Effects of Photoperiod on Reproduction and the Gonadotropin-Releasing Hormone-Immunoreactive Neuron System in the Postpubertal Male Djungarian Hamster

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ABSTRACT

The present study tested the hypothesis that photoperiodic control of reproductive function in the postpubertal Djungarian hamster is associated with changes in the number, morphology, or distribution of GnRH-immunoreactive cell bodies in the brain. To initiate or arrest sexual maturation, males were reared in long (LD, 16L:8D) or short (SD, 10L:14D) days from birth. In two other groups that were chronologically past the normal onset of puberty, males were transferred at 30 days of age from LD to SD or from SD to LD to arrest or initiate reproductive function, respectively. At 40, 60, or 90 days of age, 4–6 hamsters in each of the four photoperiod treatment groups were killed by intracardiac perfusion. Testes weights were significantly increased in males exposed to long days (LD and SD-to-LD groups) compared to those treated with short days (SD and LD-to-SD groups). Serum FSH concentrations at 40 days of age were also increased in the two groups of males in long days compared to those in both groups in short days (p < 0.05, ANOVA); LH concentrations were unaffected by photoperiod treatments. Brain sections (60 μm) from the corpus callosum decussation to the suprachiasmatic nucleus in the anterior hypothalamus were processed for GnRH immunocytochemistry. In brain regions that contained the majority of GnRH neurons, i.e., the medial preoptic area and diagonal band of Broca, the numbers of GnRH-immunoreactive cell bodies were the same among the four treatment groups. Similar numbers and a comparable ratio of unipolar to bipolar GnRH somata were observed whether reproductive development had been stimulated by long days or blocked by short days. The findings indicate that a relatively stable population of GnRH-immunoreactive neurons is present in the postpubertal male hamster and contrast with previous observations that increased numbers of unipolar GnRH-immunoreactive cell bodies are associated with sexual maturation.

INTRODUCTION

Questions about the neuroendocrine control of reproduction ultimately focus upon GnRH-producing neurons that directly regulate pituitary gonadotropin secretion. In rodents, GnRH neurons are diffusely distributed in the forebrain along a ventral and medial continuum from the rostral hypothalamus to the olfactory tubercle [1–3]. The majority of GnRH cell bodies are found in the medial preoptic area and diagonal band of Broca. Most, but not all, GnRH perikarya project to the median eminence to directly control gonadotropin hormone release [4–8]. In addition to the heterogeneity of projections, there is diversity in the cellular morphology of GnRH neurons [9, 10]. GnRH cell bodies have either smooth contours or a rough, irregular outline; some of these somata are unipolar with a single dendritic-like process while others are fusiform-shaped and bipolar.

In the Djungarian hamster, the GnRH neuron system presents a cytoarchitecture similar to that in other rodents [11]. GnRH cell bodies are morphologically unipolar or bipolar, but all have smooth contours. Moreover, evidence suggests a dynamic plasticity in GnRH neuron numbers during the process of sexual maturation. At the onset of puberty, between 15 and 25 days of age, increased numbers of unipolar GnRH-immunoreactive cell bodies are found in the medial preoptic area and diagonal band of Broca [12]. This developmental increase in the number of GnRH-immunoreactive neurons is associated with rapid growth of testes and a sustained rise or peak in gonadotropin secretion [13]. By comparison, the arrest of reproductive maturation by postnatal exposure to a short photoperiod specifically blocks the increase in unipolar GnRH perikarya that normally occurs at the onset of puberty [14, 15].

