Photoperiod and Testosterone Regulate Androgen Receptor Immunostaining in the Siberian Hamster Brain

Eric L. Bittman, David A. Ehrlich, Justyne L. Ogdahl and Amy E. Jetton

Department of Biology, Center for Neuroendocrine Studies, and Programs in Neuroscience and Behavior and Molecular and Cellular Biology; University of Massachusetts, Amherst, Massachusetts 01003

ABSTRACT

Day length regulates the effects of gonadal steroids on gonadotropin secretion and behavior in seasonal breeders. To determine whether this influence of photoperiod results from changes in androgen receptor expression in Siberian hamster brain regions that regulate neuroendocrine function, androgen receptor immunostaining was examined in castrated animals given either no androgen replacement or one of three doses of testosterone (T) resulting in physiological serum concentrations. Half of the animals were housed under inhibitory photoperiod conditions, and immunostaining was quantified 11 days later. Measurement of serum gonadotropin and prolactin concentrations confirmed that androgen exerted graded effects on pituitary function but that the animals were killed before photoperiodic influences had fully developed. T significantly increased the numbers of androgen receptor-immunoreactive cells in every brain region examined. Photoperiod exerted no significant influence on androgen receptor-immunoreactive cell number in the arcuate nucleus, bed nucleus of the stria terminalis (BNST), medial preoptic nucleus, or in medial amygdala. An interaction between T and photoperiod was observed in the BNST and in the rostral and middle portions of the arcuate nucleus. Although increasing concentrations of T resulted in more intense cellular immunostaining in the BNST and arcuate, this effect was not influenced by day length. These results indicate that relatively short-duration (11 days) exposure to inhibitory photoperiod triggers localized and regionally specific changes in androgen receptor expression.

androgen receptor, hypothalamus, melatonin, pineal, seasonal reproduction

INTRODUCTION

Mammals of the temperate zone commonly restrict reproduction to a particular season so that young are born when food is plentiful. Day length (photoperiod) provides a reliable indication of time of year, and seasonal breeders utilize this signal to regulate reproduction [1]. In Siberian hamsters (Phodopus sungorus) raised on summer day lengths, gonads regress and testicular steroidogenesis ceases over the course of approximately 8 wk following transfer to short photoperiods [2]. The collapse of the gonads is precipitated by drastic reductions in serum concentrations of LH and FSH, and prolactin (PRL), which are in turn triggered by a reduction in GnRH secretion [3–5]. The effects of day length are dependent upon the integrity of the pineal gland, which encodes photoperiod in the nightly duration of melatonin secretion [6].

Day length regulates the secretion of gonadotropins in both intact and castrated seasonal breeders. Thus, serum FSH levels are significantly higher in long-day than in short-day orchidectomized hamsters [3, 7]. This phenomenon, which is well documented in a variety of seasonal breeders [8–10], has been termed a steroid-independent effect of day length. However, short photoperiods also enhance the negative feedback influence of gonadal steroid hormones upon gonadotropin secretion. Thus, serum FSH concentrations are also significantly lower in short- than in long-day castrated Siberian hamsters implanted with capsules that release constant physiological doses of androgen [7]. The administration of low doses of testosterone (T) completely reverses the effect of castration on FSH concentrations in short-day animals but only partially suppresses FSH in long-day animals. In other species, photoperiod also modulates the induction of male and female sexual behavior by gonadal steroid hormones [11–16].

