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A systematic immunohistochemical survey of the distribution patterns of GH, prolactin, somatolactin, β–TSH, β–FSH, β–LH, ACTH, and α–MSH in the adenohypophysis of *Oreochromis niloticus*, the Nile tilapia

Abstract Fish pituitary plays a central role in the control of growth, development, reproduction and adaptation to the environment. Several types of hormone–secreting adeno-hypophyseal cells have been characterised and localised in diverse teleost species. The results suggest a similar distribution pattern among the species investigated. However, most studies deal with a single hormone or hormone family. Thus, we studied adjacent sections of the pituitary of *Oreochromis niloticus*, the tilapia, by conventional staining and immunohistochemistry with specific antisera directed against growth hormone (GH), prolactin (PRL), somatolactin (SL), thyrotropin (β–TSH), follicle–stimulating hormone (β–FSH), luteinising hormone (β–LH), adrenocorticotropic hormone (ACTH) and melanocyte–stimulating hormone (α–MSH). The pituitary was characterised by a close interdigitating neighbourhood of neurohypophysis (PN) and adenohypophysis. PRL–immunoreactive and ACTH–immunoreactive cells were detected in the rostral pars distalis. GH–immunoreactive cells were present in the proximal pars distalis (PPD). A small region of the PPD contained β–TSH–immunoreactive cells, and β–LH–immunoreactive cells covered approximately the remaining parts. Centrally, β–FSH–immunoreactive cells were detected in the vicinity of the GH–containing cells. Some of these cells also displayed β–LH immunoreactivity. The pars intermedia was characterised by branches of the PN surrounded by SL–containing and α–MSH–immunoreactive cells. The ACTH and α–MSH antisera were observed to cross-react with the respective antigens. This cross–reactivity was abolished by pre–absorption. We present a complete map of the distinct localisation sites for the classical pituitary hormones, thereby providing a solid basis for future research on teleost pituitary.

Keywords Pituitary · Hormones · Localisation · Immunohistochemistry · Mapping · Teleost

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Introduction

As in mammals, fish pituitary has been described to play a central regulatory role in the control of growth, development, reproduction and adaptation to environmental challenges, such as changes in salinity, temperature and stress. Bony fish represent the largest non-mammalian vertebrate group and provide valuable models for basic research on vertebrate physiology. In addition, numerous bony fish species, such as salmonids, seabream and tilapia, have high commercial value and are used in aquaculture, the fastest growing area of food production. Therefore, the scientific results on the regulatory mechanisms involved in growth, development and reproduction that have emerged are of major impact not only in basic science, but also in the aquaculture industry (for reviews, see Reinecke and Collet 1998; Reinecke et al. 2005). The susceptibility of fish kept in pond culture or other farming conditions to environmental challenges, including pollutants, is an important research field that is increasingly under investigation.

In teleosts, the pituitary is characterised by a close interdigitating neighbourhood between the neurohypophysis (pars nervosa, PN) and the adenohypophysis. The latter consists of three main parts: the rostral pars distalis (RPD), the proximal pars distalis (PPD), and the pars intermedia

(PI). Unlike mammals, teleost fish lack a hypothalamo–hypophyseal portal system for the transport of neurohormonal regulators. Instead, a direct axonal transport exists between hypothalamic neurons and pituitary endocrine cells via the hypophyseal stalk and the PN (Weltzien et al. 2004).

The hormone–producing cells of the adenohypophysis have been investigated for several decades by means of standard staining procedures and immunocytochemical and molecular biological methods (for a review, see Agulleiro et al. 2006). With these methods, hormone–secreting adenohypophyseal cells have been characterised and localised in diverse teleost species. Whereas the hormone–producing cell types are arranged in a mosaic pattern in the pituitary of adult tetrapods, teleost pituitary preserves the embryonic compartment organisation, i.e. each specific hormone–producing cell type is located in a particular compartment. Thus, teleost fish represent interesting models for the study of pituitary ontogeny and phylogeny (for a review, see Weltzien et al. 2004).

The potential impact of environmental pollutants on growth hormone (GH) and adrenocorticotropic hormone (ACTH) peptide expression has been pointed out in tilapia (*Oreochromis niloticus*) indicating the need for further studies (Mousa and Mousa 1999a). However, few systematic data exist on teleost pituitary as the central regulatory organ of numerous physiological processes. Most studies deal with single hormones or hormone families (e.g. Amemiya et al. 1999; Amano et al. 2005; Dores et al. 1996; García Ayala et al. 2003; Huang and Specker 1994; Joss et al. 1990; Kagawa et al. 1998; Kawauchi et al. 1986, 1989; Mousa and Mousa 1999a,b; Nagahama et al. 1981; Naito et al. 1983; Nishioka et al. 1993; Nozaki et al. 1990; Olivereau and Rand-Weaver 1994; Parhar et al. 2003; Power 1992; Rand-Weaver et al. 1991; Saga et al. 1993; Shimizu et al. 2003; Siegmund et al. 1987; Weltzien et al. 2003a). A few more extensive studies have been performed (e.g. García-Hernández et al. 1996; Grandi and Chicca 2004; Laiz-Carrión et al. 2003; Naito et al. 1993; Parhar et al. 1998, 2002; Sánchez Cala et al. 2003; Segura-Noguera et al. 2000; Weltzien et al. 2003b; Yan and Thomas 1991) but no thorough investigation has localised all pituitary hormones by immunohistochemistry on consecutive sections.

In detail, during the ontogeny of chum salmon (*Oncorhynchus keta*), cells expressing ACTH, prolactin (PRL), GH, α–melanocyte–stimulating hormone (α–MSH) and β–thyroid–stimulating hormone (β–TSH) have been localised by using immunocytochemistry (Naito et al. 1993). In the protogynous teleost wrasse *Thalassoma duperrey*, all adenohypophyseal hormones were investigated but cross-reactivities between β–TSH and the gonadotropin GTH II (β–LH) antisera had to be considered (Parhar et al. 1998); however, the study mainly characterised endocrine cells at the ultrastructural level and only selected hormones were presented by light microscopy. In an ontogenic study on sturgeon, *Acipenser naccarii*, the appearance and distribution of all adenohypophyseal hormones including β–endorphin were monitored but, as the main focus was laid on early development, the demand for investigations of the hormone expression pattern in adults was stressed (Grandi and Chicca 2004).

