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Seasonal change in luteinizing hormone subunit mRNA in Japanese quail and effects of short daylength and low temperature

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Abstract

Changes in pituitary mRNA levels of LHβ-subunit (LHβ) and glycoprotein hormone α-subunit (common α) were investigated in male Japanese quail under natural and laboratory conditions to clarify the mechanisms of seasonal regulation of luteinizing hormone (LH) secretion. In Experiment 1, birds were kept in outdoor cages under natural conditions from August for 12 months. Both LHβ and common α mRNA levels decreased rapidly from August to September, and after a period of low levels from October through January, they began to increase in February and continued to increase until July. There were more pronounced seasonal changes in testicular weight and cloacal protrusion width with large decreases from August to September and increases from March to May. In Experiment 2, birds were kept on laboratory conditions and transferred from long to short daylengths at 20 or 9 °C and held for 14 days. Although common α mRNA levels, plasma LH concentrations, testicular weight, and cloacal protrusion area decreased on short days without low temperatures, levels of LHβ mRNA did not change. Short daylengths combined with low temperatures induced testicular regression and caused decrease in all the parameters measured. Low temperatures under long days did not induce any change in the parameters significantly. These results suggest that (1) synthesis as well as secretion of LH is regulated seasonally, (2) short daylength does not suppress LH synthesis completely unless combined with low ambient temperature, and (3) the effect of photoperiod on the endocrine system regulating LH secretion is predominant over the effect of ambient temperature but ambient temperature acts as an environmental cue to terminate reproductive activities at late summer to early autumn in Japanese quail.

Keywords: Japanese quail; LH; mRNA; Seasonal change; Daylength; Ambient temperature

1. Introduction

The mechanisms controlling seasonal breeding have been studied in various avian species and many prior investigations have indicated that photoperiod plays a dominant role controlling seasonal reproduction in birds that breed at mid- to high-latitudes by regulating secretion of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (for review see Follett, 1984; Wingfield and Farner, 1993). Long daylengths not only stimulate secretion of gonadotropin but also initiate processes leading to "absolute photorefractoriness" when the reproductive system ceases to respond to continued long days. Refractoriness leads to the termination of breeding well before the summer solstice in many avian species (for review see Dawson et al., 2001; Nicholls et al., 1988).

In Japanese quail, the mechanism that induces LH secretion at the beginning of the breeding season is well established. Birds measure daylength by receiving light in the photoinducible phase, leading to an increase in circulating concentration of LH (Hatanaka and Wada, 1988; Wada, 1979, 1981). However, they do not usually

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express photorefractoriness until daylength has decreased substantially in late summer when daylength is still longer than that required for reproductive development. This is explained as "relative photorefractoriness" (Robinson and Follett, 1982; Urbanski and Follett, 1982), although its mechanism is not fully understood.

In previous studies in our laboratory, low ambient temperature combined with short daylength decreased plasma LH concentrations and caused cloacal and testicular regression, while short daylength without low ambient temperature decreased plasma LH concentration only to a certain level which could maintain cloacal and testicular development in Japanese quail (Tsuyoshi and Wada, 1992; Wada, 1993; Wada et al., 1990). A rapid decrease in plasma LH under natural conditions from late summer to early autumn also occurred when ambient temperature was declining (Wada et al., 1992, in preparation). These results suggest that a decrease in ambient temperature is required to terminate LH secretion and reproductive activities when daylength is declining.

Recently, the interests of endocrinologists have been focused on the gene expression of gonadotropin subunit precursor molecules under various environmental conditions. The measurement of mRNAs encoding these molecules is a useful approach to examine questions about gene expression. Kubokawa et al. (1994) reported that pituitary LHβ-subunit mRNA significantly increased in photostimulated white-crowned sparrows, and decreased in photorefractory birds. Kikuchi et al. (1998) reported that pituitary FSHβ-subunit mRNA increased in breeding Japanese quail kept in an outdoor cage. These reports suggest that not only secretion but also the synthesis of gonadotropins change seasonally, and also suggest that low temperature combined with short daylength might reduce pituitary LH subunit mRNA in Japanese quail.

The aims of the present study were (1) to elucidate changes in mRNA levels of LHβ-subunit and pituitary glycoprotein hormone α-subunit in the pituitary gland under natural conditions and (2) to determine the effects of low ambient temperature and short daylength on the mRNA levels in Japanese quail.

2. Materials and methods

2.1. Animals

Male Japanese quail (*Coturnix japonica*) were purchased from a commercial source (Tokai Yuki, Aichi Prefecture, Japan). Food (NQ-1, Nippon Institute for Biological Science, Tokyo, Japan) and water were provided ad libitum throughout the experimental period. No selection of birds was made on the basis of gonadal changes by photoperiodic manipulation before the start of the experiments.

2.2. Experimental schedules

2.2.1. Experiment 1: seasonal change

Birds (8 weeks old) were obtained in June 1993 and were housed collectively in cages placed on the roof of a building on the campus of Waseda University in Tokyo (35°42′N, 139°43′E) under natural daylength and ambient temperature. Four to six birds were selected randomly and sacrificed on the 15th day of every month from August through December 1993. In December 1993, newly 8-week-old birds were obtained. These birds were kept in the above conditions and used for sample collection from January 1994. Sample collection was continued until July 1994.

