Expression of hypothalamic GnRH-I mRNA in the female turkey at different reproductive states and following photostimulation

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Received 4 February 2005; revised 7 September 2005; accepted 9 October 2005
Available online 19 January 2006

Abstract

In birds, changes in hypothalamic gonadotropin-releasing hormone-I (GnRH-I) content and release are correlated with reproductive stages. This study examined the distribution and expression level of GnRH-I mRNA in anatomically discrete hypothalamic nuclei throughout the turkey reproductive cycle and following photostimulation. GnRH-I mRNA expression was determined using in situ hybridization in non-photostimulated (NPS), egg-laying (LAY), incubating (INC) and photorefractory (REF) hens. Overall, GnRH-I mRNA expression was greatest in the nucleus commissurae pallii (nCPa) and around the organum vasculosum lamina terminalis (OVLT), with less expression observed in the nucleus septalis lateralis (SL), cortico-habenula cortico-septum area, and within the nucleus preopticus medialis. GnRH-I mRNA expression was significantly increased in nCPa, OVLT, and SL after NPS hens (6L:18D) were exposed to a 30 or 90 min pulse of light beginning 14 h after first light (dawn). GnRH-I mRNA abundance within nCPa, OVLT and SL was greater in LAY than in NPS and INC hens, while mRNA expression was least in REF hens. These results indicate that GnRH-I mRNA expression in birds is sensitive to light stimulation during the photosensitive period and can be used to more precisely characterize their different reproductive stages.

Keywords: GnRH-I mRNA expression; Turkey reproductive cycle; Photostimulation; Avian hypothalamus; In situ hybridization

1. Introduction

In birds, the secretion of luteinizing hormone (LH) is controlled by gonadotropin releasing hormone-I (GnRH-I) (Sharp et al., 1998). The distribution and changes in GnRH immunoreactivity has been reported in brain areas of several avian species (Deviche et al., 2000; Katz et al., 1990; Kuenzel and Blahser, 1991; Mikami and Yamada, 1984; Mikami et al., 1988; Millam et al., 1993; Parry et al., 1997). Although there are species differences, significant populations of immunoreactive perikarya are located in the septal, preoptic and anterior hypothalamus, lying close to midline (Kuenzel and Blahser, 1991). Several studies have reported the distribution of GnRH-I mRNA and protein in avian brains (Dawson et al., 2002; Dunn and Sharp, 1999; Millam et al., 1989; Sun et al., 2001). However, the topographical distribution pattern of avian GnRH-I mRNA has not been well coordinated with the reproductive conditions. Photic stimuli play a pivotal role in the initiation of sexually-related neuroendocrine and behavioral changes in birds (Peczely and Kovacs, 2000). The effects of season and photostimulation on the hypothalamic-gonadal axis are well characterized, while the mechanisms that transduce relevant photic information to neuroendocrine effector neurons are not well established (Cho et al., 1998; Dawson and Goldsmith, 1997; Dunn and Sharp, 1999; Peczely and Kovacs, 2000). Time-course analysis of changes
in basal hypothalamic GnRH-I content during photostimulation in the male starling provides the explanation for the photo-induced gonadal cycle (Dawson et al., 2002). However, the mechanisms that transduce relevant photic information into GnRH-I expression are far from clear.

Therefore, the present study was designed to: (1) determine the distribution of GnRH-I mRNA in the turkey hypothalamus; (2) compare hypothalamic expression of GnRH-I mRNA between the different reproductive stages; and (3) determine whether or not the expression of GnRH-I mRNA is photo-sensitive.

