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An immunocytochemical study of the pituitary gland of the white seabream (*Diplodus sargus)*

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Summary

The adenohypophysis of the white seabream (*Diplodus sargus*) was studied using histochemical and immunocytochemical techniques. The adenohypophysis was composed of rostral pars distalis, proximal pars distalis and pars intermedia. Prolactin (anti-chum salmon prolactin positive) and adrenocorticotropic (anti-human ACTH positive) cells were found in the rostral pars distalis. Prolactin cells were organized into follicles, while ACTH cells were arranged in cords around neurohypophyseal tissue branches that penetrated the rostral pars distalis. In the proximal pars distalis, somatotropic (anti-chum salmon and anti-gilthead seabream growth hormone positive), gonadotropic (anti-chum salmon β-gonadotrophin II and anti-carp β-gonadotrophin II positive, but anti-chum salmon β-gonadotrophin I negative) and thyrotropic (anti-human β-thyrotropin positive) cells were observed. Growth hormone cells were restricted to the dorsal and ventral part of the proximal pars distalis. They were clustered or surrounded the neurohypophyseal branches. Only one type of gonadotrophin cell was identified and they were clustered or isolated in the proximal pars distalis. Scattered groups of thyrotropin cells were located throughout the proximal pars distalis. In the pars intermedia somatolactin (anti-chum salmon and anti-gilthead seabream somatolactin positive) and melanotropic (anti-α-melanotropic hormone positive) cells were localized. In addition, gonadotrophin cells surrounded the pars intermedia or distributed evenly between somatolactin and melanotropic hormone cells. Somatolactin cells were periodic acid-Schiff negative and surrounded the neurohypophyseal branches intermingled with melanotropic cells. These cells were also immunoreactive to anti-human ACTH antiserum.

Introduction

The identification and the distribution of the different cell types in the pituitary gland of teleosts has 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 (Follenius & Doerr-Schott 1978, Nagahama *et al.* 1981, Batten 1986, Cambré *et al.* 1986, Farbridge & Leatherland 1986, Quesada *et al.* 1988, Yan & Thomas 1991, Huang & Specker 1994, García-Hernández 1996, Rendón *et al.* 1997, Vissio *et al.* 1997, Parhar *et al.* 1998). 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 included gonadotrophins (GTHs) and thyrotropin (TSH); and (iii) proopiomelanocortin-derived hormones such as adrenocorticotropic (ACTH) and melanotropic hormone (MSH) (Batten & Ingleton 1987).

According to the immunocytochemical studies, PRL and ACTH cells occupy the rostral pars distalis (RPD), GTH and TSH cells the proximal pars distalis (PPD), and MSH and SL cells the pars intermedia (PI). SL has been found only in teleosts but not in other vertebrates (Rand-Weaver *et al.* 1991, Olivereau & Rand-Weaver 1994). On the other hand, two forms of GTH (GTH I and GTH II) have been demonstrated in some teleosts (Nozaki 1990, Magliulo-Cepriano *et al.* 1994) but not in others (Yan & Thomas 1991, García-Hernández *et al.* 1996).

The white seabream (*Diplodus sargus* L.) is a protandrous hermaphrodite euryhaline teleost that lives in environments of different salinities (Micale *et al.* 1987, Arias & Drake 1990). Recently, studies on feeding, growth and reproduction of *Diplodus sargus* have improved culture of this species (Divanach *et al.* 1982, Mazzola *et al.* 1983, Cejas *et al.* 1993, Abellan *et al.* 1995, Mordenti *et al.* 1996). 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 histological and immunocytochemical approaches. The identification of pituitary cell types will provide useful information for future physiological studies on pituitary gland function in *Diplodus sargus*.

Table 1. Primary antisera used in this study.

Antisera raised against	Source	Dilution
Chum salmon PRL	Dr. H. Kawauchi[a]	1 : 10000
Human ACTH	Peninsula Laboratories[b]	1 : 3000
Chum salmon GH	Dr. H. Kawauchi[a]	1 : 10000
Sea bream GH	Dr. M. M. Valdívia[c]	1 : 1000
Human β-TSH	NHPP[d]	1 : 200
Carp α,β GTH II	Dra. E. Burzawa-Gerard[e]	1 : 1000
Carp β GTH II	Dra. E. Burzawa-Gerard[e]	1 : 8000
Chum salmon β GTH I	Dr. H. Kawauchi[a]	1 : 500
Chum salmon α, β GTH II	Dr. H. Kawauchi[a]	1 : 1000
Chum salmon β GTH II	Dr. H. Kawauchi[a]	1 : 5000
Bovine α-MSH	Dr. Wendelaar Bonga[f]	1 : 3000
Chum salmon SL	Dr. H. Kawauchi[a]	1 : 1000
Sea bream SL	Dr. M. M. Valdívia[c]	1 : 1000

[a]Kitasato, Japan. [b]Belmont, CA, USA. [c]Cádiz, Spain. [d]NHPP National Hormone and Pituitary Program, Torrance, CA, USA. [e]Paris, France. [f]Nijmegen, Holland.

