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Immunocytochemical characterization of adenohypophyseal cells in the greater weever fish (*Trachinus draco*)

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Abstract

The adenohypophysis of the greater weever fish (*Trachinus draco*) was studied using histochemical and immunocytochemical methods. The adenohypophysis comprised the rostral pars distalis (RPD), the proximal pars distalis (PPD), and the pars intermedia (PI). Neurohypophysis showed a patent hypophyseal stalk which was divided into several branches intermingled with the adenohypophysis. Salmon prolactin (PRL)-immunoreactive (ir) cells, arranged in follicles, resided in the RPD and the most rostral part of the ventral PPD. Human adrenocorticotropin (ACTH)-ir cells were located in the RPD between PRL-ir cells and the neurohypophyseal processes. Salmon and seabream somatotropin (GH)-ir cells were located in both the dorsal and the ventral PPD. Some GH-ir cells were seen in surrounding and in contact with neurohypophyseal branches, whereas other isolated or clustered GH-ir cells were embedded in adenohypophyseal cells of the PPD. In addition, isolated or clustered GH-ir cells were also detected in the tissue of the PPD covering the most rostral part of PI. Only one class of salmon and carp gonadotropin (GTH)-ir cells was detected. Isolated or clustered GTH-ir cells resided in both the dorsal and the ventral PPD and were seen surrounding the PI and in the tissue of the PPD covering the most rostral part of PI. In addition, a few scattered GTH-ir cells were observed in the ventral RPD. Scattered groups of thyrotropin (TSH)-ir cells were present in the anteroventral PPD. Salmon and seabream somatolactin (SL)-ir and bovine melanotropin (MSH)-ir cells were intermingled surrounding the neurohypophyseal tissue. SL-ir cells were negative to periodic acid-Schiff technique. MSH-ir cells showed a very weak immunoreactivity to anti-human $ACTH_{(1–24)}$ serum. In addition to the PI location, few isolated or clustered SL- and MSH-ir cells were observed in the dorsal PPD.

Keywords: Immunocytochemistry; Adenohypophysis; Teleost

1. Introduction

The adenohypophyseal cells have been characterized in several teleost species using histochemical and immunocytochemical techniques. In general, the teleostean adenohypophysis comprises three subdivisions: the rostral pars distalis (RPD) containing prolactin (PRL) and adrenocorticotropic cells; the proximal pars distalis (PPD) containing somatotropic, gonadotropic, and thyrotropic cells; and the pars intermedia (PI), including somatolactin (SL) and melanotropic cells (Ball and Baker, 1969; Holmes and Ball, 1974; Follénius et al., 1978; Farbridge and Leatherland, 1986).

On the basis of molecular biochemistry, adenohypophyseal hormones in teleosts are grouped into three families: (a) somatotropin (GH)/PRL family, including PRL, GH, and SL hormones; (b) glycoprotein hormones containing gonadotropins (GTHs) and thyrotropin (TSH); and (c) proopiomelanocortin (POMC)-derived hormones, such as adrenocorticotropin (ACTH) and melanotropin (MSH) (Batten and Ingleton, 1987). All these hormones, except SL, are present in other vertebrates (Rand-Weaver et al., 1991; Kaneko, 1996). In some fish species, two forms of GTHs and/or gonadotropic cells have been reported (Kawauchi et al., 1989; Nozaki et al., 1990; Swanson et al., 1991; Okada et al., 1994; Elizur et al., 1996; García-Hernández et al., 1996, 1997; Gen et al., 2000).

The greater weever fish (*Trachinus draco* L.) is a venomous marine teleost. This species is widely distributed in the Atlantic Ocean and Mediterranean and Adriatic Seas. Populations of the fish live on sandy, muddy or gravelly bottoms, ranging from a few to about 150 m deep. At night, fish swim around freely, even pelagically. Spawnings usually take place from June to August. During this period, greater weever fish are quite aggressive and the number of stings to humans increases (Maretic, 1988).

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Venom has been characterized biochemically in a few species belonging to genera *Trachinus*, including *T. draco* (Chhatwal and Dreyer, 1992a,b). In addition, there have been several studies on venom apparatus and the clinical treatment of the stings of the greater weever fish (Skeie, 1962; Lemus and Boada, 2001). However, there is no information about the adenohypophyseal cell types in the greater weever fish. In this study, these cells were first characterized using histochemical and immunocytochemical approaches.

2. Material and methods

Ten greater weever fish (*T. draco* L.) (100–130 g body weight and 21–27 cm body length) were collected by gillnet from the Málaga coast (Mediterranean Sea) of Spain. Fish were transported to the aquarium of Aulas del Mar (Málaga), where they were held for 1 month under natural photoperiod and temperature for full acclimatation (May–June 1999). Afterwards, they were kept in 500 l tanks with constant water turnover and oxygen supply and fed daily on frozen mussels.

Fish were anaesthetized with 2-phenoxyethanol dissolved in water (1 ml/l water) and killed by decapitation. The brains were dissected, placed in Bouin's fluid for 48 h, dehydrated, and embedded in paraffin. Sagittal and transverse (8 μm thick) sections were obtained. The sections were stained with hematoxylin–eosin, periodic acid-Schiff technique (PAS; McManus, 1948), and Alcian blue-PAS-Orange G (AB-PAS-OG; Adams and Sweetenham, 1958) prior to conventional histological examinations. For the immunocytochemical study, the tissue sections were immunostained according to the unlabeled enzyme method of Sternberger (1986). The primary rabbit antisera and working concentrations used in this study are shown in the Table 1.

