Cell Tissue Res (1986) 246:23–31

Cell and Tissue Research

Immunocytochemical demonstration of growth hormone, prolactin and somatostatin-like immunoreactivities in the brain of larval, young adult and upstream migrant adult sea lamprey, *Petromyzon marinus*

Glenda M. Wright
Department of Zoology, University of Toronto, Toronto, Ontario, Canada

Summary. Growth hormone, prolactin and somatostatin-like immunoreactivities were demonstrated in the brains of larval, young adult (parasitic) and upstream migrant adult sea lampreys, *Petromyzon marinus*, by means of immunoperoxidase techniques. Growth hormone (GH) and prolactin (PRL) were observed within separate perikarya in the nucleus praeopticus, within fibers in the commissura praeinfundibularis, and in nerve endings within the neurohypophysis of larval and adult-stage lampreys. Cell bodies demonstrating immunoreactive growth hormone were more numerous than those reactive for prolactin. Unlike in the upstream migrant adult lamprey, no GH or PRL was demonstrated in the adenohypophysis of larval or parasitic lamprey.

Somatostatin (SRIF)-like immunoreactive neurons were demonstrated in the nucleus commissurae praeinfundibularis, anterior and posterior pars ventralis hypothalami, pars dorsalis thalami, and the tegmentum motorium rhombencephali of larval, parasitic and upstream migrant adult lampreys. Many of the SRIF containing neurons within the hypothalamus were cerebrospinal fluid (CSF)-contacting cells. SRIF fibers were found throughout most of the brain predominating within the nucleus praeopticus, pars ventralis hypothalami, and the nucleus interpeduncularis. No SRIF immunoreactivity was found within the neurophyophysis. The possible functions of these peptides within the brain of the lamprey are discussed.

Key words: Growth hormone – Prolactin – Somatostatin – Immunocytochemistry – Lamprey

Very little is known about the function of the hypothalamus and pituitary of the agnathans (lampreys and hagfishes) and the regulatory influences they may exert within the endocrine system of these primitive vertebrates. Although lampreys (both larval and adult stages) and hagfishes have a pituitary that, from ultrastructural studies, appears to be secretory (Larsen and Rothwell 1972; Percy et al. 1975; Tsuneki and Gorbman 1975a, b; Patzner et al. 1982; Wright 1983), they lack a vascular portal system or sufficient innervation which, in more advanced vertebrates, is necessary for hypothalamic control of the hypophysis (Gorbman et al. 1983). This unusual anatomical relationship that exists between the brain and pituitary of the agnathans suggests that the hypothalamus has little or no regulatory influence on the adenohypophysis. However, immunocytochemical studies have described the occurrence and localization of mammalian-like neuropeptides such as gonadotropic releasing hormone (LHRH) in the brain of lampreys (Crim et al. 1979a, b; Nozaki et al. 1984) and somatostatin (SRIF) in the brains of hagfish (Nozaki and Gorbman 1983) and upstream migrant adult (prespawning) lamprey (Nozaki et al. 1984). The demonstration of these substances in the agnathan brains indicate or allude to the evolutionary antiquity of these peptides and may also suggest a possible similarity in hypothalamic-hypophysial function to those of phylogenetically more advanced vertebrates.

Although SRIF has been localized within the brains of a wide variety of vertebrates including the prespawning adult European river lamprey, *Lampetra fluviatilis* (Falkmer et al. 1984), and prespawning sea lamprey, *Petromyzon marinus* (Nozaki et al. 1984), there has been little morphological detail or description of the actual SRIF containing neurons and no study of SRIF occurrence and distribution in the brain of larval and young adult (parasitic) lamprey.

SRIF, which inhibits the release of growth hormone (GH) from the adenohypophysis (Sétáló et al. 1978) has, more recently, been suggested to be involved in the regulation of several other endocrine functions including inhibition of the release of prolactin (PRL) (Grau et al. 1985). A study of the distribution of immunoreactive SRIF within the brain of the larval and adult lampreys is of particular interest in understanding the possible relationships between hypothalamus and pituitary since it has been demonstrated that mammalian-like immunoreactive GH and PRL are present within the adenohypophysis of the prespawning sea lamprey (Wright 1984).

In addition to hypophysiotropic releasing and inhibiting hormones (or factors), a number of hormones commonly associated with the anterior pituitary, including GH and PRL, have been identified within neurons in the brains of a variety of tetrapods (Palkovits 1980) and a teleost (Hansen and Hansen 1982). Since mammalian-like GH and PRL activity is present within the pituitary of adult lamprey (Wright 1984) it might be expected that these pituitary hormones, like those in higher vertebrates, may also be present in the brain of the lamprey.

Send offprint requests to: Dr. G.M. Wright, Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario, Canada M5S 1A1

The aim of the present investigation is to determine by means of immunocytochemical methods the occurrence, morphology and general distribution of GH-, PRL- and SRIF-containing neurons in the brains of larval, young adult, and upstream migrant adult sea lampreys, *Petromyzon marinus*.

Materials and methods

Larval anadromous sea lampreys, *P. marinus* were collected, by means of electroshockers, from various streams and rivers in New Brunswick, Canada, and were maintained at $10 \pm 2°$ C in tanks containing river silt and aerated river water. Young parasitic adult *P. marinus* were caught in Lake Washedamoak, New Brunswick while feeding on gaspereau, *Alosa pseudoharengus*, and shad, *A. sapidissima*. Prespawning landlocked adult *P. marinus* were caught during their upstream migration in the Humber River, Ontario, Canada. All adult animals were kept at $10 \pm 2°$ C in tanks containing continuously flowing, aerated, dechlorinated water. Parasitic adults were provided with rainbow trout, *Salmo gairdneri*, on which they fed. Larval lamprey used ranged from 85 to 110 mm in length and 1.0 to 1.9 g in weight; young adults ranged from 128 to 178 mm in length and 2.0 to 10 g in weight; and prespawning adults ranged from 420 to 540 mm in length and 170 to 230 g in weight.

Animals were sacrificed at various times between May and August. All were anesthetized in a 0.05% solution of tricaine methanesulfonate prior to sacrifice. The brains from all animals were quickly removed and fixed in Bouin's fluid for 24 to 48 h, washed in 70% ethanol, dehydrated in a series of ethanols and embedded in Tissue Prep. Serial sections, 5 to 8 μm thick, were cut in transverse or sagittal planes and mounted on glass slides. Sections of rat hypothalamus were used in preliminary experiments to serve as a positive control of the antisera and other reagents to be used on lamprey tissue. Sections were stained with either the unlabeled peroxidase antiperoxidase (PAP) method or the indirect peroxidase-labeled method with peroxidase conjugated secondary antiserum (Sternberger 1979).

Preparations of anti-rat GH antiserum (NIAMDD anti-rGH-S-4) raised in monkey and anti-rat PRL antiserum (NIAMDD anti-rPRL-1c-1) raised in rabbits, as well as the corresponding antigens rGH (NIAMDD-rGH-1-4) and rPRL (NIAMDD-rPRL-1-5), were supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases through the National Pituitary Agency and were prepared by Dr. A.F. Parlow. Anti-SRIF antiserum was obtained from Dr. Phillip Smith (State University of New York Upstate Medical Center, Syracuse, New York) and was produced in rabbits against synthetic SRIF-14. Synthetic SRIF-14, insulin, LHRH, vasotocin, methionine enkephalin and β-endorphin for testing the specificity of the antisera were obtained from Sigma Chemical Co.