2.2.2. Experiment 2: effect of artificial low ambient temperature and short photoperiod

Birds (10 weeks old, reared under constant dim light) were obtained and kept in individual cages under constant conditions of long photoperiod (16 h light:8 h darkness, 16L8D) at ambient temperature of 20 °C for a week before the start of experiments.

In Experiment 2-1, birds were randomly divided into three groups. Birds in two groups were transferred either to a short photoperiod (8L16D) at 20 °C (Group SD20), or to 8L16D at 9 °C (Group SD9). Birds of the remaining group were maintained on the initial conditions (Group LD20). In Experiment 2-2, birds were randomly divided into two groups. Birds of one group were transferred to 16L8D at 9 °C (Group LD9). Birds of the other group were maintained on the same initial conditions (Group LD20) as the group in Experiment 2-1.

Birds of Group SD20 and SD9 in Experiment 2-1 and Group LD9 in Experiment 2-2 were sacrificed 3, 7, and 14 days after the start of experiment. Birds of Group LD20 in both experiments were sacrificed on the 0 day and then on the same time schedule as the other groups. Eight to 12 birds were selected randomly and sacrificed from each group at each sampling time.

2.3. Sample collection

The size of the cloacal protrusion was measured with calipers. In Experiment 1, only the width of the protrusion was measured, but in Experiment 2 width and height were measured to obtain the area of the protrusion. Pituitary glands were excised, then frozen immediately in liquid nitrogen, and stored at −85 °C until RNA extraction. Both testes were dissected out and weighed to the nearest milligram. In Experiment 2, trunk blood was also collected just before

sacrifice and plasma was stored at −30 °C until radioimmunoassay.

2.4. RNA extraction

Total RNA was extracted from each pituitary gland using a commercial kit (ISOGEN, Nippon Gene, Toyama Prefecture, Japan). Extracted RNA was dissolved in nuclease-free water and stored at −85 °C until electrophoresis.

2.5. Hybridization probes

To estimate contents of mRNAs by Northern blotting, the following cDNAs were used as hybridization probes: luteinizing hormone β-subunit (LHβ) cDNA (pQL119 of Ando and Ishii, 1994), pituitary glycoprotein hormone α-subunit (common α) cDNA (pQA312 of Ando and Ishii, 1994), and chicken β-actin cDNA (Oncor, Gaithersburg, MD). All these cDNAs were labeled with [α-^{32}P]dCTP (AA0005, Amersham Biosciences, Piscataway, NJ) using the random prime labeling system (*redi*prime DNA labeling system, Amersham Biosciences).

2.6. Northern blotting

Contents of mRNAs for LHβ and common α were measured by Northern blotting as described by Kobayashi and Ishii (2002). A total RNA sample extracted from the pituitary glands of a number of sexually mature adult males Japanese quail was applied to every plate as the internal reference standard. After hybridization, hybridization signals were analyzed and quantified in a BAS-2000 II Bio-Imaging analyzer (Fuji Photo Film, Japan). Hybridization signals for all the mRNAs were expressed as relative values to the signal of the reference standard in each electrophoretic run and were standardized among different electrophoresis plates. Contents of the LH subunit mRNAs were expressed as ratios of the values of the LH subunit precursors to the values of β-actin mRNA in the pituitary gland.

2.7. Radioimmunoassay

Plasma concentrations of LH were estimated by double-antibody RIA for chicken LH as described originally by Hattori and Wakabayashi (1979) with slight modifications. A different batch of purified chicken LH (AGMS1122F, Miya and Ishii, unpublished) was used for radioiodination, and a crude chicken LH fraction (AGC112B, Kikuchi and Ishii, 1989) was used as the reference standard. Assay results were expressed in terms of the mean weight of purified chicken LH (IRC-2, Gunma). All plasma samples were assayed in a single run. The intraassay coefficient of variation was 5.49%.

2.8. Meteorological data during experimental period

Daylength was calculated from a table of sunrise and sunset times at Tokyo 35°41.2′N listed in ''Chronological Scientific Tables,'' edited by Tokyo Astronomical Observatory, and published by Maruzen, Tokyo, as the light period including civil twilight of dawn and dusk. Monthly mean ambient temperature at the same location was also listed. These meteorological data were used for figure and discussion.

2.9. Statistical analyses

In Experiment 1, Kruskal–Wallis tests were used. If they were significant, Scheffé tests were applied to compare different sampling times. In Experiment 2, two-way analysis of variance (ANOVA) was applied. If there were significant differences between groups, one-way ANOVA or Kruskal–Wallis tests followed by Scheffé tests were used to compare groups on the same sampling day. A P value less than 0.05 was regarded to be statistically significant.

