GENERAL AND COMPARATIVE ENDOCRINOLOGY 85, 424–429 (1992)

Termination of LH Secretion in Japanese Quail Due to High- and Low-Temperature Cycles and Short Daily Photoperiods

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Accepted June 1, 1991

Mature male Japanese quail were transferred from 16L:8D (19°) to one of the following combinations of daily light–dark and temperature cycles, 8L:16D (12 hr, 19°:12 hr, 9°), 12L:12D (12 hr, 19°:12 hr, 9°) and 12L:12D (16 hr, 19°:8 hr, 9°). The low temperature is for the middle of the dark period in each treatment. In the control groups, birds were transferred to the same photoperiodic conditions as the experimental groups, but without changes in ambient temperature. Blood samples were collected every other day for 30 days and circulating levels of plasma LH were estimated by radioimmunoassay. Both the change in conditions from 16L:8D to 8L:16D with the temperature lowered for 12 hr and that from 16L:8D to 12L:12D with temperatures lowered in one case for 12 hr and in the other for 8 hr caused a lowering in plasma LH levels in all the birds to reproductively quiescent levels. The cloacal protrusion of all these birds regressed completely. In control groups, however, most if not all the birds remained in active breeding states although the levels of circulating LH decreased to basal breeding levels of 1–2 ng/ml. The results indicated that in addition to a change from long to short days an alternation of high and low temperatures was sufficient supplementary information in causing termination of LH secretion and inducing regression of the gonads and the accessory sex organs in this species.

As in other avian species living in midlatitudes, Japanese quail start breeding in early spring and terminate in late summer or early autumn (Robinson and Follett, 1982; Wada *et al.*, 1992). The mechanism of the LH increase at the beginning of the quail breeding season is well established. They measure day length by receiving light in the photoinducible phase in early spring (Wada, 1979, 1981; Hatanaka and Wada, 1988), which induces LH release through the hypothalamic LH-RH neurosecretory system (Hattori *et al.*, 1986). The process of the termination of the breeding season is also related to changes in day length. It is said that the bird becomes "relatively refractory" to a long photoperiod so that LH release is suppressed at a time when the day length is still longer than that which stimulated LH release in early spring (for review see Follett, 1984). It is not very clear, however, how relative photorefractoriness is regulated.

There are many studies that examine photoperiodic control of breeding cycles, but few which examine whether other environmental factors such as ambient temperature are involved in this species. Oishi and Konishi (1978) showed that the testes in quail became regressed when the animals were subjected to both a short photoperiod and a low temperature. In natural environments the annual breeding cycle of this species is very clear (Follett and Maung, 1978; Robinson and Follett, 1982; Wada *et al.*, 1992). The decrease in plasma levels of LH begins in late summer when the ambient temperature starts to decrease. Under laboratory conditions, we could also terminate breeding activity by transferring the birds from 16L:8D to 8L:16D at continuous exposure to 8° for 24 hr (Wada *et al.*, 1990). A

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0016-6480/92 $1.50

constant low temperature of 8° is, however, not very likely in natural environments. Thus in the present study, we employed more natural conditions of lowering temperatures in addition to a change of photoperiods from long to short days to clarify the possible involvement of low temperature in the termination of the breeding season of Japanese quail.

MATERIALS AND METHODS

Animals

Male Japanese quail (*Coturnix coturnix japonica*), 3 weeks old, were purchased from a commercial source. The birds were kept in environmental chambers with a constant ambient temperature (19 ± 1°). They were raised under a daily photoperiod of 8L:16D (lights on at 0800 hr) until 5–6 weeks of age. At that point they showed full somatic growth but were still sexually immature. They were then transferred to a daily photoperiod of 16L:8D (lights on at 0800 hr) until 9–10 weeks of age. Food and water were continuously available.

The birds were then transferred to the experimental conditions described below. Fluctuations of temperatures were within 0.5° under the experimental conditions. No selection on the basis of responsiveness of gonadal activity to alternating long and short photoperiods was made beforehand.

