IMMUNOCYTOCHEMICAL IDENTIFICATION AND DISTRIBUTION OF THE CELL TYPES IN THE PITUITARY GLAND OF THE NILE PERCH, LATES NILOTICUS (TELEOSTEI, CENTROPOMIDAE)

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Key words: Immunocytochemistry, pituitary gland, *Lates niloticus* (Teleostei).

ABSTRACT

Immunocytochemical identification of the different cell types in the pituitary gland of the Nile perch (Lates niloticus) was performed using antisera against mammalian (human and rat) and piscine hormones. The adenohypophysis was composed of rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Prolactin and adrenocorticotrophic cells were located in the rostral pars distalis of the pituitary. Gonadotrophic and growth hormone cells were distributed in the proximal pars distalis, but gonadotrophic cells appear also at the border of the pars intermedia. Somatolactin cells, as well as alpha-melanotrophic cells were located in the pars intermedia of Lates niloticus pituitary. The prolactin (PRL) cells were distributed in the RPD stained with orange G and showed strong immunoreactivity with antiserum to chum salmon. The adrenocorticotrophic (ACTH) cells were lead hematoxylin-positive (PbH⁺) and showed strong immunoreactivity with anti-human ACTH; these cells bordered the neurohypophysis and islets between PRL cells in the RPD. Growth hormone (GH) cells were densely distributed and associated with the neurohypophysis in the PPD. They were orange G positive and reacted with antiserum to chum salmon. Gonadotrops were located in the central area of the PPD and in the external border of the PI. These cells were alcian blue and PAS positive, and immunostained with anti-chum salmon GTH Iβ and anti-chum salmon GTH IIβ. The gonadotrophic cells were observed in all maturity stages of L. niloticus. In addition, antiserum to rat TSHβ reacted positively to the gonadotrophic cells. These results suggest that GTH I, GTH II and TSH are synthesized in the same cells in the pituitary of L. niloticus. The PI was composed mainly of PbH⁺ cells and a PAS⁺ cell layer adjacent to the neurohypophysis. The PAS⁺ cells from the PI bound specifically to anti-chum somatolactin. Anti-alpha-MSH stained only the PbH⁺ cells (melanotrops) of the PI.

INTRODUCTION

The hypophyseal-gonadal axis is the controlling system for the reproduction of fishes. The identification and the distribution of the different cell types in the pituitary gland of teleosts have been studied using histochemical and different physiological techniques (Ball & Baker, 1969; Holmes & Ball, 1974). In addition, the adenohypophyseal cells have been characterized by immunocytochemical techniques using antisera against mammalian and piscine hormones (Batten, 1986; Cambré *et al.*, 1986; Quesada *et al.*, 1988; Yan & Thomas, 1991; García-Hernández, 1996; Rendón *et al.*, 1997; Mousa, 1998; Parhar *et al.*, 1998; Segura-Noguera *et al.*, 2000; Pandolfi *et al.*, 2001; Rodriguez-Gomez *et al.*, 2001). Seven different classes of hormones, grouped into three main families have been described: (i) growth hormone (GH)/prolactin (PRL) family, containing PRL, GH and somatolactin (SL); (ii) glycoprotein hormones including gonadotrophins (GTHs) and thyrotropin (TSH); and (iii) proopiomelanocortin-derived hormones such as adrenocorticotropic (ACTH) and melanotropic hormone (MSH) (Batten & Ingleton, 1987).

The "Nile perch" *Lates niloticus* (Linneaus, 1758) is an important food fish in tropical and semitropical waters. It grows fast but is less salt-tolerant than *Lates calcarifer*, and attains 190 cm in length with maximum weight of 200 kg. *Lates niloticus* is locally known as " Keshr Bayad" and is so far one of the most important economic species as food fish in Egypt and finds good marketing in the different parts of Egypt. During the past years with the development of industries and modern civilization, large amount of pollutants have been discharged into the Nile and the surrounding lakes. These pollutants not only affect the growth, health, and nutritional value of fishes but also the distribution (i.e., survival) and reproduction of economically important fishes. *Lates niloticus* is one of those fishes, which affected by environmental factors and its distribution was restricted only to Lake Nasser, Wadi El-Rayyan and sometimes the Nile River. On the other hand it disappeared from the most of north lakes; Manzalah and Borollus and most of the Nile River.

In spite of the extensive importance of Nile perch, it is somewhat surprising that such limited information concerning its reproduction is available. However, a detailed description of the cell types in the pituitary of this species has not yet been done. The aim of the present study was to localize and characterize the different pituitary cell types using histochemical and immunocytochemical approaches. Special attention is paid to GTH I and GTH II.

MATERIAL AND METHODS

Animals :

From Lake Nasser, *Lates niloticus* (both immature and mature of both sexes) with standard length larger than 20 cm, were collected alive during the prespawning and spawning season (July to September).

Histological and histochemical methods :

. Prior to dissection, the fishes were anesthetized in a solution (100 mg/l) of tricaine methanosulfonate (MS222, Sandoz) and then perfused via the ascending aorta with 20 ml of normal saline, followed by 50 ml of Bouin's fluid at 4 °C. Immediately after the dissection, the pituitary gland, attached to the brain, was postfixed in Bouin's fluid for 24 hr at 4 °C. The fixed brain and pituitaries were thereafter dehydrated through graded ethanol solution, cleared and embedded in paraplast (M.P.: 56-58 °C).

