Photoperiodic Regulation of Testis Function in Rats: Mediation by a Circadian Mechanism

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ABSTRACT

Laboratory rats traditionally are classified as nonphotoperiodic because variations in daylength have little or no effect on their gonadal function. After olfactory bulbectomy, however, rats show clear evidence of photoperiodic regulation of the gonads. The present study demonstrates, by means of resonance experiments, that the testicular response to daylength in rats is mediated by a circadian photoperiodic time measurement system similar to that of photoperiodic rodents. Olfactory-bulbectomized rats were maintained in fixed photoperiods in which a 6 h light period was coupled with dark periods of 18, 30, 42, or 54 h (6L:18D, 6L:30D, etc.). A fifth group was maintained in a 14L:10D photoperiod. Rats from the 6L:30D, 6L:54D and 14L:10D photoperiods had testes and seminal vesicle weights, plasma testosterone titers and spermatogenesis indices indicative of functional reproductive status. Rats exposed to the 6L:18D and 6L:42D photoperiods had reduced testicular and seminal vesicle weights, lower testosterone levels and reduced spermatogenesis. We hypothesize that photo- and nonphotoperiodic rodent species use similar mechanisms for distinguishing long from short photoperiods, but differ in the extent to which discrimination of short daylengths is transduced into altered gonadal activity.

INTRODUCTION

Seasonal changes in daylength regulate reproduction among many temperate zone rodents (Elliott, 1976; Sadlier, 1969; Turek and Campbell, 1979; Zucker et al., 1980). Exposure to daylengths less than 12 h causes gonadal atrophy and cessation of gametogenesis in hamsters, voles and several species of mice (Zucker et al., 1980). Traditionally, laboratory rats (Rattus norvegicus) have been classified as nonphotoperiodic because maintenance in short daylengths or constant darkness, as well as blinding, have little or no effect on reproductive competence (Hoffmann, 1967; Reiter, 1980).

Recently, we demonstrated that removal of the olfactory bulbs unmasks photoperiodic responsiveness in rats (Nelson and Zucker, 1981). Bulbectomized males exposed to short daylengths (8 h of light per day, 8L:16D) had significantly reduced body weights, lower seminal vesicle and testicular weights and decreased plasma testosterone levels as compared to bulbectomized animals maintained in long daylengths (14L:10D) or to intact rats exposed either to long or short photoperiods.

The manner by which olfactory-bulbectomized rats discriminate long from short daylengths remains to be determined. Bulbectomized animals could use an hourglass timer to monitor accumulation of some chemical end product during the light or dark phase of the illumination cycle. Once a critical threshold of this product is exceeded the photoperiodic response occurs. For example, when daylength is greater than some minimum value the gonads remain functional. Such interval timers mediate photoperiodic responsiveness in some insects (Beck, 1980). Alternatively, bulbectomized rats could use an endogenous circadian clock to discriminate long from short days (Bünning, 1973; Elliott and Goldman, 1981). Circadian mechanisms have been implicated in photoperiodic time measurement of several mammalian species (Elliott, 1976). The hypothesis that circadian clocks mediate photoperiodism, first proposed by Bünning forty-five years ago (1936), since has undergone revisions and refinements. In its modern form (cf. Pittendrigh and Minis, 1964) the circadian cycle is divided into two approximately 12 h periods during which the photoperiodic animal (or plant) is respectively sensitive and insensitive to the stimulatory effects of light. Photostimulation of the gonads is hypothesized to occur if, and

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only if, light extends into the photo-inductive portion of the endogenous rhythm. Light has two roles in Bünning's scheme; it entrains the circadian rhythm of photosensitivity/photoinsensitivity and it induces the photo-periodic response when it extends into the photosensitive phase (Elliott, 1976; Zucker et al., 1980).

