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STUDY OF PITUITARY CYTOLOGY AND FUNCTIONS IN SOME MAMMALIAN SPECIES

Dr. S. D. Kamble
B.S.S Art, Science & Commerce College Makani, Tq. Lohara,
Dist. Osmanabad (M.S).

ABSTRACT

Two varieties of acidophil (non-mucoid) cells and four varieties of basophil (mucoid) cells were observed in the anterior pituitary of some mammals by using various staining techniques. To each cell types has been tentatively assigned an endocrine function. By N.S.B. staining method two acidophilic cell types could be demonstrated separately. These cell types were identified as STH and LTH tentatively. All basophill cells were PAS positive. The AF/OG/LG and AB/PAS/ OG stainingmethods differentiate two types ofbasophils. The cells which specifically obtained with AF and AB were considered as TSH Cells. Two types of ganodotrophs (FSH & ICSH) are distinguished from each other after using PAS/ OG/MB preparation. The FSH cells are PAS and MB positive (Purple) and the ICSH cells are stained (red) only with PAS. The site of secretion of ACTH is till obscure.

KEYWORDS : *demonstrated separately , N.S.B. staining method.*

INTRODUCTION

The CYTOLOGY of the anterior pituitary (adenohypophyisis) gland of some mammal (e.g. bandicota, Rat) The pituitary being one of the master organ of the body. Where it is responsible for the secretion and regulation of hormonal outflow along with the hypothalamus, it's study anatomical, morphological and hiatochemical.

The study of cytological peculiarities peculiarities and Historical properties of various cell type in the anterior pituitary gland are related to its function at the present time the functions of adenohypophysis (anterior pituitary) are sufficiently well defined to make it possible to relate cytological character to specific function with some confidence. Cytological features which are of functional significance have been clarified in recent years. Consideration of this data in conjunction with the evidence derived from physiologic observation's does assists in the construction of acceptable hypothesis concerning the endocrine function of the anterior pituitary.

MATRIAL AND METHOD

Source of Material collected :

The pituitaries of different animals were collected from the following sources:

1) Bandicota (B-female: and B- male) pituitaries was donated by Mr. Sawane, fixed in formal sublimate.

2) Rat was collected from the localities of Vayu S ena Nagar, Nagpur Pitutaries of was.fixed in Helly's fixative.
3) Guinea pig was brought from the institute of Vaccine, Nagpur Pitutaries of these animals was also fixed in Helly's fixative.
4) Goat brain along with pituitary was purchased from local shop and was then preserved in 10 formalin.
5) Pituitary of human had been procured from Gvt. Medical College, Nagpur fixed infomialin.
6) Sained slides of the pituitary of Giraffe was collected from Dr. Sawne, Nagpur University Nagpur.

METHOD FOLLOWED: -

Above collected pituitaries were fixed in their respective fixative over night. The pituitaries fixed in formal sublimate and Holly fluid were washed for 5 to 6 hours in ranning tap water. The material was then dehydrated, embedded in 56-580c paraffin and then sectioned serially at 6 to 8 .The sections thus obtained were mounted separately and divided into three groups. The first group of sections of each Specimen was obtained with periodic acid Shiff/Orange G Method. The second section of the second group was stained with PAS/OG/MB. Third groups of section was stained with AB/PAS/OGAb paintained atPH 0.3 or0.2.

STAINING PROCEDURE

Periodic acid Sciff/orange GMethod (PAS/OG, Method) :

1) Sections to water.
2) Per Iodic acid-2 minutes.
3) Running tap water -5 minutes.
4) Rinse in distilled water.
5) Sniffs Reagent-15 min. to 20 min. in a coupling jar.
6) Sulphurous acid rinse- 2 min.
7) Sulphurous acid rinse - 2 min.
8) Sulphurous acid rinse - 2 rnin.
9) Running tap water- 5 to 10 min.
10) Carazzi's Haemotoxylin-1 min.
11) Running tap water- 5 to 10 min.
12) Orange G-10 to 20 min.
13) Differentiate in running tap water using microscopical control until acidophil cells stand out clearly (15 to 45 seconds).

