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Ultrastructural Detection of Secretory Function of the Pineal Organ of the Ice Goby, *Leucopsarion petersi*

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Abstract

The pineal organ of the ice goby, *Leucopsarion petersi*, was examined electron microscopically in order to search for indications of its secretory function. The pineal of the fish is a compact organ which is mainly composed of centrally located photoreceptor cells and peripherally arranged supportive cells. Photoreceptor cells existing in the middle and proximal parts of the pineal end-vesicle are of a typical structure with well-organized outer segments and denser cytoplasm, while those distributed in the distal part are modified to have irregular outer segments and clearer cytoplasm. The modified photoreceptor cells contain slightly dilated rough endoplasmic reticulum and dense-cored vesicles of two different sizes in the perinuclear cytoplasm. Dense-cored vesicles of the smaller size-class are scattered throughout the perinuclear cytoplasm, while those of the larger size-class are always found to be associated closely with the rough endoplasmic reticulum, often forming peculiar structures consisting of several layers of parallel granulated cisternae accompanied by dense-cored vesicles. The cytoplasm of supportive cells is characteristically packed with abundant tubular cisternae of the smooth endoplasmic reticulum. Electron-dense material is accumulated in some interstices among the microvilli of the supportive cells. Unmyelinated nerve fibers running along the periphery of pineal end-vesicle sometimes contain both clear and dense-cored vesicles. Furthermore, nerve terminals containing both clear and dense-cored vesicles of similar sizes to those found in the nerve fibers occur bordering on the basal processes of photoreceptor cells. The results may suggest that the pineal organ of the ice goby has not only a photosensory function but also a secretory function.

Introduction

It has been well established that the pineal organ of teleost fishes has a photosensory function (cf. Oksche and Hartwig, 1979; Meissl and Dodt, 1981). In addition, a possible secretory function of the pineal organ has also been suggested by the detection of melatonin, its precursor serotonin, and the enzyme hydroxy-indole-*O*-methyltransferase (HIOMT) in the organ (Fenwick, 1970; Owman and R  deberg, 1970; Hafeez and Zerihun, 1976; Smith and Weber, 1976a, b; Meissl et al., 1978; van Veen et al., 1980; Birks and Ewing, 1981a, b).

There have thus far been a few ultrastructural observations in several fish species suggesting that pineal parenchymal cells may have a high metabolic activity associated with synthesis and secretion of a certain material (*Nezumia lolepis*; McNulty, 1976; *Typhlogobius californiensis*; McNulty, 1978a; *Chologaster agassizi*; McNulty, 1978b; *Typhlichthyes subterraneus*; McNulty,

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1978c). Herwig (1979) proposed that some morphological features such as dense-cored vesicles, lipid droplets and intercellular material might suggest an endocrine activity of the pineal organ of fishes. More recently, Omura and Ali (1981) observed a dark-stimulated accumulation of dense-cored vesicles in the pineal organ of the killifish, *Fundulus heteroclitus*, and emphasized that these vesicles were involved in the synthesis and/or secretion of proteinaceous materials and indoleamines. In many other teleosts studied so far, however, hardly any ultrastructural information has been given about secretory function of the pineal organ, and it seems necessary to assemble much more evidence in order to provide sufficient bases for discussing the problem.

While studying the physiological functions of the pineal gland of various species of fresh water teleosts, the present writers found some ultrastructural characteristics indicating possible secretory activities of pineal parenchymal cells in the ice goby, *Leucopsarion petersi*, which will be dealt with in the present paper.

Material and Methods

The ice goby, *Leucopsarion petersi*, which had just come up the river Hekireji located in the suburbs of Hakodate for spawning, were collected in May 1979 and 1980 at the mouth of the river. A total of 52 adult fish of both sexes, ranging from 37 to 56 mm in standard length, were used as material in the present study. They were transported to the laboratory soon after the capture, measured and killed by quick decapitation in the early afternoon of the day of the capture. The pineal organ with a part of the cranium and brain tissue *in situ* was taken out, immersed immediately in Karnovsky's glutaraldehyde-paraformaldehyde mixture in 0.2 M cacodylate buffer (pH 7.4) for about 1 hour at room temperature, and postfixed in 1% OsO_4 in the same buffer for 2 hours at 4°C. The fixed specimens were dehydrated in a graded alcohol series and embedded in Epon. Ultrathin sections, cut with glass knives on a Porter-Blum MT-1 ultramicrotome, were stained with uranyl acetate and lead citrate, and then examined with a Hitachi HU-12 electron microscope. Semithin sections of Epon-embedded specimens were also examined light microscopically after staining with methylene blue.

