Photoperiodic Control of LH Secretion in Japanese Quail with Special Reference to the Photoinducible Phase

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Repetitive blood sampling from Japanese quail showed that plasma LH concentration rose during the first dark period after the birds were transferred from 8L:16D to 16L:8D. In the second light period of long days, LH concentration decreased slightly (not significant) and maintained this lower level until the second dark period of long days. During the second dark period LH again increased. This suggests that at the first stage of LH release by long photoperiod, photoinduction can occur at least during 8 to 16 hr after lights are turned on. To test the hypothesis that LH secretion induced by long days is dependent on the photoinducible phase, 0.5-hr light pulses (0.5L) were given to quail kept under 8L:16D during the dark periods. Activity was also recorded for each bird. After 10 days of treatment, testicular growth was induced when 0.5L pulses were given at 13 or 19 hr after dawn. Testicular growth induced by 0.5L pulses given at 19 hr after dawn was less than that in birds given light 13 hr after dawn and had greater individual variation compared with other groups. The activity records of these birds revealed that some remained entrained to the primary light pulse (8 hr) while others phase shifted to the short pulse (0.5 hr). The latter were, in effect, then exposed to a 13-hr photoperiod while the former were exposed to 8L:11D: 0.5L:4.5D. LH increase by 0.5L given during the dark period was detected after 2 days of treatment when the pulses were delivered at 15 hr after dawn. These results suggest that LH release is induced when light impinges on the circadian photosensitive phase which is set by external lighting schedules.

Luteinizing hormone (LH) secretion following photostimulation is a rather rapid response in quail (Follett *et al.*, 1977) that eventually plateaus with no hint of daily cyclicity in birds maintained on long days, though episodic release can be detected (Gledhill and Follett, 1976). To determine the role of the circadian system in the photoperiodic response (see Follett, 1973), experiments must be focused on events occurring during the first few days.

The critical period for the first sudden LH release which occurs at 20 hr after dawn lies about 14.7 hr after onset of lighting (Follett *et al.*, 1977). The neuroendocrine mechanisms which change photic information to hormonal secretion (see Kobayashi and Wada, 1973; Follett and Davies, 1975) must function at this interval. However, we cannot determine from the results of Follett *et al.* (1977) whether con-tinued lighting to the critical period is required or a certain photosensitive phase is present around the critical period. Classical night interruption experiments showed that there is no need for continued lighting during the entire long day to stimulate testicular growth (Hamner, 1964; Farner, 1965; Follett and Sharp, 1969; Lofts, 1975). However, testicular growth is only detectable after 10–14 days of night interruptions and in these experiments animals may phase shift: Generally in night interruption two peaks of response of testicular growth are identified one of which is assumed to be due to phase shift and entrainment to a pulse photoperiod given during the dark period. Again it is necessary to obtain results in the first few days. There have been few previous attempts to test the effect of night interruption on LH secretion except that by Follett *et al.* (1974)

141

142   MASARU WADA

which showed the effect of night interruption on LH levels. If LH changes are detected after the first few treatments with night interruption, this would be a good evidence for the presence of a photoinducible phase for LH secretion without any disturbance of phase shifting.

The purposes of the present experiments are (1) to describe the LH secretion pattern during the first 2 days of photostimulation, (2) to determine how quail detect long days, that is, whether there is a photoinducible phase for LH secretion, and (3) to determine whether the photoinducible phase is circadian.

MATERIALS AND METHODS

Male Japanese quail (*Coturnix coturnix japonica*) were purchased from a commercial source at the age of 3 weeks and kept under nonphotostimulatory photoperiods of 8L:16D (lights on from 0900 to 1700) for about 2 weeks before the start of the experiments. Birds were given pelletted quail food and water *ad libitum*.

Experiment I. To follow the photoinduced LH secretion and testicular growth, groups of birds were transferred to stimulatory long photoperiods of 16L:8D (lights on from 0900 to 0100). After 0, 2, 5, 10, and 20 days of treatment, quail were killed at 1300 hr by decapitation and blood was collected in test tubes. Several birds were held under short photoperiods and killed 10 and 20 days after the start of the experiment. All individual blood samples were centrifuged and the sera were stored at −20° until assay. Testes were removed and weighed to the nearest 0.1 mg.