The mechanisms underlying these photoperiod-induced changes in responses to T are not understood. Attention has been focused on the possibility that photoperiod regulates the expression of specific high-affinity intracellular steroid hormone receptors in brain regions that govern gonadotropin secretion and sexual behavior. In light of the ability of gonadal steroids to regulate expression of their own receptors, experiments could be conducted in seasonal breeders in which circulating concentrations of these hormones are manipulated to be comparable between long- and short-day treatment groups. Initially, such experiments utilized in vitro binding assays to assess either the affinity or the number of steroid receptors in homogenates of dissected brain areas. Results obtained using this approach generally failed to demonstrate significant effects of photoperiod in the brains of castrated and steroid-replaced seasonal breeders [15, 17–20]. With the development of antibodies to such receptors, however [21], it became possible to utilize immunocytochemical techniques to investigate steroid hormone receptor expression with greater anatomical resolution [22–24]. Such methods have seen limited application in experiments designed to study the basis of seasonal breeding. Melatonin injections reduced the number of estrogen receptor-immunoreactive cells [25] and short days...
suppressed the induction of progestin receptors by estrogen in the forebrain of female Syrian hamsters [16]. In contrast, the possible impact of photoperiod upon androgen receptor (AR) immunoreactivity (AR-ir) in seasonally breeding male mammals has received little attention. Despite extensive work on the neurobiology of photoperiodic responses in the Siberian hamster [2, 5, 7, 26, 27], the effect of day length on AR expression has not been explored in this species. The present experiments were undertaken to test the hypothesis that photoperiodic regulation of the neuroendocrine effects of androgen depends on effects of day length on the number of AR-ir cells in brain regions that are believed to mediate feedback and behavioral actions of T in Siberian hamsters. For changes in AR expression to participate in the development of seasonal changes in reproductive function, we expected that these changes would be evident before short photoperiod elicits gonadal regression. Furthermore, we assessed the effects of day length on the sensitivity of Siberian hamsters to androgen, as indicated both by gonadotropin secretion and by AR-ir. Thus, we examined serum LH, FSH, and PRL secretion and AR-ir in specific neuroendocrine regions in castrated T-implanted Siberian hamsters given a range of low doses of T and examined the effects after exposure to only 11 short days, before gonadal regression is evident in intact hamsters.

MATERIALS AND METHODS

Animals and Treatments

Young adult male Siberian hamsters (age, 5–11 wk), born and raised under 16L:8D conditions (lights-on 0415 h), were used in these experiments. Hamsters were weighed and castrated under sodium pentobarbital anesthesia (60 mg/kg). All procedures were approved by the University of Massachusetts institutional animal care and use committee and conformed to the NIH Guidelines for Animal Care and Use.

At the time of castration, hamsters were implanted s.c. with 1 cm Silastic capsules (0.145 cm inside diameter, 0.193 cm outside diameter) containing a suspension of T (Sigma, St. Louis, MO) in sesame oil. The T was suspended at concentrations of 0.25, 2.5, or 25 mg/ml. Control hamsters received capsules containing only sesame oil. The groups receiving oil, 0.25, and 2.5 mg/ml T contained 20 hamsters each, whereas 18 hamsters were implanted with the 25 mg/ml capsules. Immediately after surgery, half the animals in each group were returned to 16L:8D conditions. The remaining animals were placed under 10L:14D conditions (lights-on 0715 h).

After a survival interval of 11 days, hamsters were weighed and anesthetized with sodium pentobarbital (100 mg/kg). The thoracic cavity was opened, a blood sample was taken, and the animal was heparinized (500 U; Rugby Laboratories, Pompano Beach, FL) and transcardially perfused with 0.1 M phosphate buffer (pH 7.4) followed by 5% acrolein containing 0.25% glutaraldehyde in phosphate buffer. The brain was removed and postfixed for 4 h before infiltration with 30% sucrose in 0.1 M phosphate buffer. Sections were cut at a thickness of 40 μm on a rotary microtome. A 1- in-6 series was collected into cryoprotectant and stored at −20°C until the time of immunostaining. Serum was harvested and frozen at −20°C until RIAs were performed.

