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Immunocytohistochemical characterization of pituitary cells of the bluefin tuna, *Thunnus thynnus* L

F.J. Rodríguez-Gómez[1], M.C. Rendón-Unceta[1], C. Piñuela[2], J.A. Muñoz-Cueto[1], N. Jiménez-Tenorio[1] and C. Sarasquete[2]

[1]Department of Animal Biology, Plant Biology and Ecology, Faculty of Marine Sciences, University of Cádiz, Cádiz, Spain and [2]Institute of Marine Sciences of Andalucía (CSIC), Polígono Río San Pedro, Puerto Real, Cádiz, Spain

Summary. In this paper we report the first complete mapping of the pituitary in a tuna species. The various different adenohypophysis cell types of the bluefin tuna, *Thunnus thynnus* L. have been identified and located using different antisera against mammalian and piscine hormones and various histochemical techniques: PAS, Alcian Blue pH 2.5 and lectins -ConA and WGA- (Neutral and Acidic Glycoproteins); Bromophenol Blue (Proteins) and Tioglycollate-Ferric-Ferricianide-FeIII (-S-S- groups). Prolactin (PRL) and adrenocorticotrophic (ACTH) cells were located in the rostral pars distalis (RPD) of the pituitary, while the proximal pars distalis (PPD) displayed gonadotrophic (GTH), thyrotrophic (TSH), somatotrophic (GH) and also a few PRL cells. Moreover, somatolactin (SL) and melanotrophic (MSH) cells were identified inside the pars intermedia (PI). Interestingly, some SL-immunoreactive fibers were also detected in the neurohypophysis. Some GTH cells were also located on the exterior surface of the PI. Glycoproteins containing mannose (Man) and/or glucose (Glc); N-acetyl-glucosamine (GlcNAc) and/or sialic acid sugar residues, as well as -S-S- groups, were observed in GTH, TSH and SL cells. The Bromophenol Blue technique stained amphiphilic SL, acidophilic GH cells and weakly ACTH cells. GH and ACTH cells were unreactive to PAS, Alcian Blue, Tioglycollate-Ferric-Ferricianide-FeIII and lectin (Con A and WGA) techniques. Finally, PAS reaction was positive in amphiphilic SL cells, which were PbH unreactive, while MSH and ACTH cells were stained with PbH technique.

Key words: Adenohypophysis, Hormones, Pituitary cells, Immunocytohistochemistry, Tuna, Teleost

Offprint requests to: Dr. Carmen Sarasquete, Institute of Marine Sciences of Andalucía (CSIC), Polígono Río San Pedro, Apdo. oficial, 11510 Puerto Real,Cádiz, Spain. e-mail: carmen.sarasquete@icman.csic.es

Introduction

Morphological and physiological aspects of the pituitary gland of teleostean fish have been studied for many years. The characterization of the different adenohyphophysial cell types is basic for later studies directed towards a better understanding of the physiological implications of this endocrine gland. The pituitary displays different families of structurally and functionally related adenohyphophysial hormones: single chain polypeptide and glycoprotein hormones (growth hormone -GH-. prolactin -PRL-; somatolactin -SL-; gonadotropins -GTH- and thyroid-stimulating -TSH- hormones) and proopiomelanocortin-derived hormones (adrenocorticotropic -ACTH- and melanophore-stimulating -MSH- hormones) (Ball and Baker, 1969; Batten and Ingleton, 1987; Rand-Weaver et al., 1991a,b; Rendón et al., 1997; Segura, 2000). In different teleost fishes, ACTH and PRL cells are generally found in the rostral pars distalis (RPD), GTH and TSH cells are observed in the proximal pars distalis (PPD), and MSH and SL cells appear in the pars intermedia (PI) (Carrillo, 1977; Olivereau and Nagahama, 1983; Batten, 1986; Cambré et al., 1986; Quesada et al., 1988; Farbridge et al., 1990; Nozaki et al., 1990; Toubeau et al., 1991; Yan and Thomas, 1991; Rand-Weaver et al., 1991a; Power, 1992; García-García et al., 1994; García-Hernández et al., 1996; Rendón et al., 1997; Sarasquete et al., 1997; Segura, 2000).