Experiment II. To determine more precise profiles of LH secretion in the first 2 days of photostimulation, serial blood collection was performed mainly at 4-hr intervals. Quail were transferred to 16L:8D and blood samples of 100–200 μl were collected into heparinized hematocrit capillary tubes from a wing vein or the vena tibialis postica. To avoid light effect of blood collection during dark periods, the quail's head was covered by a light-tight hood. At the end of the experiment, birds were killed by decapitation, blood collected for the last samples. The capillary tubes were set to centrifuge and after centrifugation the individual plasma samples were introduced to small tubes used for assay. These tubes were stored at −20° until assay. Essentially, values of LH concentrations were not different from each other in sera and plasma samples.

Experiment III. To establish a photosensitive phase for LH secretion in Japanese quail, a so-called

"asymmetrical skeleton photoperiod" (night interruption) experiment was carried out. Each quail was housed singularly in a cage, the floor of which moved as a seesaw to record daily locomotor activity. Each deflection of the floor triggered a microswitch and the event was recorded as a single pen deflection on a 15-channel event recorder (OPK-15, Shimadzu Denki Keisokuki Co., Kyoto). For each animal, the activity record from a single day was pasted beneath that of the previous day. In this way, a continuous activity record of 14–15 days for each bird allowed visualization of the onset of activity and the phase shift of activity onset. After a few days in the recording cage under short days, short pulses of 0.5-hr light were given during the dark periods for 10 days. Time of a pulse given was changed at 2-hr intervals from group to group, i.e., 8L:1D: 0.5L:14.5D (group B), 8L:3D: 0.5L:12.5D (C), 8L:5D: 0.5L:10.5D (D), 8L:7D: 0.5L:8.5D (E), 8L:9D: 0.5L:6.5D (F), 8L:11D: 0.5L:4.5D (G), 8L:13D: 0.5L:2.5D (H), and 8L:15D: 0.5L:0.5D (I). Group A did not receive night interruption (8L:16D) and served as controls. Each group consisted of 7 or 8 birds. On Days 0, 1, and 2 of treatment, blood was collected from a wing vein into heparinized capillary tubes at 1300–1400. After 10 days of treatment, quail were killed by decapitation, blood was collected, and testes were removed for weighing.

Radioimmunoassay. Immunoreactive LH concentrations were determined in 200-μl serum sample in Experiment I or in 20-μl plasma sample in Experiments II and III, all in duplicate, by the method described by Follett *et al.* (1972) with slight modification (Hattori and Wakabayashi, submitted). Anti-LH serum (AL-MH#1) was anti-chicken LH fraction IRC-2 (Gunma) raised in a rabbit by Dr. Hattori, and chicken LH fraction IEF-1 was used for iodination (Hattori and Wakabayashi, submitted). LH concentration was expressed in terms of a chicken LH fraction IRC-2 (Gunma) nanograms per milliliter (Hattori *et al.*, 1975).

Statistics. Statistical analysis was carried out by means of Student's *t* test.

RESULTS

Experiment I

The first experiment was undertaken to establish LH secretion induced by photoperiodic stimulation. LH concentrations were determined with standard RIA (200-μl sample). Results are shown in Fig. 1. Before photostimulation, serum LH concentration was always about 0.5 ng/ml or less. Two days after photostimulation serum LH concentration increased to 1.23 ng/ml. After 5 days, LH concentration reached a maxi-
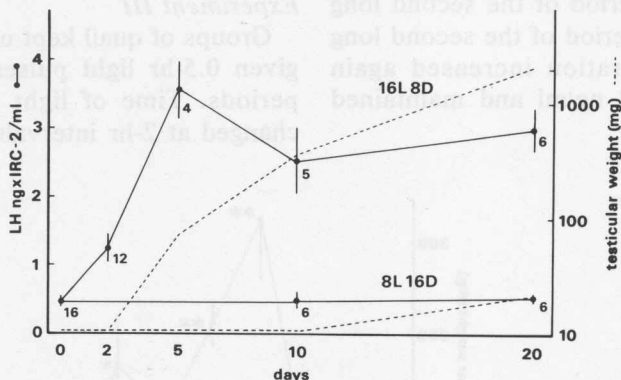
FIG. 1. Changes in serum LH concentrations and testicular weights during photostimulation (16L: 8D) in Japanese quail. Each point is the mean of variable number of birds (indicated by small number) and vertical bars represent standard errors of the means. Standard errors of the testicular weights are not shown in the figure.

mum value of about 3–4 ng/ml and then leveled off. Testicular growth started after 2 days of lag time and continued during the experimental period up to 20 days. On the other hand, LH concentration in short day controls never increased measurably from the initial value, nor did testicular weight increase.

