General and Comparative Endocrinology 113, 23–31 (1999)
Article ID gcen.1998.7189, available online at http://www.idealibrary.com on IDEAL®

Possible Gonadotropin Cells in the Lamprey Pituitary: Colocalization of Mammalian LH-like Immunoreactivity and Glycoconjugate in Adult Sea Lampreys (*Petromyzon marinus*)

Masumi Nozaki,*,[1] Kunihiro Ominato,* Akiyoshi Takahashi,† Hiroshi Kawauchi,† and Stacia A. Sower‡

**Sado Marine Biological Station, Niigata University, Tassha, Aikawa, Sado, Niigata 952-2135, Japan; †Laboratory of Molecular Endocrinology, School of Fisheries Sciences, Kitasato University, Sanriku, Iwate 022-0101, Japan; and ‡Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham, New Hampshire 03824*

Accepted September 25, 1998

In lampreys, although gonadotropin (GTH) has not yet been isolated from the pituitary gland, the presence of GTH has been strongly suggested. To detect possible GTH in the sea lamprey (*Petromyzon marinus*) pituitary, two different cytochemical probes were tested: One was the use of antibodies to GTHs, and the other was the use of lectin-screening kits for demonstration of glycoconjugate in hormonal molecules. GTH-like immunoreactivity was found in cells distributed in the ventral half of the proximal pars distalis. These cells were stained intensely by all four lots of anti-ovine LH including LH**β**, and were stained moderately or weakly by several other antibodies to LH-related GTHs, such as human LH**β**, hCGβ, amphibian LH, and sturgeon GTH II**β**. On the other hand, there were no positive reactions in the sea lamprey pituitary using the antibodies to FSH-related GTHs, thyrotropin (TSH), or pituitary glycoprotein hormones of teleost origin. Thus, GTH-like material in the sea lamprey pituitary seems to be more closely related to mammalian-like LH, rather than to FSH or TSH, as far as immunocytochemical determinants. A total of 21 kinds of lectins was tested. Among those, GTH-positive cells were also stained positively by concanavalin A and *Vicia villosa* agglutinin. Thus, the present study demonstrates colocalization of LH-like immunoreactivity and glycoconjugate in cells in the ventral half of the proximal pars distalis of the sea lamprey pituitary. It is suggested that those cells are most likely to be GTH cells in the sea lamprey pituitary.

Lampreys and hagfish are the only two extant representatives of the oldest class of vertebrates, Agnatha (jawless fishes). The lamprey pituitary consists of the same two principal elements that are found in all other vertebrates, an adenohypophysis and a neurohypophysis. The lamprey adenohypophysis is divided into three regions, the rostral pars distalis, the proximal pars distalis and the pars intermedia (Gorbman, 1980). The neurohypophysis has two regions, the anterior neurohypophysis (homologue of the infundibulum) and the posterior neurohypophysis (homologue of the pars nervosa). This overt morphological similarity between pituitaries of lampreys and gnathostome fish suggests early establishment of the endocrine and neuroendocrine functions of the tissue in vertebrates (Ball and Baker, 1969).

Among adenohypophysial hormones, adrenocorticotropin (ACTH) and melanotropins (MSHs) have only

[1] To whom correspondence should be addressed. Fax: +81-259-75-2870. E-mail: f10048@ccfsv.cc.niigata-u.ac.jp.

0016-6480/99 $30.00

been isolated from the lamprey pituitary (Takahashi *et al.*, 1995a). Unlike ACTH and MSH in gnathostome vertebrates, they were found to be encoded in two distinct genes in the lamprey (Heinig *et al.*, 1995; Takahashi *et al.*, 1995b). One gene encoded ACTH and endorphin, and the other gene encoded MSH-A, MSH-B, and another endorphin. Both MSHs differed significantly from gnathostome MSHs, and could not be assigned as α-MSH, β-MSH, or γ-MSH. Nevertheless, the topographic distributions of ACTH and MSHs in the lamprey pituitary were similar to those of gnathostome vertebrates: Immunoreactive (ir)-ACTH cells were found in the pars distalis and ir-MSH cells in the pars intermedia (Nozaki *et al.*, 1995). As yet there is no convincing evidence that the lamprey pituitary secretes thyrotropin (TSH), prolactin, or growth hormone (for review, see Hardisty and Baker, 1982).