An early study of adult Atlantic croaker (*Micropogonias undulatus*), spotted sea trout (*Cynoscion nebulosus*) and red drum (*Sciaenops ocellatus*) investigated the cells expressing GH, PRL, ACTH, β–TSH and the gonadotropins but the distinct localisation of the gonadotropins remained unclear (Yan and Thomas 1991). An immunocytochemical study in Mediterranean yellowtail (*Seriola dumerilii*) characterised the same hormonal cell types, plus SL and α–MSH. Again, the gonadotropins could not be definitively localised (García-Hernández et al. 1996). Similarly, in young white seabream (*Diplodus sargus*), the data for the gonadotropins also remained unclear (Segura-Noguera et al. 2000). In a more recent study in adult male Atlantic halibut (*Hippoglossus hippoglossus*), a map was created for the distinct localisations of all hormone cell types, whereby β–FSH was localised by in situ hybridisation (Weltzien et al. 2003b). However, a clear interpretation of the potential co–localisation of the gonadotropins was not possible. In a study on gonadotropin–releasing hormone receptors recently performed in tilapia, the gonadotropins and cells of the GH/PRL family were also investigated by using in situ hybridisation and immunohistochemistry; whereas β–LH–containing cells were displayed at the gene and peptide expression level, β–FSH–expressing cells were again detected exclusively at the mRNA level (Parhar et al. 2002). The contradictory results led to the explanation that the antisera against salmon β–FSH might recognise an epitope of tilapia β–LH (Parhar et al. 2003), a proposal that gives new support to the importance of a definite localisation of the gonadotropins, especially with respect to the differences in sexual regulation between salmonids and non–salmonids.

Thus, to our knowledge, no study has as yet succeeded in demonstrating all the hormone-producing cells in the entire pituitary in adjacent or, at least similarly oriented, sections by using the same technique consistently throughout the investigation. However, only this approach can create a basis for investigations of diverse hormone–producing cells under various experimental settings. Furthermore, no complete mapping has been performed in tilapia, a species widely used in aquaculture. Another problem that has not been sufficiently addressed to date is the dual existence and localisation of the gonadotropins, i.e. GTH I and GTH II, more recently shown to be β–FSH and β–LH in higher teleosts (Rosenfeld et al. 2001; Quérat et al. 1990). The obtained contradictory results (see above) have been attributed not only to species differences, but also to antisera–dependent differences (e.g. García-Hernández et al. 1996; Segura-Noguera et al. 2000; Parhar et al. 2003) and need to be clarified.

In order to provide a basis for future experiments, we wanted to elaborate a defined morphological knowledge of teleost pituitary and thus explored the pituitary of the Nile tilapia (*O. niloticus*) as a representative model species. Thus, the distribution pattern of GH, PRL and SL, of the POMC family members ACTH and α–MSH, and of β–TSH and the gonadotropins β–FSH and β–LH was

Table 1 Primary antisera used for immunofluorescence on Paraplast sections (all antisera raised in rabbit)

Antiserum against	Specificity	Dilutions	Source	Reference
ACTH (1–23)	Rat	1:2,000	Peptide Inst., Osaka (Japan), cat. no. Y352	–
FSH, β–subunit	Chum salmon	1:400	Dr. H. Kawauchi, Kitasato University (Japan), lot 8510	Nozaki et al. 1990
FSH, β–subunit	Human	1:100	Biogenesis, Poole (England), cat. no. 4560–8055	Grandi and Chicca 2004
GH	Chum salmon	1:8,000	Dr. H. Kawauchi, Kitasato University (Japan), lot 8502	Kawauchi et al. 1986
LH, β–subunit	Chum salmon	1:4,000	Dr. H. Kawauchi, Kitasato University (Japan), lot 8506	Nozaki et al. 1990
LH, β–subunit	Human	1:200	DAKO A/S, Glastrup, Denmark, cat. no. N1543	Siegmund et al. 1987
MSH, α–subunit	Human	1:1,500	Biogenesis, Poole (England), cat. no. 6045–0400	Amano et al. 2005
PRL	Chum salmon	1:12,000	Dr. H. Kawauchi, Kitasato University (Japan), lot 8206	Naito et al. 1983
SL	Chum salmon	1:4,000	Dr. H. Kawauchi, Kitasato University (Japan), lot 8906	Rand-Weaver et al. 1991
TSH, β–subunit	Human	1:300	Biogenesis, Poole (UK), cat. no. 8926–0004	Grandi and Chicca 2004

analysed on consecutive Paraplast and semi-thin sections. Special emphasis was laid on elucidating the localisation sites of the gonadotropins β–FSH and β–LH. Based on the results obtained, we finally obtained a complete map of the distinct localisation sites of all pituitary hormones.
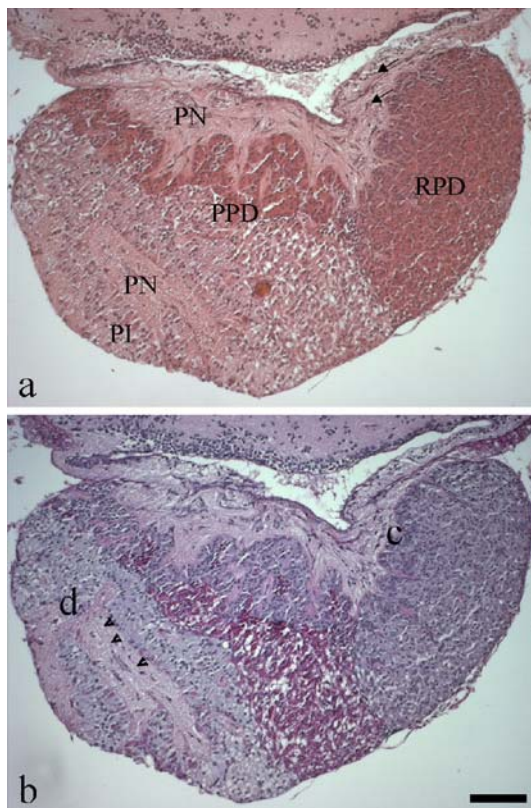

