Histochemical Journal **29**, 813–822 (1997)

An immunocytochemical study of pituitary cells of the Senegalese sole, *Solea senegalensis* (Kaup 1858)

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Received 8 May 1997 and in revised form 8 October 1997

Summary

Different antisera directed against mammalian and piscine pituitary hormones, as well as a battery of various conventional histochemical techniques (PAS, Alcian Blue pH 2.5, Bromophenol Blue) and lectins, were used to identify the different hormonal cell types in the pituitary of the Senegalese sole, *Solea senegalensis*. Prolactin and adrenocorticotrophic cells were located in the rostral pars distalis of the pituitary. Gonadotrophic, thyrotrophic 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 α-melanotrophic cells were located in the pars intermedia of the *Solea senegalensis* pituitary. The PAS reaction was positive in somatolactin cells, which were unreactive with the lead–Haematoxylin technique, whereas melanotrophic cells were positive. Glycoproteins containing mannose and/or glucose, as well as N-acetyl-glucosamine and sialic acid sugar residues, are synthesized and secreted by gonadotrophic, thyrotrophic and somatolactin cells. Adrenocorticotrophic cells and, especially, the amphiphilic somatolactin and acidophilic growth hormone cells were stained with the Bromophenol Blue technique that identifies proteins in general, but adrenocorticotrophic and growth hormone cells were unreactive towards PAS, Alcian Blue pH 2.5 and lectins (Con A and WGA).

Introduction

The pituitary gland of teleosts has been the subject of research for many years. Three main families of adenohyphophysial hormones, structurally and functionally related, have been described: trophic hormones, namely growth hormone (GH), prolactin (PRL) and somatolactin (SL); glycoprotein hormones, particularly gonadotrophins (GTH) and thyroid-stimulating hormone (TSH); and the proopiomelanocortin-derived hormones, adrenocorticotrophic hormone (ACTH) and melanophore-stimulating hormone (MSH) (Ball & Baker, 1969; Batten & Ingleton, 1987; Rand-Weaver *et al*., 1991a,b). Advances in immunocytochemical and histochemical techniques have been particularly useful for identifying these pituitary hormones and their localization in segregated pituitary cell types. Generally, ACTH and PRL cells are found in the rostral pars distalis, GTH and TSH cells in the proximal pars distalis and MSH and SL cells in the pars intermedia (Carrillo, 1977; Olivereau & Nagahama, 1983; Batten, 1986; Cambré *et al*., 1986; Quesada *et al*., 1988; Farbridge *et al*., 1990; Nozaki *et al*., 1990; Rand-Weaver *et al*., 1991a; Toubeau *et al*., 1991; Yan & Thomas, 1991; Power, 1992; García-García *et al*., 1994; García-Hernández *et al*., 1996; Sarasquete *et al*., 1997).

Antibodies prepared against mammalian pituitary hormones often cross-react with their homologous piscine hormones and have been used to localize different fish pituitary cell types (Follenius *et al*., 1978; Margolis-Kazan & Schreibman, 1981). This is true, especially for small and conserved peptides, such as MSH or ACTH, but sometimes no cross-reaction or heterologous cross-reactions are observed for large and/or less conserved hormones (Olivereau *et al*., 1976; Margolis-Kazan & Schreibman, 1981; Cambré *et al*., 1986). This problem was resolved in part by the preparation of antibodies against purified fish pituitary hormones. Recently, the cloning of some fish adenohypophysial hormones, such as GH and SL from *Sparus aurata* and *Solea senegalensis*, have provided the tools to obtain large amounts of recombinant hormones, which

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have been used to prepare specific antibodies (Martínez-Barberá *et al*., 1994; Pendón *et al*., 1994a,b, 1996; Astola *et al*., 1996).

Senegalese sole, *Solea senegalensis* (Kaup, 1858), a species adapted to temperate waters, was described for the first time on the Mediterranean coasts by Rodríguez and Rodríguez (1980). This species is commonly exploited in extensive aquaculture in some southern European countries, such as Spain (Drake *et al*., 1984) and Portugal (Dinis, 1992), and also on the African coast in Tunisia (Fehri-Bedoui, 1997). Recently, some papers concerning its biology and pathology (Rodríguez, 1984; Sarasquete *et al*., 1993a), immunocytochemical and cytochemical aspects of its oogenesis (Gutiérrez *et al*., 1985; Sarasquete *et al*., 1993b), larval development (Mourente & Vázquez, 1996; Sarasquete *et al*., 1996) and isolation and cloning of somatotrophin (GH) and somatolactin (SL) pituitary hormones (Pendón *et al*., 1994a,b) have been published. However, at present, there is little information on the reproductive physiological aspects of this species, such as its reproduction in captive conditions or under hormonal treatments and the distribution and changes of hypophysial hormones during the annual cycle, etc.

