Exogenous T3 mimics long day lengths in Siberian hamsters

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Freeman DA, Teubner BJ, Smith CD, Prendergast BJ. Exogenous T3 mimics long day lengths in Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 292: R2368–R2372, 2007. First published February 1, 2007; doi:10.1152/ajpregu.00713.2006.—Siberian hamsters (Phodopus sungorus) exhibit seasonal cycles of reproduction driven by changes in day length. Day length is encoded endogenously by the duration of nocturnal melatonin (Mel) secretion from the pineal gland. Short-duration Mel signals stimulate reproduction and long-duration signals inhibit reproduction. The mechanism by which Mel signals are decoded at the level of neural target tissues remains uncharacterized. In Siberian hamsters, exposure to short day lengths or injections of Mel in long days results in a decrease in hypothalamic expression of type 2 iodothyronine deiodinase (Dio2) mRNA. Dio2 catalyzes the conversion of the thyroid hormone thyroxine to triiodothyronine (T3). Thus exposure to short and long day lengths should decrease and increase hypothalamic T3 concentrations, respectively. We tested the hypothesis that exogenous T3 administered to short-day hamsters would mimic exposure to long day lengths with respect to gonadal stimulation. Hamsters gestated and raised in short day lengths that exhibited photoinduction of the testes were given daily subcutaneous injections of T3 or saline vehicle for 4 wk beginning at week 12 of life. The results indicate that exogenous T3 induced gonadal growth in short-day hamsters and delayed spontaneous gonadal development by an interval equal to the number of weeks during which T3 was administered. T3 injections delayed gonadal regression if given coincident with the transfer of hamsters from long to short day lengths. These results suggest that T3 mimics long day exposure in Siberian hamsters and may serve as an intermediate step between the Mel rhythm and the reproductive response.

Photoperiod; melatonin; thyroid hormone; reproduction

Animals inhabiting temperate climates experience seasonal fluctuations in food availability, temperature, and other factors. These animals adjust their physiology and behavior to cope with these changes. Siberian hamsters breed seasonally so that offspring are born when environmental conditions are conducive to both parent and offspring survival. Many animals use changes in day length, or photoperiod, as a cue for time of year. Day length is represented endogenously via the secretion of melatonin (Mel) from the pineal gland. The duration of Mel release is directly proportional to night length, and Mel is only secreted at night. In Siberian hamsters (Phodopus sungorus), long-duration Mel inhibits reproduction by acting at Mel receptors within the thalamic reuniens and paraventricular nuclei and the hypothalamic suprachiasmatic nucleus (2, 8). These brain sites are all capable of inducing reproductive inhibition if given localized, long-duration Mel infusions (2).

The duration of Mel secretion from the pineal gland encodes day length information (21). Mel then modulates the release of hypothalamic neurohormones [e.g., gonadotropin-releasing hormone (GnRH)] and pituitary hormones (luteinizing hormone, follicle-stimulating hormone, and prolactin) involved in reproduction, energetics, behavior, and pelage color (9). At present, we remain ignorant regarding the critical steps by which the Mel signal modulates reproductive neuroendocrine function. Because Mel receptors are not localized to the anterior pituitary or GnRH neurons in the hypothalamus, the Mel message must act on some target tissue upstream of these reproductive neuroendocrine tissues (13). Recent evidence suggests that regulation of thyroid hormone availability in the brain may be involved in the response to the Mel rhythm (12, 27). In long-day breeders, including Japanese quail (Coturnix coturnix japonica), Syrian hamsters (23), and in a single study in Siberian hamsters, exposure to short day lengths has been shown to result in decreased expression of the type 2 deiodinase (Dio2) gene in the brain (27). Interestingly, in goats, which are short-day breeders, exposure to long day lengths decreases hypothalamic Dio2 expression (28). In both long- and short-day breeders, the season of reproductive quiescence is correlated with inhibition of Dio2 expression. Because Dio2 catalyzes the conversion of thyroxine (T4) to triiodothyronine (T3), by outer ring deiodination of T4, a reduction in the expression of this gene results in tissue-level reductions in T3 (31). A second enzyme, type 3 deiodinase (Dio3), inactivates T4 and T3 by inner ring deiodination; the expression of this enzyme is increased by exposure to short day lengths (29). In Japanese quail, treatment with exogenous T3 mimics exposure to long days, i.e., it stimulates growth of the gonads, whereas inhibiting Dio2 activity using iopanoic acid results in a reduction in testis size of Japanese quail under long day conditions (31).