Fig. 1 Histology of tilapia pituitary. Consecutive sagittal Paraplast sections stained with (**a**) HE and (**b**) PAS (see Table 2). **a**, **b** The adenohypophysis is divided into three main parts, the rostral (*RPD*) and proximal (*PPD*) pars distalis and the pars intermedia (*PI*). Sections display the neural connection of the hypothalamus to the pituitary (**a**, *arrows*) and the interdigitating branches of the PN reaching the PI (**b**, *arrowheads*). **b** Regions containing cells expressing ACTH (*c*) and α–MSH (*d*) investigated by the pre–absorption experiment (see Fig. 4). *Bar* 150 μm

Materials and methods

Fish culture

Twelve individuals of *Oreochromis niloticus* (125 days old) kindly provided by Prof. N. Maclean (School of Biological Sciences, University of Southampton, South-ampton, UK), and Dr. J.-F. Baroiller (CIRAD, Montpellier, France) were used. The fish were kept in fresh-water tanks at 27±1°C under a 12 h/12 h light/dark cycle and fed to satiation.

Tissue sampling and preparation

Fish were anaesthetised with 2–phenoxy–ethanol (Sigma, St. Louis, Mo, USA) added to the water and killed. Pituitaries were immediately excised and, for conventional and immunofluoresence microscopy, attachment to the brain was conserved to simplify later orientation. Tissue samples were fixed by immersion in acetic-acid-free Bouin's solution for 4 h at room temperature. Specimens were dehydrated in an ascending series of ethanol and routinely embedded in Paraplast Plus at 58°C. Sections were cut at 4 μm, mounted on Super Frost Plus slides (Menzel–Gläser, Germany) and dried overnight at 42°C. After being dewaxed in xylol, they were rehydrated in a descending series of ethanol (100%, 96%, 70%).

For semi-thin sections, pituitaries were fixed by immersion in a solution containing 2.5% paraformaldehyde, 0.1%

Table 2 HE and PAS staining (*PI* pars intermedia, *PN* pars nervosa, *PPD* proximal pars distalis, *RPD* rostral pars distalis)

| Hormone	Region	HE stain	PAS stain
ACTH	RPD	Basophilic	Negative
β–FSH	PPD	Basophilic	Positive
GH	PPD	Strongly eosinophilic	Negative
β–LH	PPD	Basophilic	Positive
α–MSH	PI	Basophilic	Negative
PRL	RPD	Strongly eosinophilic	Negative
SL	PI	Eosinophilic	Negative
β–TSH	PPD	Basophilic	Positive

glutaraldehyde and 0.01% picric acid for 4 h. Thereafter, specimens were dehydrated in an ascending series of ethanol and routinely embedded in LR White (Polysciences, Warrington, USA). Serial semi-thin sections (1 μm) were cut on an Ultracut E (Reichert-Jung, Zürich, Switzerland) and placed on glass slides.

Haematoxylin–eosin and periodic–acid–Schiff stain

For haematoxylin–eosin (HE) staining, slides were immersed in Mayer`s haematoxylin for 10 min, briefly treated with HCl solution, rinsed in tap water for 10 min and subsequently immersed in eosin for another 10 min. For periodic–acid–Schiff (PAS) staining, slides were treated with periodic acid for 5 min and rinsed in tap water. Treatment with Schiff's reagent for 15 min was followed by exposure of the sections to sodium bisulfite (0.52%) for 10 min. After a rinse in tap water, slides were immersed in Mayer's haematoxylin. After another wash step, differentiation in HCl ethanol was performed followed by a rinse in flowing water. Slides were dehydrated in 100% ethanol and xylol (all reagents obtained from Fluka, Switzerland) and covered with Eukitt (Merck, Switzerland).