This observation during development raises the question whether plasticity in the GnRH neuron system is associated with a general photoperiodic effect on reproduction or a specific action upon the mechanism controlling the onset of puberty. In the postpubertal male Djungarian hamster, gonadotropin secretion is also modulated by photoperiod. Characteristics of the reproductive response to stimulatory long days or inhibitory short days, except for a longer response latency, are reminiscent of effects observed during sexual maturation [16, 17]. Thus the study reported here was a test of the hypothesis that increased numbers of GnRH cell bodies, associated with sexual maturation or blocked by exposure to short days, are unrelated to the process of puberty but depend upon a general photoperiodic regulation of reproductive development. Contrary to this hypothesis, evidence in the present report indicates that after puberty the GnRH neuron system in the male Djungarian hamster is composed of a diverse but stable population of neurons that is not affected by photoperiodic regulation of the onset and offset of reproduction.
tion was scanned at 10x, and prospective GnRH-stained cells was graciously provided by Dr. Robert Benoit. Every section was scanned at 10X, and prospective GnRH-stained cells identified the GnRH decapeptide and precursor molecules, whereas testes weights were increased by long days in the SD-LD group (Fig. 1). In the LD-SD group, paired testes weights were significantly greater than those of males transferred to SD at 30 days of age (28.3 ± 1.4 g, 24.6 ± 1.3 g, 27.8 ± 0.2 g, respectively; p < 0.05 vs. LD). By 90 days of age, the body weights of males in LD (33.8 ± 2.4 g) were significantly greater than those of males transferred to SD at 30 days of age, reared in SD, or moved from SD to LD at 30 days of age (28.3 ± 1.4 g, 24.6 ± 1.3 g, 27.8 ± 0.2 g, respectively; p < 0.05 vs. LD). By 90 days of age, the body weights of males reared in LD or after 30 long days (LD: 38.4 ± 1.6 g; SD-LD: 39.8 ± 1.1 g) were significantly greater than those of males exposed to short days (SD: 27.9 ± 0.5 g; LD-S: 33.6 ± 1.5 g; p < 0.05).

**RESULTS**

**Body Weight**

Photoperiod profoundly affected growth. At 40 days, the body weights of males in LD (33.8 ± 2.4 g) were significantly greater than those of males transferred to SD at 30 days of age, reared in SD, or moved from SD to LD at 30 days of age (28.3 ± 1.4 g, 24.6 ± 1.3 g, 27.8 ± 0.2 g, respectively; p < 0.05 vs. LD). By 90 days of age, the body weights of males reared in LD or after 30 long days (LD: 38.4 ± 1.6 g; SD-LD: 39.8 ± 1.1 g) were significantly greater than those of males exposed to short days (SD: 27.9 ± 0.5 g; LD-S: 33.6 ± 1.5 g; p < 0.05).

**Testes Weights**

At 40 days, postpubertal males had attained adult testes weights while in SD-reared males the testes were atrophic (Fig. 1). In the LD-SD group, paired testes weights were significantly reduced after exposure to 10 days of short days compared to those in males reared solely in LD.

**Materials and Methods**

Djungarian hamsters (i.e., Siberian, Phodopus sungorus) were derived from a laboratory breeding colony in long days (LD, 16L:8D; lights-on 0200 h PST). Food and water were always available. On the day of birth, litters were transferred to short days (SD, 10L:14D; lights-on 0700 h PST) or maintained in LD. Hamsters were weaned at 18 days of age and housed in groups of 3–8 individuals per cage. At 30 days of age, one group of males was transferred from LD to SD, and another was moved from SD to LD. Exposure to ten short days significantly reduced testes weights in the LD-SD group at 40 days (p < 0.05 vs. LD same age) whereas testes weights were increased by long days in the SD-LD group at 60 days (p < 0.05 vs. SD same age). See Methods for details of experimental design.

Serum LH and FSH concentrations were determined by RIA with a system validated for use in this species by Dr. Jon E. Levine [19]. For each hormone, all samples (0.07 ml in duplicate) were shipped to Northwestern University and processed in a single assay (intraassay coefficients of variation were < 12%) in the core assay facilities associated with Dr. Levine's laboratory. With the RP-2 standard, assay sensitivity was 0.14 ng/ml for LH and 1.43 ng/ml for FSH.

Data (mean ± SE) for body and testes weights, gonadotropin concentrations, and number of GnRH-immunoreactive cell bodies were analyzed by ANOVA. Individual comparisons were made with Duncan's Multiple Range test; p < 0.05 was considered significant.
FIG. 3. Number of GnRH-immunoreactive neurons (mean ± SE, n = 4–6/age group) in the four photoperiod treatment groups at 90 days of age in the medial preoptic area and diagonal band of Broca that were morphologically bipolar (dark bar) or unipolar (shaded bar). The number and ratio of GnRH cell subtypes are representative of and not significantly different from data in 40- and 60-day-old hamsters (p > 0.05, two-way ANOVA). See Figure 1 legend and Materials and Methods for further details of the experimental design.

