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HISTOMORPHOLOGICAL IDENTIFICATION OF THE TINCTORIAL CELLS IN THE PITUITARY GLAND OF CHANNA PUNCTATUS (BLOCH)

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RESUMO: Com base nas propriedades tintoriais sete tipos de células foram identificadas na glândula pituitaria da Channa punctatus. O RPD contem dois tipos de células: células C1 PbH-positiva (ACTH) e células acidofílicas A1 (Prolactina) O PPD inclui uma célula A2 acidofílica (STH ou GH) e dois tipos de células cianofílicas C2 e C3 (tireotrópicas e gonadotrópicas) Os cianófilos são PAS, AF e anilina azul positiva. O PI possui dois tipos de células: células C4 PbH-positiva (MSH) e células C5 PAS positiva (células sensíveis a Ca++)

ABSTRACT: On the basis of tinctorial properties seven cell types have been identified in the pituitary gland of Channa punctatus. The RPD contains two cell types: PbH-positive C1 cells (ACTH) and acidophilic A1 cells (Prolactin) The PPD comprises an acidophilic A2 cell (STH or GH) and two types of cyanophils C2 and C3 cells (thyrotrops and gonadotrops) The cyanophils are PAS, AF and aniline blue-positive. The PI possesses two cell types: PbH-positive C4 cells (MSH) and PAS-positive C5 cells (Ca++-sensitive cells)

INTRODUCTION

Several reviews are now available on the pituitary gland of teleostean species (Ball and Baker, 1969; Sage and Bern, 1971,; Schreibman et al , 1973; Holmes and Ball, 1974; Doerr-Schott, 1976; VanOordt, 1979; Ball, 1981) However, diversities in their structure behaviour and cell types are also evident. According to Schreibman et al , (1973) "fishes represent the most diverse group of vertebrates providing a wealth of forms variable not only in morphology, but also in their habitat and the endocrine-regulated physiological problems imposed by their diverse environments" Information on the pituitary cytology of tropical fishes inhabiting

Indian waters and showing adaptation to various environmental fluctuations are still meagre (Baker et al , 1974; Srivastava et al 1977; Joy and Sathyanesan, 1980; Srivastava and Swarup, 1980, Thomas and Sathyanesan, 1984) Therefore in the present study an attempt has been made to study in detail the cell types of pituitary gland of Channa punctatus, in order to provide background for study the physiological aspects of the cells in the subsequent studies.

MATERIAL AND METHODS

Specimens of adult Channa punctatus were collected locally After decapitation the brain along with pituitary gland was fixed in Bouin s solution. After dehydration, the material was embedded in paraffin wax and cut to 5 µm thickness in the sagittal plane and stained with Heidenhain s Azan Gomori s Aldehyde fuchsin (AF), periodic acid Schiff with orange G (PAS-OG), MacConaill's lead hematoxylin combined with PAS (PbH-PAS)

OBSERVATIONS

The pituitary gland of Channa punctatus is oval and devoid of any neurohypophysial stalk It consists of two parts neurohypophysis (NH) and adenohypohysis. The latter is divisible into rostral pars distalis (RPD), proximal pars pars distalis (PPD) and pars intermedia (PI) All the glandular parts are arranged in an anteroposterior plan (Figs. 1 and 2) The neurohypophysis mainly composed of loosely arranged nerve fibres spreads over the dorsal part of the glandular surface and the nuclei of neuroglia cells are interspersed in it. A thick bundle of fibres extend ventrally into the pars intermedia which gets closely associated with the cells of that region (Figs. 1 and 2)

By means of several staining techniques, seven tinctorial cell types were identified in the adenohypophysis. These are tentatively named C1 and A1 cells in the RPD, A2, C2 and C3 cells in the PPD, and C4 and C5 cells in the PI (Table I and II)

i) **C1 cells (ACTH):** They are columnar cells and form a band between the RPD and ramifications of neurohypophysis. These cells display poor cytoplasm with a fine powdery granulation, faintly stained with aniline blue, PAS and AF and are

selectively stained dark blue with PbH (Fig. 3) Their nuclei are rounded or oval with distinct nucleolus (Table I and II)

ii) **A1 cells (Prolactin cells):** They are dominating cells and form a compact mass packed in the ventral region of RPD. They are rounded cells with evenly granulated cytoplasm, stained lightly with azocarmine and Orange G. Their nuclei are rounded with a distinct nucleolus (Fig. 4, Table I and II) Small blood capillaries were also observed amidst these cells.