For the detection of ganglion cells by the acetylcholinesterase method, total brain with the pineal organ was fixed in 5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 2 hours in an ice bath. The specimens were then washed in cold 0.1M phosphate buffer with 0.2 M sucrose for 16 hours, and frozen sections cut sagittally or frontally were incubated for 2 hours at 4°C in Karnovsky and Roots' medium (1964) containing acetylthiocholin iodide as substrate. Following incubation, the specimens were washed in 0.44 M sucrose solution, dehydrated with alcohol, mounted and observed light microscopically.

Results

The pineal organ of the ice goby, *Leucopsarion petersi*, is located just beneath the transversal cartilage underlying the dorsal cranium. Its end-vesicle is thick disk-shaped, measuring about 100 μm in length, and is connected at its base to the dorsal roof of the diencephalon by a short, thick stalk region (Fig. 1). In light

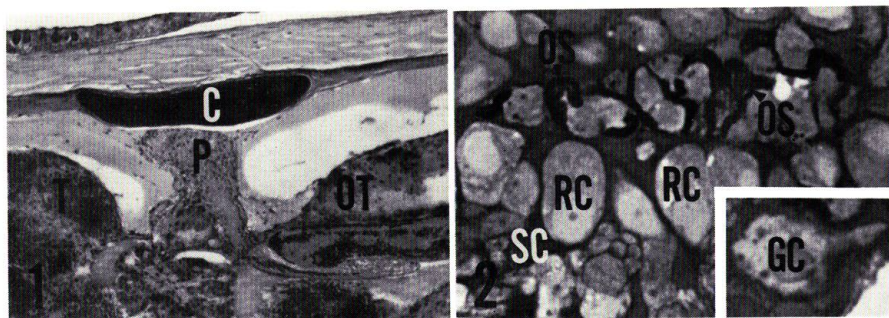


Fig. 1. A median sagittal section through the pineal organ of the ice goby, *Leucopsarion petersi*. The compact pineal organ (P) lies just beneath the cartilage (C). OT, optic tectum; T, telencephalon. Hematoxylin-eosin stain. $\times 90$.

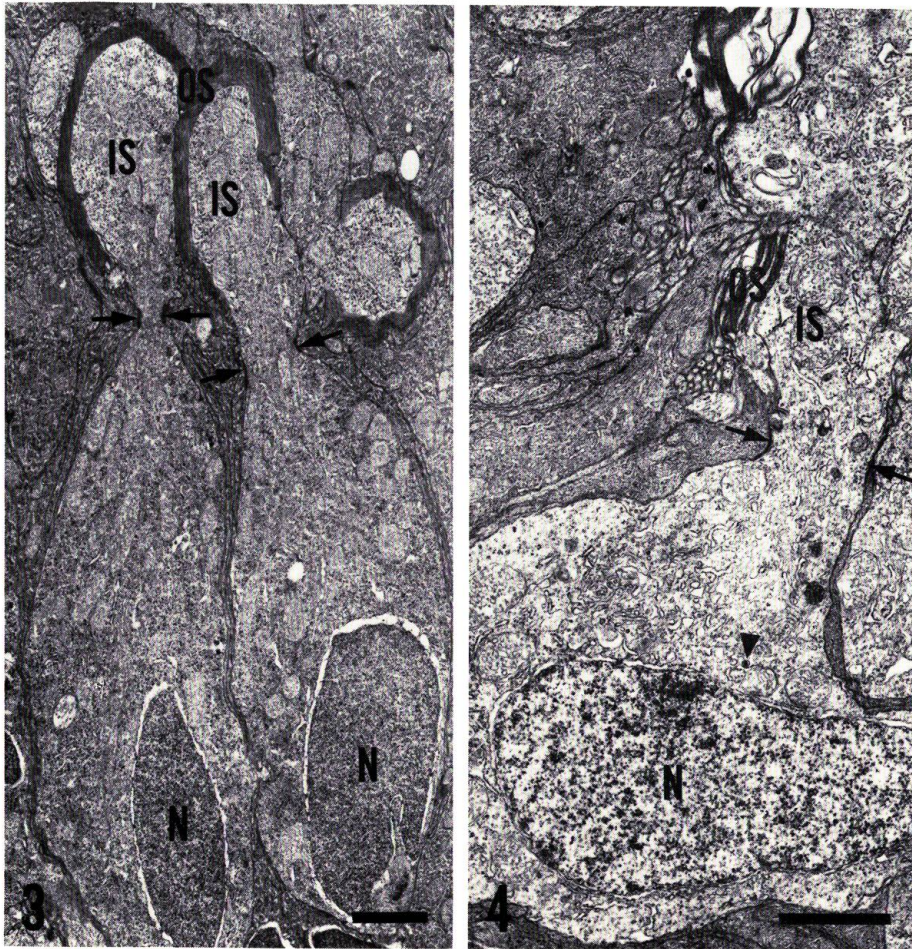
Fig. 2. An Epon-embedded section of the pineal end-vesicle, showing the arrangement of light-stained photoreceptor cells (RC) and dark-stained supportive cells (SC). OS, outer segment. Methylene blue stain. $\times 1100$. Inset: a ganglion-like cell (GC) found in the peripheral region of pineal end-vesicle. $\times 1100$.

microscopic sections, the entire organ appears to be a compact mass of cells and lacks an evident lumen in it. Gourd-shaped cells with apical structures stained deep with methylene blue, which appear to be the outer segments of photoreceptor cells, are distributed in the inner part of pineal end-vesicle and stalk regions (Fig. 2).

