HISTOPHYSIOLOGICAL ASPECTS OF THE INTERRENAL GLAND OF THE PACU FEMALE, *Piaractus mesopotamicus* (TELEOSTEI, CYPRINIFORMES)

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ABSTRACT

This paper reports the morphology and microscopical structure of interrenal gland of females of ***Piaractus mesopotamicus*** during the reproductive cycle and injected with Luteinizing Hormone Releasing Hormone analog (LHRHa-DAla[6]).Based on the developmental stage of the ovary,: the fishes were grouped into the following categories: resting, early maturation, advanced maturation, and regression. In all pacu females the interrenal gland lies within the hematopoietic head kidney, forming a collar of cells around the branches of postcardinal veins. Chromaffin cells are interspersed with the interrenal cells. There was not apparent hypertrophy or hyperplasy in interrenal cells of the animals studied. The interrenal cells showed more cytoplasmic vacuolization during the resting stage, more cytoplasmic acydophylia in the advanced maturation stage and higher cytoplasmic vacuolization during induced ovulation, indicating that the injection of LHRHa may promote significant changes in the cytoplasm of interrenal cells.

Key words: pacu, ***Piaractus mesopotamicus***, Teleostei, interrenal gland.

RESUMO

Aspectos histofisiológicos da glândula interrenal de fêmeas de pacu, ***Piaractus mesopotamicus*** *(Teleostei, Cypriniformes)*

A morfologia e estrutura microscópica da glândula interrenal de fêmeas de pacu ***Piaractus mesopotamicus*** foram analisadas durante o ciclo reprodutivo e sob experimentação de desova induzida com o análago do Hormônio Liberador do Hormônio Luteinizante (LHRHa-DAla^6). Os exemplares coletados no decorrer do ciclo gonadal foram agrupados de acordo com o estádio de desenvolvimento ovariano em repouso, maturação inicial, maturação avançada e regressão. O tecido interrenal encontra-se no interior do rim cefálico, em meio ao tecido homocitopoético, formando cordões celulares ao redor dos ramos principais das veias cardinais posteriores.As células cromafins distribuem-se em pequenos grupos de 2 a 5 células entre as células interrenais. Não foram observadas hipertrofia e/ou hiperplasia do tecido interrenal durante os diferentes estádios gonadais, nem após a indução. As modificaçõpes visualizadas foram maior vacuolização e menor acidofila citoplasmática no estádio de repouso, e menor vacuolização e maior acidofila citoplasmática na maturação avançada. Nas fêmeas induzidas à desova, foi observada uma intensa vacuolização nas células interrenais, o que sugere que o LHRHa pode promover alterações citoplasmáticas nestas células.

Palavras-chave: pacu, ***Piaractus mesopotamicus,*** Teleostei, glândula interrenal

INTRODUCTION

The structure and distribution of interrenal gland and chromaffin cells have been established in a number of teleosts and it has been suggested that the interrenal gland is homologous with the mammalian adrenal cortex and the chromaffin cells are homologous with the mammalian medulla. In Teleostei, the homologue of the adrenal gland (cortex and medulla) mainly resides in the head kidney. The cephalic part of head kidney shows hematopoietic activity and is not arranged as a glandular compact mass. It is composed of interrenal steroidogenic cells and chromaffin adrenergic cells grouped in islets, cords, or strands and sometimes intermingled with each other (Chester Jones and Phillips, 1986).

The histology of the interrenal cell conforms to the tetrapod pattern, though with less display of lipid droplet. The ultrastructure is very similar to that occuring in a typical cell of eutherian zona fascicu-

lata (Chester Jones and Phillips, 1986). The morphology of interrenal cells of fish has been shown to change in response to hormones, drugs, stress or salinity changes (Matty, 1985). In salmonid species, e.g., during the smoltification, some scientists reported hypertrophy of interrenal gland and increased plasma corticosteroid levels (Young, 1986). In the migration of fishes to spawn, which is accompanied by considerable motor activity, there is also hypertrophy of interrenal tissue and intensified secretion of corticosteroids into the blood stream (Chester Jones and Phillips, 1986; McBride *et al.*, 1986).

