Structural changes in the pars intermedia of the cichlid teleost *Sarotherodon mossambicus* as a result of background adaptation and illumination

III. The role of the pineal organ

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Summary. The MSH producing cells in the pars intermedia of *Sarotherodon mossambicus* have been shown to be involved in background adaptation processes. Reflected light received by the eyes affects the activity of these cells. In the present study the hypothesis has been tested that also the pineal organ, as a second photoreceptor, is involved in regulation of the metabolic activity of the MSH cells. The pineal organ appears to contain photoreceptor cells and is considered to be capable of transferring information about light conditions to the animal. Removal of the pineal organ of fish kept on a black background has no effect on activity of MSH cells, whereas the activity of these cells in fish kept in darkness is increased. Thus it seems that the pineal organ exercises its influence on MSH cells only in darkness and that this influence results in a reduced activity of these cells. It is therefore concluded that the metabolic activity of MSH cells is inhibited not only by reflected light received by the eyes, but also by the action of the pineal organ as a result of the absence of illumination.

No structural signs of secretory activity can be observed in the pineal, which might indicate synthesis or release of substances like melatonin. However, administration of melatonin reduces the activity of MSH cells. Neither pinealectomy nor treatment with melatonin has any influence on the second cell type of the pars intermedia, the PAS positive cells.

Key words: Pineal organ – MSH – Background adaptation – *Sarotherodon mossambicus* – Ultrastructure

The pars intermedia of teleosts contains two distinct endocrine cell types, the predominant MSH producing cells and the PAS positive cells. In *Sarotherodon mossambicus* the MSH cells are activated in fish on a black background and have low metabolic activity in fish on a white background. In darkness, the activity is...
also reduced. In blinded fish on a white background and under normal day-night conditions, the activity of the MSH cells is as high as in black background adapted fish, whereas activity is low when blinded fish are kept in darkness (Van Eys 1980a). From these findings it has been concluded that MSH cells are activated by a high ratio between reflected light (background) and direct light (illumination), rather than by the absence of reflected light. The results obtained with blinded fish point towards the existence of a second photoreceptor involved in the regulation of metabolic activity of MSH cells. As such a second photoreceptor the pineal organ has been suggested (Van Eys 1980a).

The PAS positive cells react to background colour in a similar way as the MSH cells. In darkness these cells are extremely active and in this respect they differ from the MSH cells. Blinding results in a state of activity similar to that of black background adapted animals, and a role of the pineal organ in the control of activity of these cells has been considered unlikely (Van Eys 1980b).

The pineal organ of several teleost species has been shown to contain photoreceptor-like structures. Electron microscopic observations provide indirect evidence for photoreceptivity (Herwig 1976, 1980; McNulty 1978a, b; Bergmann 1971; Ueck et al. 1978). Morita (1966), working with *Salmo irideus*, has provided electrophysiological evidence for photoreceptive properties of the pineal organ. In this last study it has been demonstrated that, like in other lower vertebrates, light perception by the pineal organ reduces the activity of this organ.

In the present study the effects of pinealectomy on the activity of MSH and PAS positive cells of the pars intermedia of *Sarotherodon mossambicus* have been investigated. Additionally, the effects of administration of melatonin have also been studied since it has been suggested that the pineal organ of teleosts contains melatonin (Fenwick 1970), and there are several reports describing a relationship between melatonin and melanophore aggregation (Ruffin et al. 1969; Reed et al. 1969). However, in adult teleosts colour change appears to be unaffected by treatment with melatonin (Abbott 1973; Bagnara and Hadley 1973; Kavaliers et al. 1980).

Materials and methods

Sexually mature male *Sarotherodon mossambicus* with a body length between 10 and 12 cm and a body weight varying from 9 to 12 g were used. Each experimental group consisted of five fish. During an adaptation period of 14 days animals were kept separately in buckets containing about 61 of tap water. Feeding and water replacement were done every other day. Room temperature was about 24°C. The groups were placed under one of the following regimes:

1. *Group B.* Non-reflecting black background with normal day-night rhythm (12 h light/12 h darkness).

2. *Groups EXB.* Fish were pinealectomized by removal of a part of the meninges and the underlying pineal organ; the operated fish were kept on a black background as described under group B. After killing the fish and dissection of the pituitary gland, the head was fixed and embedded in paraplast, and sections were examined with the light microscope to ascertain whether the pineal organ had been removed.

3. *Group D.* Non-reflecting black background in total darkness.
4. Group EXD. Pinealectomy as described for group EXB. Operated fish were kept in total darkness as described under group D.

5. Group MB. Non-reflecting black background as described for group B. An Alzet osmotic minipump, model 1701 (Alza, Palo Alto), was implanted in the abdomen. The minipump contained 170 μl of an aqueous solution of synthetic melatonin (1 mg/ml). The minipump released approximately 0.7 μl of fluid per hour over a period of ten days. The presence of hormone-free minipumps has no influence on the metabolic activity of MSH and PAS positive cells (Van Eys and Peters 1981).