Electron microscopically, photoreceptor cells existing in the middle and proximal parts of the end-vesicle have a well organized outer segment which consists of a stack of parallel saccules averaging about 25 in number (Fig. 3). In the cytoplasm of the inner segment and the nucleated part, they contain mitochondria with tubular cristae, a large number of well-developed Golgi bodies, short and flat cisternae of the rough endoplasmic reticulum and abundant free ribosomes.

On the other hand, photoreceptor cells often found in the distal part of the end-vesicle have much clear cytoplasm and irregular outer segments (Fig. 4). They are remarkably larger in size than the typical photoreceptor cells mentioned above. In their inner segment and nucleated part, oval mitochondria with tubular cristae, short cisternae of the rough endoplasmic reticulum and free ribosomes are present throughout the cytoplasm (Figs. 4–7). Cisternae of the rough endoplasmic reticulum are slightly dilated and contain amorphous material of low electron density.

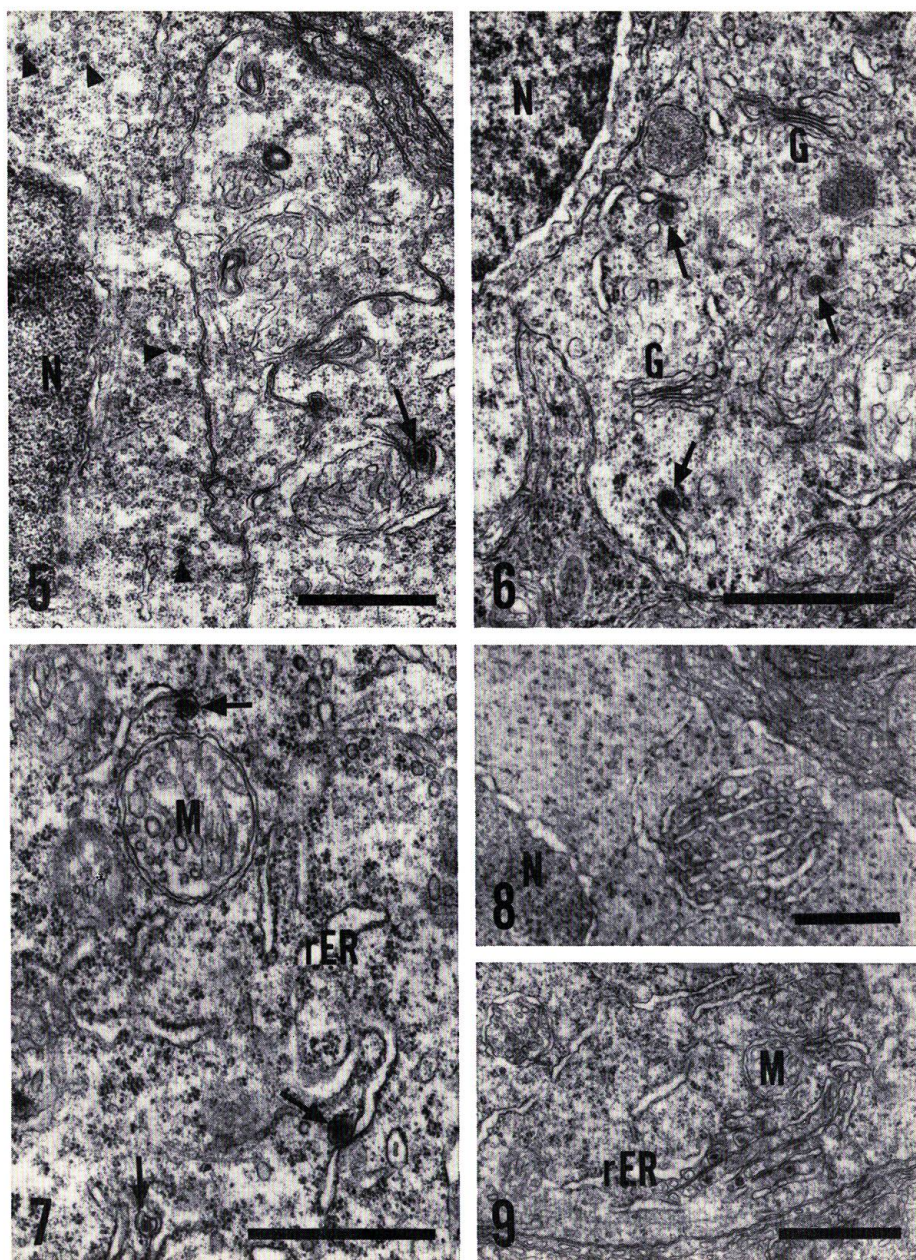
It is remarked that, in the cells of the modified photoreceptor type, dense-cored vesicles of various sizes often exist in the perinuclear cytoplasm (Figs. 5–7). The dense-cored vesicles of smaller sizes, 50–80 nm in diameter, which have an electron-dense core of clear outline, are found scattered throughout the cytoplasm, while those of larger sizes, 120–180 nm in diameter, appear more frequently than the smaller vesicles and are always encountered close to cisternae of the rough endoplasmic reticulum. Cores of the larger vesicles are rather obscure in their outline when compared with those of the smaller vesicles. Cisternae of the rough endoplasmic reticulum generally show a constriction at the place where the dense-cored vesicle is seen to be attached to their membrane. In addition, peculiar