Although gonadotropin (GTH) has not yet been isolated from the lamprey pituitary gland, there is strong evidence for the presence of GTH in the lamprey, as well as gonadotropic functions (see for review, Larsen and Rothwell, 1972; Hardisty and Baker, 1982; Sower, 1990). In river lampreys (*Lampetra fluviatilis*), hypophysectomy and substitution therapy with pituitary extracts or mammalian GTHs indicated pituitary regulation of the gonads (Evennett and Dodd 1963; Larsen 1965, 1969, 1987). Moreover, injection of salmon gonadotropin preparation into adult spawning sea lamprey (*Petromyzon marinus*) advanced ovulation by several weeks and elevated plasma estradiol levels (Sower *et al.*, 1983). Immunoreactive LH has been reported in the proximal pars distalis of the sea lamprey pituitary by use of anti-rat LHβ (Wright, 1983). Another study using *in vitro* autoradiography demonstrated specific gonadotropin-releasing hormone (GnRH) binding in the proximal pars distalis of sea lamprey pituitary (Knox *et al.*, 1994). Thus, we may propose that the chemistry of the lamprey GTH broadly resembles that of higher vertebrates.

The aim of this study was to localize possible GTH cells in the sea lamprey pituitary. For this purpose, two different cytochemical probes were tested: One was the use of antibodies to glycoprotein hormones or their subunits of various gnathostome vertebrates, and the other was the use of lectin-screening kits for demonstration of glycoconjugate in hormonal molecules. Since all the GTHs that have been isolated thus far from pituitaries are glycoproteins, the lamprey GTH may also be a glycoprotein.

MATERIALS AND METHODS

Animals

Twenty adult sea lampreys (*P. marinus*) of both sexes were used. They were collected in May and June 1997 during their upstream spawning migration from the sea, from traps located at the upstream end of a salmon ladder on the Cocheco River in Dover, New Hampshire. The animals were transported to the Fish Hatchery of the University of New Hampshire and were kept in a freshwater flow-through system supplied with ambient local reservoir water, under natural photoperiod up to 1 month. They were approximately 65 cm in total length and weighed approximately 900 g at the time of sacrifice.

Tissue Preparations

The animals were killed by decapitation after being anesthetized by immersion in ethyl *m*-aminobenzoate methanesulfonate (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 days. Tissues were embedded in Paraplast, and serial sagittal sections of 6 μm were mounted on glass slides.

Antisera and Lectin

The primary antisera used are shown in Table 1, together with sources, lot numbers, and working dilutions. The biotinylated lectins used are listed in Table 2, together with concentrations applied and inhibitory sugars. Those lectins were purchased from

TABLE 1
Results on Immunostaining in the Pituitary of Adult Sea Lampreys

Antibody to	Source	Lot no.	Working dilution	Immunoreactivity
Ovine LH	NIDDK	AFP-192279	×12,000	+++[a]
Ovine LH	Mori, Y.	YM18	×2,000	++
Ovine LH β	NHPP	AFP-697071P	×8,000	+++
Ovine LH β	Wakabayashi, K.	HAC-OV27 β-01RBP85	×3,000	++
Human LH β	NIAMDD	AFP-54372	×3,000	++
Human FSH β	NIAMDD	AFP-3710194	×4,000	−
Human TSH β	NIAMDD	AFP-62423473	×3,000	−
Rat TSH	Wakabayashi, K.	HAC-RT29-01RBP86	×20,000	−
Human CG β	Wakabayashi, K.	HAC-HM51 β-03RBP84	×3,000	+
Human CG	Nozaki, M.		×6,500	−
Chicken LH	Wakabayashi, K.	HAC-CH27-01RBP75	×9,000	−
Chicken FSH	Ishii, S.	ACF1S	×3,000	−
Toad LH	Ishii, S.	L-2	×2,000	+
Bullfrog LH	Wakabayashi, K.	MOR-BF27-RBP75	×4,000	+
Bullfrog LH	Ishii, S.	2	×9,000	−
Bullfrog FSH	Wakabayashi, K.	MOR-BF28-RBP86	×10,000	−
Salmon GTH	Swanson, P.	6404	×3,000	−
Salmon GTH	Kobayashi, M.		×2,000	−
Salmon TSH	Swanson, P.	6350	×1,500	−
Salmon GTH I β	Swanson, P.	8622	×3,000	−
Salmon GTH II β	Swanson, P.	8624	×2,000	−
Salmon GTH α	Kawauchi, H.	9009	×8,000	−
Silver carp GTH	Kobayashi, M.		×2,500	−
Sturgeon GTH II β	Kawauchi, H.	9205	×4,000	+
Sturgeon GTH II β	Kawauchi, H.	9206	×4,000	−

[a] +++, Intense immunoreaction; ++, moderately intense immunoreaction; +, weak immunoreaction; −, no immunoreaction.

Vector Laboratories, Inc., and/or from Wako Inc., Japan.

Cytochemistry

Immunocytochemical 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 close 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. The sections were then counterstained with hematoxylin, washed in running water, dehydrated through an increased ethanol series, and mounted in Entellan (Merck).