The antisera against salmon (s) 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 seabream (sb) GH and SL were kindly provided by Dr. M. Valdivia, Cádiz, Spain (Martínez-Barberá et al., 1994; Astola et al., 1996). The anti-human (h) $ACTH_{(1–24)}$ serum was obtained from the Peninsula Laboratories (California, USA). The anti-bovine monoacetyl α-MSH kindly provided by Dr. S.E. Wendelaar-Bonga has shown a very weak cross-reaction with the ACTH cells of the PDR in *Xenopus* (van Zoest et al., 1989). The anti-carp (c) α,β-GTH II and anti-carp β-GTH II sera were kindly provided by Dr. E. Burzawa-Gerard (Dubourg et al., 1985). The anti-human (h) β-TSH was kindly provided by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Hormone and Pituitary Program (NHPP) (California, USA).

All sections were first treated with 0.3% H_2O_2 in Tris buffer for 15 min at 22 °C in order to inactivate endogenous peroxidase activity, and then incubated in the primary antiserum for 18 h at 22 °C. Next, the sections were applied with the second antiserum (anti-rabbit IgG raised in goat, kindly provided by Dr. P. Fernández-Llebrez, Málaga, Spain) at a dilution of 1:40 for 45 min at 22 °C, followed by rabbit–PAP complex (Dakopatts, Copenhagen, Denmark) at a dilution of 1:100 for 45 min at 22 °C. Sections were rinsed three times in Tris buffer after H_2O_2, antisera, and PAP incubation. All antisera and the PAP complex were diluted in Tris buffer, pH 7.8, containing 0.7% non-gelling seaweed gelatin lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma), and 0.02% sodium azide (Merck, Germany). The reagent of 0.025% 3.3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma) in Tris buffer, pH 7.8, and 0.007% H_2O_2 (Merck) was used as an electron donor in the dark for 15-min incubation at 22 °C. Coplin jars were used for incubation in the first and the second antisera, whereas PAP incubation was carried out in a moist chamber. In order to monitor the immunoreactive (ir) procedure, contiguous sections went through all the above steps except the incubation in the primary antiserum. Also, normal rabbit serum was used instead of primary antiserum. No positive structures or cells were found in these sections.

Table 1
First antisera used in this study

Antisera raised against	Source	Dilution
Chum salmon PRL	Dr. H. Kawauchi[a]	1:10000
Human $ACTH_{(1–24)}$	Peninsula Laboratories[b]	1:3000
Chum salmon GH	Dr. H. Kawauchi[a]	1:10000
Seabream GH	Dr. M.M. Valdivia[c]	1:1000
Human β-TSH	NHPP[d] and NIDDK[e]	1:200
Carp α,β-GTH II	Dr. E. Burzawa-Gerard[f]	1:1000
Carp β-GTH II	Dr. E. Burzawa-Gerard[f]	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. S.E. Wendelaar-Bonga[g]	1:3000
Chum salmon SL	Dr. H. Kawauchi[a]	1:1000
Seabream SL	Dr. M.M. Valdivia[c]	1:1000

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[b] Belmont, CA, USA.
[c] Cádiz, Spain.
[d] National Hormone and Pituitary Program (NHPP), USA.
[e] National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), USA.
[f] Paris, France.
[g] Nijmegen, Holland.

3. Results

The pituitary of *T. draco* presented two well-outlined areas: adenohypophysis and neurohypophysis. Adenohypophysis showed the typical subdivisions of teleosts: the RPD, the PPD, and the PI. Neurohypophysis displayed a patent hypophyseal stalk and several processes that protruded the adenohypophysis (Fig. 1). Seven different cell types were identified in the adenohypophysis: PRL- and ACTH-ir cells