Materials and methods

Immature specimens of white seabream (*Diplodus sargus* L.) (n =10, 100–150 g body weight) were provided by a fish culturing centre (El Toruño, Consejería de Agricultura y Pesca, Junta de Andalucía, El Puerto de Santa María, Cádiz, Spain). Fish were transferred to the wet laboratories of the Faculty of Marine Science, Puerto Real, Cádiz; where they were kept for 1 month under natural photoperiod and temperature until full acclimatization (April–May 1998). The fish were housed in 1,500 L tanks with permanent water turnover and oxygen supply. They were fed twice a day with *Illex* sp.

The fish were anaesthetized with 2-phenoxyethanol dissolved in the water (1 ml/L water) and killed by decapitation. The brains were dissected out, placed in Bouin's fluid for 48 h, and then were dehydrated and embedded in paraffin wax. Sagittal and transverse (8 μm thick) sections were obtained. The sections were stained with haematoxylin–eosin, periodic acid-Schiff technique (PAS, Mc-Manus 1948) and Alcian blue-PAS-Orange G (AB–PAS–OG, Adams and Swettenham 1958) for histochemistry. For immunocytochemistry, tissue sections were immunostained according to the unlabelled enzyme method of Sternberger (1986). The primary rabbit antisera and working concentrations shown in Table 1 were used. Sections were examined by white field microscopy.

The antisera against chum salmon PRL, GH, SL, β-GTH I, β-GTH II and α,β-GTH II were kindly provided by Dr. H. Kawauchi, Kitasato, Japan (see Kawauchi *et al.* 1983, 1986, Suzuki *et al.* 1988a,b, Kaneko *et al.* 1993). The anti-recombinant sea breamGH and anti-sbSL were kindly provided by Dr. M. Valdívia, Cádiz, Spain (Martínez-Barberá *et al.* 1994, Astola *et al.* 1996). The recombinant seabream antisera used showed good and specific cross-reaction with GH- and SL-producing cells of other teleost fishes (Rendón *et al.* 1997, Sarasquete *et al.* 1997). The anti-human ACTH serum was provided by Península Laboratories (California, USA). This antiserum showed cross-reactivity with the MSH cells of PI. The anti-bovine mono-acetyl α-MSH were kindly provided by Dr. S.E. Wendelaar Bonga and it showed a very weak cross-reactivity with the ACTH cells of the RPD (van Zoest *et al.* 1989). The anti-carp α,β-GTH II and anti-carp β-GTH II were kindly provided by Dr. E. Burzawa-Gerard (Dubourg *et al.* 1985). The anti-human β-TSH was kindly provided by NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) and NHPP (National Hormone and Pituitary Program) (California, USA).

All sections were incubated for 18 h at 22 °C in the primary antiserum. The second antiserum (anti-rabbit IgG, raised in goat and kindly provided by Dr. P. Fernández-LLebrez, Málaga, Spain) was used at a dilution of 1 : 40 for 60 min at 22 °C and the PAP complex (1 : 100) (Sigma) for 45 min at 22 °C. 3,3′-Diaminobenzidine tetrahydrochloride (DAB; Sigma) was used as electron donor. All antisera and the PAP complex were diluted in TRIS buffer, pH 7.8, containing 0.7% non-gelling seaweed gelatin, λ-carrageenan (Sigma), 0.5% Triton X-100 (Sigma), and 0.02% sodium azide. Coplin jars were used for incubation in the first and the second antisera, whereas PAP incubation was carried out in a moist chamber. To enhance the immunoreaction, 0.04% DAB plus 0.04% ammonium nickel sulphate hexahydrate (Fluka) was used.

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 rabbit serum was used instead of primary antiserum. No positive structures or cells were found in these sections.

