

Retinal Modulation of the Hypothalamic Sensitivity to Testosterone Feedback in Photoperiodism of Quail

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Accepted November 5, 1984

This experiment was performed on two groups of male Japanese quail. One had been maintained in our laboratory as a closed colony (S-group), and the other had been obtained from a commercial source (R-group). Different responses of gonadal function were found between two groups following either testosterone treatment or exposure to short days. Immature birds of these groups responded to long days with rapid gonadal growth, but after sexual maturity, exposure to short days for 3 weeks induced testicular atrophy only in S-group. Involvement of the feedback effect of androgen in the photoperiodic response was then examined. Under long-day conditions, intraperitoneal placement of testosterone propionate (TP)-filled Silastic tube for 2 weeks decreased testicular weights in S-group but not in R-group. Apparently, sensitivity to short days is closely correlated with sensitivity to testosterone in the adult male. By bilateral enucleation, quail of S-group became less sensitive to both gonad inhibitory effect of short days and the negative feedback effect of TP. These results suggest that the photoperiodic mechanisms that are primarily mediated by the retinal system play a role in altering sensitivity to steroid feedback at the hypothalamus. © 1985 Academic Press, Inc.

The reproductive system of Japanese quail is under the control of day length. Exposure of male birds to long days induces a rapid increase of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels concomitant with the testicular growth (reviewed by Farner, 1975). Since Benoit's pioneering experiments (1936, 1937) have shown that light applied on the hypothalamus results in a marked growth of testes, it has been confirmed that the photostimulation of the encephalic photoreceptors during the photoinducible phase is crucial to bring about gonadal growth (Menaker and Keatts, 1968; Homma and Sakakibara, 1971; Oliver and Baylé, 1976; Homma *et al.*, 1979). However, the role of the eyes in photoperiodism remains to be studied. Sexually mature enucleated quail do not respond to short days with gonadal regression (Homma *et al.*, 1972). This observation suggests that, at sexual maturity, an intervening system in-

cluding the eyes and related hypothalamic structures develops, which enables the birds to respond to short days with gonadal regression. However, Oishi and Konishi (1983) have reported that the eyes are not always necessary for the gonadal regression by short-days. The reason for this discrepancy remains to be elucidated.

In mammals, it has been established that a change in sensitivity of the hypothalamic-pituitary axis to gonadal hormones is an important mechanism in seasonal breeders (Legan *et al.*, 1977; Legan and Karsch, 1979; Ellis and Turek, 1979). Urbanski and Follett (1982), however, reported that photoperiods could control gonadotrophin release irrespective of the gonadal status in quail, which casts doubt on whether the negative feedback effect of gonadal steroids is participatory in photoperiodic response in aves. A major problem in elucidating the role of the steroidal feedback effect in photoperiodism of birds re-

sides in the difficulty in differentiating the effects of the direct photostimulation and the steroid feedback on plasma level of gonadotrophins. In the first experiment described here, we employed a group of quail of which the hypothalamus was insensitive to the gonadoinhibitory action of androgen, in an attempt to see whether the negative feedback effect of the gonadal steroids plays an important part in photoperiodism. Second, the effect of enucleation of the eyes on the steroidal negative feedback was determined using sighted and enucleated quail.

MATERIALS AND METHODS

Animals. Two groups of male Japanese quail (*Coturnix japonica*) were used. One group had been maintained in our laboratory since 1969 as a closed colony (originated in Fukui Quail Farm, Toyohashi, Japan: S-group), and the other had been obtained from a commercial source (Johnan Uzura Center, Tokyo, Japan: R-group) at the age of 3 weeks. Although we do not know the genetical background of the latter, the two groups were phenotypically distinguishable. We found that the birds of the two groups responded differently to photoperiods: S-group was sensitive to the inhibitory effect of short days, while R-group was resistant. Experimental birds of the two groups were housed separately under short days of L:D 8:16 until the age of 6 weeks. Through the experimental period, quail were individually caged in light-controlled rooms lit with white fluorescent light. Food and water were provided *ad libitum*.

Surgery. Testosterone propionate (TP, Sigma) was administered via Silastic tube (1.85 mm in i.d., 3.00 mm in o.d.) sealed with glass beads at both ends. The tube length employed in this study (low dose, 5 mm; high dose, 10 mm) was determined according to the results of preliminary experiments, where the tube of 10 mm in length was the most effective in the inhibition of the gonadal activity and a longer tube was less inhibitory than the 10-mm tube, presumably because of the direct action of androgen at a high level on the testes (Brown and Follett, 1977; Desjardins and Turek, 1977). All surgeries were performed under anesthesia with the solution pentobarbital sodium 9.6 g, chloral hydrate 42.6 g, magnesium sulfate 21.2 g in 1 liter of water; 2.5 ml/kg BW. Castrations and Silastic tube implantation were performed through a lateral incision between the last two ribs. The eyes were removed with a pair of dental forceps after cutting the optic nerve with Wecker scissors. At the end of experiments, blood samples were obtained by cardiac punc-

ture to measure serum LH, FSH, or testosterone (T) levels, and testes were weighed fresh.

