Mechanism Controlling Photostimulated Luteinizing Hormone Secretion Is Different from Preovulatory Luteinizing Hormone Surge in Japanese Quail (Coturnix coturnix japonica)

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A so-called "night-interruption" experiment with a 15-min light pulse showed that a sensitive phase for the photoperiodic LH secretion in male Japanese quail extended over a period of 2 hr from 12.5 to 14.5 hr after dawn. Exposure of a 1-hr light pulse at this photosensitive phase to male quail kept under 8L:16D induced the increase of plasma LH concentrations just the same as quail transferred to 16L:8D. In the first few days of photostimulation either by night interruption or by long days, LH concentrations increased at several hours after the photosensitive phase and decreased to the basal levels before daybreak. The amplitude and duration of this LH surge was somewhat like a preovulatory LH surge in females. However barbiturate anesthesia (pentobarbital and phenobarbital) administered on, before, or after the photosensitive phase did not block the LH increase by photostimulation. On the other hand, an injection of phenobarbital 14 hr before the expected ovulation blocked a preovulatory LH surge, even though the same drug failed to block photoinduced LH increase in females. These results indicate that the neuroendocrine mechanism involved in photostimulated LH release is different from that for an LH surge during an ovulatory cycle. © 1988 Academic Press, Inc.

Photostimulated gonadotropin secretion has been well studied in several avian species (for review see Follett, 1984). Long days induce a rapid release of luteinizing hormone (LH) in photosensitive birds. In Japanese quail, circulating LH increases stepwise for the first few days following transfer from short days to long days (Follett et al., 1977; Wada, 1979). During the first long day, LH increases from a basal level late at night, and LH rises again during the second night (Follett et al., 1977; Wada, 1979). Thus the actual LH increase occurs several hours after photostimulation.

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To stimulate LH release, continuous light is not required throughout a long day but only a short light pulse is enough if it impinges on the critical period that exists 13–15 hr after dawn (Wada, 1979). An injection of sodium phenobarbital prior to the expected ovulation blocks spontaneous preovulatory surges of LH in rats and hamsters (Everett and Sawyer, 1950; Stetson and Watson-Whitmyre, 1977). The critical sensitive period to block the ovulation exists over a period of about 2 hr on the afternoon of proestrus. Thus an injection of phenobarbital anesthesia out of the critical period fails to prevent the expected ovulation. In chickens phenobarbital injection 14 hr before the expected ovulation also prevents spontaneous and progesterone-induced ovulation possibly due to blockade of a LH surge (Fraps, 1955,
Tanaka et al., 1970). Because the administration of phenobarbital at the proestrous critical period causes the delayed ovulation for 24 hr, it is hypothesized that a circadian neural clock times this critical period in rats and hamsters (Everett and Sawyer, 1950; Stetson and Watson-Whitmyre, 1977). In Japanese quail, a neural clock mechanism is also suggested to control the photoinducible phase (Follett et al., 1974; Wada, 1979, 1981).

These studies indicate the existence of a neuroendocrine mechanism among a clock, photoreceptor, and LH release. However, there are few investigations on the effect of barbiturate blockade on photostimulated LH release to clarify the mechanism. Thus the purposes of the present study were (1) to establish a photoinducible phase for LH secretion with a more detailed experimental schedule of night interruption than the previous studies by Wada (1979, 1981), (2) to elucidate a LH secretion pattern in quail exposed to a light pulse during the photoinducible phase to compare to that under 16L:8D, and (3) to investigate effects of barbiturates (pentobarbital and phenobarbital) on LH secretion after photostimulation in comparison with the barbiturate effect on a preovulatory LH surge.

MATERIALS AND METHODS

Animals

Male and female Japanese quail (Coturnix coturnix japonica), 3 weeks old, were purchased from a commercial source. They were raised under a short day of 8L:16D (lights on at 0800) until 5 weeks of age, when they showed full somatic growth but were still sexually immature. They were then transferred to each experimental lighting schedule. Food and water were available at all times.

Female birds, transferred to 16L:8D (lights on at 0800), showed first oviposition when they were 8–9 weeks old. They were used for experiments to examine the effect of phenobarbital on the preovulatory LH surge at 13–17 weeks of age. Time of egg laying in each bird was recorded for all experimental periods with an operation recorder (Shimadzu Keisokuki Co. Ltd., Kyoto, Japan) and time of ovulation was estimated.