Results

The adenohypophysis of *Diplodus sargus* showed the three major subdivisions typical of teleost: rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). According to the histochemical and immunocytochemical behaviour, seven different cells were identified: PRL and ACTH cells in the RPD; GH, GTH and TSH cells in the PPD; and SL, MSH and GTH cells in the PI (Figure 1). Moreover, neurohypophysial processes penetrated the different adenohypophyseal areas while a pituitary stalk was patent (Figure 2A).

Using the AB–PAS–OG method, orange-stained cells were observed at RPD. In this same location, cells immunostained with the anti-sPRL antiserum were organized in follicles and occupied almost all the RPD. No PRL cells were found outside the RPD (Figure 2B). Most of these cells were ovoid and displayed round nuclei (Figure 2C). Some of them were located close to the neurohypophyseal extensions and displayed cytoplasmatic processes that crossed the layer of ACTH cells and contacted the neurohypophysial tissue (Figure 2D). In some specimens (n = 2), the RPD showed a decrease in intensity of immunoreaction with the anti-sPRL with respect to the intensity shown by PRL cells in other specimens. The PRL cells presented heterogeneous immunostaining with a weakly immunoreactive cytoplasm

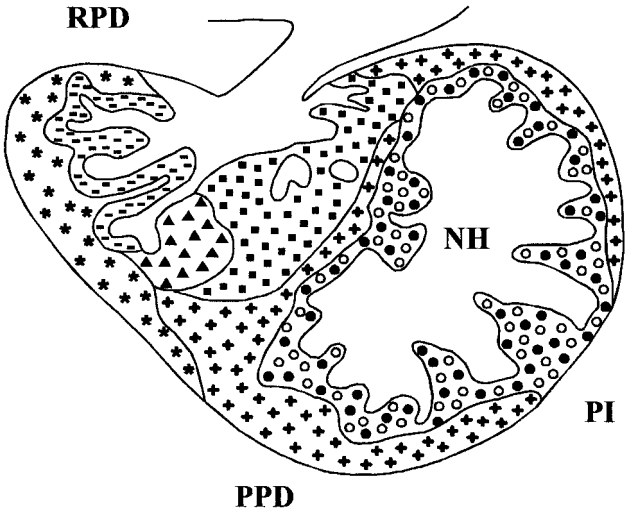

Figure 1. Schematic sagittal representation of the pituitary of *Diplodus sargus*, showing the distribution of adenohypophyseal cells. RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pas intermedia; NH, neurohypophysis. PRL (∗), ACTH (−), GH (■), GTH (+), TSH (▲), SL (●), and MSH (○) cells.

showing some areas strongly immunostained. These cells also showed a spherical and patent nucleus (Figure 2E).

Anti-sGH and anti-sbGH antisera labelled cells in the dorsal and ventral region of the PPD. No differences were observed between the two antisera used. Isolated or clustered GH cells were found near to the neurohypophyseal processes. Individual GH cells were also found inside the RPD and PI (Figure 2F). The GH cells were orange-coloured with the AB–PAS–OG method and had a spherical or oval shape with an irregular oval nucleus (Figure 2G).

In the PI, immunopositive cells to anti-sSL and anti-sbSL were found. The labelling pattern was similar with the two antisera. No cross-reactivity was observed between SL antisera and PRL or GH cells (Figure 2H). In addition, SL positive cells were not stained with PRL or GH antisera. SL cells were negative to the PAS method and they surrounded and contacted the neurohypophyseal processes intermingled among MSH cells. SL cells showed an oval or elongated shape with a patent nucleus (Figure 2I).

The use of anti-human ACTH (1-24) serum revealed in sagittal sections of the pituitary two types of immunoreactive cells: one in the RPD and another in the PI. In addition, scarce isolated or clustered immunopositive cells were observed in the PPD (Figure 3A). In the RPD, ACTH immunoreactive cells were located between the PRL cells and the neurohypophyseal projections, arranged in cords, around the neural tissue. These cells showed homogeneous immunoreactivity and were round or elongated in shape with an irregular nucleus (Figure 3B). Cells in the same location as ACTH cells were stained purple with the AB-PAS-OG method.

In the PI, anti-α-MSH positive cells occupied the same region as that stained with anti-human ACTH (1-24) antiserum. In addition, anti-α-MSH antiserum also immunostained isolated cells in the PPD and very weakly the ACTH cells of the RPD (Figure 3C). Cells located in the same region as MSH cells were stained orange with the AB–PAS–OG method. These cells showed an elongated shape and surounded the neurohypophysial processes, intermingled with SL cells (Figure 3D).