3. Results

3.1. Experiment 1: seasonal change

The annual change in daylength in Tokyo is between about 11 and 16 h with the longest one in June and the shortest in December (Fig. 1A). However, the mean ambient temperature became highest in August and lowest in January (Fig. 1A).

The mean width of the cloacal protrusion and combined testicular weight changed significantly over the 12 months studied (Figs. 1B and C, $H = 45.946$ and 43.101, respectively, $P < 0.0001$). The width of cloacal protrusion was large in August. It decreased rapidly in September to nearly the basal size, reached the minimum size in October, and remained regressed until March. From March it increased and reached full size in May and maintained the maximum levels until July with a slight decrease in July. Combined testicular weight showed a similar pattern, but the decrease from August to September and increase from March to May were more pronounced.

From October to February, the testes were completely regressed (less than 30 mg), and the cloacal protrusion at its smallest size. Therefore, we designated these months as nonbreeding months, and compared the mean of the nonbreeding months with other months. The mean cloacal width and testicular weight in the months in which they were developing or developed were significantly higher than the values in nonbreeding months ($P < 0.0001$; except testicular weight of July $P < 0.001$).

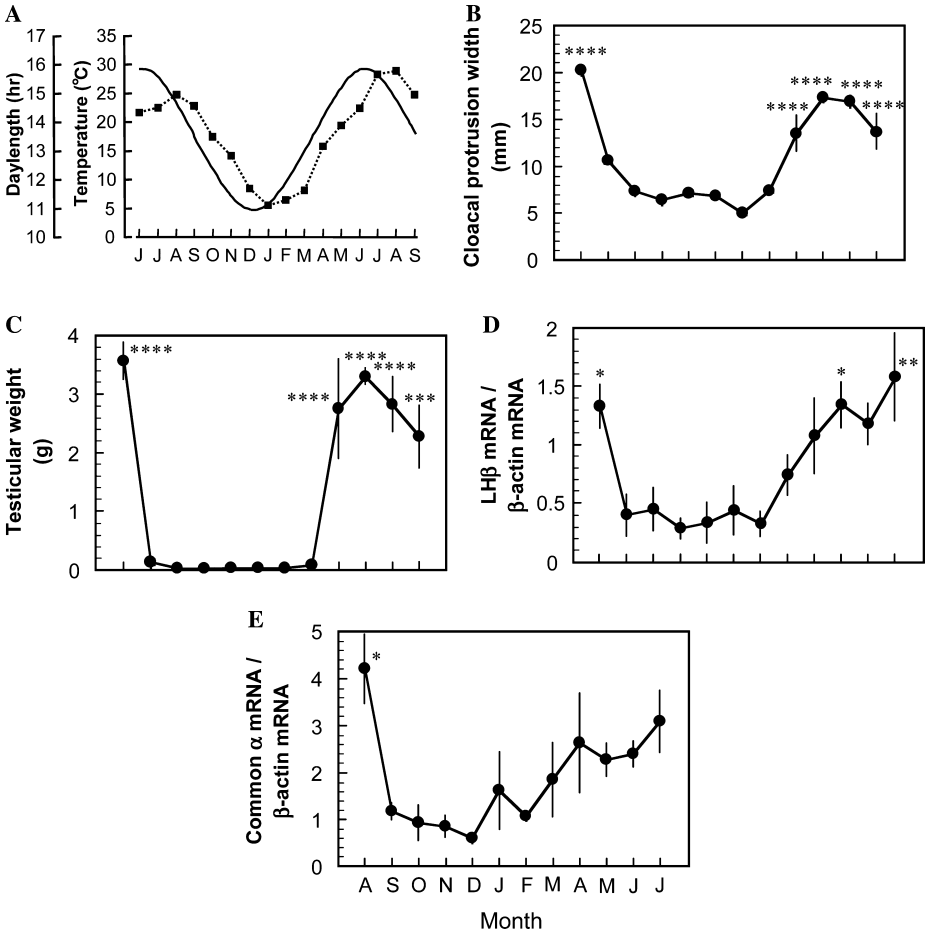

Fig. 1. Changes in daylength (solid line) and ambient temperature (solid square with dotted line) in Tokyo from June 1993 to September 1994 (A), and the width of cloacal protrusion (B), testicular weight (C), LHβ mRNA (D), and common α mRNA (E) levels in Japanese quail kept in outdoor cages under natural conditions. Circles and bars represent the mean and SEM, respectively. Significant differences from nonbreeding months (from October to February, see Section 3) are indicated at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$(****).

The mean levels of LHβ mRNA changed significantly (Fig. 1D, $H = 33.507$, $P < 0.001$). LHβ mRNA was highest in August, and then decreased rapidly in September. It remained low with some fluctuations during winter then increased gradually from February to July. LHβ mRNA levels in August, May, and July were significantly higher than those in nonbreeding months (August and May vs. nonbreeding months, $P < 0.05$, July vs. nonbreeding months, $P < 0.01$). The mean levels of common α mRNA showed similar changes to those of LHβ mRNA (Fig 1E, $H = 28.642$, $P < 0.01$). However, the fluctuations in common α mRNA were somewhat larger than those of LHβ mRNA and a significant difference from nonbreeding months was observed only in August ($P < 0.05$).