Blood samples of 0.2–0.3 ml were taken at 1000 hr through venipuncture at the wing vein into heparinized capillary tubes. The plasma was separated and stored at −20° until assay.

To confirm the end of breeding activity, cloacal protrusion, an androgen-dependent structure in male quail, was estimated by eye and recorded at the time of blood collection. Molting was also assessed by checking fallen body feathers and primaries on the cage floor.

Experiment Schedules

Experiment 1. Eight birds were transferred from 16L:8D (19°) to 8L:16D (lights on from 0800 to 1600 hr) with a temperature cycle of 9° for 12 hr and 19° for 12 hr (low temperature from 1800 to 0600 hr). For the control group eight birds were also transferred from 16L:8D to 8L:16D but at a constant temperature of 19°. Blood samples were collected 2 days before the transfer and every other day for 30 days after the start of the experiment.

Experiment 2. Eight birds were transferred from 16L:8D (19°) to 12L:12D (lights on from 0800 to 2000 hr) with a temperature cycle of 9° for 12 hr and 19° for 12 hr (low temperature from 2000 to 0800 hr). For the control group eight birds were also transferred from 16L:8D to 12L:12D but at a constant temperature of 19°. Blood samples were collected according to the same schedule that was used in Experiment 1.

Experiment 3. Ten birds were transferred from 16L:8D (19°) to 12L:12D (lights on from 0800 to 2000 hr) with a temperature cycle of 9° for 8 hr and 19° for 16 hr (low temperature from 2200 to 0600 hr). For the control group 10 birds were also transferred from 16L:8D to 12L:12D again at a constant temperature of 19°. Blood samples were collected according to the same schedule that was used in Experiment 1.

Radioimmunoassay

Plasma concentrations of LH were determined in 50-μl sample volumes, in duplicate, using the radioimmunoassay method described by Hattori and Wakabayashi (1979). Chicken LH (fraction IRC-2, Gunma) was used for reference preparations and a preparation of chicken LH (fraction AGCHDS112312A) was used for iodination. The antiserum (AH-MH No. 1) was raised against chicken LH (fraction IRC-2, Gunma). Results are expressed in terms of nanograms per milliliter of a chicken LH fraction IRC-2, Gunma.

Intraassay and interassay coefficients of variation were 4.87 and 5.94%, respectively.

Statistics

Mann–Whitney's U test and paired t test were employed for statistical analysis. Differences were considered significant when $P < 0.05$. Throughout the text, means are given with ±1 SEM.

RESULTS

Changes in Plasma LH

Experiment 1. Changes in circulating LH after transfer from 16L:8D (19°) to 8L:16D with a temperature cycle of 9° for 12 hr are shown in Fig. 1. Levels of plasma LH on Day 0 were not significantly different for the experimental and control groups. In the experimental group, levels of plasma LH began to decrease significantly as soon as the birds were transferred to the experimental condition (paired t test $0.01 < P < 0.05$, between Day 0 and Day 2). In the control group, differences from Day 0 in levels of LH became apparent on Day 8 ($0.01 < P$

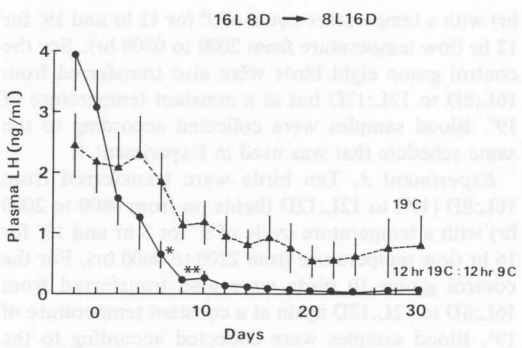

FIG. 1. Change in circulating LH after transfer from 16L:8D, 24 hr at 19° to 8L:16D, 12 hr, 19°:12 hr, 9° (solid line, $n = 8$) or to 8L:16D, 24 hr at 19° (broken line; control group, $n = 8$). The first significant difference detected from the control group is indicated as *, $0.01 < P < 0.05$ and **, $P < 0.01$. Differences are the same thereafter.

