Influence of short days on diurnal patterns of serum gonadotrophins and prolactin concentrations in the male Djungarian hamster, *Phodopus sungorus**

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Summary. Exposure to short days for 8 weeks suppressed mean serum concentrations of FSH, LH and prolactin compared to hamsters kept in long days. Hamsters in short days exhibited a small afternoon rise in serum FSH, but serum LH and prolactin did not exhibit 24-h variations. In hamsters under long days, a late afternoon–early evening increase was evident for circulating prolactin but none was detected for the gonadotrophins. A fall in testes weights rapidly occurred by 14–28 days after transfer to short days. This was accompanied or preceded by a decrease in serum gonadotrophins and prolactin. Reductions in serum FSH and LH occurred in short days in blood samples taken at 09:00 h or 15:00 h. However, the nadir in serum prolactin was first achieved (at 09:00 h), at least 7 days before that at 15:00 h (i.e. Day 14 versus Day 21 of short photoperiod, respectively). The ability to secrete gonadotrophins was further tested in hamsters that had undergone gonadal regression. Castration of hamsters exposed to short days or injected with melatonin in the afternoon, a treatment known to mimic short day effects, induced a 3- to 5-fold increase in serum gonadotrophins. However, this rise in FSH and LH was significantly attenuated compared to the 10-fold response in controls in long days. The results indicate that gonadal involution induced by short days may be mediated by the decline in mean gonadotrophin secretion which, in turn, is regulated by responsiveness to steroids, as well as a mechanism independent of the negative feedback action of gonadal steroids.

Introduction

In a variety of seasonally breeding rodents, reproductive activity is inhibited by short daylengths and maintained under long daylengths (Gaston & Menaker, 1967; Hoffmann, 1973, 1981; Zucker *et al.*, 1980). Testicular atrophy, induced by short photoperiod, is correlated with a decline in circulating gonadotrophin concentrations (Berndtson & Desjardins, 1974; Turek *et al.*, 1975) and preceded by a decrease in serum prolactin (Reiter & Johnson, 1974; Goldman *et al.*, 1981). The Syrian hamster, under long days, or in response to short days, shows no evidence of a 24-h variation in serum FSH or LH (Tamarkin *et al.*, 1976). Therefore, a decreased secretion of pituitary gonadotrophins probably accounts for gonadal regression induced by short daylengths. By comparison, the Djungarian hamster demonstrates a marked 24-h rhythm in circulating testosterone concentrations under long days (Hoffmann & Nieschlag, 1977). The gonadal regression induced by short days could result from disruption of a diurnal pattern in gonadotrophin release underlying the testosterone rhythm in this species. The present report examined the 24 h patterns of circulating

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FSH, LH and prolactin in the male Djungarian hamster to determine whether diurnal changes in these hormones accompanied the transition from long to short days. In addition, the ability to secrete gonadotrophins in response to castration was determined in hamsters that had undergone testicular atrophy.

Materials and Methods

Animals. Djungarian hamsters (*Phodopus sungorus*) were obtained from our laboratory breeding colony which is kept in a long photoperiod of 16 h light:8 h dark (lights on at 02:00 h). A weekly sunflower seed provision supplemented an ad-libitum diet of pellets (Purina F-6 chow) and water. After weaning at 18 days of age, males were group housed (5/cage) and moved to a separate room with the same long daylengths. In all studies, adults (60 days of age or older) were either maintained in long days or transferred to short days (10L:14D, lights on at 05:00 h). At the time of day and treatment specified below, hamsters were decapitated and the body trunk exsanguinated. In all studies, total body weight and paired testes weights were measured with a triple beam or torsion bar balance, respectively. Blood was allowed to clot for about 24 h at 4°C, and, after centrifugation, serum was stored at -20°C. Serum samples were assayed for FSH, LH and prolactin, as previously described (Yellon & Goldman, 1984). The reference standard for the prolactin assay was a serial dilution of a lactating hamster sera pool (1 µl = 1 U). The interassay and intra-assay coefficients of variation for these assays were <12%, respectively. Assay sensitivities were 75 ng FSH/ml (150 µl serum), 11 ng LH/ml (150 µl serum), and 7 U prolactin/ml (50 µl serum).

