prior to visualization of Fos/FRA immunoreactivity. The brain sections were developed with 3, 3'-diaminobenzidine (0.2 mg/mL), nickel sulfate (25 mg/mL), and hydrogen peroxide (0.83 µL of 3% solution/mL) in the same acetate buffer for 20 min.

After the visualization of c-Fos signals, KPBS-rinsed brain sections were treated again with 0.6% hydrogen peroxide in methanol and with 1% normal horse serum in KPBS. Sections were then incubated with a mouse monoclonal TH antibody (Chemicon Inc.) diluted at 1:100,000 in KPBS containing 2% normal horse serum, 0.4% Triton X-100, and 0.01% thimerosal for 24 h at room temperature. The antibody has a broad species cross-reactivity that includes the rat. After incubation with the TH antibody, sections were incubated serially with biotinylated horse antimouse immunoglobulin (1:100, vectastain Elite Kit) and with avidin DH-biotinylated horseradish peroxidase-H complex (45 µL of each A and B reagents/10 mL KPBS with 0.4% Triton X-100, Elite Kit) for another hour at room temperature. At this time, the sections were rinsed in Tris buffer (0.05 *M*, pH 7.25) before visualization of the immunoreactivity. 3,3'-Diaminobenzidine (0.2 mg/mL) and hydrogen peroxide (0.83 µL of 3% solution/mL) in the same Tris buffer were used to visualize TH signals. Finally, sections were rinsed in distilled water and KPBS, and cover slipped with Aqueous/Dry Mounting Medium (Biomeda Corp. Foster City, CA). c-Fos-positive signals were confined to the nucleus of the cell. Each area of interest was defined according to the rat brain atlas. The number of c-Fos, TH, c-Fos/TH dual-labeled neurons were counted by visual inspection with the aid of a microscope. The arcuate nucleus was divided into rostral and caudal portions according to the rat brain atlas. The rostral region extended from Bregma-2.12 mm to -3.60 mm, whereas the caudal region extended from Bregma-3.60 mm to -4.30 mm. The appearance of the infundibulum was the demarcation between the two areas.

Statistics

Results are expressed as the mean ± standard error of the mean. Two-way repeated measures ANOVA was used to analyze plasma PRL levels from rats treated with SAL or NAL. Two-way ANOVA was used to analyze c-Fos expression, total TH neurons, and c-Fos /TH dual-staining. Post-hoc comparisons were performed with Fisher's PLSD. Unpaired *t*-test was used to test the difference between treated and control animals at each time point. The statistics program, STAT VIEW 512+ (Brainpower, Agoura Hills, CA), was used to analyze all data.

References

1. Grattan, D. R., Pi, X. J., Andrews, Z. B., et al. (2001). *Horm. Behav.* **40,** 115–124.
2. Ben-Jonathan, N. (1985). *Endocrine Rev* **6,** 564–589.
3. Ben-Jonathan, N. and Hnasko, R. (2001). *Endocrine Rev.* **22,** 724–763.
4. Bjorklund, A., Moore R. Y., Nobin, A., and Stenevi, U. (1973). *Brain Res.* **51,** 171–191.
5. Rivier, C., Vale, W., Ling, N., Brown, M., and Guillemin, R. (1997). *Endocrinology* **100,** 138–141.

6. Ferland, L., Kledzik, G. S., Cusan, L., and Labrie, F. (1978). *Mol. Cell Endocrinology* **12,** 267–272.
7. Ieiri, T., Chen, H. T., Campbell, G. A., and Meites, J. (1980). *Endocrinology* **106,** 1568–1570.
8. Mansour, A., Fox, C. A., Akil, H., and Watson, S. J. (1995). *Trends Neurosci.* **18,** 22–29.
9. Minami, M. and Satoh, M. (1995). *Neurosci. Res.* **23,** 121-145.
10. Everitt, B. J., Meister, B., Hokfelt, T., et al. (1986). *Brain Res.* **396,** 97–155.
11. Callahan, P., Baumann, M. H., and Rabii, J. (1996). *J. Neuroendocrinology* **8,** 771–776.
12. Mezey, E., Kiss, J. Z., Mueller, G. P., Eskay, R., O'Donobue, T. L., and Palkovits, M. (1985). *Brain Res.* **328,** 341–347.
13. Moore, K. E. and Lookingland, K. J. (1995). In: *Psychopharmacology: the fifth generation of progress.* Bloom, F. E. and Kupfer, D. J. (eds.). Raven Press: New York.
14. Horvath, T. L., Naftolin, F., and Leranth, C. (1992). *Endocrinology* **131,** 1547–1555.
15. Morel, G. and Pelletier, G. (1986). *Peptides* **7,** 1197–1199.
16. Sagrillo, C. A. and Voogt, J. C. (1991). *Endocrinology* **129,** 925–930.
17. Hou, Y. and Voogt, J. C. (1999). *Endocrine* **10,** 145–152.
18. Erskine, M. S. (1995). *Endocrine Rev.* **16,** 508–528.
19. Neill, J. D. and Nagy, G. M. (1994). In: *The physiology of reproduction.* 2nd ed. Knobil, E. and Neill, J. D. (eds.). Raven Press: New York.
20. Brown, B., Deffmar, P. W., Dobson, P. R., Lynn, A. G., Metcalf, G., and Morgan, B. A. (1978). *J. Pharm. Pharmacol.* **30,** 644–645.
21. Selmanoff, M. and Gregerson, K. A. (1986). *Neuroendocrinology* **42,** 255–259.
22. Arbogast, L. A. and Voogt, J. L. (1998). *Endocrinology* **139,** 2857–2862.
23. Wang, H. J., Hoffman, G. E., and Smith, M. S. (1993). *Endocrinology* **133,** 1657–1663.
24. Pape, J. R., Ciofi, P., and Tramu, G. (1996). *J. Neuroendocrinology* **8,** 375–386.
25. Van Loon, G. R., Ho, D., and Kim, C. (1980). *Endocrinology* **106,** 76–80.
26. Gudelsky, G. A. and Porter, J. C. (1979). *Life Sci.* **25,** 1697–1702.
27. Arita, J. and Kimura, F. (1988). *Endocrinology* **123,** 694–699.