2. Materials and methods

2.1. Experiment 1

Somatically mature, large, white, female turkey hatchmates of Nicholas strain were used. The birds were housed under a day/night regime of 6 h of light and 18 h of dark (6L:18D). Three groups, of 20 birds each, were photostimulated (15L:9D) at successive four week intervals. Birds in the fourth group were not photostimulated (6L:18D). This allowed us to obtain birds of the same age in four reproductive states: (1) non-photostimulated (NPS), (2) hens that had been laying eggs (LAY) for 3–4 weeks, (3) hens that had just stopped laying eggs and completed molting. The four physiological groups included in each batch. However, sections from Experiments 1 and 2 were included in each batch. Therefore, the present study was designed to: (1) determine the distribution of GnRH-I mRNA in the turkey hypothalamus; (2) compare hypothalamic expression of GnRH-I mRNA between the different reproductive stages; and (3) determine whether or not the expression of GnRH-I mRNA is photo-sensitive.

2.2. Experiment 2

In the light-induced GnRH-I mRNA expression study, NPS hens were maintained on a 6L:18D lighting schedule (light on at 8 am and off at 2 pm) for at least 8 weeks to ensure photosensitivity and then were exposed to a pulse of light of 30 or 90 min duration beginning 14 h after first light. Control hens (that did not receive the light pulse) were euthanized at 14 h after first light while the hens receiving the 30 or 90 min light pulse were perfused immediately after the light pulse ended. There were eight hens per treatment group.

2.3. Tissue preparation

Turkey heads were pressure-perfused via the carotid arteries for 5 min with 0.1 M phosphate-buffered saline (PBS), and for 30 min with 4% formaldehyde solution in 0.1 M phosphate buffer (pH 7.4). The brain, removed from the skull, was soaked in 20% sucrose in PBS at 4 °C for four days, frozen on dry ice, and stored at –80 °C. Brains were sectioned in a cryostat at a thickness of 16 μm and mounted onto microscope slides (Probe-On; Fisher Scientific, Minneapolis, MN) and then were stored desiccated at –80 °C until further processed.

2.4. Cloning of turkey GnRH-I cDNA and preparation of GnRH-I cRNA probes

Turkey hypothalamic tissue was homogenized in Trizol and total RNA was extracted based on the manufacturer’s protocol (Ambion). SuperScript II RT was then used to transcribe 2 μg of total RNA into cDNA, following the manufacturer’s protocol (Invitrogen). A portion of the cDNA synthesis reaction was amplified using a thermocycler and the following cycling protocol: a 2 min denaturation at 94 °C followed by 34 cycles of a 1 min hold at 94 °C, a 1 min primer annealing step at 60 °C and a 1 min extension step at 72 °C. The primers were based on the known chicken GnRH-I cdNA (GenBank Accession Number: X09491); exon 1, 5’-GAA GAG TTG GAG CGA TTC TGC-3’; exon 2, 5’-ATC TTG GTC GGT GTC CTC CTG TTC ACC GCA-3’; and exon 4, 5’-GAT CGA AGG AGA AGC CAG AAG A-3’. The amplified products were cloned into pgEM-T Easy vector, and transformed into JM109 cells according to manufacturer’s protocol (Promega). Nucleotide sequence analysis of positive clones was performed on both strands by automated DNA sequencing (Advance Genetics Analysis Center, University of Minnesota, St. Paul, MN). A 250 bp cdNA clone in pgEM T Easy vector was used to make antisense GnRH-I cRNA using an in vitro transcription kit (Ambion). The cRNA probe was purified by a G-25 RNA purification column (Roche).

2.5. In situ hybridization

To localize gene expression of GnRH-I within the hypothalamus, in situ hybridization (ISH) was performed as previously described (Bhatt et al., 2003; Chaisenha et al., 2003). As all sections could not be processed in a single set, sections were processed in several batches, with sections representing all treatment groups and all pertinent parts of the hypothalamus included in each batch. However, sections from Experiments 1 and 2 were processed separately. Briefly, tissue sections were thawed to room temperature before use. The slides were hybridized with 200 μl of hybridization solution containing 33P-UTP-GnRH-I cRNA probe (0.25 × 106 cpm/slide) incubated at 52 °C for 16 h in a humid chamber. The hybridization solution contained 50% formamide; 300 mM NaCl; 10 mM Tris–HCl, pH 8.0; 1 mM EDTA; 0.02% polyvinylpyrrolidone; 0.02% Ficoll 400; 0.02% bovine

