NEUROSECRETORY ACTIVITIES IN THE ANTERIOR MEDIAN EMINENCE IN RELATION TO PHOTOPERIODIC TESTICULAR RESPONSES IN YOUNG JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA)

TAKAO KONISHI

Department of Zoology, Faculty of Science, Kyoto University, Kyoto

SYNOPSIS

Relationships between photoperiodically controlled testicular responses and the amount of neurosecretory materials in the anterior median eminence of Japanese quails have been investigated. Birds reared under 8-hour daily photoperiods from hatch to 25 days of age had a smaller amount of neurosecretory materials in the median eminence than those reared under continuous light. At 25 days of age testes showed much growth in most birds under continuous light, but less under 8-hour photoperiods. There was a correlation between testicular weight and the amount of neurosecretory materials accumulated in the median eminence. After shortening the photoperiod from continuous light to 8 hrs. per day at 43 days of age, the neurosecretory material in the median eminence had increased remarkably by 48 days of age. Alteration of light period was accompanied by testicular involution. This suggests that the release of neurosecretory materials from the median eminence was restrained by the shortened daily photoperiods. A hypothetical role of the neurosecretory material accumulated in the median eminence in the testicular development of Japanese quails is discussed.

The neurosecretory system which is considered to have an effect, directly or indirectly, on avian gonadal growth has been extensively investigated in various species (see Ishii and Kobayashi, 1962; Farner et al., 1964). More recently, the neurosecretory materials in the anterior median eminence and in the pars nervosa of the Japanese quail under various photoperiodic conditions have been studied quantitatively by the author (Konishi, 1965). It was concluded that the material appeared to be richer in quails under long daily photoperiods than in those subjected to short days. These results are in agreement with the data in Zonotrichia albicollis (Wolfson and Kobayashi, 1962) and Zosterops pulpebrosa japonica (Hirano et al., 1962; Ishii et al., 1962a; Uemura and Kobayashi, 1963), but are incompatible with the results in Zonotrichia leucophrys gambelii (Oksche et al., 1959) and Anas platyrhynchos platyrhynchos (Ishii et al., 1962b). However, various differences in experimental conditions make these comparisons complicated.

Whether the Gomori positive neurosecretory materials are identical with the "gonadotropin releasing factors" has not yet been determined. Apart from the investigations of the hypothalamic gonadotropin releasing factors (Guillemin, 1964; Igarashi and McCann, 1964; McCann and Ramirez, 1964), it is first required to establish definite relationships between activity of the neurosecretory system and testicular development. If the neurosecretory material in the anterior median eminence controls the synthesis and/
or release of the hypophysial gonadotropins, the amount should be different in the median eminence according to the gonadal conditions induced by photoperiodic manipulation. The results reported in this paper indicate well-defined relationships between the amount of neurosecretory materials in the anterior median eminence and the photoperiodic testicular responses.

MATERIALS AND METHODS

Japanese quails (Coturnix coturnix japonica) were reared from hatch in cages (45×55×12 cm) with heat supplied by an electric foot-warmer (Experiment 1) or a 100-watt incandescent light bulb (Experiment 2). Temperature during the first week was 31~35°C and was gradually reduced thereafter. At two weeks of age birds were transferred as a group to wire-floored pens (50×80×15 cm) and kept at a temperature of 15~27°C. Food and water were provided ad libitum. Illumination was by daylight fluorescent tubes which gave a light intensity of 200~1,000 lux on the floor of the aviaries. The details of the experimental schedules are illustrated in Figure 1. In the first series the quails were divided into two groups and exposed to 8-hour daily photoperiods (8L) and continuous light (24L) respectively. In 8-hour photoperiods, light was on between 10:00 A.M. and 6:00 P.M. At 25 days of age they were weighed and sacrificed by decapitation. In the second series of the experiment quails of another hatch were reared under continuous light. At 43 days of age 14 birds out of about 60 were selected randomly, weighed and transferred to individual mouse cages with wire-floors (10×15×15 cm). Seven birds were subjected to 8-hour daily photoperiods (10:00 A.M.~6:00 P.M.) (L-S), and the others remained under continuous light (L). After being weighed they were sacrificed at 48 days of age. The time of sacrifice was between 3:30 P.M. and 5:30 P.M. in Experiment 1 and between 1:00 P.M. and 3:00 P.M. in Experiment 2. The birds were sacrificed at random in order to avoid errors caused by difference in killing time. In these two experiments testes were removed and weighed immediately after the brain was dissected. For histological examination the left testis was fixed in Bouin’s fluid, sectioned at 8 μ and stained with hematoxylin and eosin. The specimens were classified according to the stages of the degree of spermatogenic development described by Bartholomew (1949).