Sections treated with anti-rPRL and anti-SRIF were stained by the PAP method as described previously (Wright 1984). Primary antiserum anti-SRIF was used at a dilution of 1:1500 and anti-rPRL was used at a dilution of 1:100. Sections treated with anti-rGH antiserum were stained by the indirect peroxidase-labeled antibody method as outlined previously (Wright 1984). Anti-rGH antiserum was used at a dilution of 1:100. Some sections were stained with aldehyde-fuschin (Gurr 1962) for comparison with immunostained sections.

Controls for specificity of the reactions were performed by 1) replacing the specific primary antiserum with normal serum, 2) using primary antiserum previously absorbed with excess homologous antigen, and 3) using primary antiserum previously absorbed with excess heterologous antigens. Terminology used to describe structures of the lamprey brain is based on that of Heier (1948).

Results

Somatostatin

Immunoreactive perikarya and nerve fibers are found in various regions of the telencephalon, diencephalon, mesencephalon and rhombencephalon. The localization of immunoreactivity for SRIF is similar in the larval, young adult and upstream migrant adult lamprey.

In the telencephalon, immunopositive nerve fibers are scattered among the cells in the nucleus olfactorius and nucleus septi lateralis in the olfactory bulbs, the primordium piriforme in the cerebral hemispheres and the nucleus praeopticus in the telencephalon medium. No immunostaining perikarya are present in the telencephalon.

In the diencephalon, immunoreactive nerve fibers are observed throughout the nucleus commissurae praeinfundibularis, pars ventralis hypothalami, pars ventralis thalami and pars dorsalis thalami. Perikarya immunostained for SRIF are found within the nucleus commissurae praeinfundibularis (Fig. 1a). The cells are spherically shaped with a large round unlabeled nucleus surrounded by a thin layer of immunoreactive cytoplasm (Fig. 1a, b). Some of the SRIF-containing perikarya border on the third ventricle among unstained ependymal cells (Fig. 1a, b). SRIF-like immunoreactive neurons and fibers are observed throughout the anterior and posterior pars ventralis hypothalami. Some fibers are found bordering on or very closely apposed to the walls of blood vessels within the pars ventralis hypothalami (Fig. 1c). Perikarya are present in the ependyma and at various levels within the subependymal region (Fig. 2a, b, c). Most of the cells appear to be bipolar; many of these immunoreactive perikarya are similar to the cerebrospinal fluid (CSF)-contacting neurons as described by Sterba (1972) and Vigh-Teichmann et al. (1983) in that they have bulb-like dendrite terminals which protrude into the third ventricle and axon-like processes extending into the wall of the hypothalamus (Fig. 2a, b, c). A compact group of SRIF-containing neurons is observed within the pars dorsalis thalami situated adjacent to the fasciculus retroflexus (Fig. 3a, b). These cells are large and apparently unipolar giving rise to thick axon-like processes that are often bifurcated at some distance from the perikarya (Fig. 3b).

A dense network of SRIF-like immunoreactive nerve fibers and perikarya is observed in the mesencephalon and rhombencephalon within the tegmentum motoricum mesencephali, the rostral portion of the commissura ventralis rhombencephali and the tegmentum rhombencephali (Fig. 4a, b). The immunostained cells appear to be pyriform with axons extending ventrally through the tegmentum (Fig. 4b). A dense mass of SRIF fibers is also found within the nucleus interpeduncularis (Fig. 4c). No immunostaining for somatostatin is found within the neurohypophysis (Fig. 5) or the adenohypophysis.

Immunostaining of all SRIF fibers and perikarya is inhibited when anti-SRIF antiserum is previously absorbed with SRIF. No inhibition of staining occurs when anti-SRIF is absorbed with insulin, LHRH, vasotocin, methionine enkephalin or β-endorphin.