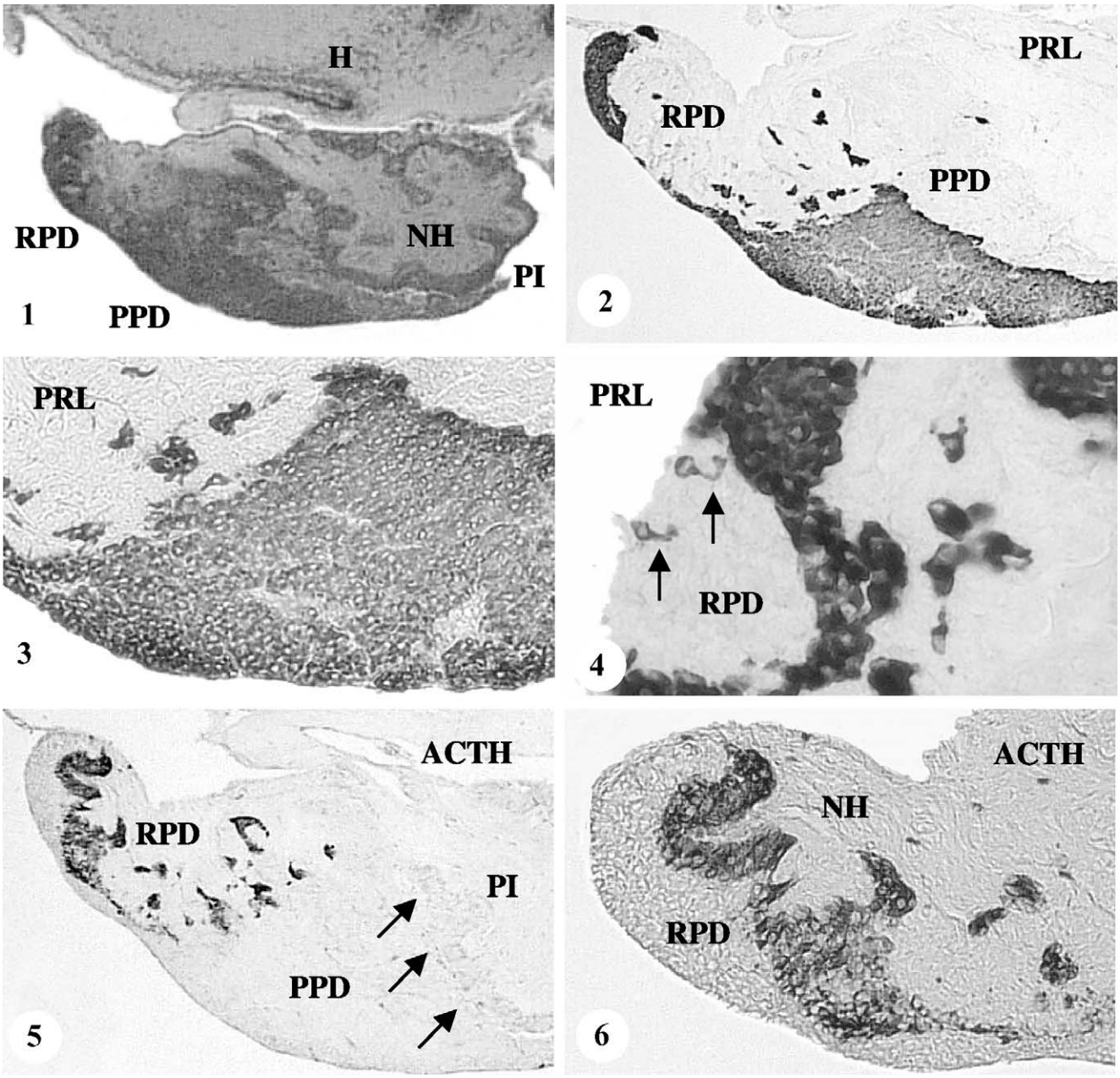
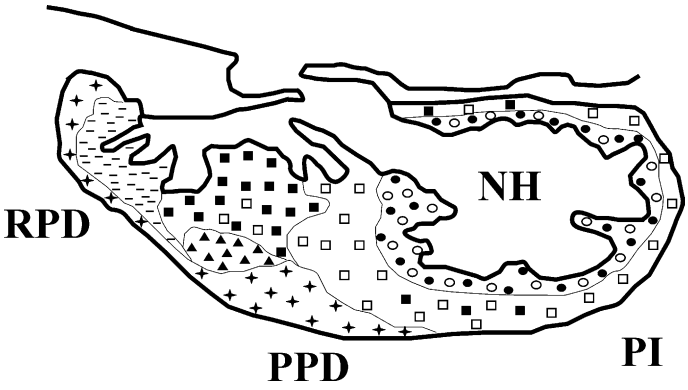


Fig. 1. Sagittal sections through the pituitary of the greater weever fish *Trachinus draco* stained with hematoxylin–eosin showing the rostral pars distalis (RPD), the proximal pars distalis (PPD), and the pars intermedia (PI). H: hypothalamus, NH: neurohypophysis. 65×. Figs. 2–4. Sagittal sections immunostained with anti-sPRL serum. PRL-ir cells were located in the RPD (Fig. 2) and the ventral PPD (Fig. 3). Fig. 4. Some PRL-ir cells showed extensions that were in contact with the neurohypophyseal branches (arrows). Fig. 2: 90×, Fig. 3: 200×, Fig. 4: 420×. Figs. 5 and 6. Sagittal sections immunostained with anti-hACTH$_{(1–24)}$ serum. Fig. 5. ACTH-ir cells were detected in the surrounding of the neurohypophysis (NH). Note a weak cross-reaction with cells in the PI that could correspond to MSH cells (arrows). Fig. 6. Details of ACTH-ir cells close to the NH had round or fusiform shapes. Fig. 5: 90×, Fig. 6: 200×.

located in the RPD; GH-, GTH-, and TSH-ir cells situated in the PPD; and SL-, MSH-, and GTH-ir cells present in the PI (Scheme 1). Occasionally, isolated or clustered cells were also observed in locations different from those described previously.

The PRL-ir cells in the RPD were stained orange using the AB-PAS-OG method and were strongly immunostained with the anti-sPRL serum (Fig. 2). This antiserum was specific and did not cross-react with other adenohypophyseal cells, in particular, those cells producing hormones belonging to the GH/PRL family. In addition, PRL-ir cells were detected in the most rostral part of ventral PPD (Fig. 3). PRL-ir cells were arranged in follicles and were ovoid-shaped with round nuclei. Some labeled cells displayed cytoplasmic extensions that were in contact with the neurohypophyseal tissue (Fig. 4).



Scheme 1. Schematic drawing of a representative sagittal section of the pituitary of the greater weever fish *Trachinus draco* showing the distribution of different adenohypophyseal cells. RPD, the rostral pars distalis, PPD, the proximal pars distalis, PI, the pars intermedia, PRL (+), ACTH (−), GH (■), GTH (□), TSH (▲), MSH (●), and SL (○) cells.