At the end of the adaptation period fish were anaesthetized with MS 222 (Sandoz) and killed. Pituitary glands of all experimental fish were quickly removed and fixed. Method of fixation for electron microscopy as well as the procedure for morphometric analysis of the ultrastructure of MSH and PAS positive cells have been described in detail elsewhere (Van Eys 1980a).

Results

Morphology of the pineal organ. Light microscopic observations on sagittal sections of the head show the pineal organ to be a long stalk with a discus-like pineal body attached to the meninges (Fig. 1). The stalk originates caudally from the telencephalon and extends almost vertically to the roof of the cranial cavity. At the place where the pineal organ is attached to the meninges a window-like thin area in the bony skull is observed. The pineal organ is surrounded by a capsule of connective tissue and a network of blood capillaries. Electron microscopic examination shows that the pineal organ is composed of three cell types: supporting cells, neurosensory receptor cells bearing an outer segment and nerve cells (Fig. 2). The supporting cells have a glia-like appearance. Their nucleus is often oval in shape and the electron translucent cytoplasm contains mitochondria, poorly developed endoplasmic reticulum (rER), Golgi areas and polysomes. The most remarkable part of the sensory cell is the outer segment. It consists of a stack of 28–38 parallel double folded membranes that are invaginations of the ciliary membrane (Figs. 3, 4). This stack is connected to the head of the sensory cell by two cilia (Fig. 5). The cell body of the sensory cell contains many large mitochondria, rER consisting of short cisternae, and relatively large Golgi areas. Dense cored
Fig. 2. Electron micrograph of the pineal organ of *Sarotherodon mossambicus*. S supporting cells, R neurosensory receptor cells, *os* outer segment. × 4,000

Fig. 3. Electron micrograph of a neurosensory receptor cell. *nu* Nucleus, *G* Golgi area, *m* mitochondrion, *os* outer segment. × 8,000
Fig. 4. Electron micrograph of the outer segment of a neurosensory receptor cell. × 40,000

Fig. 5. Electron micrograph of the cilia that connect the outer segment with the head of the neurosensory receptor cell. × 40,000
granules, that could be indicative of secretory activity, are observed from these cells. Nerve cells are scarce but when present are located in the periphery of the organ and are characterized by electron translucent cytoplasm. Nerve fibers are observed in the proximal part of the stalk.

*Effects of experimental manipulation on the pars intermedia cells.* Influence of background, pinealectomy and administration of melatonin on the metabolic activity of the pars intermedia cells have been investigated by morphometric analysis. In MSH, as well as in PAS positive cells, a high relative cytoplasmic volume for mitochondria, rough endoplasmic reticulum and Golgi area as well as a low relative cytoplasmic volume for the granules, is considered to be indicative of a high activity.

Activity of the MSH cells of black background adapted fish is high, whether the fish is intact (group B) or pinealectomized (group EXB; Fig. 6). Activity of the MSH cells of fish kept in darkness (group D) is significantly lower than in black background adapted fish. However, pinealectomy of fish kept in darkness (group EXD) results in an activity of the MSH cells significantly higher than in fish of group D. The activity of group EXD is similar to that of fish of group B and EXB (Fig. 6).
The pineal organ and background adaptation in fish

Administration of melatonin to fish placed on a black background results in a decreased activity of the MSH cells when compared to untreated controls (group B).

Neither pinealectomy nor administration of melatonin influences the activity of the PAS positive cells. The activity in group B does not differ noticeably from that in groups EXB and MB, similarly no significant differences are found between groups D and EXD (Fig. 7).

Discussion

In a previous paper a hypothesis for the regulation of the activity of MSH cells was suggested which involved a second photoreceptor in addition to the eyes (Van Eys 1980a). It was stated that the ratio between reflected light received by the eyes and the intensity of illumination perceived by a second photoreceptor was the factor determining the activity of the MSH cells. A low ratio was correlated with low activity and a high ratio with high activity. The pineal organ was suggested to be that second photoreceptor.

Our observations of the pineal organ of Sarotherodon mossambicus show that its general structure corresponds with that of most teleosts investigated so far.
capable of converting received light stimuli into nervous or endocrine signals. The presence of nerve cells and the absence of dense-core granules in the photoreceptor cells suggest a transfer of information by neural pathways.

Influence of the pineal organ on the activity of the MSH cells was only exercised in darkness. This may be deduced from the observed similarity in activity between intact and pinealectomized fish on a black background on the one hand, and the difference in activity between intact and pinealectomized fish in darkness on the other hand. Such a conclusion is in line with considerable evidence in lower vertebrates, that shows a reduction of the pineal activity as a result of illumination (Morita 1966; Morita and Bergmann 1971; Smith and Weber 1976; Firth et al. 1979; Joss 1977; Vivien-Roels et al. 1979). The increased activity of the MSH cells observed after pinealectomy of S. mossambicus kept in darkness, demonstrates that the effect of the pineal organ on these cells is inhibitory.