Antibodies prepared against mammalian pituitary hormones often show cross-reaction with their homologous piscine hormones (Follenius et al., 1978; Margolis-Kazan and Schreibman, 1981; Rendón et al., 1997; Segura, 2000). This cross-reaction is frequent for small and more conserved peptides, such as MSH or ACTH, whereas large and/or more evolved hormones show no cross-reaction or show heterologous cross-reactions (Olivereau et al., 1976; Margolis-Kazan and Schreibman, 1981; Cambré et al., 1986). Nowadays, molecular techniques of purification, cloning and sequencing of some piscine adenohypophysial

hormones, such as somatotropin, somatolactin and gonadotropins from *Sparus aurata*, *Solea senegalensis* and *Thunnus thynnus* (Martínez-Barberá et al., 1994; Pendón et al., 1994a,b, 1996; Okada et al., 1994; Astola et al., 1996; Kagawa et al., 1998) make it possible to obtain large amounts of new recombinant hormones, which have also proved very useful for the production of specific piscine antibodies.

Tuna is one of the most important groups of species in the fishery industry, caught in large numbers and well-accepted in the market. Bluefin tuna, *Thunnus thynnus* is the largest species of tuna, reaching more than 3 m in total length, 500 Kg in body weight, and having a particularly high economic value. In recent years, the exploitation of natural stocks of the bluefin tuna has increased to dangerous levels through pressure of increased fishing. The scarcity of this species and its high commercial value, together with its very high growth rates, makes it promising for marine aquaculture purposes (Davila, 1985). The complete control of sexual maturation and spawning represents an important objective for fish farming. Success in this obviously depends on understanding the mechanisms involved in the hormonal control of spawning under conditions of captivity, in which pituitary hormones play a fundamental role. The gonadotropin-releasing hormone (GnRH) is the main cerebral factor responsible for the secretion of gonadotropins (GTHs) from the pituitary (Breton et al., 1972). The presence of GnRH immunoreactive fibers has been reported in the pituitary of different fishes (Parhar and Iwata, 1994; Parhar, 1997; Rodríguez-Gómez et al., 1999: Stefano et al., 1999), establishing the role of this hormone in the release of GTHs, and suggesting that GnRH-innervation in the pituitary could also regulate PRL, GH and SL cells.

The aim of this paper is the characterization of the adenohyphophysial cell types present in the pituitary gland of the bluefin tuna, *Thunnus thynnus* L., by means of histochemical and immunohistochemical techniques, by using antisera directed against 3 hormone types: mammalian (anti-human ACTH), piscine (anti-carp α , ß GTH II, anti-carp ß GTH II and anti-salmon PRL), and recombinant piscine (anti-recombinant seabream GH and anti-recombinant sole SL) hormones. The results and conclusions reported in this paper could form a useful basis for future endocrine studies of the bluefin tuna (*Thunnus thynnus*) reproduction.

Material and methods

Adult specimens of bluefin tuna, *Thunnus thynnus* (150 Kg average weight) were captured in Barbate Almadrabe (Cádiz, SW Spain). All male and female specimens studied (May and June) were in active spermatogenesis and vitellogenesis phase (personal observation). Brains with the pituitary attached were then carefully removed and fixed with Bouin's solution (4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4, 0.2% picric acid) for 5 days in darkness at 4 °C. After fixation, tissues were cryoprotected in 15% sucrose in 0.1M phosphate buffer for 12 hours, and finally, embedded in tissue-tek (Sakura) and kept at -80 °C until processing. Parasaggital serial sections (16 μm) were obtained in a cryomicrotome (Cryocut-E, Reichert-Jung) and mounted on gelatin-coated glass slides.