Experimental Schedules

Determination of the photoinducible phase for LH release and comparison with the LH release pattern in 16L:8D. Ten groups of 9 to 13 male quail were used for this experiment. One group of quail (Group 0) remained under a short day photoperiod of 8L:16D (lights on at 0800) as controls. To the others (Groups 1–9) we gave a single 15-min light pulse between 11 and 15 hr after the beginning of the main photoperiod for 4 days. Thus, in Group 1, a 15-min light pulse was delivered at 11 hr after the onset of the main photoperiod, in Group 2 a light pulse was delivered at 11.5 hr, and so on so that in Group 9 a light pulse was delivered at 15 hr after dawn.

Blood samples of 0.3 ml were collected at 1000 only for the first 4 days of treatment to avoid the possible phase shift caused by interpreting a light pulse as an entrainment time cue after longer treatments. The plasma was separated and stored at −20° until assay.

To determine and compare detailed patterns of plasma LH changes for the first 2 days after transfer to a stimulatory skeleton photoperiod of 8L:4.5D:1L:10.5D and a long day of 16L:8D, respectively, blood of 0.1 ml was repeatedly collected at 2- to 4-hr intervals.

Effects of barbiturates on photoperiodic LH secretion in males and females. To examine the effect of barbiturate blockade on the photosensitive phase, phenobarbital (7.5 mg/100 g body wt (BW) in 0.5 ml saline) was injected intraperitoneally to male quail transferred to a skeleton photoperiod of 8L:4.5D:1L:10.5D at just before 2030 for the first 2 days. To keep birds anesthetized for 1 hr of a light pulse, 1–2 mg of phenobarbital was administered at 1830 and at 2230 for the first 2 days in the second and the third group, respectively. Control birds were injected with saline. Blood (0.1 ml) was collected at 2- to 4-hr intervals for 2 days.

In the following experiments, photosensitive males and females were transferred to a long day of 16L:8D and phenobarbital, a long acting barbiturate, was injected at just before 2000 to anesthetize quail during the photosensitive phase for the first 4 days. The administered dose was 19 mg/100 g BW in 0.5 ml saline on Day 1, while on Days 2, 3, and 4, 24 mg/100 g BW in 0.5 ml saline was injected to maintain the anesthesia effect for about the same period of time each day. Controls were injected with saline. Blood of 0.1 ml was collected serially at 2- to 8-hr intervals for 4 days in males and daily at 1000 in females.

Effect of phenobarbital on the preovulatory LH surge. In laying quail, phenobarbital (17 mg/100 g BW in 0.1 ml saline) was injected intraperitoneally 14 hr before an expected ovulation. Saline was injected in controls. Blood samples of 0.1 ml were taken at 1- to 4-hr intervals during next 16 hr.
Radioimmunoassay

Plasma concentrations of LH were determined in 20-, 25-, or 50-µl sample volumes in duplicate depending on the volumes of collected blood, using the radioimmunoassay method described by Hattori and Wakahayashi (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 6.97 and 9.89%, respectively.

Statistics

Mann–Whitney’s U test, the two-way layout analysis of variance (ANOVA), and Fisher’s exact probability test were employed for statistical analysis when applicable.

RESULTS

Determination of the Photoinducible Phase for LH Release and Comparison with the LH Release Pattern in 16L:8D

A 15-min light pulse was given 11–15 hr after the onset of main photoperiod to quail kept under a short day of 8L:16D. On Day 1 of treatment, LH increased only in Group 8 (light pulse 14.5 hr after dawn). On Day 2 of treatment, however, LH concentrations increased in quail given a 15-min light pulse at 12.5, 13, 14, and 14.5 hr after dawn (Groups 4, 5, 7 and 8, respectively; Fig. 1) (Mann–Whitney U test). On Days 3 and 4, LH increase was apparent in all the groups in which the pulses were given between 12.5 and 14.5 hr after dawn (Groups 4–8).

Figure 2a shows the patterns of plasma LH increase during the first 2 days of exposure to a long day of 16L:8D and a skeleton photoperiod in which a 1-hr light pulse was given at the photosensitive phase (8L:4.5D:1L:10.5D). In both groups, plasma LH concentrations changed in a similar pattern and a difference was not detected by two-way layout ANOVA (P > 0.05). LH increased very slightly during the first dark period and at the beginning of the second day it decreased to basal levels (<0.5 ng/ml). In the dark period of the second day, LH increased again to 1–4 ng/ml and slightly decreased at the third day. As suggested by variances about mean LH values in Fig. 2a, LH increased abruptly and soon decreased to the basal level at a slightly different time of day in each individual (Fig. 2b).

The number of individuals which showed an apparent LH increase on Days 1 and 2, respectively, was not different between the two groups (Fisher’s exact probability test).