Previously, it was reported that reflected light received by the eyes reduces the activity of the MSH cells (Van Eys 1980a). The present study shows that the pineal organ in the absence of light reduces the activity of these cells. Therefore, it is concluded that high activity of the MSH cells is the result of low signal transference from the eyes as well as from the pineal organ (Fig. 8). Activation of either the eyes by reflected light from a bright background, or of the pineal organ by lack of illumination, results in a reduced activity of the MSH cells. This conclusion differs considerably from the hypothesis proposed by Hogben and Slome (1936) for amphibians and modified for teleosts by Waring (1963) and Fujii and Novales (1972). The hypothesis proposed by these authors is based on the supposition that the retina consists of two functionally different zones, one receiving reflected light, the other receiving direct light. Differences in quantities of light received by the two zones should be responsible for the regulation of MSH synthesis and/or release, and subsequently for the skin colour of the animal. To our knowledge no histological data support this “split-retina” concept. On the contrary, morphological measurements on the activity of the MSH cells in S. mossambicus have demonstrated the untenability of the “split-retina” concept for the control of these cells (Van Eys 1980a). The present results point towards a regulation of the activity of the MSH cells by integration of information transmitted by lateral eyes as well as the pineal organ (Fig. 8).

Several other authors have connected the pineal organ with background adaptation processes in fish and amphibians. However, they consider the pineal as the origin of a “melanophore concentrating hormone”, that counteracts the pigment dispersing activity of MSH (Charlton 1966; Hafeez and Quay 1970; Fujii and Novales 1972; Kavaliers et al. 1980). Although some investigators have indicated a possible influence of the pineal on the hypothalamus and subsequently on the pars intermedia, no experimental evidence for such a connection has been given (Oshima and Gorbman 1969; Dawson and Ralph 1971; Charlton 1966; Kavaliers et al. 1980). However the influence of the pineal organ and/or melatonin on the synthesis and release of other pituitary hormones, have been demonstrated (De Vlaming and Vodicnik 1978; Vodicnik and De Vlaming 1978).

Melatonin reduces the activity of the MSH cells in S. mossambicus. This might indicate that the pineal influence on the MSH cells is mediated by melatonin. However, electron microscopic observations revealed no signs of endocrine
Fig. 8. Suggested regulatory mechanism of the MSH cell activity. On a white background MSH cell activity is decreased by reflected light (W), while on a black background, when no reflected light is received by the eyes (B) a high activity of the MSH cells is observed. The difference found in activity of the MSH cells between fish kept in darkness (D) and blinded fish (Bl) indicates the involvement of a second photo receptor; the pineal organ was suggested (Van Eys 1980a). In darkness no light is received by the eyes nor by the pineal organ. MSH cells are inactive when the pineal organ is intact (D), but active when the fish is pinealectomized (EXD). In fish kept under normal lighting condition pinealectomy has no effect on the MSH cells (EXB). Thus, the pineal organ exercises its influence on the MSH cells only in darkness and this influence has a negative effect on the activity of the MSH cells. Therefore, it is concluded that the absence of light reception by the eyes results in an activation of the MSH cells, but only when the activity of the pineal organ is reduced by the presence of direct light. E eye, P pineal organ, pi pars intermedia
activity. This result is similar to that of other structural studies (Bermann 1971; Ueck et al. 1978; Herwig 1976, 1980). Fenwick (1970) demonstrated the presence of melatonin in salmon pineal organ, a finding that is supported by some other studies that show a disruption of diurnal variations in plasma melatonin levels after pinealectomy (Delahunty et al. 1978; Vodicnik and De Vlaming 1978). In addition, melatonin has been shown to have pigment aggregating properties in vitro and is suggested to control the changes between nocturnal and diurnal pigmentation patterns in fish (Reed et al. 1969; Kavaliers et al. 1980; Hafeez and Quay 1970; Joss 1973; Ruffin et al. 1969; Finnin and Reed 1970). These investigations suggest that melatonin is able to influence skin melanophores directly. However, the present results suggest that the influence of melatonin during background adaptation is mediated by the MSH cells. This conclusion is supported by the lack of correlation between plasma melatonin levels and background adaptation observed in trout (Owens et al. 1978). In addition, in Fundulus heteroclitus pinealectomy as well as hypophysectomy abolish the rhythmic colour changes without affecting physiological background adaptation processes (Kavaliers and Abboth 1977; Kavaliers et al. 1980).

Removal of the pineal organ had no influence on the activity of the PAS positive cells of Sarotherodon mossambicus. Thus, the effect of background and darkness on these cells is most likely mediated by the eyes only.

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