3.2. Experiment 2: effect of artificial low ambient temperature and short photoperiod

ANOVA analysis indicated the significant effects of low ambient temperature and short photoperiod on parameters measured among groups in Experiment 2-1 (Fig. 2; cloacal protrusion area: $F = 16.155$, $P < 0.0001$; testicular weight: $F = 17.462$, $P < 0.0001$; plasma LH: $F = 30.540$, $P < 0.0001$; LHβ mRNA: $F = 14.884$, $P < 0.0001$; common α mRNA: $F = 16.769$, $P < 0.0001$).

The area of the cloacal protrusion of birds in Group LD20 did not change throughout the 14 days of the experiment (Fig. 2A). In Group SD20, it remained constant at 7 days, then decreased, and was lower than Group LD20 at 14 days ($P < 0.01$). In Group SD9, the area gradually decreased and was smaller than Group LD20 at 7 and 14 days ($P < 0.05$ and $P < 0.001$) and was smaller than Group SD20 ($P < 0.05$) after 14 days.

Combined testicular weight in Group LD20 did not change significantly, although there was an increase from 3 to 7 days (Fig. 2B). In Group SD20, testicular weight decreased slightly from 7 to 14 days and was significantly lower than Group LD20 at 14 days ($P < 0.05$). In Group SD9, testicular weight declined slightly at 7 days, and was significantly smaller than that of Group

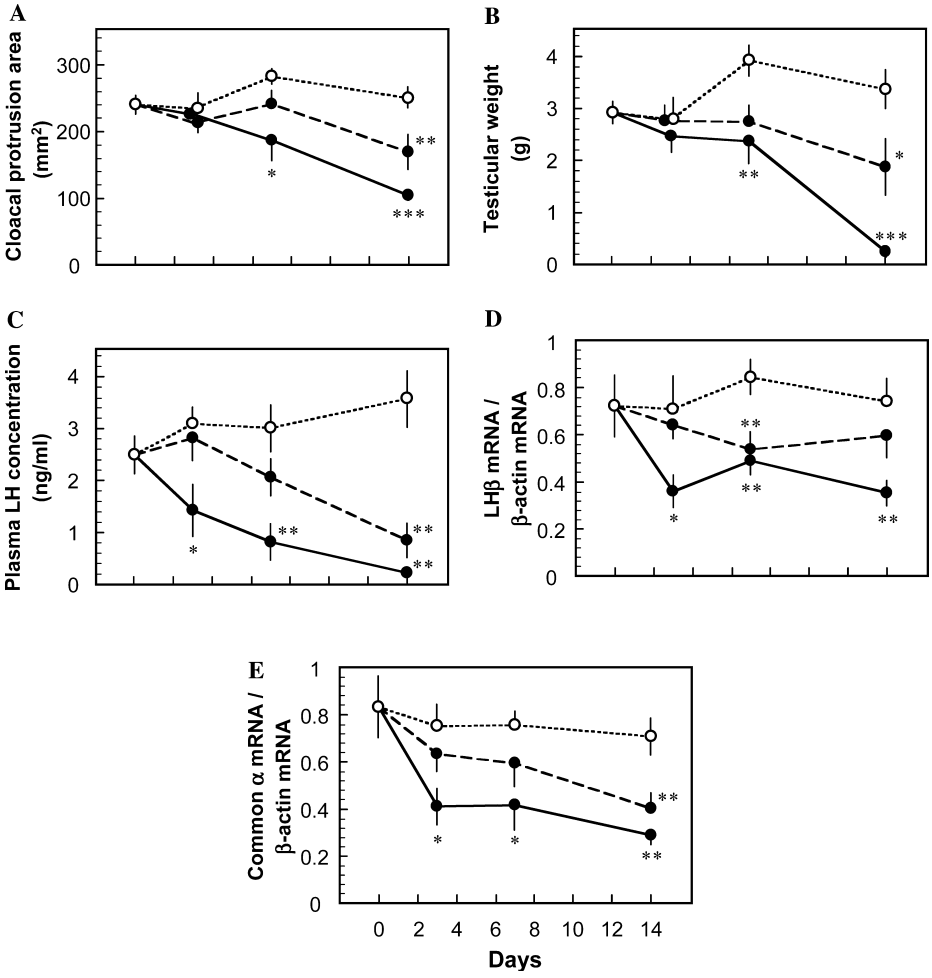

Fig. 2. Changes in the cloacal protrusion area (A), testicular weight (B), plasma LH concentration (C), LHβ mRNA (D), and common α mRNA (E) levels in Japanese quail in Group LD20 (open circle with dotted line), SD20 (solid circle with broken line), and SD9 (solid circle with solid line). Circles and bars represent the mean and SEM, respectively. Significant differences from the Group LD20 are indicated at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

LD20 which increased from the initial level ($P < 0.01$). It decreased markedly by 14 days and was significantly smaller than Group LD20 ($P < 0.001$) and Group SD20 ($P < 0.01$).

