Ultrastructural Study of Post-Hatching Development in the Pineal Gland of the Japanese Quail

Koji OHSHIMA and Kohzy HIRAMATSU

Laboratory of Functional Anatomy, Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano-ken 399-45, Japan

(Received 18 March 1993/Accepted 15 July 1993)

ABSTRACT. The pineal gland of the Japanese quail (*Coturnix coturnix japonica*) retained numerous follicular structures, each of which was composed mostly of pinealocytes and supporting cells radially arranged around the follicular lumen during the first 6 weeks of age. In the pinealocytes, paraboloids and regular stacks of lamellar disks, which were demonstrated at 1 day of age in a previous study [14], rapidly decreased in number after 1 week of age. From 1 to 6 weeks of age, the pinealocytes had more developed lamellar whorls in the follicular lumen with the increased numbers of mitochondria in their apical protrusions and synaptic ribbons in their basal processes. In addition to these photosensory elements, pigment-containing cells occasionally accumulated in the basal side of the follicular structure. Their pigment granules, which were considered as melanin, gradually became larger and more roundish in shape after 1 week of age. The histological elements associated with the secretory function of the quail pineal were dense-cored vesicles and lysosomes in the pinealocytes and dense bodies in the supporting cells. The synaptic ribbons and the dense-cored vesicles in the pinealocytes rapidly increased in number at 1 week of age, scarcely exhibiting distinct changes thereafter. Thus, it is clarified that the foregoing photosensory and secretory elements change during the post-hatching development. Furthermore, it is suggested that, in respect of these elements, the pineal gland of the quail assumes more active appearances than that of the chicken.—**KEY WORDS:** Japanese quail, pineal gland, post-hatching development, ultrastructure.

J. Vet. Med. Sci. 55(6): 945–950, 1993

It is widely recognized that the pineal organ progressively declines in photosensitivity, whereas its secretory function becomes more pronounced during phylogenetic development [17, 22].

Usually, the avian pineal gland has a rudimentary outer segment producing lamellar whorls [1, 6, 16, 18, 19]. In chickens, the pineal structure shows considerable changes with age during the post-hatching development, assuming photosensory and secretory appearances [2, 20, 25]. This pineal development may reflect a sequence of changes leading from “pineal sense organ” to “pineal gland” [24].

To our knowledge, there are scarce observations regarding functional morphology of the quail pineal, with the exception of those by Bischoff [1]. He studied the pineal glands of the adult quail and chicken by electron microscopy, but failed to demonstrate distinct morphological differences between them.

However, more recently, we demonstrated paraboloids in the pinealocytes of 1-day-old quails [14]. Moreover, in addition to the lamellar whorls, regular stacks of lamellar disks occasionally appeared in the follicular lumen of the quail pineal. Such structures are considered to be typical photoreceptive elements which have been scarcely observed in any avian pineal. Therefore, it may be that the quail pineal has a photosensory capability at 1 day of age. Furthermore, recent immunocytochemical studies using specific antibodies have demonstrated that several photoreceptor proteins are also localized in the rudimentary photoreceptor cells of the avian pineal [5, 26, 27]. The present investigation was undertaken to demonstrate age-dependent changes in respect of the photosensory and secretory elements in the pineal gland of the quail. These observations are discussed in comparison with those

reported previously in other avian species, especially in chickens.

MATERIALS AND METHODS

Twenty-four pineal glands from 1, 3, and 6-week-old male Japanese quails (*Coturnix coturnix japonica*) were used in the present investigation. All quails were reared in a LD 12:12 lighting regime (light on at 6:00) after hatching. They were sacrificed at the mid-point of the light period (12:00) at the respective ages and their pineal glands were removed.

For electron microscopy, each of five pineal glands at the respective ages was fixed in a mixture of equal amounts of 2 % osmium tetroxide and 3 % glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) containing 3.5 % sucrose at 4°C for 2 hours. It was dehydrated with a graded series of ethanol and embedded in epoxy resin. Thin sections were cut with a Sorvall ultra-microtome, and were stained with uranyl acetate and lead citrate. The preparations were observed under a Hitachi HS-9 electron microscope at 75 kV.

In addition, for light microscopic demonstration of paraboloids and pigment granules, each of three pineal glands at the respective ages was fixed with Bouin's solution for 24 hours, dehydrated, and embedded in paraffin. Periodic acid-Schiff (PAS) staining was performed on 6 μ m thick paraffin sections with occasional counter staining with hematoxylin. Some sections were exposed to saliva before the PAS reaction for identification of glycogen. Non-stained sections were treated with 10 % hydrogen peroxide for 48 hours for identification of melanin.