A significant number of researches about the distribution and structure of interrenal gland were conducted between 1960-1970 (Nandi, 1962, 1965; Banerji, 1971).The morphologic studies in a variety of teleosts have revealed enormous diversity in the distribution and organization of interrenal and chromaffin cells. A diversity of morphologic types is frequently observed among species from a single family or genus, although other families and even some higher taxonomic groups appear to be homogeneous with respect to the morphology of the entire kidney. The interrenal morphology has been particularly well studied in the family Cyprinidae. However, for South American fishes there is only one report about the interrenal gland of Prochilodus scrofa (Farias *et al.*, 1989).

Cortisol is a metabolic hormone in fishes. It provides energy by increasing peripheral proteolysis, fat release and utilization, and hepatic gluconeogenesis, thereby playing a significant role in the spawning migration and during stress (Sundararaj *et al.*, 1982). In addition cortisol may promote stimulatory effect on the gonadotropin-stimulated secretion of 17-20-dihydroxiprogesterone from mature ovarian follicles, suggesting that the teleost interrenal gland is involved in the oocyte maturation process (Jalabert, 1976; Jalabert and Fostier, 1984).

The pacu, ***Piaractus mesopotamicus***, a South American fish of great economic importance whose natural habitat is the rivers of Central Brazil, has been introduced into cultivation over the last one and a half decades..The pacu reproduces annually after migrating upstream in the native rivers. When held captive in pond culture, reproduction takes place only after hormonal induction (Cury and Tsukamoto, 1988). In the last years, the pacu has received considerable attention from scientists, but there are few data about the relationship between the endocrine glands and the reproduction process. This information may lead to a great improvement on its culture.

The objective of this study was to determine the distribution and microscopical structure of interrenal gland of pacu females under two conditions: (1) during the reproductive cycle and (2) after induced ovulation by a luteinizing hormone releasing hormone analog (LHRHa-DAla^6).

MATERIAL AND METHODS

Animals

Pacu females, ***Piaractus mesopotamicus***, (n=24), reared in captivity were captured at the Center of Research and Training in Aquaculture (CEPTA), in Pirassununga, SP, Brazil (subtropical zone, 22°02'S, 47°0'W). The fishes collected during the reproductive cycle were grouped based on the development stage of the ovary into the following categories: resting, (April-June), early maturation (July-September), advanced maturation (October-December), and regression (January-March) (Lima *et al.*, 1991).The fish were sacrificed and the gonads and head kidneys containing interrenal tissue were excised. Pacu females (n=6) in advanced maturation stage were used for the study of interrenal gland during induced ovulation. After capture, the females were weighed and injected with Gly10[D-Ala^6] LH-RH-ethylamide (Syndell Laboratories, Vancouver, Canada) at a dosage of 10 μg/Kg weight, intramuscularly, near the base of dorsal fin. At ovulation time (18-20 hours after LHRHa injection) all females were sacrificed and the head kidneys containing the interrenal tissue were excised.

Histological Techniques

Immediately after collection of the animals, samples of gonads were fixed in Bouin's solution (18 hours) and later embedded in paraffin. Sections of 5-6 μm were stained with hematoxylin-eosin. The head kidneys were fixed in Bouin's fluid (24 hours) for histological routine procedures.

After embedding in paraffin, representative 5-6 μm sections thick from different regions of the head kidneys, or in some instance, serial sections, were obtained and stained with hematoxylin-eosin.

RESULTS

In the pacu female, the head kidney appears as two lobes of lymphatic tissue located ventrolateral to the spinal column in the re-

gion of the second, third and fourth vertebrae and the anterior portion of the swim bladder. A large number of colloid-filled epithelial follicles, assumed to be thyroidean, is present throughout the lymphoid head kidney tissue. The interrenal tissue appears as a collar of two to ten layers surrounding the major and medium-size branches of the postcardinal veins which pass through the head kidneys. The cells are tightly packed together and lie just beneath of the endothelium of the vein (Fig. 1 and 2). A moderate number of pale cells occurs in small clumps among these interrenal cells. The appearance of these pale cells indicates that they are chromaffin (Fig. 2). The interrenal cells have fine-granular acidophylic cytoplasm and vacuoles may be present or not. The nuclei is usually large, spheric, central with a granular chromatin (Fig.2).

