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Immunoneutralization of Prolactin Prevents Stimulatory Feedback of Prolactin on Hypothalamic Neuroendocrine Dopaminergic Neurons

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We have found that exogenous prolactin (PRL) stimulates all three populations of hypothalamic neuroendocrine dopaminergic neurons. In this study, we investigated the effects of immunoneutralization of endogenous PRL on the activity of these neurons. Injection of 17β-estradiol (E_2) (20 μg subcutaneously) 10 d after ovariectomy induced a proestrus-like increase in PRL in peripheral plasma the following afternoon. At 1000 h the day after E_2 injection, rats received either rabbit antirat PRL antiserum (PRL-AS) (200 μL) or normal rabbit serum (NRS, 200 μL, controls) intraperitoneally. Groups of rats were then decapitated every 2 h from 1100 h to 2100 h. Trunk blood was collected and serum extracted with protein A to remove the PRL-AS/PRL complex, and the remaining free PRL was measured by radioimmunoassay. Sites of neuroendocrine dopaminergic nerve terminals, the median eminence (ME), and intermediate and neural lobes of the pituitary gland were excised and stored for determination of dopamine (DA) and 3,4-dihydroxyphenyl acetic acid (DOPAC) concentrations by high-performance liquid chromatography electrochemical detection (EC). In addition, the anterior lobe of the pituitary gland, the locus of DA action, was collected. The concentration of PRL in NRS-treated animals increased by 1500 h, peaked by 1700 h, and returned to low levels by 2100 h. PRL-AS prevented the increase in PRL secretion in response to E_2. The turnover of DA (DOPAC:DA ratio; an index of dopaminergic neuronal activity) in the ME of NRS-treated animals increased at 1500 h and rapidly returned to basal levels. Treatment with PRL-AS prevented the increase in DA turnover in the ME. DA turnover in the intermediate lobe increased coincident with the peak of PRL in serum of NRS-treated rats. PRL-AS administration prevented increased DA turnover in the intermediate lobe. The turnover of DA in the neural lobe increased by 1300 h and decreased steadily through 2100 h. However, administration of PRL-AS minimally suppressed the turnover of DA in the neural lobe. Moreover, administration of PRL-AS attenuated the rise of DA in the anterior lobe associated with the waning phase of the E_2-induced PRL surge. These results clearly indicate that endogenous PRL regulates its own secretion by activating hypothalamic neuroendocrine dopaminergic neurons.

Key Words: Prolactin; immunoneutralization; tuberoinfundibular dopaminergic; tuberohypophyseal dopaminergic; periventricular-hypophyseal dopaminergic.

Introduction

Three populations of hypothalamic neuroendocrine dopaminergic neurons provide dopamine (DA), the physiological inhibitor of the secretion of prolactin (PRL), to the pituitary gland *(1)*. Tuberoinfundibular dopaminergic (TIDA) neurons arise throughout the arcuate nucleus and terminate in the external zone of the median eminence *(2)* and release DA into long portal vessels *(3)* through which it is transported to the anterior lobe of the pituitary gland, the site of dopaminergic action. Tuberohypophyseal dopaminergic (THDA) neurons arising from the rostral portion of the areuate nucleus terminate in the intermediate and neural lobes of the pituitary gland *(4)*. Periventricular-hypophyseal dopaminergic (PHDA) neurons arise from the periventricular nucleus of the hypothalamus and terminate exclusively in the intermediate lobe *(5,6)*. While most work has focused exclusively on the contribution of DA from TIDA neurons *(7)*, the now classic work of Ben-Jonathan and colleagues *(8–11)* has characterized the contribution of the neurointermediate lobe of the pituitary gland to the regulation of PRL secretion. We also have demonstrated the significance of THDA and PHDA neurons in the regulation of the secretion of PRL *(1,12–17)*.