Table 1

The expression of GnRH-I mRNA within individual hypothalamic areas (nucleus septalis lateralis, SL; organum vasculosum laminae terminalis, OVLT; nucleus commissurae pallii, nCPa; cortico-habenula cortico-septum CHCS), and plasma PRL and LH concentration (ng/ml) of turkey hens at different reproductive stages (i.e., non-photostimulated, NPS, egg-laying, LAY; incubating, INC; photorefractory, and REF)

<table>
<thead>
<tr>
<th>Hypothalamic area</th>
<th>Reproductive group</th>
<th>NPS PRL/LH</th>
<th>LAY PRL/LH</th>
<th>INC PRL/LH</th>
<th>REF PRL/LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL</td>
<td>7.68 ± 1.364</td>
<td>2.06 ± 0.438</td>
<td>162.34 ± 34.96</td>
<td>7.61 ± 1.954</td>
<td>375.29 ± 71.59</td>
</tr>
<tr>
<td>OVLT</td>
<td>38.0 ± 6.84</td>
<td>100.0 ± 21.24</td>
<td>51.6 ± 10.04</td>
<td>34.6 ± 5.24</td>
<td>23.6 ± 4.84</td>
</tr>
<tr>
<td>nCPa</td>
<td>43.6 ± 10.44</td>
<td>182.8 ± 31.68</td>
<td>54.8 ± 12.46</td>
<td>32.4 ± 5.24</td>
<td>32.4 ± 5.24</td>
</tr>
<tr>
<td>CHCS</td>
<td>75.2 ± 12.34</td>
<td>260.8 ± 33.6</td>
<td>96.3 ± 18.74</td>
<td>34.6 ± 5.24</td>
<td>58.1 ± 6.59</td>
</tr>
<tr>
<td></td>
<td>32.4 ± 5.24</td>
<td>130.0 ± 19.6</td>
<td>40.4 ± 9.64</td>
<td>26.8 ± 6.84</td>
<td></td>
</tr>
</tbody>
</table>

Values of mRNA expression represent mean ± SEM of total integrated pixels (×103). Values with different superscripts are significantly different (P < 0.05, n = 8).
serum albumin; 10 mM dithiothreitol; 500 ng/ml yeast tRNA; 1% SDS, and 10% dextran sulfate. The slides were paired together instead of using coverslips before incubation. After hybridization, the slides were washed once with 2× SSC for 15 min at 52 °C, once with 1× SSC for 15 min at 52 °C, twice with 0.5× SSC for 15 min at 52 °C, and twice with 0.1× SSC for 15 min at 52 °C. After the final wash, the sections were allowed to slowly cool to room temperature in the washing solution. The sections were then dehydrated through graded alcohol (50–100%) and quickly air dried. The sections were then dipped in NTB; nuclear track emulsion (Kodak, Rochester, NY) diluted 1:1 with distilled water, dried, and stored in a light-proof box at 4 °C until developing (6 h). The dipped sections were developed at room temperature with D-19 developer (Kodak) diluted 1:1 with water for 3 min, followed by a 20 s rinse in water, and fixed in a rapid fixer (Kodak) for 3 min. The sections were then rinsed with water for 3 min. Following the development of autoradiographic grains, the sections were counterstained with the fluorescent dye Hoechst 33258 (0.001% bis-benzimide in 0.2 M KCl–HCl buffer, pH 2.0; Sigma, St. Louis, MO) as described by Schnell and Wessendorf (1995) and co Coverslipped with DPX Mountant (Fluka Chemical, Ronkonkoma, NY). Autoradiographic grains and counterstain were visualized with a florescence microscope equipped with a darkfield condenser (Nikon Eclipse TE200; Nikon, Japan).