Figs. 3, 4. Pineal photoreceptor cells with well-organized outer segment (Fig. 3) and with modified outer segment (Fig. 4). Arrows show junctional complexes between photoreceptor and supportive cells, and an arrow head in Fig. 4 indicates a dense-core vesicle in the perinuclear cytoplasm. *IS*, inner segment; *N*, nucleus; *OS*, outer segment. Bars indicate 2 μ m.

structures assembling dense-core vesicles of 80–120 nm in diameter and several parallel layers of cisternae of the rough endoplasmic reticulum occur in the cytoplasm of the nucleated part (Figs. 8, 9). Cores of smaller vesicles in that structure appear generally to be lower in electron density than those of larger ones. No dense-core vesicles can be observed to exist in the basal process within the neuropile zone. Golgi bodies are present near the nucleus and are not so developed as those of typical photoreceptor cells (Fig. 6).

Besides the photoreceptor cells, cells with darkly stained nucleus and cytoplasm are found distributed exclusively along the periphery of the compact pineal organ



Figs. 5-7. Perinuclear cytoplasm of modified photoreceptor cells. Mitochondria (*M*), Golgi bodies (*G*), slightly dilated rough endoplasmic reticulum (*rER*) and dense-cored vesicles of two size-classes are present. Dense-cored vesicles of smaller size-class (arrow head) in Fig. 5 are scattered throughout the perinuclear cytoplasm, whereas those of larger size-class (arrow) in Figs. 5-7 are closely associated with the rough endoplasmic reticulum. *N*, nucleus. Bars indicate 1 μ m.

Figs. 8, 9. Peculiar structures consisting of parallel cisternae of the rough endoplasmic reticulum and dense-cored vesicles in the perinuclear cytoplasm of modified photoreceptor cells. *M*, mitochondria; *N*, nucleus; *rER*, rough endoplasmic reticulum. Bars indicate 1 μ m.