Using the anti-hACTH$_{(1–24)}$ serum, ACTH-ir cells were detected in the RPD between PRL cells and the neurohypophyseal processes (Fig. 5). One or more layers of ACTH-ir cells were seen in the area surrounding the neural tissue. Furthermore, isolated or small ACTH-ir cell aggregates were found dispersed in the RPD (Fig. 6). These cells were stained purple using the AB-PAS-OG method and presented round- or fusiform-shaped with round nuclei. The anti-α-MSH serum immunoreacted weakly with cells in the same area of the RPD that could correspond to ACTH cells (Fig. 16).

After applying the AB-PAS-OG staining method, orange-stained cells were observed in both the dorsal and ventral PPD. These cells were specifically immunostained with anti-sGH and anti-sbGH sera (Fig. 7), but not with the antisera against PRL or SL (Figs. 2 and 14). In the dorsal PPD, GH-ir cells were seen surrounding and in contact with the neurohypophyseal processes. Isolated or clustered GH-ir cells were observed in the ventral PPD (Fig. 7). GH-ir cells presented round- or oval-shaped with irregular oval-shaped nuclei. GH-ir cells in contact with neurohypophyseal processes showed less immunoreactivity with respect to isolated or clustered GH-ir cells. Isolated or clustered GH-ir cells were also observed in the tissue of PPD covering dorsally the most rostral part of the dorsal PI (Fig. 8). The GH-ir cells from this area showed similar morphology and immunocytochemical characteristics to GH-ir cells from the central PPD.

In the dorsal and ventral PPD, cells were PAS-positive and stained blue using the AB-PAS-OG method. After the immunocytochemistry, these cells showed strong immunoreactivity to anti-carp α,β-GTH II, anti-carp β-GTH II (Fig. 9), anti-salmon α,β-GTH II, and anti-salmon β-GTH II. However, these cells never showed any immunoreactivity to anti-salmon β-GTH I serum (Table 2). Isolated or clustered GTH-ir cells, differing in size and shape, were present in the dorsal and ventral PPD. Similarly to GH-ir cells, isolated or clustered GTH-ir cells were detected in the PPD that dorsally covered the rostral most part of the PI. In addition, GTH-ir cells were seen in the area surrounding the entire PI (Fig. 11), while a few scattered GTH-ir cells were observed in the ventral RPD (Fig. 10).

The use of anti-human β-TSH serum revealed isolated or clustered TSH-ir cells in the anteroventral PPD (Fig. 12). These cells were stained purple using the AB-PAS-OG method and were PAS-positive. The anti-carp and anti-salmon α,β-GTH II sera immunostained cells in these same area that could correspond to TSH cells (see Table 2), and were smaller and round-shaped (Fig. 13).

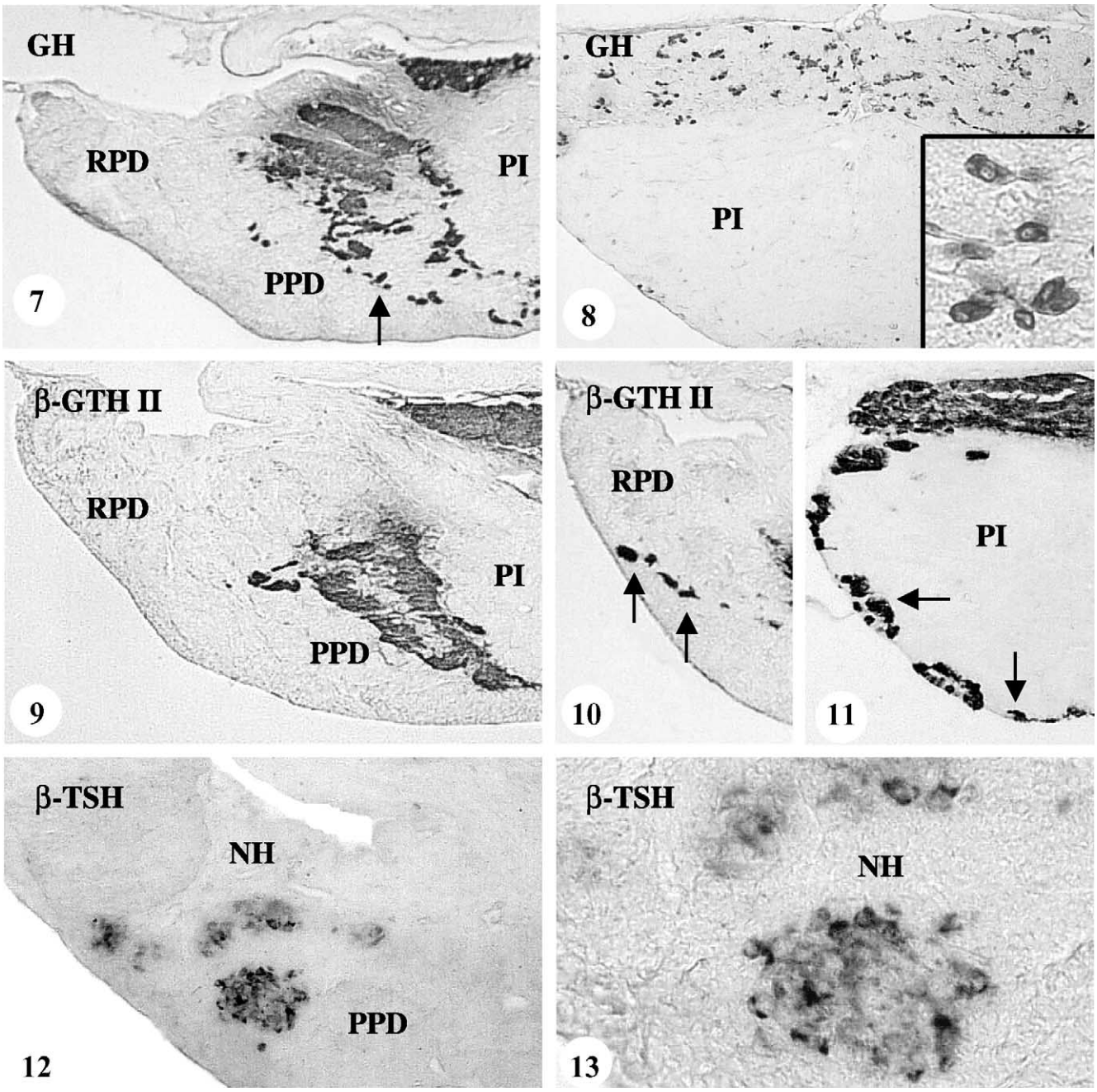
In the PI, ir cells to anti-sSL and anti-sbSL sera were detected and showed similar immunoreactions (Fig. 14). SL-ir cells were negative to PAS method (data not shown) and to antisera developed against other hormones belonging to the GH/PRL family (Figs. 2 and 7). SL-ir cells were seen surrounding the neurohypophyseal tissue and intermingled with immunonegative cells that could correspond to MSH cells (Fig. 15). They displayed either ovoid or elongated shapes, and had ovoid-shaped and central nuclei. Occasionally, a few isolated or clustered SL-ir cells were observed in the dorsal PPD (Fig. 14).