Plasma LH concentrations gradually increased through the 14 days of experiment in Group LD20 (Fig. 2C). In Group SD20, LH was the same level as Group LD20 after 3 days, then decreased gradually and was significantly lower than Group LD20 at 14 days ($P < 0.01$). In Group SD9, it decreased gradually after the onset of experiment, was significantly lower than Group LD20 after 3 days ($P < 0.05$), and continued to decline to lower levels than Group LD 20 thereafter ($P < 0.01$).

LHβ mRNA level did not change through the 14 days of experiment in Group LD20 (Fig. 2D). In Group SD20, it decreased and was significantly lower than Group LD20 at 7 days ($P < 0.01$), but then increased slightly and did not differ significantly from Group LD20 at 14 days. In Group SD9, it decreased rapidly, was significantly lower Group LD20 at 3 days ($P < 0.05$), and remained low thereafter ($P < 0.01$).

Common α mRNA level did not change markedly through the experimental period in Group LD20 (Fig. 2E). In Group SD20, it decreased gradually and became significantly lower than Group LD20 at 14 days ($P < 0.01$). In Group SD9, it decreased rapidly and was significantly lower than Group LD20 at 3 days ($P < 0.05$) and thereafter (7 days, $P < 0.05$; 14 days, $P < 0.01$).

In contrast to Experiment 2-1, no significant effects were detected by ANOVA on the birds given low ambient temperature combined with long days in the parameters measured (Fig. 3; cloacal protrusion area: $F = 2.914$, $P = 0.942$; testicular weight: $F = 0.119$, $P = 0.732$; plasma LH: $F = 2.096$, $P = 0.154$; LHβ mRNA: $F = 0.071$, $P = 0.791$; and common α mRNA: $F = 0.025$, $P = 0.875$). Cloacal protrusion area, testicular weight, LHβ mRNA, and common α mRNA fluctuated within a small range in both Group LD20 and LD9

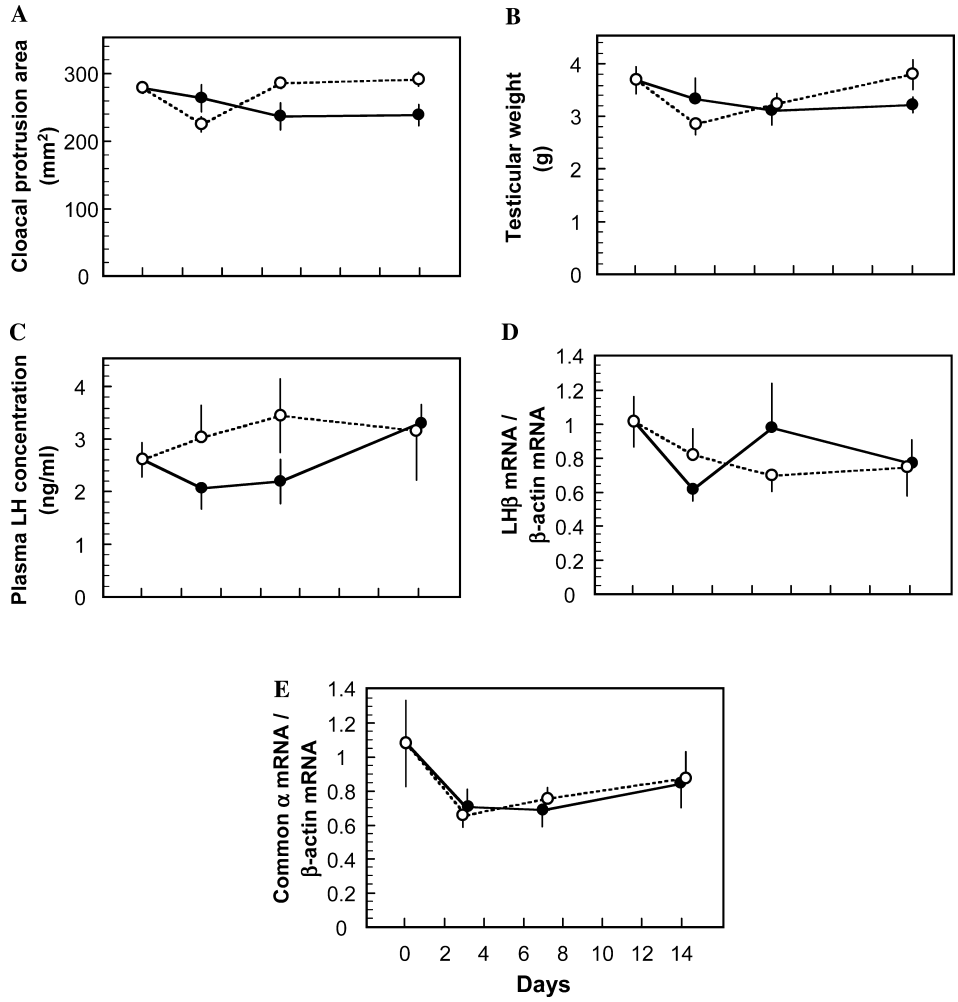

Fig. 3. Changes in the cloacal protrusion area (A), testicular weight (B), plasma LH concentration (C), LHβ mRNA (D), and common α mRNA (E) levels in Japanese quail in Group LD20 (open circle with dotted line) and LD9 (solid circle with solid line). Circles and bars represent the mean and SEM, respectively.

throughout the experimental period (Figs. 3A, B, D, and E, respectively). Plasma LH increased slightly after the start of experiment in Group LD20, while it decreased slightly in Group LD9 (Fig. 3C). However, plasma LH in Group LD9 then increased and reached the level of Group LD20 on Day 14.