Immunocytochemical staining was performed using a Streptavidin-biotin-peroxidase complex method. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in Coons buffer (0.01M Veronal, 0.15M NaCl) with 0.1% Triton X-100 (CBT) for 30 minutes. Before immunostaining, sections were transferred for 5 minutes to CBT and saturated in CBT with 0.5% casein for 30 min. Sections were incubated overnight in a moist chamber at room temperature with different primary rabbit antisera: anti-carp α, ß GTH-II and anti-carp ß GTH II, kindly provided by Dr. Burzawa-Gerard (both 1:3000 dilution), anti recombinant seabream growth hormone (GH, 1:1000 dilution) and anti sole somatolactin (SL, 1:4000 dilution), donated by Dr. Valdivia, anti salmon prolactin (PRL), donated by Dr. Kawauchi (1:10000 dilution), anti α melanotrophic stimulating hormone (α MSH), provided by Dr. Tramu (1:1000 dilution), as well as anti human adrenocorticotropic hormone (ACTH, 1-24) (1:3000 dilution) purchased from Peninsula Laboratories Inc. California. Anti-carp ß GTH II antiserum was revealed as specific for GTH cells and did not cross-react with TSH cells (Burzawa-Gerard, personal communication). Sections were washed in CBT and incubated for 1 hour at room temperature with biotinylated anti rabbit-IgG (Jackson) diluted 1:1000 in CBT-0.5% casein. After washing in CBT, sections were incubated 1 hour at room temperature with streptavidin-peroxidase complex (Jackson) diluted 1:1000 in CBT. Finally, sections were washed with CBT followed by Tris-HCl (0.05M, pH 7.4) and peroxidase activity was confirmed visually in Tris-HCl 0.05M, pH 7.6 containing 0.025% 3,3'-diaminobenzidine tetrahydrochlroride (DAB, Sigma, St Louis, MO) and 0.05% hydrogen peroxide. To distinguish TSH and GTH cells, contiguous sections were incubated with anti-carp α, ß GTH-II and anti-carp ß GTH II respectively. The specificity of the immunostaining was confirmed with controls that were performed by replacement of primary antisera with normal rabbit serum and with omission of primary antisera.

Haematoxylin-Eosin and Haematoxylin-Gutiérrez´V.O.F (light green-orange G-acid fuchsin) morphological staining techniques were performed according to Gutiérrez et al. (1985) and Sarasquete et al. (1993b, 1996). In this study, a variant of V.O.F. trichromic staining (Type II Gutiérrez´V.O.F polychrome), in which light green dye was substituted by methyl blue dye, was used. Histochemical tests such as: PAS, saponification-PAS, diastase-PAS, Alcian Blue pH 2.5, neuraminidase-type V from *Clostridium perfringens* (Sigma, St Louis, MO), chlorhydric hydrolysis-Alcian Blue, and Bromophenol Blue

Table 1. Histological and histochemical characteristics of the bluefin tuna, *Thunnus thynnus* secretory pituitary cells.

	H-E/H-VOF-I/II	PbH	PAS	AA	BFB	ConA	WGA	TFF-FeIII
GH	Acidophilic	-	-	-	+	-	-	-
PRL	Acidophilic	-	-	-	-	-	-	-
SL	Amphiphilic/Acidophilic	-	+	+	+	+	+	+
ACTH	Amphiphilic/Acidophilic	+	-	-	+/-	-	-	-
MSH	Basophilic	+	-	-	-	-	-	-
GTH	Basophilic	-	+	+	-	+	+	+
TSH	Basophilic	-	+	+	-	+	+	+

H-E: Haematoxylin-eosin; H-VOF-I: Haematoxylin-VOF I (Light green-Orange G-Acid fucsin); H-VOF-II: Haematoxylin-VOF II (Methyl Blue-Orange G-Acid fucsin); PbH: Plumb-Haematoxylin; PAS: Periodic acid-schiff: neutral glycoproteins; AA: Alcian Blue pH 2.5: Acidic glycoproteins; BFB: proteins; ConA: Glycoproteins containing mannose and/or glucose residues; WGA: Glycoproteins containing N-acetyl-glucosamine and/or sialic acid sugar residues; TFF-FeIII: Tioglycollate-Ferric-Ferricianide-FeIII.

reactions, as well as the Lead-Haematoxylin (PbH) technique, were used according to Pearse (1985) and Bancroft and Stevens (1990). In order to determine the existence of glucidic residues of the glycoproteins, the sections were washed (3x5 min) in Tris buffer saline (TBS), after endogenous peroxidase blockage, and then incubated in a moist chamber for 2 hours, at room temperature, in horseradish peroxidase (HRP)-conjugated lectins (20 μg/ml TBS). They were then tested for ConA, evidencing mannose and/or glucose, and WGA, evidencing N-acetyl-glucosamine and/or N-acetyl-neuraminic acid. After three washes in TBS, the peroxidase activity was confirmed visually with TBS containing 0.05% 3,3´-DAB and 0.015% hydrogen peroxide. Then the sections were washed in running tap water (10 min), dehydrated, cleared and mounted in Eukitt. Substitution of lectin-HPR conjugates by TBS was used as control. Tioglycollate-Ferric-Ferricianide-FeIII staining technique was also used in order to identify -S-S- residues.

