Morphological and cytochemical characterization of cell types of the adenohypophysis of Manjuba, Anchoviella lepidentostole (Fowler, 1911) (Osteichthyes, Engraulidae).

INTRODUCTION

The Anchoviella lepidentostole, commonly known as manjuba, is a bone anadromous fish which inhabits temperate and hot waters, and has a vast geographical distribution from Guianas to Parana State/Brazil. It is specially abundant in the coast of Sao Paulo State (Lopes et al., 1984). Among the engraulidaes, manjuba is the species of greatest economical importance in the south-east of Brazil (Figueiredo; Menezes, 1978; Suzuki, 1983).

The capture of manjuba in the Ribeira do Iguape River from its estuary area and along its course, occurs from October to April. According to Bendazoli; Froshi (1990) the fishing production nowadays reaches 500 tons annually. However, this study also shows that there has been a clear fall in the manjuba fishing in the latter years owing to predatory capture in the estuary region, thus preventing the animals from reaching the areas where spawning takes place.

The evident economical value of A. lepidentostole, as well as the imminent extinction risk have led to a great number of researches. Some of them aimed systematic reviews (Ihering, 1930; Carvalho, 1950; Figueiredo; Menezes, 1978) while others are related with the ichthyological, nutritional and fishing aspects (Furuya, 1959; Nomura, 1962; Nomura, 1964; Mandelli; Giamas et al., 1984; Paiva Filho et al., 1986; Bendazoli; Rossi-Wongstchoski, 1990). Nevertheless, there are few studies which dealt with the gonadal characterization and reproductive cycle of this species (Giamas et al., 1983; Lopes et al., 1984; Giamas et al., 1990), and no research on other endocrine features of the manjuba.

As cytochemical methods have been largely used for the characterization of cells from the adenohypophysis in Osteichthyes under normal or experimental conditions (Oliveirau, 1976; Burns, 1991), our goal was to characterize morphologically and cytochemically these cell types in manjuba. By doing so, we would like to contribute to future investigations which relate endocrinology of A. lepidentostole with its reproductive cycle. This knowledge and a more adequate fishing technology will allow the survival and reproduction of the manjuba, either in nature or in captivity.

MATERIAL AND METHODS

The pituitary glands used in this study were taken from 40 adult manjubas—Anchoviella lepidentostole specimens—of both sexes, captured along the whole Ribeira de Iguape River in Sao Paulo State/Brazil. A special device named “manjubeira” net was used.

The specimens were sacrificed by asphyxia, decapitated and fixed in Bouin’s liquid for 24 hours at room temperature. Then they were decalcified for 15 days in EDTA solution neutralized at 10%, which was daily changed. Afterwards, they were washed in tap water for 24 hours and processed for histological paraffin embedding.
Seven μm serial cephalic sections, oriented in a sagittal and frontal manner, allowed localization of the pituitary gland. Sections which contained hypophysial areas for morphological analysis were stained with haematoxylin-eosin (HE) and with Mac Conaill’s lead haematoxylin (HPb) (Mac Conail57, 1947), Halmi’s Trichromic (HT) (Behmeret al., 1976) and Mallory’s Trichromic (MT) (Mallory28, 1942). For the cytochemical study the Periodic Acid of Schiff (PAS) (Mac Manus11, 1946) Alcian Blue pH 2.5 (AB) (Steedman53, 1950), and Alcian Blue pH 0.5 (Lev; Spicer24, 1964) methods were used.

In an attempt to have a better control and more details on the polysaccharides cytochemistry, the following methods were used: acetylation+PAS (Mc Manus; Cason12, 1950) and acetylation+saponification+PAS (Mc Manus; Cason12, 1950). PAS after salivary amilase treatment (Lison56, 1960), metilation+AB pH 2.5 (Terner; Lev63, 1963) metilation and saponification+AB pH 2.5 (Terner; Lev63, 1963), and acid hydrolysis followed by AB pH 2.5 (Quintarelli et al.46, 1961).

RESULTS

In the manjuba the average diameter of the pituitary gland is about 1 mm, and it is placed in the sella turcica, found rostrally to the saccus vasculosus and caudally to the palate muscle (Fig. 1).

The pituitary gland is divided in adenohypophys and neurohypophys which branches largely as it invades the adenohypophysis in a way that enters all regions.

The adenohypophysis is formed by two regions: the pars distalis (PDi divided in pars distalis rostralis (PDR) and in pars distalis proximalis (PDP), and the pars intermedia (PI) (Fig. 1).

PARS DISTALIS ROSTRALIS: The PDR is the most developed adenohypophysary region. It is invaded by large neurohypophysary branches and is organized in follicles which contain stainable material in the lumen. These follicles have irregular shape and are ventrally elongated in the middle of the palate muscles, and turn thinner as they advance farther from the gland.