Consecutive median sagittal sections of the pituitary gland were made at 4 μm thickness. For each specimen, selected sections were stained with the following techniques:

1- Harris's alum hematoxylin, according to Conn (1953) and aqueous solution of eosin (1%) was used as a counterstain.
2- Periodic Acid-Schiff-Lead hematoxylin (PAS-PbH): Lead hematoxylin "PbH" (McConail, 1947) combined with PAS (Pearse, 1949).
3- Performic acid-Alcian blue (PFAAB)- Periodic Acid- Schiff (PAS)- Orange G (OG) stain, according to Heath (1965).

Immunocytochemical procedures:

Antibodies: Rabbit antisera directed against human ACTH and rat thyrotropin (rBetaTSH) was obtained from National Institute of Health. The α-MSH antiserum was kindly provided by Dr. R. M. Dores (University of Denver, USA). Antisera to chum salmon (*Oncorhynchus keta*) hormones; chum salmon GTH Iβ subunit (Lot No.8707), chum salmon GTH IIβ subunit (Lot No.8506), chum salmon somatotropin (chum GH) (Lot No.8208), chum salmon prolactin (Lot No.8502) and chum salmon somatolactin (Lot No. 8906) were obtained from Dr. H. Kawauchi (School of Fisheries Science, Kitasato University, Iwate, Japan).

Immunocytochemical reactions: Immunocytochemical staining for the sections of the pituitary gland was generally performed with a vectastain ABC (Avidin-biotin peroxidase complex) Kit (Vector Laboratories) as described previously (Mousa and Mousa, 1999). In brief, sections were deparaffinized in xylene, rehydrated through graded ethanol, washed in phosphate-buffered saline (PBS; pH 7.4) for two times 10 min each. All incubations were done at room temperature and PBS was used for washing after each step. Sections were incubated with the antisera to the various hormones for 12-18 hr. The dilution of the hormone antisera was determined empirically and their working dilutions were shown in table (1). Thereafter, the sections were incubated with the biotinylated secondary antibody (Vector Laboratories) for 1h. and with avidin- biotin- conjugated peroxidase for 45 min. Finally, the sections were washed and stained with 3, 3`-diaminobenzidine tetrahydrochloride (DAB) (Sigma) including 0.01 % H_2O_2 in 0.05 M Tris-buffered saline (pH 7.6) for 3-5 min. After the enzyme reaction, the sections were washed in tap water, dehydrated in alcohol, cleared in xylene and mounted in DPX.

In order to confirm the specificity of the immunoreactive procedures, adjacent sections were stained according to the above described protocol but incubation in the primary antisera was omitted. In addition, normal bovine serum was used instead of primary antiserum. No positive structures or cells were found in these sections.

RESULTS

The pituitary gland of *Lates niloticus* consists of the neurohypophysis, and the adenohypophysis, which showed the three major subdivisions typical of teleost; an anterior-dorsal rostral pars distalis (RPD), a medium proximal pars distalis (PPD) and ventral posterior pars intermedia (PI). Moreover, neurohypophysial processes penetrated the different adenohypophyseal areas.

According to the histochemical and immunocytochemical behavior, six endocrine cell types were identified in the pituitary gland of *L. niloticus*. In the RPD two cell types could be distinguished, the prolactin (PRL) cells and the ACTH cells. Somatotrops (GH cells) and gonadotropins (GTH Iβ and GTH IIβ) and thyrotropin (TSH)-secreting cells have been identified in the PPD and in the external border of the PI. The PI was mainly composed of two cell types; the somatolactin (SL) cells and the melanotrops (MSH cells).

Prolactin (PRL) cells. The PRL cells occupy the major part of the RPD (Fig. 1a). These cells are small in sizes, with irregular shapes and have spherical nuclei with a distinct nucleolus (Fig. 1b). Some of them were located close to the neurohypophysial extensions. The PRL cells form a compact mass being stained orange with orange G stain (Fig. 1c) and are accordingly termed "orangeophilous acidophils", and also stained with eosin (Fig. 1b). The PRL cells showed strong immunoreactivity to anti-chum salmon PRL (Table 1 and Figs. 1d and 1e).

Adrenocorticotrops (ACTH cells). The ACTH cells appear as cords bordering the PRL cells or as islets among PRL cells and the neurohypophysis (Figs. 1a, 2a and 2b). They are small in size, spherical or oval in shape and with small eccentric nuclei (Figs. 2a and 2b). These cells stained positively with lead hematoxylin (PbH⁻) and have no affinity for PAS (Fig. 2b). Anti-serum to human ACTH bound strongly to the ACTH cells (Figs. 2c and 2d). Also, anti-hACTH showed cross-reaction with the PbH⁻ cells of the PI (Fig. 2c and Table 1).

Somatotrops (GH cells). The GH are restricted to the posterior part of the PPD and are in close contact with neurohypophysis (Figs. 1a). They are frequently arranged in cords bordering the neurohypophysis and are also dispersed between the basophils. The cytoplasm of these cells is densely granulated and their nuclei were oval or rounded (Fig. 2e). These acidophilic cells were strongly stained with eosin and orange

G (Figs. 2e and 2f) but negative to PAS. The somatotrops showed strong and specific immunoreactivity to anti-chum salmon GH which showed no cross-reaction with other cell types (Figs.2g and 2h).

Gonadotropins (GTH Iβ and GTH IIβ) and thyrotropin (TSH)-secreting cells. The GTH cells are present in the central part of the PPD (Figs. 1a), and the external border of the PI. These basophilic cells manifest variable shapes and sizes and exhibit spherical nuclei (Fig. 3a). The GTH cells contained numerous basophilic granules, which were positive to PAS and alcian blue (Fig. 3b). They also exhibited a large unstained intracytoplasmic vacuole. However, the number, size and intensity of those granulations of these basophils represented certain seasonal variations, being concomitant with the gonad maturation and spawning. Anti-sera to chum salmon GTH Iβ and chum salmon GTH IIβ bound strongly and specifically to the GTH cells (Figs. 3c and 3d). In addition, antiserum to rat TSHβ subunit showed strongly and specifically immunoreaction to the gonadotropins (GTH Iβ and GTH IIβ)-secreting cells (Figs. 3e-3g and Table 1).