Lectin cytochemistry was also performed by use of a Vectastain avidin-biotin peroxidase complex (Elite ABC) kit. The staining procedures followed those of the above-mentioned immunostaining, but in the case of Con A staining, 0.05 M Tris-HCl-buffered saline (pH 7.6, TBS) was used as a substitute for PBS. Thus, biotin-conjugated lectins were applied to sections for about 12 h (Table 2), instead of using primary antibodies and biotinylated secondary antibody. Some dewaxed sections were incubated with 12.5 mg of trypsin

(from porcine pancreas, 12,000–15,000 BAEE units/mg, Wako Inc.) and 100 mg of calcium chloride in 100 ml of TBS for 10 min before lectin cytochemistry.

To test the specificity of the immunostaining, the following control stains were done: (1) replacement of primary antisera with normal rabbit serum, and (2) absorption of primary antisera with ovine LHβ (AFP-35B; 20 μg/ml antibody at working dilution) or human LHβ (AFP-3477A; 20 μg/ml antibody at working dilution). In control experiments of lectin cytochemistry, biotinylated lectins were preincubated with 0.1 M of the specific inhibitory sugars (Table 2) for 1 h before cytochemistry.

TABLE 2
Biotinylated Lectins Used in the Present Study

Acronym of lectins	Concentration (μg/ml) Vector: Tested range	Vector: Optimum	Wako: Tested range	Wako: Optimum	Inhibitory sugar (0.1 M)
BSL-I[a]	1	1			D-galactose
BSL-II	1	1			D-galactose
Con A	0.08–1	0.1	0.02–10	0.1	D-Mannose
DBA	0.3–1	0.5	0.1–10	1	Gal-NAc
DSL	1	1			Glc-NAc
ECL	0.5–1	0.5			D-galactose
Jac	1	1			Gal-NAc
LCA	0.1–10	0.2	0.1–10	0.2	D-Mannose
LEL	0.5–1	0.5			Glc-NAc
PHA-E	1	1	0.02–10	0.1	
PHA-L	0.5–1	0.5			
PNA	0.07–1	0.1	0.02–10	0.1	D-Galactose
PSA	0.2–1	0.25			D-Mannose
RCA-I	0.5–1	0.5			D-Galactose
SBA	0.25–1	0.5	0.1–10	1	Gal-NAc
SJA	1	1			Gal-NAc
STL	0.5–1	0.5			Glc-NAc
UEA-I	1	1	0.1–10	1	L-fucose
VVA	0.3–1	0.5			Gal-NAc
WGA	0.1–1	0.16	0.1–10	0.3	Glc-NAc
S-WGA	0.12–1	0.16			Glc-NAc

[a] BSL-I, *Bandeiraea simplicifolia* lectin I; BSL-II, *Bandeiraea simplicifolia* lectin II; Con A, concanavalin A; DBA, *Dolichos biflorus* agglutinin; DSL, *Datura stramonium* lectin; ECL, *Erythrina cristagalli* lectin; Jac, Jacalin; LCA, *Lens culinaris* agglutinin; LEL, *Lycopersicon esculentum* (tomato) lectin; PHA-E, *Phaseolus vulgaris* Erythroagglutinin; PHA-L, *Phaseolus vulgaris* Leucoagglutinin; PNA, peanut agglutinin; PSA, *Pisum sativum* agglutinin; RCA-I, *Ricinus communis* agglutinin I; SBA, soybean agglutinin; SJA, *Sophora japonica* agglutinin; STL, *Solanum tuberosum* (potato) lectin; UEA-I, *Ulex europaeus* agglutinin I; VVA, *Vicia villosa* agglutinin; WGA, wheat germ agglutinin; S-WGA, succinylated wheat germ agglutinin.

TABLE 3
Results on Lectin Staining in the Pituitary of Sea Lampreys

Lectins[a]	RPD[b]	PPD	PI
Con A	−[c]	++	−
VVA	+	++	+
WGA	+++	+	−
S-WGA	+++	+	−
LCA	+++	+	−
ECL	+++	+	−
SBA	+++	+	−
PSA	+++	+	+++
DBA	+	+	+++
PNA	−	−	+++

[a] No specific reaction for BSL-I, BSL-II, DSL, Jacalin, LEL, PHA-E, PHA-L, RCA-I, SJA, STL, or UEA-I.

[b] RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia.

[c] +++, Most cells were stained positively. ++, About half cells were stained positively. +, Some cells were stained positively. −, No cells were stained positively.

RESULTS

Results obtained are summarized in Tables 1 and 3.

Immunocytochemistry

All four lots of anti-ovine LH including LHβ gave an intense immunoreaction in the proximal pars distalis, where most cells distributed in the ventral half were stained positively (Figs. 1a and 1b). These cells were also stained moderately with anti-human LHβ (Figs. 1c and 1d). The same cells were stained weakly with several other antibodies to LH-related gonadotropins, such as hCGβ, toad LH, bullfrog LH, and sturgeon GTH IIβ (Figs. 1e–1h), though the results were inconsistent using different antibody lots (Table 1). On the other hand, none of the antibodies to FSH-related gonadotropin or TSH gave positive reactions in the pituitary. Similarly, any antibodies to pituitary glycoprotein of teleost origins did not give any positive reaction in the pituitary.