In Experiment 2-1, cloacal protrusion area and testicular weight in 4 out of 10 quail in Group SD20 had regressed to the level of Group SD9 14 days after the start of the experiment. Therefore, we designated these four quail that had regressed cloacal protrusion and testes as Group SD20(R) and the other quail that had developed cloacal protrusion and testes as Group SD20(D). These two groups were considered separately, and compared with Group LD20 and SD9 (Fig. 4). Cloacal protrusion areas and testicular weights in Group SD20(D) and SD20(R) were comparable to those in Group LD20 and SD9, respectively (Figs. 4A and B, respectively). Those in Group SD20(R) were significantly lower than those in Group LD20 and SD20(D) ($P < 0.0001$). Plasma LH in Group SD20(R) was also lower than in Group SD20(D) and slightly lower than in Group SD9 (Fig. 4C). However, the difference between Group SD20(D) and SD20(R) was statistically insignificant, although the levels in these two groups were significantly lower than Group LD20 (LD20 vs. SD20(D), $P < 0.01$; LD20 vs. SD20(R), $P < 0.0001$). Both LHβ and common α mRNA levels in Group SD20(R) were lower than those in Group SD20(D), while the differences between the two groups were not statistically significant (Figs. 4D and E, respectively). However, the common α mRNA level in Group SD20(R) was significantly lower than that in Group LD20 ($P < 0.05$).

4. Discussion

The present study clearly showed seasonal changes in both LHβ and common α mRNA levels. There were rapid decreases of both LHβ and common α mRNA levels

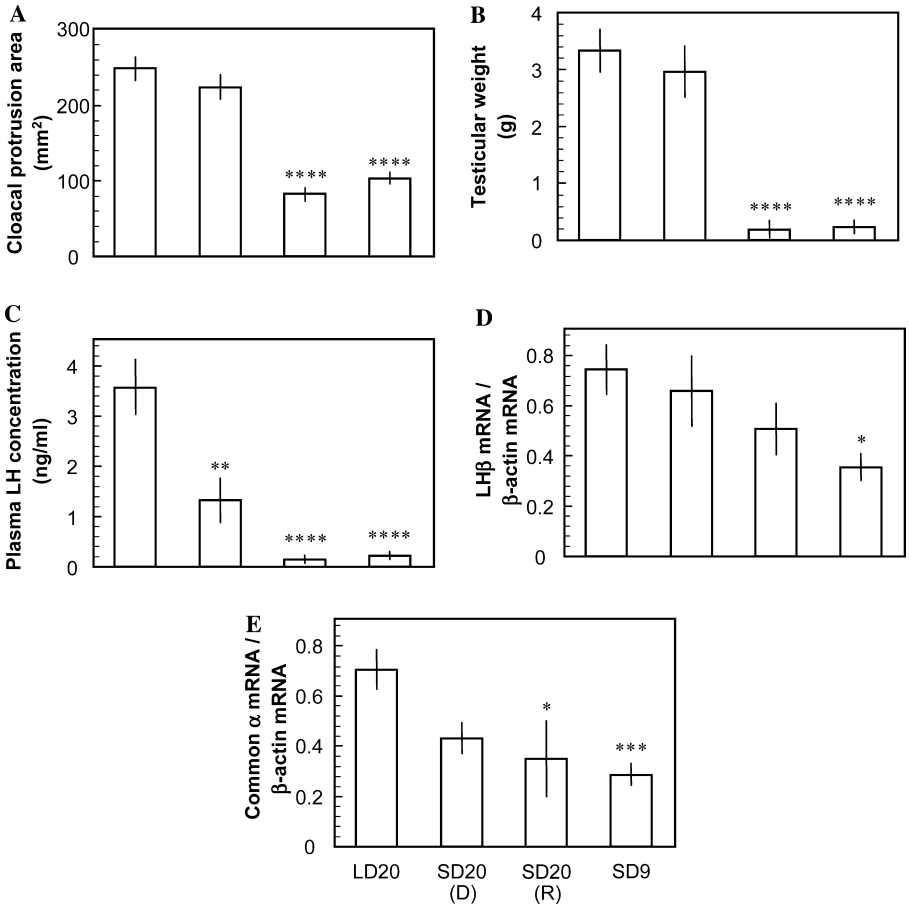

Fig. 4. The cloacal protrusion area (A), testicular weight (B), plasma LH concentration (C), LHβ mRNA (D), and common α mRNA (E) levels in Japanese quail in Group LD20, SD20(D), SD20(R), and SD9 on Day 14. Column and bars represent the mean and SEM, respectively. Significant differences from the Group LD20 are indicated at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****).

from August to September, and a period of low levels from October to January. Then they began to increase from February throughout July. These changes were accompanied by cloacal and testicular changes. Plasma LH was not estimated in these birds in Experiment 1, but these results agree well with previous studies in which plasma LH levels were measured in quail kept outdoors (Follett and Maung, 1978; Robinson and Follett, 1982; Wada et al., 1992; Wada et al., in preparation). The data suggest that not only secretion but also the synthesis of LH is seasonally regulated.