Effects of Barbiturate on Photoperiodic LH Release

A daily pentobarbital injection either at 1830, 2030, or 2230 did not block normal LH release, and LH release which mainly occurred during the dark period was found as in the saline control groups (Fig. 3) (P > 0.05, ANOVA).

Figure 4 shows the effect of phenobarbital anesthesia on the rise of plasma LH by photoinduction. A single injection of phe-
FIG. 2. (a) Changes in plasma LH concentrations during the first 2 days of exposure from 8L:16D to 16L:8D (solid line) and to 8L:4.5D:1L:10.5D (broken line). Each point is the mean of 11 or 17 birds, respectively. Vertical lines indicate standard error of the means. (b) Two typical examples of LH profiles in an individual bird under the conditions mentioned above.

Fig. 3. Changes in plasma LH concentrations during the first 2 days in saline- (solid line) and pentobarbital-injected (broken line) male quail after transfer from 8L:16D to 8L:4.5D:1L:10.5D. Pentobarbital was injected 12.5 hr after dawn (at 2030). Each point is the mean of 9–12 birds and vertical lines indicate standard error of the means.

FIG. 4. Changes in plasma LH concentrations in saline (solid line) and phenobarbital-injected (broken line) male quail during the first 4 days of exposure to 16L:8D. Arrows represent the time of injection. Each point is the mean of six birds and vertical lines indicate standard error of the means.

Pentobarbital at 2000 anesthetized the birds for more than 4 hr, completely covering the photoinducible phase. This anesthetic effect is longer than that of pentobarbital. However, phenobarbital also failed to prevent the normal LH rise for the first 4 days of photostimulation in male and female quail (P > 0.05, ANOVA).

Effects of Phenobarbital on Preovulatory LH Release

Phenobarbital administration significantly reduced the level of the preovulatory LH surge and blocked ovulation in eight out of nine quail (Fig. 5). In the control group, plasma concentrations of LH started to increase 6–7 hr before ovulation and reached maximum levels (3.07 ± 0.39 ng/ml, n = 9) at 4–5 hr before ovulation; seven out of nine quail ovulated (Fig. 5). The pattern of LH release was significantly different (P < 0.01, ANOVA) and rates of ovulation between the two groups was also significantly different (P < 0.01, Fisher’s exact probability test).

DISCUSSION

The existence of the photoinducible phase in Japanese quail was demonstrated by the use of “resonance” and “interrupted-night” experiments (Follett and
PHOTOPERIODIC AND OVULATORY LUTEINIZING HORMONE RELEASE

Fig. 5. (a) Plasma LH concentrations in relation to the time of expected ovulation in saline (solid line) and phenobarbital-injected (broken line) quail. Time of injections (arrow) was 14 h before expected ovulation. Each point is the mean of nine birds and vertical lines indicate standard error of the means. (b) Typical examples of LH profiles during successive 2 days in experimental (left) and control (right) birds. On the first day no injection was given and an LH surge and following ovulation (short solid bar by graph) was observed in both birds while on the second day phenobarbital injection (thick arrow) 14 h before expected ovulation blocked the LH surge and subsequent ovulation (bar with cross) whereas saline injection (thin arrow) did not.

Sharp, 1969). From previous studies using a 30-min light pulse changing at 2-hr intervals from group to group, Wada (1979, 1981) argued that the photoinducible phase was initially short and it gradually extended from 13 to 15 hr after dawn with increasing number of days of photostimulation. However, data from the present study, using a more precise night-interruption schedule, show that the photoinducible phase for LH secre-

tion exists for a relatively long duration, about 2 hr from 12.5 to 14.5 hr after dawn, from the beginning of photostimulation.

When the external light coincided with this photoinducible phase, LH release occurred several hours later as shown in Fig. 2. The profile of increase is similar in form to the LH surge for ovulation. The time lag between light stimulation and LH increase suggests a duration which is required for the environmental information of light to be processed and transmitted to the neuroendocrine pathways involved in the activation of LH-RH neurons in the hypothalamus. The idea is supported by the fact that LH-RH injection induces immediate release of LH both in vivo and in vitro (Hattori et al., 1986). Thus LH-RH release from the median eminence would likely cause immediate LH secretion in the normal in vivo process.