The anti-α-MSH serum specifically immunostained cells in the PI (Fig. 16). A very weak immunoreaction was seen in the PI using anti-hACTH$_{(1–24)}$ serum (Fig. 5). These MSH-ir cells were PAS-negative (data not shown) and stained orange using the AB-PAS-OG method. MSH-ir

Table 2
Immunoreactivity of GTH and TSH cells to different antisera used in the study

Antiserum	GTH cells	TSH cells
Anti-carp α,β GTH II	+	+
Anti-carp β-GTH II	+	−
Anti-salmon α,β-GTH II	+	+
Anti-salmon β-GTH I	−	−
Anti-salmon β-GTH II	+	−
Anti-human β-TSH	−	+

(+) positive and (−) negative immunoreactivity.

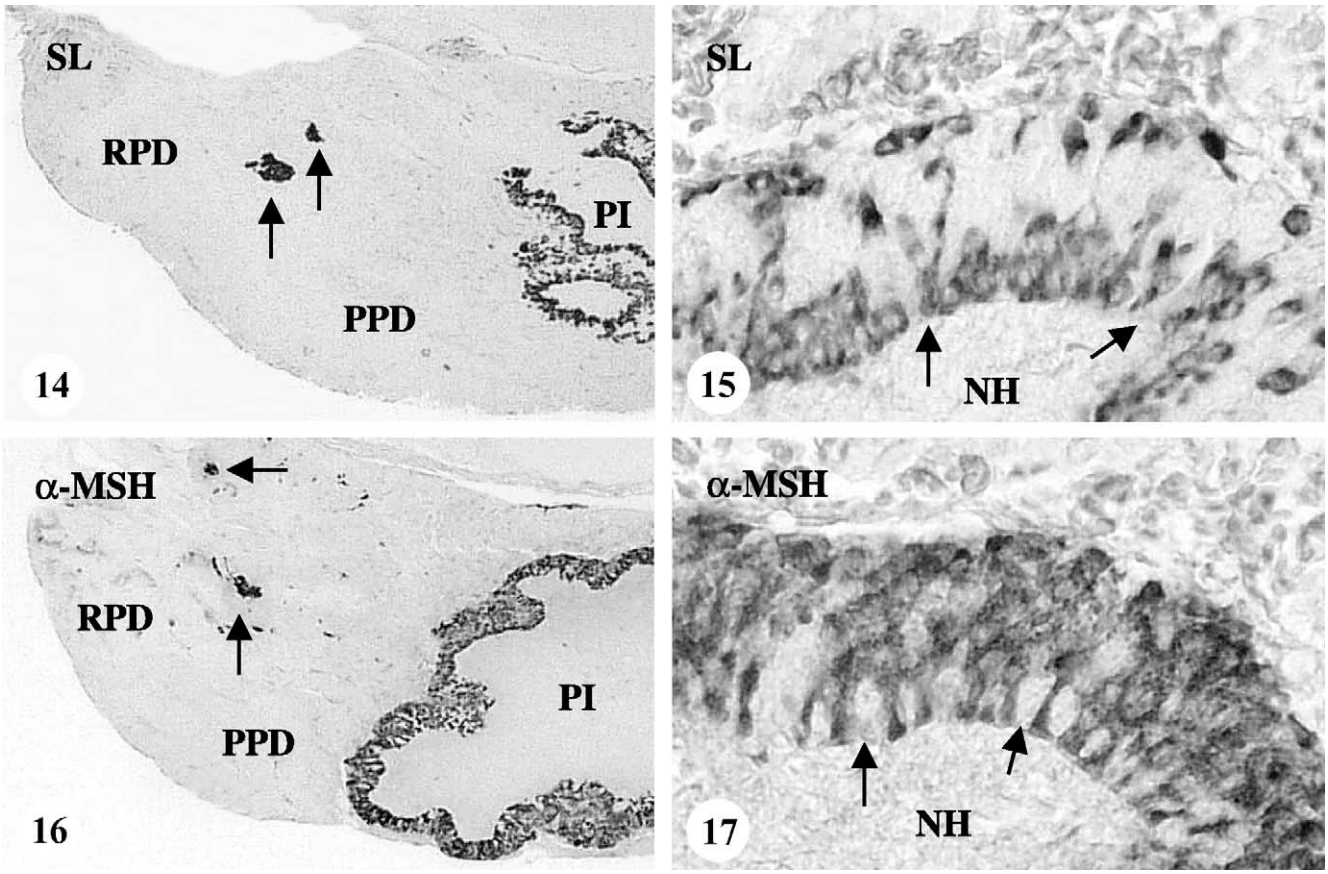


Figs. 7 and 8. Sagittal (Fig. 7) and transverse (Fig. 8) sections through the most rostral PI immunostained with anti-sbGH serum. GH-ir cells were observed surrounding and in contact with the neurohypophyseal processes in the dorsal PPD. Isolated or clustered GH-ir cells were seen in the ventral PPD (arrow) (Fig. 7) and in the tissue of PPD covering the dorsal PI intermingled with cells that could correspond to GTH cells (compare with Fig. 11). Inset: detail of GH cell. Fig. 7: 90×, Fig. 8: 200×, inset: 420×. Figs. 9–11. Sagittal (Figs. 9 and 10) and transverse (Fig. 11) sections immunostained with anti-carp β-GTH II serum. Isolated or clustered GTH II-ir cells of different sizes and shapes are visible in both the dorsal and the ventral PPD (Fig. 9) and in the ventral RPD (Fig. 10, arrows). In the tissue of PPD covering the most rostral PI, isolated or clustered GTH-ir cells were intermingled in the dorsal region with cells that could correspond to GH cells (see Fig. 8). GTH-ir cells were seen all over the PI (Fig. 11, arrows). Figs. 9 and 10: 90×, Fig. 11: 200×. Figs. 12 and 13. Sagittal sections immunostained with anti-hTSH serum. Fig. 12. TSH-ir cells were located in the anteroventral PPD. Fig. 13. Details of round and small TSH-ir cells. Fig. 12: 200×, Fig. 13: 420×.

cells were fusiform-shaped, in contact with the neurohypophyseal tissue and intermingled with immunonegative cells that could correspond to SL-ir cells. Few isolated or clustered MSH-ir cells were observed in the dorsal PPD (Fig. 17).

4. Discussion

Overall, the adenohypophysis and the distribution of adenohypophyseal cells in *T. draco* were similar to those described in a number of teleost species (Ball and Baker, 1969;



