Identity and distribution of immunoreactive adenohypophysial cells in the pituitary during the life cycle of sea lampreys, *Petromyzon marinus*

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Abstract

Adrenocorticotropin (ACTH), melanotropins (MSHs), growth hormone (GH) and gonadotropin (GTH) have been identified or cloned from the pituitary gland of sea lampreys (*Petromyzon marinus*). The present study was designed to gain insights into the functional significance of these hormones through a description of changes in the occurrence and distribution of cells immunoreactive to their antibodies at several different stages of the sea lamprey life cycle. ACTH-like cells and MSH-like cells were distributed in the rostral pars distalis and the pars intermedia, respectively, throughout the life cycle from ammocoetes (larvae) to pre-spawning adults. A large number of ACTH-like cells were observed during the pre-spawning period when animals may experience the highest stressful conditions. On the other hand, the number of MSH-like cells increased markedly during metamorphosis, in accordance with the completion of eye development. A small number of GH-like cells were present in the proximal pars distalis during the larval and metamorphic phases, but the number of cells increased markedly during the parasitic period, which corresponded well with the rapid somatic growth. GTH-like cells were not observed in the pituitary during the larval and metamorphic phases, but were present in the proximal pars distalis of immediately post-metamorphosed animals. Since there was a high accumulation of GTH-like cells in pre-spawning adults, these cells appeared to be involved in gonadotropic functions. Given that lampreys represent the most ancient group of vertebrates, it is most likely that these hormones have been conserved for their functions throughout vertebrate evolution.

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1. Introduction

Lampreys and hagfish are only two extant representatives of the oldest class of vertebrates, Agnatha (jawless fishes). The agnathans probably arose as the first vertebrates about 530 million years ago, immediately after the evolutionary explosion of multicellular organisms in the Cambrian period (Forey and Janvier, 1993). The pituitary gland of agnathans consists of the same two principal elements that are found in all other vertebrates, an adenohypophysis and a neurohypophysis. Furthermore, the adenohypophysis of the lamprey pituitary gland resembles to that of gnathostome fishes, and is divided into three regions, the rostral pars distalis (RPD), the proximal pars distalis (PPD) and the pars intermedia (PI). To date, four adenohypophysial hormones have...
been identified or cloned from the pituitary gland of sea lampreys: adrenocorticotropic hormone (ACTH), melanotropins (MSHs), growth hormone (GH) and gonadotropin (GTH) (Takahashi et al., 1995a; Kawauchi et al., 2002; Sower et al., 2006). These four hormones have been suggested to be the original adenohypophysial hormones that appeared first during the evolution of the earliest vertebrates (Kawauchi et al., 2002; Kawauchi and Sower, 2006; Sower et al., 2006).

Our previous immunohistochemical studies in the pituitary gland of the sea lamprey (Petromyzon marinus) have revealed that ACTH-like cells are distributed in most parts of the RPD as well as some scattered cells in the PPD, whereas MSH-like cells are present in almost all parts of the PI (Nozaki et al., 1995). In the PPD, GH-like cells occupy the dorsal half (Kawauchi et al., 2002), while GTH-like cells are present in the ventral half (Nozaki et al., 1999; Sower et al., 2006). Thus, these four kinds of adenohypophysial cells occupy most parts of the pituitary gland of adult sea lampreys (Nozaki et al., 2001). However, such immunohistochemical studies have only been completed in pre-spawning anadromous adult sea lampreys. Information using homologous antisera in lampreys during other periods of the lamprey life cycle is completely lacking, excepting for some studies on the post-translational products of proopiomelanocortin (POMC) in larvae (P. marinus, Sower et al., 1995; Geotria australis and Mordacia mordax, Takahashi et al., 2006). Moreover, Northern blotting and in situ hybridization have been employed to show that the two lamprey POMC genes, proopiocortin (POC) and proopiomelanotropin (POM) are differentially expressed during the lamprey life of at least two species of lampreys (Ficle et al., 1998; Heinig et al., 1999; Youson et al., 2006). However, we know little about changes in the activity of the post-translational products of these genes, such as MSH and ACTH, over lamprey development.