Annual Cycle

The interrenal cells of pacu females show little changes during the gonadal cycle. Apparently no hypertrophy or hiperplasy of interrenal tissue is observed in the various ovarian stages. During the resting stage of gonadal cycle more vacuolization and smaller acidophylia is seen in the cytoplasm of interrenal gland (Fig. 3). In advanced maturation stage the cytoplasm of these cells is more acidophylic and less vacuolated (Fig. 4). In early maturation and regression stages the interrenal cells show similar aspects with yhe cytoplasm exhibiting eosinophylic fine granules and moderate vacuolization (Fig. 5).

Induced Ovulation

In pacu female injected with LHRHa the interrenal tissue has the same distribution and structure of the females in gonadal cycle. However, the interrenal cells show a pronounced vacuolization in cytoplasm indicating that the injection of LHRHa may promote significant cytoplasmic changes (Fig. 6).

DISCUSSION

The interrenal gland of teleost fishes is homologous to the mammalian adrenal cortex and is well established as the source of adrenocortical steroids (Chester Jones and Phillips, 1986). In teleost, the interrenal cells and the chromaffin cells frequently lie within the anterior portion of the kidney. However, there is diversity in the distri-

bution and organization of interrenal and chromaffin cells, as well as in the anatomic relationships to each other and to the other tissues comprising the anterior head kidney (Nandi, 1965). In ***Heteropneustes fossilis***, e.g., the adrenal gland is a remarkably large area occupying almost a three-fourth area of the head kidney and, in ***Periophthasmus viridis***, the interrenal tissue is a narrow epithelium that only appears by the wall of the posterior cardinal vein (Banerji, 1971).

To simplify the description of the morphology and location of teleost interrenal tissue, Nandi (1962) has classified the tissue in four types(I, II, III and IV). According with Nandi's classification the interrenal tissue of pacu belongs to type I, where the interrenal gland occurs surrounding the post-cardinal veins or their largest branches. The morphology of interrenal gland of pacu resembles the cypriniformes and, in general, the associated tissues appear to be uniform within the same group (Nandi,1965; Banerji ,1971).

There was no change in the distribution and structure of interrenal tissue of pacu females during gonadal cycle. However, modifications in acidophylia and vacuolization of cytoplasm were verified and they were more pronounced in interrenal cells of pacus collected during the resting stage of reproductive cycle. This fact suggests great stock of lipids in the cytoplasm of the cells, probably, a steroid precursor during this stage of the cycle. The stores of lipids are extracted by the hystological technique of dehydration-clarification employed.There were no changes in morphology and structure of interrenal tissue of pacu females injected with LHRHa as well. However, a higher cytoplasmatic vacuolization is observed in interrenal cells of induced females when compared with all others females studied. This fact is probably related to the output of corticosteroid hormone from cellular cytoplasm to circulating blood after hormonal induction by LHRHa. Hibiya (1982) and Chester Jones and Phillips (1986) reported that interrenal cells of teleosts contain significant amounts of lipid droplets, cholesterol, ascorbic acid, glucose-6-phosphate-dehydrogenase, 5-ene-3β-01-steroid dehydrogenase, which are important precursors for the biosynthesis of steroid hormones.

Robertson and Wexler (1960) reported pronounced changes in the body structure of salmonid fish during the migration to spawning mainly in stomach, liver, spleen, thymus, thyroid, gonads, pituitary, kidney and cardiovascular system, including atrophy or not. In addition McBride *et al.* (1986) related that some organs as adrenal,

pancreatic islets and skin may exhibited hypertrophy and hyperplasy. The comparison with Cushing's syndrome, experimental hyperadrenocorticism and aging, histological alterations suggest that many of the tissue changes found in the salmonids could be ascribed to the effects of the hyperactivity of adrenal gland (Chester Jones and Phillips, 1986).

