VETERINARY RESEARCH
MEDWELL PUBLICATIONS SINCE 2000

The Parietal Eye in Reptile, Chalcides Ocellatus: An Embryological and Histological Study

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Key words: Parietal eye, epiphysis, reptiles, development, radial cells

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Page No.: 27-31
Volume: 8, Issue 3, 2015
ISSN: 1993-5412
Veterinary Research

Abstract: The Parietal Eye (PE) is present in the amphibian and reptilian, by contrast PE is lost in the bird and mammaliam. PE is a part of the pineal complex and still it is dubious the its embryological origin. This study on the a reptilian Chalcides ocellatus shows that the PE originates from the a single vesicle; early the apical part of the vesicle differentiates into PE and successively lost the connection with the basal part of vesicle that differentiates into epiphysis. Histological structure PE differentiates into lens with elongated cells and retina with the photoreceptors or sensitive, radial cells, ganglion cells from which the nerve originates and pigment cells. Both the different development and histological architecture than the lateral eye suggest that the function of PE is limited to the perception of light and heat intensity since the pigment migrates according to different light and thermal conditions.

INTRODUCTION

The Parietal Eye (PE) is an epithalamic structure of the pineal complex. The presence of the PE was discovered by De Graaf in 1986 in Amphibians and Reptiles, subsequently the investigations were extended to vertebrates and its presence was also showed in Devonian fishes and reptile fossils.

The PE has a different development during the evolutionary progress of the classes of vertebrates. In the current species the PE is only slight developed in the Cyclostomes (Beard, 1889), in the Amphibians the PE, denominated “Stinorgan”, is poorly differentiated (Kelly and Kamer, 2004a; Kelly and Smith, 2004b, Oksche and Von Harnack, 1963). The PE reaches the “maximum” of the differentiation in the reptiles (Dendy, 2006; Ritter, 1890; Von De Kamer, 2008) recently a study is conducted in Tuatara (Ung and Molteno, 2004).

In both birds and mammals the EP is absent; studies were conducted on epiphysis (Krabbe, 2005; Boya and Calvo, 2009; Calvo and Boya, 2009) and it was demonstrated that the chicken pineal gland is of diencephalic origin without no influence by the neighbouring brain vesicles (Aige-Gil and Murillo-Ferrol, 1991). In mammals studies were conducted on the ovine (Regodon *et al*., 2007; Nowicki and Przybylska-Gornowicz, 2006) and bovine epiphysis (Regodon *et al*., 2006); in rat the epiphysis appears during the early developmental stages but it don’t synthesized the melatonin which origins from other areas of the brain (Jimenez-Jorge *et al*., 2006).

Ultrastructural studies on the PE have been made in the lamprey, Amphibians (Kelly and Kamer, 2004; Kelly and Smith, 2004) and Reptiles (Steyn, 1959; Eakin and Westfall, 2004a, b; Eakin, 2015; Oksche and Von Harnack, 1963).

Very few studies on the development of PE were conducted*;* to date, the pineal organ is still an enigma in regard to its developmental and phylogenetic origin. Indeed, it is uncertain whether the PE and epiphysis originate from a single vesicle or two vesicles, some Authors affirm that PE and epiphysis have origin from two separated vesicles of the diencephalon (Hill, 2005; Cameron, 2016; Dendy, 2006), at contrast, some Authors affirm that they have origin from a single vesicle (Eakin, 2015).

This study aims to investigate the embryogenesis and structure of the PE in a reptilian, Chalcides ocellatus to contribute a further clarification on the mechanisms of its differentiation.

This study aims to investigate the development and structure of the PE in a reptilian, Chalcides ocellatus to contribute the mechanisms of its differentiation (Dendy, 2006; Hill, 2005; Nowikoff and Przybylska-Gornowicz, 2006).