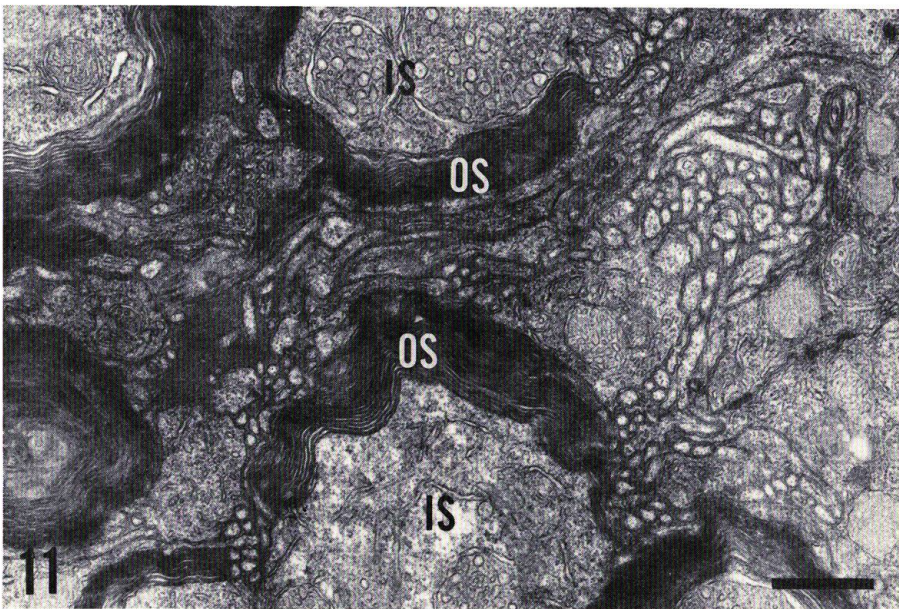
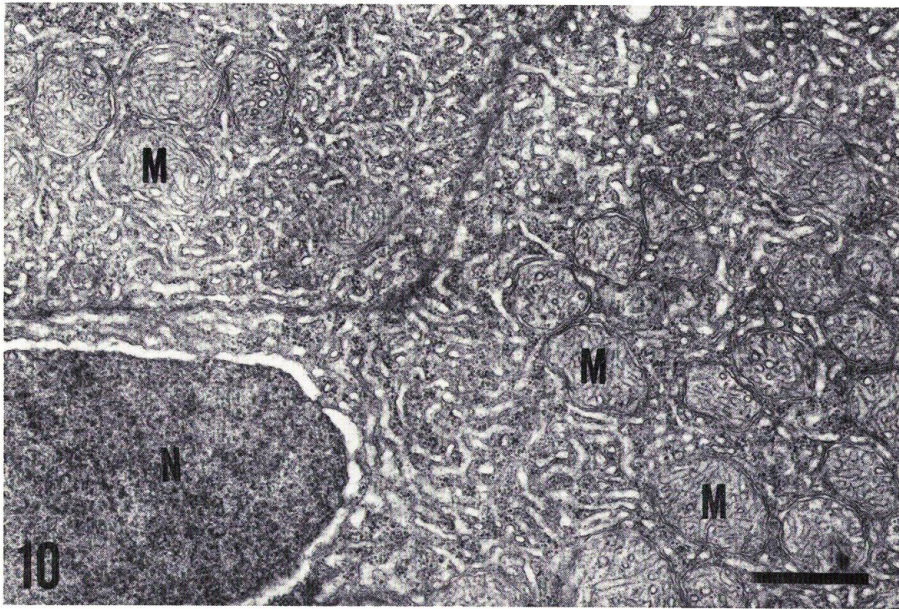


Fig. 10. Cytoplasm of pineal supportive cells packed with abundant tubular cisternae of the smooth endoplasmic reticulum. *M*, mitochondria; *N*, nucleus. Bar indicates 1 μ m.

Fig. 11. Interdigitating cytoplasmic processes extending from supportive cells around the inner (*IS*) and outer (*OS*) segments. Electron-dense material is accumulated in some interstices among the microvilli of supportive cells. Bar indicates 1 μ m.

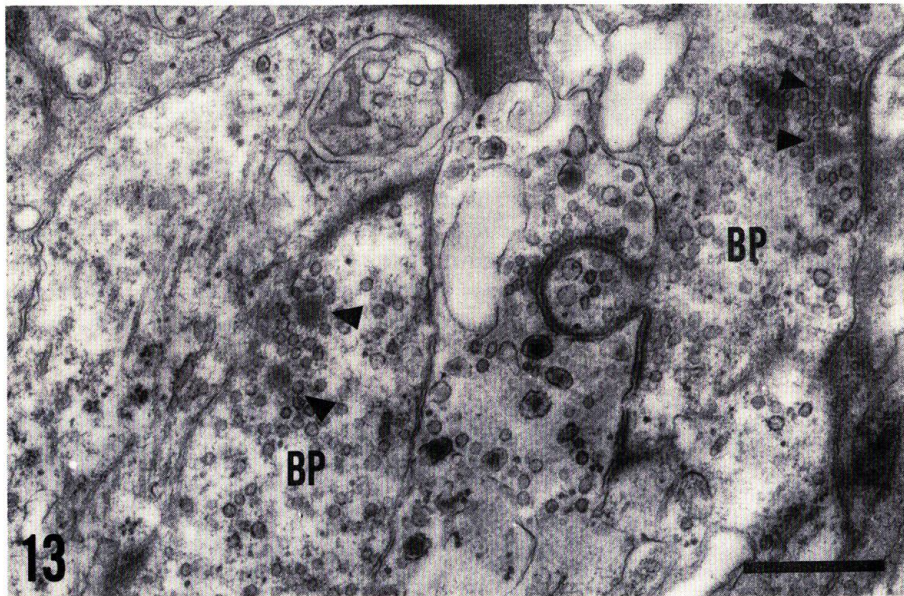
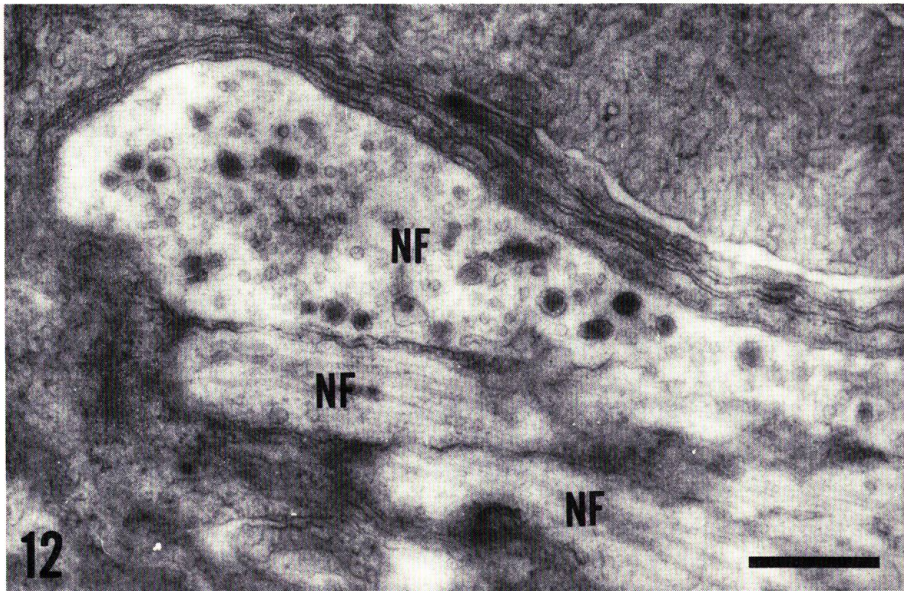