Experiment 1. This study determined whether a 24-h pattern of mean circulating concentrations of gonadotrophins and prolactin was influenced by daylength. Adult males, under long days (N = 46) or after 8 weeks of exposure to short days (N = 45), were killed at various times over a 24-h period. In the short-day groups, only males with regressed testes (<50 mg) were included. Serum was harvested and assayed for FSH, LH and prolactin as described above.

Experiment 2. This study examined whether photoperiod-mediated gonadal atrophy induced a change in the diurnal pattern of gonadotrophins or prolactin concentrations in circulation. Adult males (N = 122) were transferred from long to short days and killed 3, 6, 7, 8, 11, 14, 21, 28, 35, 42, 49 or 56 days later at 09:00 h or 15:00 h (N = 3–8), as previously described. These times approximated nadir and peak gonadotrophin concentrations in Exp. 1. As an additional control for the first 2 weeks of the study, males kept in long days were killed at the same time of day as those exposed to short days. Serum was assayed for FSH, LH and prolactin as mentioned above.

Experiment 3. This study determined whether exposure to short days or melatonin treatment impaired the ability of hamsters to secrete gonadotrophic hormones or prolactin after castration. Six groups, consisting of 5–8 adult males each, were used. Under long days, hamsters were injected daily with saline (9 g NaCl/l) (0.1 ml with 1% propylene glycol, i.p.) or melatonin (2 µg in 0.1 ml saline with 1% propylene glycol, i.p.) at 09:00 h or 17:00 h. For comparison, two additional groups received no injections: one remained under long days while the other was transferred to short days. After 60 days, the testes were removed (under light ether anaesthesia), and weighed. After another 7 days (67 days), blood was obtained from the body trunk following decapitation at about 09:00 h; serum was harvested for later assay of serum hormones as described earlier.

Data analysis. Results for body weight, testes weight and serum hormones were analysed by one-way ANOVA. Other hormone results (Exps 1 and 2) were evaluated by two-way ANOVA which compared photoperiod treatment versus time effects. Individual comparisons were then made with Duncan's multirange test. In each analysis, a value of $P < 0.05$ was considered significant.

Results

Experiment 1

Over the 24-h sampling period, serum FSH and prolactin concentrations were significantly higher in hamsters in long days than in those in short days (Fig. 1). Serum LH concentrations were frequently below the limits of detection in hamsters kept in short days, thus precluding statistical comparisons with males in long days (Fig. 1). A significant 24-h variation in circulating prolactin was evident in long days; peak concentrations occurred 3 h after dark followed by a nadir 3 h after lights on. A 24-h pattern was not evident for circulating FSH or LH in hamsters maintained in long days or for serum LH or prolactin concentrations in males kept in short days. However, a small but significant 24-h variation in serum FSH was noticed in short-day hamsters, with an afternoon peak 3 h before lights off.

Fig. 1. Concentrations of serum FSH, LH, and prolactin over 24 h in adult male Djungarian hamsters kept in long days (closed circles) or short days (open circles). Each datum point represents the mean $\pm$ s.e.m. of 3–8 individuals. Arrows indicate that the majority of the group had concentrations below the limit of detection. The hours of darkness, relative to lights off ($t = 0$), are on the abscissa. Nighttime is shown for long days (16L:8D, lights off 18:00 h) as the dark bar and for short days (10L:14D, lights off 15:00 h) as the hatched bar.

Experiment 2

Exposure to short days resulted in a small but significant decline in total body weight ($P < 0.001$, Day 0 compared to Days 42 and 44; Fig. 2). Paired testes weights rapidly decreased after 14–28 days of exposure to short daylengths (Fig. 2). However, in 4 males, the testes were still large (> 480 mg) at 28 and 35 days of exposure to short days; data from these outliers were not included in the analysis of the results. Testes weights for hamsters killed on the same day, whether at 09:00 or 15:00 h, were not significantly different.

