SHORT PHOTOPERIOD EXPOSURE ALTERS THE TESTOSTERONE SURGE DURING PUBERTAL DEVELOPMENT IN MALE MONGOLIAN GERBILS (*MERIONES UNGUICULATUS*)

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ABSTRACT

Prepubertal male Mongolian gerbils (*Meriones unguiculatus*) were exposed to short photoperiods (10L:14D) for up to 15 weeks to determine the photoperiodic involvement in the onset of reproductive activity. Gerbils were killed at the beginning of the experiment and after 3, 4, 7, 8, 11 or 15 weeks of photoperiodic exposure. Short photoperiod-exposed gerbils were compared to age-matched long photoperiod-exposed (14L:10D) animals. Serum testosterone levels were depressed after 3, 4 and 8 weeks of short photoperiod exposure but were not different from long photoperiod exposed gerbils after 7, 11 or 15 weeks of photoperiod exposure. Testes weights increased throughout the pubertal period with a slight depression observed after 4 weeks of short photoperiod exposure. These data indicate that short photoperiod exposure alters the testosterone surge which occurs during pubertal development in the gerbil without major modifications in testicular development. It appears that short photoperiod exposure depresses or modifies the rhythm of pubertal testosterone production in male gerbils.

Photoperiodic control of pubertal development has been examined previously in a number of rodent species (2, 4-6, 11, 12). In Djungarian hamsters and deer mice, short photoperiod exposure appears to be responsible for prevention of pubertal development (7, 11). However, short photoperiod exposure has no effect on pubertal development in Syrian hamsters (5, 6). In general, it is believed that activation of the pineal gland by short photoperiod exposure is responsible for the delay or prevention of reproductive function (2). Recently, plasma testosterone levels, plasma luteinizing hormone levels and testes weights were determined from birth to 24 months of age in male gerbils (3). Testosterone levels increased dramatically throughout puberty and declined to moderate levels within a two month period (3). The present study was con-

ducted to examine this time frame and to determine if the photoperiod controlled the onset of puberty or the testosterone surge observed during puberty in male gerbils.

MATERIALS AND METHODS

Prepubertal male gerbils (*Meriones unguiculatus*) at 8 weeks of age were purchased from Tumblebrook Farms, MA, U.S.A., with an average body weight of 43 g (1). The gerbils were exposed to long photoperiods (14L:10D, lights on at 0500) or short photoperiods (10L:14D, lights on at 0700) in an air conditioned room. Food and water were available *ad libitum*.

In the first experiment, the gerbils were subdivided into five groups of 10 gerbils per group. One group of gerbils was killed by de-

capitation at the onset of the experiment. Two groups were exposed to short photoperiods (SP) and two groups remained under long photoperiods (LP). One group of gerbils from each photoperiod regimen were killed 4 weeks or 8 weeks after the beginning of the experiment. In the second experiment, the gerbils were subdivided into nine groups of 8 gerbils per group. One group of gerbils was killed by decapitation at the onset of the experiment. Four groups were exposed to short photoperiods and four groups remained under long photoperiods. One group of gerbils from each photoperiod regimen were killed at 3, 7, 11 and 15 weeks after photoperiod exposure.

At the end of each experiment, the gerbils were weighed and organ weights (testes, adrenals and spleens) and sera were collected. The sera were stored frozen at -20°C until the time of assay. The organs collected were prepared for histological examination by fixation in Bouin's fluid, embedding in paraffin, sectioning ($7\text{ }\mu\text{m}$) and staining with hematoxylin and eosin. The tissues from the age-matched, long or short photoperiod exposed gerbils were examined for cytological differences.

Sera testosterone and thyroxine were determined using kits from Diagnostic Products Corporation, Los Angeles, CA, U.S.A. The least detectable concentration of testosterone and thyroxine in these assays was $0.052\text{ ng testosterone per ml}$ and $0.20\text{ }\mu\text{g thyroxine per dl}$. The inter-assay and intra-assay variability was below 3% for both assays. Significant differences were calculated using a one-way analysis of variance followed by a Newman-Keuls test for differences among means. Only significant differences between long photoperiod and short photoperiod groups at each time point are presented.

RESULTS

Age-related increases were observed in adrenal and testes weights, but not in spleen weights. Relative spleen weights (mg%) declined with age while serum thyroxine levels were unaltered by the age of the gerbils.