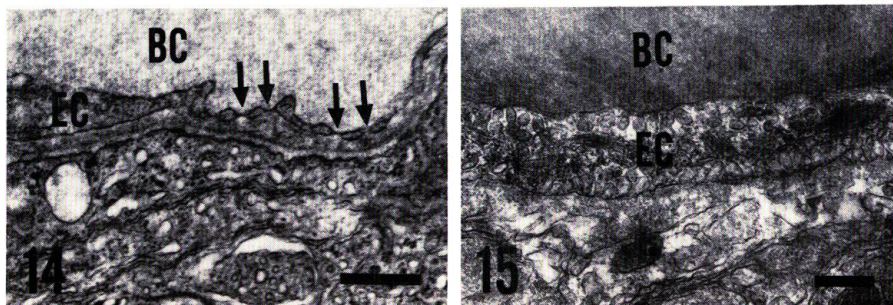
Fig. 12. Unmyelinated nerve fibers (*NF*) running along the periphery of the pineal end-vesicle. Both clear and dense-cored vesicles are seen in one of the fibers. Bar indicates $0.5\ \mu\text{m}$.

Fig. 13. A part of the neuropile zone. Arrow heads indicate short synaptic ribbons surrounded by synaptic vesicles. A nerve terminal bordering on the basal processes (*BP*) of photoreceptor cells is provided with both clear and dense-cored vesicles. Bar indicates $0.5\ \mu\text{m}$.

of the ice goby (Fig. 2). In general, they spread their cytoplasm between centrally located photoreceptor cells and have a junctional complex of the desmosomal type at the neck region of neighbouring photoreceptor cells (Figs. 3, 4), thus representing the nature of pineal supportive cells. The cytoplasm of the supportive cells is characteristically packed with abundant tubular cisternae of the smooth endoplasmic reticulum (Fig. 10). Oval mitochondria with tubular cristae, measuring about $1\mu\text{m}$ in length, are randomly distributed throughout the cytoplasm. Lysosome-like bodies with granular contents, free ribosomes and lipid droplets are also present in the cytoplasm. Golgi bodies are found only on rare occasions. At their apical part the supportive cells extend their microvilli which form compact interdigitations with each other to closely surround the inner and outer segments of photoreceptor cells (Fig. 11). It is remarked that electron-dense material is accumulated in some interstices among the microvilli of supportive cells. The basal surface of supportive cells bordering on the pericapillary space scarcely showed signs of infoldings. No cell type other than supportive cells can be observed to border on the pericapillary space surrounding the pineal organ of the ice goby.

A large roundish cell with a spherical nucleus and a thick cytoplasmic protrusion is observed to exist in the periphery of the pineal end-vesicle (inset in Fig. 2). The cell is likely to be a ganglion cell. However, an application of the acetylcholinesterase method, which has been shown to be effective in demonstrating pineal ganglion cells in several fishes (Ohba et al., 1979; Falcon, 1979a; Omura, 1980), failed to evidently locate the cells in the pineal organ of the ice goby, though the method could exactly demonstrate nerve cells in various brain regions in the present study.