Results

The hypophysis of the bluefin tuna, *Thunnus thynnus* L. was of the anterior-posterior type and displayed a highly developed neurohypohysis reaching all regions of the adenohypophysis. The pituitary could be divided into an anterior portion, the pars distalis (PD), and a posterior portion, the pars intermedia (PI). Within the PD, a rostral pars distalis (RPD) and a proximal pars distalis (PPD) could be recognized. Fig. 1 and Table 1 show the immunocytochemical and histochemical results.

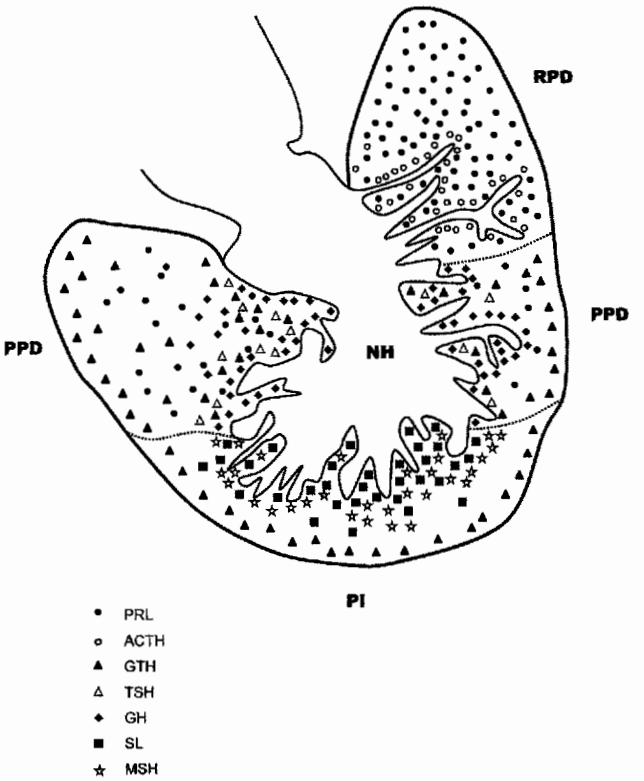

Fig. 1. Representation of the pituitary of bluefin tuna, *Thunnus thynnus*, showing the location of the adenohypophysis cells. NH: neurohypophysis; RPD: rostral pars distalis; PPD: proximal pars distalis; PI: pars intermedia.

Rostral pars distalis (RPD)

The RPD was located in the anterior portion of the pituitary and was occupied principally by PRL cells (Figs. 1, 2a) immunostained with anti-salmon-PRL antiserum. Most of these cells were ovoid in shape, although some were round. PRL cells exhibited tinctorial affinity for eosin/light green/methyl blue (Haematoxylin-Eosin/Haematoxylin-V.O.F´Gutiérrez I and II) and these acidophilic cells formed compact groups within RPD, though a few scattered individual cells could be identified in the internal part of PPD (Figs. 1, 2a). Furthermore, these cells were negative to PAS, Alcian Blue, Bromophenol Blue, Lectins (ConA and WGA) and Tioglycollate-Ferric-Ferricianide-FeIII techniques (Table 1).

Between PRL cell groups, and organized in cordons bordering the neurohypophysis, ACTH cells were located by using an anti-human ACTH antiserum (Figs.

1, 2b). This immunoreactive staining exhibited cross-reactivity with presumed MSH cells located in PI. ACTH cells were both round and ovoid in shape, and showed tinctorial affinity for lead-Haematoxylin (PbH). However, PAS, Alcian Blue, lectins (Con A and WGA) and Tioglycollate-Ferric-Ferricianide-FeIII techniques were all negative in these cells, which were weakly stained with Bromophenol Blue technique for protein identification (Table 1).