Lectin Screening

Three different types of glycoconjugate-positive cells were revealed by lectin cytochemistry. The first type of

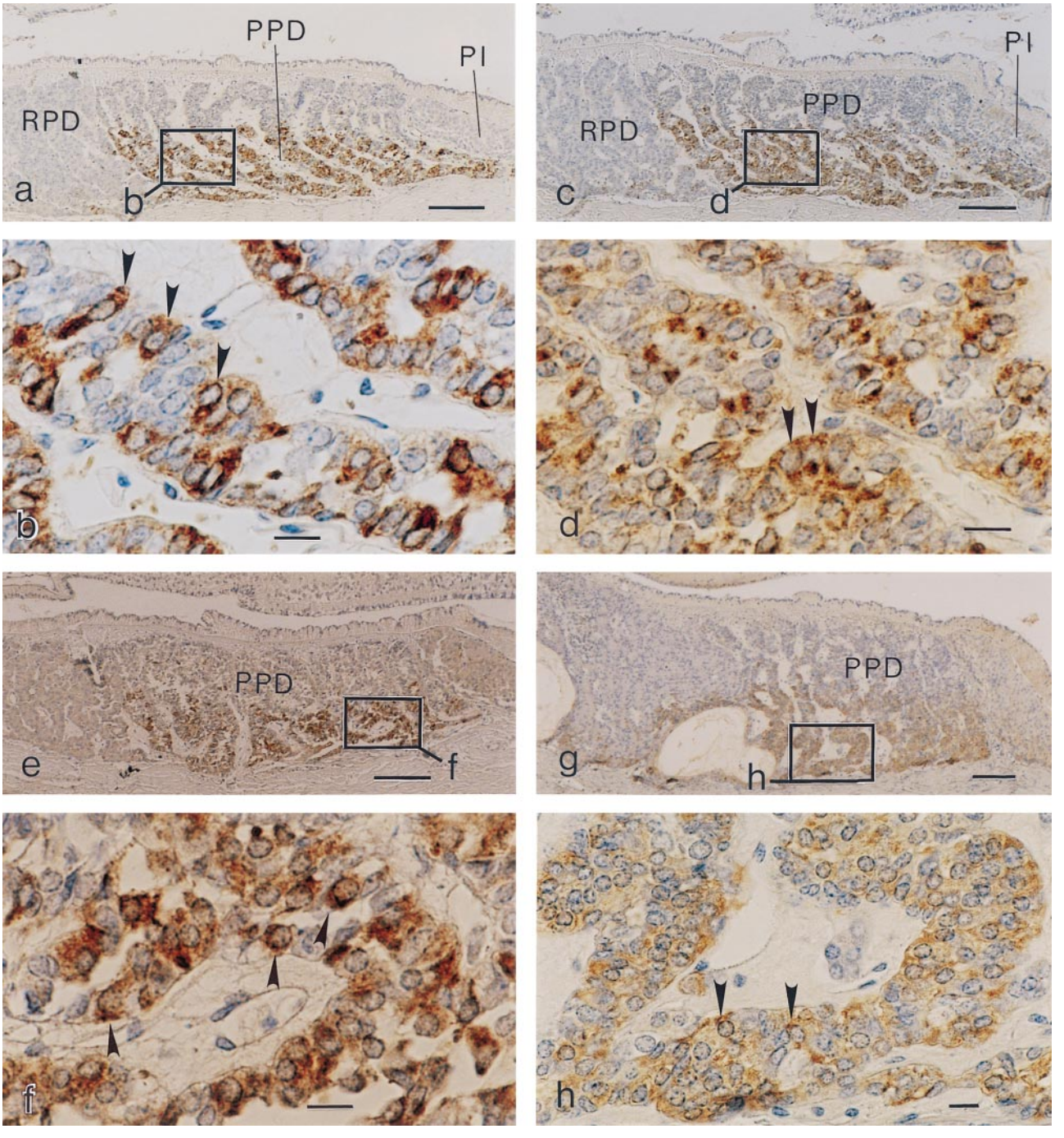

FIG. 1. (a, c, e, g) Four sagittal sections of the pituitary gland of adult sea lampreys, stained with anti-ovine LH (a), anti-human LHβ (c), anti-toad LH (e), and anti-sturgeon GTH IIβ (g), respectively. The areas outlined by rectangles (part of proximal pars distalis, PPD) are enlarged and shown in b, d, f, and h. Arrowheads show typically stained cells by respective antibodies. PI, pars intermedia; RPD, rostral pars distalis. Scale bars: a, c, e, g, 100 μm; b, d, f, h, 10 μm.

cells was stained positively by Con A and VVA and was found in the ventral half of the proximal pars distalis (Figs. 2a–2d). These cells were also stained positively by several anti-LH-related GTH antibodies (see Figs. 1a–1h). The second type of cells was stained positively by WGA, s-WGA, LCA, SBA, PSA, and VVA. Using these lectins most cells of the rostral pars distalis and a few scattered cells of the proximal pars distalis were stained positively. The third type of cells was stained positively by DBA, PNA, PSA, and VVA and was found in the pars intermedia, where almost all cells were stained positively. The second and the last types of cells appeared to correspond with ACTH cells and MSH-B cells in our previous studies, respectively (data not shown, see Sower *et al.*, 1995; Nozaki *et al.*, 1995). The remaining lectins gave no specific reaction in the adenohypophysial cells of the sea lamprey pituitary (Table 3).