Earlier experimental studies including partial hypophysectomy have demonstrated the pituitary involvement in the regulation of several biological events such as pigmentation changes, metamorphosis and reproduction in the lamprey (for reviews, see Larsen and Rothwell, 1972; Hardisty, 1979). However, at the time of the earlier research none of adenohypophysial hormones had been identified, and interpretation in those studies was not always correct in the light of the present knowledge on the lamprey pituitary hormones.

Since we now have a complete profile of all the major adenohypophysial hormones in the lamprey pituitary gland, it is pertinent to study the pituitary cytology at different points of the life cycle to establish whether there is any relationship of the cytology to events such as metamorphosis and reproduction. For this purpose, occurrence and distribution of immunoreactive adenohypophysial cells were studied using sea lampreys at several different stages of the life cycle.

2. Materials and methods

2.1. Animals

Sea lampreys (Petromyzon marinus) at different stages of the life cycle were used. Larval lampreys (ammocoetes) were collected by electrofishing several streams in southern New Hampshire (anadromous variety) during the summer of 1999 and 2000, and in the Great Lakes region, near Toronto, Ontario, Canada (landlocked variety) during the summer of 1995 and 1998. Animals were transported back to the laboratories of the University of New Hampshire and University of Toronto at Scarborough, respectively. The New Hampshire larvae were maintained in a fresh water flow-through system supplied with either 12 °C well water, or ambient local reservoir water, under natural photoperiod, yeast was provided three times per week. Larvae in Canada were housed in filter-glass aquaria containing a layer of 5–10 cm of river silt and flow-through, dechlorinated water at 17–20 °C under natural photoperiod. Prior to the tissue preparations, they were examined for external signs of metamorphosis, and those with the following stages were used. (1) A total of 20 nonmetamorphosing animals were sampled in June of the collected year from both the anadromous (n = 14) and the landlocked varieties (n = 6). They were 11.0–15.0 cm in total length and weighed 1.2–2.3 g. (2) Eighteen metamorphic animals at stages 3–5 (Youson and Potter, 1979) were sampled in July to August of the collected year from the anadromous (n = 6) and the landlocked varieties (n = 12). They were 12.6–14.3 cm in total length and weighed 2.0–5.2 g. (3) Twelve immediately post-metamorphic animals were sampled in October to November of the collected year from the anadromous (n = 8) and the landlocked varieties (n = 4). They were 11.0–13.2 cm in total length and weighed 1.6–2.3 g. Apart from the larvae, (4) six parasite stage landlocked lampreys were collected by the staff of Hammond Bay Biological Station, Michigan, in June 1999, and were shipped to the University of New Hampshire. They were 26–30 cm in total length, weighed 22–25 g, at the time of sacrifice. (5) Ten adult pre-spawning landlocked animals were collected by the staff of Hammond Bay Biological Station, Michigan, in June 1999, and were shipped to the University of New Hampshire. They were 43–55 cm in total length, weighed about 170 g, at the time of sacrifice. Both parasitic and adult lampreys were kept in a freshwater flow-through system supplied with ambient local reservoir water, under natural photoperiod for up to one week.

2.2. Tissue preparations

Animals were killed by decapitation without anesthetization or following anesthetization by immersion in ethyl m-amino benzonate methane-sulfonate (MS222). After rapid removal of the dorsal fibrocranium and exposure of the dorsal surface of the brain, the dissected brain and the attached pituitary were immersed in Bouin–Hollande sublimate solution (Romeis, 1948) for about 24 h. The fixed tissues were dehydrated through a series of solutions with increasing concentrations of ethanol. Deposited mercuric chloride was removed by treatment with iodine–potassium iodide in 90% ethanol for 1–2 day. Tissues were embedded in Paraplast, and were sectioned sagittally at 6 μm in thickness. Every one or two sections were mounted on different gelatin-coated glass slides, so that adjacent sections were stained with different antisera.