FIG. 2. Number of GnRH-immunoreactive cell bodies (mean ± SE, n = 4–6/age group) in the medial preoptic area and diagonal band of Broca in 40-, 60-, and 90-day-old male Djungarian hamsters reared in LD or SD or transferred at 30 days of age from LD to SD or SD to LD (p > 0.05, two-way ANOVA).

these gonadal weights were still increased in comparison to those in SD or SD-LD males. By 90 days, testes weights in males exposed to long days, i.e., LD and SD-LD groups, were typical of those in adults while in the SD and LD-SD groups the gonads were regressed.

Serum Gonadotropins

At 40 days of age, serum LH was not significantly different among the four treatment groups; concentrations ranged from 0.16 to 0.37 ng/ml (n = 3–7/group). For FSH, serum concentrations in males in LD (3.44 ± 0.26 ng/ml) were increased compared to those in hamsters reared solely in SD (1.87 ± 0.15 ng/ml) or transferred from LD to SD at 30 days of age (2.51 ± 0.28 ng/ml) (p < 0.05, ANOVA). Furthermore, in males shifted from SD to LD at 30 days of age, the concentrations of FSH in serum (7.68 ± 0.79 ng/ml) was significantly increased compared to concentrations in each of the other three treatment groups.

Distribution and Number of GnRH Cell Bodies

GnRH-immunoreactive cell bodies were diffusely distributed in ventral and medial portions of the medial preoptic area and along the midline in the vertical limb of the diagonal band of Broca. GnRH-stained perikarya were also found at the ventral boundary of the medial and lateral preoptic area and in the horizontal limb of the diagonal band of Broca. Few GnRH somata were observed in the anterior hypothalamus, i.e., regions surrounding or caudal to the suprachiasmatic nucleus.

The neuroanatomical distribution and number of GnRH-immunoreactive cell bodies in the medial preoptic area and diagonal band of Broca were unaffected by photoperiod treatment or the status of gonadal development (Fig. 2). In 40-day-old hamsters, GnRH-immunoreactive soma numbers were similar among the four treatment groups (p > 0.05). At 60 and 90 days of age, the number of GnRH perikarya remained the same, and no differences were observed relative to photoperiod treatment or gonadal weight. Thus in hamsters that were chronologically past puberty, the number and distribution of GnRH cell bodies were similar whether reproductive development had been stimulated by long days or blocked by exposure to short days.

Analysis of the morphology of GnRH cell body subtypes revealed few differences in unipolar/bipolar soma numbers or their ratio relative to photoperiod treatment or age. By 90 days of age, the number of unipolar and bipolar GnRH-stained perikarya had not been affected by the photoperiod treatments even though gonadal development was stimulated by long days or suppressed by exposure to short days (Fig. 3). The ratio and numbers of GnRH cell subtypes were
also comparable in hamsters at 40 and 60 days of age (p > 0.05, data not shown).

Colchicine treatment failed to enhance the number of GnRH neurons visualized by immunocytochemistry in males reared in either long or short days. In 40-day-old hamsters that had 24 h previously received an injection of colchicine into the cerebral ventricle, the numbers of GnRH-immunoreactive somata in the medial preoptic area and diagonal band of Broca were not statistically different in males reared in long compared to short days (110 ± 7, n = 3 vs. 109 ± 5, n = 6, respectively). In addition, similar numbers of GnRH-stained perikarya were found in these brain regions in controls that had received an intracerebral ventricular injection of saline (LD: 115 ± 11, n = 3; SD: 110 ± 12, n = 3, respectively). The ratio of unipolar to bipolar GnRH subtypes was also the same as that found in the four photoperiod treatment groups.