Figs. 14 and 15. Sagittal sections immunostained with anti-sbSL serum. Fig. 14. SL-ir cells were seen surrounding the neurohypophyseal tissue of the PI. A few isolated or clustered cells were seen in the dorsal PPD (arrows). Fig. 15. Ovoid- or elongated-shaped SL cells were intermingled with cells that could correspond to MSH cells (arrows). Fig. 14: 90×, Fig. 15: 420×. Figs. 16 and 17. Sagittal sections immunostained with anti-α-MSH serum. Fig. 16. MSH-ir cells were located mainly in the PI. A few isolated or clustered MSH-ir cells were also found in the dorsal PPD (arrows). Fig. 17. Details of MSH-ir cells in the PI intermingled with immunonegative cells that could correspond to SL cells (arrows). Fig. 16: 90×, Fig. 17: 420×.

Holmes and Ball, 1974; Follénius et al., 1978) with a few differences. The adenohypophysis was comprised of three different regions: the RPD, the PPD, and the PI, but the border was unclear in *T. draco*. In particular, tissue of the RPD was overlapped with the most rostral part of the dorsal PPD, while tissue of the PPD overlaid on the most rostral part of the dorsal PI. In *T. draco*, seven different cell types were identified in the adenohypophysis: PRL- and ACTH-ir cells located in the RPD; GH-, GTH-, and TSH-ir cells situated in the PPD; and SL-, MSH-, and GTH-ir cells present in the PI.

4.1. PRL-ir cells

It appears that the antiserum against salmon PRL provides a specific immunoreaction with putative PRL cells of *T. draco*. The same antiserum has been useful previously in the identification of PRL cells in other teleosts (Batten, 1986; Cambré et al., 1986; Quesada et al., 1988; Rendón et al., 1997; Parhar et al., 1998; Rodríguez-Gómez et al., 2001) except the carp *Cyprinus carpio* (Farbridge and Leatherland, 1986) and the ciclid fish *Cichlasoma dimerus* (Pandolfi et al., 2001), in which it showed a weak cross-reactivity with GH cells. Our results showed that PRL-ir cells of *T. draco*, as in other teleosts, were mainly located in the RPD. However, and because tissue of the RPD was overlapped with the most rostral part of PPD, PRL-ir cell clusters were observed below tissue of the PPD. A similar feature has been also observed in *Plecoglossus altivelis* (Saga et al., 1999) and *Diplodus sargus* (Segura-Noguera et al., 2000). In addition, isolated or clustered PRL-ir cells have been described in the anterior PPD of the stripped bass *Morone saxatilis* (Huang and Specker, 1994) and the Mediterranean yellowtail *Seriola dumerilii* (García-Hernández et al., 1996).