Experiment II

A more detailed analysis of the LH rise during the first 2 days of photostimulation was performed. Samples were collected from 15 quail at 2- or 4-hr intervals (three independent series are combined) and assayed using 20-μl plasma sample volumes. The results are shown in Fig. 2. It is apparent that the mean LH concentration (about 0.5 ng/ml) did not rise during the first long day. During the first dark period of the long day, LH concentration began to increase ($P < 0.05$) and at the beginning of the second long day LH concentration reached about 1.6 ng/ml. Then LH concentration decreased slightly but not significantly and maintained this value until the
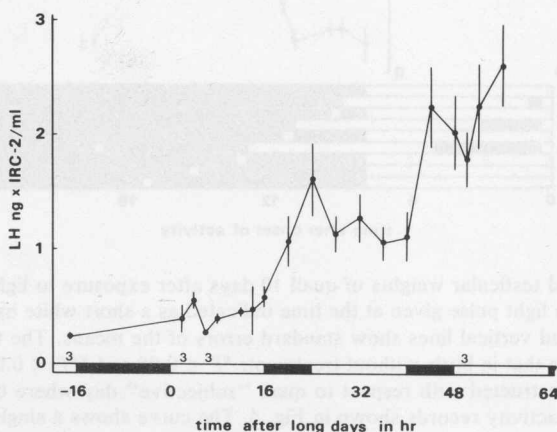

FIG. 2. Plasma LH concentrations in Japanese quail at various times in the first 2 days after transfer from short days (8L:16D) to stimulatory long days (16L:8D). Each point is the mean of 12–15 birds if not otherwise indicated and vertical bars designate standard errors of the means. Increases are statistically significant ($P < 0.05$, paired t test) between 16 and 20 hr as well as between 40 and 44 hr after transfer.

end of the light period of the second long day. In the dark period of the second long day, LH concentration increased again ($P < 0.05$) to 2.2 ng/ml and maintained around this value.

Experiment III

Groups of quail kept under 8L:16D were given 0.5-hr light pulses during the dark periods. Time of light pulses given was changed at 2-hr intervals from group B to
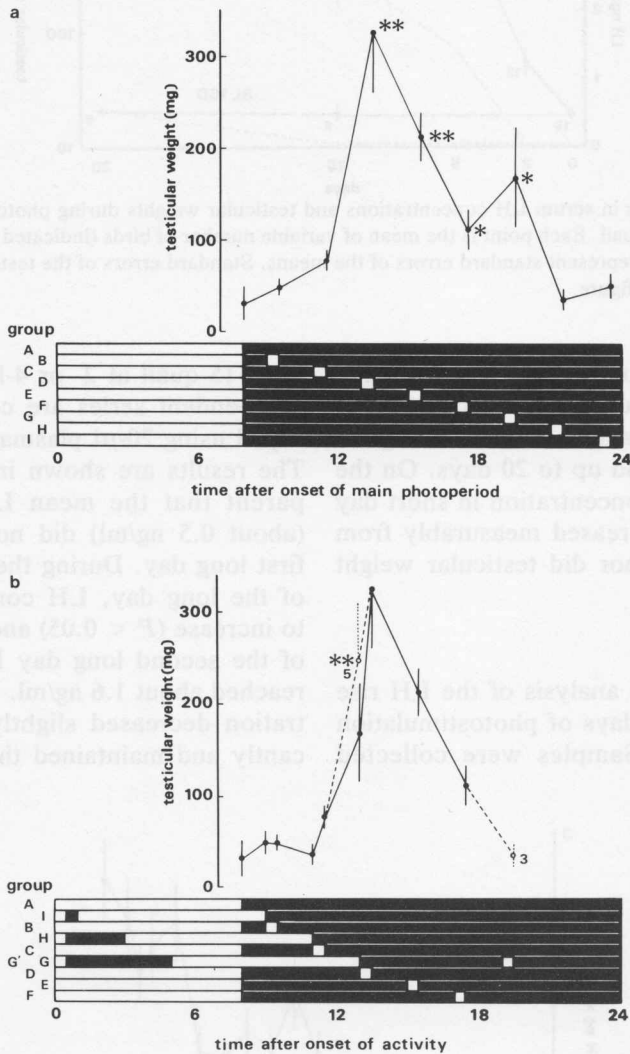