Fig. 2 Localisation of hormones in tilapia pituitary by the use of immunofluorescence. Eight consecutive sagittal Paraplast sections adjacent to Fig. 1 were stained with specific antisera raised in rabbit (see Table 1) and visualised with an FITC-coupled anti–rabbit IgG (*PI* pars intermedia, *PN* pars nervosa, *PPD* proximal pars distalis, *RPD* rostral pars distalis). **a**–**c** Antisera directed against chum salmon (**a**) PRL, (**b**) GH, (**c**) SL. **d** Antibody directed against human TSH β–subunit. **e** Anti-chum-salmon β–LH antiserum. **f** Anti-human β–FSH antiserum. **g**, **h** Antibodies directed against (**g**) rat ACTH (amino acids 1–23) and (**h**) human MSH α–subunit. *Bar* 200 μm
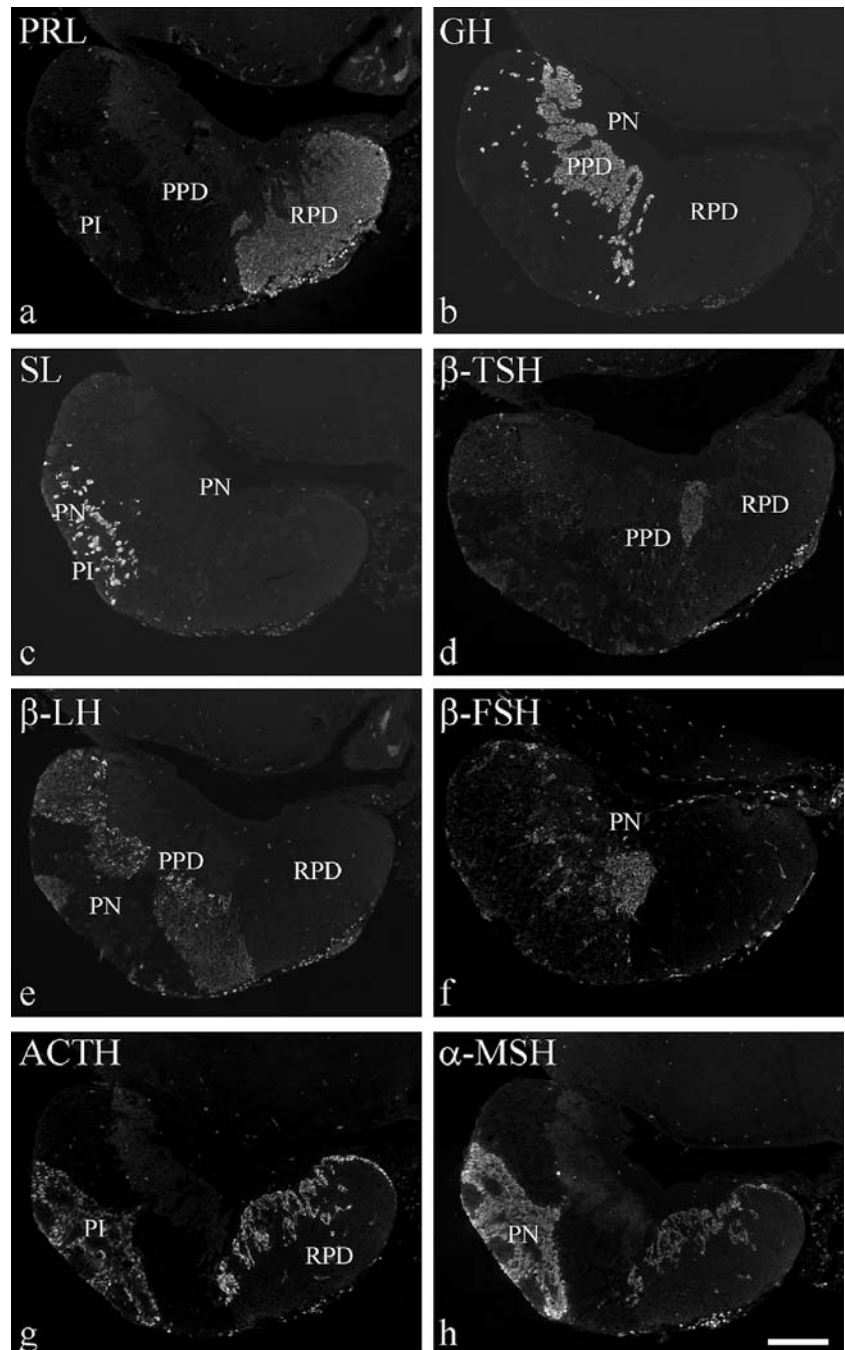
Immunocytochemistry

To allow unmasking of the antigen for immunofluorescent stain, microwaving (350 W, 5 min) in 0.01 M citrate buffer (pH 6) was performed. Unspecific binding was reduced by treatment with phosphate-buffered saline (PBS, pH 7.4) containing 2% bovine serum albumin (BSA) for 30 min at room temperature. Thereafter, sections were incubated overnight at 4°C with the diverse antisera specific for the various pituitary hormones (diluted in PBS/2% BSA; for dilutions see Table 1). After repeated rinses in PBS/2% BSA, the antisera were detected by incubation in the dark for 30 min at room temperature with a fluorescein–isothiocyanate (FITC)–conjugated goat anti–rabbit IgG (Bioscience Products, Emmenbrücke, Switzerland) diluted 1:100 in PBS. Specificity of the antisera (Table 1) had been previously shown by other groups. For instance, pre–absorptions of the antisera against chum salmon GH, SL and PRL with their respective antigens had been shown to abolish immunoreactivity (Mousa and Mousa 1999b; Ominato and Nozaki 2002). In contrast, for the anti-chum-salmon gonadotropin antisera, the reported cross–reactivities (see Introduction) could not be abolished by pre–absorption (Nozaki et al. 1990), whereas no immuno-staining was observed after pre–incubation of the anti-human β–FSH and β–TSH antisera with an excess of the appropriate antigens (Grandi and Chicca 2004). Thus, in the present study, specificities were further examined by omitting the primary antiserum and by the choice of different antisera (see Table 1). In addition, the specificities of the antisera directed against rat ACTH and human α–MSH were tested by pre–incubation for 24 h with 40 or 400 μg recombinant human α–MSH (Biogenesis, UK) and ACTH (fragment 1–24, Sigma), respectively, per millilitre of diluted antiserum.

The semi–thin sections were treated with 10 mM gelatin in 10 ml PBS (pH 7.4) containing 0.2 g BSA for 30 min to reduce non–specific binding. After repeated washes in PBS, consecutive semi–thin sections were processed for immunohistochemistry. The first antisera directed against human and chum salmon β–LH and β–FSH, respectively (see Table 1; all diluted 1:30 in gelatin–PBS–BSA) were incubated in a humid chamber for 12 h at 4°C. After repeated washes in PBS, the sections were treated with biotin-coupled goat anti-rabbit IgG (Bio–Science Products; diluted 1:100 in gelatin–PBS) for 30 min at room temperature, followed by incubation with a streptavidin–gold–5–nm complex (Amersham, Little Chalfont, UK; diluted 1:100 in gelatin–PBS) for 1 h at room temperature. After repeated washes in double-distilled water, the sections were treated with the IntenSE M silver enhance-ment kit (Amersham) for 10 min at room temperature and counterstained with methylene blue/azure.

Imaging

Microscopy and photography were performed with a Zeiss Axioscope and Axiovision 3.1. software (Zeiss, Zürich, Switzerland). The fluorochromes were visualised with a fluorescence module for FITC (BP 450–490 nm, FT 510, LP 515–565 nm).