Somatolactin (SL) and α-MSH cells. Two types of cells were found in the PI of *L. niloticus*; one is stained with periodic acid-Schiff (PAS+ cells) and the other cell type is stained with lead hematoxylin (PbH⁺ cells) (Figs. 4a-4c). The PAS⁺ cells of the PI have variable sizes and shapes and were found either singly or in-groups (Figs. 4a-4c). The PAS⁺ cells showed strong immunoreactivity to anti-chum salmon SL (Figs. 4d and 4e). The number, size and intensity of the immunoreaction of the SL-cells represented seasonal variations, being concomitant with the development of the gonads and spawning.

Anti-αMSH stained only the PbH⁺ cells of the PI (Figs. 4f and 4g). In addition, anti-αMSH positive cells showed cross-reaction with the anti-hACTH (Fig.2c). Alpha-melanotropin (MSH)-immunoreactive cells were weakly orange-stained in the AB-PAS-OG-stained section (Fig. 4c). These cells showed variable shapes and sizes and surrounded the neurohypophysial processes, intermingled with SL cells (Figs. 4a and 4b).

DISCUSSION

The *L. niloticus* adenohypophysis was studied by both histochemical and immunocytochemical methods, the general structure conforming to that described for other teleosts. Six cell types were identified in the adenohypophysis of *L. niloticus*. The distribution of the different types of cells identified in the present work is in a good agreement with previous studies in teleost fish (García-Hernández, 1996; Mousa, 1998; Segura-Noguera *et al.*, 2000). The secretory cells show segregation into the different zones of the adenohypophysis.

PRL cells

In the present study we have used an antiserum against chum salmon PRL that showed a quite good and specific immunoreaction with PRL cells of *L. niloticus*, as has been reported for other teleosts (Batten, 1986; Cambré *et al.*, 1986; Quesada *et al.*, 1988; Yan & Thomas, 1991; Rendón *et al.*, 1997; Mousa, 1998; Parhar *et al.*, 1998; Segura-Noguera *et al.*, 2000). Moreover, in contrast to other PRL antisera (Kawauchi & Yasuda, 1989), it did not cross-react with GH cells. As for other freshwater and marine teleosts, *L. niloticus* PRL cells are exclusively localized in the RPD (Munro, 1985; Toubeau *et al.*, 1991; Yan & Thomas, 1991; Segura-Noguera *et al.*, 2000; Pandolfi *et al.*, 2001; Rodriguez-Gomez *et al.*, 2001). The osmoregulatory role of PRL in hypo-osmotic environments is well established in teleosts, and especially in euryhaline species (Mancera *et al.*, 1993; Auperin *et al.*, 1995; Mousa *et al.*, 1999). In addition to an osmoregulatory role, PRL has been related to stress (Avella *et al.*, 1991; Wendelaar Bonga, 1997). Furthermore, there is increasing evidence that GH and PRL possess steroidogenic and gonadotropic activity in some teleosts such as: *Fundulus heteroclitus* (Singh *et al.*, 1988) and *Pleuronectes platessa* (Power, 1992). However, the significance of these effects of teleost GH and PRL is not known.

GH cells

In this study we used anti-chum salmon GH that has been utilized to reveal GH cells of salmonids (Kawauchi *et al.*, 1986) and non-salmonid species (Mancera *et al.*, 1995; Mousa, 1998; Parhar *et al.*, 1998; Segura-Noguera *et al.*, 2000). This antiserum strongly stained GH cells of *L. niloticus* that were restricted, similarly to other teleost fish, to the dorsal and ventral parts of the PPD (Batten, 1986; Quesada *et al.*, 1988; Toubeau *et al.*, 1991; Yan & Thomas, 1991; Rendón *et al.*, 1997; Mousa, 1998; Parhar *et al.*, 1998; Segura-Noguera *et al.*, 2000; Pandolfi *et al.*, 2001; Rodriguez-Gomez *et al.*, 2001).

The physiological role of GH as a growth-promoting hormone has been well established in teleosts (McLean & Donaldson, 1993). Also GH has been involved in metabolism, reproduction and immune response (Björnsson, 1997). In addition, an osmoregulatory role of GH has been reported in salmonids and non-salmonid species (Sakamoto *et al.*, 1993; Mancera & McCormick, 1998; Mousa *et al.*, 1999). Thus, in some euryhaline species it has been found that GH cells were activated in fish acclimatized to brackish or fresh water (Benjamin, 1978; Mancera *et al.*, 1995; Mousa *et al.*, 1999).

SL cells

Two types of cells were found in the PI of *L. niloticus*; one is stained with lead hematoxylin (PbH+ or MSH cells) and the other cell type is stained with periodic acid-Schiff (PAS+ cells). Immunocytochemical studies indicated that PAS-negative were MSH cells while PAS-positive were SL cells.

The SL is the latest pituitary hormone of the GH/PRL family described (Rand-Weaver & Kawauchi, 1993). In the last few years, several studies have analyzed the structure, localization and physiological role of SL. However, the function of this hormone remains unclear (Kanko, 1996). In this study, we used antiserum against chum salmon SL. The SL genes seem to be highly conserved and the protein homology is high among the different teleost species (Rand-Weaver & Kawauchi, 1993; Kaneko, 1996). Our results showed that anti-SL reacted only with the SL cells of *L. niloticus*. The distribution and localization of SL in this species is similar to those in other teleosts (Rand-Weaver *et al.*,1991; Kaneko *et al.*, 1993; Kaneko, 1996; Mousa, 1998; Mousa and Mousa, 1999; 2000; Segura-Noguera *et al.*, 2000; Pandolfi *et al.*, 2001; Rodriguez-Gomez *et al.*, 2001).