There are four cell types in the stratified epithelium, according to morphology and stain affinity to the applied methods.

The I-PDR cell type covers the lumen of the follicles (Fig. 2, Fig. 3). They are pavementous cells with elongated nucleus and loose chromatin. The cytoplasm is poor and basophilic. These cells do not show positive reaction for any cytochemical applied method.

The second cell type, II-PDR, shows a palisade arrangement and is characterized by having one of its faces turned to the neurohypophysis. They are polyedric, big cells with rounded nucleus, frequently eccentric and with loose chromatin. The cytoplasm is acidophilic when stained by HE and stains in black by HPb method (Fig. 1, Fig. 2).

The III-PDR cell type is scattered among the other follicular cell types. They are spindle shaped, the nucleus is rounded, usually central and the chromatin is loose. The cytoplasm shows thin granules which are stained by AB pH 2.5 (Fig. 3), by aldehyde-fuccsin of the Halmi’s trichromatic method, and by PAS. The polysaccharides analysis showed that this cell type is positive for the following methods: PAS after salivary amilase reaction, acetylation followed by saponification+PAS and finally by metilation followed by saponification+AB pH 2.5.

The fourth cell type from PDR, IV-PDR, seems to be more abundant. It is characterized by an elongated shape, round and big nucleus which is generally central, and with loose chromatin. The cytoplasm is acidophilic when stained by MT and orange by the HT. These cells are chromophobes when submitted to the other applied methods (Fig. 2).

PARS DISTALIS PROXIMALIS: In this region two cell types can be distinguished according to morphology, staining affinity and topography.

The first cell type, I-PDP, prevails in the lateral and ventral portions of the PDP (Fig. 4) and it is represented by globular, big cells with rounded and generally eccentric nucleus. The chromatin is loose and the nucleolus is evident. The cytoplasm has rough granules which are stained by aldehyde-fuccsin of the Halmi’s trichromatic method (Fig. 4), and by blue in Mallory’s trichromatic. The I-PDP cells are still positive for AB pH 2.5 (Fig. 3). PAS (Fig. 5), PAS after salivary amilase action, acetylation followed by saponification+PAS and finally by metilation followed by saponification+AB pH 2.5.

The second cell type of this hypophysary region is distributed mainly in the areas near the PI, are arranged in cord-like structures which surround the neurohypophysary branches, and are still scatterly present among the I-PDP cells. They are prismatic or pyramidal with oval or round nucleus, generally eccentric. The chromatin is granular. The cytoplasm has delicate granules which are clearly acidophilic by HE and MT methods, and there is no positive reaction for the other applied methods (Fig. 5).

PARS INTERMEDIA: This hypophysary region also has cell types which differ in staining affinity with the HPb method.

The I-PI cells are polyedric, the nucleus is oval or rounded and eccentric. The chromatin is loose and the nucleus is conspicuous. The cytoplasm has thin granules which are slightly positive for HPb (Fig. 6). In this cell population there can still appear cells with nucleus and cytoplasm clearly enlarged. They are scatterly distributed in the PI.

The second cell type is represented by II-PI type cells which appear less than the I-PI type and show a polyedrical shape, oval nucleus and are characterized by cytoplasmatic chromophobia (Fig. 6).

Mitosis figures between the two cell types can still be found in the PI (Fig. 6).

**Figure 1**
Anchoviella lepidentostole. Pituitary gland. Sella turcica (ST), Saccus vasculosus (SV), diencephalon (D), Pars Distalis Proximais (PDP), Pars Distalis Rostralis (Dark arrow), where the II-PDR cells are HPB+, Pars Intermedia (clear arrow) and the neurohypophysis (N). 130 x Hpb.

**Figure 2**
Anchoviella lepidentostole. Pituitary gland. Pars Distalis Rostralis (PDR). I-PDR cells (arrowhead), II-PDR cells (dark arrow) and IV-PDR cells (clear arrow). Follicular lumem (L) 800x. Hpb.

**Figure 3**
Anchoviella lepidentostole. Pituitary gland. Pars Distalis Rostralis (PDR) and Pars Distalis Proximais (PDP). I-PDR cells (arrowhead), II-PDR cells (long arrow) and I-PDP cells (star) 800x Alcian Blue pH2.5.

**Figure 4**
Figure 5
Anchoviella lepidentostole. Pituitary gland. Pars Distalis Proximalis (PDP) I-PDP cells (star) PAS positive; II-PDP cells (white arrow). 800X PAS.