Unmyelinated nerve fibers are found to run along the periphery of the pineal end-vesicle. Sometimes, these nerve fibers contain many clear vesicles, approximately 40 nm in diameter, and dense-cored vesicles, 80–120 nm in diameter (Fig. 12). Furthermore, nerve terminals of unknown origin are situated in the neuropile zone, bordering on the basal processes of photoreceptor cells in which short synaptic ribbons with synaptic vesicles are present. It is noted further that the



Figs. 14, 15. Endothelial cells of a capillary (Fig. 14) and of a thick blood vessel (Fig. 15). Endothelial cells of the capillary have fenestrations (arrows), while those of the thick blood vessel have many pinocytotic vesicles. BC, blood capillary; EC, endothelial cell. Bars indicate $0.5\mu\text{m}$.

terminals are always provided with both clear and dense-cored vesicles of similar sizes to those found in the nerve fibers (Fig. 13).

The pineal organ of the ice goby is surrounded by capillaries and a thick blood vessel running along its ventral side. Endothelial cells of the capillaries are furnished with many fenestrations (Fig. 14), while those of the thick blood vessel have no fenestrations but numerous pinocytotic vesicles on the periphery of the cytoplasm (Fig. 15). The pinocytotic vesicles contain moderately electron-dense material.

Discussion

The present study indicates that, in the pineal organ of the ice goby, *Leucopsarion petersi*, two types of photoreceptor cells show their respective regional distributions: the cells with well organized outer segments exist mainly in the proximal and middle regions of the pineal end-vesicle and those with modified outer segments are situated in the distal region. In the northern pike, *Esox lucius*, Falcon (1979b) noted well-differentiated photoreceptor cells located in the distal and proximal regions and rudimentary ones distributed mainly in the middle region of the pineal organ, and suggested a regional difference in the photoreceptive function of the pineal organ of the fish. The author was not convinced of secretory activity in the pineal organ of the pike, but noticed a rather rare occurrence of dense-cored vesicles, 60–100 nm in diameter, in both the typical and rudimentary photoreceptor cells. In the present study, photoreceptor cells, particularly those with modified outer segments, have ultrastructural characteristics denoting their synthetic activity. These cells contain slightly dilated endoplasmic reticulum and dense-cored vesicles of various sizes in the cytoplasm of the nucleated part.

The presence of dense-cored vesicles, which are believed to contain indoleamines and/or peptides as secretory products (Pévet et al., 1976, 1977; Collin, 1979), has been ultrastructurally demonstrated in pineal parenchymal cells of several species of teleosts. In the deep-sea fish, *Nezumia liolepis* (McNulty, 1976), the blind goby, *Typhlogobius californiensis* (McNulty, 1978a), and the seahorse, *Hippocampus hudsonius* (Herwig, 1980), occasional dense-cored vesicles are present in supportive cells of the pineal organ. In the organ of the cave-dwelling fishes, *Chologaster agassizi* (McNulty, 1978b) and *Typhlichthyes subterraneus* (McNulty, 1978c), both photoreceptor and supportive cells are shown to be metabolically active and produce dense-cored vesicles as well as clear vesicles. In *C. agassizi*, dense-cored vesicles occurring in photoreceptor cells range in diameter from 70 to 110 nm and those in supportive cells from 100 to 140 nm, whereas in *T. subterraneus* they are similar in size measuring 50–90 nm in photoreceptor cells and 70–90 nm in supportive cells. In the goldfish, *Carassius auratus*, too, dense-cored vesicles with a diameter of 70–90 nm are occasionally encountered in both the photoreceptor and supportive cells (McNulty, 1981). In the ice goby of the present study, dense-cored vesicles of two size-classes, one measuring 50–80 nm and the other 120–180 nm in diameter, are coexistent in the perinuclear cytoplasm of photoreceptor cells but not in supportive cells.

The exact cause of the differences in distribution of the possible secretory vesicles among distinct cell types of the pineal organ is still uncertain, but might be sought in different states of synthetic and secretory activities of the organ rather than in interspecific differences of the fishes studied thus far. It is interesting to note in this context that, in *Hyphessobrycon scholzei*, cytoplasmic protrusions accumulating large dense-cored vesicles appear in the neuropile area of the pineal organ of the fish kept in complete darkness for several days (Herwig, 1979). Prominent occurrence of dense-cored vesicles in pineal photoreceptor cells was also recently revealed in killifish, *Fundulus heteroclitus*, that had been adapted to darkness (Omura and Ali, 1981). Since it has been emphasized repeatedly that some ultrastructural features of pineal parenchymal cells may depend on physiological states of animals, examinations of the pineal organ in fishes under experimentally controlled conditions seem to be necessary to substantiate further the secretory function of the organ.