In this paper, we present an histochemical and immunocytochemical distribution of the different cell types in the pituitary gland of the Senegalese sole, *Solea senegalensis*, using several histochemical techniques and antisera directed against mammalian (anti-human ACTH), piscine (anti-carp α, β GTH II; anti-carp β GTH II; anti-salmon PRL), synthetic (anti-α-MSH) and recombinant piscine (anti-recombinant seabream GH; anti-recombinant sole SL) pituitary hormones.

Materials and methods

Adult specimens of Senegalese sole, *Solea senegalensis*, were purchased from a local fishery (Cupimar, San Fernando, Spain) and kept in the laboratory in running seawater. Specimens were anaesthetized with 2-phenoxiethanol (Sigma, St Louis, MO, USA) and perfused via the aortic bulb with 0.6% saline solution followed by Bouin fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, 0.2% picric acid). Brains with the pituitary attached were then carefully removed and further post-fixed in the same fixative for 6 h. After fixation, tissues were washed in distilled water and embedded in paraffin. Parasaggital serial sections (7 μm) were mounted on gelatin-coated glass slides and deparaffinized through xylene–ethanol–water.

Immunocytochemical staining was performed using a streptavidin–biotin–peroxidase complex method. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in Coons buffer (0.01 M Veronal, 0.15 M NaCl) with 0.1% Triton X-100 (CBT) for 30 min. Before immunostaining, sections were transferred to CBT for 5 min 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, β gonadotrophin (GTH) II and anti-carp β 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, 1:8000 dilution), donated by Dr Valdivia, anti-salmon prolactin (PRL), donated by Dr Kawauchi (1:10 000 dilution), anti-α melanotrophic stimulating hormone (α-MSH), provided by Dr Tramu (1:8000 dilution), as well as anti-human adrenocorticotrophic hormone (ACTH) 1–24 (1:3000 dilution), purchased from Peninsula Laboratories (CA, USA). Anti-carp β GTH II antiserum was revealed as specific for GTH cells and did not cross-react with TSH cells (E. Burzawa-Gerard, personal communication). Sections were washed in CBT and incubated for 1 h at room temperature with biotinylated anti-rabbit IgG diluted 1:1000 in CBT–0.5% casein. After washing in CBT, sections were incubated for 1 h at room temperature with streptavidin–peroxidase complex diluted 1:1000 in CBT. Finally, sections were washed with CBT followed by Tris-HCl (0.05 M, pH 7.4), and peroxidase activity was visualized in 0.05 M Tris-HCl, pH 7.6, containing 0.025% 3,3 diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis) and 0.05% hydrogen peroxide or 0.03% 4-chloro-1-naphthol (Sigma) and 0.03% hydrogen peroxide. Double immunocytochemical reactions were performed on the same sections to distinguish TSH and GTH cells. First, the sections were incubated with anti-carp β GTH II, processed as previously described and developed using DAB (brown staining). Subsequently, the antibodies were eluted, and the sections were incubated with anti-carp α, β GTH II, processed as indicated previously, and peroxidase activity was detected using 4-chloro-1-naphthol (blue staining). To confirm the specificity of the immunostaining, controls were performed by replacement of primary antisera with normal rabbit serum and omission of primary antisera.

Haematoxylin–Eosin and Haematoxylin–Gutiérrez' VOF (Light Green–Orange G–Acid Fuchsin) morphological techniques were performed according to Gutiérrez *et al*. (1985) and Sarasquete *et al*. (1993b, 1996). In this study, a variant of VOF trichromic (type II Gutiérrez' VOF polychrome), in which Light Green dye was substituted by Methyl Blue dye, was used. Histochemical tests, such as PAS, saponification–PAS, diastasa–PAS, Alcian Blue pH 2.5, neuraminidase type V from *Clostridium perfringens* (Sigma), chlorhydric hydrolysis–Alcian Blue, Bromophenol Blue reactions, as well as lead–Haematoxylin (PbH) techniques, were used according to Pearse (1985) and Bancroft *et al*. (1990). In order to determine the existence of some glucidic residues of the glycoproteins, the sections were washed (3×5 min) in Tris-buffered saline (TBS) after endogenous peroxidase blockage and then incubated in a moist chamber for 2 h at room temperature in horseradish peroxidase (HRP)-conjugated lectins (20 μg ml^{-1} TBS): ConA, showing mannose and/or glucose; and WGA, showing *N*-acetyl-glucosamine and/or *N*-acetyl-neuraminic acid. After three washes in TBS, the peroxidase activity was visualized with TBS containing 0.05% 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 a control.