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PRL receptors (PRL-Rs) have been found on all three populations of neuroendocrine dopaminergic neurons *(18–24)*. PRL, acting through a short feedback loop, stimulates the activity of TIDA neurons *(25–37)*. In addition, we have recently found that PRL also stimulates THDA and PHDA neuronal activity *(14)*. Moreover, our laboratory has shown that PRL-Rs on neuroendocrine dopaminergic neurons are activated following administration of exogenous PRL *(38)*, thus suggesting an anatomical and physiological basis for direct PRL activity on these neurons.

The majority of previous studies addressing the issue of the regulation of neuroendocrine dopaminergic neuron activity by PRL have relied on either exogenous PRL or DA antagonists to dramatically alter the concentration of PRL. In the present experiments, we used antisera to immunoneutralize circulating PRL and provide evidence that endogenous PRL plays an important role in regulating hypothalamic neuroendocrine dopaminergic neurons.

Results

Serum PRL

The concentration of PRL in serum of normal rabbit serum (NRS)-treated rats began to increase by 1500 h, peaked by 1700 h, and returned to baseline levels by 2100 h in response to an sc injection of 17β-estradiol (E_2) given at 1000 h the previous day. Administration of PRL antiserum (PRL-AS) attenuated the E_2-mediated peak concentration of PRL to 20% of that of NRS-treated rats (Fig. 1).

DA Turnover in Median Eminence (TIDA Neurons)

The turnover of DA in the median eminence of NRS-treated animals (Fig. 2A) increased, as indicated by the increase in the DOPAC:DA ratio, coincident with the initiation of the PRL secretory increase (1500 h) and returned to low levels thereafter. However, in PRL-AS-treated animals, there was no significant change in the DOPAC:DA ratio throughout the day (Fig. 2A). Much of the change in ratio was due to greater changes in DOPAC rather than DA.

DA Turnover in Intermediate Lobe (THDA and PHDA Neurons)

In NRS-treated controls, the turnover of DA in the intermediate lobe (Fig. 2B) decreased significantly ($p < 0.05$) coincident with the initiation in the increase of PRL at 1500 h, subsequently increased with the peak of PRL secretion (1700 h) and remained elevated through the descending phase of the PRL surge (1900 h). Administration of PRL-AS prevented all the changes in the DOPAC:DA ratio observed in the intermediate lobe of NRS-treated rats (Fig. 2B). Much of the change in ratio was due to greater changes in DOPAC rather than DA.

DA Turnover in Neural Lobe (THDA Neurons)

The turnover of DA in the neural lobe of NRS-treated rats was greatest at 1300 h and 1500 h and slowly decreased thereafter (Fig. 2C). Following PRL-AS administration, the turnover of DA in the neural lobe during the initiation and peak of the PRL surge (1500 and 1700 h, respectively) was slightly but significantly ($p < 0.05$) lower than that of NRS-treated rats (Fig. 2C). Much of the change in ratio was due to greater changes in DOPAC rather than DA.

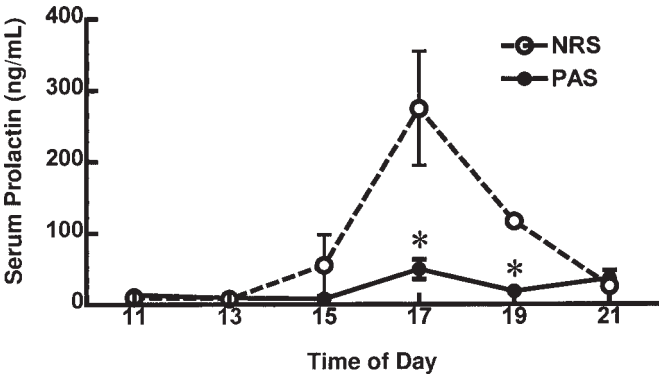

Fig. 1. Effect of NRS and anti-rat PRL-AS on the concentration of immunoassayable PRL in the serum of ovariectomized E_2-treated rats. Sera were ip injected at 1000 h * $p < 0.05$ for NRS- vs PRL-AS-treated rats. Each point is the mean ± SEM for five rats. Time of day = h × 10^{-2}.

