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Gel Electrophoretic Separation of Pineal Gland Proteins of the Iropical Rodent *Funambulus pennantii*

Sweta Arora, Ratna Sarkar and Chandana Haldar*

Pineal Research Laboratory, Centre of Advanced Study in Zoology, Banaras Hindu University, Varanasi - 221005, Uttar Pradesh, India; chaldar2001@yahoo.com

Abstract

The investigation of pineal-specific proteins is not new but offers scope for identification of antigonadotropic compound(s). There is difference in the activities of the reproductively active and inactive phase pineal homogenates of seasonally breeding animals, e.g., squirrels. The present study aimed at checking the squirrel pineal proteins adopting gel-electrophoresis technique. Homogenate of the reproductively quiescent phase pineal homogenate separated into 14 fractions whereas that of reproductively active phase pineal presented 17 protein fractions (3 additional fractions). It is assumed that these three protein bands (which were not noted for the squirrel in reproductively inactive phase) are responsible for the antigonadal/ antigonadotropic effect of the pineal gland. The present study, though very preliminary in nature, has brought out the difference in the pattern of proteins of two different phases of the pineal gland- reproductively active and quiescent. The data throw open scope for extensive biochemical studies to decipher the physical and chemical nature and the properties of an anti-gonadotropic protein.

Keywords: Electrophoretic Separation, Pineal, Proteins

1. Introduction

The investigation of pineal-specific proteins is not new[1] but has opened a recent area of newer pineal research for antigonadotropic compounds. It was demonstrated that bovine pineal gland contains a serologically specific, heat-labile and alcohol-insoluble protein, which was obtained from gel electrophoresis of supernatant or water-soluble fractions from pineal homogenates. Seventeen protein fractions (stained with Amido black B in 7.5% acetic acid) have been observed when supernatant of pineal homogenate (1-1.5 mg tissue in 0.25 M sucrose) of 12 week old female rat was electrophoresed on polyacralamide gel[2]. Separation of pineal proteins has been reported by other investigators but without evidences for pineal specificity[3,4].

Biologically active pineal peptides and proteins, not yet shown to be specific or peculiar to pineal tissue or having distinctive activities, are very imperfectly known chemically. In mammalian pineal gland the presence of/ production of active principle(s) (proteic or peptidergic substances) have been widely investigated, but in the absence of data on the chemical structure and a possible hormonal role of the specific protein, the' pinealin hypothesis" was proposed5. It was suggested that the precursor(s) of the protein secretion(s) stored in Dense-Cored Vesicles (DCV) is synthesized by ribosomes, and segregated into the cisternal space of rough endoplasmic reticulum from where it migrates towards the Golgi apparatus.

Recently, diurnal patterns in rat pineal protein synthesis and secretion were assessed by incubating individual pineal glands with (^{35}S)-metheonine and analyzing the secreted (medium) and cellular (tissue) proteins by one- and two-dimensional polyacrylamide gel electrophoresis6. It was suggested that pineal protein synthesis and secretion is subject to the influence of photoperiod and has been implicated with melatonin as a mediator for this action.

In our laboratory, we have evaluated the pineal activity of the squirrel Funambulus pennantii by determining the protein content along with the other biochemical con-

*Author for correspondence

stituents during both the phases (active and inactive) of reproductive cycle[7]. However, we were unable to detect the type of proteins, their molecular weights and amino acid sequences at terminals because of the lack of specific markers and technical problems. On the other hand, it was indeed possible for us to separate them electrophoretically.

2. Materials and Methods

The method followed in the present study was adopted essentially from Laemmli[8] with slight modifications i.e., Sodium Dodecyl Sulphate (SDS) PAGE electrophoresis when (3-mercaptol was not used in sample buffer or in tank buffer for the separation of pineal proteins. The following tables present the details of preparation of solution, gel, buffer, stain, etc.