**DISCUSSION**

Results in the present study do not support the hypothesis that the number of GnRH-immunoreactive cell bodies in the postpubertal male Djungarian hamster brain is related to a general photoperiodic mechanism that regulates the reproductive system. In hamsters that were chronologically past puberty, GnRH-immunoreactive neuron numbers and the ratio of morphological subtypes were not affected by treatments that altered gonadal function. Also in terms of the neuroendocrine regulation of gonadotropin secretion, GnRH cell numbers were the same whether serum FSH concentrations were increased by long days or suppressed by exposure to short days. For LH, mean serum concentrations were less responsive to the inhibitory effects of short photoperiod than was the case for FSH, a result comparable to those in other reports on this species [16, 17]. Therefore, evidence suggests that after puberty there is a stable and diverse population of GnRH-immunoreactive neurons whose cell body numbers are not affected by photoperiodic regulation of the onset or offset of reproduction.

This conclusion is further supported by a preliminary study that compared the GnRH neuron system in aged and adult male Djungarian hamsters [20]. In aged males greater than 12 mo of age, the number, subtype ratio, and neuronal anatomical distribution of GnRH-immunoreactive cell bodies were comparable to those in adult males. Moreover, the overall cytoarchitecture of the GnRH neuron system and morphology of individual soma appeared unaffected by melatonin-induced suppression of testsis function. By extending the duration of increased melatonin in circulation, this melatonin treatment mimics the endocrine mechanism that mediates information about short daylengths [21]. Thus the effect of melatonin—inhibiting reproductive function without affecting specific characteristics of the GnRH-immunoreactive neuron system—further suggests a reduced plasticity of the adult GnRH neuron system in responding to treatments that affect reproductive function.

The absence of a photoperiodic or melatonin effect on the GnRH-immunoreactive neuron system during adulthood is in contrast with effects during the critical period for sexual maturation. At the onset of puberty, the number of unipolar GnRH-immunoreactive cell bodies is increased in the medial preoptic area and diagonal band of Broca of 25-day-old males in long days compared to prepubertal males at 15 days of age [12]. This increase is specifically blocked by exposure to short days or timed melatonin injections and is associated with arrested sexual maturation [14]. Comparison of studies of adult and of developing Djungarian hamsters suggests that the neuroendocrine mechanism of sexual maturation in juvenile hamsters may be fundamentally different from the photoperiodic control of adult reproduction.

In the Syrian hamster, photoperiod effects on adult reproductive function are similar to that in the Djungarian hamster. Although there is a longer response latency, short-day-induced suppression of gonadotropin secretion and gonadal regression was not associated with a change in the number or subtype ratio of GnRH perikarya in brain regions that extended from the septal-diagonal band to the medial preoptic area compared to long-day controls [22]. Characteristics of the GnRH-immunoreactive neuron system were also unchanged throughout sexual maturation [23], although in this species photoperiod appears to play no role in the process initiating puberty [24]. Thus in this hamster species as well, specific immunoreactive characteristics of the GnRH neuronal system appear to be independent of the photoperiodic regulation of adult reproduction.

Ultimately, the ability of long days to sustain reproduction or for short days to suppress serum gonadotropins and testis function is mediated by effects on GnRH secretory activity, which for the adult appears to be distinct from specific immunoreactive characteristics of the GnRH neuron system. The LR1 antiserum used in the present study detects not only the GnRH decapeptide but precursor molecules [25]. Within the neuron, the concentration of all molecules that contain the sequence for GnRH may not be altered by photoperiod treatment, but posttranslational modification of those molecules could still be inhibited. Conceivably, a decline in the releasable pool of GnRH decapeptide could be masked by an unchanged or increased concentration of precursor molecules; as a consequence, the number of GnRH-immunoreactive neurons would not be changed. Other antisera, selective for the GnRH precursor or secretory molecule, may be useful to resolve whether photoperiod influences posttranslational processing within the GnRH neuron. Although transcription may be another locus for the effects of photoperiod on the GnRH neuron, reproductive quiescence induced by short days is not associated with suppression of GnRH message or synthesis [26]. Therefore, the continued presence of substantial numbers of GnRH-immunoreactive neurons that contain message, precursors, and the releasable form of GnRH sug-
gests that alterations in GnRH secretion may be the critical factor underlying the photoperiodic regulation of gonadal function in the adult male hamster.

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REFERENCES