The physiological function of SL is still unknown. This hormone has been related to reproductive maturation, calcium metabolism, stress, acid-base regulation, fat metabolism, background adaptation and osmoregulation (Kaneko, 1996; Mousa and Mousa, 2000). The possible involvement of SL in gonadal maturation was determined by correlating changes in cell number and distribution, and intensity of staining (using immunocytochemistry) with gonadal maturation and spawning of *O. niloticus* and *M. cephalus* (Mousa and Mousa, 1999; 2000). Olivereau and Rand-Weaver (1994) obtained similar immunocytochemical observations in *Oncorhynchus nerka* and *O. keta*. In addition, these immunocytochemical results received a good support from biochemical studies, which showed that in *Oncorhynchus kisutch* SL stimulates gonadal steroidogenesis *in vitro* (Planas *et al.*, 1992). Also, in *O. kisutch* during the period of gonadal growth, plasma SL levels increased and were highly correlated to oestradiol levels in females and 11- ketotestosterone levels in males (Rand-Weaver *et al.*, 1992 and 1995).

ACTH and α-MSH cells

The ACTH cells of *L. niloticus* were immunostainesd by an antiserum against human ACTH, the same one used for other teleost species (Follenius & Dubois, 1980; Munro, 1985; Cambré *et al.*, 1986; Quesada *et al.* 1988; Garcia- Hernández *et al.*, 1996; Mousa and Mousa, 1997; Rendón *et al.*, 1997; Parhar *et al.*1998; Mousa, 1998; Segura-Noguera *et al.*, 2000; Pandolfi *et al.*, 2001). This antiserum also showed cross-reactivity with the MSH cells present in the PI of these species and also in *L. niloticus* as depicted by our results. Only in the barble *Barbus barbus* has such a cross-reactivity been reported not to exist (Toubeau *et al,.* 1991).

The family of proopiomelanocortin (POMC)-derived hormones includes ACTH, MSH, β-endorphin and LPH. These hormones proceeded from differential processing of a common precursor molecule POMC and the amino acidic sequence of α-MSH is identical to the 13 first amino acids of the ACTH molecule (Follenius & Dubois, 1980; Dores, 1990). This may account for the cross-reactivity observed in *L. niloticus* using anti-human ACTH.

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The ACTH cells were located in the RPD, forming a palisade between PRL cells and the branches of neurohypophysial tissue. According to their locations, both ACTH cells in the RPD and MSH cells in the PI stained with lead hematoxylin (PbH⁺) and were PAS-negative. This result agrees with previous reports for other teleosts and suggests that teleosts do not have the capacity to glycosylate the precursor proopiomelanocortin (Iturriza & Estivariz, 1986).

With respect to the melanotropic cells, immunocytochemical studies have shown that α-MSH antiserum specifically immunostained melanotropic (MSH) cells in all the teleost species so far studied (Munro, 1985; Batten, 1986; Cambré et al., 1986; Quesada et al., 1988; Toubeau et al., 1991; Garcia- Hernández et al., 1996; Rendón et al., 1997; Segura-Noguera et al., 2000) including *L. niloticus*. In *L. niloticus* the MSH cells surround the neuroyhpophysial branches protruding into the PI, intermingled with SL cells. This is the typical distribution for MSH and SL cells reported for other teleosts (Munro, 1985; Batten, 1986; Cambré et al., 1986; Quesada et al., 1988; Toubeau et al., 1991; Mousa and Mousa, 1997; Mousa, 1998; Segura-Noguera et al., 2000). However, in *Solea senegalensis* (Rendón et al., 1997) and *Thalassoma duperry* (Parhar et al., 1998) MSH cells appear to surround the SL cells.

The physiological role of ACTH is the stimulation of synthesis and release of cortisol from the inter-renal tissue (Henderson & Garland, 1980). In teleosts, cortisol has been related to different physiological processes, such as stress, metabolism and osmoregulation (Wendelaar Bonga, 1997). In addition, Jalabert and Fostier (1984) reported that high cortisol levels enhance the GTH stimulation of $17a,10\beta$-Dihydroxyprogesterone secretion from mature oocyte follicles of *Oncorhynchus mykiss*, this being in line with earlier *in vitro* studies suggesting stimulation by cortisol of oocyte maturation of *O. rhodurus* (Young et al., 1982). On the other hand, MSH has been related to adaptation to a different background colour (Baker et al., 1984; Zhu and Thomas, 1996) and also in stress response (Wendelaar Bonga, 1997).

TSH and GTH cells

The family of adenohypohyseal glycoprotein hormones includes TSH and GTH. Both hormones have an identical α-subunit but different β-subunit (Farmer & Papkoff, 1979; Pierce & Parsons, 1981). Thus, the use of specific antiserum against β-subunit of TSH and GTH is necessary for the specific immunocytochemical detection of TSH or GTH cells. To our knowledge, there is no specific antibody against the piscine β-subunit of TSH. However, antiserum raised against human-βTSH have been demonstrated to cross-react selectively with the TSH-producing cells of several teleost species (Munro, 1985; Cambré et al., 1986; Siegmund et al., 1987; Garcia- Hernández et al., 1996; Segura-Noguera et al., 2000), although a weak immunoreactivity to this antiserum was observed in the gonadotrops of some teleosts (Batten, 1986; Nozaki *et*

al.,1990; Yan & Thomas, 1991). In our study, anti-human β-TSH showed a strong immunoreactivity with the gonadotrops of *L. niloticus*.