Table 1. Stock solutions

S No	Stock	Conc.	W/Vol.	Final vol.
	HCl	IN	22.75 mL	250 mL
	NaOH	IN	10 g	250 mL
	Acr:Bis	30:08	30+0.8 g	100 mL
	Agarose	0.8	0.8 g	100 mL
	Tris-HCl pH 8.6	1.5 M	18.1629 g	100 mL
	Tris-HCl pH 6.8	0.5 M	6.052 g	100 mL

Table 2. Separating gel (For 15 mL)

S No	Reagent	Stock conc.	Final conc.	Final vol.
	Acr. Bis	30%	8%	4 mL
	Tris-HCl pH 8.6	1.5 M	0.375 M	3.75 mL
	APs	10%	0.025%	30 |iL
	TEMED	-	0.025	15 |iL
	Distilled water	-	-	7.2 mL

Table 3. Stacking gel (5 mL)

S No	Reagent	Stock conc.	Final conc.	Final vol.
	Acr. Bis	30%	4%	0.66 mL
	Tris-HCl pH 6.8	0.5 M	0.125 M	1.25 mL
	APs	10%	0.025%	20 |iL
	TEMED	-	0.025%	10 |iL
	Distilled water	-	-	3.06 mL

Table 4. Sample buffer (5 mL)

S No	Reagent	Stock conc.	Final vol.
	Tris-HCl pH 8.6	0.625 M	1.25 mL
	Glycerol	10%	1 mL
	Bromophenol blue	0.01%	0.02 mL
	Distilled water	-	2.75 mL

Table 5. Electrophoretic buffer (500 ml)

S No.	Reagent	Stock conc.	Final vol.
	Tris-HCl pH 8.6	1.5M	33.33 mL
	Glycine	0.1M	3.75 g
	Distilled water		4.66.67 mL

Table 6. Staining solution (200 mL)

S No	Reagent	Stock conc.	Final vol.
	Coomassie blue	0.2%	0.4 g
	Methanol	50%	100 mL
	Acetic acid	10%	20 mL
	Distilled water	-	80 mL

Table 7. Destaining solution I (200 mL)

S No	Reagent	Stock conc.	Final vol.
	Methanol	10%	20 mL
	Acetic acid	10%	20 mL
	Distilled Water	-	80 mL

Table 8. Destaining solution II (200 mL)

S No	Reagent	Stock conc.	Final vol.
	Methanol	10%	20 mL
	Acetic acid	10%	20 mL
	Distilled Water	-	160 mL

The adult male squirrels (100-120 g body weight) were obtained during the month of October (reproductively active phase animal) and April (reproductively quiescent phase) for the electrophoretic study. The animals were sacrificed by decapitation under anesthesia and the pineal gland was quickly removed. The tissues were weighed and 0.9% NaCl (in chilled condition) was added in a ratio of 15 pL/4mg tissue. The tissue was homogenized for 1 minute in a specially designed micro-glass homogenizer and centrifuged at 2°C and 10,000 rpm for 15 minutes. The clear supernatant was transferred onto

a chilled test tube and mixed with an equal volume of the sample buffer containing 0.0625 M Tris-HCl (pH 8.6),10% glycerol and bromophenol blue as the dye.

Figure 1. Photograph showing the electropherogram of pineal protein fractions of *F. pennantii* during active phase of pineal gland. Note 17 bands.

3.1 Polyacrylamide Gel Electrophoresis

Polyacrylamide gel was prepared to a final concentration of 0.375 M Tris HCl (pH 8.6):0.025% APS and 0.025% TEMED for separating gel and 0.125 M Tris HCl (pH 6.8): 0.025% APS and 0.025% TEMED for stacking gel.

Electrophoresis was performed on 8% slab gel (130 x 100 x 3 mm) at 20 mA and 4°C for 5 hours. After electrophoretic run, gels were stained overnight at room temperature in 0.25% Coomassie brilliant blue (50% methanol, 10% acetic acid), and destained following two changes in Destain I and Destain II. Finally, the gels were photographed.