To date, investigations of the neuronal system involved in regulating LH-RH secretion have exploited to affect the pharmacological techniques on an ovulatory LH surge in mammals (see for review Everett, 1964, 1972; Barraclough and Wise 1982; Barraclough et al., 1984). Barbiturate anesthesia blocks the preovulatory LH surge and ovulation in rats and hamsters (Everett and Sawyer, 1950; Stetson and Watson-Whitmyre, 1977). Experiments employing more specific agonists and antagonists for neurotransmitters indicate that the noradrenergic system is essential for regulation of LH release (Barraclough and Sawyer, 1957; Karla and McCann, 1974). Catecholamine turnover rates in discrete hypothalamic areas also correlate with circulating gonadotropins (Rancer et al., 1981). In birds, catecholamine involvement in preovulatory LH release was also demonstrated by barbiturate blockade (Fraps, 1953, 1955; Tanaka et al., 1970), by injection of specific monoamine antagonists (van Tienhoven et al., 1954; Buonomo et al., 1981), and by changes in catecholamine
turnover rates in the hypothalamus (Knight et al., 1982a, b).

Involvement of adrenergic neural systems in the photoperiodic gonadotropin release is also supported by pharmacological studies; Davies and Follett (1974) demonstrated that 6-hydroxydopamine, which causes degeneration of adrenergic neurons, caused a suppression of photoperiodically induced testicular growth in Japanese quail. Reserpin, an inhibitor of catecholamine reuptake, and pentobarbital anesthesia blocked the rise of LH in quail on the first long day of photostimulation (Follett et al., 1977). El Halawani et al. (1980) showed in quail that photoinduced LH release was prevented by α-MT injection, which causes depletion of brain norepinephrine (NE), epinephrine (E), and dopamine by acting as a competitive inhibitor of tyrosine hydroxylase (El Halawani and Burke, 1975). Recent in vitro experiments show that NE, E, or isoproterenol added to the superfusion medium cause the release of LH-RH from quail hypothalamic tissue (Millam et al., 1984). El Halawani et al. (1978) and Sakurai et al. (1986) also indicated that serotonergic neural circuits were involved in photoinduction in Japanese quail and preovulatory LH release in hens.

In the present experiments, however, we failed to demonstrate the blockade of photostimulated LH release by either pentobarbital or phenobarbital. We cannot explain why our results conflict with the results of Follett et al. (1977). Probably the discrepancy is derived from the different state of birds in the two investigations; we used maturing quail detecting the first response to a stimulatory long day while Follett et al. (1977) used recrudescent adult quail in their experiments. Since a phenobarbital injection of the same dose caused a complete suppression of the preovulatory LH surge and the expected ovulation (Fig. 5), different mechanisms in the neural system are involved in modulating LH release between sexually maturing quail and mature laying quail.

The pharmacological studies mentioned above seem to indicate that monoamines and serotonin are involved in photoinduced LH secretion and the preovulatory LH surge. However, the present results suggest that the processes involved in photostimulated LH release and in preovulatory LH release are different in their neuronal bases. Actually, under experimental conditions used here, injections of either α-MT or p-chlorophenylalanine did not block photoinduced LH secretion (unpublished results). A careful survey of the experimental conditions in the experiments mentioned above indicate that they were designed to detect LH changes after circulating LH became rather high. We postulate that LH release at the transition from short to long days, before the hypothalamo–hypophysial–gonadal axis is established in a “long-day mode” is different from that at the later stages of photostimulation.

The most significant factor for activating the central neuroendocrine system regulating photoperiodic LH release seems to be photic information itself. Encephalic photoreception has been demonstrated in Japanese quail (Follett et al., 1975; Oliver et al., 1979; Homma et al., 1979). Direct illumination of the medial basal hypothalamus through optic fibers induced testicular growth in white-crowned sparrows (Yokoyama et al., 1978) and LH secretion as well as testicular growth in Japanese quail (Ohta et al., 1984). These results indicate that the basal hypothalamus has the capacity to perceive photic information. Recently, a rhodopsin-like photopigment in the hypothalamus was observed by spectral analysis (Foster and Follett, 1985).

In this regard, the results by Ohta et al. (1984) are interesting. They show an intrinsic rhythm of sensitivity to weak, brief electric stimulation which resembles the rhythm for photoinducibility. Photic stimu-
lation as well as weak electric stimulation seem to activate a system which finally induces LH-RH release after hours of lag time. The process may differ from LH surges during ovarian cycles which are steroid-dependent and neurally mediated.

Even if the speculation is correct, the relation between adrenergic and serotonergic neural systems and steroid feedback mechanisms in regulating LH secretion must be investigated. At present, we feel that LH secretion in photostimulated naive birds is regulated directly by light whether in more experienced birds LH release is regulated and modified by the neural system, after sexual maturation is completed and ovulatory cycles start.

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