Two different GTHs (GTH I and GTH II) have been reported in salmonids and non-salmonid species (Nozaki *et al.*, 1990; Copeland & Thomas, 1993; Okada *et al.*, 1994). In some species, two different types of GTH have been reported (Nozaki *et al.*, 1990; Natio *et al.*, 1991; Kagawa *et al.*, 1998). However, in other species it is not clear whether two different types of GTH cells exists or a single polymorphic GTH cell type gives rises to both molecular forms depending upon the physiological stages of the fish (Quesada *et al.*, 1988; Yan & Thomas, 1991; Garcia-Hernández *et al.*, 1996).

The present study showed that GTH Iβ and GTH IIβ immunoreactivities were colocalized in the same cells in the pituitary of *L. niloticus*. Similarly, in *S. dumerilii*, from which GTH I and GTH II have been obtained (Garcia-Hernández *et al.*, 1997), chum salmon βGTHI and βGTHII immunoreactivities were colocalized in the same cells (Garcia-Hernández *et al.*, 1996). The use of antisera against human-β-TSH and chum salmon β-GTHs (GTH I and GTH II) on consecutive sections showed that β-TSH and β-GTHs (GTH I and GTH II) immunoreactivities were colocalized in the same cells located in PPD and PI in the pituitary of *L. niloticus*. The gonadotrophic cells were observed in all maturity stages of *L. niloticus*. These results suggest that GTH I, GTH II and TSH are synthesized in the same cells in the pituitary of *L. niloticus*.

The distribution of GTH cells in *L. niloticus* is similar to that reported in other teleosts, the GTH cells being located in the dorsal and ventral portions of PPD (Munro, 1985; Batten 1986; Toubeau *et al.*, 1991). In addition, GTH were found around the PI of *L. niloticus*. This distribution has been also reported in other teleosts (Cambré *et al.*,1986; Quesada *et al.*, 1988; Yan & Thomas, 1991; Garcia- Hernández *et al.*,1996; Mousa, 1998; Segura-Noguera *et al.*, 2000).

Gonadotropin produced by gonadotrops is most closely associated with reproduction, stimulating steroid production, uptake of vitellogenin, oocyte maturation and ovulation, and spermiation (Wallace and Selman, 1981; Goetz, 1983). In female teleosts, sexual maturation is driven by a gonadotropin-induced increase in plasma estradiol-17β (E$_1$) levels (Nagahama, 1987). E$_1$ stimulates the synthesis and secretion of vitellogenin (VTG), which is a yolk protein precursor, by the liver while gonadotropin (s) stimulates vitellogenin uptake by the oocyte (Tyler *et al.*, 1987; Mommsen and Walsh, 1988).

EXPLANATION OF FIGURES

Fig. (1): Sagittal section of the pituitary gland of L. niloticus: (a) Stained with Harris's hematoxylin and eosin, showing the rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) which comprise the adenohypophysis, and neurohypophysis (NH).X20. (b) A magnified portion of (a) displaying the prolactin (PRL) cells with irregular shapes and have spherical nuclei with a distinct nucleolus. X500. (c) Stained with AB-PAS-OG, showing the PRL cells stained with orange G, in addition to nerve fibers (arrowhead). X500. (d) Immunostained with anti-chum salmon prolactin antiserum. PRL positive cells are restricted in the rostral pars distalis. X20. (e) A magnified portion of (d) showing the immunostained PRL cells. X1000.

Fig. (2): Sagittal section of the pituitary gland of L. niloticus: (a) Stained with Harris's hematoxylin and eosin, showing ACTH cells (arrowheads), beside the PRL cells and the neurohypophysis (NH). X500. (b) Stained with PAS-PbH combined stain, displaying the PbH-positive ACTH cells (arrowheads). X500. (c) Immunostained with anti-human ACTH antiserum. The ACTH cells appear as cords bordering the PRL cells or as islets between PRL cells and the neurohypophysis (arrows), beside cross-reaction of the PbH+ cells in the PI (arrowheads). X20. (d) A magnified portion of (c) showing the immunostained ACTH cells. X1000. (e) Stained with Harris's hematoxylin and eosin, displaying the somatotrops (STH), beside nerve fibers (NF). X500. (f) Stained with PAS-PbH - OG combined stain, showing the somatotrops (STH) stained orange with OG, beside nerve fibers (NF). X500. (g) Immunostained with anti-chum salmon growth hormone (anti-chum GH) antiserum. The GH-positive cells are distributed throughout the PPD in close contact with the neurohypophysis. X20. (h) A magnified portion of (g) displaying the somatotrops immunostained with anti-chum salmon GH antiserum. They are arranged in cords surrounding the exteriorities of nerve fibers (NF). X1000.

Fig. (3): Sagittal section of the pituitary gland of L. niloticus: (a) Stained with Harris's hematoxylin and eosin, showing the gonadotrops (GTH cells) manifest variable shapes and sizes. X500. (b) Stained with AB-PAS-OG, displaying the granulated GTH cells stained with both alcian blue and PAS. X500. (c-d) Two successive sections immunostained with anti-chum salmon GTH IIβ subunit (c) and anti-chum salmon GTH Iβ subunit (d). GTH Iβ and GTH IIβ immunoreactivity are localized in same cell types occupying most of the PPD and in the external border of the PI. X20. (e-g) Three successive sections through the external border of the PI immunostained with anti-GTHIβ (e), anti-GTH IIβ (f) and anti-βTSH (g).