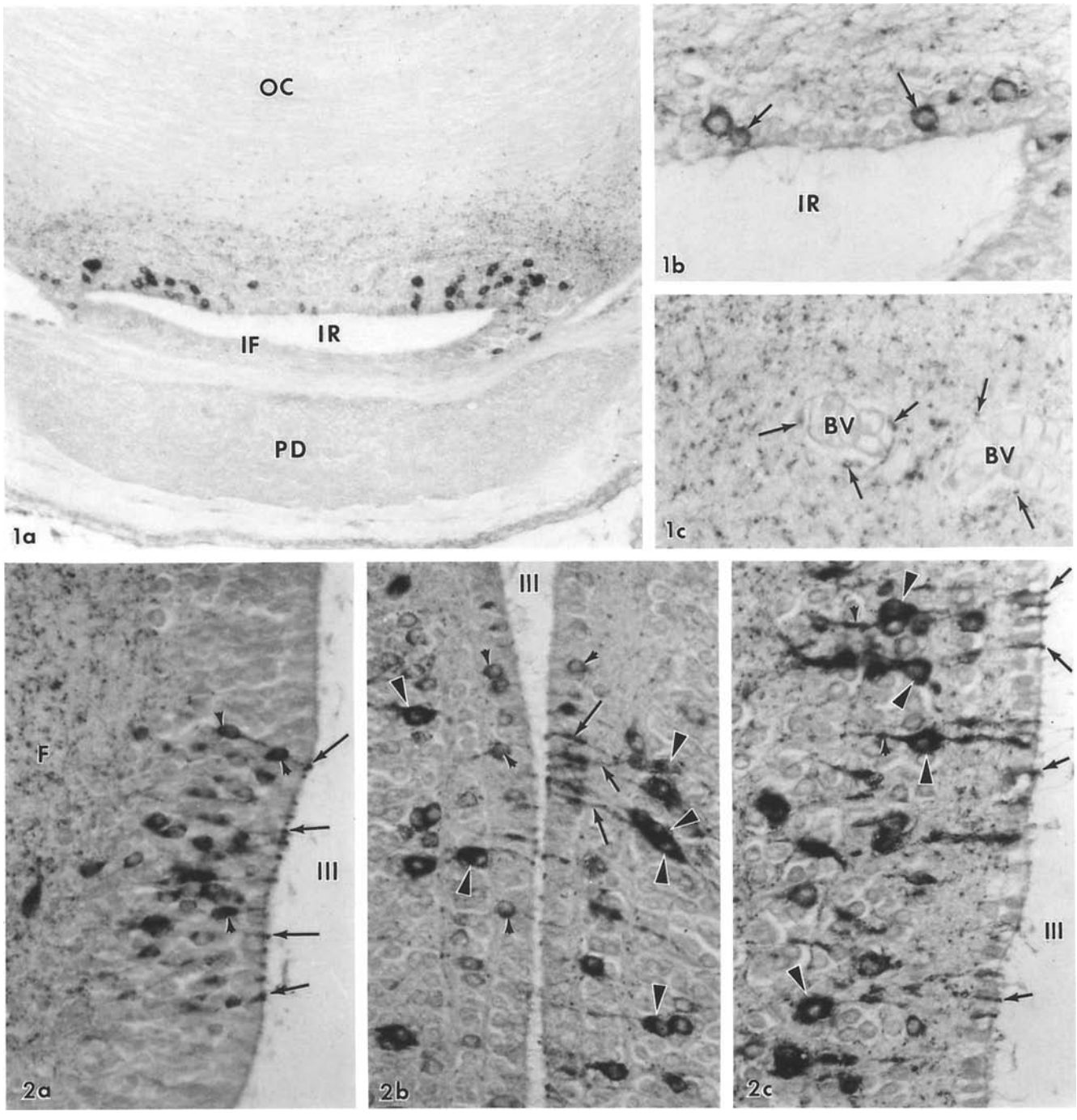


Fig. 1. a SRIF-like-immunoreactive perikarya and fibers in nucleus commissurae praeinfundibularis of young adult (parasitic) lamprey. *OC* optic chiasma; *IR* infundibular recess; *IF* infundibulum; *PD* pars distalis. ×180. **b** Some SRIF-like-immunoreactive perikarya (*arrows*) in nucleus commissurae praeinfundibularis bordering onto infundibular recess (*IR*) in an upstream migrant adult lamprey. ×440. **c** SRIF-containing fibers (*arrow*) surrounding blood vessels (*BV*) in hypothalamus of upstream migrant adult lamprey. ×500

Figs. 2a–c. SRIF immunoreactivity in pars ventralis hypothalami of larval (**a**), young adult (**b**) and upstream migrant adult lamprey (**c**). **a** Immunoreactive CSF-contacting dendritic protrusions (*arrows*) and perikarya (*arrowheads*). F immunoreactive fiber plexus; III third ventricle. ×480. **b** Immunoreactive perikarya intraependymal (*small arrowheads*) and within subependymal regions (*large arrowheads*) some with long dendritic processes (*arrows*) that protrude into third ventricle (III). ×420. **c** Strong immunoreaction in CSF-contacting dendritic processes (*arrows*), perikarya (*large arrowheads*) and axon-like processes (*small arrowheads*). *III* third ventricle. ×420

Growth hormone

GH-like-immunoreactive perikarya and fibers are present throughout the anterior and middle regions of the nucleus praeopticus in all stages of lampreys studied but are most abundant in the upstream migrant adult lampreys (Fig. 6a, b). Immunostained cells range from spherical to pyriform in shape with their axon-like processes extending ventro-laterally and then posterior-laterally into the infundibulum. None of the GH containing neurons appeared to have any

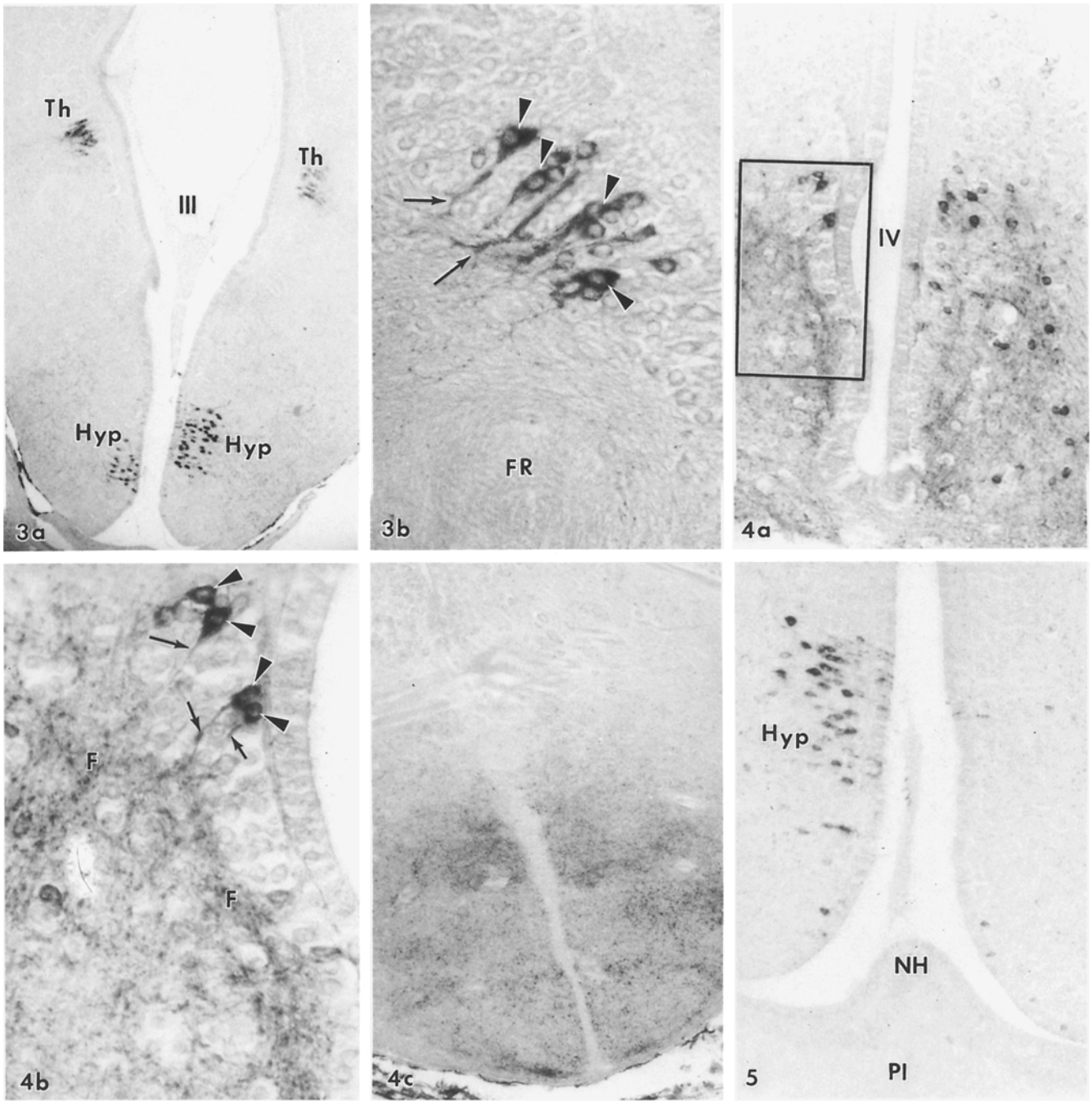


Fig. 3. **a** SRIF immunoreactivity in pars ventralis hypothalami (*Hyp*) and pars dorsalis thalami (*Th*) of young adult lamprey. *III* third ventricle. ×61. **b** Higher magnification of immunoreactive neurons within pars dorsalis thalami showing group of intensely stained perikarya (*arrowheads*) with bifurcating axon-like processes (*arrows*) near fasciculus retroflexus (*FR*). ×380

Fig. 4. **a** SRIF immunoreactivity in tegmentum mesencephali of upstream migrant adult lamprey. *IV* fourth ventricle. ×190. **b** Higher magnification of rectangular area in **Fig. 4a** showing immunoreactive perikarya (*arrowheads*) with axon-like processes (*arrows*) that extend ventrally into a fiber plexus (*F*). ×420. **c** SRIF-containing fibers in nucleus interpeduncularis of upstream migrant adult lamprey. ×170

Fig. 5. Note lack of SRIF immunoreaction within neurohypophysis (*NH*) and pars intermedia (*PI*) in comparison to that in pars ventralis hypothalami (*Hyp*) of young adult lamprey. ×170

contact with the CSF. Immunostaining fibers are also present within the ventral portion of the neurohypophysis adjacent to the pars intermedia of the adenohypophysis (Fig. 6c). In sagittal sections, the GH containing fibers could be traced from the immunostained perikarya in the nucleus praeopticus through the praeoptico-hypophysial tract to the neurohypophysis. Although GH containing cells are found in the caudal pars distalis of the upstream migrant adult lamprey no GH immunostaining cells are observed in the adenohypophysis of larval or young adult lamprey.