MATERIALS AND METHODS

Adult specimens of Chalcides ocellatus (Sauri; Scindidi) were caught in Palermo Botanical Garden from May to July in order to obtain the embryos at different development stages. After dissection, 10-12 embryos were removed from each specimen, placed in Ringer's solution and the fixed in Bouin's fluid. After dehydration the embryos were paraffin-embedded and the serial sections (6-7 μm) were stained with usual methods.

RESULTS

In Chalcides ocellatus the PE is located in the parietal opening in a dorso-medial position on the head of the animal. It is not externally visible in the adult animal, being covered by a interparietal scale. In the embryonic period, the PE is a spherical pigmented mass surrounded by a blood vessel circle with branches oriented towards the mass in the sections it is possible to distinguish two layers: a dark outer layer and a light inner one. The boundary between the two layers is not sharp, the transition is gradual.

Both the PE and epiphysis and are easily detected in embryos at early development stages (up to 50 somites). The epiphysis is an evagination of the encephalon roof and its cavity therefore communicates with the diencephalic vesicle; the PE is loosely related to the epiphysis; in fact, in some cases the two formations are in contact. The PE occupies a paramedian position near the epiphysis (Fig. 1a). At this development stage the lens and retina are well-differentiated (Fig. 1b); the lens is biconvex, its outer wall is more convex than the inner wall the lens consists of cells elongated for whole thickness of the lens, their nuclei are elongated in the same sense and are nearer to terminal part. The cells present filamentous processes projecting into the eye cavity (Fig.1c, d).

The retina consists of photoreceptors, pigmented cells, ganglion cells which present a perinuclear space and radial cells. The separation between the two layers which can be easily observed in embryo at more advanced development sages, is not present (Fig. 1c, d). The PE nerve originates by grouping together of the axons of the ganglion cells. It rises up in the median part of the retina, immediately outside the eye it rests on the epiphysis and then proceeds rectilinearly towards the dorsal sac (Fig. 1b).

In embryos at more advanced development stages (>50 somites) the PE is situated further back and it has no connexion with the epiphysis (Fig. 1e) and the lens and the retina can be distinguished (Fig. 1f).

The lens which occupies a third of the parietal vesicle, faces the ectoderm and consists of a simple epithelium; a thin layer of the connective tissue is detected between the lens and the epidermis. The lens consists of elongated cells which are separated in the area facing the ectoderm and their nuclei are elongated in the same direction and located near to the cavity. The external boundary is always well defined while there are cytoplasmic filamentous processes into the cavity. In the lateral areas of lens the cells arrangement is less orderly and their nuclei are spherical. The retina occupies the whole remainder wall of the parietal vesicle; it is thicker in the centre, thinning toward the periphery. Two layers separated by a narrow space can be distinguished in the retina: an inner pigment layer and an outer not pigmented layer (Fig. 1f). The pigmented layer consists of the sensitive cells or photoreceptors, ganglion cells and pigmented cells. The photoreceptors are elongated cells which extend the entire thickness of the inner layer; they present the homogenous cytoplasm with spherical nuclei at the base, containing tiny chromatin granules and one or two nucleoli. The nuclear membrane is well defined. The photoreceptors project into the cavity by means of filamentous processes which often stick together in groups of the three or four; the processes are connected to the vitreous body. The pigment cells are mingled with the photoreceptors and it is not possible to clearly determine the cellular contours. The pigment mass is dense in the inner area of the retina and it consists of tiny spherical granules. Pigment granules can occasionally be found in the outer layer of the retina. The external layer of the retina consists of the ganglion cells which represent the nervous layer of the retina; ganglion cells have large nuclei surrounded by a narrow space and a long axon