Hormone Assays

T was measured using a kit from ICN Biomedicals (Costa Mesa, CA), which included 125I-T as a tracer. FSH was measured in two assays using a kit from the National Pituitary Agency; the primary antibody was rabbit antirat FSH S-1, and rFSH RP-2 was used for iodination and as the standard. Mean assay sensitivity was 1.5 ng/ml; the mean intra-assay coefficient of variation (CV) was 6.7%, and the interassay CV was 6.6%. LH was measured in three assays using rLH I9 for iodination and the standard; the primary antibody was anti rLH RIA-11. The mean assay sensitivity was 0.66 ng/ml, the mean intra-assay CV was 3.0%, and the mean interassay CV was 2.9%.

Western Blot

Although the PG-21 antibody has been used for immunostaining in several species, it has not previously been characterized in Siberian hamster. Accordingly, homogenates of long-day Siberian hamster hypothalamic preoptic area (HPOA), pituitary, and testis were prepared in Lammeli buffer using a Polytron. To determine whether castration influences the molecular weight of immunoreactive AR, HPOA and pituitary were collected from an adult long-day Siberian hamster 5 days after orchidectomy. For comparative purposes, homogenates of HPOA, pituitary, and testis were also prepared from a C57/B10J mouse. Homogenates were boiled for 5 min to denature proteins and reduce proteinase activity and were centrifuged at 5000 × g for 5 min to pellet undissolved debris. Protein concentrations of supernatants were quantified with an assay kit using BSA for a standard curve (Pierce, Rockford, IL), and approximately 60 μg total protein was loaded on an 8% polyacrylamide gel. SDS-PAGE was conducted at 80–100 mV for 2 h. All brain and testicular tissue was run on one gel, and all pituitary samples were run on another. Proteins were transferred to nitrocellulose membranes by electroblotting, after which membranes were blocked using 5% dry milk in 1× PBS-Tween buffer. Blots were incubated overnight in PG-21 primary antisera at a concentration of 0.1 ng/ml, with or without 25-fold molar excess of blocking peptide (generously provided by Dr. Gail S. Prins, University of Illinois at Chicago, Chicago, IL), to determine specificity of binding. Blots were treated with horseradish peroxidase-conjugated anti-rabbit IgG (1:2000; Jackson Laboratories, Bar Harbor, ME) in PBS-Tween with 5% dry milk, washed in PBS-Tween, and reacted with enhanced chemiluminescence detection reagent (New England Nuclear, Boston, MA). Blots were viewed by autoradiography.

Immunostaining

All steps in the immunostaining procedure were carried out at room temperature. Sections were thoroughly rinsed in 0.05 M Tris-buffered 0.9% saline, pH 7.6 (TBS) at the beginning of the procedure and between the incubation steps. Sections were incubated in 0.5% NaBH4 and 1.0% H2O2 for 10 min each to reduce unreacted aldehydes and neutralize endogenous peroxidases. Sections were then incubated for 1 h in 20% normal goat serum diluted in TBS containing 0.03% Triton-X 1000 and 0.02% Na2S, followed by overnight incubation in rabbit anti-AR (PG-21; generously supplied by Dr. Geoffrey Greene, Ben-May Institute, University of Chicago, Chicago, IL) diluted 1:1000 in TBS containing the same concentration of Triton X-100 and 1% normal goat serum. The next day, sections were thoroughly rinsed with TBS and incubated with biotinylated goat anti-rabbit (1:500, Vector Laboratories, Burlingame, CA) and avidin-biotin peroxidase (Vector Elite Kit) for 30 min each at room temperature. Immunostaining was visualized by incubating the sections in 0.15% 10 nm nickel ammonium sulfate, 2 M sodium acetate, 49 mM ammonium chloride, 0.3% β-mercaptoethanol, and 10 μM glucose oxidase with 0.05% diaminobenzidine (DAB) in TBS for 5–10 min. Sections were rinsed three times in TBS, dried, mounted on subbed slides, and coverslipped with Permount.