Within 2 mins. after decapitation the skull was trimmed away and the brain was immersed in fresh Bouin’s fluid. Tissues were embedded in paraffin through methyl benzoate and xylol. Sagittal serial sections were cut at 10 μ. The serial sections were attached to a coverslip (24×50 mm) of 0.17 mm in thickness. Those coverslips were chosen randomly, erected in a staining cage, and stained by aldehyde-thionin (Paget, 1959; Konishi, 1965). Deparaffinized sections were re-fixed for about 20 hrs. with Bouin’s fluid. After 5 mins’ oxidation with 0.15% potassium permanganate-sulfuric acid solution and 30 secs.’ bleaching with 3.0% sodium bisulfite, they were stained for 15 mins. with properly ripened aldehyde-thionin. In these two experiments all of the specimens were stained with the solution of the same batch held at room temperature for 7~10 days.

The neurosecretory materials stained with aldehyde-thionin in the median eminence were measured following the microphotometrical method (Konishi, 1965). The instrument used was a microspectrophotometer (MSP, type A-
IV) manufactured by Olympus Co. Ltd. Measurement was made at a wave length of 600 mλ, where the stained specimens as well as the solution of aldehyde-thionin show maximum absorption. Mean optical density of a section was obtained by scanning the stained region several times with a circular spot of 57.6 μ in diameter. The number of measurements varied from 5 to 15 times depending on the size of the stained area. Photographs of the median eminence were enlarged to a certain magnification, projected on printing paper (Mitsubishi Paper Mills Ltd., No. V-3), and an outline of the stained region was traced. The paper was cut along the line and weighed within an accuracy to 1 mg on a torsion balance. Then the area was calculated with the standard exchange table of weight (mg) and area (mm²). The measurements of optical density and area were carried out on every other specimens of serial sections.

The total amount (M) of nerosecretory materials was calculated from the equation;

\[ M = \sum_{i=1}^{l} B_i \left( \log \frac{T_i}{i} \right) \]

(-log Ti: optical density of a section i, Bi: area of the stained region of a section i, l: number of sections stainable with aldehyde-thionin).

RESULTS

Birds used in Experiment 1 were variable with respect to body weight (Tables 1 and 3). However, the mean body weight of continuously illuminated quails (24L) did not differ (0.05 < p < 0.20) from those under 8-hour daily photoperiods (8L). Quails reared under 8-hour daily photoperiods from hatch to 25 days of age showed little testicular growth. Under continuous light, however, significant testicular development was observed (Tables 1 and 3). The difference in the mean testicular weight of 8L and 24L birds was statistically significant (p < 0.05). Testes of all 8L birds and 5 birds of the 24L group were histologically at stage I (resting spermatogonia only), but the testis of one bird (No. 2) in 24L was at stage II (only a few spermatocytes), and two other birds (No. 4 and No. 7) were at stage III (many spermatocytes). These data are in agreement with the results previously reported (Abplanalp et al., 1962; Wilson et al., 1962; Konishi et al., 1965; Tanaka et al., 1965; Follett and Farner, 1966).

In Experiment 2 there was no considerable change in body weight during the experimental period. Also, no distinguishable difference was observed in body weight between groups L-S and L at 43 and 48 days of age (p > 0.20). The average testis weight is usually about 500 mg at an age of 43 days, although testes of birds at 43 days of age were not investigated in this experiment. It has been confirmed that under continuous light as well as long daily photoperiods, testes are in the growing state at 43 days of age and almost attain maximum weight at 48 days of age (Konishi et al., 1965). In Experiment 2 the mean testicular weight of L group at an age of 48 days was 983 ± 121 mg and all had reached spermatogenic stage VI (Table 3). These results indicate that testicular growth proceeded from 43 to 48 days of age under continuous light. The birds subjected to 8-hour photoperiods for 6 days from 43 days of age, showed a mean testicular weight of 358 ± 108 mg. The testicular regression was observed histologically in all testes of L-S birds; even the largest testis (No. 1) showed degradation of spermatogenic cells, breakdown of sperm bundles, and reduction of lumen in a tubule.