FIG. 3. (a) Combined testicular weights of quail 10 days after exposure to lighting schedules consisting of 8L:16D and a light pulse given at the time indicated as a short white bar. Each point is the mean of 7 or 8 birds and vertical lines show standard errors of the means. The two peak values are significantly higher than that in birds without treatment: *$P < 0.05$ and **$P < 0.01$ (paired t test). (b) Results of (a) are reconstructed with respect to quail "subjective" day where 0 represents activity onset according to the activity records shown in Fig. 4. The curve shows a single peak at 13 hr after onset of activity. Broken lines and circles indicate situation when group G is divided into two subgroups according to activity records. Numbers by the circles are the number of birds divided. See Figs. 4G and 4G'.

group I. Testicular growth of quail in these groups is shown in Fig. 3a. In this figure, the abscissa is time in hours after onset of main photoperiod. In group D where light pulses were given at 13 hr after onset of main photoperiod, testicular growth was comparable with that seen after 10 days under 16L:8D. Testicular growth in groups E and F was also significantly greater compared with that in nontreated controls but the amount of growth was less than that in group D. In group G, testicular growth was induced greater than those in group F and reached a second peak. However, the variance of testicular weight of 8 birds in group G was large compared with the other groups. Activity records of each bird of group G revealed that there were apparently two subgroups: one had phase shifted to the pulse photoperiod of 0.5 hr given at 19 hr after dawn and the other had not (Figs. 4G' and G, respectively). This idea that greater variance in group G is due to individual difference of phase shifting is strongly supported by the fact that in groups H and I all birds had phase shifted and entrained to the pulse photoperiods of 0.5 hr (Figs. 4H and I). Considering the activity records of each bird, results of testicular growth were reconstructed following the circadian time where 0 hour means time of activity onset. As shown in Fig. 3b after conversion, the curve of Fig. 3a shows a single peak at 13 hr after circadian time 0. Broken lines and circles indicate the situation when group G is subdivided into groups G and G'.

From the RIA measurements of LH concentration in each bird 0, 1, 2, and 10 days after treatment, changes of LH concentration from Day 0 are calculated. The means in each group at each day are shown in Fig. 5. One-day treatment induced no significant changes in any group but there was a tendency to gradual increase according to given pulse photoperiods from 9 to 15 hr after dawn. Two days after night interruption, this tendency became clearer to show

an increase significant in group E ($P < 0.05$) (15 hr after dawn). Groups F, G, H, and I had mean values which were negative indicating that LH secretion was inhibited in these groups. Ten days after treatment, increases were more prominent but significant increases were found in Groups C, D, and E. Reconstruction of the results in LH concentration is not present here, since it produces no essentially different curves after conversion following circadian time.

DISCUSSION

From these results, it appears that LH release induced by photostimulation occurs after only one long photoperiod and that photoinducible phase for LH secretion is present during 13−15 hr after dawn. This photoinducible phase is daily and may be driven by the circadian oscillator which is set by external lighting schedules, suggesting that an "external coincidence" model (see Pittendrigh and Minis, 1964) is applicable to explain the phenomenon.

The pattern of LH concentrations following photostimulation (Fig. 2) is quite similar to the results in quail by Nicholls and Follett (1974) and Follett et al. (1977). Recently, Scanes et al. (1978) showed increases of LH concentrations around 17−19 hr after dawn following 10−12 weeks of photostimulation in chickens of both sexes. The results in chickens are roughly comparable with those in quail that LH concentrations increase 20 hr after dawn when birds are transferred from a short day to a long day (Follett et al., 1977; this study).