RESULTS

Pinealocytes: The pineal glands of 1, 3, and 6-week-old quails retained numerous follicular structures. The pinealocytes were radially arranged around the follicular

lumen, accompanied by supporting cells (Figs. 1a, 6), and composing a great majority of the quail pineal parenchyma during the first 1 to 6 weeks of age.

The pinealocyte usually had an apical protrusion which was spherical in shape, projecting into the follicular lumen

(Figs. 1a, 1b, 6). In 1-week-old quail pineals, this protrusion contained a number of mitochondria and free ribosomes, having a bulbous cilium of the sensory type with 9+0 axial configuration (Fig. 1b), and occasionally forming lamellar whorls in the follicular lumen (Fig. 1a). In the supranuclear cytoplasm, there were numerous mitochondria, lysosomes, moderately developed Golgi complexes, rough endoplasmic reticulum and numerous free ribosomes, with scarce lipid droplets (Fig. 1c). The pinealocyte usually had a round to oval nucleus with centralized distinct chromatin and one or two nucleoli. Occasionally, a large number of synaptic ribbons and dense-cored vesicles about 120–160 nm in diameter appeared in the basal process of the pinealocyte in 1-week-old quails (Figs. 2, 3). These synaptic ribbons and dense-cored vesicles tended to be localized separately in the different processes, scarcely coexisting (Fig. 2).

A few PAS-positive reaction products, completely digestible by saliva, were encountered in the follicular lumen and follicular wall in 1-week-old quail pineals (Fig. 4a). These PAS-positive products probably corresponded to paraboloids which consisted of a number of glycogen granules and a poorly-developed network of smooth endoplasmic reticulum in the pinealocytes (Fig. 4b). In 3-week-old quail pineals, such paraboloids in the pinealocytes completely disappeared or were retained some dilated tubules of smooth endoplasmic reticulum, accompanied by no glycogen granules (Fig. 5).

With advancing age, the quail pinealocytes exhibited structural changes in such a way that their apical protrusions became large and had an increased number of mitochondria (Fig. 6). Their supranuclear cytoplasm also had an increased number of mitochondria and large lysosomes (Fig. 6). The synaptic ribbons and the dense-cored vesicles did not distinctly change after 1 week of age. In a few cases, a part of those basal processes which contained numerous dense-cored vesicles extended into the interlobular perivascular space (Fig. 3) and the follicular lumen (Fig. 7).

Supporting cells: The supporting cell had an oval to

oblong, relatively dense nucleus with a narrow band of pale supranuclear cytoplasm. It contained a small number of mitochondria which were slightly larger than those found in the pinealocyte, poorly-developed Golgi complexes and some small, dense bodies about 200–300 nm in diameter (Figs. 1a, 6). From the apical end of the supporting cell, microvilli extended and lined the follicular lumen (Fig. 1a). With advancing age, the dense bodies gradually increased in number, whereas the apical microvilli became ill-defined, not totally vanishing (Fig. 6).

Pigment-containing cells: Pigment-containing cells occasionally accumulated in the basal side of the pineal follicular structures (Figs. 8a,b). Pigment granules in these cells in 1-week-old quails were round (0.4–0.6 μm in diameter) or elongated in shape, having an extremely high electron density (Fig. 8a). The pigment-containing cell also had an irregular-shaped nucleus and a few cell organelles, such as a number of large, round mitochondria and moderately-developed Golgi complexes (Fig. 8a). Occasionally, a few pigment granules were scattered in the supranuclear region of the pinealocyte and the supporting cell (Fig. 1). With advancing age, pigment granules in the pigment-containing cell gradually became large and roundish (1.0–1.5 μm in diameter in 6-week-old quail pineal) (Fig. 8b). They were considered as melanin because of their characteristic form and because they were bleached by treatment with 10 % hydrogen peroxide for 48 hours.

DISCUSSION

The pineal gland varies widely in size, form and structure among the avian species [18, 22]. In chickens, the pineal structure shows a considerable variation, assuming appearances from follicular to solid lobular depending on age during the post-hatching development [2,20,25]. Present observations revealed that the quail pineal retained numerous follicular structures during the first 1 to 6 weeks of age. Also, 1-day-old quail pineals showed similar structures [14].