Quantification of Immunostaining

To quantify AR-immunostained cells, relevant brain areas were inspected at 200× magnification on an Axioscope (Zeiss, Thornwood, NY). The observer was blind to the experimental treatment of the animals. Images were captured using an MTK CCD72 camera and a Power Macintosh 7110 computer (Apple, Cupertino, CA). The NIH Image program was utilized to count the number of cells stained to densities ≥3 SDs above background as previously described [7]. Validation experiments employing linear regression verified that application of this criterion resulted in close correlation between number of cells obtained with image analysis and the number of cells estimated by eye (r2 = 0.92). In addition, the integrated density of a number of randomly selected cells in the arcuate nucleus and the bed nucleus of the stria terminals (BNST) of each animal was assessed to determine whether the intensity of staining was affected by photoperiod or androgen treatment.

Matched sections from long- and short-day hamsters were processed simultaneously to quantify AR-ir in brain regions that participate in feedback and behavioral actions of T. The results are shown in Figure 1. Various subdivisions of the BNST have been recognized in rats and Syrian hamsters by several authors [29–31]; we quantitated AR-ir in...
the portion corresponding to the anteromedial BNST of Ju and Swanson [31] and Ratiere et al. [32]. AR-ir was also quantified in the anterior and posterior portions of the medial amygdaloid nucleus, as described by Gomez and Newman [33] in the Syrian hamster, in the medial preoptic nucleus (MPN), and in the rostral, middle, and caudal portions of the arcuate nuclei corresponding to the rat brain atlas of Paxinos and Watson [34: plates 26–28, 29–32, and 33–36, respectively]. In general, immunostained cells of each hamster were counted in two consecutive sections of the 1-in-6 series containing the same anatomical region. A standard shape (square or oval, Fig. 1) fitting the approximate dimensions of the region of interest was applied to each section for purposes of quantification; the length of the side or the major axis of the shape varied among nuclei but was approximately 125–175 µm.

**Statistics**

The Levene test was used to assess homogeneity of variance of the mean cell counts in each brain area prior to use of ANOVA to evaluate significance (SAS 8; SAS Institute, Cary, NC). Where variability of cell counts did not differ significantly, data were analyzed by ANOVA for the main effects of T dose and photoperiod and for their interaction. The Student-Newman-Keuls test was used for post hoc comparisons. Where significant differences in the variability of cell counts were indicated, the nonparametric Kruskal-Wallis test was applied to evaluate main effects of photoperiod and T dose. We also assessed the effects of photoperiod within levels of T dose and of T dose within photoperiod. Differences were considered significant at \( P < 0.05 \). Because the results of the ANOVA and Kruskal-Wallis tests were entirely consistent and because nonparametric tests are not available for evaluation of interactions, the parametric ANOVA was used to evaluate the interaction between photoperiod and androgen.

**RESULTS**

**Hormone Concentrations**

Serum androgen concentrations were similar in long- and short-day hamsters bearing capsules containing the same concentration of T. Androgen concentrations were below the level of assay detectability (i.e., <0.02 ng/ml) in all oil-treated castrated hamsters and in all hamsters given 0.25-mg capsules. Serum T concentrations in several animals implanted with 2.5-mg capsules were also undetectable but averaged 0.043 ± 0.004 ng/ml in long-day and 0.046 ± 0.005 ng/ml in short-day hamsters (mean ± SEM). Only the 25-mg capsules resulted in uniformly detectable circulating levels of T. Serum concentrations were 1.03 ± 0.18 and 1.03 ± 0.23 ng/ml in long- and short-day hamsters, respectively.

Assay of pituitary hormones in serum confirmed that 11 short days triggered only the initial stages of the neuroendocrine response to photoperiod. Serum LH concentrations were significantly affected by T dose (\( P = 0.0001 \)) but not by photoperiod (\( P = 0.47 \)), and there was no interaction between androgen dose and day length (\( P = 0.16 \); Fig. 2A). The effect of T was apparent from the profound reduction in LH values at the highest dose, which was evident for both day lengths (Fig. 2A). In short-day hamsters, serum LH concentrations were also significantly reduced from those of oil-implanted animals by both the 0.25-mg and 2.5-mg doses (\( P = 0.02 \) and 0.0001, respectively). In contrast, the effect of androgen upon LH in long-day hamsters was significant only at the highest dose (\( P = 0.02 \)).

