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# Norepinephrine Content in the Paraventicular Nucleus of the Hypothalamus as a Function of Photoperiod and Dopaminergic Tone

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**The decline in the release of various anterior pituitary hormones, particularly prolactin (PRL), as a result of exposure to inhibitory, short-day photoperiods in long-day breeders is a well-documented phenomenon. This alteration in the hypothalamic–hypophyseal–gonadal axis is largely controlled by changes in the duration of the nocturnal melatonin pulse secreted by the pineal gland. Increased duration of melatonin secretion serves to increase the inhibitory activity of the tuberoinfundibular dopaminergic (TIDA) neurons, which in turn suppress PRL release and synthesis. However, other hypothalamic factors also appear to stimulate anterior pituitary function, working in concert with changes in TIDA activity to modulate circulating PRL levels under different photoperiod conditions. Past work from this laboratory has suggested that neurochemical changes at the level of the hypothalamic paraventricular nuclei (PVN), particularly changes in norepinephrine (NE) activity, may represent one of these modulatory influences.**

**The current study investigated potential alterations in NF, content within the PVN during the early stages of short-day exposure in Siberian hamsters. In addition, the interaction of NE content with the modulatory dopamine (DA) system was investigated via administration of a DA agonist (CB154) or antagonist (pimozide). Hamsters received CB 154 (500 μg), pimozide (45 μg), or control solution via the drinking water under either long days (16L:8D) or short days (10L: 14D), and were sacrificed at 1, 3, or 5 wk of photoperiod and drug treatment. The brains were removed, and the PVN microdissected from the hypothalamus and analyzed for catecholamine content using high-pressure liquid chromatography with electrochemical detection (HPLC-EC). The results revealed the expected stimulation of circulating PRL under both photoperiods following pimozide administration, as well as the expected trend toward an accelerated suppression of PRL under short days induced by CB 154. For control animals, there were no changes in NE content, or the ratio of 3-methoxy-4-hydroxyphenyglycol (MHPG)/NE in long-day exposed animalx, but for short-day animals, both NE content and the ratio of MHPG/NE declined over time. Administration of CB 154 had only a marginal effect on NE content and the ratio of MHPG/NE, but pimozide administration resulted in a decline in NE content and an increase in the MHPG/NE ratio under short days, and an increase in NE content and the MHPG/NE ratio over time for long-day animals. These results suggest that both photoperiod and administration of a DA antagonist that increases circulating PRL levels induce alterations in NE terminal activity within the hypothalamic PVN.**

**Key Words:** Siberian hamster; HPLC; pimozide; bromocryptine; prolactin.

## Introduction

The reproductive status of the Siberian hamster is influenced by seasonal effects on both neuroendocrine and neurochemical systems. Exposure of hamsters to a long-day photoperiod (e.g., 16L:8D) is conducive to activation of the hypothalamic–pituitary axis, which stimulates reproductive physiology and behavior, whereas exposure to a short-day photoperiod (e.g., 10L:14D) decreases pituitary function, induces gonadal regression, and results in a cessation of the display of reproductive behavior *(1,2)*. The neuroanatomical pathway by which photoperiod information alters reproductive physiology is well characterized (*see 3* for a review). The primary signal for day-length information is contained within the duration of the nocturnal melatonin pulse secreted by the pineal gland *(4)*. However, the mechanisms by which melatonin alters the neuroendocrine axis remain to be elucidated.

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One pituitary hormone that is greatly influenced by ambient photoperiod is prolactin (PRL). PRL levels decline under short-day photoperiods in all seasonal mammals, regardless of gonadal status (*see 5* for a review). Some research suggests that the pineal melatonin signal is not fully responsible for the seasonal fluctuations that occur for this hormone. For example, some inhibitory effects of short days on PRL synthesis and release are still evident in pinealectomized female Syrian hamsters *(6)*. Furthermore, hypothalamic knife cuts in the paraventricular nucleus region that abolish the rhythm in pineal melatonin activity result in declines in circulating PRL regardless of ambient photoperiod *(7)*. These findings suggest that pineal-independent anatomical pathways may exist that function to modulate circulating PRL under some physiological conditions.