All immunostaining is abolished when anti-rGH antise-

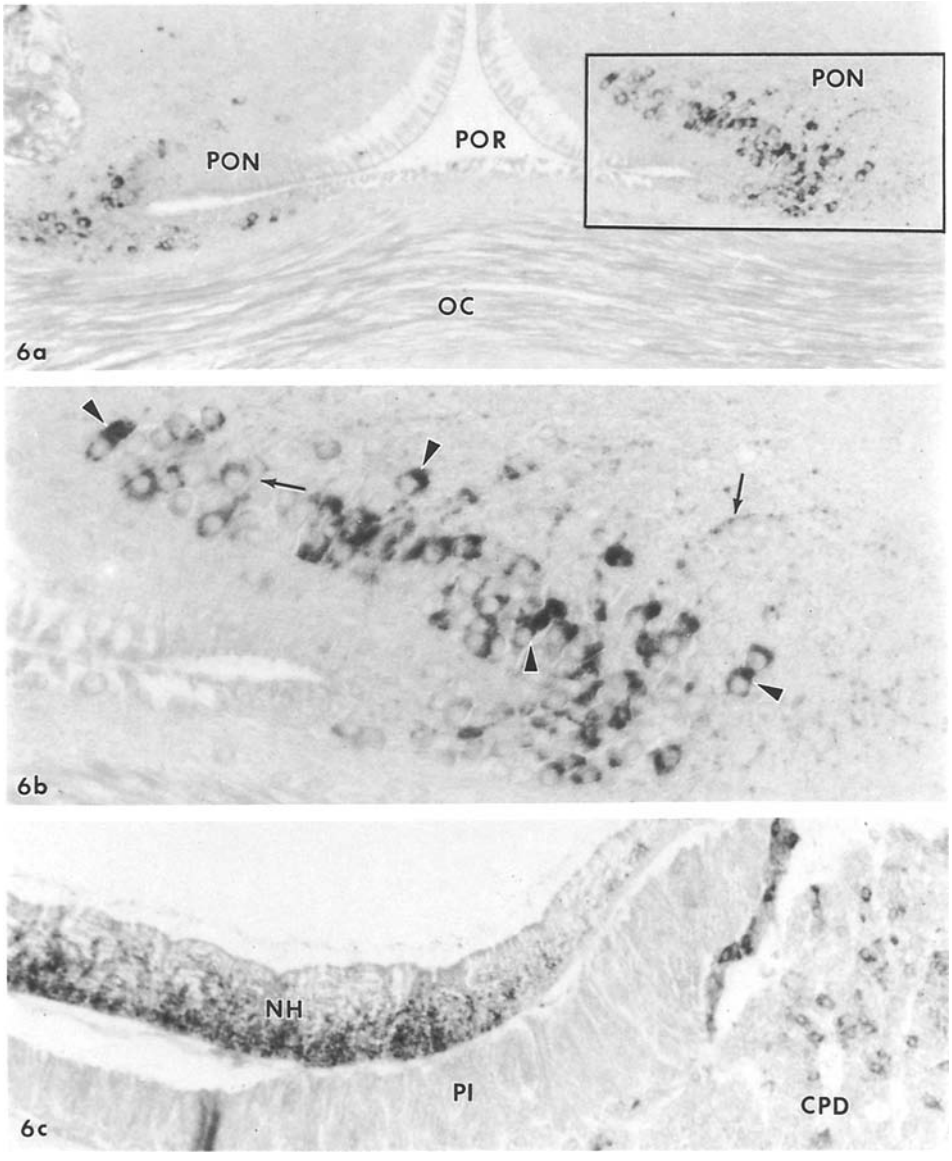


Fig. 6. a GH-like immunoreactivity within nucleus praeopticus (*PON*) of upstream migrant lamprey. *POR* preoptic recess; OC optic chiasma. ×180. **b** Higher magnification of rectangular area in Fig. 6b showing immunoreactive perikarya (*arrowheads*) and fibers (*arrows*). ×140. **c** Sagittal section through portion of neurohypophysis (*NH*), pars intermedia (*PI*) and caudal pars distalis (*CPD*) of upstream migrant adult lamprey showing GH-like immunoreactivity within neurohypophysis and caudal pars distalis. ×230

rum is previously absorbed with rGH; however staining is not inhibited when anti-rGH antiserum is preabsorbed with rPRL or any other heterologous antigens tested, i.e. LHRH, vasotocin, methionine enkephalin or β-endorphin.