Results

The pituitary gland of the Senegalese sole, *Solea senegalensis*, is of the anterior–posterior type. The neurohypophysis interdigitates profusely within all regions of the adenohypophysis, which can be divided further into an anterior portion, the pars distalis (PD), and a posterior portion, the pars intermedia (PI). Based on its histology, two regions are recognized in the PD (Figs 1 and 2a): the rostral pars distalis (RPD) and the proximal pars distalis (PPD). Immunocytochemical and histochemical results obtained in the pituitary gland of *Solea senegalensis* are summarized in Fig. 1 and Table 1 respectively.

Rostral pars distalis (RPD)

In *Solea senegalensis*, the RPD is located in the anterior portion of the hypophysis. The prolactin cells, immunostained with the anti-salmon-PRL antiserum, occupied the major part of the RPD (Fig. 2b). Most of these cells were round in shape, although some were polygonal. These acidophilic cells, which form a compact cell mass, showed tinctorial affinity for Eosin/Light Green/Methyl Blue (Haematoxylin–Eosin/Haematoxylin–Gutiérrez' VOF I and II). These cells were negative to PAS, Alcian Blue and Bromophenol Blue techniques (Table 1, Fig. 3a and b).

A smaller number of round and polygonal cells with tinctorial affinity for lead–Haematoxylin (PbH) appear to be organized in cordons bordering the PRL cells or as islets between PRL cells and the neurohypophysis. These cells showed positive immunoreactivity against the anti-human ACTH antiserum and also exhibited cross-reactivity with presumptive MSH cells located in the pars intermedia (Fig. 2c). ACTH cells had no affinity for PAS or Alcian Blue and lectin reactions, but they were weakly stained with the general protein technique, Bromophenol Blue (Table 1, Fig. 3a and b).

Proximal pars distalis

Three types of cells can be observed in the PPD of *Solea senegalensis*: GTH, presumptive TSH and GH cells. GTH cells appear in the dorsal and ventral aspects of PPD, extending caudally to form the external border of the PI. These cells were immunoreactive against anti-carp α, β GTH-II (Fig. 2d and f) and anti-carp β GTH-II (Fig. 2e). They are basophilic and show granules positive for PAS and Alcian Blue