Regulation of T3 may be involved in decoding the Mel message in several photoperiodic species (25, 27, 28, 31). Evidence in birds, sheep, and Syrian and Siberian hamsters suggests that thyroid hormones are involved in photoperiodism (5, 14, 20, 23, 24, 26, 27, 31). Seasonal regulation of hypothalamic T3 may be a critical step between the Mel rhythm and regulation of the reproductive response, and perhaps other seasonal responses, in Siberian hamsters. The circulating concentration of thyroxine is not regulated by photoperiod in Siberian hamsters; rather, thyroid-binding proteins and nuclear transporters are under photoperiodic control, which may alter the tissue-level availability of thyroid hormone to the brain (20). In Siberian hamsters, Dio2 expression is evident within the ependymal cell layer lining the third ventricle and is inhibited by short day-like Mel profiles (27). We tested the hypothesis that exogenous T3 mimics exposure to long day...
lengths with respect to reproduction. We also explored whether T3 mimics long days with respect to the endogenous interval timer responsible for triggering neuroendocrine photofractoriness and the subsequent onset of spontaneous puberty in Siberian hamsters housed from birth in short day lengths. This provides an ecologically relevant model in which to examine the role of T3 in the different phases of the photoperiodic response, since the midwinter onset of photofractoriness is thought to result in spontaneous puberty in hamsters born the preceding fall (10, 19).

**MATERIALS AND METHODS**

Siberian hamsters (originally supplied by Irving Zucker at the University of California, Berkeley) were gestated and raised in either a 10:14- or 16:8-h light-dark cycle [10L and 16L, respectively; lights off at 1800 Central Standard Time (CST)], weaned, separated by sex at 17 days of age, and singly housed in polypropylene cages (29 × 18 × 13 cm) at 22 ± 1°C for the duration of the experiment. Animals had access to ad libitum food (mouse chow no. 5015; Purina Mills, St. Louis, MO) and tap water. All procedures involving animals were approved by the Animal Care and Use Committee at the University of Memphis. Estimated testis volume (ETV) was used to assay the role of T3 in the different phases of the photoperiodic response, since the midwinter onset of photofractoriness is thought to result in spontaneous puberty in hamsters born the preceding fall (10, 19).

**RESULTS**

**Experiment 1.** Siberian hamsters (n = 16) gestated and raised in a 10L photoperiod (light offset 1800 CST) that were responsive to short day lengths, as indicated by ETV > 600 mm³, were separated into two body mass- and ETV-matched groups at ~90 days of age (n = 8/group). One group of animals received daily T3 injections, whereas the other group was subject to saline injections. Treatments were administered for 4 wk; body mass, pelage coloration, and ETV were determined weekly during the treatment period and for an additional 8 wk. The onset of the spontaneous gonadal development (i.e., spontaneous puberty) is defined as the 1st wk of two consecutive days of age. One group of animals received daily T3 injections, whereas the other group was subject to saline injections. Treatments were administered for 4 wk; body mass, pelage coloration, and ETV were determined weekly during the treatment period and for an additional 8 wk. The onset of the spontaneous gonadal development (i.e., spontaneous puberty) is defined as the 1st wk of two consecutive increases of 25% over the postinjection ETV. This was used as a marker to indicate the phase of the endogenous interval timer that has been hypothesized to trigger neuroendocrine refractoriness to short day lengths (10).

**Experiment 2.** Siberian hamsters (n = 16) gestated and raised in 16L (light offset 1800 CST) that exhibited large gonads (ETV > 600) were separated into two groups, body mass and ETV matched, at ~90 days of age. One group of animals received daily T3 injections, whereas the other group received saline injections. Treatment was administered in 16L for 2 wk, with body mass and ETV determined weekly. Injections in long day were given for 2 wk because maximal testis volume response was seen after 2 wk of injection in the short day-housed animals (experiment 1). After the 2 wk of injections in 16L, animals were transferred to 10L, and injections were continued for 4 wk. Body mass and ETV were obtained weekly.

**Statistics.** Body mass and ETV were both analyzed using one-way repeated-measures ANOVA in each experiment; Fisher’s protected least significant difference (PLSD) post hoc analysis was performed if allowed by the repeated-measures analysis. Differences were considered significant at P < 0.05.

**Fig. 1. A:** estimated testis volume (ETV) response (means ± SE) of short day-housed Siberian hamsters that were injected daily with saline or triiodothyronine (T3). Solid black bars indicate the interval during which injections were administered. T3-injected, but not saline-injected, hamsters exhibited a significant increase in ETV over the treatment period. Upward arrows depict the week at which spontaneous development of the testis began for each group; the T3-injected group initiated gonadal development 4 wk later than the saline-injected control group. *P < 0.05. B: T3 had no significant effect on body mass compared with saline-injected control hamsters. Both groups initiated refractoriness of the body mass response at week 15.
ETV after 3 wk of injections was significantly decreased compared with the week 1 and 2 ETV measures in the saline-injected group ($P \leq 0.02$).