Neuroendocrine regulation of PRL is achieved largely via the inhibitory actions of the tuberoinfundibular dopaminergic (TIDA) neurons whose cell bodies are present in the arcuate nucleus, and that release dopamine (DA) into the portal vasculature via their terminals in the median eminence *(8)*. However, under many physiological conditions, the inhibitory TIDA neurons appear to work in concert with other hypothalamic factors that stimulate PRL release (*see 9* for a review). One neuroanatomical locus of origin for these factors is the paraventricular nucleus (PVN), which synthesizes and secretes several putative PRL-releasing factors. Knife cuts that disrupt the afferent connections to the PVN attenuate PRL responses under such physiological conditions as lactation and stress *(10)*, and induce declines in PRI that are independent of photoperiod exposure *(7)*. One putative releasing factor within the PVN region that may be involved in seasonal effects on PRL secretion is vasoactive intestinal peptide (VIP). Intrahypothalamic administration of VIP induces increases in PRL, whereas administration of a specific VIP antagonist results in decreased circulating PRL *(11)*. However, the neurochemical inputs that may modulate VIP activity, or that of other putative PRL-releasing factors in the PVN region have not yet been thoroughly investigated.

One potential neurotransmitter that may carry afferent information to the PVN is norepinephrine (NE). Both the parvocellular and magnocellular divisions of the PVN receive dense innervation from noradrenergic cells residing in the ventrolateral medulla and nucleus of the solitary tract *(12–14)*. This input appears to be functionally differentiated with regard to its effects on the neurosecretory neurons that ultimately modulate PRL release. NE can inhibit basal PRL release through its action on the α-2 receptor, whereas NE activation of the α-l receptor leads to stimulation of pulsatile PRL release with no effects on basal PRL levels *(15)* Moreover, circadian variations in NE release and turnover within the PVN have been described *(16–19),* raising the possibility that this neurotransmitter could have modulatory effects on PVN outputs that also regulate seasonal endocrine responses.

The current study investigated the possible role of NE in modulating seasonal fluctuations in PRL release in animals that received either a potent DA agonist, bromocryptine (CB154), or a selective D2 antagonist, pimozide (PIH), under different photoperiod conditions, or exposure to the photoperiods alone. These drugs were administered to evaluate NE content in the PVN of animals with both high and low DAergic tone, and how resulting NE may be related to both photoperiod condition and circulating PRL. This species has been shown to be relatively resistant to increased DAergic tone for inhibition of PRL under a long-day photoperiod *(20)*, and thus, provides the opportunity to assess NE content under a condition of high DAergic tone and high PRL. Furthermore, the early stages of short-photoperiod exposure were evaluated, since PRL levels begin to decline significantly by 5 wk of exposure to short days, thus allowing for any changes in NE content that might precede this reduction in circulating PRL to be detected.

## Results

### *Body Weight and Circulating Prolactin*

There were no significant changes in body weight among groups or across sampling time. There was a significant three-way interaction of photoperiod × drug × weeks on circulating PRL levels ($F_{(4,145)} = 5.55$, $p < 0.0003$). Follow-up analyses revealed that collapsing across photoperiod, this interaction was the result of differences between responsiveness to the drug condition over time. Specifically, PRL levels increased under both photoperiods for females receiving PIM, but this increase was significantly faster for short-day exposed animals ($p < 0.01$ for all comparisons). PIM also increased PRL levels under both photoperiods in males, but there was no difference between photoperiods on the rate of this increase. However, overall, PIM-induced increases in PRL were lower for males than females ($p < 0.001$ for all comparisons). There were no differences overall for CON animals under either photoperiod. However, for CB154 animals, there was a significant decrease in circulating PRL in females exposed to short days by 5 wk of treatment ($p < 0.01$), but this decrease was not seen in males. Figures 1 and 2 show PRL levels over time for females and males, respectively.

### *NE Content*

For NE, four cases were dropped because of undetectable peaks (*see* Table 1). There were no gender effects detected at any time-point; thus, subsequent analyses were collapsed across gender in order to increase statistical power (0.6–0.8). There was a significant two-way interaction of photoperiod × weeks ($F_{(2,141)} = 5.45$, $p < 0.005$). Follow-up analyses revealed that for the CON group, there was no change in NE content over time for long-day exposed animals, but there was a decrease in NE over time

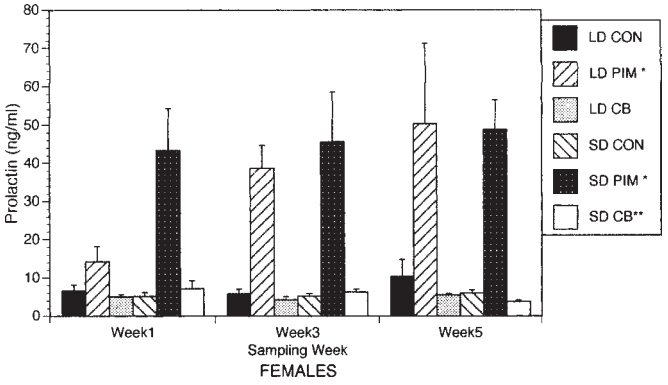


**Fig. 1.** Mean ± SEM PRL levels for females across groups and sampling times. *Significant increase over time, $p < 0.01$; **significant decrease over time, $p < 0.01$.