Proximal pars distalis (PPD)

Four cell-types: GTH, presumed TSH, GH and PRL cells, were identified. GTH cells were located in the PPD of the bluefin tuna, *Thunnus thynnus*, and were identified by using anti-carp ß GTH-II antiserum. These cells were located in the dorsal and ventral regions of PPD, in both the interior and exterior areas. These cells also extended caudally to constitute the exterior surface of the PI (Figs. 1, 2c). Histochemical techniques showed that GTH cells were basophilic, displaying PAS and Alcian Blue pH 2.5 positive granules in their cytoplasm. Bromophenol Blue technique was negative in these cells, which were positive to WGA and ConA lectins and Tioglycollate-Ferric-Ferricianide-FeIII techniques, confirming the presence of glycoproteins containing GlcNAc and/or sialic acid, mannose and/or glucose residues, as well as -S-S- groups (Table 1).

By using anti-carp α, ß GTH-II antiserum, only a few presumptive TSH cells were located in the internal part of the PPD, among the GTH cells (Figs. 1, 2e). Anti-carp α, ß GTH-II antiserum immunostained both

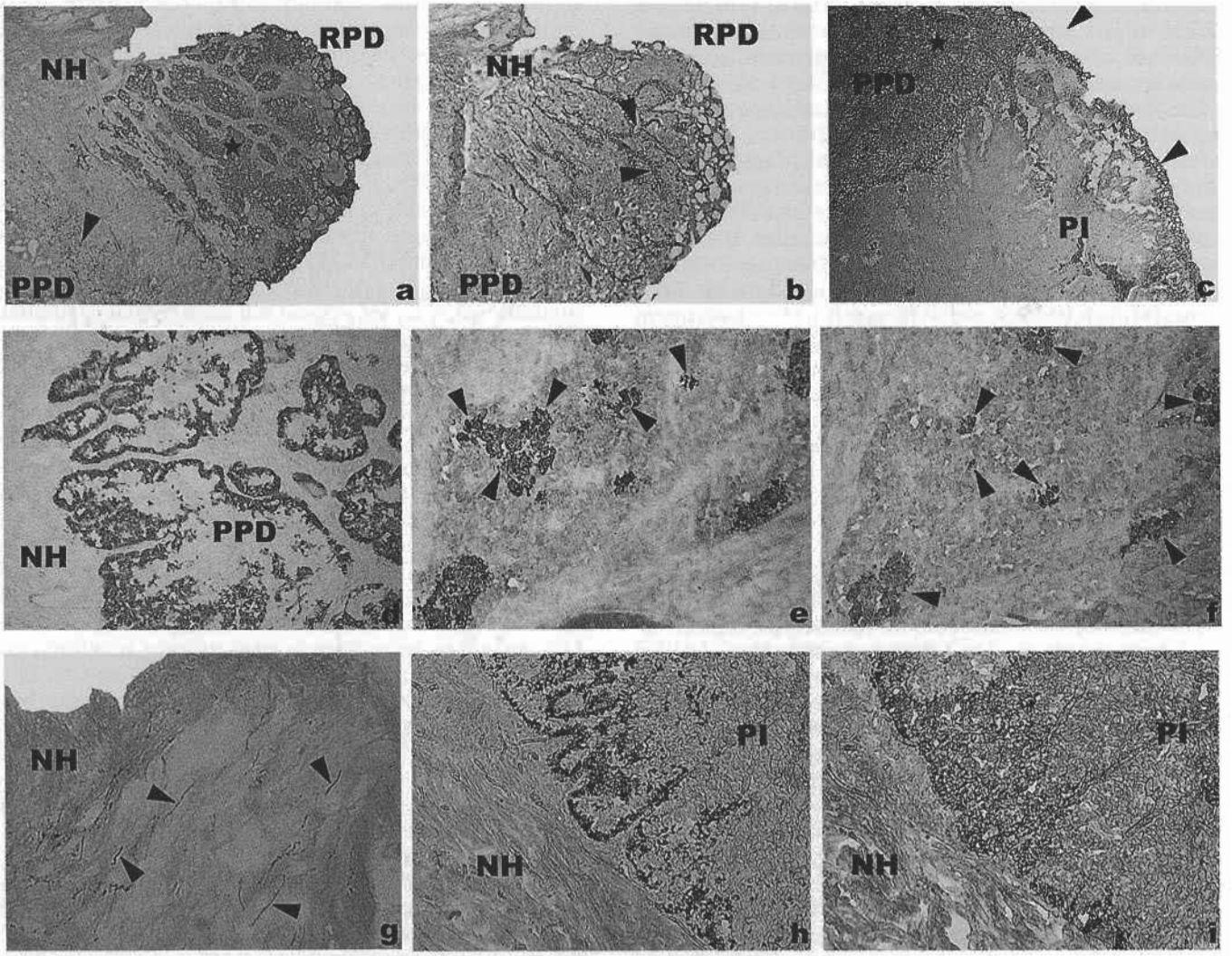

Fig. 2. Pituitary of *Thunnus thynnus*. **a.** PRL cells. Anti-salmon PRL. x 20. **b.** ACTH cells. Anti-human ACTH. x 20. **c.** GTH cells. Anti-carp ß GTH-II. x 20. **d.** GH cells. Anti-recombinant seabream GH. x 64. **e.** Detail of GTH and presumed TSH cells in the PPD. Note unspecific immunostaining of presumed TSH cells (arrowheads). Anti-carp α ß GTH-II. x 80. **f.** Detail of GTH cells in the adjacent slide. Anti-carp ß GTH-II. x 80. **g.** Detail of SL immunoreactive fibers in the neurohypophysis. Anti-recombinant sole SL. x 200. **h.** SL cells. Anti-recombinant sole SL. x 80. **i.** MSH cells in the adjacent slide. Anti-α-MSH. x 80

GTH and TSH cell types. The identification of TSH cells was made by comparison of contiguous sections stained with anti-carp ß GTH-II and anti-carp α, ß GTH-II. Anti-carp ß GTH-II antiserum reacted with GTH cells but did not react with presumed TSH cells (Figs. 2e, 2f), which displayed the same histochemical characteristics as those described for GTH cells (Table 1).

Anti-recombinant seabream growth hormone antiserum was used to identify specifically the GH cells. These cells were more abundant in the deepest interior of the PPD, in close contact with the neurohypophysis (Figs. 1, 2d). These round cells were acidophilic, with tinctorial affinity for Light Green or Methyl Blue (VOF-I/II´Gutiérrez polychromes). GH cells were stained by Bromophenol Blue (proteins) and they were negative to PAS, Alcian Blue, lectins (ConA and WGA) and Tioglycollate-Ferric-Ferricianide-FeIII techniques, suggesting the absence of neutral and acid glycoproteins and -S-S- groups (Table 1). Furthermore, some scattered PRL cells could also be identified in the interior of the PPD (Figs. 1, 2a).