Fig. 1a,b,c. Pineal follicular cells of a 1-week-old quail. a: Apical protrusions (arrow heads) of pinealocytes (P) and apical microvilli (arrows) of supporting cells (S) extend into a follicular lumen (L). Note well-developed lamellar whorls (LW) in the follicular lumen, and lysosomes (LY▶) and a pigment granule (PG▶) in the pinealocyte. $\times 4,000$. b: Higher magnification of an apical protrusion of a pinealocyte. Note a cilium of 9+0 type (arrow), a number of mitochondria and free ribosomes. Arrow heads: cross sections of cilia in a follicular lumen (L). $\times 8,500$. c: Higher magnification of supranuclear cytoplasm of a pinealocyte. Note numerous free ribosomes, rough endoplasmic reticulum and Golgi complexes (GC▶). $\times 8,500$.

Fig. 2. Synaptic ribbons (arrows) and a dense-cored vesicle (V▶) in basal processes of pinealocytes of a 1-week-old quail. Arrow heads: basal lamina, asterisk: perivascular space. $\times 19,000$.

Fig. 3. Dense-cored vesicles (arrows) in basal processes of pinealocytes of a 1-week-old quail. A part of these processes (arrow heads) extend into the perivascular space (asterisks). $\times 17,000$.

Fig. 4a,b. a: Light microscopic demonstration for prominent PAS-positive paraboloids (arrows) in follicular lumina and follicular walls of a 1-week-old quail. PAS-hematoxylin staining. $\times 280$. b: A paraboloid (PA) in perinuclear cytoplasm of a pinealocyte of a 1-week-old quail. It consists of some dilated tubules of smooth endoplasmic reticulum and a number of glycogen granules. $\times 18,000$.

Fig. 5. A degenerated paraboloid (arrows) in an apical protrusion of a pinealocyte of a 3-week-old quail. Note some dilated tubules of smooth endoplasmic reticulum, accompanied by no glycogen granule. L: follicular lumen, S: supporting cell. $\times 11,000$.

Fig. 6. Follicular cells in a pineal gland of a 6-week-old quail. Compare with Fig. 1a. The pinealocytes (P) have increasing numbers of mitochondria in their apical protrusions (arrow heads) and in their supranuclear cytoplasm, and lysosomes (LY▶) in their supranuclear cytoplasm: lysosomes are larger than those in the 1-week-old quail pineal. Small dense bodies (arrows) in the supporting cells (S) are more in number than those in the 1-week-old quail pineal. $\times 4,000$.

Fig. 7. Dense-cored vesicles (arrows) in processes of pinealocytes located in the close vicinity of the follicular lumen (L) of a 6-week-old quail. $\times 15,000$.

Fig. 8a,b. Accumulations of pigment-containing cells in basal region of pineal follicles of a 1-week-old quail (a) and a 6-week-old quail (b). These pigment granules with a high electron density in the 6-week-old quail pineal are larger and more roundish in shape than those in the 1-week-old quail pineal. Note a few mitochondria (arrows) and moderately-developed Golgi complexes (GC▶) in these cells. N: Nucleus. $\times 6,000$.

As reported previously [14], paraboloids, which consist of a large number of glycogen granules lying among a well-developed network of tubular smooth endoplasmic reticulum, are frequently found in the pinealocyte of the 1-day-old quail. Furthermore, regular stacks of lamellar discs, which closely resemble those in the cone-type outer segment of the retinal photoreceptor cell, are demonstrated in the follicular lumen of the 1-day-old quail pineal, adjacent to the paraboloid-containing cytoplasmic processes. Therefore, it has been suggested that these characteristic photoreceptive structures compose morphological evidence of the photoreceptive capability in the 1-day-old quail pineal [14]. In the present investigation, it was demonstrated that these photoreceptive structures rapidly decreased in number after 1 week of age, and that the outer segments in particular showed modified lamellar whorl-like structures. Since recent immunocytochemical studies using specific antibodies, however, have been demonstrated that several photoreceptor proteins are localized in the rudimentary photoreceptor cells of the avian pineal [5, 26, 27], the young and adult quail pinealocytes may also have a photoreceptive capability.

Another striking feature of the quail pinealocyte was the presence of a number of mitochondria in its apical protrusion, which increased with advancing age. Phylogenetically, it has been suggested that such a feature corresponds to a similar one in the ellipsoid in the inner segment of the retinal photoreceptor cell [6, 10, 16, 17, 20, 22]. A characteristic accumulation of mitochondria has been demonstrated in the apical process of the pinealocyte in the adult house sparrow [16–18], quail [1], white-crowned sparrow [19] and goose [8]. Such a feature is absent in young and adult chicken pinealocytes [6, 11, 20]. Also in the supranuclear cytoplasm of the quail pinealocyte, mitochondria and large dense bodies gradually increased in number with advancing age. Oksche and Vaupel-von Harnack [17] observed an accumulation of mitochondria in the supranuclear region of the frog pinealocyte and called "Ersatzellipsoid", suggesting a functional relationship with the ellipsoid of the inner segment.