GTH Iβ, GTH IIβ and βTSH immunoreactivity are localized in the same cell types. X400.

Fig. (4): *Sagittal section of the pituitary gland of L. niloticus*: (a) Stained with Harris's hematoxylin and eosin, showing the cell types of the pars intermedia; PI$_1$ stained with eosin and have small sizes and the PI$_2$ stained with haematoxylin and have large variable sizes and shapes. X500. (b) Stained with PAS-PbH, showing the cell types in the pars intermedia (PI); the PAS-positive cells (arrowheads) and PbH-positive cells (arrows), beside the gonadotrops (GtH). X500. (c) Stained with AB-PAS-OG, displaying the PAS-positive cells (arrowheads) and weakly orange-stained PbH+ cells (arrows), beside GtH cells. X500. (d) Immunostained with anti-chum salmon somatolactin (SL) antiserum. Note the immunoreactive SL cells (PAS+ cells) in the pars intermedia. X20. (e) A magnified portion of (d), displaying the intensely stained SL cells in the PI. X1000. f) Immunostained with anti- α-MSH antiserum. Note, the immunoreactive MSH (PbH+ cells) in the PI. X20. (g) A magnified portion of (f), displaying the intensely stained MSH cells in the PI. X1000.

Table (1): Immunocytochemical staining of the adenohypophysis of L. niloticus.

Antiserum to	Dilution	RPD		PPD		PI	
		P	C	S	G	PAS$^+$	PbH$^+$
chum PRL	1:5000	++	-	-	-	-	-
h ACTH	1:500	-	++	-	-	-	+
chum GH	1:5000	-	-	++	-	-	-
chum GTH Iβ	1:1000	-	-	-	++	-	-
chum GTH IIβ	1:5000	-	-	-	++	-	-
rβTSH	1:500	-	-	-	++	-	-
Chum SL	1:5000	-	-	-	-	++	-
α-MSH	1:1000	-	-	-	-	-	++

Note. RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia; P, PRL cells; C, ACTH   cells; T, thyrotrops; S, somatotrops; G, gonadotrops; PAS$^+$, periodic acid-Schiff reaction positive cell; PbH$^+$, lead hematoxylin positive cell; PRL, prolactin; ACTH, adrenocorticotropin; TSH, thyrotropin; GH, growth hormone; GTH, gonadotropin; SL, somatolactin; chum, chum salmon; h, human; r, rat; -, +, ++, negative, weak and strong immunostaining responses, respectively.