PAS-positive cells were observed in the PPD. According to their location, and using immunocytochemical methods, these cells were often gonadotropic and thyrotropic cells. Both anti-carp β-GTH II and anti-chum salmon β-GTH II stained cells in the PPD (Figure 3E); in contrast, anti-chum salmon β-GTH I did not stain any structure. Gonadotropic cells differed in size and shape and were found isolated or clustered in the dorsal and ventral part of the PPD. According to their location, they were stained blue with the AB–PAS–OG method. GTH cells were also found around the PI and some among the SL and MSH cells inside the PI (Figure 3F).

In addition to cells immunoreactive for β-GTH II only, anti-carp α,β-GTH II and anti-chum salmon α,β-GTH II showed stained cells located in the anterior dorsal part of PPD. These cells were of small size, round shape and appeared to be stained purple with the AB–PAS–OG method. Similar cells were specifically immunostained with anti-human β-TSH (Figure 3G–H).

Discussion

PRL cells

In this study we have used an antiserum against chum salmon PRL that showed a quite good and specific cross-reaction with PRL cells of *Diplodus sargus*, as has been reported for other teleosts (Batten 1986, Cambré *et al.* 1986, Quesada *et al.* 1988, Yan & Thomas 1991, Rendón *et al.* 1997, Parhar *et al.* 1998). 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, *Diplodus sargus* PRL cells are exclusively localized in the RPD (Nagahama *et al.* 1981, Munro 1985, Farbridge & Leatherland 1986; Toubeau *et al.* 1991, Yan & Thomas 1991, Huang & Specker 1994).

Our results showed that some specimens had a relative reduction of immunoreactivity in PRL cells with respect to other fish, indicating a decrease in the amount of antigen present in the cytoplasm. According to some authors, a reduced immunoreactivity may be correlated to an activated release of this hormone (see Ruijter & Creuwels 1988, Mancera *et al.* 1993, 1995). The reason for this putative activation is not known. In addition to an osmoregulatory role (see below), PRL has been related to stress (Avella *et al.* 1991, Wendelaar Bonga 1997). If these fish were stressed and presented an activation of PRL cells, this could explain the atypical pattern of immunoreactivity in PRL cells. However, further studies will be needed to confirm this suggestion.

The osmoregulatory role of PRL in hypo-osmotic environments is well established in teleosts, and especially in euryhaline species (Bern 1983, Hirano 1986, Mancera *et al.* 1993). *Diplodus sargus* is an euryhaline teleost that lives in