The results of the night interruption experiment indicate that the photoinducible phase is present under the nonphotostimulatory short photoperiod. These are consistent with the results of earlier experiments in finches (Hamner, 1964), in white-crowned sparrows (Farner, 1965), in Japanese quail (Follett and Sharp, 1969), and in sparrows (Menaker, 1965; Lofts,

time of day

09   17

0

days

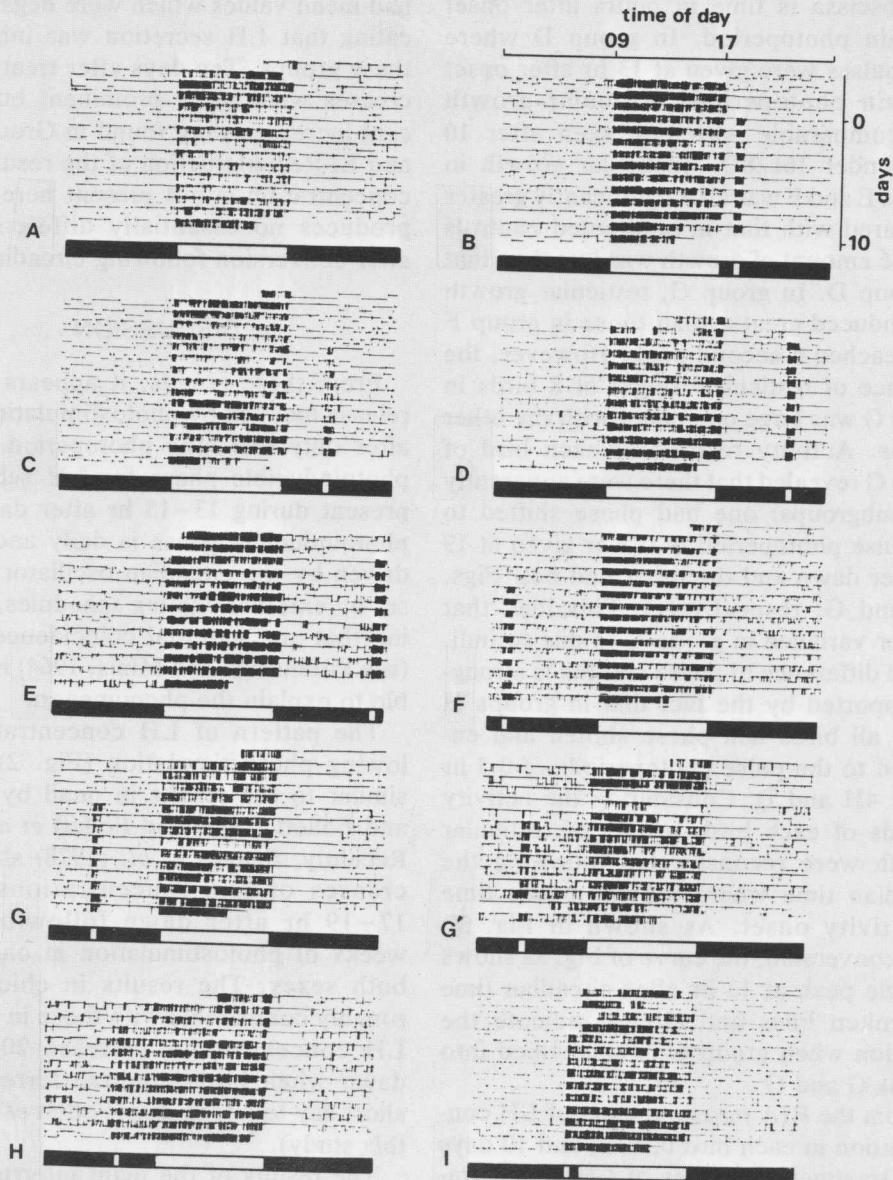10

A

B

C

D

E

F

G

G'