Pars intermedia

Three cell types could be observed in the PI of the bluefin tuna, *Thunnus thynnus*: GTH, SL and α MSH cells. As previously described, GTH cells were found on the outer surface of the PI (Figs. 1, 2c). However, both SL and a MSH cells were located in the interior part; the groups of SL cells are distributed in close contact with the neurohypohysis interdigitations, enveloping the MSH cells (Figs. 1, 2h, 2i). Amphiphilic SL cells were identified using anti-recombinant sole somatolactin antiserum and showing a PAS-positive reaction. They were also stained with Bromophenol Blue, Con A and WGA lectins, and contained -S-S- groups (Table 1). Further, with respect to the SL cells in the PI of the adenohypophysis, a few SL immunoreactive fibers were identified along the neurohypophysis, suggesting a brain source (Fig. 2g). MSH cells were located using anti-α MSH antiserum, which cross-reacted slightly with the ACTH cells of the RPD. These cells were basophilic, displaying negative results to the rest of the histochemical techniques used (Table 1).

Discussion

An immunocytochemical and histochemical study of *Thunnus thynnus* L. adenohypophysis has been designed in order to identify the different endocrine cell types. The general morphology of the pituitary of bluefin tuna species is in accordance with that described for other teleosts. Three distinct zones profusely interdigited by the neurohypophysis were observed: the RPD, PPD and PI.

PRL cells have been generally described in the RPD of both freshwater and marine teleostean fish (Naito et al., 1983; Toubeau et al., 1991; Yan and Thomas, 1991; Power, 1992; Rendón et al., 1997; Segura, 2000). However in bluefin tuna, PRL cells were found in the PPD, as in other species, e.g. *Morone saxatilis* (Huang and Specker, 1994), *Seriola dumerilii* (García-Hernández et al., 1996) and *Plecoglosus altivelis* (Saga et al., 1999). In a recent study using histochemical techniques exclusively (Kagawa et al., 1998), these cells were not identified in the *Thunnus thynnus* L. PPD-pituitary. The migration of PRL cells from the RPD to other zones of the pituitary in the early stages of life has been suggested to explain this particular distribution in different teleost fishes (Naito et al., 1983; Farbridge and Leatherland, 1986).