Results

Structure of the pituitary of adult *O. niloticus*

Consecutive sagittal Paraplast sections of tilapia pituitary attached to the brain were stained with HE (Fig. 1a) and PAS (Fig. 1b). The neurohypophysis (PN) and the ade-
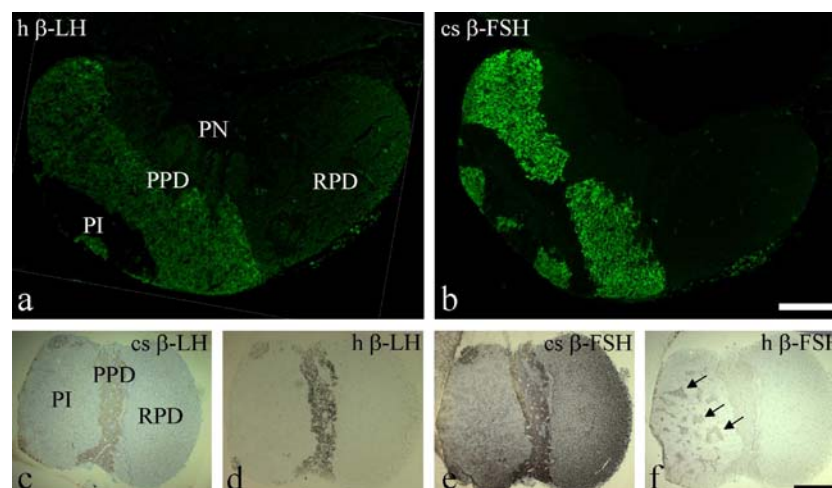

Fig. 3 Localisation of gonadotropins in tilapia pituitary (*PI* pars intermedia, *PN* pars nervosa, *PPD* proximal pars distalis, *RPD* rostral pars distalis). **a**, **b** Consecutive Paraplast sections to those in Figs. 1, 2 immunostained by rabbit antisera (see Table 1) directed against (**a**) human (*h*) β–LH, and (**b**) chum salmon (*cs*) β–FSH visualised with an FITC-coupled anti–rabbit IgG. *Bar* 200 μm. **c–f** Consecutive horizontal semi-thin sections of tilapia pituitary immunostained with antisera directed against (**c**) cs β–LH, (**d**) h β–LH, (**e**) cs β–FSH and (**f**) h β–FSH (see Table 1) visualised with biotin-conjugated anti–rabbit IgG followed by a streptavidin–gold–5–nm complex (*arrows* branches of the PN within the PI). *Bar* 300 μm

nohypophysis presented an interdigitating neighbourhood. The adenohypophysis was divided into three main parts: the RPD, PPD and PI. Branches of the PN were detected throughout the adenohypophysis, especially in the PI. The hormone–containing cells displayed differential staining behaviour with both techniques (see Table 2).

Distribution pattern of tilapia adenohypophyseal hormones analysed by immunofluorescence microscopy

Consecutive sections to Fig. 1 were investigated by using antisera (Table 1) specific for the classical adenohypophyseal hormones, i.e. members of the GH/PRL family (GH, PRL, SL), the glycoprotein hormone group constituted by the gonadotropins and thyrotropin (β–FSH, β–LH, β–TSH) and members of the proopio–melanocorticotropin (POMC) family (ACTH, α–MSH).

GH/PRL family

The members of the GH/PRL family were detected in all three parts of the adenohypophysis and showed a strong eosinophilic staining behaviour (Fig. 1a, Table 2). PRL–immunoreactive cells filled the major part of the RPD (Fig. 2a). GH–immunoreactive cells were arranged in regular chains along the branches of the PN within the PPD (Fig. 2b). SL–containing cells surrounded the nerve fibres of the PN (Fig. 2c).

Glycoprotein hormones

The glycoprotein hormone–containing cells reacted to PAS (Fig. 1b, Table 2). Near to the border seam between RPD and PPD, a small region contained cells immunoreactive for β–TSH (Fig. 2d). Cells immunoreactive to the anti-chum-salmon β–LH antiserum filled the whole PPD except for the