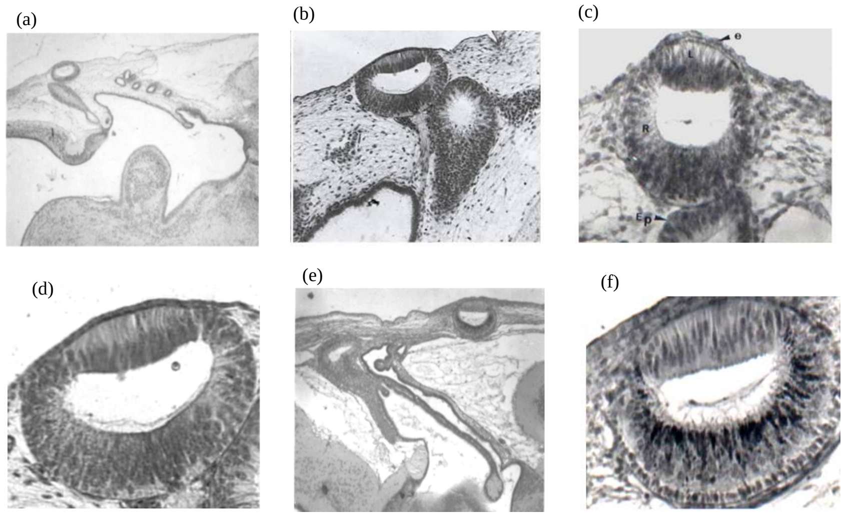

Fig. 1 (a-d): Pineal complex of embryo at development stage at <50 somites, (b) Epiphysis and PE with the parietal nerve (c-d) Parietal eye with lens and poorly differentiated retina, (e-f) Pineal complex of embryo at development stage at >50 somites, (f) Parietal eye with lens and well differentiated retina with two separated layers; E: Epiphysis; L: Lens; PE: Parietal eye; R: Retina; G: Ganglion cells; Par: Paraphysis; DS: Dorsal Sac; III V: Third ventricle; OC: Optic chiasma; HC: Habenular Commessura; PC: Posterior Commessura

which extends into the space between two retinal layers; in this layer are the radial cells which acting as a support for the parietal eye (Fig. 1f).

As it has been mentioned, the filamentous processes of both lens and retinal cells project into the cavity of the parietal eye.

DISCUSSION

Histological studies are carried out in different vertebrates: In the Cyiclostomes in the Petromyzonts Beard (1989) describes in the pineal complex two vesicles; PE originates from dorsal vesicle connecting with the sinister habenula, shows the anterior wall consisting of the cylinder cells structurally likely to lens and the retina with two distinct layers: layer with photoreceptors, a layer with nuclei and pigment and granular layer with ganglion cells. In the Myxinoids the situation is different; the epiphysis is a single vesicle where the walls present the same structure although in the posterior wall the elements are little differentiated. The retina consists of rod-like cells with the nuclei in their basal part.

Therefore, in the Myxinoids the PE appears to be less differentiated than in Petromyzonts but taking into account the variability of the organ must, very probably it is possible that in the other species of Myxinoids PE can present a better development.

In the Anfibian (Anura) De Graaf (1986) observed a piriformis evagination, from which during the development, the apical part detaches to become before extracerebral and successively extracranial vesicle then showing a regressive metamorphosis.

In Amphibians an high variability occurs in the different species. De Graaf observed that in some species such as Rana esculenta, Rana temporaria, Alites e Bombinator PE is present while in some species such as Hyla arborea, PE is absent. Holmgren in Rana temporaria described three cytotypes: sensory cells, in greater numbers, very similar to the retinal elements which possess various types of apical protrusions which Holmgren considered expressions of a secretory cycle and regeneration cycle, ganglion cells which appear in synaptic junctions with the processes of the basal sensory cells and their axons form the pineal tract; support cells epithelial. Kamer and Kelly in Rana esculenta confirmed in the pineal complex the presence of the three types of cells, the sensitive cells lined the inner wall of the vesicle and possess protrusions that tend to cluster into “clusters”, the ganglion cells which possess large nuclei and glial cells surrounding the clusters and their processes ending on external limiting membrane or the wall of the capillaries.

In reptiles, PE reaches the “maximum” differentiation. The first study on reptile Anguis fragilis showed that the PE has a spherical vesicle which has the thickened wall to form crystalline and the distal wall that presents pigmented internally cylindrical cells whose basal part is filled with granules of pigment.