2.3. Antisera

Antisera used were: (1) Anti-lamprey naso-hypophysial factor (NHF; lot No. 9207-09-08; optimal working dilution, 1:20,000) for demonstration of ACTH-like cells (Sower et al., 1995; Nozaki et al., 1995). NHF is the N-terminal peptide of the lamprey ACTH precursor, POC (Heinig et al., 1995; Takahashi et al., 1995b). Anti-lamprey NHF was used as the substitution for anti-lamprey ACTH, since the former antiserum does not show any cross-reaction to MSH-like cells, while latter antiserum exhibited a slight crossreaction to MSH-like cells (Nozaki et al., 1995; Ominato and Nozaki, 2002a). (2) Anti-lamprey MSH-B (lot No. 9311, optimal working
2.4. Immunohistochemistry

Immunohistochemical staining was performed by use of a Vectastain avidin–biotin peroxidase complex (Elite ABC) kit. In brief, sections were deparaffinized in xylene, hydrated in a graded ethanol series and washed in phosphate-buffered saline (10 mM sodium phosphate, 0.15 M sodium chloride, pH 7.5; PBS). All procedures were performed at room temperature, and incubations were performed in closed humid chambers. First, the tissue sections were incubated for 30 min in methanol containing 0.3% hydrogen peroxide to block endogenous peroxidase activities, and washed in PBS. To reduce nonspecific staining, sections were treated with diluted normal goat serum for 30 min according to the manufacturer’s instruction. Primary antisera were applied to the sections for about 12 h, and the biotinylated secondary antibody solution and ABC reagent were each applied for 1 h. The final reaction product was visualized with 3,3′-diaminobenzidine tetrahydrochloride in 10 mM Tris–HCl containing 0.003% hydrogen peroxide to block endogenous peroxidase activities, and washed in PBS. To reduce nonspecific staining, sections were treated with diluted normal goat serum for 30 min according to the manufacturer’s instruction. Primary antisera were applied to the sections for about 12 h, and the biotinylated secondary antibody solution and ABC reagent were each applied for 1 h. The final reaction product was visualized with 3,3′-diaminobenzidine tetrahydrochloride in 10 mM Tris–HCl containing 0.03% hydrogen peroxide. The sections were then counterstained with hematoxylin, washed in running water, dehydrated through an increased ethanol series and mounted in Entellan New (Merck).

2.5. Measurement of the size of the RPD, PPD and PI

For measurement of the size (area) of the RPD, nearly mid-sagittal sections stained with anti-lamprey NHF were used. A part of the pituitary gland, which contained the whole part of the RPD, was taken by photographs under a microscope with a digital camera. The resulting images were processed with a computer-aided image analyzer (Image J, NIH, USA), and the area of the RPD was measured by the enclosure of the outer border of the RPD. Then, the area of the RPD was calibrated as mm² from the number of pixels. In each animal, at least three sections were examined, and the mean value was adopted as the size of the RPD of that animal. The size of the PPD was measured in the same way using sections stained with anti-lamprey NHF, anti-lamprey GH or anti-oLH. The size of the PI was also measured in the same way using sections stained with anti-lamprey MSH-B.

2.6. Measurement of the area occupied by each cell type

For measurement, nearly mid-sagittal sections stained with respective antisera were used. For example, for ACTH-like cells, sections stained with anti-lamprey NHF, were examined in a microscope, and images of the part of the pituitary gland which contained both the RPD and the RPD was taken with a digital camera. The images were processed with a computer-aided image analyzer (Image J, NIH, USA). The area occupied by whole population (area) of NHF-positive cells was calculated by adjusting the threshold levels of the figure contrast to subtract the background stain. Then, the area of NHF-positive cells was calibrated as mm² from the number of pixels. In each animal, at least three sections were examined, and the mean value was adopted as the area of ACTH-like cells of that animal. Similar methods were employed for measurement of the remaining cell types.

2.7. Statistics

All data obtained were expressed as the group means ± SEM. Differences between two means were evaluated with Student t-test or Cochran-Cox test. All statistical tests with \( P < 0.05 \) were considered significant.

3. Results

There was no apparent difference on the occurrence and distribution of immunoreactive adenohypophysial cells between the samples from landlocked and anadromous larval, metamorphic and immediately post-metamorphosed periods. Thus, data from the two varieties were incorporated in the various intervals of the life cycle.