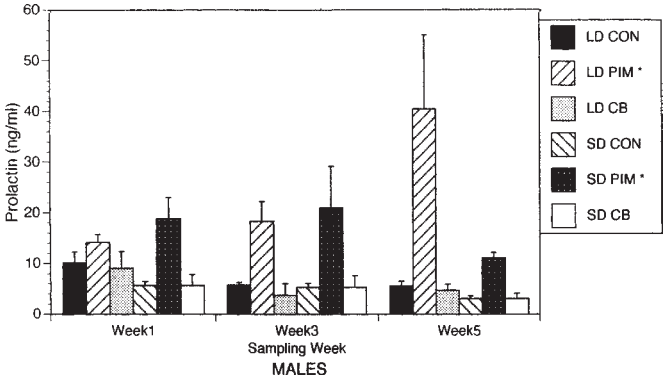


**Fig. 2.** Mean ± SEM PRL levels for males across groups and sampling times. *Significant increase over time, $p < 0.01$.

for short-day exposed animals $p < 0.05$). For CB 154 animals, no differences were seen across photoperiod or time. However, for PIM-treated animals, there was a significant increase in NE content over time for long-day exposed animals, and a significant decrease over time for short-day exposed animals ($p < 0.05$ for all comparisons). Figure 3 summarizes NE content combined across gender for each treatment group at each sampling period.

### *MHPG/NE Ratios*

For the MHPG/NE ratio, 22 subjects were dropped owing to undetectable MHPG or NE levels (*see* Table 1). There were no gender effects detected at any time point; thus, subsequent analyses were collapsed across gender in order to increase statistical power. No significant interactions were detected; however, there was a significant main effect of drug ($F_{(2,123)} = 3.09$, $p < 0.05$). Follow-up comparisons revealed that there was no change for long-day CON or CB 154 animals over time; however, there was a significant increase in the MHPG/NE ratio for PIM animals under both photoperiods, and a significant decrease for short-day CON and CB154 animals over time ($p < 0.05$ for all comparisons). Figure 4 summarizes the MHPG/NE ratios for all groups, combined across gender, over time.

## Discussion

As previously reported *(20)*, DAergic blockade via administration of the D2 receptor antagonist, PIM, was permissive for increases in PRL secretion irrespective of photoperiod condition. Furthermore, increased DA tone by administration oi the DA agonist, CB 154, did not alter PRL levels under the long-day photoperiod, but did induce a near-significant trend toward accelerated declines in short-day exposed animals. PRL levels were not sign)ficantly altered by short-day exposure in the present study, and this finding is consistent with previous work from our lab that has shown PRL levels in this species start to decline between 3 and 6 wk of short-day exposure *(11,20)* and return to long-day levels by 25 wk of short-day exposure, even in CB154-treated animals *(20)*. Thus, the PRL data confirm that these animals were tested during the early stages of photoperiod exposure prior to significant declines in PRL.

There was no clear-cut relationship between total NE content and PRL levels seen in this study. While total tissue content of NE appeared to show an effe ct of photoperiod, with increased levels being seen for animals exposed to long days and decreases for animals in short days, these data are unlikely to bear physiological significance, as it is difficult to determine whether these significant differences reflect changes in NE storage or release. However, the data are indicative of changes in the activity of NEergic terminals. Many studies have suggested that the ratio of MHPG/NE is more useful, since it has been used as an index of turnover rates, which reflect the activity of neural terminals in this region. In this case, the ratio of MHPG/NE did bear a close relationship to circulating PRL levels, with increases in this ratio being seen for PIM animals with high PRL under both photoperiods. Although significant declines in PRL were not found during the first 5 wk of short photoperiod exposure in this study, there were significantly lower MHPG/NE ratios for both CON and CB154 groups, suggesting that a decrease in NE activity may precede the short-day induced declines in PRL.