The onset of spontaneous gonadal development occurred at week 16 in the saline-injected animals and at week 20 in the T3-injected group.

Short-day hamsters exhibited a pelage color change from winter white to a summer-like color patch $\sim 10$ mm$^2$, surrounding the injection site in the T3-injected but not the saline-injected hamsters (data not shown).

Experiment 2. One-way repeated-measures ANOVA performed on body mass approached significance ($P = 0.084$; body mass data not shown); thus, we corrected for body mass in our subsequent analysis of ETV [ETV (mm$^3$/body mass (g) = testis index)], thereby analyzing ETV as a proportion of body mass. The ETV response between the T3- and saline-injected hamsters did not differ in 16L ($P > 0.05$; Fig. 2). There was a significant interaction of time and treatment with regard to the testis response between T3- and saline-injected animals in 10L; the saline-injected hamsters exhibited reduced testis volume in 10L, whereas the T3 group did not ($P < 0.03$; Fig 2).

There was a significant decrease in body mass for both groups over the 2 wk in 16L ($P < 0.001$); PLSD revealed a difference between the initial body mass and after weeks 1 and 2 of receiving injections ($P < 0.001$).

**DISCUSSION**

The results of these experiments suggest that exogenous T3 mimics long day lengths with respect to reproduction in Siberian hamsters. Injections of T3 administered to photoinhibited hamsters resulted in significant gonadal growth within 1 wk, and this stimulation was maintained throughout the 4 wk of injection. Testicular growth in response to T3 injections in short day-housed hamsters in the present experiment did not result in complete gonadal development, as would be elicited by prolonged exposure to long day lengths. The magnitude of the stimulation by T3 (e.g., ETV $\sim 250$ mm$^3$) was comparable to that obtained in previous work in which 2 wk of long day length exposure at week 10 in short day-housed hamsters also resulted in ETV measures of $\sim 250$ mm$^3$ (15). In that experiment, spontaneous gonadal development was also delayed. Several possibilities exist to explain the lack of complete gonadal development in the T3-injected group. First, it is possible that the dose of T3 administered was insufficient to trigger complete development of the testes; second, it is unknown how much of the exogenous T3 crossed the blood-brain barrier, although T3 administered peripherally does gain access to the central nervous system (18); third, it is possible that, to attain summer-like testis size, prolactin secretion must also be stimulated. Inhibition of prolactin is necessary for the expression of complete testicular regression in short day lengths; thus, long day-like prolactin concentrations may be necessary to attain full testicular growth (1, 3). Although serum prolactin concentrations were not obtained in this experiment, it is unlikely that T3 injections stimulated release of prolactin from the anterior pituitary, since none of the hamsters exhibited a complete molt to summer-type pelage, which is dependent on increased prolactin secretion (6); last, it is possible that increasing hypothalamic T3 production is only one part of the mechanism by which long day lengths stimulate gonadal growth. An alternative hypothesis regarding the mechanism by which exogenous T3 elicited testicular growth in short day-housed hamsters posits that T3 alters entrainment of the circadian system to short day lengths in a manner resulting in a long day-like response. The strongest evidence against this possibility is that, if T3 was acting to stimulate gonadal growth by altering entrainment, then T3 injections should also have elicited long day-like alterations in body mass and pelage coloration; these changes did not materialize. It is difficult to imagine how changes in entrainment induced by T3 would only impact the reproductive response, since all of these photoperiodic responses are downstream of the circadian-regulated pattern of Mel secretion (9).

Data from experiment 2 suggest that exogenous T3 is sufficient to delay gonadal regression in hamsters transferred from 16L to 10L. The present experimental design does not allow us to determine whether this effect of T3 would have persisted beyond 4 wk of 10L exposure; it is possible that the testes would have regressed with continued 10L exposure despite exogenous T3 administration.

Four weeks of T3 injections delayed the timing of spontaneous gonadal development by 4 wk compared with the saline-injected group. This suggests that T3 has acute effects, stimulating gonadal growth, as well as actions on the endogenous interval timer responsible for timing spontaneous puberty; the latter is part of the photoperiodic mechanism. The effects of T3 appear to be restricted to reproductive responses, since the body mass response did not differ between T3 and saline-injected control hamsters. T3 did appear to have local, but not systemic, effects on pelage; other types of photoperiodic responses were not examined. The gonadal response to T3 appears to be specific to photoinhibited hamsters, since identical injections administered to long day-housed hamsters were without effect. These results suggest that photoperiod and Mel regulation of Dio2 and Dio3 expression may act to modulate
reproduction via seasonal alterations in the tissue-level availability of the hormone T₃.