In non-salmonid species, changes in structure of adrenal were related associated with the reproductive cycle. In ***Channa gachua***, e.g., the interrenal cells exhibit clear seasonal change, the interrenal becomes hyperactive or inactive during the breeding and non-breeding period of the fish, respectivly (Verma and Misra, 1992). In ***Heteropneustes fossilis***, e.g., when the spawning period is reached, the cytoplasm of interrenal cells becomes vacuolated and the nuclei enlarges (Yadov *et al.*, 1970). In a South American species ***Prochilodus scrofa***, Farias *et al.* (198' .ported that the changes in interrenal cells during the reproductive cycle were less pronounced than in others migrating teleostei species. McBride and Van Overbeeke (1969) attributed the strong hypertrophy of the interrenal tissue during the period of sexual maturation and spawning in salmonid fish to gonadal hormones which may act directly on the adrenal homologue. However, Schreck *et al.* (1989) suggested that the hypophyseal gonadotropin plays a role in the regulation of the interrenal, besides the direct effect of the pituitary ACTH proposed by Donaldson (1981).

Several studies have characterized steroid secretion by the interrenal tissue. Cortisol is the main corticosteroid secreted by the interrenal gland of the teleosts studied (Chester Jones and Phillips, 1986). It is reported that the cortisol may be important to the maturation process in salmonids and Cyprinids species (Nagahama, 1994). The oocyte maturation in ***Heteropneustes fossilis***, e.g., involves the gonadotropin-ovarian axis besides the gonadotropin-interrenal axis. In this species the maturating action of gonadotropin is mediated by an extra-ovarian relay, and the interrenal gland, presumably, is involved (Sundararaj and Goswami, 1985). In pacu females Gazola *et al.* (1995), reported that the plasma cortisol levels were significantly higher during the ovulation time concomitantly with the emergence of 17α-20β-dihydroxy-4-pregnen-3-one, indicating that the cortisol may have a relevant role in oocyte maturation. Oocyte maturation in teleosts is thus a complex phenomenon involving the interaction between gonadotropin and steroid hormones, the latter being synthesized either in the oocyte follicle or in the interrenal tissue (Sundararaj and Goswami, 1985).

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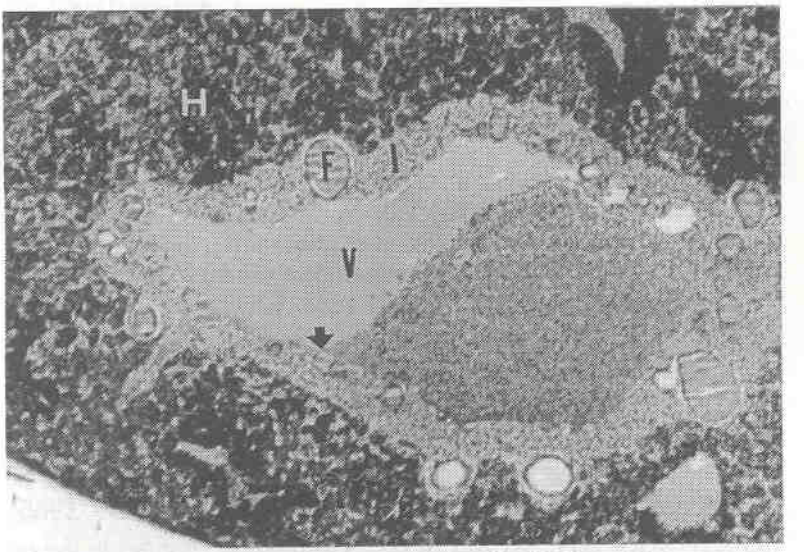

Fig. 1. ***Piaractus mesopotamicus***. Female. Head Kidney. Early maturation stage. Chromaffin cells (seta). Thyreoidean follicle (F). Hematopoietic tissue (H). Interrenal tissue (I). Vein (V). Hematoxylin-eosin. 92.5 x.