3.1. Changes in the size of RPD, PPD and PI during the life cycle

Results on the size of the regions of the adenohypophysis are summarized in Fig. 1 (for details, also see Figs. 3–6). In larvae, the RPD and the PPD were similar in size, and were larger than the PI. On the other hand, in the metamorphic animals the RPD was smaller than the PPD, but was of similar size to the PI. Such changes in the metamorphic animals appeared to be due to more rapid growth of the PPD than the RPD and the PI during the early stages of metamorphosis. The adenohypophysis changed its shape during the progress of metamorphosis mainly due to the expansion of the bottom of the third ventricle. The expansion was more evident at the level of the posterior neurohypophysis and the PI. Thus, the RPD and the PPD exhibited little growth during the latter stages of metamorphosis, whereas the PI increased its size greatly during this same interval resulting in the largest lobe in the adenohypophysis.
at the end of metamorphosis. During the parasitic period, the RPD and PPD grew more rapidly than the PI, and became similar to the PI in size. Finally, the RPD and the PPD became larger than the PI in size in the pre-spawning adults. The PI exhibited apparently continuous growth after the onset of metamorphosis until the pre-spawning adult period.

3.2. ACTH-like cells

At all stages examined, an intense ACTH-like immunoreactivity was found in most cells of the RPD and in a few scattered cells of the PPD (Figs. 2 and 3). Indeed, GH-like or GTH-like cells were rarely observed in the RPD throughout the life cycle (see Figs. 5 and 6). Thus, there was a strong positive correlation between the size of the RPD and the area occupied by whole population (=area) of ACTH-like cells throughout the life cycle (Figs. 1 and 2).

The area of ACTH-like cells per histological section was small in the larvae (Figs. 2 and 3a). The area increased significantly in the metamorphic animals in comparison with that of larvae (Figs. 2 and 3b). However, it was not clear whether the difference was a consequence of the onset of metamorphosis or was mainly due to a slight difference of age of animals, since some metamorphic animals may have been older than the larvae. There was no remarkable change in the area occupied by ACTH-like cells between metamorphic and immediately post-metamorphosed animals (Figs. 2 and 3b, c). The area of ACTH-like cells increased greatly during the parasitic period, and reached to a maximum in the pre-spawning adults (Figs. 2a and 3d, e).

The area occupied by ACTH-like cells in the RPD was 93.5% (larvae), 93.9% (metamorphic animals), 86.9% (recently metamorphosed animals), 78.1% (parasitic animals) and 82.3% (adult animals), respectively. On the other hand, the area occupied by ACTH-like cells in the PPD was 2.3% (larvae), 7.8% (metamorphic animals), 13.1% (recently metamorphosed animals), 4.9% (parasitic animals) and 13.7% (adult animals), respectively.

3.3. MSH-like cells

At all stages examined, an intense MSH-like immunoreactivity was found in almost all cells of the PI (Figs. 2 and 4). Thus, there was also a strong positive correlation between the size of the PI and the area of MSH-like cells throughout the life cycle (Figs. 1 and 2). The area of MSH-like cells per histological section was the smallest in the ammocoete larvae (Figs. 2 and 4a). Thereafter, the area of MSH-like cells exhibited an apparent continuous increase throughout the life cycle, and reached a maximum in the adults (Figs. 2 and 4b–e). The area occupied by MSH-like cells in the PI was 83.4% (larvae), 39.1% (metamorphic animals), 82.3% (recently metamorphosed animals), 85.0% (parasitic animals) and 97.8% (adult animals), respectively.

3.4. GH-like cells

A small number of scattered GH-like cells were observed in the PPD of the larvae (Figs. 2 and 5a). Small number of GH-like cells were still present in the metamorphic and immediately post-metamorphosed animals (Figs. 2 and 5b, c). Thus, even in the post-metamorphosed animals, most cells in the PPD remained unstained with any antisera to lamprey pituitary hormones (Fig. 5c). The marked increase in the area of GH-like cells was observed in the parasitic lampreys, in which GH-like cells were distributed throughout the PPD (Figs. 2 and 5d). In the pre-spawning adult lampreys, a large number of GH-like cells were accumulated in the dorsal half of the PPD (Figs. 2 and 5e). The area occupied by GH-like cells in the PPD was 3.9% (larvae), 4.5% (metamorphic animals), 19.9% (recently metamorphosed animals), 30.7% (parasitic animals) and 32.7% (adult animals), respectively.
Fig. 3. Representative nearly mid-sagittal sections through the adenohypophysis of sea lampreys stained with anti-lamprey NHF. (a) Larval animal (L); (b) metamorphic animal (M-ing); (c) immediately post-metamorphosed animal (M-ed); (d) parasitic stage landlocked animal (P), and (e) adult pre-spawning landlocked animal (LL). All pictures were taken at the same magnification. PI, pars intermedia; PPD, proximal pars distalis; RPD, rostral pars distalis. Scale bars: 50 μm (200×).