Although other brain regions were not evaluated in this study, the present data suggest a role for NE within the PVN as a modulator of circulating PRL. Under conditions of low PRL, as seen in short-day exposed animals with high exogenous or endogenous DAergic tone, the turnover rates of NE within the PVN are also low. However, when PRL levels are increased by blockade of endogenous DA activity, the NE turnover rates are significantly elevated. Although these photoperiod-associated changes in NE terminal activity may not be specific to the PVN alone, the presence of such alterations in activity in this region provides a putative mechanism for modulation of PVN-specific PRL-regulatory factors. It is unlikely that NE alters the activity of PRL-releasing factor neurons directly, since NE activation of the α-2 receptor has been shown to decrease basal PRL

**Table 1**

Number of Animals in Each Group for Analysis of NE and of MHPG/NE Ratios

| LD group | Week | NE | MHPG/NE | SD group | Week | NE | MHPG/NE |
|---|---|---|---|---|---|---|---|
| CB154 | 1 | 10 | 8 | CB154 | 1 | 8 | 7 |
| CON | 1 | 10 | 7 | CON | 1 | 10 | 9 |
| PIM | 1 | 10 | 9 | PIM | 1 | 10 | 10 |
| CB154 | 3 | 9 | 9 | CB 154 | 3 | 10 | 8 |
| CON | 3 | 9 | 8 | CON | 3 | 10 | 8 |
| PIM | 3 | 10 | 10 | PIM | 3 | 10 | 10 |
| CB154 | 5 | 10 | 9 | CB154 | 5 | 10 | 10 |
| CON | 5 | 10 | 10 | CON | 5 | 10 | 9 |
| PIM | 5 | 10 | 9 | PIM | 5 | 10 | 8 |

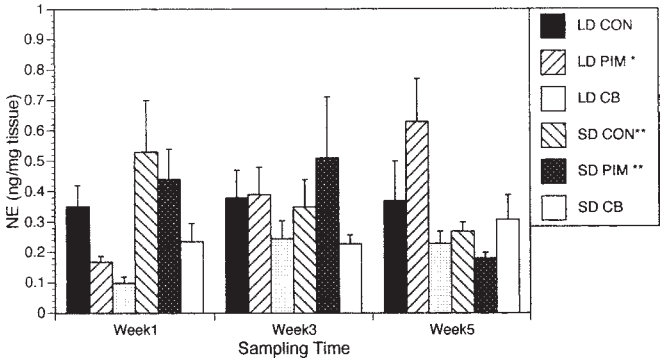


**Fig. 3.** Mean ± SEM NE content for males and females combined across groups and sampling times. *Significant increase over time, $p < 0.01$; **significant decrease over time, $p < 0.05$.

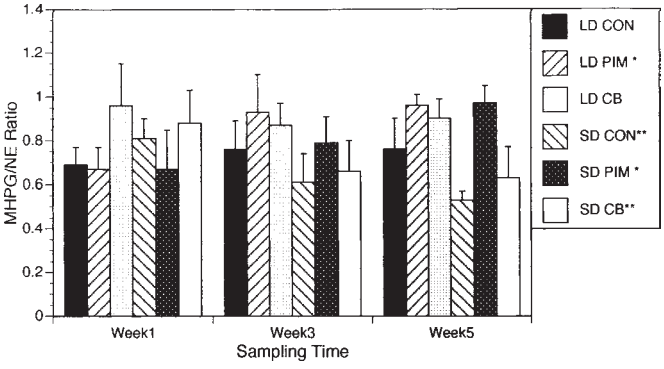


**Fig. 4.** Mean ± SEM MHPG/NE ratios for males and females combined across groups and sampling times. *Significant increase over time, $p < 0.05$; **significant decrease over time, $p < 0.05$.

(*see 15*). NE may have an indirect modulating effect within the PVN by altering the activity of an inhibitory interneuron or afferent projection. Increased NE release would thus disinhibit PRL-releasing activity, resulting in increased PRL secretion. Conversely, this species may show a predominance of α-1 receptor activation as a function of photoperiod exposure. Although work in rats has shown the α-1 receptor to be related to stimulation of pulsatile PRL release, with no effects on basal PRL levels (*see 15*), the predominance and functional significance of these receptor types may differ in photoperiodic mammals. Future research will address the receptor subtypes involved in these changes in NE content and their potential modulation by photoperiod. Furthermore, a direct assessment of NE release, rather than whole-tissue content, is necessary before the functional significance of this neurotransmitter to modulation of photoperiod-dependent fluctuations in circulating PRL can be fully ascertained.