Fig. 4 Specificity test of anti-rat ACTH antiserum on consecutive Paraplast sections (*PI* pars intermedia, *PN* pars nervosa, *PPD* proximal pars distalis, *RPD* rostral pars distalis). **a–c** Immunostaining of ACTH in the RPD (compare Fig. 1a,b, region c). **a** With the anti-rat ACTH antiserum not pre-absorbed. **b,c** After 24 h pre–incubation with α–MSH peptide at 40 µg/ml (**b**) and 400 µg/ml (**c**) antiserum. **d–f** Cross-reactive staining of α–MSH in the PI (compare (Fig. 1a,b, region d). **d** With the ACTH antiserum not pre-absorbed. **e, f** After 24 h pre–incubation with α–MSH at 40 µg/ml (**e**) and 400 µg/ml (**f**) antiserum. *Bar* 50 µm. **g, h** Immunostaining of ACTH in the RPD. **g** With the anti-rat ACTH antiserum not pre-absorbed. **h** After 24 h pre–incubation with human ACTH 1–24 fragment at 40 µg/ml antiserum. *Bar* 150 µm. **i** Amino acid sequences for tilapia ACTH (*1st line*), human/rat ACTH (*2nd line*) and α–MSH (*3rd line*). Differences are indicated in *red*, insertions and deletions in *blue*. *Green vertical lines* indicate amino acids 1–23, the epitope of the rat ACTH peptide to which the antiserum is directed
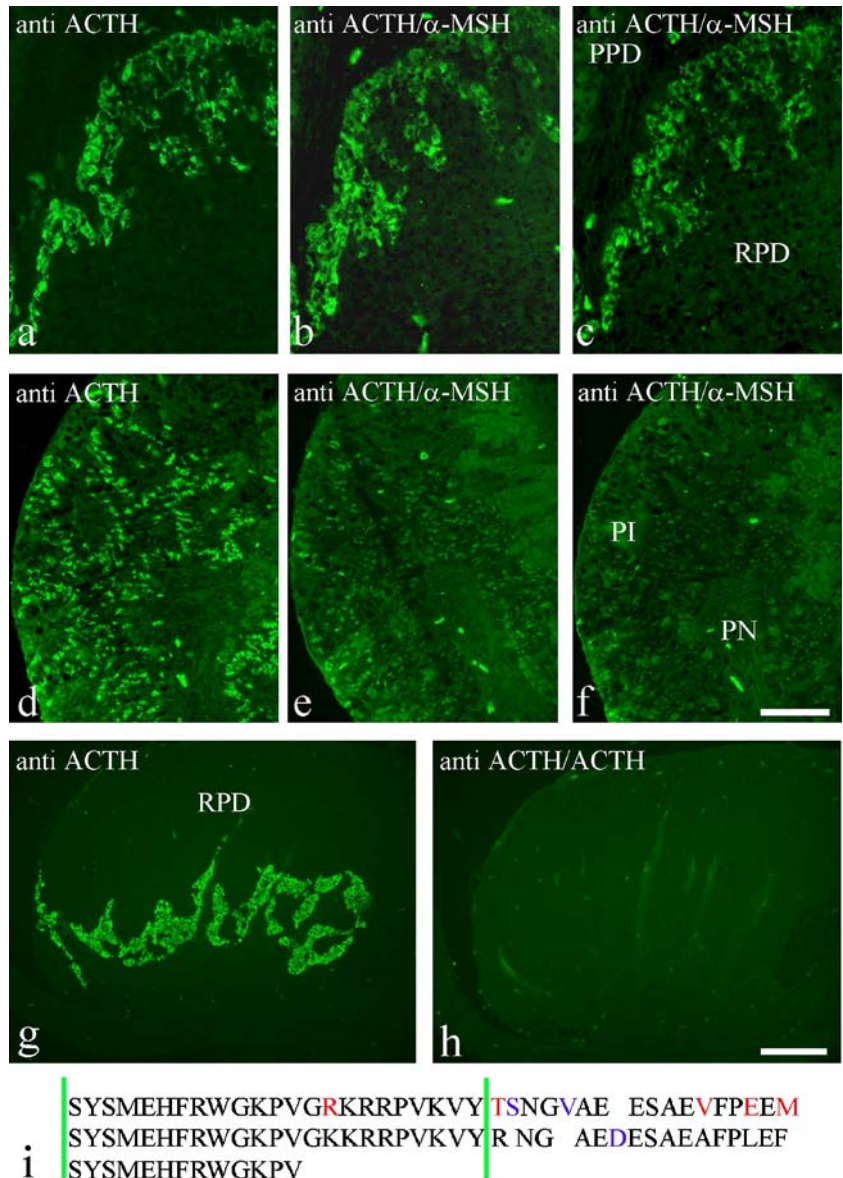
GH and β–TSH regions (Fig. 2e). Similar results were obtained with an antiserum directed against human β–LH (Fig. 3a). On using the antiserum against chum salmon β–FSH (Fig. 3b), an almost identical distribution pattern as with the β–LH–antisera (Figs. 2e, 3a) was detected, whereas an antiserum directed against human β–FSH recognised a cell population near to the somatotrophs, mainly in the region of the β–LH–expressing cells (Fig. 2f). Similar results were achieved by immunocytochemistry on horizontal semi-thin sections (Fig. 3c–f): anti-chum-salmon (Fig. 3c) and anti-human (Fig. 3d) β–LH antisera displayed an identical staining pattern in the PPD, and the anti-chum-salmon β–FSH antiserum (Fig. 3e) again recognised the same regions as the β–LH antisera. In horizontally oriented pituitary sections, even the anti-human β–FSH antiserum stained the same regions, indicating a partial co–localisation of GTH I (β–FSH) and GTH II (β–LH) in the centre of the PPD (Figs. 2e,f, 3c–f). In addition, distinct staining of the PN branches in the PI was observed with both anti-β–FSH antisera (Fig. 3e,f). A similar but markedly weaker staining pattern in the PI was observed for β–LH (Fig. 3c,d).

POMC family

The members of the POMC family were only faintly basophilic after HE staining and not stained by PAS (Fig. 1, Table 2). ACTH-immunoreactive cells formed a fine seam of cuboid to columnar cells in the RPD (Figs. 2g, 4a) along the region containing the PRL-immunoreactive cells (Fig. 2a). In the PI, a large cell population immunoreactive for α–MSH was detected around the PN branches (Figs. 2h, 4d).

The antisera used for detecting ACTH and α–MSH reacted with both cell types, i.e. with ACTH–containing cells of the RPD and with α–MSH–containing cells of the PI (Fig. 2g,h), although with different staining intensity. Because this reaction pattern might have indicated cross–reactions, we performed preabsorption assays: the antiserum directed against ACTH was preincubated with α–MSH to saturate potential α–MSH-binding sites. Accordingly, cross-reactivity of the ACTH antiserum to α–MSH cells (Fig. 4d) in the PI was strongly reduced by preincubation of the anti–ACTH antiserum with α–MSH at 40 μg/ml (Fig. 4e) and completely abolished at 400 μg/ml (Fig. 4f) antiserum. On the contrary, immunostaining for ACTH–containing cells (Fig. 4a,g) in the RPD was not impaired by preabsorption of the antiserum with α–MSH (Fig. 4b,c), whereas it was completely abolished (Fig. 4h) after pre–incubation with the human ACTH amino acid fragment 1–24. The anti-rat ACTH antiserum used was thus specific for amino acids 1–23, which are almost identical for human, rat and tilapia ACTH (Fig. 4i).