2.6. RIA

Plasma prolactin (PRL) and LH concentration were determined by the methods of Proudman and Opel (1981) and Bacon and Long (1996), respectively. The PRL and LH concentrations from blood samples for the four different reproductive states were each measured within a single RIA. Intra-assay variability was 5.1% for the PRL RIA and 12.1% for the LH RIA.

2.7. Quantitative analysis of GnRH-I mRNA

The localization of GnRH-I mRNA expression within the turkey hypothalamus was selected and identified according to atlases of the turkey hypothalamus (Younghren, unpublished) and the chicken brain (Kuenzel and Masson, 1988). The microscopic images of the brain sections were visualized at 4 and 20× magnification using a videocamera (CoolCam 2000 color) fitted to a fluorescent microscope (Nikon TE200 Eclipse, Nikon, Japan). The images were then captured and stored by ImagePro-Plus (Media Cybernetics, Silver Spring, MD, USA) with a fixed setting for videocapture. Eight microscopic fields on pairs of adjacent sections (four microscopic fields/section) from each bird were chosen for area imaging in each reproductive group or photostimulated group (PS: 30 and 90 min).

These microscopic fields correspond to the following brain areas: (1) The nucleus septalis lateralis (SL), where GnRH mRNA is scattered throughout its extent, but mainly concentrated at the rostral-caudal midpoint. (2) The organum vasculosum laminae terminalis (OVLT), where GnRH mRNA is grouped in the rostral-dorsal POM immediately adjacent to OVLT. (3) The nucleus commissurae pallii (nCPa), where GnRH mRNA is clustered at midline surrounding this structure. (4) The tractus cortico-habenularis et cortico-septalis (CHCS), where GnRH mRNA is located in the anterior dorsal medial thalamus near the CHCS.

2.8. Statistical analysis

Images were inverted for measuring integrated pixels. Densitometric analysis of in situ autoradiographs was performed with the Scion Software (NIH Image version for PC, http://www.scioncorp.com) to determine the integrated pixels of the radiolabel in hypothalamic regions (SL, OVLT, nCPa, and CHCS). Background was measured from eight nonhybridizing tissue fields, averaged, and subtracted from the integrated pixels of hybridization signals. Values corresponding to the expression of GnRH-I mRNA in the defined areas were calculated for each bird by summing the integrated pixel values from eight microscopic fields from two consecutive sections. Results were expressed as mean ± SEM of total integrated pixel. A two-way fixed ANOVA, using the general linear model procedure, was used to evaluate GnRH-I mRNA, PRL, and LH plasma levels (SAS ver 8.2, SAS Institute, 2001). A P < 0.05 was considered statistically significant.

3. Results

Two different sized GnRH-I cDNAs were cloned from turkey hypothalamus mRNA using the sequence information from chicken GnRH-I (Fig. 2; Dunn et al., 1993). The overall nucleotide and deduced amino acid sequence of turkey cDNA (GenBank Accession No. AY632693) is 91% homologous with those of chicken GnRH-I cDNA (Dunn et al., 1993). Riboprobe L fragment consists of a 40-bp 5′ UTR, a 70-bp signal sequence, a 30-bp coding region of GnRH-I, and a 42-bp in exon 2, a 97-bp in exon 3, and a 29-bp in exon 4 of GnRH associated peptide (GAP). The probe used in this study, which showed high specificity in ISH, did not contain the 5′ UTR. Therefore, we used the smaller fragment (Riboprobe S in Fig. 1) for this study in order to discriminate the subtypes of GnRH-I and -II, and because of the specificity of the probe.