H

I

FIG. 4. Representative activity records of each group. Activity record of each day is pasted beneath that of the previous day. Black and white bars under each activity record indicate lighting time schedule in which white bars mean light periods and black ones dark periods. Beginning on Day 0, a pulse photoperiod of 0.5L is given. Note that in group G', H and I, activity onset has undergone phase shift and entrained to the onset of the pulse photoperiods of 0.5L as "dawn."
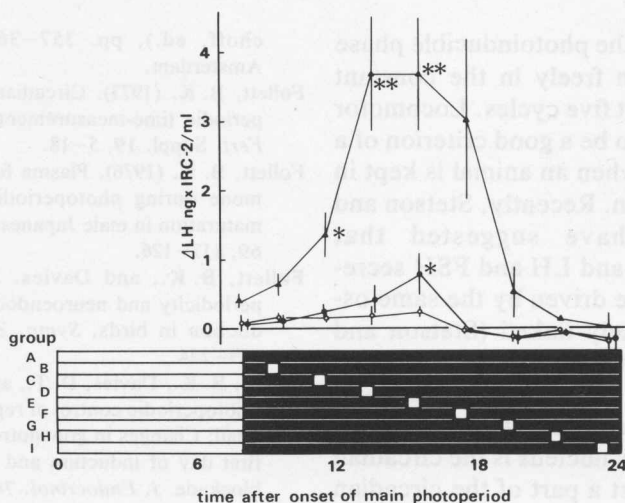
FIG. 5. Increase and decrease of plasma LH concentrations after 1 (○), 2 (●), and 10 (▲) days of night interruption. Significant differences from the control group A are indicated as *($P < 0.05$) and **($P < 0.01$).

1975), which demonstrate that gonadal growth can be induced by night interruption. However, a bimodal pattern is uncommon in avian species where the photoinducible phase is usually expressed as a single peak (Hamner, 1964; Farner, 1965; Menaker, 1965; Follett and Sharp, 1969). Lofts (1975) showed that the pattern of testicular growth induced by night interruption is bimodal in mountain sparrows. He suggested that this bimodality is due to phase shift and entrainment to the light pulse as a false dawn, so that the photoinducible phase is coincident with the main photoperiod of following day. This is the case which is clearly demonstrated using the activity record in this study. In a similar experiment by Follett and Sharp (1969) using the same species, Japanese quail, the curve for testicular growth induced by night interruption had a single peak. Differences in the results of the two experiments are not explicable at present.

As to LH release, increases of LH concentrations by night interruption was only detectable at 15 hr after dawn at the beginning. But after 10 days' treatment significant increases were found in groups in which pulse photoperiods were given after

11, 13, and 15 hr after main lights were turned on. It seems to indicate that the photoinducible phase becomes lengthened in time during photostimulation. This is also suggested by the fact that the second increase of LH concentration following photostimulation occurs earlier than the first increase in Experiment II (Fig. 2). This readiness of LH release might be attributable to change in physiological condition for LH release at the level of the hypothalamus or the pituitary after initiation of LH secretion, although no conclusive evidence is at hand.

Unfortunately FSH was not measured in this study but if it is assumed that testicular growth is a good reflection of FSH released at the first stage of photostimulation (Follett, 1976), the inducible phase for FSH and LH secretion are different from each other (Figs. 3 and 5).

Is the photoinducible phase circadian in this species? In the present experiment, there is no direct evidence, but the phase shift in locomotor activity observed in groups G', H, and I, and the bimodality of the testicular growth curve induced by night interruption suggest it. In the white-crowned sparrow, Follett et al. (1974) have

demonstrated that the photoinducible phase for LH release ran freely in the constant darkness for at least five cycles. Locomotor activity is proved to be a good criterion of a circadian rhythm when an animal is kept in a constant condition. Recently, Stetson and his colleagues have suggested that locomotor activity and LH and FSH secretion in hamsters are driven by the same oscillator or are closely linked (Stetson and Gibson, 1977; Stetson and Watson-Whitmyre, 1977; Watson-Whitmyre and Stetson, 1977). In hamsters, they argue that the suprachiasmatic nucleus is the circadian oscillator or at least a part of the circadian oscillatory system. In avian species, there is no anatomical evidence which indicates the presence of the oscillator as an organ. Even though all of these observations may indicate that photoinduced gonadotropin release involves time measurement by the circadian oscillator, much experimental evidence is needed before this conclusion can be accepted.

A simple external coincidence model seems to be replaced by an internal coincidence model to explain locomotor activity in rodents (Pittendrigh and Daan, 1976). However, to explain the photoinduced LH release in avian species, an external coincidence model still seems plausible. One reason is that only one or two pulses can induce LH secretion in quail kept on short days. Another reason is that even with continuous lighting LH secretion can be induced 22 hr after lights are turned on (Follett *et al.*, 1977).

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