iii) **A2 Cells (GH or STH):** The A2 cells are the most prominent acidophils and occupy the centro-dorsal region between the PPD and the ramifications of the neurohypophysis. They are cuboidal or ovoidal cells with cytoplasm containing distinct and dense granules stained deeply with azocarmine and orange G. In almost all the cells nuclei are eccentrically placed (Fig. 5, Table I and II)

iv) **C2 Cells (TSH):** The C2 cells are located in the anterodorsal region of the PPD intermingled with the A2 cells. They are rounded cells with rounded nuclei situated apically. Their cytoplasm contains fine secretory granules stained brightly with aniline blue PAS and AF (Fig. 5, Table I and II)

v) **C3 Cells (GTH):** The C3 cells are generally concentrated in the ventral part of the PPD. These cells possess distinct granules which exhibit strong affinity with aniline blue. PAS and AF and characterized by eccentrically placed distinct rounded nuclei (Fig. 6, Table I and II) The appearance of C3 cells show distinct morphological and cytological changes during gonadal cycles. During spawning season some cells were also encountered in the RPD and PI regions of the adenohypophysis.

vi) **C4 Cells (MSH):** They are polygonal or clavate cells bordering the neurohypophysial protrusions that invade deeply into the PI The cells are large with distinct nucleus and display dense granulation and stained bluish grey with PbH. Nuclei are rounded with distinct nucleolus (Fig. 7, Table I and II)

vii) **C5 Cells:** These cells are intermingled with PbH-positive cells of PI They are rounded cells with scanty cytoplasm and distinct rounded nuclei, stained lightly with PAS (Fig. 7, Table I and II)

Both the cells (C4 and C5) display poor response to aniline blue.

DISCUSSION

The pituitary gland of C. punctatus is devoid of any stalk and is therefore, classified as a platybasic type (BRETSCHNEIDER and DUYVENE DE WIT, 1947) On the basis of cell types and their staining affinity to different stains adenohypophysis has been divided into three parts: (i) rostral pars distalis (RPD), (ii) proximal pars distalis (PPD), and (iii) pars intermedia (PI) According to VanOordt and Peute (1983) this subdivision also reflects the predominant localization of functionally different cell types in these three regions. The neurohypophysis innervates all the three lobes of adenohypophysis though its main branch ramifies into the PI, a feature characteristic of teleosts. At light microscopical level seven tinctorial cell types were identified in the adenohypophysis of C. punctatus. They were tentatively named: C1 and A1 cells in the RPD; A2, C2 and C3 cells in the PPD and C4 and C5 cells in the PI

C1 Cells:

The C1 cells are identified as lightly stained cyanophils arranged with the long axis perpendicular to the adeno-neuyrohypophysial interface in RPD. On their staining affinity with different stains these cells have been identified as chromophobes (Olivereau and Ball, 1964); light cyanophils (Srivastava et al , 1977; Srivastava and Swarup, 1980); acidophils (Aoki and Uemura, 1970; Chiba and Honma, 1973; 1974) and amphiphils (Wai-Sum and Chan, 1974) In C. punctatus these cells exhibit weak staining affinity with PAS, AF and aniline blue and are identified as light cyanophils. In addition, like other teleosts (Srivastava and Swarup, 1980; Olivereau and Callard, 1985) these cells show special affinity for MacConaill s lead hematoxylin. Several experimental studies and immunocytochemistry prove that PbH-positive cells are corticotrops (Yoshie and Honma, 1978; Joy and Sathyanesan, 1979; Olivereau and Callard 1985) On the basis of morphological similarity and staining affinity the C1 cells in C. punctatus have been identified as ACTH cells reported in other species of teleosts.

Histomorphological identification of the tinctorial cells

A1 Cells:

In C. punctatus the A1 cells occupy major part of the RPD, stained lightly with azocarmine and are identified as acidophils. In other teleosts these cell have been identified as chromophobes (Ball and Baker 1969; Benjamin 1975; 1978) The acidophils which constitute the major part of the RPD are generally believed to secrete the prolactin (Ball and Baker 1969); VanOordt and Peute, 1983) Experimentally the physiological role of these cells have been demonstrated in osmoregulation (Wendelaar Bonga and Van der Meij 1981; Ogasawara and Hirano, 1984; Wendelaar Bonga et al , 1984a, b; Olivereau and Olivereau, 1987a) In C. punctatus the similarity in staining properties of A1 cells with the prolactin cells of other teleosts suggests them as prolactin cells.