Hormone assay. Serum T levels were measured by radioimmunoassay according to the method of Coyotupa *et al.* (1972). Immunoreactive LH concentration was determined according to the methods described by Hattori and Wakabayashi (1979). Immunoreactive FSH concentration was determined according to the method of Sakai and Ishii (1980).

Experiment. The experimental design is shown in Fig. 1. In experiment 1, quail of S- and R-groups were subjected to treatments of either short days or TP administration. In experiment 2, birds of S-group either intact or castrated were enucleated and subjected to the same treatments as in experiment 1. In the experiment of TP administration, we performed two separate series of experiments. In one series, serum LH, FSH levels, and testicular weights were measured, and in the other, serum T levels and testicular weights were measured.

Statistics. Statistical significance of intergroup and intertreatment differences was determined by Mann-Whitney *U* test, because samples after short days or TP treatment are not normally distributed.

RESULTS

Experiment 1. The Effects of TP and Short Days on the Male Gonadal Activity in S- and R-groups

Serum T levels after intraperitoneal placement of TP-filled Silastic tube were 3.59 ± 0.64 and 4.82 ± 0.67 ng/ml in low- and high-dose groups. Administration of a

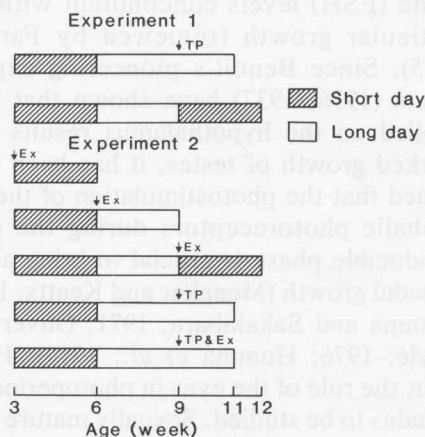


FIG. 1. Experimental schedule. Arrows indicate the time of surgery. In the experiment of TP treatment, some quail were castrated at 6 weeks old. TP: implantation of TP-filled Silastic tube. Ex: Enucleation.

low dose of TP for 2 weeks to male quail that were maintained under long days (L:D 14:10), caused different effects in the two groups. Combined testicular weights of S-group were significantly ($P < 0.002$) decreased by TP treatments including some that failed to respond to TP. In contrast, none of R-group responded to T with gonadal regression (Fig. 2).

Both quail of S- and R-groups possessed small testes at the age of 6 weeks, when they were maintained under short days. Both responded to 3 weeks of long days with a marked increase of gonadal weight (Fig. 3). There is no significant difference in the combined testicular weights between the two groups under these circumstances. Once the sexual maturation had been attained, gonadal responses to short days became different between the two groups. Combined testicular weights were significantly ($P < 0.002$) reduced by short-day

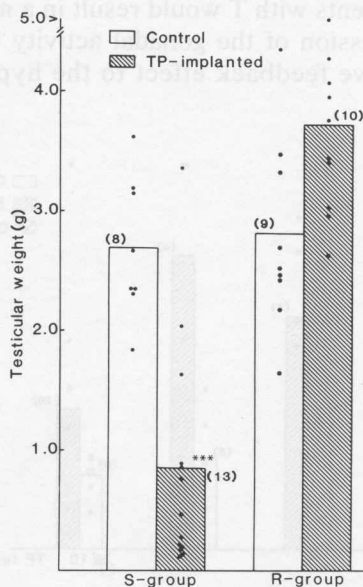


FIG. 2. Mean paired testis weight of S- and R-groups in the presence or absence of TP-filled Silastic tube 5mm in length (individual values are plotted by the filled circles). Number of birds in each experimental group is indicated in parentheses. Birds were kept under long days. $***P < 0.002$ vs control.