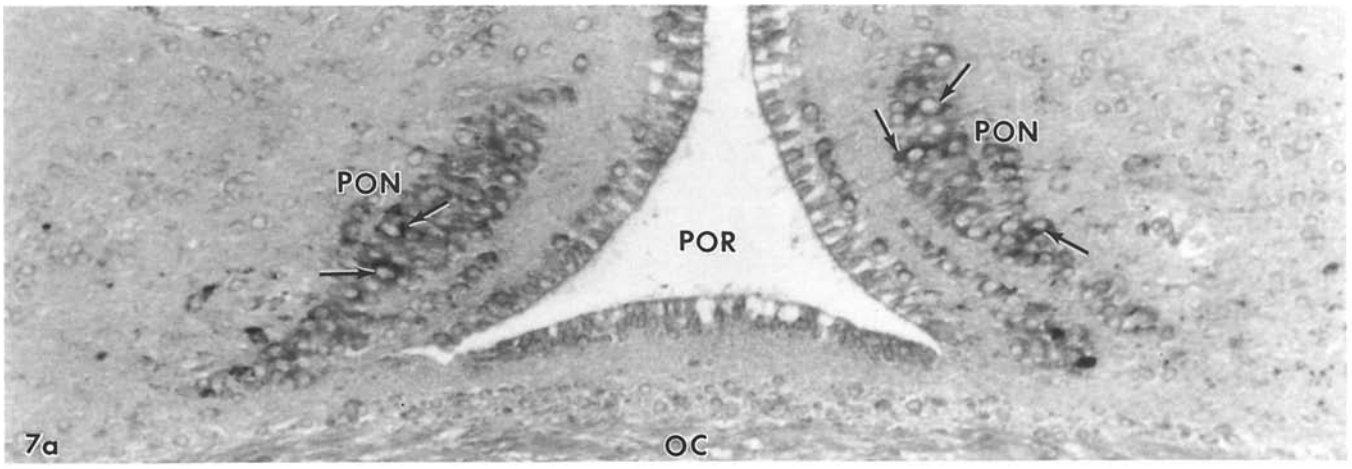
Prolactin

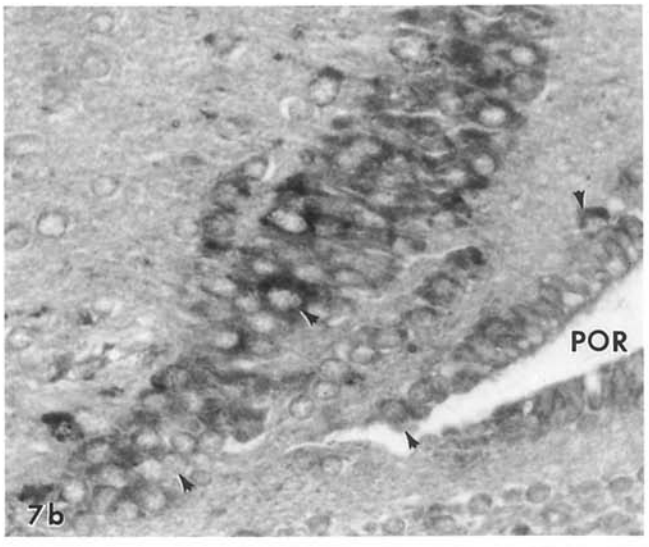
A few perikarya and fibers demonstrating immunoreactivity for PRL are also present within the nucleus praeopticus of larval, young adult and upstream migrant lamprey although they are far lower in number than the GH-like immunoreactive cells (Fig. 7a, b). Immunostaining of adjacent sections demonstrated that perikarya immunoreactive for PRL are different than those that show the presence of GH-like material (compare Fig. 7b, c). Like the GH containing neurons, none of the PRL immunoreactive neurons appear to be CSF-contacting cells. PRL-like immunoreactive fibers are observed within the ventral portion of the neurohypophysis (Fig. 8). Although PRL containing cells are present in the caudal pars distalis of the upstream migrant adult lamprey, no PRL cells are found in the adenohypophysis of the larval or young adult lamprey. Immunostaining is abolished when the anti-rPRL antiserum is absorbed with rPRL, but not when the antiserum is absorbed with rGH, LHRH, vasotocin, methionine enkephalin or β-endorphin.

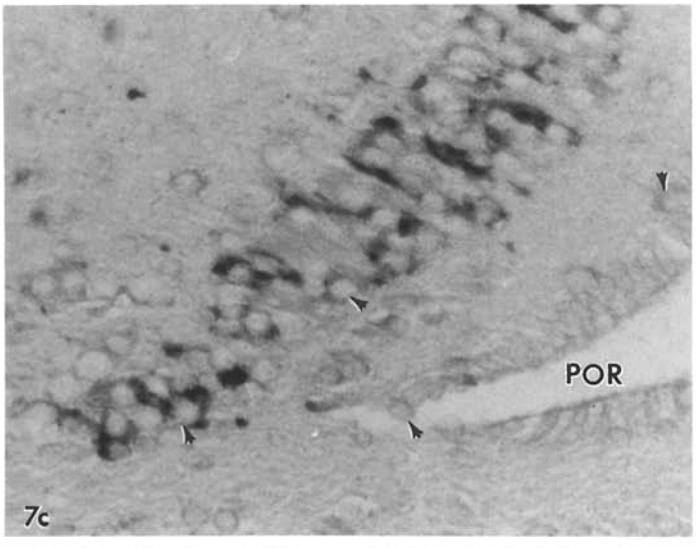
Figures 9 and 10 represent summary diagrams of the distribution of SRIF, GH, and PRL within the brain of upstream migrant adult lamprey.

Discussion

The present immunocytochemical observations demonstrate that GH-, PRL- and SRIF-like-immunoreactive material exists within the brains of larval, young adult and upstream migrant adult sea lampreys, *P. marinus*. Absorption tests showed that the anti-SRIF used was specific for SRIF demonstrating no cross-reactivity with methionine enkephalin, β-endorphin, vasotocin or LHRH, which are also known to be present within the brain of lamprey (Crim et al. 1979a, b; Nozaki and Gorbman 1984). The distribution of immunoreactive SRIF-containing perikarya and







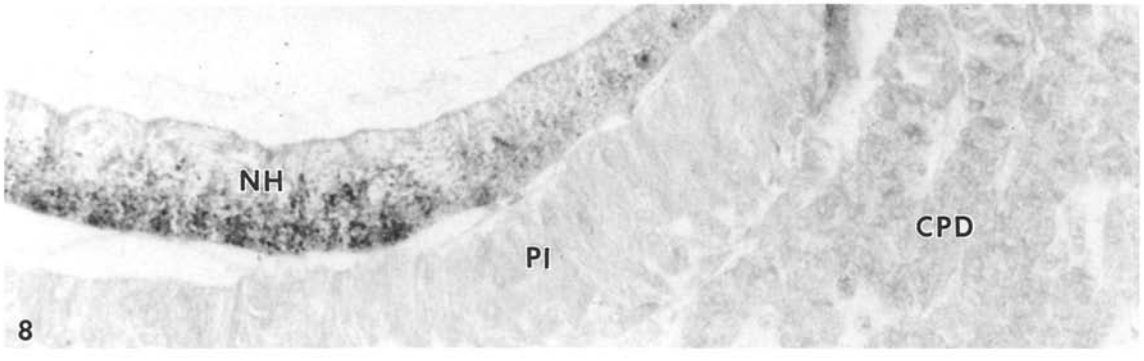


Fig. 7. a PRL-like immunoreactivity (*arrows*) within nucleus praeopticus (*PON*) of upstream migrant adult lamprey. *POR* preoptic recess; *OC* optic chiasma. × 270. **b–c** Adjacent sections treated with anti-rPRL antiserum **(Fig. 7b)** and with anti-rGH antiserum **(Fig. 7c)** showing the same field of nucleus praeopticus at level of preoptic recess (*POR*). *Arrowheads* represent common reference points. Comparison of sections shows immunostaining of different neurons by the two antisera. × 500

Fig. 8. Sagittal section through neurohypophysis (*NH*), pars intermedia (*PI*) and caudal pars distalis (*CPD*) showing PRL-like immunoreactivity within neurohypophysis and weak reaction in some cells of caudal pars distalis. × 230

fibers within the brain of the larval and the parasitic sea lamprey is consistent with that previously found in the brain of the upstream migrant (prespawning) adult sea lamprey (Nozaki et al. 1984). Therefore, there appears to be no change in distribution of SRIF with age of the animal.

Although the general localization and appearance of the SRIF perikarya and fibers in the brains of larval and adult stage lampreys resemble that observed in the brains of a variety of teleosts (Vigh-Teichmann et al. 1983; Olivereau et al. 1984) two significant differences are found between the distribution of SRIF in the lamprey and that in the teleost brain; no SRIF-positive perikarya are present within the nucleus praeopticus and no immunostained fibers are found within the neurohypophysis of the lamprey.