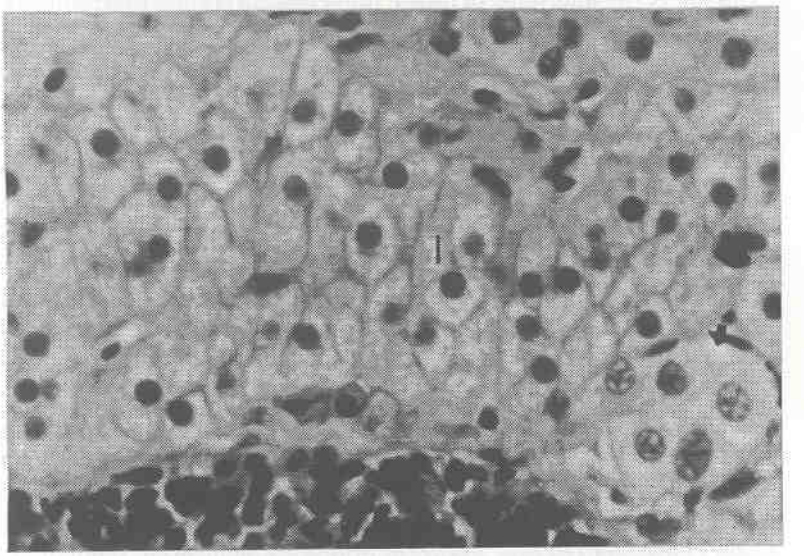

Fig. 2. ***Piaractus mesopotamicus***. Female. Head kidney. Resting stage. Chromaffin cell (seta). Interrenal cell (I). Hematoxylin-eosin. 925 x.

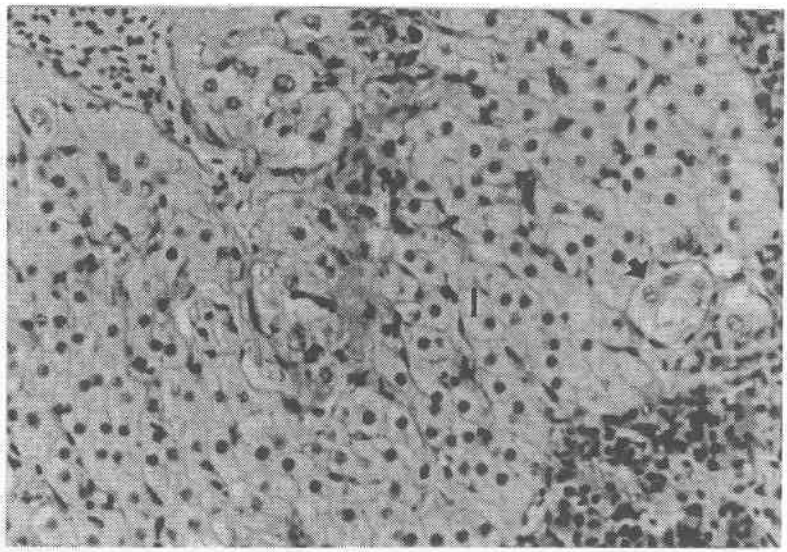

Fig. 3. ***Piaractus mesopotamicus***. female. Head Kidey. Resting stage. Chromaffin cell (seta). Interrenal cell (I). Hematoxylin-eosin. 465.5 x.