One of the major means of testing circadian involvement in photo-periodic time measurement makes use of the resonance paradigm. Groups of animals are maintained on photoperiod cycles that couple a fixed light phase (e.g. 6 h of light) with dark periods of different duration (cf. Elliott, 1976; Elliott and Goldman, 1981). The present experiment used this procedure to test the hypothesis that a circadian mechanism mediates the photo-periodic gonadal response of olfactory-bulbectomized rats; males were exposed to 6L:18D, 6L:30D, 6L:42D and 6L:54D photoperiods. If an hourglass timer is used for photoperiodic time measurement, then rats maintained in each of these photoperiods should have regressed (or undeveloped) reproductive systems; the duration of light is in each instance less than 12 h per day. On the other hand, if bulbectomized rats employ a circadian timer for photoperiodic time measurement then only animals in the 6L:18D and 6L:42D photoperiods should have regressed reproductive systems since only during these photo-regimens is light restricted to the photo-insensitive phase of the circadian cycle. Animals exposed to 14L:10D, 6L:30D and 6L:54D photoperiods ought to have comparably sized reproductive systems as light would coincide with the photo-inducible phase every day, every other day or every third day, respectively (cf. Elliott, 1976).

MATERIALS AND METHODS

The experiment was conducted in two parts that were similar except for the photoperiods employed.

Housing Conditions

Ninety male Sprague-Dawley rats, born in our laboratory to animals obtained from Simonsen Laboratories (Gilroy, CA), were weaned at 21 days of age and kept in wire cages (21 x 33 x 20 cm) with same-sexed cohorts (approximately 8 per cage) at a room temperature of approximately 23°C. Food pellets (Simonsen Rat Maintenance Diet) and tap water were freely available. All rats were exposed to a 14L:10D photoperiod (lights on 7.00 to 21.00 h, Pacific Standard Time) from birth to 26 days of age. Postoperatively, animals were housed individually in wire cages (same dimensions as above) and exposed to one of the following photoperiods: 6L:18D (n=14), 6L:30D (n=13) or 14L:10D (n=10) (Part I), or 6L:18D (n=14), 6L:42D (n=12) or 6L:54D (n=12) (Part II). For the 14L:10D photoperiod, lights came on at 0700 h; in all other photoperiods the initial light phase began at 1100 h. Cages were housed in one of three cabinets (10 to 14 animals per cabinet) in the same room.

Surgical Procedures

At 26 days of age, rats were anesthetized i.p. with a chloral hydrate (60 mg/ml) and a sodium pentobarbital (6.5 mg/ml) mixture (L.Atheia; Haven-Lockhart Labs, Shawnee, KS) in a dosage of 0.35 ml/100 g of body weight. The olfactory bulbs were removed as described previously (Nelson and Zucker, 1981). Upon postoperative recovery, animals were assigned at random to one of the cabinets programmed with its own photoperiod (Flexopulse 60 h timers; Eagle-Signal Manufacturing Co., Davenport, IA).

At 75 days of age, rats were anesthetized with Lphoto 1 in and approximately 2 ml of blood withdrawn using a modified version of the retro-orbital bleeding technique described by Riley (1960). Blood was centrifuged and plasma stored frozen until testosterone titers were assayed with the radioimmunoassay method described by Frankel and co-workers (1975). The interassay coefficient of variation was 3.8%.

After bleeding was completed, rats were laparotomized; the left testis was exposed and its maximum length and width determined to the nearest 0.1 mm. The testis was replaced, irrigated with saline, the tissue covering the abdominal cavity was sutured and the skin closed with wound clips. Rats were returned to their respective cabinets after recovery from anesthesia.

Autopsy and Histological Procedures

Rats were sacrificed at 90 days of age by administering a lethal dose of sodium pentobarbital. Body weights, paired testes weights and seminal vesicle weights were recorded. Testes were stored in Bouin's solution for 24 h, dehydrated, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin. The functional state of the testes was determined by two methods. First, the number of elongated spermatids in five randomly chosen sections of seminiferous tubules was recorded. Slides of testes from all rats also were rated according to the criteria established by Grocock and Clarke (1974). In this system ratings ranging from 5 to 0 were assigned, where 5=large seminiferous tubules with complete spermatogenesis; 4=complete spermatogenesis, but spermatozoa and elongated spermatids were decreased in number; 3=the number of spermatozoa was further reduced with elongated spermatids present; 2=elongated spermatids were absent; 1=only Sertoli cells, spermatoctonia and primary spermatocytes were present; and 0=only Sertoli cells and spermatoctonia were observed.