Fig.1
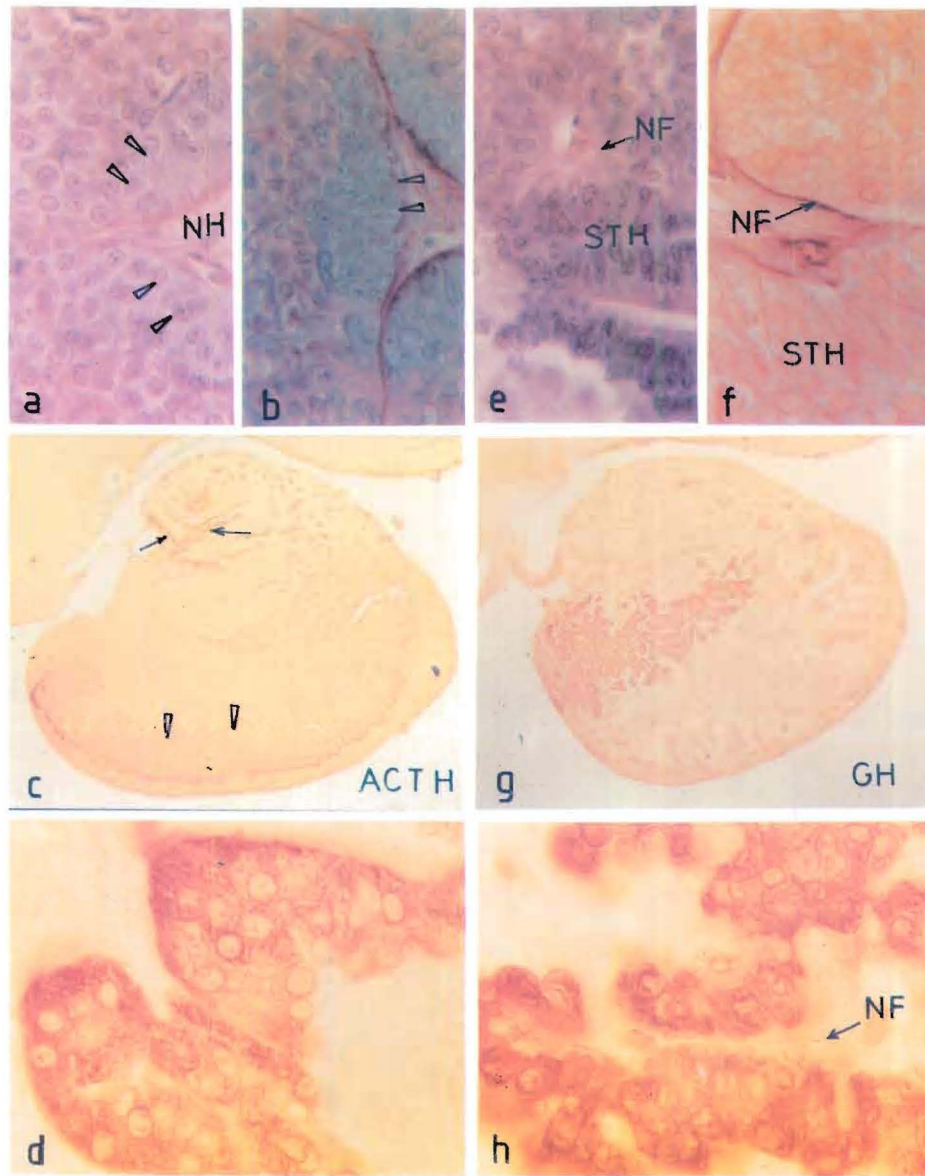
Fig.2

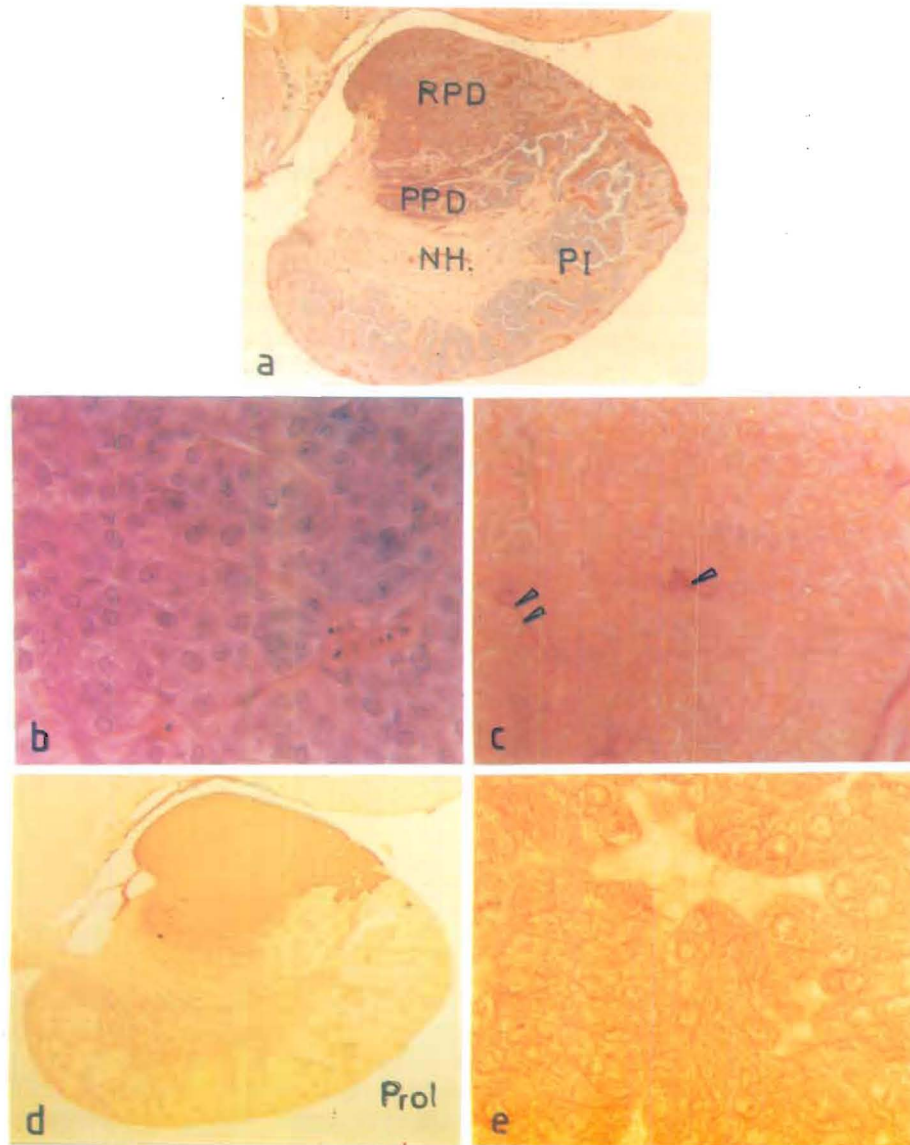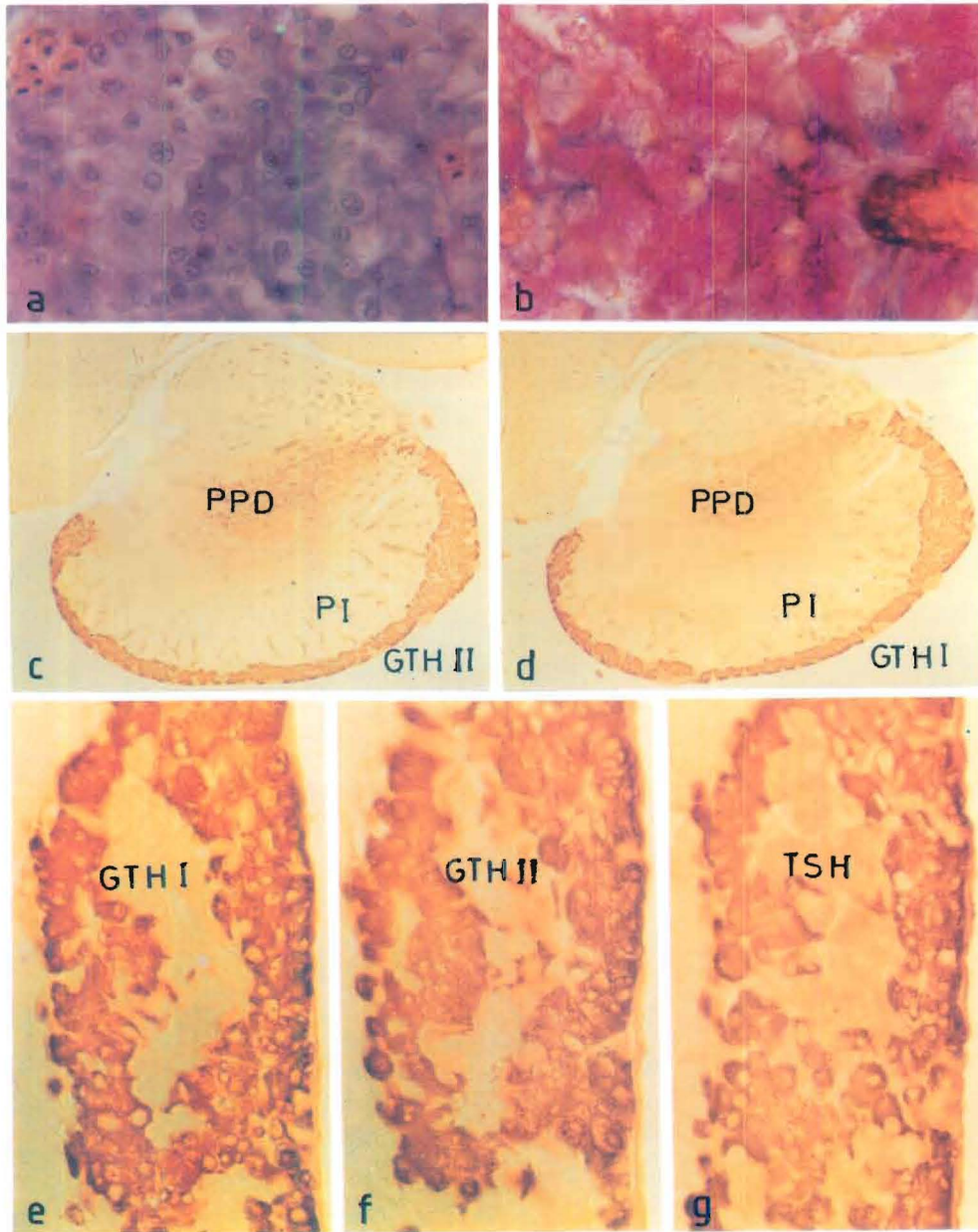