Previous experiments in birds, hamsters, and goats indicate that the enzymes involved in T₃ production and inactivation (Dio2 and Dio3, respectively) are sensitive to day length and Mel (27, 29). In hamsters, exposure to short day lengths results in a decrease in the expression of Dio2, whereas expression of Dio3, which inactivates T₃, is increased. This expression pattern presumably results in lower T₃ availability in short day lengths. A hypothesis consistent with these findings is that T₃ supports the long-day phenotype, whereas the absence of T₃ results in the short-day phenotype. The present results support this hypothesis in Siberian hamsters; namely, T₃ is capable of mimicking long day lengths with regard to reproduction. A previous experiment that employed long-day-length exposure rather than T₃ in short-day housed hamsters resulted in similar patterns of response with regard to both body mass and testis response; both measures exhibited significant increases in response to 2 wk of long day exposure (15). In the present experiment, T₃ resulted in significant testicular growth but did not affect the body mass response. Furthermore, Kauffman and Zucker (15) reported that exposure to long day lengths delayed the interval timer that triggers refractoriness for both the body mass and testis responses; in contrast, the present results suggest that the interval timer was delayed with respect to testis development, but not body mass, in response to T₃ injections. This result suggests that these two responses are mediated by separate neural pathways and that recrudescence of each is triggered by a separate interval timer.

In long days, hamsters are likely producing T₃ via the relatively high expression of Dio2 (27). Thus injections of additional T₃ in long day-housed hamsters failed to further stimulate gonadal growth. In short-day hamsters, Dio2 expression is decreased and Dio3 expression is increased, resulting in lower hypothalamic T₃ concentrations. Therefore, injections of exogenous T₃ in 10L resulted in a significant increase in T₃ over the presumably low endogenous concentrations at this time within the hypothalamus, thereby triggering testis growth. Systemic N-methyl-D-aspartate (NMDA) injections, like the T₃ injections here, are capable of stimulating gonadal growth in photoinhibited Siberian hamsters (7). Unlike NMDA, T₃ injections were able to alter the timing of spontaneous puberty, which is thought to mark the onset of neuroendocrine refractoriness to short day lengths. This indicates that T₃ does not merely mask the short-day response as do injections of NMDA (7); rather, this suggests that alterations in T₃ may comprise part of the photoperiodic mechanism.

A role for thyroid hormones in photorefractoriness has been suggested in previous research. Prendergast et al. (20) revealed a decrease in the hypothalamic expression of several thyroid hormone-binding proteins and indicated a decrease in hypothalamic uptake of thyroid hormone in photorefractory Siberian hamsters. Thyroidectomy results in the failure of sheep to enter the photorefractory state; thyroid hormone replacement reverses this effect (25). Syrian hamsters exhibit decreased Dio2 expression in the hypothalamus in short days, and this inhibition persists after gonadal recrudescence (23).

The subcutaneous T₃ injections in the present experiments caused a localized change in pelage color, from winter-type white to a summer-type yellowish-brown, surrounding the injection site. Previous experiments have implicated thyroid hormones in pelage growth and molt in both mammalian and avian species. Thyroidectomy results in a retardation or cessation of fur growth in both beagle dogs and male European badgers (4, 16); in both cases, replacement of T₄ reinstated fur growth. Several avian species require thyroid hormones to accurately time feather molt (17, 22). The exogenous T₃ injections in the present experiment may have stimulated the surrounding fur to molt via local actions at the level of the follicle, whereas the concentration of circulating prolactin may have been sufficient to result in summer-type fur color in the newly grown fur. However, the present results do not allow for a direct test of T₃ injections in the regulation of prolactin secretion.

In conclusion, the present results provide a direct test of the role of T₃ in the regulation of reproduction by photoperiod. The results support the hypothesis that regulation of T₃ serves as an intermediate step between the Mel rhythm and regulation of reproduction. Exogenous T₃ stimulated gonadal growth and delayed the endogenous interval timer responsible for triggering spontaneous puberty; these responses have previously been attributable only to either long day lengths or short-duration Mel treatment (15, 30). Exogenous T₃ also delayed gonadal regression in hamsters transferred from 16L to 10L. In addition, these results suggest that the reproductive and body mass responses are mediated by separate neural mechanisms and that separate endogenous interval timers trigger recrudescence of each.

REFERENCES