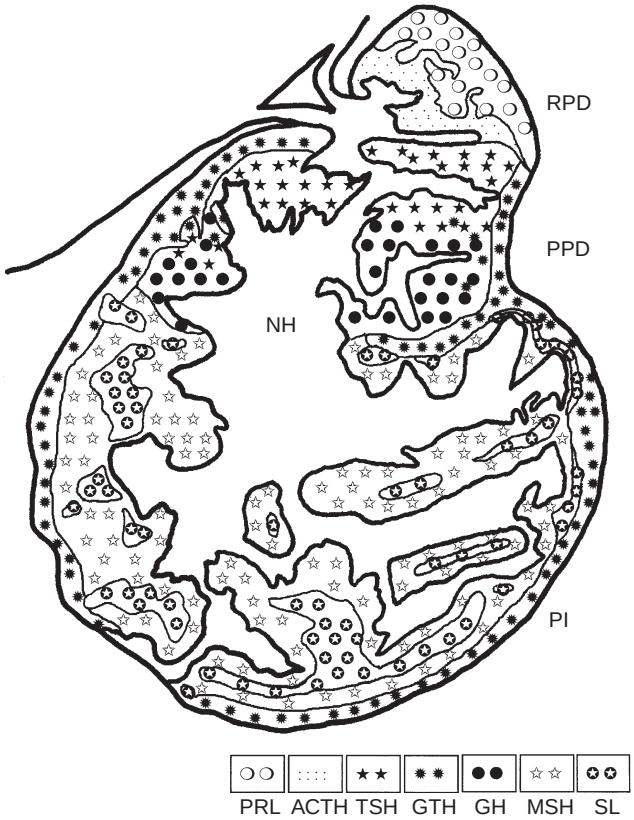
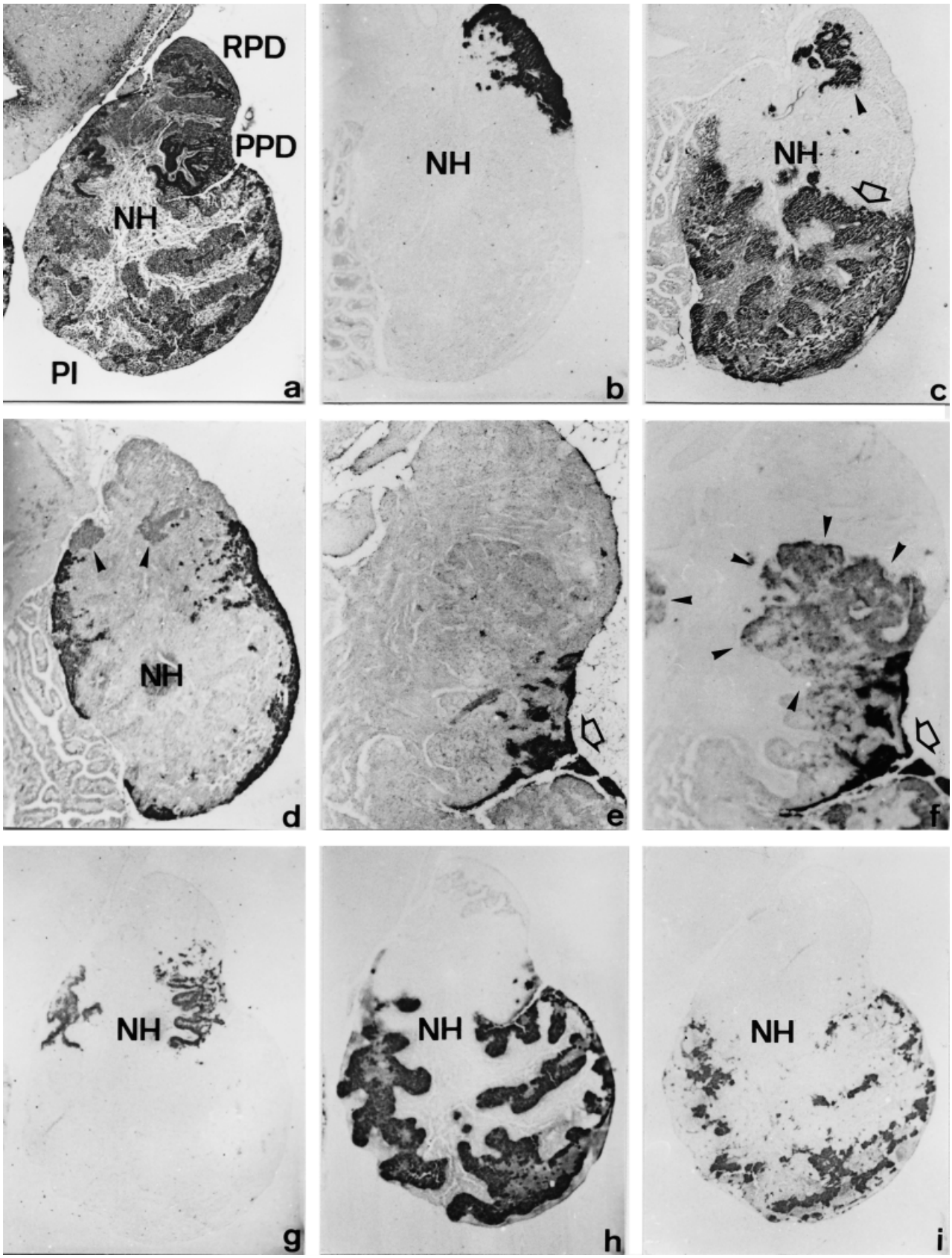


Fig. 1. Diagrammatic representation of the pituitary of *Solea senegalensis*, showing the distribution of secretory cell types. NH, neurohypophysis; RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia.