A negative correlation between testicular weight and the amount of neurosecretory materials in the median eminence was observed in Experiment 1 (Table 1). In 24L group, birds Nos. 1, 3, 5, 6, and 8 which had relatively smaller testes showed a high accumulation of neurosecretory materials, whereas birds of larger testes (Nos. 2, 4, and 7) had little substances in the median eminence. This indicates that at about 25 days of age the release of neurosecretory material
from the median eminence began actively in proportion to testes development under continuous light. In general, birds of 24L group had a larger amount of neurosecretory materials in the median eminence than those of 8L group (p<0.05) (Table 3). The synthesis of neurosecretory material may be more active at 25 days of age under continuous light than under 8-hour daily photoperiods.

In No. 3 of L-S group and No. 2 of L group of Experiment 2, total numbers of stainable sections were not clearly defined, by accident, so that the minimum and maximum values are presented in Table 2. The results indicate that the amount of neurosecretory materials in the median eminence remarkably increased after shortening the daily photoperiod (Tables 2 and 3). The M in all of L-S birds exceeded the values in L birds and the difference in the mean values between groups L-S and L was highly significant (p<0.01).

Average optical density (av. OD) and average area (av. Area) are shown in Tables 1 and 2, and their mean values are given in Table 3. The value of av.OD may be considered to be an indicator of the concentration of neurosecretory materials in the median eminence and may be comparable to the scores which have been employed as criteria for assessment of neurosecretory activity (Oksche et al., 1959). It was ascertained that the value of av.OD is not always proportional to the value M (Tables 1 and 2; see Konishi, 1965). The difference in av. OD between groups 8L and 24L was small (0.05<p<0.20), but highly significant between L and L-S (p<0.01). The mean area of the median eminence stained by aldehyde-thionin was slightly larger (0.05<p<0.20) in 24L group than in 8L, whereas no significant difference was found between that of L-S and L (p>0.20). The result in av.OD of L-S and L birds further confirms the con-

<table>
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<th>av. Area (mm²)</th>
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B.W: body weight, T.W.: mean weight of left and right testes, No.: number of sagittal sections of the median eminence stainable with aldehyde-thionin, av. OD: average optical density, av. Area: average area, Total: amounts of neurosecretory materials in the median eminence.
Table 2. Effects of photoperiodic conditions on body weight, testicular weight, and amount of neurosecretory materials in the median eminence in Experiment 2

<table>
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<th>B.W. (g)</th>
<th>T.W. (mg)</th>
<th>No.</th>
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<th>av. Area (mm²)</th>
<th>Total (M)</th>
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<td>.173</td>
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<td>89.0</td>
<td>142</td>
<td>&gt;46</td>
<td>.195</td>
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<td>.165</td>
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<td>.165</td>
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For abbreviations, see the explanation in Table 1.

Table 3. Comparisons of the neurosecretory activities on four experimental groups of Japanese quails

<table>
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<tr>
<th></th>
<th>B.W. (g)</th>
<th>T.W. (mg)</th>
<th>av. OD (–logT)</th>
<th>av. Area (mm²)</th>
<th>Total (M)</th>
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<tr>
<td>8L</td>
<td>51.4±2.93</td>
<td>3.8±0.36</td>
<td>0.077±0.004</td>
<td>1.94±0.123</td>
<td>7.1±0.86</td>
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<tr>
<td>24L</td>
<td>56.8±1.21</td>
<td>19.6±5.84</td>
<td>0.101±0.033</td>
<td>2.21±0.079</td>
<td>10.0±0.89</td>
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<td>L – S</td>
<td>94.4±3.00</td>
<td>358±108</td>
<td>0.168±0.007</td>
<td>2.67±0.132</td>
<td>21.1±0.63</td>
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<tr>
<td>L</td>
<td>98.1±2.03</td>
<td>983±121</td>
<td>0.127±0.007</td>
<td>2.48±0.075</td>
<td>15.1±0.41</td>
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Values in the table are mean ± standard error.

Conclusion that the accumulation of neurosecretory materials in the median eminence was caused remarkably by the shortened photoperiods.