The survey of GnRH-I expression in the hypothalamus of the turkey hen (Experiment 1) demonstrates that GnRH-I mRNA is expressed in the nucleus preopticus medialis (POM), nucleus septalis lateralis (SL), organum vasculosum lamina terminalis (OVLT), nucleus commissurae pallii (nCPa), and tractus cortico-habenularis et cortico-septalis (CHCS) (Fig. 2). The greatest density of GnRH-I mRNA for all areas measured is found in the nCPa area where GnRH-I immunoreactive neurons were observed (Millam et al., 1993). The next highest density of GnRH-I mRNA-expressing cells is observed in the SL, OVLT, and CHCS (Table 1). The least amount of GnRH-I mRNA is found in cells in the POM (Fig. 2A). The expression of GnRH-I mRNA is significantly greater in the nCPa of LAY hens (Fig. 3), and markedly less in NPS, INC, and REF hens when compared with LAY hens (Fig. 4). The most dense GnRH-I mRNA, found in the nCPa, is 1.3-, 4.5-, and 1.7-fold greater in NPS, INC, and REF hens, respectively, as compared with that of REF hens, the lowest group, though expression in REF hens is not statistically different from NPS hens (Table 1). These data also show differential expression of GnRH-I in a specific area of the hypothalamus and reveal that this
Differential expression is highly correlated with the reproductive states and with the circulating LH levels of the hens (Table 1). In Experiment 2, which was designed to test the photosensitivity of GnRH-I mRNA expression in the turkey hypothalamus, photosensitive NPS hens were exposed to a 30 or 90 min pulse of light beginning 14 h after first light (dawn). The areas which expressed GnRH-I mRNA were compared with short day controls. After 30 min photostimulation, GnRH-I mRNA levels around OVLT and nCPa area of NPS hens significantly increased (Figs. 5A and B), but not in SL and CHCS (data not shown). However, 90 min photostimulation has affected the stimulation of GnRH-I mRNA in SL area as well as OVLT and nCPa area (Fig. 5B).
4. Discussion

The avian reproductive cycle is strongly regulated by the VIP and GnRH systems in the hypothalamus, and there are several reports delineating the distribution of immunoreactive GnRH neurons and fibers in the avian brain (Kuenzel and Blahser, 1991; Mikami et al., 1988; Millam et al., 1993). In the turkey, it has been suggested that GnRH-I and -II occur in separate neuronal systems and that GnRH-II does not directly promote pituitary gonadotropin secretion (Millam et al., 1993). Therefore, this study addressed, for the first time, the distribution of GnRH-I mRNA in specific areas of the turkey hypothalamus during different reproductive stages and the activation of GnRH-I mRNA expression by photostimulation.