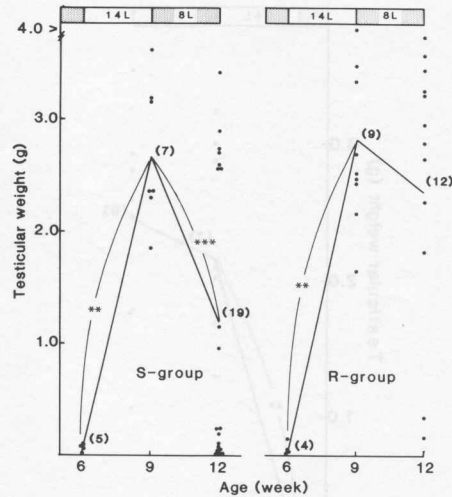


FIG. 3. Changes in mean paired testis weight of S- and R-groups in alternate short and long days (individual values are plotted by the filled circles). Number of birds in each experimental group is indicated in parentheses. $**P < 0.05$. $***P < 0.002$.

treatments in S-group. Meanwhile, quail of R-group resisted short-day treatments, without showing gonadal regression. According to the response to short days, birds were divided into two groups. In one group, testes were regressed ($< 1.5g$), while in the other group, testes remained active. The number of birds with regressed testes was 12 of 19 in S-group, and 2 of 12 in R-group.

These results indicate that R-group, which was insensitive to the inhibitory effects of TP, was also capable of resisting the gonadoinhibitory effect of short days.

Experiment 2. The Effect of the Orbital Enucleation in Gonadal Activity

In this experiment, the quail of S-group were used. In birds of this group, short-day exposure resulted in retarded testicular growth, and long-day exposure enhanced testicular growth irrespective of the presence or absence of eyes. However, after sexual maturity, all of them except one became resistant to short days by enucleation, and maintained mature testes (Fig. 4).

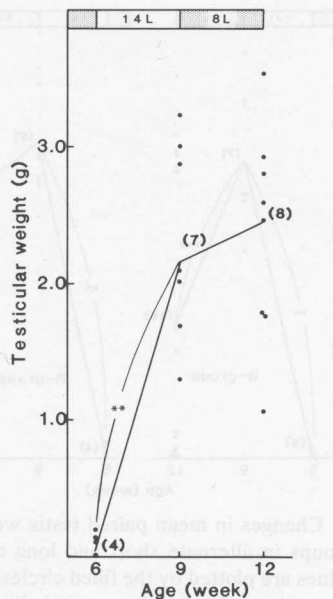


FIG. 4. Changes in mean paired testis weight of enucleated S-group quail in alternate short and long days (individual values are plotted by the filled circles). Number of birds in each experimental group is indicated in parentheses. $**P < 0.05$.

Administration of high or low doses of TP for 2 weeks into sighted birds that had been kept under long-day conditions caused a marked reduction in testicular weights in a dose-dependent fashion. The combined testicular weights of enucleated birds were significantly ($P < 0.002$) heavier than those of intact birds at a high-dose TP treatment (Fig. 5). In accordance with this, both serum LH and FSH levels were higher in enucleated birds than in sighted birds near the significant level ($P < 0.1$) (Figs. 6, 7). These results indicate that the negative feedback effect of T is attenuated by enucleation.

DISCUSSION

It has been documented that the eyes play a gonadoinhibitory role in quail after sexual maturation (Homma *et al.*, 1972; Siopes and Wilson, 1980b), and in chickens (Siopes and Wilson, 1980a), and white crowned sparrows (Yokoyama and Farner,

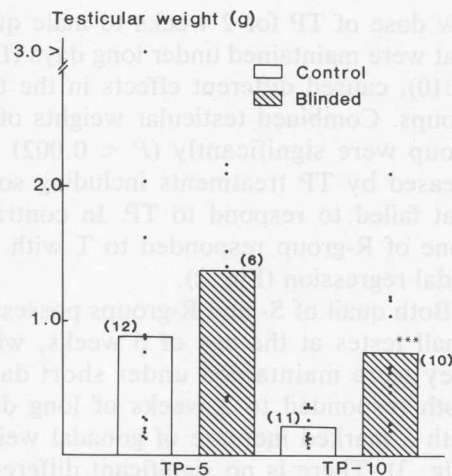


FIG. 5. Mean paired testis weight of sighted (control) and blinded quail of S-group after implantation of TP-filled Silastic tube either 5 or 10 mm in length for 2 weeks (individual values are plotted by the filled circles). Number of birds in each experimental group is indicated in parentheses. Birds were kept under long days. $***P < 0.002$ vs control.