It has been reported that the timing of changes in plasma FSH concentrations was nearly identical with changes in plasma LH concentrations in Japanese quail (Follett and Maung, 1978; Wada et al., in preparation). Kikuchi et al. (1998) also reported that FSHβ-subunit mRNA level in Japanese quail kept under similar conditions to the present experiment increased in the breeding season. It is suggested that seasonal changes in mRNA levels encoding gonadotropin subunits in Japanese quail are regulated by gonadotropin-releasing hormone (GnRH) neurons, or related neuroendocrine systems in the hypothalamus. In white-crowned sparrows, which show absolute photorefractoriness, 30 days of photostimulation increased pituitary LHβ mRNA. However, it decreased significantly when photostimulation was prolonged up to 60 days (Kubokawa et al., 1994). Moreover, absolute photorefractoriness is thought to be mediated via GnRH systems in white-crowned sparrow (Meddle et al., 1999) and European starling (Dawson et al., 1985; Foster et al., 1987; Parry et al., 1997).

In previous studies in our laboratory, rapid decrease in plasma LH under natural conditions was observed from late August to early September when ambient temperature was declining (Wada et al., 1992; Wada et al., in preparation). Our present result in pituitary LH subunit mRNA also agrees with these previous results. Low ambient temperature combined with short daylength is required to decrease plasma LH concentrations markedly and cause cloacal and testicular regression in Japanese quail in laboratory conditions (Tsuyoshi and Wada, 1992; Wada, 1993; Wada et al., 1990). Therefore, Experiment 2 in the present study was conducted to investigate whether artificial low temperature combined with short daylength reduces LHβ and common α

mRNA levels in the pituitary gland, as observed in natural conditions.

As expected, low temperature combined with short daylength significantly decreased LHβ and common α subunit mRNA levels and caused marked decreases in plasma LH concentration, cloacal protrusion area, and testicular weight in Group SD9 in Experiment 2-1. Although short daylength without low ambient temperature induced significant decreases in cloacal protrusion area, testicular weight, plasma LH concentration, and common α subunit mRNA level in Group SD20, the rates of decrease of these parameters were less than those in Group SD9. Also, LHβ mRNA in Group SD20 decreased only temporarily, and the level in Group SD20 after 14 days did not significantly differ from the level in Group LD20. These results suggest that low temperature combined with short daylength is required to suppress not only LH secretion but also its synthesis. These results agree with previous studies and support the idea that ambient temperature as well as daylength acts as an environmental cue to terminate reproductive activities in Japanese quail (Tsuyoshi and Wada, 1992; Wada, 1993; Wada et al., 1990).

The discrepancy between changes in plasma LH concentration and LHβ mRNA level in Group SD20 may be explained by a large store of LH in the pituitary grand. Hattori et al. (1986) studied changes in gonadotropin release in vitro in Japanese quail and suggested that the pituitary gland in Japanese quail has a relatively large store of LH. It is suggested that short daylengths affect LH secretion more profoundly than its synthesis.

On the other hand, low ambient temperature under long days did not reduce the parameters measured in Experiment 2-2. This result agrees with previous study where low ambient temperature did not decrease plasma LH concentration in Japanese quail under long day photoperiod and could not prevent increasing plasma LH induced by long day photostimulation (Wada et al., 1990). It is suggested that the effect of photoperiod on the neuroendocrine system regulating reproduction is predominant over ambient temperature.

Four of 10 quail in Group SD20 on 14 days, which were designated as Group SD20(R), had reduced not only plasma LH concentrations but also cloacal protrusion area and testicular weight that decreased markedly to the levels of quail in Group SD9. The present results are quite comparable to those in which short daylength without reducing ambient temperature caused marked cloacal regression in about 30–40% of Japanese quail (Oishi and Konishi, 1983). This phenomenon has been mentioned in our previous studies (Tsuyoshi and Wada, 1992; Wada, 1993; Wada et al., 1990). By contrast, 6 of 10 quail in Group SD20 on 14 days, which were designated as Group SD20(D), maintained large developed cloacal protrusion areas and testicular weights comparable to those in Group LD20, while the level of plasma LH in Group SD20(D) was half of that found in Group LD20 or somewhat below. The maintenance of a large developed cloacal protrusion area in Group SD20(D) suggests that there is a threshold level in circulating LH concentrations for the testicular functions associated with androgen production. It was mentioned by Wada et al. (1990) that the highest level of circulating LH induced by long day photostimulation was not required to maintain large developed cloacal protrusions.