Concentration of DA in Anterior Lobe

Prior to the initiation of the increase of PRL at 1500 h, the concentration of DA in the anterior lobe in E_2-treated rats decreased by 55% (Fig. 3). Coincident with the waning phase of PRL secretion at 1900 h, there was a rise in the concentration of DA in the anterior lobe. Although the decrease in DA in the anterior lobe prior to the initiation of the PRL surge occurred in PRL-AS-treated animals, the increase in DA following the peak of PRL (1900 –2100 h) in PRL-AS-treated rats was diminished by 50% compared with NRS-treated controls (Fig. 3).

Discussion

DA, released in the ME from TIDA neurons, is considered the primary inhibitor of the secretion of PRL *(7)*. However, we have recently shown that DA from THDA and PHDA neurons also plays a significant physiological role in the regulation of the secretion of PRL *(1,12–15)*. PRL, in turn, activates TIDA *(14,25–32,34,36,37,39,40)*, THDA, and PHDA *(14)* neuronal activity. Moreover, hyperprolactinemia increases whereas hypoprolactinemia decreases the expression of TH mRNA in the hypothalamus *(41)*. In most studies of the regulation of neuroendocrine dopaminergic neurons, hyperprolactinemia was induced by administering exogenous PRL *(14,35,38)* or D_2 DA receptor antagonists *(25,36,41)*. A recent report by Hentschel et al. *(42)* has shown that endogenous PRL plays a role in regulating the activity of TIDA neurons terminating in the ME. Specifically, they report that administration of PRL-AS inhibits the increased activity of TIDA neurons in haloperidol-induced