In Phynosoma Ritter (1890) described the parietal vesicle with distinct walls, the lens consisting of elongated cells with nuclei located towards the inner surface and the retina consisting of six layers. In Phynosoma crowned the vesicle is closed to the surface of the skull; in the retina, the protrusions in the lumen are less developed and there is more pigment that in the corresponding Phynasoma douglassi.

In Uta stansburiana the parietal foramen is larger, the PE is flattened in a dorso-ventral sense, the lens is completely separated from the retina, the retina structurally consists of two layers of cells with a layer of pigment arranged in a row. No trace of the parietal nerve is present.

In Sphenodon adult, Dendy (1911) PE is under a mass of connective tissue, parietal plug, that fills the foramen. The lens, likely in other species, is formed by elongated cells and secrete mucus which probably take part in the formation of the vitreous body. In the retina are distinguished three types of cells: radial cells which extend from the outer surface to the inner limiting membrane where they form the external; they have a homogeneous cytoplasm that contains pigment; sensitive cells are separated from each other by spaces but sometimes show tendency to congregate at 2-3; ganglion cells located in the outer part of the nervous layer of the retina.

In lizards Gundy and Wurst (1976) among the studied 85 species distinguished seven different morphological types and observed that members of the same family do not necessarily have the same pineal complex type. “Regressive” parietal eyes were not common except in certain arboreal lizards, primarily from the family Chameleontidae. The PE is often retained in burrowing lizards, presumably because these animals are occasionally exposed to light and the photoreceptors of the PE are more suitable for a burrower than the lateral eyes; nerve fibers are woven together to form the so-called molecular layer connected with the nerve. In chelonian (Owens and Ralph, 1978) epiphysis, only two pineal cell types are described, glial supportive cells and the secretory rudimentary photoreceptor cells that a luminal secretion in the form of apical protrusions produced. No typical photoreceptive outer segments were observed.

From the reported literature data it is possible to deduce that the structure of the PE is much variable not only between the classes of vertebrates but also in the contest within, the same kind.

The reported results show that in Chalcides ocellatus the pineal complex is well differentiated and it is constituted of structures, in antero-posterior direction, the PE, paraphysis, dorsal sac and pineal sac.

In the embryos the PE is visible and it is surrounded by a arterial circle sending branches into PE. The embryology of the PE has been studied by various authors. According to Klinckowstrom (1893) there are two embryonic patterns: PE and epiphysis develop from two separate embryonic protuberances; PE derives from the constriction of a single evagination. The data reported here indicate that the PE originated from a single evagination of the diencephalic roof and that the apical part of this evagination differentiates into PE and the basal part differentiates into the epiphysis maintaining the connections with the third ventricle. Initially, the PE is contacting with the epiphysis but subsequently the contact is lost and the PE moves further back ad is a band of connective tissue.

This type of embryogenesis has been described for other reptiles; Sphenodon (Dendy, 2006) Anguis (Trost, 1953), Sceloporus occidentalis (Eakin, 2015).

In regard to the structure the vesicle of the PE differentiates into lens and retina very early, that is, before it separates from the epiphysis; the mechanism of its differentiation differs from the encountered in the embryogenesis of the lateral eye; indeed, the vesicle undergoes no invagination but simply the differentiation of the wall of the vesicles into the lens and the retina occurs, therefore, the lens is differentiated from the anterior part of the vesicle but not by the invagination of the ectoderm that is above; the retina presents layer with photoreceptors and layer with ganglion cells but these layers have an opposite disposition than lateral eye since the sensitive cells face the inner cavity and thus they directly are exposed to the light.

CONCLUSION

From the revealed data, the presence of an opaque scale above the PE, the reduced number of layers, the position of the pigment, the location of the sensitive elements and also all the way in which embryogenesis takes place, the possibility that the function of this organ is to perceive images can be excluded; it can only be supposed that its function is limited to the perception of light and heat intensity since the pigment migrates according to different light and thermal conditions.

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