1976), and there is evidence that chronic treatments with T would result in a marked suppression of the gonadal activity by the negative feedback effect to the hypothal-

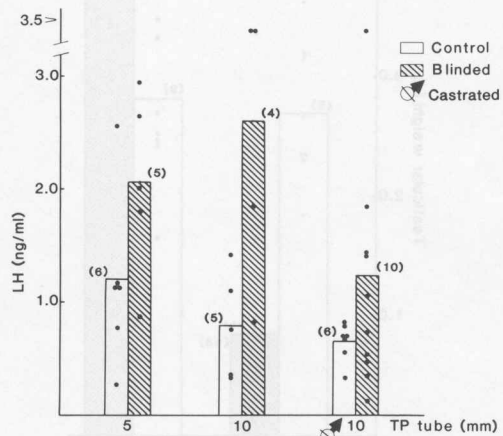


FIG. 6. Mean serum LH levels of sighted (control) and blinded quail of S-group after implantation of TP-filled Silastic tube either 5 or 10 mm in length for 2 weeks (individual values are plotted by the filled circles). Number of birds in each experimental group is indicated in each parentheses. Birds were kept under long days.

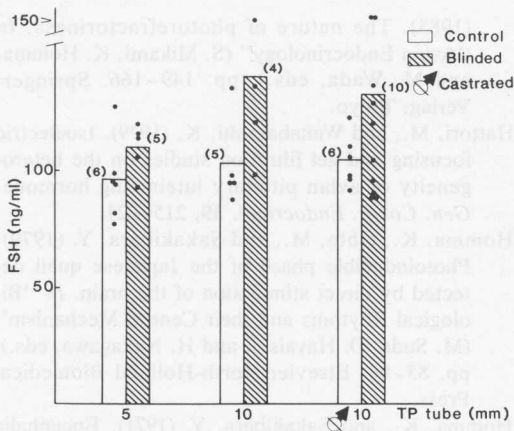


FIG. 7. Mean serum FSH levels of sighted (control) and blinded quail of S-group after implantation of TP-filled Silastic tube either 5 or 10 mm in length for 2 weeks (individual values are plotted by the filled circles). Number of birds in each experimental group is indicated in parentheses. Birds were kept under long days.

amus in male quail (Stetson, 1972; Wada, 1972).

Our findings that quail became less sensitive to the feedback effect of T after enucleation might indicate a functional link between the ocular and steroidal gonadoregulatory systems, i.e., the effect of short days transmitted by the eyes may be expressed by lowering the threshold for steroidal feedback at the hypothalamus in mature birds.

In our results, quail of R-group (a) as well as enucleated quail of S-group (b) were less sensitive to the inhibitory effect of T than sighted quail of S-group (c). To avoid confusion, we tentatively designated (a) and (b) groups together as nonresponders and (c) group as responder to T. Since no practical differences in photoperiodic response were noted between (a) and (b) groups, the possible cause of different response to short-day treatments among these groups (Figs. 3 and 4) may be reasonably explained by the difference in threshold level of T necessary for feedback action. In other words, the negative feedback effect of T may be important when gonadal regression is in-

duced by short days, although such effect of T is not always necessary. We found 3 of 20 nonresponders to T [(a) and (b)] exhibited gonadal regression by short days.

Because plasma T levels were too low to be effective in immature birds, it is not unreasonable to assume that the steroidal feedback plays a minor role in photoperiodic response before sexual maturation. Where gonadal steroid hormone levels are extremely low, direct action of the light on the brain may play a dominant role in the regulation of the gonadal activity (Urbanski and Follett, 1982).

It is known that, in many avian species, the gonads regress in the summer season due to photorefractoriness, prior to the beginning of short days (Farner, 1983). Although we were unable to induce photorefractoriness under laboratory conditions of L:D 14:10, Robinson and Follett (1982) reported that seasonal breeding of quail in natural photocycles is terminated by photorefractoriness. All our experiments of T implants had been carried out under long days. It is then suspected that a high plasma T level may be facilitatory for the induction of photorefractoriness in sighted but not in blinded birds. Future studies will clarify whether the eyes play a role in the induction of photorefractoriness.

Although our data do not show the detailed mechanism how the eyes enhanced the gonadoinhibitory effects of T, it is reported that enzymatic activity of 5β -reductase, which inactivates T, changes seasonally in the brain of starlings (Bottoni and Massa, 1981). As 5β -reductase activity changes during sexual maturation in the brain of quail (Balthazart and Schumacher, 1984), the functional coupling between the retinal system and this enzyme is suspected.

We propose that gonadal regression by either short days or seasonality is not the reversal of the process of gonad stimulation under long days. Lowering the hypothalamic threshold to T by the retinal system

while plasma T level is still high would be the most plausible explanation for the mechanism which determines the time of termination of the breeding activity.

ACKNOWLEDGMENTS

The authors are grateful to Dr. M. Hattori and Professor Wakabayashi for their kind supply of chicken LH RIA kit and Dr. H. Sakai and Professor S. Ishii for their kind supply of chicken FSH RIA kit. This work was supported by a Grant-in-Aid for Scientific Research (57560261) from the Ministry of Education, Science and Culture.

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