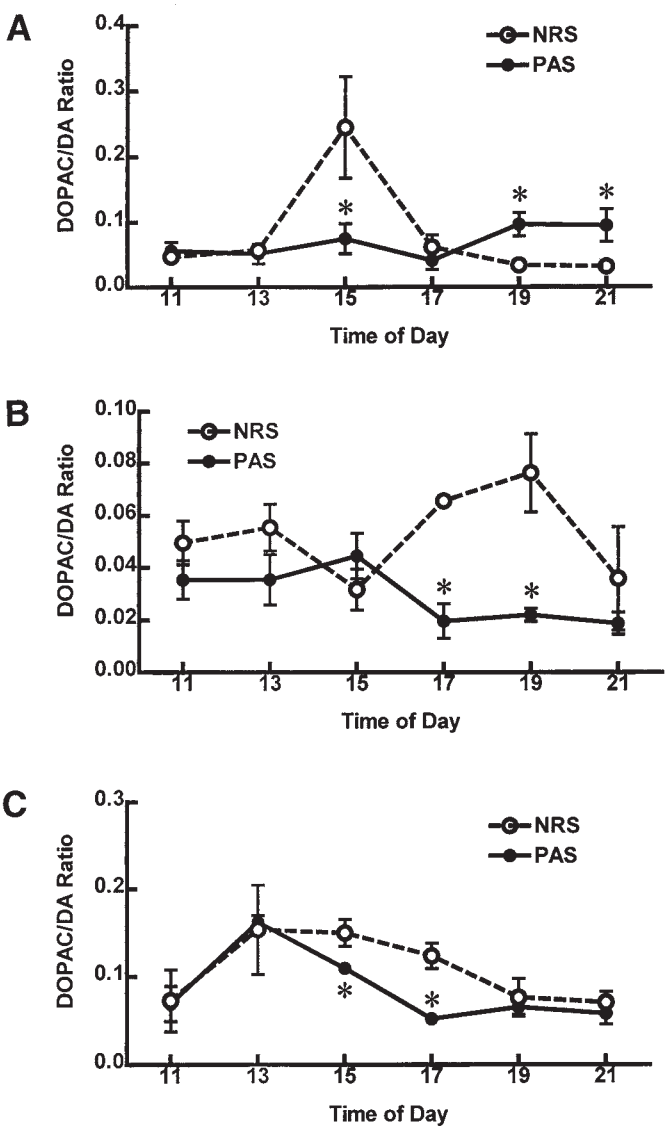

Fig. 2. DOPAC: DA (as an index of neuronal activity) in the **(A)** median eminence, **(B)** intermediate lobe of the pituitary gland, and **(C)** neural lobe of the pituitary gland after NRS or PRL-AS administration (as described in the text). These regions represent the terminal areas of the (A) TIDA, (B) THDA and PHDA (C) THDA neurons, respectively. $^{*}p < 0.05$ for NRS- vs PRL-AS-treated rats. Each point is the mean ± SEM for five rats. Time of day = h × 10^{-2}.

hyperprolactinemia. However, they did not report effects on THDA and PHDA neurons. In the present experiments, we have shown that endogenous PRL, released in response to E_2, plays a considerable role in governing the activity of all three populations of neuroendocrine dopaminergic neurons.

Injection of ovariectomized rats with E_2 at 1000 h induced a proestrus-like increase in the concentration of PRL the following afternoon. This model thus provides an increase in endogenous PRL that can be experimentally manipulated. Injection of PRL-AS decreased the peak concentrations of PRL in the circulation by 80%.

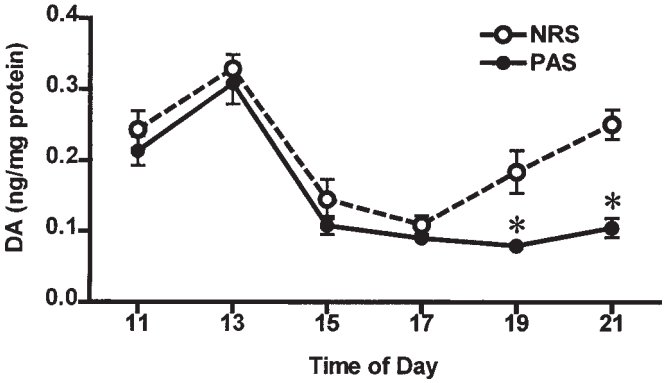

Fig. 3. The concentration of DA in the anterior lobe of the pituitary gland after NRS or PRL-AS administration (as described in the text). $^{*}p < 0.05$ for NRS- vs PRL-AS-treated rats. Each point is the mean ± SEM for five rats.

We have demonstrated that, in addition to stimulating the activity of TIDA neurons, increased levels of PRL stimulate the activity of THDA and PHDA neurons *(14)*. Likewise, administration of exogenous PRL activates PRL-Rs localized on the cell bodies of hypothalamic neuroendocrine dopaminergic neurons *(38)*. In addition, our laboratory has previously published results implicating all three populations of hypothalamic neuroendocrine dopaminergic neurons in the regulation of PRL secretion in various physiological and pharmacological states *(1,12–17,24,38)*. These data underscore the importance of including all three populations of hypothalamic neuroendocrine dopaminergic neurons when evaluating the dopaminergic regulation of PRL secretion.

In the present experiments, administration of PRL-AS depressed the concentration of DA in the anterior lobe following the peak of PRL in circulation. However, the concentration of DA in the anterior lobe is not a direct measure of the activity of neuroendocrine dopaminergic neurons *(43)*. Neuroendocrine dopaminergic neuronal activity is best monitored by measuring the turnover of DA in the individual terminal areas (TIDA: ME; PHDA: intermediate lobe; THDA: neural and intermediate lobes) *(44,45)*. The concentration of DA in the anterior lobe is an indirect measure of dopaminergic activity, because it is the sum of the activities of all three populations after release and transport through portal blood to the target tissue. The relative contribution of each of the three populations cannot be ascertained from these or our previous experiments *(1,12–17,24,38)*. However, because neurointermediate lobe denervation results in attenuation of DA concentration in the anterior lobe and an elevation of serum levels of PRL *(1)*, there seems to be little doubt that all three populations contribute DA for regulation of PRL secretion.