Statistical Analyses

Two-tailed t-tests for independent samples were used to evaluate differences between treatments for testicular weight, body weight, seminal vesicle weights, testosterone titers and spermatid counts. Average histological ratings were evaluated with chi-square
Seminal vesicle and testes weights and testosterone levels were lower in rats from the 6L:18D photoperiod than in rats from the 6L:30D photoperiod (Figs. 1–3). Seminal vesicle and testes weights, but not testosterone levels were lower in 6L:18D vs 14L:1OD groups (Table 1). Neither seminal vesicle nor testes weights were significantly different in 6L:30D vs 14L:1OD groups; however, testosterone levels were significantly lower in the latter group (Table 1). Body weight differences between groups (Fig. 4 and Table 1), in general, paralleled those for testes weights.
TABLE 1. Level of statistical significance of differences between groups maintained in various photoperiods.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Seminal vesicle weight</th>
<th>Testes weight</th>
<th>Testosterone</th>
<th>Body weight</th>
<th>Spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>6L:18D vs 6L:30D</td>
<td>.001</td>
<td>.003</td>
<td>.05</td>
<td>n.s.</td>
<td>.05</td>
</tr>
<tr>
<td>6L:18D vs 14L:10D</td>
<td>.001</td>
<td>.002</td>
<td>n.s.</td>
<td>.001</td>
<td>.05</td>
</tr>
<tr>
<td>6L:18D vs 6L:42D</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>.05</td>
</tr>
<tr>
<td>6L:30D vs 14L:10D</td>
<td>n.s.</td>
<td>n.s.</td>
<td>.004</td>
<td>n.s.</td>
<td>.05</td>
</tr>
<tr>
<td>6L:42D vs 6L:54D</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
<td>n.s.</td>
<td>.001</td>
</tr>
</tbody>
</table>

n.s. = differences not statistically significant.

Part II

Differences between 6L:18D and 6L:42D groups were not significant; each of these groups had significantly lower organ weights and testosterone levels than did the 6L:54D animals (Figs. 1–3 and Table 1). The effects of photoperiod on body weight were similar to those on testes weight (Table 1).

Spermatogenesis

The number of elongated spermatids was significantly reduced in 6L:18D and 6L:42D groups (Table 1) as compared to rats in the 6L:30D, 6L:54D or 14L:10D photoperiods (Parts 1 and 2, respectively, Fig. 5, A–D). There also was a significant difference in the overall chi-square for ratings of slides ($\chi^2 = 29.62$, p<0.001). However, there were too few animals to fulfill requirements for pairwise $\chi^2$ comparisons (Marascuilo and McSweeney, 1977). Nevertheless, pronounced inhibition of spermatogenesis (ratings of 0, 1 or 2) occurred only in the 6L:18D and 6L:42D groups.

DISCUSSION

Photoperiodic regulation of the testes in rats is accomplished by means of a circadian mechanism. Bulbectomized animals maintained in 6L:30D and 6L:54D photoperiods had functional reproductive systems, whereas those housed in 6L:18D or 6L:42D photoperiods manifested reduced spermatogenesis, reduced testes and seminal vesicle weights and lower plasma testosterone levels. These results are inconsistent with an hourglass model of photoperiodic time measurement; i.e., bulbectomized rats are not measuring the duration of the light or dark phases or the ratio of light to darkness. Instead, the present findings support one of several circadian models of photoperiodic time measurement (Beck, 1980; Elliott and Goldman, 1981).

Between 20% and 25% of bulbectomized rats in nonstimulatory photoperiods (6L:18D and 6L:42D) showed little or no evidence of reproductive system involution. This observation is similar to findings with traditional photoperiodic species of rodents. Between 20% and 30% of male and female white-footed mice (Peromyscus leucopus) did not regress their gonads during many weeks of short-day treatment (Johnston and Zucker, 1980; Beasley, et al., 1981). These nonphotoperiodic individuals may be responsive to other proximate factors.