Following the trypsinization of dewaxed sections, none of the lectins showed significant changes in the stainability of the first type of cells, though significant differences were found in the second and last types of cells in some lectins (data, not shown).

DISCUSSION

The present study demonstrated GTH-like immunoreactivity in cells distributed in the ventral half of the proximal pars distalis of the sea lamprey pituitary. These cells were stained intensely by all four lots of anti-ovine LH including LHβ and were stained moderately or weakly by several other antibodies to LH-related GTHs, such as human LHβ, hCG, amphibian LH, and sturgeon GTH IIβ. On the other hand, there were no positive reactions in the sea lamprey pituitary using the antibodies to FSH-related GTHs, TSH, or glycoprotein hormones of teleost origin. Thus, GTH-like material in the sea lamprey pituitary seems to be more closely related to mammalian-like LH, rather

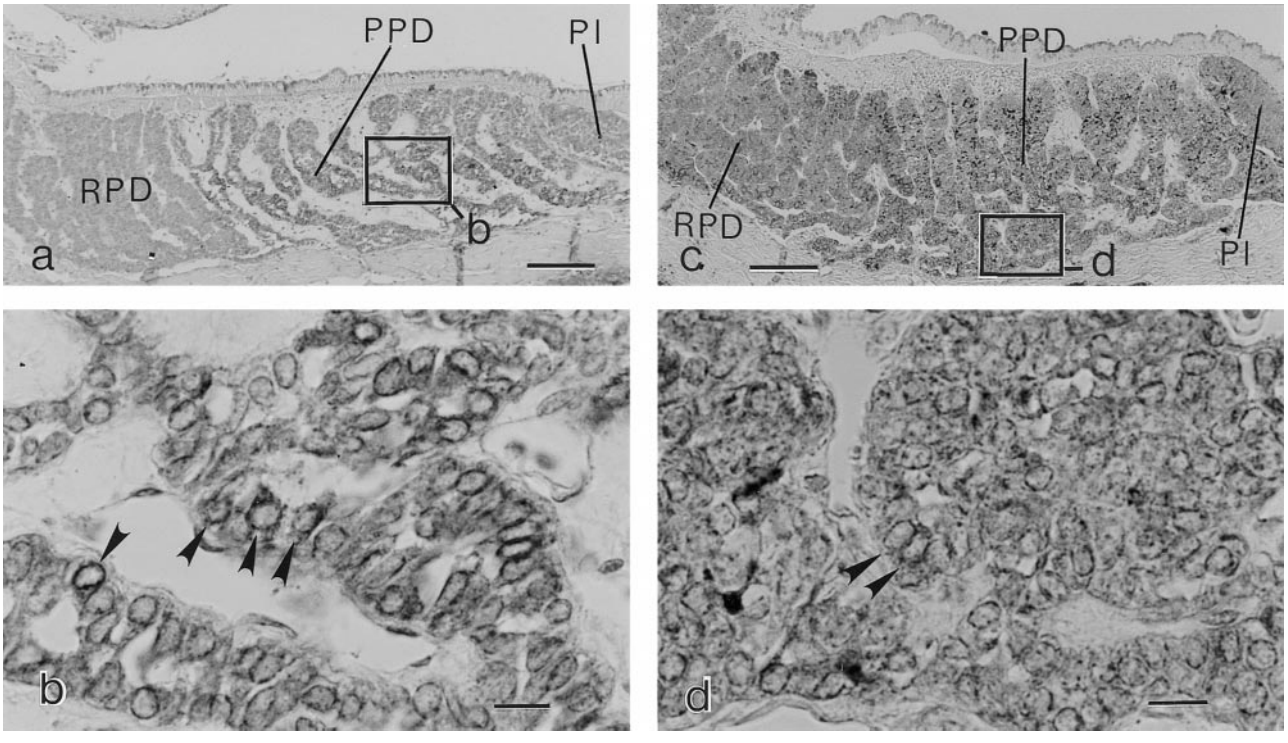

FIG. 2. (a, c) Two sagittal sections of the pituitary gland of adult sea lampreys, stained with (a) biotin-conjugated concanavalin A (Con A), and (c) biotin-conjugated *Vicia villosa* agglutinin (VVA), respectively. The areas outlined by rectangles (part of proximal pars distalis, PPD) are enlarged and shown in b and d. Arrowheads show typically stained cells by respective antibodies. PI, pars intermedia; RPD, rostral pars distalis. Scale bars: a, c, 100 μm; b, d, 10 μm.

than to FSH or TSH, as far as immunocytochemical determinants.