Similarly, overall ANOVA indicated a significant effect of androgen treatment (\( P = 0.0001 \)) but not of photoperiod (\( P = 0.53 \)) on serum FSH concentration (Fig. 2B). There was no significant interaction between photoperiod and T treatment (\( P = 0.8 \)). Pairwise comparisons indicated that the 25-mg dose resulted in a significant suppression of serum FSH below concentrations observed in hamsters given oil or lower doses of androgen (\( P = 0.0001 \)).

Although short days tended to decrease PRL concentrations at middle and high doses of androgen, this effect was not significant (0.10 > \( P > 0.05 \); Fig. 2C). The effect of T dose on serum PRL concentrations was significant, however (\( P < 0.0001 \)); in both photoperiods, hamsters given the highest concentration of androgen had significantly higher serum levels of PRL than did the other three groups (\( P < 0.02 \)). The interaction between T dose and photoperiod was not significant (\( P = 0.22 \)).
FIG. 2. Effects of photoperiod, castration, and androgen treatments on serum concentrations of LH, FSH, and PRL in Siberian hamsters. Animals were castrated 11 days prior to death and exposed to either 16L:8D (LD, open bars) or 10L:14D (SD, shaded bars). Hamsters received s.c. Silastic capsules that contained only oil or concentrations of 0.25, 2.5, or 25 mg/ml T; these treatments resulted in graded levels of circulating androgen (none, low, middle, high, respectively). Values depicted are mean ± SEM. †, Significantly different from groups given lower doses of T or castrated without androgen replacement.

AR Immunostaining

Western blotting indicated multiple immunoreactive bands in homogenates of Siberian hamster HPOA and testis (Fig. 3). Predominant bands of 63 and 68 kDa were detected in brain and appeared quite similar in castrated and intact Siberian hamster and mouse. A 98-kDa band found in testis of both species was barely detectable in HPOA.

Increasing concentrations of T significantly elevated the number of AR-ir cells in all areas of the brain examined in this study. The influence of photoperiod, however, was restricted to the arcuate nucleus. Nevertheless, androgen and photoperiod interacted in the regulation of AR-ir in the arcuate nucleus and the BNST.

In the BNST, androgen dose exerted a significant effect upon the number of AR-ir cells (P = 0.0001; Kruskal-Wallis test). Photoperiod did not have a significant effect (P = 0.75), but ANOVA indicated an interaction between photoperiod and androgen dose (P = 0.002; Fig. 4). The number of AR-ir cells was significantly increased above that for castrated animals by the 2.5-mg/ml concentration of androgen in long days and rose further upon treatment with 25 mg/ml T (P < 0.01 for both comparisons). Increasing doses of androgen also elevated the number of AR-ir cells in
short-day hamsters, but only the highest dose of T produced a significant rise above values for castrated animals (P = 0.002).

All three planes of the arcuate showed a similar pattern (Fig. 5). The main effect of photoperiod failed to achieve statistical significance in the rostral, middle, or caudal arcuate (P = 0.02). The overall effect of androgen dose was significant in all three levels of the arcuate (P = 0.0001), and ANOVA indicated a significant interaction between photoperiod and androgen dose in the rostral and middle arcuate (P = 0.0006 and 0.03, respectively). Only the highest concentration of androgen caused a significant increase in the number of cells in the rostral arcuate in either photoperiod (P = 0.0001), but in the middle and caudal arcuate the 2.5-mg dose was sufficient to precipitate a significant increase in cell count above values for castrated animals (P < 0.03). Only in hamsters given the highest dose did long days increase the number of immunostained cells. This effect achieved significance in the rostral arcuate nucleus (P < 0.02, Student-Newman-Keuls test).