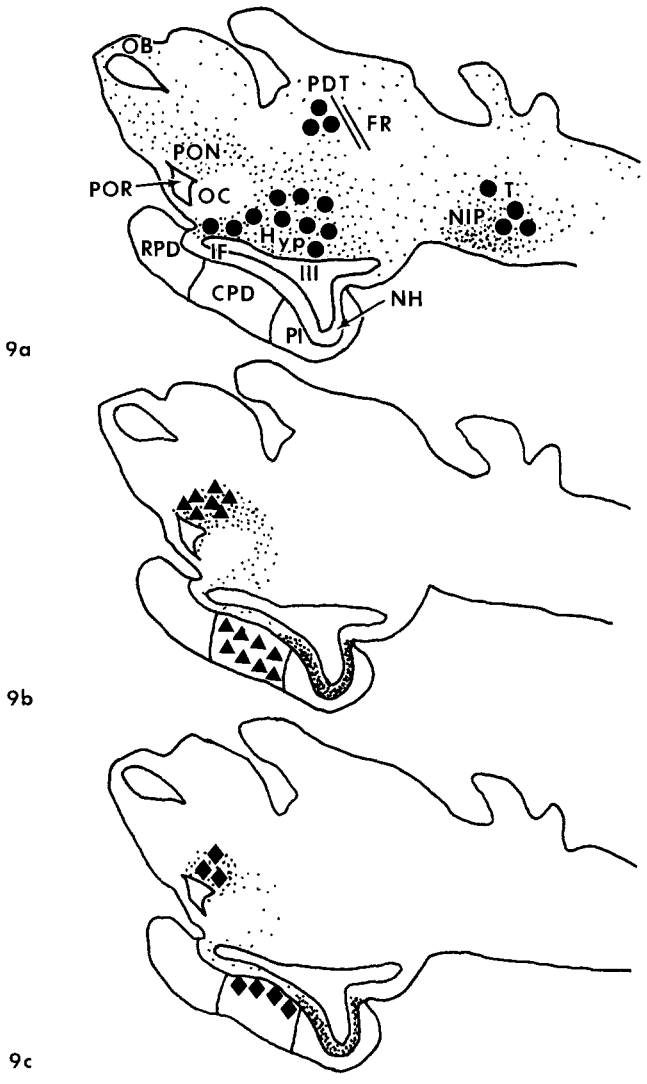


Fig. 9. Diagrammatic representation of sagittal sections of brain and pituitary of upstream migrant adult lamprey showing distribution of **a** SRIF-positive perikarya (*filled circles*) and fibers (*dots*), **b** GH-positive perikarya (*filled triangles*) and fibers (*dots*), and **c** PRL-positive perikarya (*filled diamonds*) and fibers (*dots*). *CPD* caudal pars distalis; *FR* fasciculus retroflexus; Hyp hypothalamus; *III* third ventricle; *IF* infundibulum; *NH* neurohypophysis; *NIP* nucleus interpeduncularis; *OB* olfactory bulb; *OC* optic chiasma; *PDT* pars dorsalis thalami; *PI* pars intermedia; *PON* nucleus praeopticus; *POR* preoptic recess; *RPD* rostral pars distalis; *T* tegmentum

SRIF in the brains of higher vertebrates acts as a neurotransmitter and/or neuromodulator (Luft et al. 1978) as well as being involved in the regulation of anterior pituitary function (Filby and Gross 1983). In several teleosts, SRIF is known to be a release inhibiting factor for GH (Setalo et al. 1978; Fryer et al. 1979) and PRL (Grau et al. 1985) secretion in the adenohypophysis. In some teleosts, SRIF is delivered directly to the cells in the pituitary via peptidergic fibers from perikarya within the hypothalamus that extend into the pars distalis (Grau et al. 1985). More commonly, SRIF in teleosts and a variety of other vertebrates is received within the pituitary via indirect peptidergic innervation. SRIF axons in the nucleus praeopticus or hypothalamus terminate on or close to blood vessels in the median emminence or within the neurohypophysis (Bern et al. 1975; Filby and Gross 1983; Yui 1983) thereby entering the hypothalamic-hypophysial portal system or systemic circulation. The abundant and widespread distribution of SRIF in the brain of larval and adult stage lampreys may reflect its function as a local neurotransmitter and/or neuromodulator but not as a regulator of pituitary acitivity since no SRIF-containing fibers are found in the pars distalis of the lamprey, and no SRIF-immunoreactive perikarya and fibers are present in the nucleus praeopticus and neurohypophysis, respectively. In addition to this, no portal system exists into which SRIF originating in the hypothalamus could enter the lamprey adenohypophysis. However, it is still possible that some of the SRIF in the brain of the lamprey may exert a regulatory effect on the pituitary by way of the CSF. There are many SRIF immunoreactive CSF-contacting neurons present within the pars ventralis hypothalami. Vigh-Teichmann et al. (1983) attribute a sensory function to SRIF-immunoreactive CSF-contacting neurons, whereby they pass information from the CSF to the SRIF system of the hypothalamus and/or other components of the neuroendocrine system. One can not exclude the possibility that some CSF-contacting neurons may be a source of neuropeptides in the CSF releasing their peptide into the CSF (Rodríguez 1976; Rodríguez et al. 1982). Many peptides including SRIF have been found within the CSF of mammals (Kronheim et al. 1977). One can speculate that the SRIF-immunoreactive CSF-contacting neurons in the lamprey hypothalamus may deliver SRIF indirectly to the pars distalis, in the absence of a portal system, by way of the CSF and the ependymal cells of the infundibulum (commissura praeinfundibularis) and neurohypophysis. Ependymal cells lining the lumen of the neurohypophysis of the hagfish (which along with the lamprey is the only other living representative of the Agnatha and like the lamprey lacks a portal system) readily absorb peroxidase (molecular weight of 44 000) when it is injected into the CSF within the hypothalamic recess of the 3rd ventricle (Nozaki et al. 1975). The peroxidase rapidly diffuses into connective tissue beneath the neurohypophysis and into connective tissue between cells in the adenohypophysis. Ependymal cells in the infundibulum and neurohypophysis of the lamprey may have a similar absorptive (transport) ability. It is possible then that a small molecular weight protein like SRIF in the CSF could be transported to the lamprey adenohypophysis. Once in the adenohypophysis it may, at least in the prespawning adult lamprey, act in regulating secretion of GH and possibly PRL cells which are present in the caudal pars distalis (Wright 1984).

PRL- and GH-like immunoreactivity has been observed in perikarya and fibers in the hypothalamus and praeoptic nucleus of the rat brain (Fuxe et al. 1977; Pacold et al. 1978). More recently both these pituitary hormones have also been found in the brain of a lower vertebrate, the African freshwater fish, *Calamoichthys calabricus,* localized in separate neurons in the praeoptic nucleus with axons terminating in the neurohypophysis (Hansen and Hansen 1982). The distribution of GH and PRL-like immunoreactivity in the brain of the lamprey is similar to that observed in *C. calabricus,* although unlike those of the teleost, none of the GH or PRL containing neurons within the praeoptic nucleus of the lamprey brain appear to be CSF-contacting cells.