Regression of the testes was accompanied or preceded by a decrease in circulating gonadotrophins and prolactin. Mean serum FSH and LH concentrations significantly declined after 6 days or 14 days of short days, respectively ($P < 0.001$, Fig. 3). No diurnal differences in serum FSH or LH occurred, and so results at 09:00 and 15:00 h were combined for each hormone. By contrast, diurnal differences were evident for prolactin (Fig. 4). Serum prolactin concentrations declined after 6 days of exposure to short days, and nadir values were evident after 14–28 days of short daylengths, at least 7 days earlier for samples taken at 09:00 h than for those at 15:00 h.

Fig. 2. Mean $\pm$ s.e.m. values for body weight and paired testes weights in adult males after transfer from long to short days. Each datum point represents 3–8 individuals except Day 0 which consisted of 32 hamsters: 10 killed on the last long day and the remainder killed over the next 2 weeks of the study at the same time as the short-day treated hamsters.

Experiment 3

Testicular regression occurred in hamsters exposed to short days or injected each afternoon with melatonin (Table 1). As controls, males kept in long days and receiving no injection, or either saline or melatonin treatment in the morning maintained large testes. After castration, serum gonadotrophin concentrations were elevated 5–10 fold compared to those in intact males kept in long days (Table 1; Fig. 1). However, the response of FSH and LH to castration was significantly attenuated by exposure to short days or treatment with melatonin in the afternoon. In hamsters with atrophic testes, prolactin concentrations were markedly reduced compared to those of other groups in long days. In addition, serum prolactin concentrations were lower in hamsters given melatonin in the morning or saline in the afternoon than in castrated or intact males kept in long days and receiving no injection.

Discussion

The present study failed to detect a 24-h rhythm in mean concentrations of gonadotrophins in the circulation of hamsters kept in long days, when the reproductive system is functional. Similarly, hamsters exposed to short days exhibit only a small diurnal change in serum FSH and no rhythm in serum LH values. Therefore, disruption of a daily pattern in mean gonadotrophin secretion does not appear to account for testicular atrophy induced by short days. Rather, the rapid phase of

Fig. 3. Concentrations of gonadotrophic hormones in serum of adult males after transfer from long to short days. Data for FSH (closed circles) and LH (open triangles) are the mean $\pm$ s.e.m. of 6–13 individuals. Data from hamsters killed at 09:00 h and 15:00 h on the same day were not significantly different and were combined for each gonadotrophin ($P > 0.05$, one-way ANOVA). Day 0 values for hamsters killed under long days (see Fig. 1 legend) represent 31 and 28 individual males for FSH and LH, respectively.

Fig. 4. Serum concentrations of prolactin in adult males after transfer from long to short days. Data are the mean $\pm$ s.e.m. of 4–8 individuals killed in the morning (09:00 h, open squares) or afternoon (15:00 h, closed squares). Long-day hamsters, Day 0 (see Fig. 2 legend), were killed at 09:00 or 15:00 h ($N = 16$ each).

Table 1. Hormonal and gonadal response to photoperiod or timed treatments with melatonin

Photo period	Treatment	Testes wt (mg)	FSH (ng/ml)	LH (ng/ml)	Prolactin (U/ml)
Long	No injection	765 ± 32 ^a (8)	2160 ± 332 ^a (4)	373 ± 137 ^{a,c} (3)	356 ± 46 ^a (4)
Long	Saline, 09:00 h	777 ± 30 ^a (5)	2695 ± 398 ^a (4)	325 ± 59 ^{a,c} (3)	277 ± 45 ^{a,b} (4)
Long	Melatonin, 09:00 h	780 ± 37 ^a (7)	2812 ± 133 ^a (6)	536 ± 127 ^a (6)	186 ± 34 ^{a,b} (6)
Long	Saline, 17:00 h	783 ± 34 ^a (6)	2677 ± 253 ^a (5)	460 ± 52 ^a (5)	152 ± 38 ^{b,c} (5)
Long	Melatonin, 17:00 h	65 ± 17 ^b (7)	1488 ± 220 ^b (6)	199 ± 47 ^{b,c} (6)	93 ± 19 ^c (6)
Short	No injection	45 ± 3 ^b (8)	758 ± 252 ^b (4)	84 ± 36 ^b (4)	88 ± 32 ^c (4)

Values are mean ± s.e.m. for the number of hamsters indicated in parentheses.