Table 1. Histological and histochemical characteristics of the Senegalese sole, *Solea senegalensis*, secretory pituitary cells

	H–E	*H–VOF-I/II*	*PbH*	*PAS*	*AA*	*BFB*	*ConA*	*WGA*
PRL	Acidophilic	Acidophilic	−	−	−	−	−	−
ACTH	Amphiphilic/Acidophilic	Amphiphilic/Acidophilic	+	−	−	±	−	−
GTH	Basophilic	Basophilic	−	+	+	−	+	+
TSH	Basophilic	Basophilic	−	+	+	−	+	+
GH	Acidophilic	Acidophilic	−	−	−	+	−	−
SL	Amphiphilic/Acidophilic	Amphiphilic/Acidophilic	−	+	+	+	+	+
	Basophilic	Basophilic	+	−	−	−	−	−

Abbreviations: H–E, Haematoxylin–Eosin; H–VOF-I, Haematoxylin–VOF-I (Light Green–Orange G–Acid Fuchsin); H–VOF-II, Haematoxylin–VOF-II (Methyl Blue–Orange G–Acid Fuchsin); PbH, lead–Haematoxylin; PAS, Periodic acid–Schiff: neutral glycoproteins; AA, Alcian Blue pH 2.5: acidic glycoproteins; BFB, general proteins; ConA, glycoproteins containing mannose and/or glucose residues; WGA, glycoproteins containing *N*-acetyl-glucosamine and/or sialic acid sugar residues.


RPD
PPD
NH
PI
a
NH
b
NH
c
NH
d
e
f
NH
g
NH
h
NH
i

pH 2.5 in their cytoplasm. These cells were unstained with the Bromophenol Blue technique. Moreover, the positive reaction of the gonadotrophs to lectins (WGA and ConA) shows the presence of glycoproteins containing *N*-acetyl glucosamine and/or sialic acid, as well as mannose and/or glucose residues (Table 1).

Presumptive TSH cells are located among the gonadotrophs and somatotrophs, being more abundant in the anterior and central region of the PPD (Fig. 1). The location of these TSH cells was determined by comparison of contiguous sections processed with anti-carp β GTH-II (Fig. 2e) and anti-carp α, β GTH-II (Fig. 2f). Anti-carp β GTH antiserum reacts with GTH cells but does not react with presumptive thyrotrophs (Fig. 2e). In turn, anti-carp α, β GTH-II antiserum immunostains both GTH and TSH cells (Fig. 2d and f). These results were corroborated using double immunocytochemical techniques on the same sections and revealing with different chromogens (data not shown).

The histochemical properties of the basophilic presumptive TSH cells were similar to those described for GTH cells (Table 1).

GH cells reacted specifically with anti-recombinant seabream growth hormone antiserum and appear to be organized as small cordons in close contact with neurohypophysis interdigitations (Fig. 2g). These acidophilic cells are round or ellipsoid in shape and show tinctorial affinity for Light Green or Methyl Blue of the VOF I or II Gutierrez polychromes (Table 1). Somatotrophs were negative to PAS and Alcian Blue reactions but were strongly stained with the Bromophenol Blue technique, which identifies general proteins (Table 1, Fig. 3a and b). Lectin techniques (ConA and WGA) were negative in GH cells (Table 1).

Pars intermedia

Three cell types were observed in the PI of *Solea senegalensis*: SL, α-MSH and GTH cells (Fig. 1). The first type includes amphiphilic/acidophilic and PAS-positive cells stained with Bromophenol Blue (Table 1, Fig. 3c). Con A and WGA lectins were positive in these cells (Table 1). SL cells were immunoreactive against anti-recombinant sole somatolactin antiserum (Fig. 2i). Around SL cells, we observed groups of cells stained with lead–Haematoxylin (Fig. 3d). These PbH-positive cells were immunoreactive against the anti-α-MSH antiserum, which cross-reacts only slightly with ACTH cells (Fig 2h). As has been described previously, GTH cells also appear at the border of the PI (Fig. 2d).