When compared with 24L group, the mean values of M and av. Area were significantly large in L group (p<0.05), while the mean value of av.OD was slightly different (0.05<p<0.20). This indicates that the amount of neurosecretory materials in the median eminence and the stainable area increased from 25 to 48 days of age under continuous light. Since the av. Area at 48 days of age (L-S and L) was conspicuously larger (p<0.001) than that at 25 days of age (24L and 8L), the region containing the neurosecretory material was clearly augmented during the growing period.

DISCUSSION

The method of measurement

It has been reported by several authors that light affects avian gonadal development, perhaps mediated by the neurosecretory materials found in the anterior median eminence (Oksche et al., 1959; Laws, 1961; Farner, 1962; Hirano et al., 1962; Ishii et al., 1962a and b; Ishii and Kobayashi, 1962; Wolfson and Kobayashi, 1962; Oksche et al., 1963; Uemura and Kobayashi, 1963; Farner et al., 1964). In these studies sub-
jective scores determined by means of microscopic observation were used for expression of the amount or concentration of neurosecretory materials (Oksche et al., 1959). This seems to make the interpretation of results indefinite. It should be required that the amount of neurosecretory materials in the median eminence is expressed objectively. An attempt to measure the amount of neurosecretory materials in the pars nervosa has been made by Tamiya et al. (1957), but it is thought to be questionable in so far as the distributional errors in microphotometry could not be eliminated. For these reasons it was devised to measure quantitatively the neurosecretory materials stained in the median eminence (Konishi, 1965). The aldehyde-thionin method was adopted to stain the neurosecretory material since the uniformity and stability of this method have been demonstrated to be superior to the usual method of aldehyde-fuchsin. The specificity of aldehyde-thionin has also been investigated. However, whether aldehyde-thionin is quantitatively identical in specificity with aldehyde-fuchsin is still obscure.

The daily photoperiod and neurosecretory material

Since both the rate of release from the median eminence and that of synthesis in hypothalamic regions will determine the amount of neurosecretory materials accumulated in the median eminence, there may be a proper time when the effect of alteration of the light condition can be reflected on the amount of neurosecretory materials in the median eminence. The results of Experiment 2 suggest that the accumulation of neurosecretory materials can be recognized distinctly a week or so after shortening the duration of the daily photoperiod. This is in agreement with the results reported by Wolfson and Kobayashi (1962). It is difficult to obtain a clear relationship between the effect of day length on gonadal growth and neurosecretory activity, when a certain duration of the photoperiod is given to Japanese quails from hatch to the time of killing (L group in Experiment 2; Konishi, 1965; Follett and Farner, 1966). There was, however, a fine correlation between testicular weight and the amount of neurosecretory materials in the median eminence in birds of 25 days of age under continuous light. This suggests the possibility that age is an important factor for the detection of a clear relationship when the same photoperiod is continuously given after hatching. The testicular growth of Japanese quails appears to be unaffected by light at least for the first two weeks after hatching (Kato and Oishi, in preparation). At 25 days of age the testicular development had been influenced by environmental light conditions for 10 days at the most. It may be identical with the case of birds receiving altered light conditions for about one week. case of birds receiving This would also help to explain why the relationship between testicular weight and the amount of neurosecretory materials in the median eminence was observed in 24L birds at 25 days of age.

The circadian rhythm in tropic hormone activities in the adenohypophysis has been demonstrated in Japanese quails (Tanaka et al., 1966), as well as in mice (Ungar and Halberg, 1963) and rats (Everret and Sawyer, 1950; Clark and Baker, 1964; Wagner and Brown-Grant, 1965). If the neurosecretory material controls the release and/or synthesis of gonadotropins in the adenohypophysis, its rhythmicity might be reflected on activities in the hypothalamic neurosecretory system. The time of day at which decapitation takes place comes to be an important factor for the interpretation of the results on the amount of neurosecretory materials in the median eminence. In fact, daily rhythmic changes have been observed in the concentration of neurosecretory materials in the median eminence (White-crowned sparrow: Oksche et al., 1959; Japanese quails: Ko-nishi, in preparation). On the other hand, it was not conspicuous in the amount of neurosecretory materials, more correctly, in the
value M. The daily rhythmic changes need not be considered in so far as the value M is used to express the amount of neurosecretory materials in the median eminence.

Hypothetical interpretation of the results

In Experiment 1 some quails of 25 days of age under continuous light showed considerable testicular development, while the others did not. In quail with developing testes the neurosecretory material in the median eminence was relatively poor, but was rich in quail with small testes. These results can reasonably be interpreted, supposing that the gonadotropin releasing factors are included in the neurosecretory material deposited in the median eminence.