Coincident with increased concentrations of PRL, the turnover of DA at the terminal fields of TIDA, THDA, and PHDA neurons is enhanced *(12,14)*. Administration of

PRL-AS attenuated the PRL-induced increases in the activity of TIDA, THDA, and PHDA neurons. These data, considered with previous work, suggest that endogenous PRL acting on neuroendocrine dopaminergic neurons is responsible for the decrease in PRL in circulation.

In addition to the regulation of neuroendocrine dopaminergic neurons by PRL, it has been reported that these neurons can be influenced by other factors, including ovarian steroids *(7)*. Indeed, it has been shown that E_2 will inhibit the activity of these neurons *(46)*, whereas progesterone (P_4) stimulates their activity *(47)* and reverses the inhibitory actions of E_2 on these neurons *(48)*. A decrease in DA delivery to the anterior lobe and the decrease in dopaminergic activity prior to the increase of PRL is most likely mediated by E_2. Whereas E_2 inhibits the activity of the neuroendocrine dopaminergic neurons, PRL may mediate the reactivation of these neurons and the consequent decrease in PRL secretion that follows.

In addition to PRL and ovarian steroids, other endogenous factors may influence the activity of neuroendocrine dopaminergic neurons. Several candidates, such as vasoactive intestinal peptide *(49,50)*, oxytocin *(51,52)*, and direct retinal input *(53)*, have been implicated. However, definitive studies elucidating the exact role of these endogenous factors in influencing the activity of neuroendocrine dopaminergic neurons and consequently the secretion of PRL have yet to be undertaken. Our laboratory has previously shown that PRL secretion is under the control of several endogenous release and release-inhibiting factors *(54–60)*. Although the influence of PRL on the activity of hypothalamic neuroendocrine dopaminergic neurons has been unequivocally proven, the role of PRL in the regulation of these other factors is less than clear.

The data presented here underscore the potent role of PRL in indirectly autoregulating its secretion from the pituitary gland by stimulating the activity of all three populations of hypothalamic neuroendocrine dopaminergic neurons.

Materials and Methods

Female Sprague-Dawley rats, from Charles River, North Carolina, weighing 200–250 g were bilaterally ovariectomized under Halothane anesthesia. Ten days after ovariectomy, rats were sc injected with 20 μg of E_2 (Sigma, St. Louis, MO) at 1000 h. The following day rats were divided into two groups that received 200 μL of either NRS (Antibodies, Davis, CA) or rabbit antirat PRL-AS (raised by G. M. Nagy) by ip injection at 10:00 h. The dose of antiserum was chosen on the basis of preliminary experiments that identified the minimal dose required to block suckling-induced PRL secretion in lactating rats. The antiserum specifically recognized rat PRL. Groups of rats were sacrificed every 2h from 1100 to 2100 h. Trunk blood was collected and excess rabbit γ globulins were extracted from serum with protein A (PanSorbin; Calbiochem, La Jolla, CA). The concentration of free PRL in the resulting supernatant was determined by radioimmunoassay, with a sensitivity of 1 ng/mL, as previously described *(61)*. The separation of bound from free hormone was accomplished with PanSorbin. The intra- and interassay coefficients of variation were 5 and 10%, respectively. Neuroendocrine dopaminergic neuron terminals in the median eminence, and the intermediate, neural, and anterior lobes of the pituitary gland were excised and stored for determination of the concentration of DA and DOPAC by high-performance liquid chromatography electrochemical detection (EC), as previously described *(1,12–14)*. The sensitivity of the HPLC system was 30 pg for DA and 33 pg for DOPAC.

All animal procedures were approved by the Florida State University Animal Care and Use Committee. Data within groups (NRS- or PRL-AS-treated) were analyzed using a one-way analysis of variance (ANOVA). Data sets comparing NRS- to PRL-AS-treated animals were analyzed using a two-way ANOVA. Tukey's post-hoc test to 95% confidence was used for both the one-way and two-way ANOVA.

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