(Remove from 90%'quickly otherwise slide distains) or two or three changes of absolute alcohol.

RESULT:

Basophil granules - Magenta red.
A- Ci dophil - Orange.
Other chromophobe cells - unstained.
Nuclei - Dark gray,
Periodic acid schiff/oranqe GV Methyl Blue. (PAS/OG/MB)
(Wilson and Ezrin -1954

1) Bring sections to distilled H20.
2) Oxidise in 0.54% aqeous periodic acid for 5 minutes.
3) Treat with Schiff s reagent for 15 minutes.
4) Wash m running water for 10 minutes.
5) Stain in 1 % aqueous Orange acid for 15 seconds.
6) Mordant in 5% phosphotungstic acid for 15 seconds.

7) Rinse mrannmgtapH20 for 15 second.
8) Stain in 1% aqueous methyl blue for 1 minutes.
9) Rinse briefly in 1 % acetic acid.
10) Dehydrate in alcohal, clear and mount in synthetic medium:

Result:
Beta granules - Magenta.
Delta granules - Purple Red.
Alpha granules - Yellow.

Alcian Blue/Periodic acid Schiff/Oranqe G (AB/PAS/OG) (Marctierlance 1966) Method :

1) Oxidise in mixture of KNno4 and H2S04 for 1 to 2 minutes.
2) Rinse in rimning H20.
3) Bleach in 5% sodium metabisulphite.
4) ' RinsemrurmmgH20(10minutes).
5) Stain in 1 % alcian blue either at ph3 'or ph2 (10-15 minutes)
6) WashinrunningH20 5to 10 minutes or 10 to 20 minutes.
7) Oxidi se in 1 % periodic acid solution.
8) Wash in tap H20 for 10 minutes.
9) Rinse for few second in distilled H20.
10) rnimersein Schiff sreagent 6 -30minutes.
11) Pass through 3 baths of sodium meta bisulphite Hcl.
12) WashmrunrimgH20foratleastT5niinutes.
13) Dehydrate and mount.
14) Stain nuclei by haemotoxylin.
15) Alpha cells pan be differentiated by Orange G or by 1 % Yeilow naphthol.

Result:
Beta
Cells-Purple
Delta Cells - Dark Blue,
Gamma - Brick Red.
Apha - Yellow.,

OBSERVATIONS

Acidophilic (Non-mucoid) Cells :

The Martius - Scarlet - Blue (M.S.B.) and Carmoisinel - Orange G - Light green (Car - OG -LG) staining methods enable to differentiate two types of acidophils in Guinea pig & Girraffe pituitary gland. Type 1 cells are stained yellow (Martiun Yellow positive and OG positive respectively), and Type 2 cell red (Scarlet & Carmoisinel positive respectively) with these methods. Type 1 and Type 2 cells differ not only Historically but also in their morphology and their distribution pattern

Type 1

These cell types are present in great number and are diffusely but evenly distributed in the anterior pituitary in all species studied. They are smaller and characterized by fine granulation. These cells are circular or ovel in outline and cell membrane is distinct. Nuclei are prominent'and they are eccentric or situated at the centre. Cytoplasmic granules are fine, Golgj body were observed only in the Type 1 cell of Bandicota indica.

Type 2

The distrubution pattern of this cell type in individual speices of animals is to some extent differ and characteristic. These cells are found in cluster and distributed evenly in the anterior pituitary gland or guinea pig. Mginaffepituitary several follicles are.

made from these cell types, however the cells can be found singly. These ceils are mainly to be found rostrally. Type 2 ceils were large and can be distinguished by thick granules and irregular in shape. Golgi body were seen frequently adj aeent to the nucleus. In rat and Goat these cells takes PAS & Orange G stain and appears orahge-red colour.

Type 3

These cells are scattered singly or in group at the central part of the anterior pituitary of human. Type 3 cells are varied considerably in shape and thickly granulated, cytoplasm were intensly haemotoxyl in positive. The granules were also reacted with PAS and appear red in PAS/OG preparation. These cells possessed large eccentric nucleus.