In the MPN, T dose had a significant overall effect (P < 0.0001), but photoperiod did not (P = 0.9; Kruskal-Wallis test; Fig. 6). The interaction between photoperiod and T fell short of significance (P = 0.056). In both photoperiods, only the highest dose of T resulted in a significant increase in the number of AR-ir cells above values for castrated animals (P = 0.0001).

The anterior and posterodorsal portions of the medial amygdala were analyzed separately but showed a similar pattern (Fig. 7). The effect of androgen dose was significant in each region (P = 0.0001), but photoperiod had no significant effect (P = 0.8, Student-Newman-Keuls test), and ANOVA indicated no significant interaction between androgen concentration and photoperiod (P = 0.9 and 0.4, respectively). In both the anterior and posterior medial amygdala of short-day hamsters, the 2.5-mg dose resulted in a significant rise in the number of AR-ir cells above values for castrated animals (P < 0.05). In contrast, this dose of T had no significant effect on AR-ir cell number in long-day hamsters (P > 0.1). In both long- and short-day hamsters, the 25-mg dose resulted in a significant rise above the number of cells recorded in hamsters implanted with 2.5-mg capsules (P < 0.0003).

In addition to assessing the dependence of the number of AR-ir cells upon photoperiod and androgen treatment, we also examined the effects of these manipulations on the mean intensity of staining of individual AR-ir cells in the midarcuate and the BNST (Fig. 8). The mean integrated density (representing the intensity of the DAB reaction product in individual cells) was significantly affected by T dose (P = 0.0001) in both areas but was not regulated by photoperiod (P > 0.09), and day length and androgen treat-
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FIG. 7. Effects of photoperiod and androgen treatment on the number of AR-ir cells in anterior (A) and postero dorsal (B) medial amygdala. Symbols as in Figure 2.

FIG. 8. Mean (±SEM) intensity of immunostaining in AR-ir cells of the middle arcuate nucleus (A) and BNST (B) of castrated Siberian hamsters exposed to various regimens of androgen replacement. Open circles, long days; closed squares, short days. Although the main effect of androgen treatment on the integrated density estimate of cellular immunostaining was significant by ANOVA (P < 0.001), values did not differ between photoperiods in groups receiving the same dose of androgen replacement. †, Significantly greater than values for castrated animals maintained in the same photoperiod (P < 0.05).

DISCUSSION

The present results indicate that in Siberian hamsters, as in other species that have received more extensive study, T increases AR-ir in androgen-responsive brain regions. More interesting is the finding that photoperiod regulates AR expression in this seasonal breeder. Both the steroidal and environmental effects are found in brain regions that regulate seasonally changing aspects of endocrine feedback and sociosexual behavior. The most robust effect of photoperiod occurs in the arcuate nucleus. Of particular interest is the finding that T and photoperiod interact to regulate the number of AR-ir cells in the BNST and the arcuate nucleus. These results may help to explain how day length modifies the influence of gonadal steroid hormones and thus should add to our understanding of environmental regulation of neuroendocrine function.

The concentrations of T maintained by the implants used in these studies spanned the physiological range of intact Siberian hamsters [7]. The highest dose was adequate to suppress LH and FSH secretion below values for castrated animals and to increase serum PRL concentrations. Nevertheless, the effect of T treatment upon FSH and PRL did not depend upon photoperiod. There was a suggestion that short-day hamsters are more sensitive to the suppressive effects of T on LH secretion than are long-day hamsters; lower doses of T were adequate to reduce LH concentrations in the photoinhibited group. We attribute the lack of dramatic interaction effects of photoperiod and T on pituitary hormone secretion to the fact that hamsters were killed after only 11 days of photoperiod manipulation. We designed the experiment to focus on the early stages of neuroendocrine events, which ultimately produce dramatic changes in pituitary function after weeks of short days. AR-ir responded more rapidly and was a more sensitive indicator of interactions between photoperiod and androgen than was the endocrine response. However, more frequent sampling of serum hormones may have revealed alterations...
in the pulsatile pattern of pituitary secretion that culminate in collapse of the gonads [4, 10, 35].