A2 Cells:

In C. punctatus A2 cells are most prominent acidophils, form cell cords between the neurohypophysial projections and PPD. They are stained darkly with azocarmine and other acid dyes as also reported in other teleosts (Srivastava et al , 1977; Joy and Sathyanesan, 1980; Srivastava and Swarup 1980; Hendee and Mueller, 1985) The acidophils of the PPD are reported to secrete somatotropin or the growth hormone (Ball and Baker, 1969, Sage and Bern 1971; Olivereau and Callard, 1985) On morphological similarities the A2 cells in C. punctatus have been shown to relate with the secretion of growth hormone in some teleosts (Olivereau and Ridgeway, 1962)

On the basis of morphological appearance, cytoplasmic granulation and staining affinity with PAS, AF and aniline blue two types of cyanophils have been identified in the PPD of C. punctatus. They have been referred to as C2 and C3 cells and are located in the dorsal and ventral zones of PPD respectively. The C2 cells lie in between the neurohypophysial interdigitation of PPD just adjacent to the layer of A2 cells. They are characterized by fine granules exhibiting positive reactions with PAS, AF and aniline blue. The C3 cells are larger than the C2 cells and are brightly stained with PAS, AF and aniline blue. Additionally, the C3 cells are also identified on account of their morphological variations with gonadal cycle. On the basis of staining affinity and morphological appearance the C2 and C3 cells in the present study show close resemblance with those cyanophils which are functionally described respectively as thyrotrops and gonadotrops in many teleosts (Ball and Baker,

1969; Aoki and Uemura, 1970; Chiba and Honma, 1973, 1974; Srivastava and Swarup, 1980) Presence of one or two types of gonadotrops in teleosts pituitary gland is a disputed and unrevealed problem. In few species two types of gonadotrops were reported (Olivereau, 1976, 1978; Ekengren et al , 1978; Ueda and Takahashi, 1980; Ueda et al , 1983) while in others only one type of gonadotrop was recognized (Srivastava, 1979, 1983; Young and Ball, 1982; Peute et al , 1984; Kaneko et al , 1986a, b) In C. punctatus only one type of gonadotrop has been identified.

The PI surrounds the posterior region and consists largest part of the adenohypophysis. The cells of the PI in C. punctatus are identified as lightly stained cyanophils whereas in other species the PI cells are described as acidophils, amphiphils, basophils or chromophobes (Ball and Baker, 1969; Holmes and Ball, 1974; Van Overbeeke and McBride. 1967) By PbH-PAS technique two cell types (C4 and C5) have been identified in the PI region of C. punctatus. The PbH-positive C4 cells bordering the NH and the PAS-positive C5 cells laying in between C4 cells. Several other workers have also differentiated two types of cells in the PI by PbH-PAS technique (Olivereau, 1964; Mattheij and VanOordt, 1967, Joy and Sathyanesan, 1980; Srivastava et al , 1977; Srivastava and Swarup, 1980; Ball and Batten, 1981; Van Eyes and Wendelaar Bonga, 1984; Olivereau et al , 1985; Olivereau and Callard, 1985) It has been suggested that two cell types in the pars intermedia secrete two hormones. The PbH-positive cells secrete melanocyte stimulating hormone (MSH) while PAS-positive cells are involved in osmoregulatory function, particularly in C++ regulation (VanOordt and Peute, 1983: van Eyes and Wendelaar Bonga, 1984: Olivereau and Olivereau, 1987b) In C. punctatus PbH-positive C4 cells and PAS-positive C5 cells appear to correspond with MSH cells and the cells involved in the calcium metabolism as described in other teleosts.

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Histomorphological identification of the tinctorial cells

Table 1: General Organization of Cell types in the Adenohypophysis of *Channa punctatus*.

Regions of Adeno-hypophysis	Cell types	Cell Shape	Cell Size (µm)	Cytoplasm Amount	Cytoplasm Granulation	Nucleus Shape	Nucleus Size	Nucleolus	Chromatin Matter
RPD	C_1	Columnar	9.2±0.12	Scanty	Fine	Rounded or oval eccentric	3.60±0.04	Distinct	Less Distinct
	A_1	Rounded or oval	7.7±0.07	Scanty	Fine	Rounded, Central	3.7±0.05	Distinct	More Distinct
	A_2	Cuboidal or ovoid	6.8±0.07	More	Dense	Rounded eccentric	4.1±0.09	Distinct 1-4 nucleoli	Less distinct
PPD	C_2	Spherical	7.3±0.17	More	Fine	Rounded eccentric	4.1±0.07	Less Distinct	Distinct
	C_3	Variable (Rounded or Oval)	Variable (7.8±0.09 to 9.5±0.11)	Variable (More or Scanty)	Variable (Dense, or fine or vacuolated	Rounded or oval	Variable (2.16±0.18 to 4.7±0.05)	More Distinct	More distinct
PI	C_4	Polygonal or clavate	7.6±0.05	More	Dense	Rounded Central	3.5±0.04	Distinct	Distinct
	C_5	Rounded or oval	6.4±0.04	Scanty	Fine	Rounded or oval, central	3.2±0.02	Distinct	Distinct