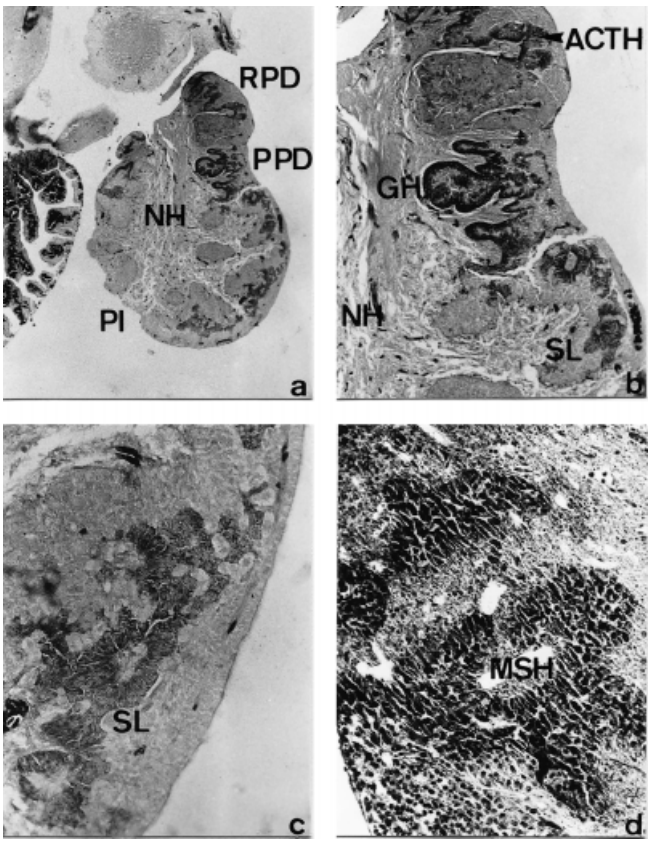


Fig. 3. Pituitary of *Solea senegalensis*. (a) Bromophenol Blue technique ×23. Positive cells to Bromophenol Blue reaction (general proteins) are magnified in b (×58) and c (×152). (d) Lead–Haematoxylin reaction showing positive MSH cells in the PI × 154. NH, neurohypophysis; RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia.

Discussion

As in many other teleosts, the adenohypophysis of the Senegalese sole, *Solea senegalensis* (Kaup, 1858), is subdivided into three distinct zones profusely infiltrated by the processes of the neurohypophysis: the RPD, the PPD and the PI, in which different cell

Fig. 2. Pituitary of *Solea senegalensis*. (a) Haematoxylin–Eosin staining ×37. (b) PRL cells. Anti-salmon PRL ×37. (c) ACTH cells (arrowhead). Note unspecific immunostaining of MSH cells in the pars intermedia (PI; open arrow). Anti-human ACTH ×37. (d) GTH cells. Note unspecific immunostaining of presumptive TSH cells in the PPD (arrowheads). Anti-carp α, β GTH-II ×37. (e) Detail of GTH cells (open arrow). Anti-carp α GTH-II ×85. (f) Detail of GTH cells (open arrow) and presumptive TSH cells (arrowheads). Anti-carp α, β GTH ×85. (g) GH cells. Anti-recombinant seabream GH ×37. (h) α-MSH cells. Anti-α-MSH ×37. (i) SL cells. Anti-recombinant sole SL ×37. The streptavidin–biotin–peroxidase complex method was used to reveal secretory cell types. NH, neurohypophysis; RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia.

types can be identified using histochemical and immunocytochemical techniques. Prolactin cells (PRL) of the *Solea senegalensis* pituitary have been identified as acidophilic cells, forming the bulk of the RPD. These cells were specifically immunostained with anti-salmon PRL antiserum. PRL cells have been detected in the RPD of both freshwater and marine teleosts (Nagahama *et al*., 1981; Naito *et al*., 1983; Yan & Thomas, 1991; Power, 1992), playing an important role in osmoregulation (Rawdon, 1979; Wendelaar Bonga *et al*., 1985; Mancera *et al*., 1993).

The rostral pars distalis (RPD) of the *Solea senegalensis* pituitary also contained corticotrophs, which were grouped into cords bordering the PRL cells or as islets between the PRL cells and the neurohypophysis. These cells were strongly immunoreactive against anti-human ACTH antiserum. The location of ACTH cells in the RPD has been observed in many teleosts (Olivereau *et al*., 1976; Munro, 1985; Cambré *et al*., 1986; Toubeau *et al*., 1991). According to Margolis-Kazan and Schreibman (1981), high dilutions of anti-ACTH antisera resulted in the immunostaining of corticotrophs alone. In *Barbus barbus*, the antiserum against human ACTH does not cross-react with the PI cells (Toubeau *et al*., 1991). However, in *Solea senegalensis*, the anti-human ACTH antiserum also stains the presumptive MSH cells located in the PI, as observed in other species (Follenius *et al*., 1978; Ball & Batten, 1981; Munro, 1985; Cambré *et al*., 1986; Yan & Thomas, 1991). ACTH and MSH cells, which have been identified in the pituitary gland of all groups of fishes, originate from a common precursor molecule, the proopiomelanocortin, and corticotrophic activity has been ascribed to α-MSH cells in both mammals and teleosts (Wendelaar Bonga, 1993). Positive reaction in MSH cells using human ACTH antisera is possibly caused by the presence of ACTH in these cells as a precursor of α-MSH (Follenius & Dubois, 1980; Krieger, 1983). However, the antisera against α-MSH used in this study permit us to distinguish between ACTH and α-MSH cells, as only the lead–Haematoxylin-positive cells of the PI were intensely immunostained with this antiserum.