In intact hamsters, the profound changes in gonadal function that occur upon exposure to short days certainly have an impact on steroid receptors in the brain. Syrian hamsters experience a dramatic decrease in AR occupancy in hypothalamus, preoptic area, and pituitary after 8 wk of exposure to inhibitory day lengths [18]. AR occupancy in brain is unaffected by photoperiod, however, in castrated hamsters given physiological levels of T or blank capsules. Stankov et al. [19] reported that short-day exposure and melatonin treatment significantly reduced the affinity and number of androgen-binding sites in homogenates of hypothalamus and pituitary of intact Syrian hamsters. In neither of these studies did the methods allow localization of the effects of day length or androgen to particular brain nuclei. In one of the few studies to employ histochemical methods with adequate anatomical resolution, Wood and Newman [23] observed that testicular regression in short-day Syrian hamsters was correlated with a marked reduction in brain AR-ir. Silastic implants of T increased AR-ir in two short-day hamsters within 1 wk, but the interaction between androgen dose and photoperiod was not systematically investigated and too few individuals were studied to permit conclusions to be drawn. To determine whether photoperiod influences AR expression, it is necessary to hold androgen levels constant while varying day length. To our knowledge, the present experiment is the first immunostaining study to follow such a protocol.

Androgen and photoperiod interact to regulate AR-ir cell number in the BNST. Higher doses of T are required to elevate AR-ir in short-day hamsters. This finding is especially provocative in light of evidence that the BNST integrates signals from the social environment with endogenous endocrine signals [28, 33, 36]. The diminished responsiveness of the BNST to AR induction by T (Fig. 4) may contribute to a reduction in the ability of androgen to activate sexual behavior in short-day animals. This phenomenon, which is well established in Syrian hamsters [12, 13], deserves further exploration in Siberian hamsters. The BNST may participate in photoperiodic regulation of testicular function in hamsters. Ratiere et al. [32, 37] reported that electrolytic lesions of the anterior BNST eliminated gonadal regression in Syrian hamsters exposed to short days. These authors suggested that cells in the BNST receive input from melatonin-sensitive neurons in the medial hypothalamus and relay information to GnRH neurons in the preoptic area. Young et al. [38] found that vasopressin

In the present study, the middle and caudal portions of the anterior BNST in hamsters was photoperiod dependent. The increased response of the hamster amygdala to androgen during long days may result from a photoperiodic modulation of AR expression within the anterior mediolateral amygdala that was too small or variable to be detected in our study. Alternatively, photoperiodic modulation of the response of the anterior mediolateral amygdala to androgen may be indirect, depending on an action of T on some other site at which ARs are photoperiodically regulated.

AR-ir in the MPN was regulated by T but not by photoperiod. The interaction of androgen and photoperiod approached significance (P = 0.056). Androgen regulates neuropeptide gene expression in the MPN of male rats [42], and responses of this AR-ir-rich and sexually dimorphic region to manipulation of gonadal steroids may parallel that of the BNST in hamsters [43]. The heterogeneity of the MPN suggests that interactions between androgen and photoperiod may be restricted to a particular cell phenotype. Multiple label experiments would be required to demonstrate this mechanism. The MPN appears to participate in the regulation of sociosexual behavior in rodents [44, 45], but this region has received little study in Siberian hamsters. The role of the MPN in regulation of GnRH secretion is best established in female rats, but our findings suggest that this area may be functionally relevant to photoperiodic regulation of gonadotropin secretion in male hamsters.