Basophils or Mucoid cells :

With M.S.B. Car/OG/LG and PAS/OG stain cytoplasmic granules ofbasophils stained blue, green, red respectively.

Type 4

Type 4 cells are distributed peripherally in the anterior pituitary of ratbandicota. These cells are round or oval in shape in bandicota but angular in rat. They possess central, sometimes hypochromatic nucleus as well as coarse cytoplasmic granules. A distinct Golgi Zone in usually present. Type 4 cells are strongly PAS positive and stain deep blue with alcian blue and aldehyde fuschin after AB/PAS/OG and AF/OG/LG staining techniques.

Type 5 & 6

In PAS/OG and AB/PAS/OG preparation Historical differentiation between type 5 & type 6 is not possible as both are stained by PAS. In rat Type 5 cell possessed coarse granulation while in Type 6 cell granulation is homogeneous, fine and more uniformly dispersed. Type 5 is peripherally distributed and type 6 centrally iocated. With the PAS/ HB/ OG method these two types of gonadotrops can be differentiated in rat, and goat. Type 5 cells stained with PAS & Type 6 stained both'with PAS and methy blue and therefore it appear violet.

DISCUSSION

The precise assignment of specific hormone production to various cell types is' not possible in this report. However, response of given ceil type to a particular staining technique in commonly related to the function of that cell type (Foster and Cameron 1965?Purves, 1965, Conclin 1966 & 68, Pooly 1971). Designation to various cell types in this report however must be considered to be tentative. In the present study two types of acidophils and four types of basophils have been found in some mammalian specie studied. Two types of acidophils can be differentiated in giraffe and guinenpigby N.S.B. and Car/CG/LG staining techniques. Patil (1974 & 75) described H.S.B. technique as a specific stain for STH & LTH cells. Using Car/OG/LG method it has been shown in number of mammalian species that STH cells stained orange, while LTH cell stained red (Brookes, 1963, N. Etreb and Gunzel 1974). Type 2 cell seemed to have affinity for PAS. The morphological characteristics and the staining response of Type 1 and type 2 cells was similar to STH and LTH described for human (Conclin 1966), rat (Purves 1966 andPooly 1971), bat (Patii 1974), Viscacha (Patil" 1976)..

The morphological characteristics and response of basophil e (mucoid) cells to the various staining procedure, indicated that the cell type 4,5 and 6 could be producer of TSH, FSH & ICSH respectively.

In almost all mammalian species as far studied (except hamster -Serber, 1958) the cell which stained with AF and alcian blue is generally considered to be thyrotrophs (rat - purves 1966, Vols - Gierke and Forsyth, 1964, Palm squirrel - Dhaliwal and Prasad 1965, Sheep - Mikami and Daimon 1968, bats and Viscacha Patil 1974-75). Purves (1966) believes that in addition to TSH cell one or more of gonadotrophe may also stain under some circumstances. My observations on the above mentioned mammalian species indicate's that type 4 cells specifically stained with AF and alcian blue, it is therefore likely that the type 4 cells maybe thyrotrophs.

The other mucoid cell types can be distinguished by PAS/MB/OG technique. One type of cells stained purple (PAS/and MB positive) and the other stained red. Wilson and Esrin (1954) and Ren els (1957) and subsequent workers using PAS/MB/ OG technique differentiated two types of gonadotrophs. The purple, gonadotrophs were considered FSH and the red cells as LH producing cells. Dhaliwal and Prasad (1965) did not notified any tinctorial differentiation of gonadotrophs of the squirrel pituitary by this staining technique. In mouse, rabbit, monkey, hamster and guinea pig, it was difficult ot differentiate between two types of gonadotrophs. Nakane (1977) showed that FSH and LH hormones were frequently found in the same cell. This suggested that, gonadotrophs should not be identified as FSH and ICSH cells by their histochemical characteristics. A further evaluation of the classification by immunohistochernical technique is worthy for consideration. A functional classification of gonadotrophs can be confirmed on the basis of solubility of secretary granules and the result ofhormone extraction, this study have still to be carried out.

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