## Materials and Methods

### *Animals and Housing*

Adult male and female Siberian hamsters (at 45 d of age) were obtained, from our long-day (16L:8D, lights off at 1800 h) breeding colony at the University of Buffalo. Animals (total N = 180) were divided into two equal groups and were maintained either under the long-day photoperiod, or a short-day photoperiod (1OL:8D, lights out at 1800 h) at a constant temperature of 25–27°C. The integrity of the light:dark cycle and colony temperatures were monitored daily by animal care personnel, and no failures were detected during the course of this experiment. All animals had access to food and water ad libitum. throughout the experiment. All procedures were approved by the State University of New York at Buffalo Institutional Animal Use Committee prior to implementation.

### *Drug Administration*

Animals under each photoperiod ($n$ = 5/group) were given one of three drug regimes: the potent dopaminergic agonist, bromocryptine (CB154; 200 µg/mL), the specific D2 receptor antagonist, pimozide (PIM; 20 µg/mL), or control solution (CON; tap water) via the drinking water. All solutions had 1 % sucrose added to increase palatability. Previous research from this lab has demonstrated that under these conditions, the animals ingest approx 500 µg CB154 and 45 µg PIM/d, and the total fluid intake does not differ among these groups *(20)*.

### *Tissue Harvesting and Preparation*

Following 1, 3, or 5 wk of drug and photoperiod treatment, the animals were sacrificed via decapitation between 0900 and 1000 h and trunk blood collected. The brains were removed, rapidly frozen, and stored at –80°C until microdissection of the PVN region. Blood was allowed to clot for 4–24 h at 4°C prior to centrifugation and serum extraction. Serum was stored frozen at –80°C until determination of PRL content via radioimmunoassay.

The brains were sliced on a freezing microtome and a 500-µm section taken through the PVN region based upon coordinates empirically determined in this laboratory. The PVN region was bilaterally microdissected from the hypothalamus of each animal, weighed, homogenized in 200 µL of 0.01 *N* perchloric acid with a sonic dismembrator, centrifuged at 10,000 rpm for 2 min, and stored at –80°C until assay for neurotransmitter content via high-pressure liquid chromatography with electrochemical detection (HPLC-EC).

### *High-Pressure Liquid Chromatography with Electrochemical Detection (HPLC-EC)*

Supernatant (20 µL) from each sample was injected with a refrigerated autosampler (ESA Model 540) onto an ESA dual-piston HPLC pump with a Coulechem II electrochemical detector and reverse-phase column optimized for detection of catecholamines. Mobile phase consisted of 75 m*M* sodium dihydrogen phosphate (monohydrate), 1.7 m*M* 1-octanesulfonic acid sodium salt, 25 µ*M* EDTA, and 10% HPLC-grade acetonitrile adjusted to pH 3.0 with phosphoric acid, and was run at 0.4 mL/min with an oxidation potential of 600 mV. Peak heights were analyzed with a Chromjet integrator and compared against standard peak chatacteristics ($10^{-7}$ *M* for all neurotransmitters). Concentrations of NE and its major metabolite, 3-methoxyhydroxyphenylglycol (MHPG), were determined in reference to tissue weight for each sample and expressed in terms of ng/mg tissue weight. Furthermore, the ratio of MHPG/NE was calculated for each subject as an index of NE activity within the PVN.

### *Radioimmunoassay (RIA)*

Serum (20 µL) from each animal was dispensed with 280 µL of 0.4% bovine serum albumin–O.1 % gelatinized phosphate-buffered saline (pH 7.0) into borosilicate tubes and analyzed for PRL content with RIA. Guinea pig antihamster PRL (final dilution, lot #AFP10302E), obtained courtesy of A. F. Parlow, served as first antibody and goat-antiguinea pig γ-globulin (final dilution = 1:108; Chemicon) was used as the precipitating secondary antibody. Purified hamster PRL (obtained from A. F. Parlow) iodinated using the chloramine T method served as trace, and serial dilutions of this hormone were used as the standard reference preparation. The lower limit of detectability of this assay was 2.75 ng and the coefficients of variance at 20, 65, and 80% bound were 4, 9, and 12%, respectively.

### *Statistics*

PRL and neurotransmitter content between groups were analyzed with between subjects' ANOVAs. Significant interactions were broken down using Analysis of Simple Main Effects, and individual post-hoc comparisons were evaluated with Scheffe's F tests where appropriate. All values were considered significant if $p < 0.05$.

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