Hypothalamic neuronal activation, as indicated by Fos-like protein expression, is shown to occur in quail in response to long day exposure. However, Fos-like immunoreactivity has not been observed in GnRH cells in association with the photoperiodically driven LH rise (Meddle and Follett, 1997). In a preliminary study, we examined the effect of a single 30 min light pulse on c-fos mRNA expression as a neuronal activation marker in the female turkey hypothalamus (unpublished data). The results showed that a single 30 min light period, given 14 h after first light to non-photostimulated (NPS) hens maintained under a short day light regimen (6L:18D), induced c-fos mRNA in hypothalamic areas known to contain GnRH-I perikarya (Millam et al., 1993). In the present study, we confirmed that a similar light segment was able to upregulate GnRH-I mRNA expression within 30 min, 14 h after the start of the daily 6 h light period. A light stimulus at this time is shown to increase LH release and to induce gonad recrudescence (Follett and Sharp, 1969). To our knowledge, the present study is the first to show an abrupt photoperiodic induction of avian GnRH-I mRNA expression and the first to demonstrate a neuronal link between hypothalamic GnRH-I and the gonad stimulating photoperiod. An enhanced GnRH-I release by hypothalamic explants was found to occur 23 h after a single long day in quail (Perera and Follett, 1992), and an increase in hypothalamic GnRH-I peptide level was observed following photostimulation in starlings (Dawson and Goldsmith, 1997; Dawson et al., 2001). Taken together, these findings are presumed to suggest that photostimulation appears to immediately stimulate GnRH-I synthesis. Studies in the hamster also show increases in hypothalamic GnRH mRNA (Porkka-Heiskanen et al., 1997) and peptide (Bernard et al., 1999) levels during the transition from short to long days. In the chicken, hypothalamic GnRH-I mRNA is measured in response to a changing photoperiod (Dunn and Sharp, 1999); the response is variable and dependent upon the age and the steroid status of the bird. It is of interest to note that chickens descended from an ancestral non-seasonally breeding tropical species, whereas domestic turkeys are descendants of a temperate zone photoperiodic species and they still do exhibit photoperiodic cycles.
The neuronal circuits that regulate GnRH-I expression and release and that are involved in the processing of the photoperiodic response are far from clear. There are indications that VIP may play a role in the seasonal reproductive transition in avian species (Chaiseha et al., 1998). In avian species, hypothalamic VIP peptide and mRNA are photoperiodically controlled (Chaiseha et al., 1998; Mauro et al., 1992). Levels are low in reproductively quiescent short day birds, increase following photostimulation, and reach their highest levels during incubation and photorefractoriness (Chaiseha and El Halawani, 1999). These levels remain elevated unless birds are subjected to a short day light regimen. Currently, very little information is available concerning mechanisms by which VIP affects the avian reproductive neuroendocrine system.

Active immunization against VIP prevents photo-induced PRL increase (El Halawani et al., 1996), upregulates LHβ and FSHβ subunit mRNAs (Ahn et al., 2001) and delays the onset of photorefractoriness (Dawson and Sharp, 1998). These findings led to the suggestion that VIP may be involved in mediating photoperiodic information concern ed with the regulation of the GnRH-I/gonadotropin system.

It is interesting to note that a light pulse similar to the one used in this study is capable of inducing iodothyronine deiodinase II expression, an enzyme responsible for converting thyroxine to triiodothyronine in the mediobasal hypothalamus (MBH) of short day photosensitive quail (Yoshimura et al., 2003). Thyroid hormones may be important for the regulation of photoperiodically induced gonad recrudescence (Dawson et al., 2001; Yoshimura et al., 2003). Treatment with thyroxine can mimic the effects of long photoperiods (Follett and Nicholls, 1988; Goldsmith and Nicholls, 1992; Wilson and Reinert, 1995). In starlings and sparrows, the prevention of photorefractoriness by thyroidec tomy is associated with maintenance of the high hypothalamic levels of GnRH-I typical of photosensitive birds (Dawson et al., 1985; Reinert and Wilson, 1996) and thyroidectomy of photorefractory birds results in an increase in GnRH-I (Dawson et al., 1986). Central administration of thyroid hormones to thyroidectomized tree sparrows restores all of the photoperiodic responses (Wilson and Reinert, 2000).

The results of the present study show that the magnitude of GnRH-I mRNA expression in the septopreoptic region is dependent upon the reproductive state of the bird. Levels of GnRH-I mRNA are highest in LAY hens, as compared to that of sexually inactive hens (i.e., NPS, INC, and REF). Even though GnRH-I mRNA expression did not vary significantly between NPS and REF hens, GnRH-I mRNA levels of INC and NPS hens were, respectively, 1.7- and 1.3-fold greater than that of REF hens. Consistent with the present findings, hypothalamic GnRH-I peptide levels are highest in LAY and depressed in REF, INC, and NPS turkey hens (Rozenboim et al., 1993a). In INC chickens, GnRH-I peptide levels are similar to that of LAY hens; however, as in the present study, GnRH-I mRNA is reduced (Dunn et al., 1996). Unlike the results of the present study, the distribution and the staining intensity of GnRH-I immunoreactive neurons were similar in short day and photostimulated starlings (Foster et al., 1987).
These findings, taken together, lead to the suggestion that photoperiodic differences in reproduction, as well as the transitions between reproductive states, likely reflect, in part, changes in GnRH-I synthesis. Furthermore, the finding that GnRH-I neurons in all hypothalamic structures investigated (nCPa, OVLT, SL, POM, and CHCS) showed similar changes in GnRH-I mRNA expression, either in response to the light pulse or among reproductive states, implies that regional specificity (i.e., different subsets may specifically mediate preovulatory LH surge, tonic LH release or photoperiodically-induced LH release) does not exist in the those GnRH-I neurons.