The physiological function of PRL-ir cells in freshwater and euryhaline teleosts has been well established (Manzon, 2002). In marine fish, PRL-ir cells have been shown to be involved in stress or reproduction (Wendelaar-Bonga, 1997). This could also be the case in *T. draco* since it is a stenohaline marine teleost that does not encounter hypoosmotic environments. However, further studies will be necessary to link PRL functions to stress and/or reproduction in *T. draco*.

4.2. GH-ir cells

Anti-sGH and anti-sbGH sera were employed in this study. These two antisera have been used for the immunocytochemical identification of GH cells in several other teleost species, in none of which cross-reactions with other cell types have occurred (see below). GH-ir cells of *T. draco* were observed in both the dorsal and ventral PPD, which agrees with previous reports in teleosts (Batten, 1986; Farbridge and Leatherland, 1986; Quesada et al., 1988; Toubeau et al., 1991; Rendón et al., 1997; Vissio et al., 1997; Parhar et al., 1998; Segura-Noguera et al., 2000; Rodríguez-Gómez et al., 2001). An interesting feature concerning the pituitary of *T. draco* is the existence of tissue of PPD covering the most rostral part of the PI. The GH-ir cells from this area showed similar morphology and immunocytochemical characteristics to GH-ir cells from the central PPD.

On the basis of morphology and immunocytochemistry, two different types of GH-ir cells have been reported in *M. saxatilis* (Huang and Specker, 1994). In *S. dumerelii* (García-Hernández et al., 1996) and *T. draco* (present results), it has been found that those GH-ir cells located close to neurohypophyseal tissue always showed less immunoreactivity than the isolated or clustered GH-ir cells in other locations. Nonetheless, all the morphological characteristics of these GH-ir cells were similar. It is thought that there is only a single type of GH-ir cell as in other teleosts. However, studies on GH cells at different times of the year or life cycle would be necessary to corroborate our suggestion.

4.3. SL-ir cells

SL is a quite conservative hormone among species (Rand-Weaver and Kawauchi, 1993; Kaneko, 1996). Two anti-SL sera (anti-salmon and anti-recombinant seabream) were used in this study. Both antisera presented similar and specific immunoreaction. SL-ir cells were seen surrounding the neurohypophyseal tissue and intermingled with MSH-ir cells in the PI. The distribution was agreeable to previous results in teleosts (Rand-Weaver et al., 1991; Kaneko, 1996). However, in some species, such as *Solea senegalensis* (Rendón et al., 1997) and *Thalassoma duperrey* (Parhar et al., 1998), SL-ir cells have been located in the periphery of the PI surrounding MSH-ir cells while in *C. dimerus* (Pandolfi et al., 2001) and *T. thynnus* (Rodríguez-Gómez et al., 2001), the distribution has been inversed. In addition to the PI location, isolated or clustered SL-ir cells have also been found in the PPD in *T. draco* as in *S. dumerilii* (García-Hernández et al., 1996) and *D. sargus* (Segura-Noguera et al., 2000). These SL-ir cells in the PPD could be derived from cell migrations during the ontogenesis of adenohypophysis (see Farbridge and Leatherland, 1986; Villaplana et al., 1997).

SL-ir cells from different species reacted distinctly to the PAS technique. SL-ir cells in several non-salmonid species were PAS-positive due to glycosylated SL (Kaneko, 1996). However, in salmonids (Rand-Weaver et al., 1991; Kaneko, 1996) and in the adults of some non-salmonid teleosts (Villaplana et al., 1997; Segura-Noguera et al., 2000) SL-ir cells were PAS-negative as a result of non-glycosylated SL. Furthermore, some teleost species are both PAS-positive and -negative indicating the presence of both glycosylated and non-glycosylated SL (*Sparus aurata*: Cavari et al., 1995; *S. senegalensis*: Pendón et al., 1998). Since SL-ir cells were PAS-negative in *T. draco*, SL would be non-glycosylated.

It has been suggested that SL is involved in reproduction, calcium metabolism, stress, acid–base regulation, fat metabolism, background adaptation, and osmoregulation (Kaneko, 1996). However, the actual function of SL has not yet been determined. Since this hormone shares a part of structural homology with PRL and GH, there may be certain physiological associations between them (Rand-Weaver and Kawauchi, 1993; Kaneko, 1996).

4.4. ACTH- and α-MSH-ir cells

In teleost, POMC is the precursor of ACTH in the corticotropic cells of the RPD and α-MSH in the melanotropic cells of the PI (Dores, 1990). Sequence analysis has revealed that the first 13 amino acids are identical between two hormones (Follénius and Dubois, 1980; Dores, 1990). Thus, the anti-hACTH$_{(1–24)}$ serum has cross-reacted strongly with native α-MSH in several teleost species (Cambré et al., 1986; Quesada et al., 1988; García-Hernández et al., 1996; Rendón et al., 1997; Vissio et al., 1997; Parhar et al., 1998; Pandolfi et al., 2001). In contrast, a very weak cross-reaction between the ACTH antiserum and MSH-ir cells in *T. draco* was seen. In the barbel *Barbus barbus*, the absence of this cross-reaction has been reported (Toubeau et al., 1991). The same batch of antiserum used in our study has cross-reacted strongly with native α-MSH in several other teleosts (*D. sargus*: Segura-Noguera et al., 2000; *T. thynnus*: Rodríguez-Gómez et al., 2001). On the other hand, it has been reported that α-MSHs contain almost an identical primary structure among different groups of vertebrates, including fish (Arends et al., 2000). Our immunocytochemical results and those of Toubeau et al. (1991) in *B. barbus* indicate that the amino acid sequences of α-MSH in *T. draco* and *B. barbus* may be different from those in humans and other teleost species. It would be interesting to sequence the amino acids of the α-MSH in *T. draco* in the future.