ACTH cells have been described in different teleosts (Olivereau et al., 1976; Munro, 1985; Cambré et al., 1986; Toubeau et al., 1991; García-Hernández et al., 1996; Rendón et al., 1997; Segura, 2000). Their distribution in cordons bordering the masses of PRL cells and in close contact with the neurohypohysis is general in all the teleostean fish studied. In the bluefin tuna, as in other species (Follenius et al., 1978; Ball and Batten, 1981; Munro, 1985; Cambré et al., 1986; Yan and Thomas, 1991; García-Hernández et al., 1996; Rendón et al., 1997; Segura, 2000), but with the exception of the barbel, *Barbus barbus* (Toubeau et al., 1991), the anti-human ACTH antiserum cross-reacted with the presumed MSH cells of the PI. This cross-reaction is due to both ACTH and MSH hormones having their origin in a common precursor molecule, the proopiomelanocortin (POMC), showing a similar molecular structure. Iturriza and Estivariz (1986) showed that the negative PAS reaction in chromophobic ACTH and MSH cells, confirmed that teleosts were unable to glycosylate POMC. Although the secretion of ACTH in MSH cells cannot be discounted (Wendelaar-Bonga, 1993), our results suggest that both groups of cells are clearly differentiated; in this sense, only PbH-positive cells of the PI were intensely stained with α-MSH antisera.

The location of TSH cells in the ventral and dorsal part of PPD is similar to that described in *Solea senegalensis* (Rendón et al., 1997); however in *Solea vulgaris* (Nuñez-Rodríguez, 1985), *Seriola dumerilii* (García-Hernández et al., 1996) and *Diplodus sargus* (Segura, 2000), presumptive TSH positive cells were only displayed in the ventral PPD; the presence of TSH cells in the dorsal PPD has also been described in *Barbus barbus* (Toubeau et al., 1991). To date, antisera raised against purified preparations of fish TSH have not yet been performed, although Yoshiura et al. (1999) have recently developed the molecular cloning of the cDNA encoding of the ß subunit of thyrotrophin in *Carassius auratus*. Previously, in several species, antiserum against human-TSH has selectively immunostained TSH cells (Ueda et al., 1983; Van Putten et al., 1983; Munro, 1985; Cambré et al., 1986; Siegmund et al., 1987; García-Hernández et al., 1996; Segura, 2000), although this was not possible in the bluefin tuna pituitary (data unshown). As previously developed in *Solea senegalensis* by Rendón et al. (1997), we have used both anti-carp α, ß

GTH-II and anti-carp ß GTH-II to distinguish the TSH and GTH cells. In glycoprotein hormones, while the α-subunit is the most conserved molecular part, the ß-subunit is specific to each hormone and seems to agree with the biological specificity (Pierce and Parsons, 1981). The α subunit of gonadotrophins is similar to the α subunit of thyrotrophins, in that antisera raised against α and ß GTH normally react with both GTH and TSH cells (Burzawa-Gerard, 1974), while the specificity of the ß subunit was improved to distinguish the GTH cells from the TSH cells using an antisera raised against ß GTH.

GTH cells are distributed in the interior of the PPD and the exterior border portions of the PPD and PI. These cells were located using anti-catfish ß GTH-II antiserum. The recent isolation of two chemically distinct GTH cells from several teleosts (Suzuki et al., 1988; Swanson et al., 1991; Van der Kraak et al., 1992; Koide et al., 1993; Copeland and Thomas, 1993; Okada et al., 1994) has made it possible to identify two GTH cell types. Kagawa et al. (1998), using anti-*Tunnus obesus* ß GTH-I and ß GTH-II antiserum, identified GTH I and GTH II cells in the pituitary of the bluefin tuna. In that study it was found that the GTH cells of the interior of the PPD corresponded to GTH I cells, while the immunostained cells of the exterior, on the surfaces of the PPD and PI, were described as GTH II cells. Although Kagawa et al. (1998) studied immature tuna fish, our results in vitellogenic fish seem to indicate a similar distribution of these two GTH cell types. In salmonids it has been proved that GTH I is important for the early phases of gonadal growth and that GTH II is involved in the control of final maturation (Nozaki et al., 1990; Swanson et al., 1991). In this context, and in the bluefin tuna, *in vitro* experiments indicate than tuna GTH II is more potent that tuna GTH I in stimulating production of 17ß estradiol and testosterone by ovarian follicles of tuna (Okada et al., 1994).