The fact that neuronal storage of PRL and GH in the

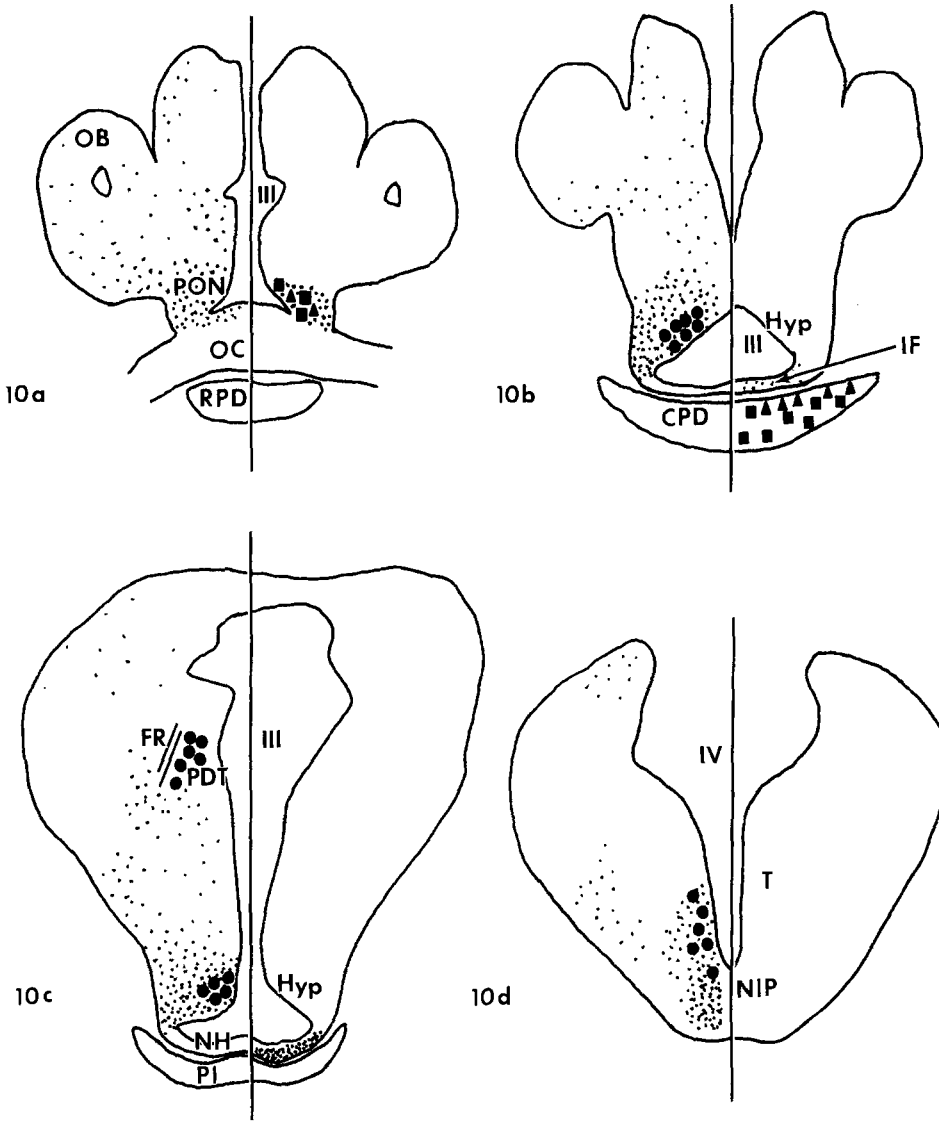


Fig. 10a–d. Diagrammatic representation of SRIF-containing perikarya (*filled circles*) and fibers (*dots*) (*left halves*), and GH (*filled squares*) and PRL (*filled triangles*) containing perikarya and fibers (*dots*) (*right halves*) in transverse sections of the lamprey brain at level of **a** anterior nucleus praeopticus, **b** anterior pars ventralis hypothalami, **c** pars dorsalis thalami, and **d** tegmentum mesencephali. *CPD* caudal pars distalis, *FR* fasciculus retroflexus; *Hyp* pars ventralis hypothalami; *IF* infundibulum; *III* third ventricle; *IV* fourth ventricle; *NH* neurohypophysis; *NIP* nucleus interpeduncularis; *OB* olfactory bulb; *OC* optic chiasma; *PDT* pars dorsalis thalami; *PI* pars intermedia; *PON* nucleus praeopticus; *RPD* rostral pars distalis; *T* tegmentum mesencephali

brain of the rat is not affected by hypophysectomy has suggested that these peptides originate in the central nervous system rather than the pituitary (Krieger and Liotta 1979). These peptides in the brains of tetrapods are believed to function as neurotransmitters or neuromodulators rather than endocrine regulators (Krieger and Liotta 1979). Although GH and PRL-like material have been observed in the caudal pars distalis of the pituitary of upstream migrant adult lamprey (Wright 1984) no GH or PRL-like immunoreactivity could be found, during this investigation, within the adenohypophysis of the larval or young adult lamprey. The only source of GH and PRL in the larval and the young parasitic adult lampreys, therefore, appears to be the praeoptic nucleus and neurohypophysis. Presumably these peptides in the brain of the lamprey, like those in tetrapods, originate in the central nervous system and function as local neurotransmitters or neuromodulators. The brain, along with the pituitary of the upstream migrant lamprey, may also supply GH and PRL for use in the body through their release into the systemic circulation via blood vessels beneath the neurohypophysis or those in the adenohypophysis, respectively. Since no GH or PRL is present in the pituitary of the larval and young adult lamprey, any GH or PRL needed for use within the body must be supplied by the brain through their release into the systemic circulation via the neurohypophysis.

It is interesting to note here that many neurons in the nucleus praeopticus, some of which may be GH or PRL containing, are surrounded by SRIF-immunoreactive fibers. The release of GH and possibly PRL from neurons in the nucleus praeopticus of the lamprey brain may be affected by SRIF through synaptic contacts with SRIF containing axons originating from perikarya in the hypothalamus or elsewhere in the brain.

The praeoptico-neurohypophysial neurons in the lamprey brain have been shown to contain separate vasotocinergic (Goossens et al. 1977) and LHRH (Crim et al. 1979a, b) immunoreactivity. The results from the present investigation suggest that there are a third and fourth type of neuron present; one somatotropinergic and the other prolactinergic. Absorption controls have demonstrated the specificity of the antisera. Preabsorption with homologous hormone eliminates staining reaction while absorption with heterologous hormones including LHRH and vasotocin does not abolish or diminish the staining reactions. However, double immunostaining tests or comparative immunocytochemical

experiments with anti-rGH, anti-rPRL, anti-vasotocin and anti-LHRH remain to be done to determine whether praeoptic neurons may contain more than one peptide.