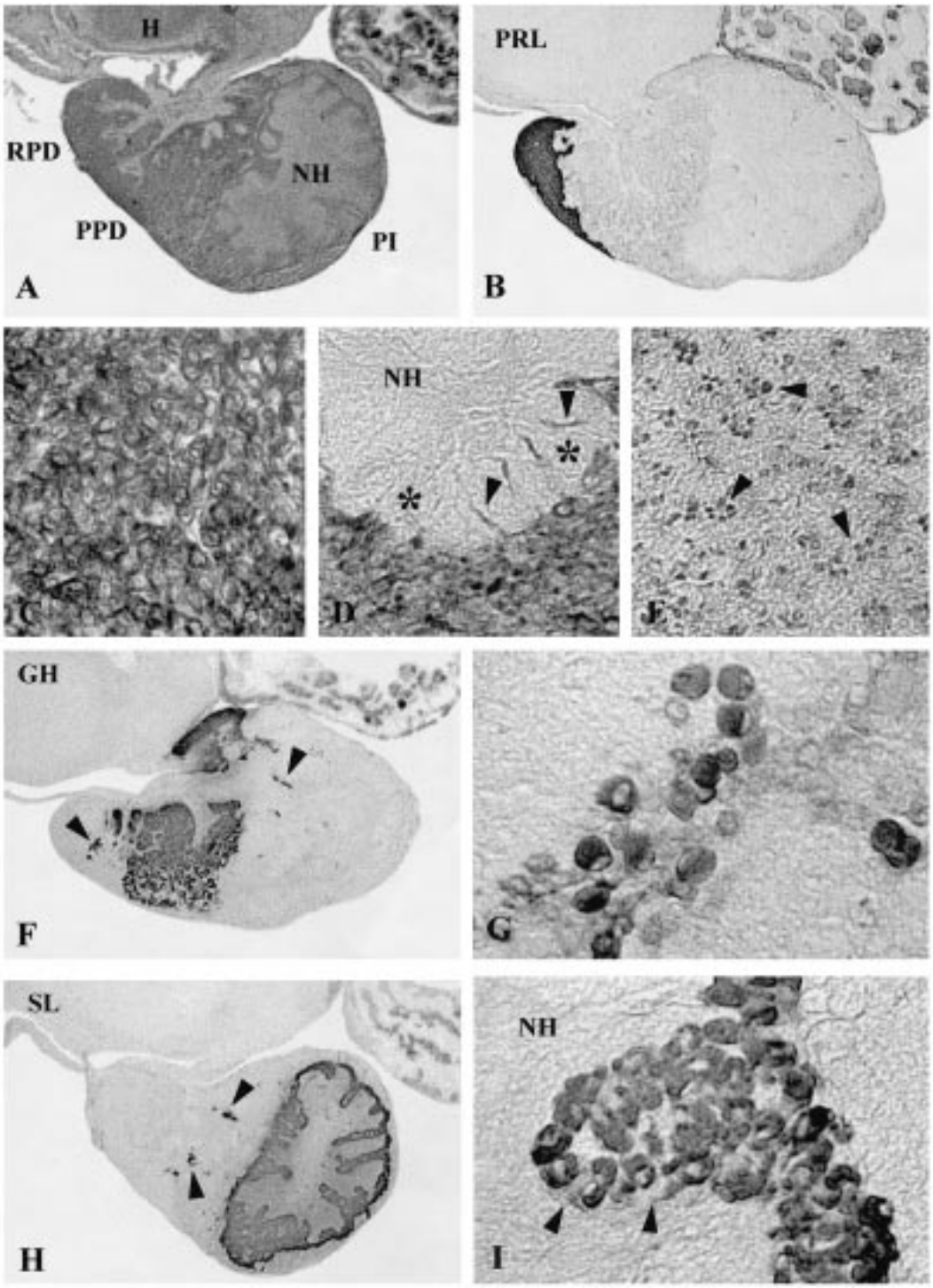

Figure 2. (A) Sagittal section of the basal hypothalamus (H) and the pituitary of *Diplodus sargus* stained with haematoxylin–eosin. The adenohypophysis shows the three typical zones: rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Note the patent pituitary stalk and the neurohypophyseal branches going into the adenohypophyseal area. ×55. (B) Section immunostained with anti-chum salmon PRL showing immunoreactive cells only in the RPD. ×55. (C) Detail of PRL immunopositive cells. ×760. (D) Note the extension of PRL cells (arrowheads) that cross the layer of ACTH cells (asterisks) and contact with neurohypophyseal branches. ×760. (E) Some specimens showed a decreased reactivity in the cells with strongly immunostained regions in the cytoplasm (arrowheads) ×760. (F) GH immunoreactive cells are located mainly in PPD but isolated cells were also observed in RPD and PI (arrowheads). ×55. (G) Detail of GH cells. ×760. (H) SL immunopositive cells are located in PI and clustered cells also are observed at PPD (arrowheads). ×55. (I) Detail of the SL cells in the PI. These cells surround the neurohypophyseal branches (NH) intermingled with MSH cells (arrowheads). ×760.