In lampreys, the presence of GTH as well as gonadotropic functions has been strongly suggested (see for review, Larsen and Rothwell, 1972; Hardisty and Baker, 1982; Sower, 1990). Both in river lampreys (*L. fluviatilis*) and in sea lampreys (*P. marinus*), hypophysectomy and substitution therapy with pituitary extracts or mammalian GTHs indicated pituitary regulation of the gonads (Evennett and Dodd, 1963; Larsen, 1965, 1969, 1987; Sower, 1990; Sower and Larsen, 1991). Administration of lamprey GnRH in adult sea lampreys *in vivo* stimulated steroidogenesis and spermiation or ovulation (Sower *et al.*, 1987). Addition of lamprey GnRH in coculture media of lamprey pituitary and gonads stimulated estradiol release, although lamprey GnRH had no direct effect on estradiol release from media of gonad cultures (Sower, 1990). Specific GnRH binding was reported in the proximal pars distalis of sea lampreys (Knox *et al.*, 1994). Immunoreactive LH was demonstrable in the proximal pars distalis by use of anti-rat LH (Wright, 1983). These studies have suggested that the chemistry of the lamprey GTH broadly resembles that of higher vertebrates.

Pituitary glycoprotein hormones, LH, FSH, and TSH, consist of α- and β-subunits. The α-subunit is considered to be identical in amino acid sequence among all these glycoprotein hormones within respective species (Pierce and Parsons, 1981; Hayashi *et al.*, 1992a,b). Both subunits contain certain amounts of oligosaccharides, which typically consist of mannose, galactose, fucose, *N*-acetylgalactosamine, *N*-acetylglucosamine, and sialic acid, although sugar composition varies among species (Green and Baenziger, 1988; Wilson *et al.*, 1990; Hayashi *et al.*, 1993). Hayashi *et al.* (1993) further reported that sugar chains bound to the bullfrog LH are shorter and simpler than those of mammals. The duality of GTHs has also been established in teleosts, in which GTH I and GTH II have been isolated, comparable to FSH and LH of tetrapods (Suzuki *et al.*, 1988a,b; Itoh *et al.*, 1988). In contrast, Idler and his associates isolated two GTHs, a maturational, high concanavalin A (Con AII) glycoprotein GTH and a vitellogenic, low (Con AI) glycoprotein GTH from pituitaries of several species of teleosts (see Idler and Ng, 1983). Maturational Con AII of Idler appears to be homologous to classical LH-like teleost GTH or GTH II, but the vitellogenic Con AI does not seem to be homologues to GTH I or GTH II, and thus the latter remains without chemical characterization. Since all GTHs so far isolated from the pituitaries are glycoproteins, the lamprey GTH, if present, may also be glycoprotein, as suggested by the present study.

Lectins are naturally occurring proteins and glycoproteins which selectively bind noncovalently to carbohydrate residues. It is for this reason that they are of interest and are used in cytochemistry. However, only a few studies have been conducted on the lectin cytochemistry in the pituitary (Komuro and Shioda, 1981; Kurosaki *et al.*, 1995). Our lectin histochemical analyses revealed glycoconjugate-positive cells in the ventral half of the proximal pars distalis of the sea lamprey pituitary. These cells were stained positively by Con A and VVA, which recognize mannose and *N*-acetylgalactosamine residues, respectively. These results may suggest the presence of mannose and *N*-acetylgalactosamine residues in the glycoconjugate. However, other mannose- or *N*-acetylgalactosamine-binding lectins were negative to those cells. The reason for such differences in responsiveness among lectins is not clear at the present time. As mentioned earlier, Con A / VVA-positive cells also contained LH-like immunoreactivity. Thus, the present study demonstrates colocalization of LH-like immunoreactivity and glycoconjugate in cells in the ventral half of the proximal pars distalis of the sea lamprey pituitary, and thus, those cells are most likely to be GTH cells in the sea lamprey pituitary.

In a previous study, Nozaki and Gorbman (1985) reported that cells in the dorsal half of the proximal pars distalis of the sea lamprey pituitary were stained positively by one of three anti-substance P antibodies. Since numbers of substance P-positive cells were few in ammocoetes, but were numerous in prespawning adults, such substance P-positive cells were suggested as possible GTH cells in the sea lamprey pituitary. However, GTH-like cells in the present study are found in the ventral half of the proximal pars distalis, and thus they are distinctly different cells. Substance P-positive cells reported by Nozaki and Gorbman (1985) appear to resemble anti-rat prolactin-positive cells reported by Wright (1984), which were also distributed in the dorsal part of the proximal pars distalis of the same species.