Localisation of the principal hormones in tilapia adenohypophysis

A map was created displaying the localisation of all principal hormones in tilapia adenohypophysis (Fig. 5).
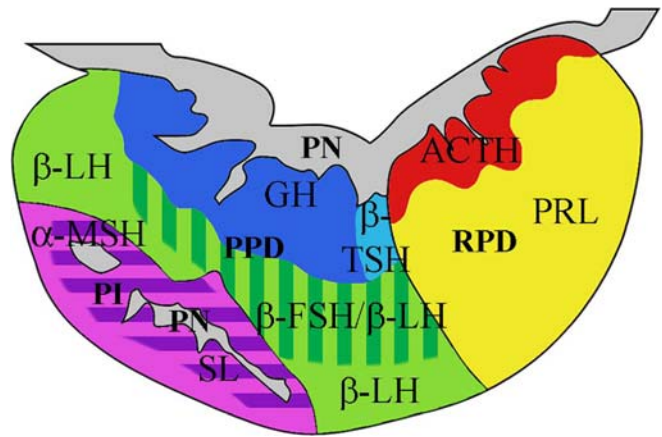

Fig. 5 Map of all pituitary hormone localisations. The rostral pars distalis (*RPD*) is given in *red* (ACTH region) and *yellow* (PRL region). The proximal pars distalis (*PPD*) is coloured in *dark blue* (GH) and *light blue* (β–TSH) regions and in *striped green* symbolising both β–LH (*light green*) and β–FSH (*dark green*) areas. The pars intermedia (*PI*) is coloured as *striped violet* representing the intermingled α–MSH (*pink*) and SL (*dark violet*) cells. Note the pars nervosa (*PN*) branches (*grey*) that reach towards the PI

Thus, PRL and ACTH (Figs. 2a,g, 4a) were localised in the RPD, β–TSH, β–FSH and β–LH (the last two mentioned in partial coexistence) in the PPD (Figs. 2d–f, 3c–f), and SL and α–MSH in the PI (Figs. 2c,h, 4d).

Discussion

An understanding of the central role of the adenohypophysis in teleosts (for a recent review, see Agulleiro et al. 2006) demands profound knowledge of the distribution of the hormone–producing cells. The present study investigating all pituitary hormones on consecutive sections in the Nile tilapia (*O. niloticus*) has revealed a structure and distribution pattern similar to those emerging when combining the results of other groups in various teleosts including tilapia (e.g. Holmes and Ball 1974; Leatherland et al. 1974; Joy and Sathyanesan 1980; Siegmund et al. 1987; Yan and Thomas 1991).

The hormones GH, PRL and SL belong to the GH–PRL family and are believed to have evolved from a common ancestral gene by duplication and divergence (Ono et al. 1990; Rand-Weaver et al. 1991). GH and PRL have been identified in most gnathostome vertebrate classes but SL is thought to be confined to osteichthyes (Nozaki et al. 2001). The general occurrence of the GH–PRL family hormones in the three parts of the teleost pituitary as found here has previously been described in the Mediterranean yellowtail and Atlantic halibut (García-Hernández et al. 1996; Weltzien et al. 2003b). In juvenile striped bass (Huang and Specker 1994) and *Anguilla anguilla* (Grandi et al. 2003), GH immunoreactivity has also been detected in the RPD, and in Mediterranean yellowtail, greater weever fish and juvenile *Acipenser naccarii* in the PI (García-Hernández et al. 1996; Sánchez Cala et al. 2003; Grandi and Chicca 2004). In young white seabream, GH–

expressing cells have been found in PPD, RPD and PI (Segura-Noguera et al. 2000). In contrast, we have not detected GH–containing cells outside the PPD of the adult tilapia pituitary. Our results are, thus, in agreement with those of the majority of groups (for a review, see Agulleiro et al. 2006). Ominato and Nozaki (2002) have reported a similar localisation in the agnathan sea lamprey in which the likely common ancestral form of GH–like cells is present (Nozaki et al. 2001). We assume that the restriction of GH cells to the PPD is characteristic for adult fish and their wide–spread occurrence for developing fish. This hypothesis agrees with recently observed differences in GH gene expression between adult and developing bony fish. In adult four–spine sculpin, *Cottus kazika* (Inoue et al. 2003) and tilapia (Caelers et al. 2005), GH gene expression has been found exclusively in the pituitary. In contrast, in developing rainbow trout, GH mRNA has been detected in the pituitary and other organs, such as brain, gills, liver and ovary (Yang et al. 1999) and, in coho salmon, GH has been found not only in the pituitary, but also in the intestine, but exclusively in small fish (Mori and Devlin 1999). The importance of GH during fish development is stressed by the observation that GH in pituitary is expressed even before the separation of the adenohypophysis into PI and PD (Grandi and Chicca 2004) and precedes the appearance of primordial germ cells and gonadotropins (Saga et al. 1993).

The defined localisation of cells immunoreactive for PRL in the RPD and for GH around the neurohypophyseal processes in the PPD as found in the present study has been observed for various teleosts (Yan and Thomas 1991; Power 1992; García-Hernández et al. 1996; Weltzien et al. 2003b; Volckaert et al. 1999; Laiz-Carrión et al. 2003), including tilapia (Nishioka et al. 1993; Nagahama et al. 1981; Mousa and Mousa 1999a,b).

In tilapia, SL is localised in epithelial cells arranged around the interdigitating branches of the PN reaching the PI. Such a regular arrangement has also been described in other developing (Villaplana et al. 1997; Laiz-Carrión et al. 2003) and adult teleosts (Weltzien et al. 2003b). In mature Pacific salmon (*Oncorhynchus nerka*), pronounced differences in SL expression have been noted during the sexual cycle (Olivereau and Rand-Weaver 1994). However, the sexual cycle of salmonids differs from that of cychlid fish, such as tilapia, which are reproductive throughout the year (Baroiller and Guiguen 2001; Kawauchi et al. 1989; Melamed et al. 1998; Santos et al. 2001; Shimizu et al. 2003; Weltzien et al. 2004). Nevertheless, in a comparative analysis of SL-related immunoreactivity in *O. niloticus*, different staining patterns were observed during sexual maturation (Mousa and Mousa 1999b). The distribution pattern of the SL–immunoreactive cells observed in our study agrees with results in mature tilapia (Mousa and Mousa 1999b; Dores et al. 1996).