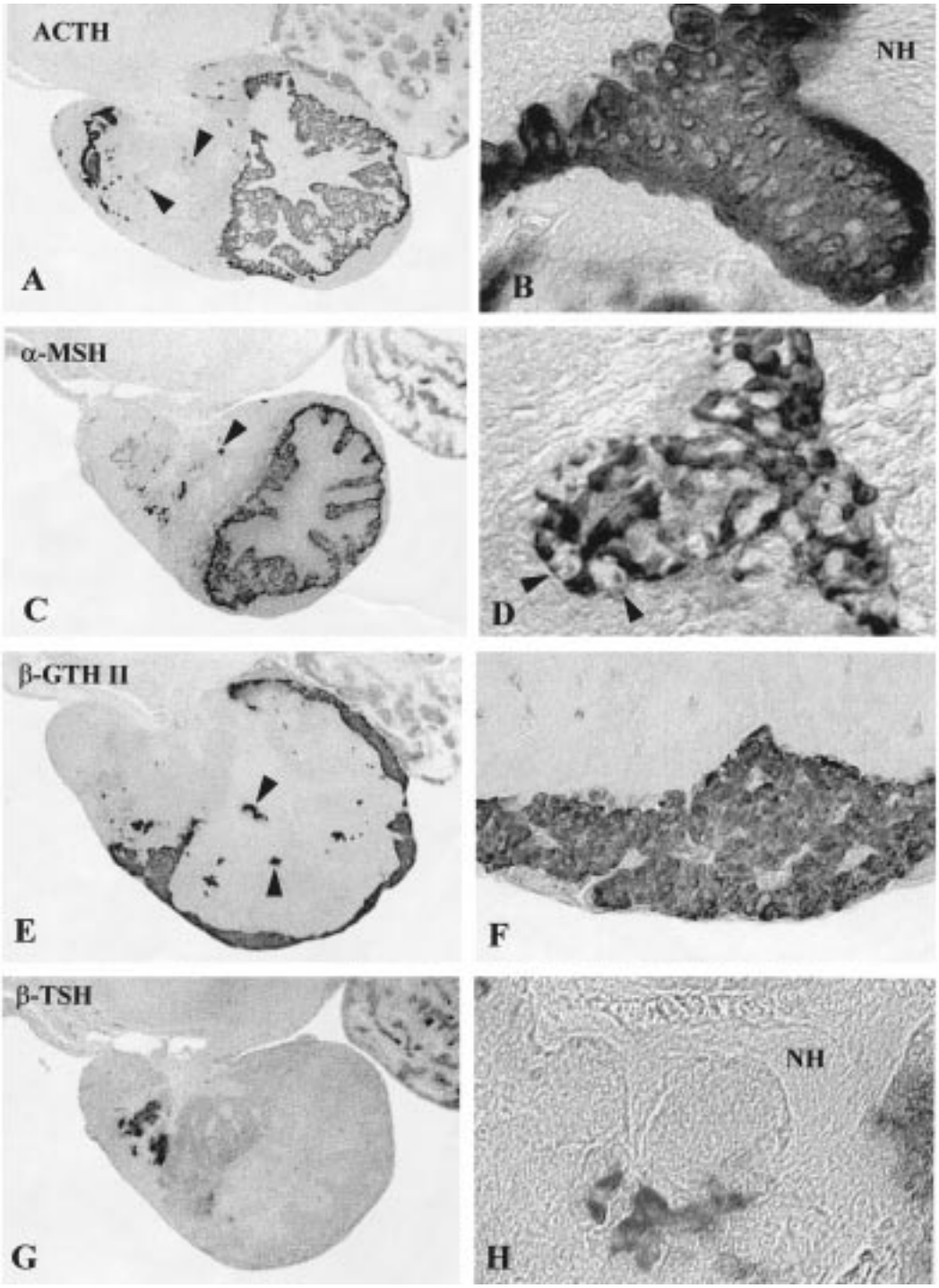

Figure 3. (A) Sagittal section immunostained with anti-human ACTH (1–24). Immunoreactive cells are localized in RPD and PI. Isolated immunoreactive cells are observed in the PPD (arrowheads). ×55. (B) Detail of ACTH cells in the RPD bordering the neurohypophyseal branches. ×760. (C) MSH immunoreactive cells are located in PI and some isolated cells are observed in PPD (arrowheads). ×55. (D) Detail of MSH cells at PI intermingled with SL cells (arrowheads) and surrounded by the neurohypophyseal processes. ×760. (E) GTH immunoreactive cells are located in PPD and bordering the PI. Isolated cells are observed between SL and MSH cells (arrowheads). ×55. (F) Detail of GTH cells. ×360. (G) TSH immunopositive cells are located at anterodorsal region of PPD. ×55. (H) Detail of TSH cells. ×760.

environments of different salinity, coastal water (sea water) and estuaries (brackish water), depending on its life cycle (Arias & Drake 1990). Studies on morphological changes in PRL cells, using immunocytochemical and ultrastructural techniques, and on changes in plasma concentration of PRL in brackish water- and sea water-adapted fishes will be useful for understanding the osmoregulatory role of PRL in this species.