DISCUSSION

Generally the fish neurohypophysis is largely related to the adenohypophysis (Olivereau, 1967). In Poecilia latipinna (Olivereau; Ball, 1964) the neurohypophysis penetrates the hypophysary region, specially the pars distalis as is seen in the manjuba. In many teleosts the adenohypophysis is formed in compact cell strings, but in more primitive species as Anguilla anguilla, the pars distalis rostralis is arranged in follicles with a stainable material in the lumen (Olivereau, 1967), as is observed in the PDR of the manjuba. However, in the A. lepidentostole we believe there is only one large follicle in the PDR, with an extremely irregular outline which in the histological sections gives the idea of many follicles. This possibility, however, has not been considered in the literature up to now.

In the PDR of the manjuba there is an elongated ventral follicle in the middle of the palate muscle, becoming gradually thinner as it goes farther from the pituitary gland. Olsson (1971) comments the existence of a primitive connection from the oral cavity with the rostral region of the pituitary gland in primitive fish. Likely, our observations for A. lepidentostole suggest the persistence of Ratke's pouch cavity, or of the cystic rest of the orophypophysary duct.

The PDP of A. lepidentostole shows its cells arranged in strings intermingled by smooth neurohyophyseal branches, as was described for Pimelodus maculatus (Fenerich, 1975) and for Prochilodus scrofa (Borella, 1987). The literature usually refers to the anatomical relation of the neurohypophysis with the pars intermedia as in the Hippocampus in which the neurohypophysis involves all the faces of the pars intermedia (Da Lage, 1958) or in the Prochilodus scrofa in which the PI contains a great number of thin, terminal branches from the neurohypophysis (Borella, 1987). In the manjuba this anatomical relation is also observed, but differs clearly from the above species since the PI involves the neurohyophyseal main branch.

In Ostichthytes the pars distalis rostralis is formed by many cell types characterized according to the cytochemical reaction. In the A. Lepidentostole four cell types were characterized: I-PDR, II-PDR, III-PDR, IV-PDR.

The I-PDR cells show negative reaction to the applied cytochemical methods. It is believed that these cells are responsible for the colloid production which occupies the follicular lumen. However, there is no evidence up to now about these cells, as well as about the nature and functions of this secretion.

The II-PDR prismatic cells in a palisade arrangement show one of their faces turned to the neurohypophysis, as it is usually described for one cell type in the pars distalis rostralis in ostechytes (Fenerich, 1975; Srivastava; Swarup, 1980; Rubal et al., 1984; Siegmund et al., 1987). In the manjuba, these cells were positive for HPb and this was also observed in the PDR cells from other species as: Chanua marulius (Srivastava; Swarup, 1980) and Plecostomus albopunctatus (Rubal et al., 1984), but in Carassius auratus (Kaul; Vollrath, 1974) and Rutulus rutulus (Jafri; Ensor, 1980) this cell type is chromophobe for HPb. According to Olivereau (1967) and Nagahama (1973) the staining affinity of these cells to HPb depends on their functional state, as verified by the fall of the HPb positivity of these cells in experiments with metapirone, which stimulates the secretion of ACTH.

Cambré et al. (1986) described cells which were similar in morphology to the II-PDR cell in D. labrax, and had the same spatial relation to the neurohypophysis and immunoreactivity to anti-ACTH. Thus, we believe that in manjuba the II-PDR cells are possibly corticotrophin secreting cells.

In manjuba the III-PDR cells, which are spindled and scatterly distributed among the other cell types, are positive for PAS, AB pH
2.5 and aldehyde-fuchsins of Halmi’s trichromic. Different authors who used immunohistochemical methods identified tireotropic cells in the PDR (Ueda et al. 1983; Batten 1986; Cambre et al. 1986; Sigmund et al. 1987; Toubeau et al. 1991) that have morphological and staining characteristics which correspond to the III-PDR cell. So, we discuss that this cell type, in *A. lepidentostole*, may possibly secrete the tireotropic hormone.

The IV-PDR cells, in acidophilic and apparently they outnumber the others in the PDR in manjuba. Schreibman et al. (1973), Srivastava; Swarup (1980), Zagha; Valsella et al. (1985), analysed specimens in different physiological conditions and verified that the acidophilic cell type and most abundant one in the PDR in these fish was the prolactin secreting cell. Batten (1986) and Toubeau et al. (1991) used immunohistochemical methods in *Poecilia latipinna* and *Barbus barbus*, respectively, and also verified that the most abundant cell type in the PDR was immunoreactive to anti-ACTH. By the comparison of the results from the literature with those made for *A. lepidentostole* we can suggest the possible secreting role for IV-PDR cells.