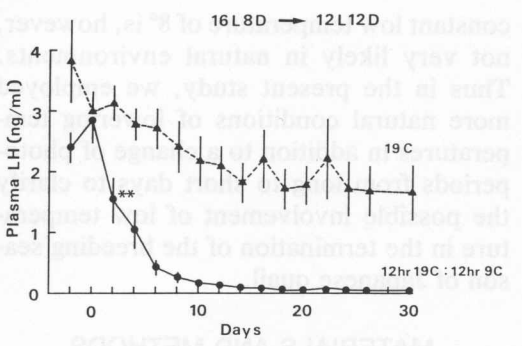

FIG. 2. Change in circulating LH after transfer from 16L:8D, 24 hr at 19° to 12L:12D, 12 hr, 19°:12 hr, 9° (solid line, $n = 8$) or to 12L:12D 24 hr at 19° (broken line; control group, $n = 8$). The first significant difference detected from the control group is indicated as **, $P < 0.01$. Differences are the same thereafter.

< 0.05), and after 10 days circulating LH leveled off until the end of experiment. The rate of LH decrease in the experimental group was more rapid than that in the control group. At Day 10, concentrations of plasma LH in the experimental group decreased to nonreproductive levels (0.26 ± 0.11 ng/ml), but those in the control group decreased to only 1.18 ± 0.36 ng/ml which is significantly higher than those in the experimental group.

Experiment 2. Changes in circulating LH after transfer from 16L:8D (19°) to 12L:12D with a temperature cycle of 9° for 12 hr is shown in Fig. 2. Levels of plasma LH on Day 0 were not significantly different between experimental and control groups. The profiles for the change in circulating plasma LH were similar to those shown in Fig. 1. In the experimental group, LH concentrations decreased to 0.17 ± 0.04 ng/ml at Day 10, while in the control group LH concentrations decreased to only 2.17 ± 0.40 ng/ml.

Experiment 3. Changes in circulating LH after transfer from 16L:8D (19°) to 12L:12D with a temperature cycle of 9° for 8 hr is shown in Fig. 3. The profiles for the change in circulating LH were similar to those shown in Figs. 1 and 2. In the experimental group, LH concentrations decreased to 0.19 ± 0.05 ng/ml at Day 10, while in the control group LH concentrations decreased to only 1.30 ± 0.24 ng/ml.

Cloacal Protrusion and Molt

The cloacal protrusions of all the birds in the experimental groups regressed completely to nonbreeding states by Day 14. In the control group regressed cloacal protrusion was found in about 30% of all the birds (3 of 8 in Experiment 1, 2 of 8 in Experiment 2, and 3 of 10 in Experiment 3). The

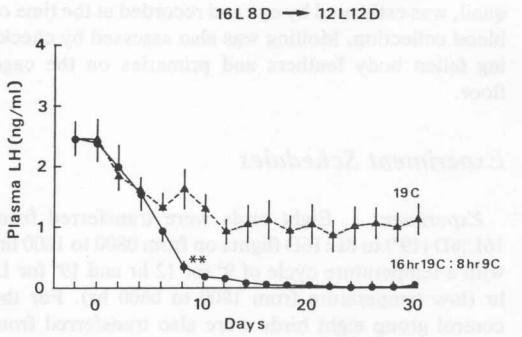

FIG. 3. Change in circulating LH after transfer from 16L:8D, 24 hr at 19° to 12L:12D, 16 hr, 19°:8 hr, 9° (solid line, $n = 10$) or to 12L:12D, 24 hr at 19° (broken line; control group, $n = 10$). The first significant difference detected from the control group is indicated as **, $P < 0.01$. Differences are the same thereafter.

cloacal protrusion in other control group birds remained at full mature size until the end of the experiment.