Fig. 4. Representative nearly mid-sagittal sections through the adenohypophysis of sea lampreys stained with anti-lamprey MSH-B. See Fig. 3 for legends and abbreviations. Scale bars: 50 μm (200×).
Fig. 5. Representative nearly mid-sagittal sections through the adenohypophysis of sea lampreys stained with anti-lamprey GH. See Fig. 3 for legends and abbreviations. Scale bars: 50 μm (200×).

Fig. 6. Representative nearly mid-sagittal sections through the adenohypophysis of sea lampreys stained with anti-ovine LHβ. See Fig. 3 for legends and abbreviations. Scale bars: 50 μm (200×).
3.5. GTH-like cells

GTH-like cells were not found in the pituitary gland in larvae or metamorphic animals (Figs. 2 and 6a, b). They were first observed in the ventral part of the PPD in immediately post-metamorphosed animals (Figs. 2 and 6c). The area of GTH-like cells increased slightly in parasitic lampreys (Fig. 2), in which GTH-like cells were scattered in the ventral half of the PPD (Fig. 6d). In the pre-spawning adults a huge number of GTH-like cells were accumulated in the ventral half of the PPD (Fig. 6e). The area occupied by GTH-like cells in the PPD was 0% (larvae and metamorphic animals), 20.2% (recently metamorphosed animals), 13.2% (parasitic animals) and 34.5% (adult animals), respectively.

4. Discussion

4.1. ACTH-like cells

The activity of ACTH-like cells, as judged from the size of the RPD and the area of positive cells, appeared to be relatively low during the larval and metamorphic phases, but increased rapidly during the parasitic and pre-spawning adult phases. These results were in excellent agreement with those of Ficele et al. (1998), Heinig et al. (1999) and Youson et al. (2006), who reported similar changes in the expression of proopio-corticotropin (POC) mRNAs during the life cycle of sea lampreys and nonparasitic lampreys.

In the present study, ACTH-like cells occupied most parts of the RPD even in the larvae. In this connection, Sower et al. (1995) have reported that NHF (=ACTH)-like immunoreactivity was found in the pars distalis of a sea lamprey larva 56 days after fertilization. Thus, ACTH-like cells appear to be active from a very early period of lamprey development, and thus they seem to be involved in providing a hormone that is critical for larval and later life. In gnathostomes, ACTH is known to stimulate the adrenal cortex or its homologue to produce corticosteroids. Mammalian ACTH has been shown to stimulate interrenal cells of lamprey in vivo (Sterba, 1955; Youson, 1973), and corticosteroids have been identified in the circulation of the sea lamprey (Weisbart and Youson, 1975). Lamprey ACTH has also been shown to stimulate in vitro steroidogenic activity (Takahashi et al., 1995a). Thus, ACTH-like cells appeared to possess corticotropic functions similar to those of more advanced gnathostome vertebrates.

The present study further showed that peak levels of activity of ACTH-like cells occurred during the pre-spawning phase when the animal is undergoing upstream migration, and preparation for reproduction. Such increase in the activity of ACTH-like cells appears to be involved in material mobilization processes to cover energy requirements during starvation as well as various stresses such as migration, reproduction and post-spawning mortality. In support of the last possibility, Larsen (1973) has shown that prolonged survival can be induced by maintaining the lampreys at constant, low temperature, by castration and more especially by hypophysectomy. The possibility that hypersecretion of the adrenocortical tissues might be involved in the post-spawning mortality of salmonid fishes has been widely discussed (Pickering and Pottinger, 1987; Maule et al., 1996). Thus, hyperactivity of ACTH-like cells in the pre-spawning adult lamprey may also be involved in the post-spawning mortality.