Usually, the distinction between cells containing other structurally related hormones, such as thyrotrophins and gonadotrophins, has also been difficult. Within a given species, the α-subunit shares similarities among the glycoprotein hormones and is highly conserved even between distantly related species, whereas the β-subunit is unique to each hormone and apparently determines the biological specificity (Pierce & Parsons, 1981). Since α chain of gonadotrophins is similar to the α chain of thyrotrophins, it might be expected that antisera raised against α, β GTH reacts with both GTH and TSH cells (Burzawa-Gerard, 1974).

In *Solea senegalensis*, anti-carp α, β GTH II antiserum revealed the presence of immunostained cells in the dorsal and ventral parts of the PPD and also forming the external border of the PI. The presence of GTH cells in the PI has also been described in other teleost species (Cambré *et al*. 1986; García-Hernández *et al*., 1996; Sarasquete *et al*., 1997; Vissio *et al*., 1997), and it has been suggested that these cells should be considered as projections from the proximal pars distalis into the PI (Cambré *et al*. 1986). Anti-carp α, β GTH II antiserum also reveals immunopositive cell groups in the anterior and central parts of the PPD, most probably representing the TSH cells as in other species (Burzawa-Gerard, 1974; Margolis-Kazan & Schreibman, 1981; García-Ayala *et al*., 1989; Yan & Thomas, 1991; Sarasquete *et al*., 1997; Vissio *et al*., 1997). These presumptive TSH cells did not appear to be immunostained in sections of pituitary incubated with anti-carp β GTH II. However, cross-reaction with TSH cells was not observed in *Solea vulgaris* pituitary using anti-carp α, β GTH and, in contrast to our results, the immunostaining pattern was similar when anti-carp α, β GTH and anti-carp β GTH antisera were used (Nunez-Rodriguez, 1985). In *Solea vulgaris,* TSH cells were restricted to the ventral part of the PPD (Nunez-Rodriguez, 1985) but, in *Solea senegalensis* pituitary, presumptive TSH cells are located in both the ventral and the dorsal zones of the PPD. The presence of TSH cells in the dorsal zone of the PPD and scattered among GH and GTH cells was also observed in *Barbus barbus* (Toubeau *et al*., 1991).

In *Solea senegalensis*, pituitary cells immunostained with α, β GTH II and not with β GTH II could also represent, at least in part, GTH I cells. However, these cells were positive with the PAS reaction (Table 1), as are TSH cells usually and contrary to that observed in salmonids, in which the PAS reaction is negative in GTH I cells (Nozaki *et al*., 1990). Up to now, the presence of GTH I and GTH II in different pituitary cells has only been demonstrated in salmonids (Nozaki *et al*., 1990). In *Seriola dumerili*, anti-chum salmon GTH I and anti-chum salmon GTH II stained the same cells in the outermost part of the adenohypophysis at the level of the PPD and the PI (García-Hernández *et al*., 1996).

On the other hand, histochemical results (PAS, Alcian Blue pH 2.5 and lectins) of GTH and presumptive TSH cells of *Solea senegalensis* pituitary were similar to those observed in *Fundulus heteroclitus* (Sarasquete *et al*., 1997), suggesting the synthesis in these cells of glycoprotein hormones containing mannose and/or glucose and *N*-acetyl glucosamine and/or sialic acid. These cytochemical tests were negative in GH cells, which were

strongly stained with Bromophenol Blue (general proteins).

Acidophilic GH cells, restricted to the dorsal and ventral parts of the PPD of *Solea senegalensis* pituitary, were specifically immunostained with anti-recombinant seabream GH antiserum (anti-rsbGH). Similar results were observed in other teleost species using this antiserum (Sarasquete *et al*., 1997) and others, such as anti-porcine GH, anti-chum and -coho salmon GH and anti-trout GH antisera (Cambré *et al*., 1986; Toubeau *et al*., 1991; Yan & Thomas, 1991; Power, 1992). In *Solea senegalensis* pituitary, anti-rsbGH does not cross-react with either PRL cells or PAS-positive cells of the PI (SL cells), in contrast to the situation observed in other teleosts with several anti-GH antisera (Batten, 1986; Cambré *et al*., 1986).