Within each column, values with the same superscript are not significantly different ($P > 0.05$, one way ANOVA).

gonadal involution, some 14–28 days after exposure to short days, is preceded by a > 50% decline in mean serum FSH and LH concentration, with FSH values being reduced at all times in short days and LH values being reduced at most times. This finding supports the hypothesis that, unless gonadotrophin secretion is sustained at 'long day' levels, full reproductive function cannot be maintained.

The lack of diurnal changes in serum FSH and LH concentrations during gonadal regression does not preclude the possibility that the mode of gonadotrophin secretion may be altered by treatment with short days or melatonin. Perhaps a change in pulsatile gonadotrophin secretion may account for the diurnal rhythm in circulatory testosterone (Hoffmann & Neischlag, 1977). In the sheep (Lincoln *et al.*, 1977; Goodman & Karsch, 1981) and ferret (Ryan & Robinson, 1985), pulsatile LH release is diminished during the photoperiod-mediated transition between breeding and non-breeding states. However, mean serum LH concentration does not change because as pulse frequency decreases a compensatory increase in amplitude occurs. Whether the 24-h pattern of pulsatile hormone secretion is also influenced by daylength in these species is not known.

There are several differences between the responses of Djungarian and Syrian hamsters to short days. Although the reproductive system is profoundly inhibited by short photoperiods, the rapid phase of testicular regression occurs earlier in Djungarian (14–28 days) than in Syrian males (50–75 days) (Turek *et al.*, 1975). Also, the decline in mean serum FSH and LH concentration is more rapid in the Djungarian male, decreasing within 6–11 days as compared to 30–60 days in the Syrian hamster.

Along with changes in gonadotrophins, fewer short days are required to suppress serum prolactin concentration in the Djungarian hamster (Fig. 4; Goldman *et al.*, 1981); in this species the prolactin response to short days occurs within 3–6 days as compared to 28–35 days in the Syrian hamster. However, in both species, the fall in circulating prolactin concentration precedes the decline in gonadotrophin secretion. This fall in serum prolactin may influence the rate of testicular regression by modulating testicular responsiveness to gonadotrophins (Bex *et al.*, 1978). The coincidence of peak and nadir values in the 24-h patterns of prolactin and testosterone in the circulation raises the possibility that these hormones are intimately related. Finally, the ability to release gonadotrophins is diminished in both species because the castration-induced rise in serum FSH and LH is attenuated by chronic exposure to short days (Table 1; Tamarkin *et al.*, 1976). This confirms

an earlier study by Simpson *et al.* (1982) in which the castration induced rise in serum FSH was attenuated by exposure to short days.

In these hamster species, the ability to secrete gonadotrophins is affected not only by short days but also by the pineal hormone melatonin. Afternoon injections of melatonin inhibit gonadotrophin release and arrest gonadal function (Table 1; Tarmarkin *et al.*, 1977). In addition, afternoon melatonin injections in the Djungarian hamster also attenuate the castration-induced increase in serum FSH and LH. This finding supports the conclusion that melatonin mediates the effects of short daylength on the reproductive system (Goldman & Darrow, 1983).

The physiological mechanism by which short days inhibit reproduction cannot be attributed in any simple way to an alteration in the 24-h pattern of mean gonadotrophin secretion in either hamster species. The effects of short days and melatonin treatment have been proposed to be, at least in part, the result of an increase in sensitivity to gonadal steroid feedback (Tarmarkin *et al.*, 1976; Ellis & Turek, 1979). However, both species also exhibit a diminished ability to secrete gonadotrophins even after castration during exposure to short days or timed melatonin injections (Table 1). Therefore, a CNS mechanism controlling gonadotrophin release, independent of gonadal steroids, appears to be directly restrained by short days and melatonin. The relative contributions of steroid-independent and steroid-dependent regulation of gonadotrophin secretion during the rapid phase of testicular regression remain to be determined.

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