Whereas the distribution of the GH/PRL family has been mostly elucidated among teleosts investigated so far, that of the gonadotropins is still under discussion. In the present study, we obtained a marked co–localisation of β–FSH and β–LH–immunoreactive cells by using the antisera directed against chum salmon FSH and LH β–subunits. These results are similar to those obtained in Mediterranean yellowtail with the same antisera. However, anti-catfish α,β–GTH antiserum has given different results (García-Hernández et al. 1996). Anti-chum-salmon antisera are widely used for investigating teleost gonadotropins and have been reported to give different results among the diverse teleost species investigated (for a review, see Agulleiro et al. 2006). The discrepancies have been ascribed either to biological variations or to the possibility that heterologous antisera might be inappropriate to investigate the occurrence of two types of gonadotropes (García-Hernández et al. 1996; García Ayala et al. 2003). Because homologous antisera are often difficult to obtain, different approaches to counter the problem have been chosen, e.g. staining for the common α–unit followed by staining for β–LH and β–TSH, whereby the remaining cells are presumed to be β–FSH–containing cells (García Ayala et al. 2003). Human β–TSH antisera have been reported to cross–react well with teleost TSH (for a review, see Agulleiro et al. 2006); this has also been found here in tilapia. We have not observed cross–reactivity between antisera to β–TSH and GTH II (β–LH) as described in several studies (García-Hernández et al. 1996; Parhar et al. 1998; Segura-Noguera et al. 2000) but between the antisera directed against GTH I and GTH II. A moderate cross–reactivity (10% and 12%, respectively) between these antisera has previously been observed (Suzuki et al. 1988). Our data obtained in tilapia with anti-chum-salmon antisera nevertheless contrast with those obtained in salmonids against which the antisera have been generated and to which they have been shown to be specific (Kawauchi et al. 1989; Nozaki et al. 1990). No co–localisation of GTH I (β–FSH) and GTH II (β–LH) immunoreactivities has been found in rainbow trout and Atlantic salmon (Nozaki et al. 1990). In our study, the antisera directed against human β–LH and chum salmon β–LH give identical staining patterns. This result is comparable to those obtained with anti-chum-salmon GTH II antiserum and an anti–ovine β–LH antiserum (Mousa and Mousa 1999a). These and our data support the postulated good conservation of β–LH throughout evolution (Yaron et al. 2001) in contrast to GTH I/β–FSH, which is poorly conserved (Kawauchi et al. 1989).

Concerning the localisation of β–FSH, the anti-chum-salmon antiserum reacting with the tilapia β–LH region gives results different from those obtained by the use of the anti-human β–FSH antiserum that has been used before in sturgeon *Acipenser naccarii* (Grandi and Chicca 2004). The β–FSH localisation sites recognised by the anti-human β–FSH antiserum in the study by Grandi and Chicca (2004) and in the present study correspond well to data obtained so far with homologous antisera, e.g. in greasy grouper *Epinephelus coioides* (Li et al. 2005) and mummichog (Shimizu et al. 2003) or with antisera against hormones of closely related species, e.g. rainbow trout *Salmo gairdneri* and *S. salar* (anti-coho-salmon antisera; Nozaki et al. 1990) and Bluefin tuna *Thynnus thynnus* (anti-*Thynnus obesus* antisera; Kagawa et al. 1998).

Similar results have been achieved even with antisera against hormones of non-closely-related species, e.g. in gilthead seabream (anti-Mediterranean-yellowtail antisera; García Ayala et al. 2003) and *Uranoscopus japonicus* (anti-chum-salmon antisera; Matsuda et al. 2005). These studies and our own have revealed a distribution pattern similar to that obtained by in situ hybridisation with species–specific probes. In tilapia, GTH-I–expressing cells are closely associated to the somatotrophs, whereas the GTH-II–containing cells are located peripherally (Melamed et al. 1998; Yaron et al. 2001). Thus, we have shown that the use of anti-salmonid β–FSH antisera in non–salmonid fish, such as tilapia, might give misleading results by suggesting a complete co–localisation of β–FSH and β–LH (compare García-Hernández et al. 1996; García Ayala et al. 2003; Parhar et al. 2002, 2003). Nevertheless, by using immunocytochemistry on consecutive semi-thin sections, a partial co–localisation of the smaller number of β–FSH-expressing cells with the major population of β–LH-containing cells has been detected in the central PPD. This result is consistent with data obtained by reverse transcription/polymerase chain reaction (RT–PCR) in seabream in which a larger amount of β–LH mRNA than of β–FSH mRNA has been measured (Elizur et al. 2000). Interestingly, both antisera strongly react with β–FSH and weakly with β–LH in the PI (this might be attributed to PN fibres). This result agrees with those of an RT–PCR study in which high β–FSH gene expression and lower β–LH gene expression were detected in the preoptic area of the brain indicating that β–FSH and β–LH are produced in the brain and subsequently delivered by axoplasmic flow via the neurohypophysis to the pituitary (Parhar et al. 2003).

The cell population within the PI that we considered as α–MSH-containing cells displayed a pattern similar to that described in the literature. However, the ACTH antiserum also clearly reacted with the α–MSH–containing cells. The cross–reactivity between ACTH and α–MSH antisera with each others' antigens is a previously described phenomenon and has been attributed to the presence of corticotropin–like intermediate peptide (CLIP) after the cleavage of ACTH to produce α–MSH and CLIP (Dores and Lecaude 2005). Thus, α–MSH possesses an amino acid sequence identical to a region of ACTH (e.g. Joss et al. 1990; Yan and Thomas 1991; Amemiya et al. 1999; Lee et al. 1999; Schally et al. 1980; Segura-Noguera et al. 2000; Weltzien et al. 2003b).

The molecular evolution of the POMC family in vertebrates has recently been further clarified (Takahashi et al. 2004; Dores and Lecaude 2005). In some studies, the cross–reactivities of the antisera have been used for staining procedures. For instance, in White sturgeon, the cross–reactivity of anti–porcine ACTH with α–MSH has been used to specify the localisation of SL immunoreactivity in cells near to the α–MSH cells of the PI (Amemiya et al. 1999). In order to answer the question of cross–reactivity, we have used a preabsorption technique to abolish cross–reactivity. We have thus been able to display α–MSH and ACTH in distinct regions.

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