In *T. draco*, ACTH-ir cells were observed in the RPD between PRL cells and the neurohypophyseal tissue, whereas MSH-ir cells detected in the PI were in contact with the neurohypophyseal tissue and intermingled with SL cells. This distribution was consistent with previous reports in teleosts (Cambré et al., 1986; Quesada et al., 1988; García-Hernández et al., 1996; Rendón et al., 1997; Vissio et al., 1997; Parhar et al., 1998; Pandolfi et al., 2001). Some scattered MSH-ir cells were also found in the anterodorsal PPD in *S. dumerilii* (García-Hernández et al., 1996) and *D.*

sargus (Segura-Noguera et al., 2000). Similar to SL-ir cells, these ectopic MSH-ir cells found beyond PI could implicate an event of cell migration during the development of adenohypophysis (Farbridge and Leatherland, 1986; Villaplana et al., 1997). The functions of ACTH and α-MSH have been linked to stress response and background color adaptation (Wendelaar-Bonga, 1997). Thus, it will be interesting to design a specific experiment to elucidate the physiological roles of these hormones in *T. draco*.

4.5. GTH- and TSH-ir cells

The family of PAS-positive glycoprotein hormones includes GTHs (GTH I and II) and TSH. These hormones contain two subunits: a constant α-subunit and a variable β-subunit specific for each hormone and responsible for hormonal activity (Pierce and Parsons, 1981). In some teleosts, two forms of GTH, GTH I and GTH II, have been described (Nozaki et al., 1990; Swanson et al., 1991; Okada et al., 1994; Elizur et al., 1996; García-Hernández et al., 1996, 1997; Gen et al., 2000). Some specific antisera against β-subunits of GTH for the selective immunodetection of these hormones have been developed.

In the present study, we used antisera against salmon β-GTH I and II and carp β-GTH II to detect gonadotropic cells containing GTH. However, anti-salmon β-GTH I serum never showed any immunoreactivity in the pituitary of *T. draco*. Similar results have been observed in *D. sargus* (Segura-Noguera et al., 2000) and *C. dimerus* (Pandolfi et al., 2001) using the same antiserum. In teleost, a high divergence in amino acid sequence of β-subunit of GTH I has been reported (Kawauchi et al., 1989; Swanson et al., 1991). Consequently, GTH I of *T. draco* could not be detected with a heterologous GTH I antiserum. Alternatively, *T. draco* could have one GTH, with high similarity to GTH II but not to GTH I.

GTH-ir cells have been found in all three divisions of the pituitary in *T. draco*. In PPD, they were seen in both the dorsal and the ventral zones (Olivereau and Nagahama, 1983; Batten, 1986; Toubeau et al., 1991). In addition, GTH-ir cells were detected intermingled with GH-ir cells in the tissue of PPD covering the most rostral part of the PI. The presence of GTH-ir cells in the border of the PI agreed with the previous reports in other teleosts (Cambré et al., 1986; Quesada et al., 1988; García-Hernández et al., 1996; Vissio et al., 1997; Rendón et al., 1997; Segura-Noguera et al., 2000; Rodríguez-Gómez et al., 2001). In *T. draco*, scattered GTH-ir cells were also observed in the RPD. The existence of GTH-ir cells in the RPD has also been described in some teleosts (Olivereau and Nagahama, 1983; Dubourg et al., 1985) and could be due to the migration of GTH cells from the PPD to the RPD during the ontogeny of the adenohypophysis (Farbridge and Leatherland, 1986; Nozaki et al., 1990).

In *T. draco*, GTH-ir cells have mainly been found in the PPD and PI. However, these GTH-ir cells always presented similar shapes and immunocytochemical characteristics. These observations suggest the existence of only one class of GTH cells in *T. draco*. However, more evidences will be needed to make this conclusion. For a complete study of gonadotropic cells in *T. draco*, it will be necessary to develop specific and homologous antisera against GTH I and II. Furthermore, the GTH profile of males and females in *T. draco* during the sexual cycle needs be established.

TSH-ir cells in teleosts have been detected with an anti-human β-TSH serum. The antiserum did not cross-react with the β-subunit of TSH in some species (*S. senegalensis*: Rendón et al., 1997; *T. thynnus*: Rodríguez-Gómez et al., 2001), whereas it immunostained both TSH- and GTH-ir cells in some other species (Batten, 1986; Quesada et al., 1988). Furthermore, it only identified TSH cells in some other teleosts (Schreibman and Margolis-Kazan, 1979; Ueda et al., 1983; Cambré et al., 1986; Quesada et al., 1988; García-Hernández et al., 1996; Vissio et al., 1997; Segura-Noguera et al., 2000). In the present study, we got specific immunodetection of TSH cells in *T. draco* using this antiserum. The location and cell morphology of TSH cells agreed with previous results from some teleosts.

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