Table II Summary of staining reaction

Stain	Cell Types							
	Rostral Pars Distalis (RPD)		Proximal Pars Distalis (PPD)			Pars Intermedia (PI)		
	C_1	A_1	A_2	C_2	C_3	C_4	C_5	
Heidenhain's Azan	Aniline blue +	Azocarmine +	Azocarmine +++	Aniline blue ++	Aniline blue +++	Aniline blue +	Aniline blue +	
PAS-OG	PAS +	OG +	OG +++	PAS ++	PAS +++	-	PAS ++	
AF	AF +	-	-	AF ++	AF +++	AF +	AF +	
PbH-PAS	PbH +++	-	-	PAS ++	PAS +++	PbH +++	PAS ++	

Histomorphological identification of the tinctorial cells

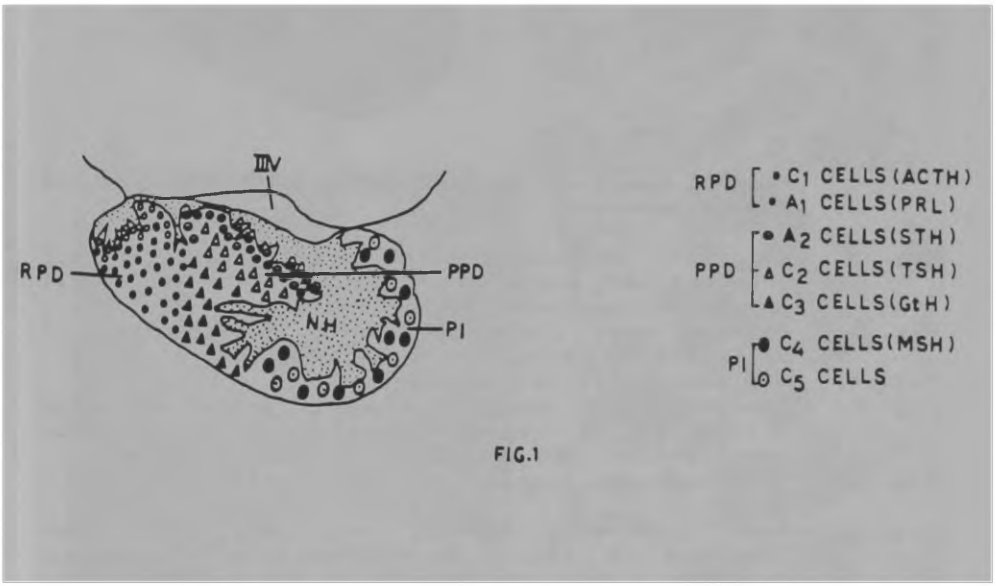

FIG.1

Fig. 1 Diagrammatic representation of Median sagittal section of the pituitary gland of Channa punctatus showing Neurohypophysis (NH) and general distribution of adenohypophysial cell types in the rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) × 50.

Median sagittal section of pituitary gland of Channa punctatus

Fig. 2 Pituitary showing rostral pars distalis (RPD), proximal pars distalis (PPD), pars intermedia (PI) and Neurohypophysis (NH) in PI Azan. x 50

Fig. 3. Rostral pars distalis showing PbH-positive C1 cells and A1 cells. PbH-PAS x 1000

Fig 4. RPD showing A1 cells. Azan x 1000

Fig. 5. Proximal pars distalis showing azocarminophilic acidophils (A2 cells) facing the neurohypohysial ramification (NF) and C2 cells. Azan. x 1000

Fig. 6. Proximal pars distalis showing C3 cells. PAS-OG. x 1000

Fig. 7 Pars intermedia showing two cell types PbH-positive (C4 cells) and PAS-positive (C5 cells). PbH-PAS. x 1000

Histomorphological identification of the tinctorial cells