The only significant differences in organ weights between long and short photoperiod exposed gerbils occurred 4 or 8 weeks after photoperiod exposure. Both testes weights and relative testes weights (mg%) were

decreased after 4 weeks of SP exposure ($P<0.05$ vs. respective LP group, Table 1). Also, relative adrenal weights were increased ($P<0.05$) after 4 weeks of SP exposure (Table 1). Relative testes weights were slightly decreased ($P<0.05$) after 8 weeks of SP exposure, while adrenal weights were increased ($P<0.05$) after 8 weeks of SP exposure (Table 1). Serum thyroxine levels and absolute and relative spleen weights were not changed by photoperiod exposure (Table 1). Cytological examination of the above tissues revealed no differences between long and short photoperiod-exposed gerbils. Spermatogenesis was absent in the pretreatment controls and was maximal 11 weeks later, irrespective of photoperiodic treatment.

Serum testosterone levels were significantly depressed ($P<0.01$) after 3, 4 and 8 weeks of SP exposure, but were not different after 7, 11 or 15 weeks of SP exposure (Fig. 1). It is not known why surge testosterone levels were not present in the gerbils exposed to 7 weeks of LP. However, each time point is represented by a different group of animals and the particular group of gerbils at 7 weeks of LP exposure may have experienced a testosterone surge at an earlier or later time point which was missed at that sampling time.

DISCUSSION

Alterations in spleen weights, adrenal weights and sera thyroxine levels have been previously observed in adult hamsters and gerbils exposed to short photoperiods (8-10). Therefore, it was of interest to determine if alterations occurred in these constituents during pubertal exposure to short photoperiods. No differences in spleen weights or thyroxine levels were found between long and short photoperiod-exposed gerbils. However, relative spleen weights decreased throughout the experiment, while adrenal weights increased throughout the experiment (Table 1). Whether the alterations in these organ weights are physiologically relevant remains to be determined. Body weights and testes weights increased throughout the duration of the experiment, but no differences in body weight were observed between age-matched, long and short photoperiod-exposed gerbils (Table 1). A slight decline in testes weights

Table 1 *Effects of Short Photoperiod (SP, 10L:14D) Exposure on Body Weight (g), Absolute (mg) and Relative (mg/100 g body weight, mg%) Organ Weights and Serum Thyroxine (μ g/dl) Levels of Pubertal Male Gerbils Compared to Long Photoperiod (LP, 14L:10D) Exposed Gerbils*