Finally, the present study clearly suggests the presence of GTH cells in the proximal pars distalis of the sea lamprey pituitary, which contains both mammalian LH-like immunoreactivity and glycoconjugate.

ACKNOWLEDGMENTS

We are grateful to National Hormone and Pituitary Program (NHPP), Torrance, California, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Reekville, Maryland, and National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD), Bethesda, Maryland, for providing antisera to ovine LH, ovine LHβ, human LHβ, human FSHβ, and human TSHβ, and antigens (ovine LHβ, AFP-35B; human LHβ, AFP-3477A); Dr. K. Wakabayashi, Hormone Assay Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan, for providing antisera to ovine LHβ, rat TSH, hCGβ, chicken LH, bullfrog LH and bullfrog FSH, Dr. Y. Mori, Laboratory of Veterinary Ethology, University of Tokyo, Tokyo, Japan, for providing antiserum to ovine LH (YM No 18), Dr. S. Ishii, Department of Biology, School of Education, Waseda University, Tokyo, for providing antisera to chicken FSH, toad LH, and bullfrog LH; Dr. P. Swanson, Northwest Fisheries Science Center, Seattle, Washington, for providing antisera to salmon GTH, salmon TSH, salmon GTHIβ, and salmon GTHIIβ; and Dr. M. Kobayashi, Department of Aquatic Bioscience, University of Tokyo, Tokyo, Japan, for providing antisera to salmon GTH and silver carp GTH.

REFERENCES

Ball, J. N., and Baker, B. I. (1969). The pituitary gland: Anatomy and histophysiology. *In* "Fish Physiology" (W. S. Hoar and D. J. Randall, Eds.), Vol. **1,** pp. 1–110. Academic Press, New York.

Evennett, P. J., and Dodd, J. M. (1963). Endocrinology of reproduction in the river lamprey. *Nature* **197,** 715–716.

Gorbman, A. (1980). Evolution of the brain-pituitary relationship: Evidence from the Agnatha. *Can. J. Fish. Aquat. Sci.* **37,** 1680–1686.

Green, E. D., and Baenziger, J. U. (1988). Asparagine-linked oligosaccharides on lutropin, follitropin, and thyrotropin. II. Distributions of sulfated and sialylated oligosaccharides on bovine, ovine, and human pituitary glycoprotein hormones. *J. Biol. Chem.* **263,** 36–44.

Hardisty, M. W., and Baker, B. I. (1982). Endocrinology of lampreys. *In* "The Biology of Lampreys" (M. W. Hardisty and I. C. Potta, Eds.), Vol. 4b, pp. 1–115. Academic Press, New York.

Hayashi, H., Hayashi, T., and Hanaoka, Y. (1992a). Amphibian lutropin and follitropin from the bullfrog *Rana catesbeiana:* Complete amino acid sequence of the alpha subunit. *Eur. J. Biochem.* **203,** 185–191.

Hayashi, H., Hayashi, T., and Hanaoka, Y. (1992b). Amphibian lutropin from the bullfrog *Rana catesbeiana:* Complete amino acid sequence of the beta subunit. *Eur. J. Biochem.* **205,** 105–110.

Hayashi, T., Hanaoka, Y., and Hayashi, H. (1993). Simple N-linked sugar chains are bound to lutropin of the bullfrog *Rana catesbeiana. Gen. Comp. Endocrinol.* **90,** 282–289.

Heinig, J. A., Keeley, F. W., Robson, P., Sower, S. A., and Youson, J. H. (1995). The appearance of proopiomelanocortin early in vertebrate evolution: Cloning and sequencing of POMC from a lamprey pituitary cDNA library. *Gen. Comp. Endocrinol.* **99,** 137–144.

Itoh, H., Suzuki, K., and Kawauchi, H. (1988). The complete amino acid sequences of beta subunits of two distinct chum salmon gonadotropins. *Gen. Comp. Endocrinol.* **71,** 438–451.

Idler, D. R., and Ng, T. B. (1983). Teleost gonadotropins: Isolation, biochemistry and function. *In* "Fish Physiology" (W. S. Hoar, D. J. Randall, and E. M. Donaldspn, Eds.), Vol. 9A, pp. 187–221. Academic Press, New York.

Knox, C. J., Boyd, S. K., and Sower, S. A. (1994). Characterization and localization of gonadotropin-releasing hormone receptors in the adult sea lampreys, *Petromyzon marinus. Endocrinology* **134,** 492–498.

Komuro, M., and Shioda, T. (1981). Localization of acid-containing hormones in GTH cells and ACTH cells of the rat anterior pituitary. *Cell Tissue Res.* **220,** 519–528.

Kurosaki, M., Hori, T., Tanaka, K., Kawakami, H., and Hirano, H. (1995). Peanut agglutinin binding to human prolactin-producing pituitary adenomas. *Acta Neuropathol.* **89,** 475–482.