The synaptic ribbons, which have been found in some sensory organs including the pineal organs of the various vertebrates, were frequently observed in the pinealocyte of the young and adult quails. Consequently, they may be also one of the photosensory elements in the quail pineal.

Little information has been available on the pigment in the avian pineal gland, except for brief remarks on the chick embryos [25]. More recently, we observed pigment-containing cells in the pineal stalk of the chick embryo by light and electron microscopy [12], and suggested functional relationships between these cells and the photoreceptive capability, because of their characteristic form and close proximity to nerve cells. In the present investigation of the quail pineal, we occasionally found that pigment-containing cells accumulated in the basal side as well as in the stalk of the follicular structure, not adjacent to nerve cells. Pigment granules in these cells became larger and

more roundish with advancing age. Judging from light and electron microscopic observations, these pigments were identified as melanin, and were also considered as one of the photosensory elements of the quail pineal.

On the other hand, the principal, morphological elements associated with the secretory function of the quail pineal are the dense-cored vesicles in the pinealocytes. The cytoplasmic processes of the pinealocytes, having a large number of these dense-cored vesicles, extended into the interlobular, perivascular space and occasionally into the follicular lumen after 1 week of age. However, exocytosis was not found in the quail pinealocyte, but it is found in the pineal gland of rats [21], golden hamsters, snakes, and parakeets [9]. It has been proposed that the pineal gland usually has two categories of hormones, i.e., indoleamines and polypeptides [23]. Although these dense-cored vesicles have been considered to contain serotonin in reptiles, birds and mammals [4, 7], our previous immunohistochemical investigation [15] showed that they scarcely contained serotonin, and suggested that their content might be proteinaceous component such as arginine vasotocin.

In young [3] and adult chickens [13], large dense bodies in the pinealocyte have been identified as lysosomes by electron microscopic demonstration of acid phosphatase (AcPase) activity. Moreover, it has been revealed that the functional form and enzyme activity of the lysosome in the pinealocytes change in relation to the environmental lighting conditions and the functional activity of the gonadal organs [13]. Since, in the present study, lysosomes of the quail pinealocyte also changed in their form and increased in number with advancing age, it may be that they are related to the pineal function, especially to the secretion.

Meanwhile, lipid droplets were scarcely observed in the quail pinealocyte, whereas they are frequently observed in the supranuclear region of the chicken pinealocyte during embryonic and post-hatching development [2, 3, 6, 10–12, 20].

In the present observations, the supporting cell of the quail exhibited an inactive appearance having a cytoplasm with a few organelles, and very much resembled that of the chicken [2, 6, 11, 20]. Although the functional aspect of the supporting cells is not clear, their dense bodies may be involved in the secretory elements. Furthermore, the pineal follicular lumen may also be concerned in the secretory process, because a part of the dense-cored vesicles of the pinealocyte and the dense bodies of the supporting cell are closely adjacent to the follicular lumen.

Thus, it is clarified that the quail pineal gland undergoes histological changes during the post-hatching development, especially in some photosensory and secretory elements. Furthermore, it is suggested that, in respect of these elements, the pineal gland of the quail assumes more active appearances than that of the chicken.