In contrast to observations in striped bass (Huang and Specker, 1994) and in Mediterranean yellowtail (García-Hernández et al., 1996), only two homogeneous populations of GH cells have been identified in the ventral and dorsal portions of the PPD of bluefin tuna pituitary; these cells were similar in shape, immunostaining intensity and organization. GH cells have previously been located using anti-recombinant seabream GH antiserum with similar results to observed in killifish, *Fundulus heteroclitus* (Sarasquete et al., 1997), Senegalese sole, *Solea senegalensis* (Rendón et al., 1997) and in white seabream, *Diplodus sargus* (Segura, 2000). In other species, and using anti-chum, anti-coho salmon, and anti-trout GH antisera (Batten, 1986; Cambré et al., 1986), groups of GH cells were also observed in the ventral and dorsal regions of the PPD. In contrast to the findings reported in those fish species, in which other anti-GH antisera were used (Batten, 1986; Cambré et al., 1986), anti recombinant seabream GH antisera did not cross-react with PRL or SL cells in the bluefin tuna pituitary.

The distribution of SL and MSH cells in bluefin pituitary, is in accordance with that described in the majority of teleost fish, where SL cells are in close contact with the neurohypophysis interdigitations in the PI, enveloping MSH cells (Rand-Weaver et al., 1991a; Olivereau and Rand-Weaver, 1994; García-Hernández et al., 1996). However, two exceptions to this general pattern have been observed in *Solea senegalensis*, where this distribution was the opposite (Rendón et al., 1997) and in *Diplodus sargus* pituitary (Segura, 2000) where both cell types (MSH and SL) were mixed in the interior of the PI. Further studies must be made to determine whether this difference in the location of SL and MSH cells could indicate differences in hormonal regulation.

A particularly interesting finding in the pituitary of the bluefin tuna was the presence of SL-immunoreactive fibers throughout the neurohypohysis. This finding could suggest the presence of SL cells in different brain areas; in this sense, Mousa and Mousa (1999) in the brain of the *Oreochromis niloticus*, by using a specific chum salmon SL antisera, described immunoreactive cells in nucleus preopticus periventricularis, habenula, midbrain tegmentum, nucleus preopticus basalis lateralis and organum vasculosum luminae terminalis. Future studies could investigate whether these SL immunoreactive fibers in the neurohypohysis of the bluefin tuna could be the result of a cross-reaction with other brain factors with a similar molecular structure, or could really correspond to SL fibers with a brain source. In this way, SL mRNA has been detected, in addition to different somatic tissues (gill, heart, kidney and liver), in the brain of the rainbow trout, *Oncorhyncus mykiss* (Yang et al., 1997, 1999), using reverse transcription-polymerase chain reaction (RT-PCR) and DNA blot hybridization. However, in spite of these results, it is too soon to confirm that these SL cells detected in the brain really innervate the hypohysis. Future studies have to be developed, specially those related with the distribution of SL cells and fibers in the brain of the bluefin tuna.

Finally, SL cells of bluefin tuna pituitary were positive to PAS, Bromophenol Blue (proteins) reactions and contain -S-S- groups. These cells were also reactive with Con A and WGA lectins suggesting the presence in SL cells of glycoproteins containing mannose and/or glucose, as well as N-acetyl-glucosamine and sialic acid sugar residues. Similar histochemical results were observed in *Solea senegalensis* pituitary (Rendón et al., 1997). Interestingly, in vitro studies performed in other teleostean species suggest that pituitary secretes both non-glycosylated and glycosylated SL forms (Pendón et al., 1998).

As bluefin tuna, *Thunnus thynnus*, a species of great economic and commercial interest, is a potential new species for mariculture, the immunocytochemical distribution of the hormone pituitary cell types presented here and other hormonal studies under consideration (i.e. hormonal receptors, distribution of GnRH and SL fibers in brain, etc.) will provide a basis for future studies on its reproductive physiology, such as variation of these

pituitary cells during the annual reproductive cycle, as well in captive conditions and/or under hormonal treatments.

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