Our data indicate that both photoperiod and androgen regulate AR-ir in the arcuate nucleus and reveal a significant interaction between these factors in the rostral and middle portions of this nucleus. The role of the arcuate in the feedback effects of androgens on gonadotropin secretion is well established, and T probably regulates GnRH secretion through its control of propiomelanocortin (POMC) gene expression in this brain region. Photoperiod and androgen interact to regulate POMC expression in the arcuate nucleus of Syrian hamsters, and this interaction differs across the rostral-caudal extent of this nucleus [46]. In the present study, the middle and caudal portions of the arcuate appeared more sensitive to androgen than did the rostral arcuate; the number of AR-ir cells increased at lower T doses. The clearest evidence for photoperiodic regulation of number of cells showing AR-ir was found in the rostral arcuate. An intricate neurochemical mechanism that regulates seasonal changes in body weight and appetite resides...
in the arcuate nucleus of the Siberian hamster. Short days induce significant decreases in POMC, melanocortin-3 receptor, and leptin receptor mRNAs in the arcuate while increasing levels of cocaine- and amphetamine-regulated transcript in this species [47]. Androgens may act within the arcuate through their cognate receptors to regulate transcription of these genes or of Neuropeptide Y, melanocortins, or their receptors. Thus our finding that the effects of androgen on AR-ir in the arcuate depend upon photoperiod may provide an explanation for seasonal differences in appetite and body weight in this species.

We did not find that the intensity of cell nuclear AR-ir depends upon photoperiod in either the arcuate or the BNST, which is consistent with evidence utilizing exchange assays in Syrian hamsters exposed to long or short days [18]. At the short survival time examined in the present study, however, the influence of short days on the negative feedback effects of androgen upon gonadotropin secretion were not yet evident. It would be useful to determine whether levels of immunoreactive nuclear AR are altered by exposure of Siberian hamsters to short days for durations sufficient to alter pituitary function or sexual behavior more profoundly. At least some of the neuroendocrine effects of androgen occur after aromatization to estrogen, and photoperiod may influence feedback or behavioral response through an effect on estrogen receptors in specific brain regions. In addition, molecules that participate in the cellular action of gonadal steroids other than the steroid receptors themselves may be modified by photoperiod. For example, day length might influence expression of members of the recently discovered family of steroid receptor cofactors [48]. Short photoperiods reduce levels of immunoreactive steroid receptor cofactor 1 in the posterodorsal medial amygdala and BNST of Siberian hamsters (E.L. Bittman and M.J. Tetel, unpublished results). The relative importance of AR and such cofactors in the modulation of effects of gonadal steroids by day length requires further examination.

Many experiments have revealed that androgens regulate expression of their own receptor in central and peripheral tissues. The decrease in AR-ir after castration may result from a combination of changes. AR gene transcription is most likely regulated by androgen, and the half-life of AR protein in some target tissues is influenced by T [49]. Within the limbic brain, cellular compartmentalization of AR is specifically regulated by androgens. Our observations are consistent with the findings of Wood and Newman [23], who reported a decrease in nuclear localization of AR-ir in castrated Syrian hamsters, and with those of Romeo et al. [43], who found that prepubertal castration selectively reduces the density of AR-ir neurons in the MPN of Syrian hamsters given T in adulthood. Although Western blotting indicated that AR protein extracted from castrated, intact, and T-treated mice has a similar molecular weight [50], we cannot exclude the possibility that conformational changes, interactions with cofactors, or other changes in the molecule that occur upon its occupation by androgen may influence its affinity for the antibody. Our findings indicate, however, that whatever changes occur in the AR molecule as a function of androgen levels, day length has a significant influence on AR expression in specific brain areas of Siberian hamsters. Further studies of this and other photoperiodic species, perhaps utilizing Northern and Western blots, will be useful in evaluating the nature and extent of influences of photoperiod and androgen on AR expression.

Both internal (gonadal steroid hormone) and external (photoperiodic) signals regulate AR-ir in brain regions that regulate seasonally changing neuroendocrine functions in male Siberian hamsters. The influence of androgen on AR-ir is regulated by day length, particularly in the arcuate nucleus and the BNST. These results suggest novel and potentially important mechanisms by which seasonal breeding is accomplished.

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