3. Result

The bands obtained after the gel electrophoresis of homogenates of reproductively active and inactive phase pineal glands are presented in Figures 1 and 2. The inactive phase pineal homogenate (April), showed up 14 protein fractions (Figure 2) while the active phase pineal (November) homogenate presented 17 protein fractions (Figure 1).

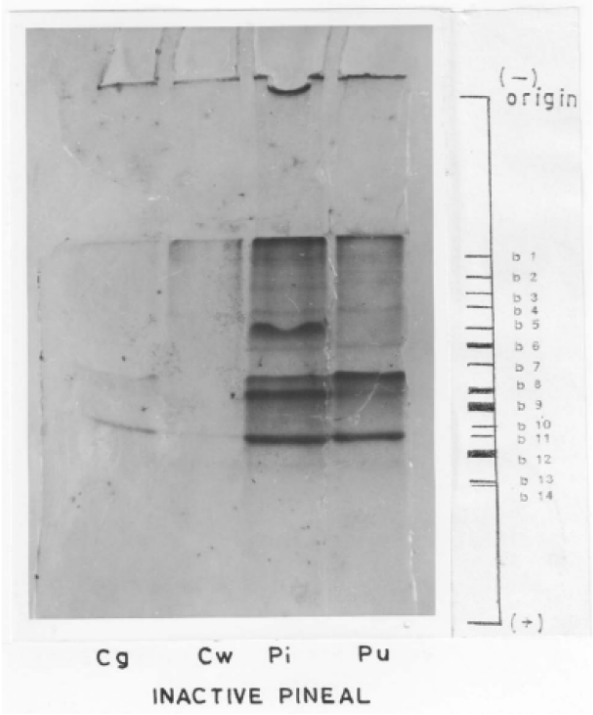

Figure 2. Photograph showing the electropherogram of pineal protein fractions of *F. pennantii* during inactive phase of pineal gland. Note only 14 bands.

4. Discussion

From a morphological point of view[9] it appears difficult to accept the premise that the mammalian pineal gland is capable of synthesizing and releasing such a large number of peptides/proteins[10,11]. Some authors, therefore, proposed that the pineal may be able to sequester and concentrate circulating hormones that are produced elsewhere. Although this idea was disputed by some authors who consider the pineal to be a supplemental source of hypothalamic hormones[12], it has been shown that a number of peptides present in the pineal are indeed produced outside this organ.

Our studies on estimation of pineal protein content (spectrophotometrically) provided results which suggested that the pineal protein content responds to different experimental conditions, especially photoperiod[7]. We specifically noted that the change in the pineal protein content during reproductively active or inactive phase of the pineal gland presented an inverse relationship with gonadal activity. A question arises as to what is the specific nature of the protein which changes during these two activity phases of pineal. As stated in the introduction, due to lack of specific markers and technical (instrumentation) problems, we were not able to decipher the amino acid content/sequence, molecular weight, etc., but at least we could present the specific gel electrophoretic changes in the fractions or pattern. The result clearly indicates that there is appearance of three specific fractions during the reproductively active phase pineal which may be responsible for pineal antigonadotropic activity. Our study, though of a preliminary nature, is able to clearly bring out a difference in the pattern of proteins of pineal of two different reproductively activity phases.

Till date, quite a few investigations have been carried out by biochemists in search of a simple protein/peptide antigonadotropin. The occurrence of Arginine Vasopressin and Oxytocin, as determined by radioimmunoassay by Dogterom et al.[13,14], and the large number of reports have substantiated the presence of neurohypophyseal hormone-like peptides in mammalian pineal glands[15–17].

It appears that the search for a proper pineal protein/peptide effector compound is still incomplete as some time the reports are without indication of a specific biological activity. It could be that isolated pineal protein/peptide effector compound, when injected into the animal, loses its biological activity due to blood proteases whereupon a protector or pro-pre-hormone-like nature is prerequisite for the expression of activity. This study opens up great lot of interest and newer avenues for research to find a role for pineal in connection with the antigonadotropic hormone/substance.

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