On the other hand, there was no remarkable change in the size of RPD or the area of ACTH-like cells during metamorphosis. Similar results were also obtained by a qualitative and quantitative in situ hybridization study of POC mRNA during the life cycle of the sea lamprey (Ficele et al., 1998). These results were somewhat strange in the light of the results from an earlier study which suggested that the pituitary is involved in metamorphosis of the lamprey. Namely, Joss (1985) performed partial hypophysectomy in the Southern Hemisphere lamprey, Geotria australis, and showed that removal of the RPD completely blocked metamorphosis, whereas removal of the PPD allowed metamorphosis to begin but not to proceed to completion. Takahashi et al. (2006) have reported that distribution of POC and POM in the pituitary gland was similar among all modern lampreys including Geotriidae. Thus, it seems likely that ACTH-like cells might be involved in metamorphosis of lampreys, however, the nature of their activity requires further study.

4.2. MSH-like cells

MSH is generally associated with pigmentation changes in vertebrates. Larval and adult lampreys normally maintain a diurnal color change rhythm (Eddy, 1972). Hypophysectomy of both larval and adult lampreys leads to permanent paling (Young, 1935; Larsen, 1965; Eddy and Strahan, 1968). Further studies with hypophysectomy have also demonstrated that melanin-dispersing hormone is secreted from the pars nervosa or the PI (Young, 1935) and not from the RPD and the PPD (Larsen, 1965). More recently, Takahashi et al. (1995a) have isolated two forms of MSHs, MSH-A and MSH-B, from the sea lamprey pituitary gland, and showed that both lamprey MSH-A and -B have melanotrophic activity in an in vitro assay of melanin-dispersing activity on frog skin, but no corticotropic activity. Immunoreactive MSH-A and -B have been demonstrated in the PI of the sea lamprey pituitary gland (Nozaki et al., 1995). These data implicate involvement of MSHs in pigmentation changes in the lamprey.

One of several conspicuous features of MSH-like cells revealed in the present study was that the size of the PI increased markedly during metamorphosis, which resulted in the largest lobe among the three adenohypophysial lobes at the time of the completion of metamorphosis. In the
lamprey, the completion of eye development occurs during metamorphosis (Youson, 1980). Thus, the timing of the rapid increase in the size of the PI corresponded well with the timing of the completion of eye development. Incidentally, larval lampreys are blind, but exhibit a diurnal color change rhythm, as already mentioned above. This color change can be completely abolished by pinealectomy, leaving the animals permanently dark, and thus the pineal organ is involved in the diurnal color change rhythm of the larvae (Eddy, 1972). It seems most likely that a diurnal information is transferred to MSH-like cells through the pineal organ in the larvae.

In the present study, the activity of MSH-like cells, as judged from the size of the PI and the area of MSH-like cells, attained maximum levels in the pre-spawning adult lamprey. Thus, MSH-like cells may have additional functions in relation to the stress response and/or reproduction. In agreement with the present finding, Heinig et al. (1999) and Youson et al. (2006) also reported the highest levels of POM expression in the pre-spawning adult sea lampreys. Moreover, Sower et al. (2006) have reported that after expressed sequence tag (EST) analysis of the sea lamprey pituitary gland, POM genes were the most actively expressed among four adenohypophysial hormone genes in the pre-spawning adults. Indeed, they reported that from the cDNA library, 2208 clones were subjected to sequence analysis from the 5’ end, of which 281 clones corresponded to pituitary hormones; 155 clones for POM cDNA, 124 clones for POC cDNA, 9 clones for GH cDNA and 3 clones for GTHβ cDNA. Since POM produces another β-endorphin as well as MSHs, it is likely that β-endorphins derived from both POC and POM are involved in the stress response in relation to reproduction.