The mechanism(s) underlying the suppressed GnRH-I mRNA expression in sexually inactive birds (i.e., NPS, INC, and REF) may vary with the reproductive state. In hyperprolactinemic INC hens, elevated PRL levels may play a prominent role in GnRH-I mRNA suppression, as the antigonadotropic effects of PRL are well established. Central administration of PRL reduces circulating LH levels and terminates egg laying (Youngren et al., 1991). And systemic PRL administration reduces hypothalamic GnRH-I peptide (Rozenboim et al., 1993b) and the photoperiodically-induced rise in circulating LH (El Halawani et al., 1991). Nest deprivation is known to suppress PRL levels and upregulate GnRH-I mRNA expression in incubating chickens (Dunn et al., 1996). The low GnRH-I mRNA content observed in short day photosensitive turkeys appears to be related to the lack of photostimulation, since providing a light pulse, presumably during the photosensitive phase, up-regulates GnRH-I mRNA (this study), increases LH release and induces gonad recrudescence (Follett and Sharp, 1969).

The results of the present study show that the synthesis of GnRH-I is at its lowest point during the initiation of photorefractoriness, when compared to that observed in the other reproductive states. These photorefractory hens will require at least 8 weeks of short day light treatment (6L:18D) in order to regain photosensitivity. Inhibition of the GnRH-I releasing mechanism(s) has also been reported to occur at the initiation of photorefractoriness in the white-crowned sparrow (Meddle et al., 1999). These findings suggest that the first neuroendocrine indication of the onset of photorefractoriness is the inhibition of, or the lack of stimulation of, GnRH-I synthesis and release. These findings further suggest that the maintenance of elevated levels of GnRH-I peptide observed at the onset of photorefractoriness (Dawson et al., 2001) is a consequence of the inhibition of its release, and not due to an increased synthesis of the peptide. Thereby, it appears that the transient fluctuations in GnRH-I peptide content observed during the photorefractory stage may be related to the degradation of the peptide and does not accurately reflect the functional activity of the GnRH-I system. It is of interest to note, at the onset of photorefractoriness, that the VIP/PRL system, as in the case of the GnRH-I/gonadotropin system, is also characterized by hypoprolactinemia (Chaiseha and El Halawani, 2005), diminished VIP mRNA expression in the infundibular nuclear complex (INF; Chaiseha and El Halawani, 1999) and elevated hypothalamic VIP peptide (Mauro et al., 1992). Nevertheless, the two systems respond positively to treatments with neurotransmitters. A glutamate agonist will override the suppressive effect of photorefractoriness on LH secretion in white-crowned sparrows (Meddle et al., 1999), and microinjection of a dopamine receptor agonist in the INF of photorefractory turkeys induces as strong a release of PRL as that observed in laying hens (Youngren et al., 2002). These findings are interpreted to suggest that a reduced neurotransmission (i.e., glutamatergic and dopaminergic) to GnRH-I/VIP systems is responsible for the suppressed gonadotropin and PRL secretions and the induction of gonad regression associated with the onset photorefractoriness.

Acknowledgments

We thank I. C. Dunn for providing of cGnRH-I cDNA, and O. M. Youngren for comments on the manuscript. This research was supported by Research Grant No. US-3399-03C from BARD, The United States–Israel Bionational Agricultural Research and Development Fund.

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