Molting was observed from Day 10 to Day 25 after transfer to experimental conditions in all the birds of the experimental groups. In the control groups, however, molting was observed in only those birds whose profiles of decreases in plasma levels of LH were similar to the birds of the experimental groups and whose cloacal protrusions also regressed completely as described above.

DISCUSSION

The decreases in levels of plasma LH were markedly different for the experimental and control groups in each treatment. In all the experimental groups, LH concentrations decreased steadily to nonbreeding levels within 10 days after transfer to the experimental conditions and this level was maintained to the end of the experiment. In the control groups, LH concentrations decreased to only 1–2 ng/ml, which we designated as basal breeding levels (Wada *et al.*, 1990), and breeding activity was maintained. The results clearly indicate that in addition to exposure to short days, cycles of high and low temperatures are enough to consistently suppress LH secretion.

Two or three birds in each control group showed a full decrease in levels of circulating LH. This indicates that there were two types of quail, each type having a different sensitivity to temperature. In other words, one type needs only a short photoperiod for termination of breeding activity, while the other requires both low temperature and short daily photoperiods. This phenomenon is also seen in the reports by Oishi and Konishi (1983) and Konishi *et al.* (1988).

The patterns of decrease in levels of plasma LH were basically the same among the three experimental groups and also among the three control groups, even though the assays of the LH were done separately for each treatment. However, there were differences among the three treatments as to the day on which a significant difference between the experimental and control groups became apparent as follows: Day 6 in Experiment 1 (Fig. 1), Day 2 in Experiment 2 (Fig. 2), and Day 8 in Experiment 3 (Fig. 3), respectively. In Experiment 3, significant differences from the control group became apparent on Day 8, which was a little later than those in the other two treatments. The levels of plasma LH decreased more slowly in the 12L:12D experimental group where the temperature was lowered for 8 hr. This condition may be a slightly milder stimulus than the other two conditions in suppressing LH secretion. Since it requires more than 1 month for the levels of circulating LH to decrease to nonbreeding levels in natural environments, the actual wild condition is milder than the condition of 12L:12D and 16 hr, 19°:8 hr, 9°.

In natural environments, Japanese quail terminate breeding activity from late summer to early autumn (Wada *et al.*, 1992). Temperature conditions used in the present experiment were still cooler than those in late summer or early autumn. However, the temperature conditions employed in this study were not so severe as to give stress to Japanese quail and result in LH suppression due to the stress. Wada *et al.* (1990) showed that low temperature of 8° did not attenuate the increases in plasma LH levels at the beginning of an annual breeding cycle, and Oishi and Konishi (1978) showed that low temperature did not induce regression of the cloacal protrusion in sexually mature birds under a long daily photoperiod.

Follett and Nicholls (1984, 1985) reported that thyroidectomized quail did not show cloacal regression under a short daily photoperiod. Dawson *et al.* (1987) reported that thyroidectomy in nestlings of starling appeared to cause neotenous sexual maturation. Temperature effects on the thyroid glands of birds were reported by several au-

thors (Oishi and Konishi, 1978; Cogburn and Freeman, 1987). In particular, plasma triiodothyronine and thyroxine levels changed in the 1st hr of exposure to low ambient temperature (Bobek *et al.*, 1980). Thus the condition used in this work, 8 hr, 9° a day, was sufficient for thyroid activation in quail. Sharp and Klandorf (1985) also reported that the change in levels of plasma thyroid hormone was related to food intake. In natural autumnal environments, thyroid hormone may be increased by interaction of temperature decrease and increased food intake, which may induce the decrease of LH. No attempt was made to identify a role for the thyroid hormone regarding decrease of LH in the present experimental conditions. Further experiments will determine a relationship between changed thyroid function and gonadotropin secretion.

ACKNOWLEDGMENTS

We express our gratitude to Dr. M. Hattori and Professor K. Wakabayashi of Gunma University for their kind gift of chicken LH antiserum. We are also indebted to Professor S. Ishii of Waseda University for his kind supply of LH for iodination and for standard preparations. This study was supported by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan, to M.W.

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