Treatment	n	Body weight (g)	Testes weight (mg)	Testes weight (mg%)	Adrenal weight (mg)	Adrenal weight (mg%)	Spleen weight (mg)	Spleen weight (mg%)	Serum thyroxine (μ g/dl)
Pretreatment controls (1)	10	47 $\pm$ 2	540 $\pm$ 51	1,145 $\pm$ 81	27 $\pm$ 1	57 $\pm$ 2	56 $\pm$ 3	120 $\pm$ 5	2.51 $\pm$ 0.26
Pretreatment controls (2)	8	40 $\pm$ 2	294 $\pm$ 26	729 $\pm$ 65	20 $\pm$ 1	49 $\pm$ 3	69 $\pm$ 4	170 $\pm$ 6	3.11 $\pm$ 0.32
LP/3 weeks	7	65 $\pm$ 2	822 $\pm$ 33	1,258 $\pm$ 31	33 $\pm$ 2	51 $\pm$ 2	78 $\pm$ 4	119 $\pm$ 4	2.69 $\pm$ 0.26
SP/3 weeks	7	60 $\pm$ 2	767 $\pm$ 42	1,265 $\pm$ 40	33 $\pm$ 2	55 $\pm$ 1	70 $\pm$ 5	117 $\pm$ 7	2.30 $\pm$ 0.17
LP/4 weeks	10	68 $\pm$ 2	1,002 $\pm$ 21	1,484 $\pm$ 37	41 $\pm$ 1	60 $\pm$ 1	67 $\pm$ 4	99 $\pm$ 6	3.59 $\pm$ 0.30
SP/4 weeks	10	64 $\pm$ 2	844 $\pm$ 36*	1,325 $\pm$ 55*	42 $\pm$ 1	67 $\pm$ 1*	57 $\pm$ 2	89 $\pm$ 2	3.33 $\pm$ 0.28
LP/7 weeks	7	74 $\pm$ 3	1,109 $\pm$ 38	1,505 $\pm$ 27	37 $\pm$ 2	51 $\pm$ 2	63 $\pm$ 1	86 $\pm$ 3	2.26 $\pm$ 0.13
SP/7 weeks	7	71 $\pm$ 1	1,098 $\pm$ 23	1,544 $\pm$ 31	41 $\pm$ 2	58 $\pm$ 2	69 $\pm$ 2	98 $\pm$ 4	2.68 $\pm$ 0.23
LP/8 weeks	8	66 $\pm$ 2	1,102 $\pm$ 18	1,669 $\pm$ 50	43 $\pm$ 2	65 $\pm$ 2	54 $\pm$ 4	81 $\pm$ 6	2.41 $\pm$ 0.16
SP/8 weeks	9	71 $\pm$ 2	1,072 $\pm$ 44	1,502 $\pm$ 38*	49 $\pm$ 1*	68 $\pm$ 1	58 $\pm$ 2	82 $\pm$ 4	2.87 $\pm$ 0.27
LP/11 weeks	7	71 $\pm$ 5	1,086 $\pm$ 59	1,528 $\pm$ 32	40 $\pm$ 3	57 $\pm$ 2	61 $\pm$ 5	85 $\pm$ 4	1.92 $\pm$ 0.21
SP/11 weeks	8	75 $\pm$ 3	1,128 $\pm$ 42	1,499 $\pm$ 17	43 $\pm$ 3	58 $\pm$ 4	64 $\pm$ 4	84 $\pm$ 2	2.36 $\pm$ 0.28
LP/15 weeks	6	82 $\pm$ 4	1,212 $\pm$ 44	1,492 $\pm$ 51	44 $\pm$ 4	53 $\pm$ 2	63 $\pm$ 7	76 $\pm$ 5	2.64 $\pm$ 0.31
SP/15 weeks	8	80 $\pm$ 3	1,224 $\pm$ 17	1,537 $\pm$ 39	48 $\pm$ 2	60 $\pm$ 2	66 $\pm$ 4	83 $\pm$ 4	2.72 $\pm$ 0.29

The data are from two separate experiments with both pretreatment control values presented. Values are mean $\pm$ SE. * P <0.05 vs. respective LP group

Fig. 1 Serum testosterone values in pubertal male gerbils exposed to long photoperiods (14L:10D, LP) or short photoperiods (10L:14D, SP). The data are from two separate experiments with both pretreatment control values given at week 0. Values are expressed as means $\pm$ SE.

was seen in gerbils exposed to 4 weeks of short photoperiods (Table 1).

The present data confirm the surge of testosterone levels observed during pubertal development in gerbils (3). Interestingly, this surge was not apparent in gerbils exposed to short photoperiods during development (Fig. 1). Perhaps the mechanisms responsible for

the rhythm of testosterone production were phase-shifted, so that the surge was moved to a time point which was not sampled in the present studies. As an alternate hypothesis, surge testosterone production may be depressed by short photoperiod exposure. Irrespective of the mechanism which produces the changes in testosterone levels in

short photoperiods, both sets of gerbils had normal testes weights for their ages with no distinguishable histological changes. It appears that the alterations in testosterone production produced by short photoperiods are not extensive enough to produce changes in testicular development.

Many rodents utilize photoperiod to delay pubertal development (2, 7, 11). It is believed that the pineal gland prevents pubertal development from occurring during the fall and winter months, when reproductive capacity would be detrimental to the survival of the animal (2). In Djungarian hamsters, prior photoperiodic exposure or melatonin treatments are vital for the regulation of pubertal development (7). If these hamsters are exposed to decreasing photoperiodic conditions, gonadal development is inhibited, while increasing photoperiod lengths stimulate gonadal development (7). On the other hand, it appears that photoperiodic exposure to prepubertal Syrian hamsters has no effect on gonadal growth and development, even though the hamster develops a highly sensitive photoperiodic response in adulthood (5, 6). From the present data, it appears that pubertal development in gerbils is not altered by exposure to short photoperiods although changes in testosterone levels are apparent. It may be that gerbils use other environmental determinants to regulate reproduction, such as water availability (13).

In conclusion, short photoperiods appear to prevent the surge in plasma testosterone levels which occurs in pubertal male Mongolian gerbils. Even though the testosterone surge is not present, testicular development in short photoperiod exposed gerbils appears to mature at the same rate as long photoperiod exposed gerbils.

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