![FIG. 4. Body weights at 90 days of age. Symbols and conventions as in Fig. 1.](image-url)
FIG. 5. Testis cross sections. A (14L:10D) and B (6L:54D) illustrate the lowest spermatid counts in their respective groups. C is from an animal in the 6L:42D photoperiod; and is representative of approximately 25% of rats in the 6L:18D and 6L:42D photoperiods had functional testes (see text). D is from an animal in the 6L:42D photoperiod; the reduced spermatogenesis is representative of that observed in approximately 75% of rats in the 6L:18D and 6L:42D photoperiods. (X149).
(e.g. temperature, food availability); clearly short daylengths, per se, under typical laboratory testing conditions are not sufficient to regress the gonads of a substantial minority of animals from photoperiodic populations (Zucker et al., 1980; Beasley et al., 1981).

Rats in the 6L:18D group (Part 1) received unscheduled light exposures on two separate occasions. Body weights, testosterone levels and seminal vesicle weights were higher for these animals than for rats maintained on the 6L:18D photoperiod during the second part of the experiment. Values of all parameters in the uncontrolled replication (Part 2) were similar to those previously obtained with bulbectomized rats maintained in short photoperiods (Nelson and Zucker, 1981) or with bulbectomized-blind rats (Reiter et al., 1971).

The response of bulbectomized rats to resonance photoperiods was identical in pattern and differed only in magnitude from that previously reported for hamsters (Elliott et al., 1972) and for voles (Grococ and Clarke, 1974). We speculate that photo- and nonphotoperiodic rodent species make use of similar circadian mechanisms to discriminate long from short daylengths. The two types of rodents (e.g. golden hamsters and laboratory rats) mainly differ in the extent to which this discrimination is transduced into altered gonadal activity. In photoperiodic species, perception of short days is translated into gonadal regression by means of physiological changes yet to be specified precisely and likely to involve altered substrate sensitivity to pineal antagononal agents. The olfactory bulbs of laboratory rats, and perhaps also other species unresponsive to photoperiod, may ordinarily override some part of the pineal transduction process or interfere with photoperiodic time measurement. The differences between photo- and nonphotoperiodic individuals (and species) do not appear to lie in their ability to discriminate short daylengths but in the uses made of this information.

The mechanisms by which the olfactory bulbs interfere with expression of photoperiodism are unknown. The relative contributions of sensory versus nonsensory components of olfactory bulb neuropil to the phenomena under study have not been assessed. Airborne chemical signals from conspecifics may override the effects of short-day exposure in nonphotoperiodic rats. Testing procedures in which nonbulbectomized rats are isolated from odors of other rats may be necessary to reveal the effects of photoperiod. In the absence of the olfactory bulbs the effects of olfactory stimulation are eliminated, perhaps thereby facilitating expression of photoperiodism. Alternatively, the suppressive effects of the olfactory bulbs on photoperiodism may be unrelated to sensory functions and dependent on changes in hormone secretion or in substrate sensitivity to hormones (cf. Nelson and Zucker, 1981).

Neurologically intact rats retain vestiges of sensitivity to short daylengths. Albino rats maintained in short photoperiods are more sensitive to negative feedback effects of testosterone than are animals maintained in long days (Wallen and Turek, 1981). Additionally, the reduction in body weight, observed in bulbectomized rats maintained in short photoperiods, also was detected in intact rats (Nelson and Zucker, 1981). Components of the neuro-endocrine system which presumably mediated responsiveness to photoperiod in ancestral species are evident in laboratory rats, despite the absence of photoperiodic regulation of reproduction. The reduction in responsiveness to daylength variations could reflect artificial selection procedures practiced in rat husbandry. Selection against out-of-season breeding can be presumed to be severe in natural populations of rodents in the temperate zones (Sadleir, 1969) and absent or relaxed in the laboratory setting. Rats commensal with human populations also may be less subject to seasonal regulation of reproduction and might advantageously suppress responsiveness to photoperiod.

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CIRCADIAN MEDIATION OF RAT PHOTOPERIODISM