Two cell types were observed in the PDP in manjuba: I-PDP and II-PDP cells. The same staining affinity reported for I-PDP cells was shown for similar cells in other osteichthyes species, and they were designated as gonadotropics by the following authors: Aoki; Uemura (1970 [Oryzias latipes]), Chiba; Honma (1974 [Fugu menticetus]), and Srivastava; Swarup (1980 [Chana marilandus]).

In *A. lepidentostole* we found the same staining affinity in I-PDP and III-PDP cells for PAS, AB pH2.5 and aldehyde-fusicn of Halmi’s trichromic methods, probably because both produce glycoprotein hormones. The two cell types, however, are different in their morphology and location, and this makes the classification easier. Many authors pointed this phenomenon in different bone fish species (Oliveareau et al. 1984; Ueda et al. 1983; Borella 1987) for gonadotropic and tireotropic cells. So, according to what has been just shown and to the immunocytochemical identification of gonadotropics cells (Oliveareau et al. 1988; Borella et al. 1990) similar to the I-PDP of the manjuba, we suggest a possible gonadotropin secreting role for I-PDP cells.

The II-PDP cells of the *A. lepidentostole* are characterised by the prismatic or pyramidal shape, cytoplasmatic acidophily when stained by HE and MT and by the chromophoby to the other cytochemical applied methods. Cells like that have been pointed by other studies as one of the PDP cell types and have been considered to be somatotropinsecreting cells after cytochemical and immunocytochemical analyses (Sage; Bern 1971; McKeown; Van Overbeke 1971; Jafri; Ensor 1980; Nagahama et al. 1981; Wagner; McKeown 1983). Then, we suppose that this cell type is the responsible for the somatotropin in the manjuba.

Many studies have pointed the presence of two cell types in the pars intermedia in osteichthyes, according to the staining affinity of these cells with Mac Conail’s haematoxylinein and to PAS (Oliveareau; Ball 1964; Leatherland 1970). However, these cells show different reactions for these two methods depending on the species studied.

In the manjuba there also were two cell types characterised: I-PI cells, positive for HPb, and II-PI cells, negative to HPb and PAS. In salmonines, similar results were obtained (Holmes; Ball 1974). Unlikely, in the *Prochilodus scrofa* PAS positive cells and chromophobes to HPb and PAS were also found (Borella 1987). The application of anti-B MSH in immunocytochemistry has revealed HPb+ cells in the pars intermedia as the metatropin-secreting ones in chondrichthyes (Pelissero et al. 1988) and osteichthyes (Cambre et al. 1986). Thus, our results suggest that I-PI cells are the melanotropic-secreting cells.

Studies on other bone fish have propose different roles for the second cell type of the pars intermedia. So, Wendelaar-Bonga et al. (1984) consider these cells as hypercalcemic in *Carassius auratus*, while Margolis-Kazana et al. (1981) and Batten (1986), studying respectively *Xiphophorus maculatus* and *Poecilia latimina* suggest the possible gonadotropic role for this cell type.

Nevertheless, Rand-Weaver et al. (1991) used a somatolactin anti-hormone in various species of fish and observed that the second cell type in the PI described as Hb positive, PAS positive, or chromophage, depending on the species, is immunoreactive for the anti-hormone. The somatolactin possibly takes part in the reproductive process (Planas et al. 1992). So, once we have identified two cellular types in the PI of manjuba, and considering that the first one has the same profile as the MSH secretory cells present in others species of fish, we can propose that the II-PI cell type has a somatolactin-secreting role in the manjuba, such as the cells described for Rand-Weaver et al. (1991). However, more studies are necessary to clarify this question.

**RESUMO**

A hipófise de *Anchoviella lepidentostole* apresenta-se dividida em neuro-hipófise e adeno-hipófise, sendo que a caracterização morfológica e citocitomica dos tipos celulares desta região foi a proposta deste trabalho. A adeno-hipófise divide-se em pars intermedia (PI) e pars distalis (PD), sendo que esta ultima se divide em pars distalis rostral (PDR) e pars distalis proximalis (PDP). As células da PDR organizam-se em folículos. No epitélio follicular foram caracterizados quatro tipos celulares: I-PDR (basofílo), II-PDR (positivo à hematoxilina-chumbo/HPb+), III-PDR (PAS+, AB pH2,5± e AF+), e IV-PDR (acidófilas). A PDP possui dois tipos celulares: I-PDP (PAS+, AB pH2,5± e AF+) e II-PDP (acidófilas). Na PI também foram caracterizados dois tipos celulares: I-PI (HPb+) e II-PI (cromofobo aos métodos empregados).

**UNITERMOS:** Pituitária anterior; Osteíctes; Citoquímica; *Anchoviella lepidentostole*