4.3. GH-like cells

Until recently, there was no convincing data for the presence of GH in the lamprey pituitary gland except for some immunohistochemical evidence (Wright, 1984; Ominato and Nozaki, 2002). However, Kawauchi et al. (2002) have reported for the first time the identification of GH and demonstration of GH-insulin-like growth factor (IGF) system in the sea lamprey. Kawauchi et al. (2002) have suggested that GH is the only member of the GH/PRL family in the lamprey, and thus GH may be the ancestral hormone that came first in the molecular evolution of the GH/PRL family. In the present study, a marked increase in the area of GH-like cells was observed in the parasitic stage lampreys. Such marked increase in the area of GH-like cells corresponded well with the rapid somatic growth during that phase. On the other hand, activity of GH-like cells, as judged from the area of positive cells, appeared to maintain at low levels during the larval and metamorphic phases, when animals exhibit very low growth rates. Thus, the present study provides support for a view of the somatotropic function of GH in the lamprey.

4.4. GTH-like cells

Very recently, a single GTHβ-like protein has been identified from the sea lamprey pituitary gland after extensive and exhaustive research of over 20 years (Sower et al., 2006; Kawauchi and Sower, 2006). In the present study, GTH-like cells, which were stained with anti-oLHβ, were densely accumulated in the ventral half of the PPD in the pre-spawning landlocked adult lamprey. These results agreed well with those of previous studies in the anadromous adult lampreys (Nozaki et al., 1999; Sower et al., 2006). Since GTH-like cells were most active in the pre-spawning adults, when sexual maturation is nearing completion, these cells are likely involved in the gonadotropic functions.

The present study further revealed that GTH-like cells were absent from the pituitary gland in larval and metamorphic animals, but were present in the PPD in immediately post-metamorphosed animals. The timing of the first appearance of GTH-like cells in the pituitary coincided well with the increase in the hypothalamic GnRH contents during the late stages of metamorphosis in sea lampreys (Youson and Sower, 1991; Youson et al., 2006). Moreover, the timing of the first appearance of GTH-like cells preceded the onset of vitellogenesis and spermatogenesis, both of which occur after the completion of metamorphosis in the parasitic anadromous lamprey (Hardisty, 1971).

4.5. Cells uncharacterized as to immunostaining affinity

In the present study, most cells in the PPD were not characterized immunohistochemically during early stages of the life cycle. In this connection, Percy et al. (1975) have reported that, in P. marinus, on the basis of electron microscopy, granulated cells can be detected in the RPD of the younger larvae within two years of hatching, while the PPD is almost completely devoid of granulated cells up to the onset of metamorphosis. Thus, most cells of the PPD during early stages of the life cycle seem to be undifferentiated cells.

To date, there is no evidence to support the presence of thyroid-stimulating hormone (TSH) in lampreys. However, two kinds of glycoprotein hormone receptors have been cloned in the sea lamprey (Freamat et al., 2006): one is GTH-like receptor located in the gonads and the other is a TSH-like receptor in the thyroid tissue. Therefore, it could be considered that TSH may exist in lampreys. Although most cells of the RPD and PPD of pre-spawning adult sea lampreys were identified as one of three adenohypophysial cells, some cells still remained uncharacterized immunohistochemically. Some of unstained cells may account for TSH-like cells.

4.6. General discussion and conclusion

The present study has revealed that each of the four adenohypophysial cells exhibit distinctly different patterns on the occurrence and distribution over the life cycle of sea...
lampreys. Such differences among cell types seemed to be well correlated with specific changes in activity or with developmental events during the life cycle. Namely, the prominence of ACTH-like cells and MSH-like cells at specific intervals of the life cycle such that they are likely involved in corticotropic and melanotropic functions, respectively. On the other hand, the timing of the prominence of GH-like and GTH-like immunoreactivity suggests that there are cell types involved in the somatotropic and gonadotropic functions, respectively. Thus, the present results imply that each of the four adenohypophyseal cells possess biological functions similar to those of more advanced gnathostome vertebrates. The present data also support a viewpoint that these four hormones are among the original pituitary hormones, and maintain their original functions throughout vertebrate evolution, as suggested by Kawauchi et al. (2002), Kawauchi and Sower (2006) and Sower et al. (2006). On the other hand, the later derived hormones, such as prolactin and TSH, arose at a later date to contribute to the expansion of vertebrates into new environments (Kawauchi et al., 2002).

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