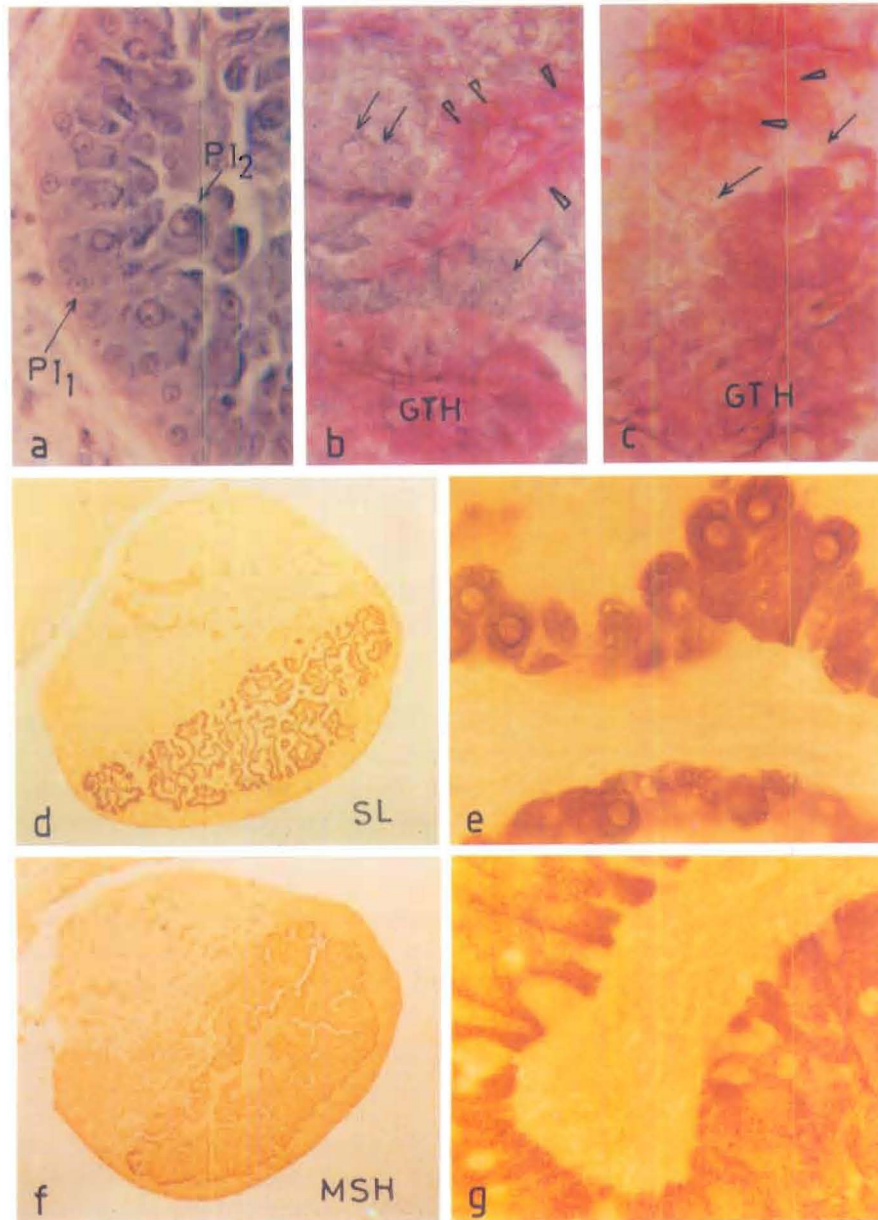Fig. 3

Fig. 4

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