The difference in testicular weight between Group SD20(D) and SD20(R) may reflect circulating FSH concentrations. It is assumed that quail in Group SD20(D) maintained high circulating concentrations of FSH, while quail in SD20(R) reduced FSH in the same manner as plasma LH. As mentioned above, it is suggested that photorefractoriness occurs at the hypothalamic level related to GnRH. The study of gonadotropin releases in vitro in Japanese quail by Hattori et al. (1986) also suggested that the secretion of LH is rigidly controlled by GnRH but the secretion of FSH is at least partly autonomous. As is well known, starvation suppresses reproduction by reducing gonadotropin secretion in vertebrates including birds. It has been suggested that starvation suppresses the release of GnRH from hypothalamus in chickens (Contijoch et al., 1992). It is also reported that the secretion of LH is more sensitive to suppression by starvation than FSH in chicken (Scanes et al., 1976) and Japanese quail (Kobayashi et al., 2002). These studies support the assumption that quail in Group SD20(D) maintained high FSH.

Different responses to short daylengths were also observed for LHβ and common α mRNA levels in Group SD20. Common α mRNA levels in Group SD20 were significantly lower than in Group LD20. Also, the level in Group SD20(R) was significantly lower than that in Group LD20. However, 14 days of short daylength did not decrease LHβ mRNA levels in Group SD20(R), SD20(D) or a combination of them (Group SD20), while the level in SD20(R) was lower than that in SD20(D). It has been reported that starvation decreased mRNA levels of common α-subunit more markedly than those of β-subunit of LH and FSH in Japanese quail (Kobayashi and Ishii, 2002; Kobayashi et al., 2002). These data suggest that the common α mRNA level in pituitary gland is more sensitive than the other β-subunit mRNA levels to changes in the environment which affect reproduction. In Experiment 1 in the present study, however, the rate of fluctuation of the mean levels of LHβ mRNA was almost the same or somewhat larger than that of common α mRNA. Different responses of LHβ and common α mRNA levels may be caused by rapid environmental changes over a short time period.

The experimental data and interpretations by us differ from those by Follett and his colleagues. They

demonstrated that short daylengths were sufficient to terminate breeding activities in Japanese quail. Their interpretations are based only on photoperiodic mechanisms, although Robinson and Follett (1982) mentioned that the timing of decreases in plasma LH in Japanese quail under natural condition differed from year to year and noted the possibility that this difference was caused by the influence of ambient temperature as a 'modifying factor' (Marshall, 1959). However, the annual change in daylength in Tokyo is from about 11 to 16 h. In our study, a change of photoperiods from 16L8D to 8L16D in the laboratory did not decrease LHβ mRNA level significantly and some birds maintained large cloacal protrusion and testicular weights. However, both LHβ and common α mRNA levels showed a clear annual cycle under natural conditions. These results suggest that environmental factors other than photoperiod are required for termination of reproductive activity in Japanese quail used in our studies.

It is assumed that there are some genetic variations in Japanese quail with respect to photoperiodic response. If we had selected quail that respond to only photoperiodic manipulation for our experiments, we would have obtained the same results as Follett's laboratory did. However, at present, it is unknown whether the physiological basis of the quail designated as Group SD20(R) in present study is the same as the quail used by Follett's laboratory. Larger number of quail would be needed to judge the effects of photoperiodic manipulation on mRNA encoding gonadotropin subunits in each line of quail.

Although photoperiod may be the predominant information for the annual reproductive cycle in Japanese quail, the degree of total dependency on photoperiod may vary among the lines in Japanese quail population. This idea could be applied to other avian species that have different strategies for reproduction at mid- to high-latitudes. This is supported by previous studies using wild, captive, and also closely related birds. Silverin and Viebke (1994) reported that effects of ambient temperature on photoperiodically induced changes in plasma LH concentration and testicular development were different between two closely related tits, great tits (*Parus major*) and willow tits (*Parus montanus*). Wingfield and his colleagues also studied the effects of temperature on photoperiodically induced gonadal development and regression in three closely related white-crowned sparrows, *Zonotrichia leucophrys gambelii* (Wingfield et al., 1996), *Zonotrichia leucophrys pugetensis* (Wingfield et al., 1997), and *Zonotrichia leucophrys oriantha* (Wingfield et al., 2003). These studies demonstrate that ambient temperature does not affects photoperiodically induced increase in plasma LH concentrations but gonadal development and regression differently among these closely related birds.

The present study confirms the conclusion of our previous studies that decrease in ambient temperature acts as environmental cues to terminate reproductive activities from late summer to early autumn in Japanese quail, and extend it in terms of mRNA encoding LH subunits in pituitary gland. However, the mechanisms of photorefractoriness remain unclear. Further experiments are required to elucidate this phenomenon. In addition, clarification of the differences in physiological bases between lines that show different responses to short daylength in our Japanese quail populations would be helpful to elucidate the mechanisms of photorefractoriness.

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