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 (Tanaka *et al.* 1995, Mancera *et al.* 1995, Vissio *et al.* 1997, Parhar *et al.* 1998), and anti-sbGH that specifically reveals GH cells in the RPD of *Sparus aurata* (Martínez-Barberá *et al.* 1994), *Solea senegalensis* (Rendón *et al.* 1997) and *Fundulus heteroclitus* (Sarasquete *et al.* 1997). Both antisera strongly stained GH cells of *Diplodus sargus* that were restricted, similarly to other teleost fish, to the dorsal and ventral parts of the PPD (Nagahama *et al.* 1981, Batten 1986, Farbridge & Leatherland 1986, Quesada *et al.* 1988, Toubeau *et al.* 1991, Yan & Thomas 1991, Huang & Specker 1994, Rendón *et al.* 1997, Vissio *et al.* 1997, Parhar *et al.* 1998).

In most of these teleosts, only one type of GH cell has been reported. However, two distinct GH cell populations with different size, shape and intensity of immunoreaction have been described in the striped bass *Morone saxatilis* (Huang & Specker 1994) and Mediterranean yellowtail *Seriola dumerilii* (García-Hernández *et al.* 1996). Our results showed that *Diplodus sargus* only presented one type of GH cells in the PPD.

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). Thus, in some euryhaline species it has been found that GH cells were activated in fish acclimatized to brackish water (Abraham 1974, Benjamin 1978, Mancera *et al.* 1995). *Diplodus sargus* is an euryhaline teleost capable of living in different environmental salinities (Arias & Drake 1990). It will be interesting to check the effect of decreased environmental salinity on the activity of GH cells in this species.

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 analysed the structure, localization and physiological role of SL. However, the function of this hormone remains unclear (see Kaneko 1996). In this study, we used antisera against chum salmon and recombinant gilthead seabream SL. The SL genes seem to be highly conserved and the protein homology is high among the different teleost species (Rand-Weaver and Kawauchi 1993, Kaneko 1996). Our results showed that both anti-SL antisera reacted only with the SL cells of *Diplodus sargus*. 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).

The PAS technique has revealed two different types of cells in the PI of non-salmonid species: PAS-positive and PAS-negative cells. Immunocytochemical studies indicated that PAS-negative were MSH cells while PAS-positive were SL cells. Biochemical analyses have shown the existence of N-glycosylation sites in the SL of several teleosts but not in salmonid species. The existence of these sites has been related to the positivity of SL cells to the PAS technique and for this reason SL cells are PAS-negative in salmonids (Rand-Weaver *et al.* 1991, Kaneko 1996). In addition, SL cells also were PAS-negative in larval stages of the gilthead seabream *Sparus aurata* (Villaplana *et al.* 1997). This observation supported previous reports on the presence of a glycosylated and a non-glycosylated form of SL in *Sparus aurata* (Cavari *et al.* 1995). Also, non-glycosylated and glycosylated forms of SL have been demonstrated in *Solea senegalensis* (Pendón *et al.* 1997). Our results for *Diplodus sargus* show that SL cells were PAS-negative and suggest that in this species, as in salmonids, SL was present only in a non-glycosylated form.

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). As mentioned above, other hormones of the GH/PRL family play a role in osmoregulation, i.e. PRL presents a hypo-osmotic role (Bern 1983, Hirano 1986) and GH promotes adaptation to sea water (Sakamoto *et al.* 1993, Mancera & McCormick 1998). The related structure of these hormones and the euryhaline pattern of *Diplodus sargus* suggest a possible role of SL in osmoregulation. However, further studies will be necessary to confirm this suggestion.

ACTH and α-MSH cells

Corticotropic cells of *Diplodus sargus* were immunostained by an antiserum against human ACTH (1-24), the same one used for other teleost species (Follenius & Dubois 1980, Munro 1985, Cambré *et al.* 1986, Quesada *et al.* 1988, García-Hernández *et al.* 1996, Rendón *et al.* 1997, Parhar *et al.* 1998). This antiserum also immunostained the MSH cells present in the PI of these species and also in *Diplodus sargus* as depicted by our results. Only in the barbel *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 *Diplodus sargus* using anti-human ACTH.

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 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 antisera specifically immunostained melanotropic cells of the teleost PI (Munro 1985, Batten 1986, Cambré *et al.* 1986, Quesada *et al.* 1988, Toubeau 1991, García-Hernández *et al.* 1996, Rendón *et al.* 1997). In *Diplodus sargus* the MSH cells surround the neurohypophysal 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). However, in *Solea senegalensis* (Rendón *et al.* 1997) and *Thalassoma duperry* (Parhar *et al.* 1998) MSH cells appear to surround the SL cells. In addition to the PI, isolated or clustered MSH cells have also been found in the PPD of *Seriola dumerilii* (García-Hernández *et al.* 1996). Our results also showed isolated MSH cells in the PPD of *Diplodus sargus*.