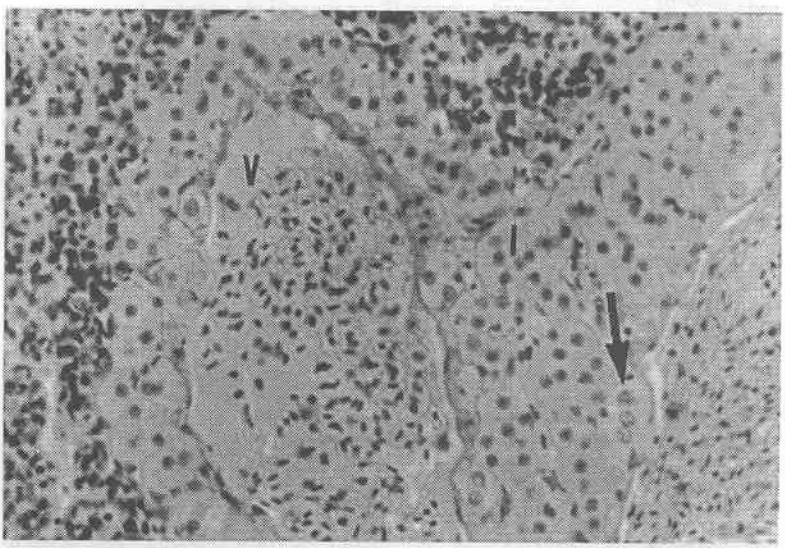

Fig. 4. ***Piaractus mesopotamicus***. Female. Head Kidney. Advanced maturation stage. Chromaffin cell (seta). Interrenal cell (I). Vein (V). Hematoxylin-eosin. 465.5 x.

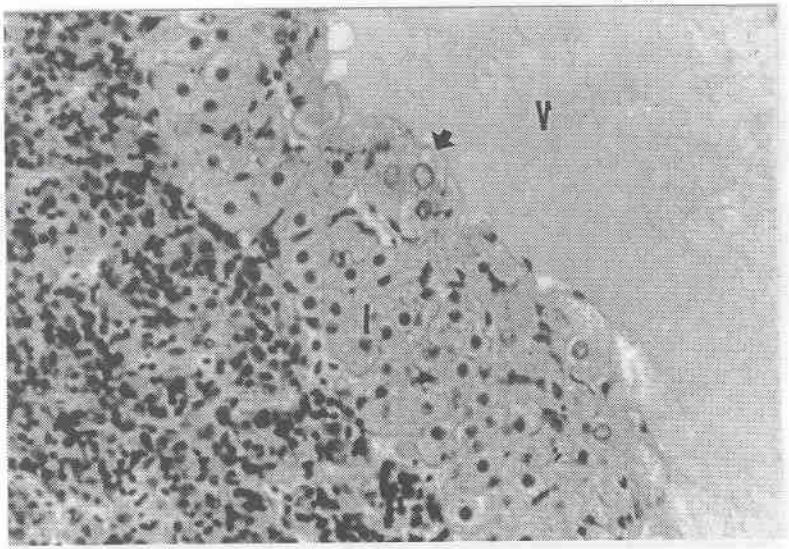

Fig. 5. ***Piaractus mesopotamicus***. Female. Head Kidney. Early maturation stage. Chromaffin cell (seta). Interrenal cell (I). Vein (V). Hematoxylin-eosin. 465.5 x.

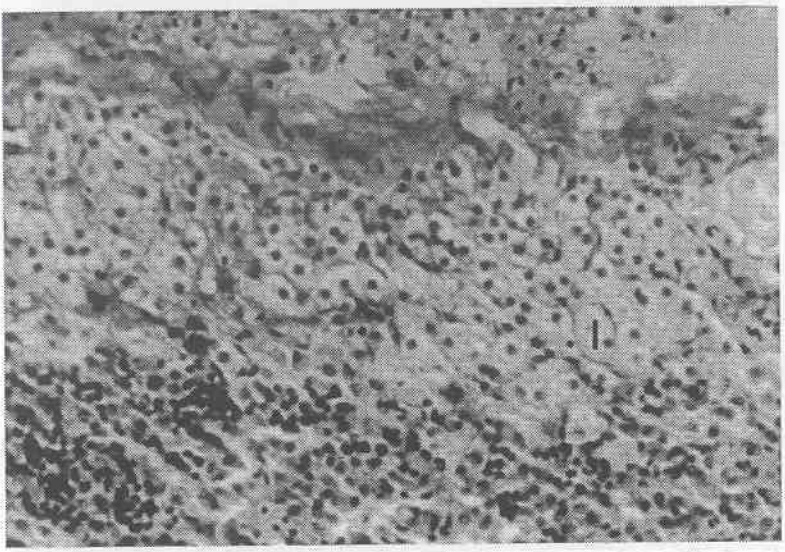

Fig. 6. ***Piaractus mesopotamicus***. Female. Head Kidney. 6 hours after LHRHa injection. Interrenal cell (I). Hematoxylin-eosin. 232.5 x.