According to Omura and Ali (1981), the appearance of dense-cored vesicles in pineal photoreceptor cells of the killifish is closely associated with well-developed rough endoplasmic reticulum in the perinuclear region and the basal process. This is also the case for dense-cored vesicles of the larger size-class occurring in the modified photoreceptor cells of the ice goby. Well-developed cisternae of the rough endoplasmic reticulum always appear to keep close contact with the large dense-cored vesicles, sometimes giving rise to peculiar accumulations of these two components. The cisternae show constrictions at the sites where the vesicles attach to their membrane. These facts may indicate a possible implication of the rough endoplasmic reticulum in the production of dense-cored vesicles. On the other hand, McNulty (1978b) has mentioned that dense-cored vesicles appearing in the pineal photoreceptor and supportive cells of *Chologaster agassizi* may derive from Golgi bodies. In the modified photoreceptor cells of the ice goby, dense-cored vesicles of the smaller size-class are seen to have no special relation to the rough endoplasmic reticulum, unlike those of the larger size-class. However, the development of Golgi bodies is not prominent and lacks any sign of their contribution to the formation of dense-cored vesicles of either size.

As to the supportive cell of the pineal organ of fishes, abundant smooth endoplasmic reticulum, well-developed Golgi bodies, a large amount of glycogen granules, dense-cored or clear vesicles and, sometimes, frequent lipid droplets have been related to their possible secretory function (McNulty, 1981). Pineal supportive cells of the ice goby are characterized primarily by a remarkable development of characteristic tubular cisternae of the smooth endoplasmic reticulum which are located all through the cytoplasm. Similar specialized development of the smooth endoplasmic reticulum has been noted also in some supportive cells of the pineal organ of the deep-sea fish, *Cyclothone signata* (McNulty, 1979). It seems highly possible that the feature is indicative of synthetic activities which may differ in nature from those of the pineal photoreceptor cell. Pineal supportive cells of the ice goby also contain a small number of lipid droplets together with lysosome-like bodies. In the pineal organ of the cave-dwelling fish, *Astyanax mexicanus*, accumulation of lipid droplets associated with lysosome-like structures is frequently seen in the cytoplasm of supportive cells (Omura, 1975; Herwig, 1976).

Herwig (1979) considers this to be one of the morphological indications for endocrine activity of the pineal organ.

Another interesting feature suggestive of a secretory activity of pineal supportive cells of the ice goby is the occurrence of electron-dense material accumulated in narrow interstices among the apical microvilli of the cells which form a compact network surrounding the outer and inner segments of photoreceptor cells. The origin of the electron-dense material is unknown at present. Based on observations of the appearance of similar intercellular material in the pineal organ of various fish species, Herwig (1979) has suggested that the material may be taken up from or released into the intercellular spaces by pineal supportive cells. An apocrine type of secretion has been suspected to occur in pineal supportive cells of *Nezumia liolepis* (McNulty, 1976). It has also been shown that, in *Hippocampus hadsonius*, large dense-cored vesicles are accumulated in apical microvilli of pineal supportive cells (Herwig, 1980). In the ice goby, however, no ultrastructural characteristics of the supportive cell could be detected in relation to the electron-dense material around the microvilli.

Endothelial cells of the blood capillaries distributed along the ventral surface of the pineal organ of the ice goby have many fenestrations like those of the organ of other teleosts such as the pike, *Esox lucius* (Owman and R  deberg, 1970; Flacon, 1979b), the medaka, *Oryzias latipes* (Takahashi and Kasuga, 1971) and the deep-sea fish, *Bathylagus wesethi* (McNulty, 1976). Moreover, endothelial cells of a thick blood vessel in the ventral side of the pineal organ of the ice goby possess many pinocytotic vesicles. These features may imply an active interchange of materials between the blood and supportive cells of the pineal organ of the ice goby which lacks the central lumen.

Unmyelinated nerve fibers running along the periphery of the pineal end-vesicle of the ice goby are seen in some cases to have both clear and dense-cored vesicles. Moreover, nerve terminals containing the vesicles of similar sizes to those seen in nerve fibers exist bordering on the basal process of photoreceptor cells in the neuropile zone. The appearance of unmyelinated nerve fibers and their terminals with many dense-cored vesicles has been noted in the pineal organ of various fishes such as the European eel, *Anguilla anguilla* (R  deberg, 1971), the minnow, *Phoxinus laevis* (Oksche and Kirschstein, 1971), *Typhlogobius californiensis* (McNulty, 1978a) and *Chologaster agassizi* (McNulty, 1978b). Omura and Ali (1980) have revealed that, in the pineal organ of the brook trout, *Salvelinus fontinalis*, and the rainbow trout, *Salmo gairdneri*, axons of unknown origin have a synaptic contact with photoreceptor cells, and have suggested the possibility of an efferent innervation into the pineal organ. On the other hand, the production of dense-cored vesicles in the ganglion cell has also been demonstrated in the pineal organ of the crucian carp, *Carassius gibelio* (Ohba et al., 1979). The significance of these neural elements in the regulation of secretory function of the pineal organ remains to be explained.

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