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). On the other hand, MSH has been related to adaptation to a different background colour (Baker *et al.* 1984) and also in stress response (Wendelaar Bonga 1997). The use of different experimental designs for the activation of ACTH or MSH cells will be useful for understanding the role of these hormones in *Diplodus sargus*.

TSH and GTH cells

The family of adenohypophyseal 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. Recently, the cloning of the cDNA of this subunit has been reported for the goldfish *Carassius auratus*, and the antiserum is under development (Yoshiura *et al.* 1999). Usually, antibodies against the β-subunit of the human TSH showed a good cross-reactivity with TSH cells in teleosts (Schreibman & Margolis-Kazan 1979, Ueda *et al.* 1983, Van Putten *et al.* 1983, Cambré *et al.* 1986, Quesada *et al.* 1988, García-Hernández *et al.* 1996, Vissio *et al.* 1997). However, in some species a weak cross-reactivity with GTH cells has been observed (Batten *et al.* 1986, Quesada *et al.* 1988, Yan & Thomas 1991). In our study, anti-human β-TSH showed a good cross-reactivity with the β-TSH subunit of *Diplodus sargus*.

In addition, the use of antisera against chum salmon or carp α,β-GTH II revealed GTH and TSH cells, while antisera against chum salmon or carp β-GTH II immunostained GTH cells specifically. The use of both antisera on consecutive sections led us to detect putative TSH cells. The distribution of these putative TSH cells is identical to the pattern obtained after the use of anti-human β-TSH. In *Diplodus sargus*, as for other teleosts, TSH cells are located in the dorsal part of PPD (Schreibman & Margolis-Kazan 1979, Ueda *et al.* 1983, Van Putten *et al.* 1983, Cambré *et al.* 1986, Quesada *et al.* 1988, García-Hernández *et al.* 1996, Vissio *et al.* 1997).

The distribution of GTH cells in *Diplodus sargus* is similar to that reported in other teleosts, the GTH cells being located in the dorsal and ventral portions of PPD (Schreibman & Magolis-Kazan 1979, Olivereau & Nagama 1983, Munro 1985, Batten 1986, Toubeau *et al.* 1991). In addition, GTH were found around the PI of *Diplodus sargus*. This distribution has been also reported in other teleosts (Cambré *et al.* 1986, Quesada *et al.* 1988, Yan & Thomas 1991, García-Hernández *et al.* 1996, Vissio *et al.* 1997, Rendón *et al.* 1997).

Two different GTHs (GTH I and GTH II) have been reported in salmonids and non-salmonid species (Nozaki *et al.* 1990, Swanson *et al.* 1991, Copeland & Thomas 1993, Okada *et al.* 1994). GTH I and GTH II each consist of a common α-subunit and a hormone specific β-subunit. The β-GTH II showed amino acid sequences quite similar in different teleosts; however β-GTH I appears to be less conserved (Kawauchi *et al.* 1989, Swanson *et al.* 1991). In some species, two different types of GTH have been reported (Nozaki *et al.* 1990, Naito *et al.* 1991). 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 stage of the fish (Quesada *et al.* 1988, Yan & Thomas 1991, García-Hernández *et al.* 1996).

In *Diplodus sargus*, anti-carp β-GTH II and anti-chum salmon β-GTH II reveal a single type of gonadotropic cell located in PPD and PI. However, the anti-chum salmon β-GTH I did not show any kind of immunoreactivity in the adenohypophysis at all. This negative result could be ascribed to the high degree of difference between amino acid sequences of β-GTH I of *Oncorhynchus keta*, used as antigen, and that of *Diplodus sargus*. In addition, it is possible that only a GTH, with high similarity to GTH II but not GTH I, was present in this species. However, the existence of two types of GTH in the pituitary of the gilthead seabream (Elizur *et al.* 1996) and red seabream (Gen *et al.* 2000) has been replaced. For this reason, the existence of GTH I and GTH II would be expected in the pituitary of the white seabream.

From our results, it is not possible to answer the question of whether one or two types of gonadotrophins/gonadotropic cells exist in *Diplodus sargus*. For the complete investigation of the gonadotropic cells in this species, it will be necessary to study the modifications of gonadotropic cells during the sexual cycle in male and female specimens, and during the sexual inversion process of this protandrous hermaphrodite species. In addition, the use of homologous and specific antisera to GTH I and GHT II will also be necessary.

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