In the birds with developing testes both the release and synthesis of neurosecretory materials is supposed to occur. The release from the median eminence can be established by testicular development, and if synthesis does not occur, the substance in the median eminence should be depleted. As for the birds with small testes, despite rearing under the stimulatory condition the release of the neurosecretory material from the median eminence has not yet been initiated. The synthesis in the hypothalamic neurosecretory cells could have been stimulated by light since the amount in the median eminence exceeded the level of those under 8-hour photoperiods. The accumulated amount of neurosecretory materials in the median eminence is suggested as a determinant of release from the median eminence in post-natal Japanese quails. As the neurosecretory material in the median eminence increases still more in amount, the release from the median eminence naturally appears to begin. However, the mechanism of the release and synthesis of neurosecretory materials may alternatively be explained by the dual innervation to the hypothalamo-hypophysial tract by non-neurosecretory neurons. The hypothalamic neurosecretory cells are considered to receive innervation by non-neurosecretory cells through the synapses to the cell body in the hypothalamic region (Murakami, 1962; Kawabata, 1964). This suggests the possibility that the neurosecretory cells respond to the stimulus of environmental light through this innervation. Further, Kobayashi and his colleagues have suggested that the secretion of neurohormones from the median eminence is regulated by other neurons through the synapses found in the hilar region of the infundibulum, where the neurosecretory axons are post-synaptic (Mouse : Oota, 1963; Bullfrog and Turtle : Kobayashi and Oota, 1964). Ishii and Kobayashi (1963) also suggested the possibility of control of the release of neurosecretory materials through the synapses of non-neurosecretory cells to the perivascular connective tissue space of the blood capillary in the median eminence. Although no synapse characterized by an aggregation of synaptic vesicles has so far been demonstrated in the median eminence, pars nervosa, or the hilar region of the infundibulum of other animals, it is probable that these innervations are involved in the mechanism of release of neurosecretory materials from the median eminence in birds.

Under 8-hour photoperiods the testes were not well developed at an age of 25 days and the neurosecretory material in the median eminence was considerably poor. In this case, it seems likely that the release from the median eminence was not yet initiated and that the synthesis in the hypothalamic neurosecretory cells was restrained by the inhibitory photoperiods, as compared with the 24L group.

This hypothetical interpretation can be applied without contradiction to the results in Experiment 2. When the duration of the daily photoperiod was shortened from continuous light to 8 hrs. at 43 days of age, the amount of neurosecretory materials in the median eminence had increased remarkably at an age of 48 days, as compared with those reared under continuous light. It might be assumed that the release of neurosecretory materials from the median eminence has
been strongly inhibited and surpassed the possible suppression of synthesis in the hypothalamic neurosecretory cells.

The relationship between the neurosecretory materials and the so-called hypothalamic gonadotropin releasing factors has not yet been determined. The results obtained by the author, however, suggest that the releasing factors of gonadotropins probably exist in the "aldehyde-thionin" positive materials in the anterior median eminence. A fine correlation between the photoperiodic testicular responses and the activities of the hypothalamic neurosecretory system could reasonably be explained when the neurosecretory material contains the gonadotropin releasing factors. Continuance of such quantitative investigation in the amount of neurosecretory materials in the median eminence will serve to examine in further detail the role of neurosecretory material in relation to the releasing factors of gonadotropins.

More recently attention has been paid to melatonin, a substance highly localized in the pineal body, which exhibits inhibitory action on the mammalian gonads (Wurtman and Axelrod, 1965; Clementi et al., 1966). In birds, however, melatonin content and the changes in activity of the related enzymes in the pineal body are not consistent with gonadal growth (Axelrod et al., 1964; Quay, 1966). Bischoff and Richter (1966) have reported that the pineal organ of the Japanese quail appears to be an intermediate form between the sensory type observed in the amphibians and the glandular type typified in mammals. The results obtained in our laboratory with the enucleated Japanese quails by pinealectomy or local illumination of the region superjacent to the pineal body suggest that the pineal organ does not play an inhibitory role on the avian gonadal growth but serves as a kind of photoreceptor (Oishi, unpublished). In any event, the role of the pineal organ remains uncertain until direct evidences for the inhibitory effect on the avian gonadal growth are produced.

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