Larsen, L. O., and Rothwell, B. (1972). Adenohypophysis. *In* "The Biology of Lampreys" (M. W. Hardisty and I. C. Potter, Eds.), Vol. 2, pp. 1–67. Academic Press, New York.

Larsen, L. O. (1965). Effects of hypophysectomy in the cyclostome, *Lampetra fluviatilis* (L.) Gray. *Gen. Comp. Endocrinol.* **5,** 16–30.

Larsen, L. O. (1969). Effects of hypophysectomy before and during sexual maturation in the cyclostome, *Lampetra fluviatilis* (L.) Gray. *Gen. Comp. Endocrinol.* **12,** 200–208.

Larsen, L. O. (1987). The role of hormones in initiation of sexual maturation in male river lampreys (*Lampetra fluviatilis* L.): Gonadotropin and testosterone. *Gen. Comp. Endocrinol.* **68,** 197–201.

Nozaki, M., and Gorbman, A. (1985). Immunoreactivity for Met-enkephalin and substance P in cells of the adenohypophysis of larval and adult sea lampreys, *Petromyzon marinus. Gen. Comp. Endocrinol.* **57,** 172–183.

Nozaki, M., Takahashi, A., Amemiya, Y., Kawauchi, H., and Sower, S. A. (1995). Distribution of lamprey adrenocorticotropin and melanotropins in the pituitary of the adult sea lamprey, *Petromyzon marinus. Gen. Comp. Endocrinol.* **98,** 147–156.

Pierce, J. G., and Parsons, T. F. (1981). Glycoprotein hormones: Structure and function. *Annu. Rev. Biochem.* **50,** 465–495.

Romeis, B. (1948). "Microskopische Technik" 15 verb. Aufl. Leibnit Verlag, Munchen.

Sower, S. A. (1990). Neuroendocrine control of reproduction in lampreys. *Fish Physiol. Biochem.* **8,** 365–374.

Sower, S. A., and Larsen, L. O. (1991). Plasma estradiol and progesterone after hypophysectomy and substitution with pituitary in female sea lampreys (*Petromyzon marinus*). *Gen. Comp. Endocrinol.* **81,** 93–96.

Sower, S. A., Dickhoff, W. W., Gorbman, A., Vale, W. W., and Rivier, J. E. (1983). Ovulatory and steroidal responses in the lamprey following administration of salmon gonadotropin and agonistic and antagonistic analogues of GnRH. *Can. J. Zool.* **61,** 2653–2659.

Sower, S. A., King, J. A., Millar, R. P., Sherwood, N. M., and Marshak, D. R. (1987). Comparative biological properties of lamprey gonadotropin-releasing hormone in vertebrates. *Endocrinology* **120,** 773–779.

Sower, S. A., Chiang, Y-C., Lovas, S., and Conlon, J. M. (1993). Primary structure of and biological activity of a third gonadotropin-releasing hormone from lamprey brain. *Endocrinology* **132,** 1125–1131.

Sower, S. A., Takahashi, A., Nozaki, M., Gorbman, A., Youson, J. H., Joss, J., and Kawauchi, H. (1995). A novel glycoprotein in the olfactory and pituitary systems of larval and adult lampreys. *Endocrinology* **132,** 1125–1131.

Suzuki, K., Kawauchi, H., and Nagahama, Y. (1988a). Isolation and characterization of subunits from two distinct gonadotropins. *Gen. Comp. Endocrinol.* **71,** 302–306.

Suzuki, K., Kawauchi, H., and Nagahama, Y. (1988b). Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. *Gen. Comp. Endocrinol.* **71,** 292–301.

Takahashi, A., Amemiya, Y., Nozaki, M., Sower, S. A., Joss, J., Gorbman, A., and Kawauchi, H. (1995a). Isolation and characterization of melanotropins from the lamprey pituitary glands. *Int. J. Peptide Protein Res.* **46,** 197–204.

Takahashi, A., Amemiya, Y., Sarashi, M., Sower, S. A., and Kawauchi, H. (1995b). Melanotropin and corticotropin are encoded on two distinct genes in the lamprey, the earliest evolved extant vertebrate. *Biochem. Biophys. Res. Commun.* **213,** 490–498.

Wilson, C. A., Leigh, A. J., and Chapman, A. J. (1990). Gonadotropin glycosylation and function. *J. Endocrinol.* **125,** 3–14.

Wright, G. M. (1983). Immunocytochemical study of luteinizing hormone in the pituitary of the sea lamprey, *Petromyzon marinus* L., during its upstream migration. *Cell Tissue Res.* **230,** 225–228.

Wright, G. M. (1984). Immunocytochemical study of growth hormone, prolactin, and thyroid-stimulating hormone in the adenohypophysis of the sea lamprey, *Petromyzon marinus* L., during its upstream migration. *Gen. Comp. Endocrinol.* **55,** 269–274.