REFERENCES

1. Bischoff, M. B. 1969. Photoreceptor and secretory structures in the avian pineal organ. *J. Ultrastruct. Res.* 28: 16-26.
2. Boya, J. and Calvo, J. 1980. Ultrastructural study of the post-hatching evolution of the pineal gland of the chicken (*Gallus gallus*). *Acta Anat.* 107: 143-168.
3. Calvo, J. and Boya, J. 1979. Ultrastructural study of the embryonic development of the pineal gland of the chicken (*Gallus gallus*). *Acta Anat.* 103: 39-73.
4. Collin, J.-P. 1979. Recent advances in pineal cytochemistry. Evidence of the production of indoleamines and proteinaceous substances by rudimentary photoreceptor cells and pinealocytes of amniota. *Prog. Brain Res.* 52:271-296.
5. Collin, J.-P., Mirshahi, M., Brisson, P., Guerlotte, J., and Faure, J. P. 1986. Pineal-retinal molecular relationships: Distribution of "S-antigen" in the pineal complex. *Neuroscience* 19:656-666.
6. Fujie, E. 1968. Ultrastructure of the pineal body of the domestic chicken, with special reference to the changes induced by altered photoperiods. *Arch. Histol. Jpn.* 29:271-303.
7. Juillard, M. T. and Collin, J.-P. 1980. Pools of serotonin in the pineal gland of the mouse: The mammalian pinealocytes as a component of the diffuse neuroendocrine system. *Cell Tissue Res.* 213: 273-291.
8. Mahon, S. and George, J.C. 1979. The structural organization of the pineal complex in the domestic goose. *J. Morphol.* 162:221-248.
9. Masson-Pévet, M., Pévet, P., and Noteborn, H.P.J.M. 1987. Ultrastructural demonstration of exocytosis in the pineal gland. *J. Pineal Res.* 4:61-68.
10. Nishiyama, H. and Mikami, S. 1974. Cytodifferentiation of the pineal body of the domestic fowl. *J. Fac. Agric. Iwate Univ.* 12:107-131(in Japanese).
11. Ohshima, K. and Matsuo, S. 1984. Functional morphology of the pineal gland in young chickens. *Anat. Anz.* 156:407-418.
12. Ohshima, K. and Matsuo, S. 1988. Cytodifferentiation of the chick pineal gland, with special reference to the photosensory and secretory elements. *J. Pineal Res.* 5: 397-410.
13. Ohshima, K. and Matsuo, S. 1989. Cytochemical demonstration of lysosomes in the chicken pineal gland: Changes induced by light-dark cycle. *J. Pineal Res.* 6:103-110.
14. Ohshima, K. and Matsuo, S. 1991. Photosensory elements in the pineal gland of the Japanese quail, with special reference to the paraboloid. *Anat. Anz.* 172:247-255.
15. Ohshima, K. and Matsuo, S. 1991. Immunohistochemical localization of serotonin in the pineal gland of the chicken during post-hatching development in relation to light-dark cycle. *Anat. Anz.* 173:65-72.
16. Oksche, A. and Kirschstein, H. 1969. Elektronenmikroskopische Untersuchungen am Pinealorgan von *Passer domesticus*. *Z. Zellforsch. Mikrosk. Anat.* 102:214-241.
17. Oksche, A. and Vaupel-von Harnack, M. 1965. Vergleichende elektronenmikroskopische Studien am Pinealorgan. *Prog. Brain Res.* 10:237-258.
18. Oksche, A. and Vaupel-von Harnack, M. 1966. Elektronenmikroskopische Untersuchungen zur Frage der Sinneszellen im Pinealorgan der Vögel. *Z. Zellforsch. Mikrosk. Anat.* 69:41-60.
19. Oksche, A., Kirschstein, H., Kobayashi H., and Farner, D.S. 1972. Electron microscopic and experimental studies of the pineal organ in the white crowned sparrow, *Zonotrichia leucophrys gambelii*. *Z. Zellforsch. Mikrosk. Anat.* 124:247-274.
20. Omura, Y. 1977. Ultrastructural study of embryonic and post-hatching development in the pineal organ of the chicken (Brown Leghorn). *Cell Tissue Res.* 183: 255-271.
21. Pévet, P. and Yadav, M. 1980. The pineal gland of equatorial mammals 1. The pinealocytes of the Malaysia rat (*Rattus sabanus*). *Cell Tissue Res.* 210: 417-433.
22. Ralph, C. L. 1970. Structure and alleged functions of avian pineals. *Am. Zoologist* 10: 217-235.
23. Reiter, R.J. and Vaughan M.K. 1977. Pineal antigonadotropic substances; polypeptides and indoles. *Life Sci.* 21:159-172.
24. Sato, T. and Wake, K. 1986. Separation of the pineal vesicles from the wall of the third ventricle during the post-hatching development of the chicken. *Cell Tissue Res.* 244: 321-326.
25. Spiroff, B. E. N. 1958. Embryonic and post-hatching development of the pineal body of the domestic fowl. *Am. J. Anat.* 103:375-401.
26. Van Veen, Th., Ostholm, T., Gierschik, P., Spiegel, A., Somers, W., Korf, H.-W., and Klein, D.C. 1986. α -Transducin immunoreactivity in retinae and sensory pineal organs of adult vertebrates. *Proc. Natl. Acad. Sci. U.S.A.* 83:912-916.
27. Vigh, B. and Vigh-Teichmann, I. 1981. Light- and electron-microscopic demonstration of immunoreactive opsin in the pinealocytes of various vertebrates. *Cell Tissue Res.* 221:451-463.