Ball and Baker (1969) described two cell types in the PI of teleosts, according to different staining properties: PIPbH cells, stained with lead–Haematoxylin; and PIPAS cells, which were stained with the PAS reaction. As a result of cytophysiological experiments, the PIPbH cells may now be realistically termed as MSH cells (Baker, 1979; Olivereau *et al*., 1976, 1981). Immunocytochemical studies have shown that PIPAS cells are immunoreactive against anti-mammalian PRL (Rawdon, 1979; Toubeau *et al*., 1991). The nature of these PIPAS cells is unknown, although they could be activated under several environmental conditions, such as black background (Ball & Batten, 1981), acid pH (Wendelaar Bonga *et al*., 1986), low calcium (Olivereau *et al*., 1981) or low osmolarity (Olivereau *et al*., 1980) of the ambient water. However, *Sparus aurata* specimens kept at different salt concentrations did not exhibit morphometric, tinctorial or ultrastructural differences in these PIPAS cells, which were negative against anti-salmon GTH, anti-salmon GH, anti-salmon PRL and anti-human ACTH (Mancera, 1991).

Recently, a pituitary hormone of the growth hormone/prolactin family, the somatolactin (SL), was isolated and/or cloned and expressed from the flounder, *Paralichthys olivaceus* (Ono *et al*., 1990), Atlantic cod, *Gadus morhua* (Rand-Weaver *et al*., 1991b), Senegalese sole, *Solea senegalensis* (Pendón *et al*., 1994a, 1996), and gilthead seabream, *Sparus aurata* (Astola *et al*., 1996). The target organs and functions of SL are unknown, although involvements in reproduction (Kawauchi, 1991; Planas *et al*., 1992; Olivereau & Rand-Weaver, 1994), calcium regulation (Kaneko & Hirano, 1993), stress (Rand-Weaver *et al*., 1993) and environmental salinity adaptation (Wendelaar Bonga *et al*., 1986) have been suggested. Antisera raised against cod SL labelled the PIPAS cells in several teleost species, such as the rainbow trout and salmon (Rand-Weaver *et al*., 1991a; Olivereau & Rand-Weaver, 1994). Similarly, in *Solea senegalensis*, the PAS-positive cells of the PI reacted specifically with the antiserum raised against recombinant sole SL (Pendón *et al*., 1996). These SL cells appear to be surrounded by MSH cells, while, in other species, SL cells mainly surround the neurohypophysis branches in the PI (Rand-Weaver *et al*., 1991a; Olivereau & Rand-Weaver, 1994; García-Hernández *et al*., 1996). Differences in the localization of SL cells within the PI might affect the regulation of these cells in different species.

On the other hand, the SL cells of *Solea senegalensis* were reactive with ConA and WGA lectins, suggesting the presence in these cells of glycoproteins containing mannose and/or glucose, as well as *N*-acetyl-glucosamine and sialic acid sugar residues. These results could be consistent with *in vitro* studies performed by Pendón *et al*. (1997), which demonstrated that *Solea senegalensis* pituitary secretes not only non-glycosylated SL but also glycosylated SL.

As *Solea senegalensis*, a species of great economic and commercial interest, is a potential new species for mariculture from the Mediterranean and south Atlantic coasts, the immunocytochemical distribution of the hormone pituitary cell types presented here will provide a basis for future studies on its physiology, such as the variation of these pituitary cells during the annual reproductive cycle and in captive conditions, as well as under hormonal treatments or different salinity conditions.

Acknowledgements

This work was supported by the Comisión Interministerial de Ciencia y Tecnología, Spain (projects DIGCYT, PB93-1209 and CYTMAR95-1888-C03-02). The authors are grateful to Dr Valdivia for providing the anti-recombinant seabream GH and anti-recombinant sole SL antisera, to Dr Burzawa-Gerard for providing anti-carp α, β GTH II and anti-carp β GTH II antisera, to Dr Tramu for providing α-MSH antiserum and to Dr Kawauchi for donating anti-salmon PRL antiserum. Thanks to Isabel Viaña and Agustín Santos for their helpful technical assistance.

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