In conclusion, the presence of GH, PRL, and SRIF-like material in the brain of lampreys suggests the involvement of these peptides in the regulation of brain function. However, one cannot dismiss the possibility that SRIF in the lamprey brain, as in the brain of higher vertebrates, may also act as a regulator of pituitary function and that the GH and PRL originating in the brain may exert some effect on other tissues in the body of the lamprey.

Acknowledgements. This research was supported by the Natural Sciences and Engineering Research Council of Canada grant U0105. The author wishes to thank Dr. P. Smith and Dr. A.F. Parlow and the NIAMDD-Sponsored National Pituitary Agency for the hormones and antisera used in this study.

References

Bern HA, Nishioka RS, Nagahama Y (1975) The relationship between nerve fibers and adenohypophysial cell types in the cichlid teleost, *Tilapia mossambica*. In: Arvy L (ed) Recherches biologiques contemporaines. Imprimérie Vagner, Nancy, pp 179–194

Crim JW, Urano A, Gorbman A (1979a) Immunocytochemical studies of luteinizing hormone-releasing hormone in brains of agnathan fishes. I. Comparisons of adult Pacific lamprey (*Entosphenus tridentata*) and the Pacific hagfish (*Eptatretus stouti*). Gen Comp Endocrinol 17:294–305

Crim JW, Urano A, Gorbman A (1979b) Immunocytochemical studies of luteinizing hormone-releasing hormone in brains of agnathan fishes. II. Patterns of immunoreactivity in larval and maturing western brook lamprey (*Lampetra richardsoni*). Gen Comp Endocrinol 38:290–299

Falkmer S, El-Salhy M, Titlbach M (1984) Evolution of the neuroendocrine system in vertebrates. A review with particular reference to the phylogeny and postnatal maturation of the islet parenchyma. In: Falkmer S, Håkanson R, Sundler F (eds) Evolution and tumour pathology of the neuroendocrine system in birds. Elsevier Science Publishers, New York

Filby AB, Gross DS (1983) Distribution of immunoreactive somatostatin in the primate hypothalamus. Cell Tissue Res 233:69–80

Fryer JN, Nishioka RS, Bern HA (1979) Somatostatin inhibition of teleost growth hormone secretion. Gen Comp Endocrinol 39:244–246

Fuxe K, Hökfelt T, Eneroth P, Gustafsson J-A, Skett P (1977) Prolactin-like immunoreactivity: localization in nerve terminals of rat hypothalamus. Science 196:899–900

Goossens N, Dierickx K, Vandesande F (1977) Immunocytochemical demonstration of the hypothalamus-hypophysial vasotocinergic system of *Lampetra fluviatilis*. Cell Tissue Res 177:317–323

Gorbman A, Dickhoff W, Vigna SR, Clark NB, Ralph CL (1983) Comparative endocrinology. John Wiley and Sons, New York

Grau EG, Nishioka RS, Young G, Bern HA (1985) Somatostatin-like immunoreactivity in the pituitary and brain of three teleost fish species: somatostatin as a potential regulator of prolactin cell function. Gen Comp Endocrinol 59:350–357

Gurr E (1962) Staining animal tissues. Practical and theoretical. Leonard Hill Ltd., London

Hansen BL, Hansen GN (1982) Immunocytochemical demonstration of somatotropin-like and prolactin-like activity in the brain of *Calamoichthys calabaricus* (Actinopterygii). Cell Tissue Res 222:615–627

Heier P (1948) Fundamental principles in the structure of the brain. A study of the brain of *Petromyzon fluviatilis*. Act Anat [Suppl] VI:3–213

Krieger DT, Liotta AS (1979) Pituitary hormones in brain: Where, how and why? Science 205:366–372

Kronheim S, Berelowitz M, Pimstone BL (1977) The presence of immunoreactive growth hormone release-inhibiting hormone in normal cerebrospinal fluid. Clin Endocrinol 6:411–415

Larsen LO, Rothwell B (1972) Adenohypophysis. In: Hardisty MW, Potter IC (eds) The biology of lampreys. Vol 1. Academic Press, New York, pp 1–67

Luft R, Efendri S, Hökfelt T (1978) Somatostatin-both hormone and neurotransmitter? Diabetologia 14:1 13

Nozaki M, Gorbman A (1983) Immunocytochemical localization of somatostatin and vasotocin in the brain of the Pacific hagfish *Eptatretus stouti*. Cell Tissue Res 229:541–550

Nozaki M, Fernholm B, Kobayashi H (1975) Ependymal absorption of peroxidase in the third ventricle of the hagfish *Eptatretus burgeri* (Girard). Acta Zool (Stockh) 56:265–269

Nozaki M, Tsukahara T, Kobayashi H (1984) An immunocytochemical study on the distribution of neuropeptides in the brain of certain species of fish. Biomed Res 4 [Suppl]:135–145

Olivereau M, Ollevier F, Vandesande F, Olivereau J (1984) Somatostatin in the brain and the pituitary of some teleosts. Immunocytochemical identification and the effect of starvation. Cell Tissue Res 238:289–296

Pacold ST, Kirsteins L, Hojvat S, Lawrence AM, Hagen TC (1978) Biologically active pituitary hormones in the rat brain amygdaloid nucleus. Science 199:804–805

Palkovits M (1980) Topography of chemically identified neurons in the central nervous system: Progress in 1977–1979. Medical Biol 58:188–227

Patzner RA, Erhart G, Adam H (1982) Cell types in the adenohypophysis of the hagfish, *Myxine glutinosa* (Cyclostomata). Cell Tissue Res 223:583–592

Percy R, Leatherland JF, Beamish FWH (1975) Structure and ultrastructure of the pituitary gland in the sea lamprey, *Petromyzon marinus* at different stages in its life cycle. Cell Tissue Res 157:141–164

Rodríguez EM (1976) The cerebrospinal fluid as a pathway in neuroendocrine integration. J Endocrinol 71:407–443

Rodriguez EM, Perra P, Rodriguez S, Aguado LI (1982) Evidence for the participation of the CSF and periventricular structures in certain neuroendocrine mechanisms. Front Horm Res 9:142–158

Sétáló G, Flerkó B, Arimura A, Schally AV (1978) Brain cells as producers of releasing and inhibiting hormones. Int Rev Cytol [Suppl] 7:1–52

Sterba G (1972) Neuro- and gliasecretion. In: Hardisty MW, Potter IC (eds) The biology of lampreys. Vol. 2. Academic Press, New York, pp 69–89

Sternberger A (1979) Immunocytochemistry. John Wiley and Sons, New York

Tsuneki K, Gorbman A (1975a) Ultrastructure of the anterior neurohypophysis and the pars distalis of the lamprey, *Lampetra tridentata*. Gen Comp Endocrinol 25:487–508

Tsuneki K, Gorbman A (1975b) Ultrastructure of the pars nervosa and pars intermedia of the lamprey, *Lampetra tridentata*. Cell Tissues Res 157:165–184

Vigh-Teichmann I, Vigh B, Korf H-W, Oksche A (1983) CSF-contacting and other somatostatin-immunoreactive neurons in the brains of *Anguilla anguilla, Phoxinus phoxinus* and *Salmo gairdneri* (Teleostei). Cell Tissue Res 233:319–334

Wright GM (1983) Ultrastructure of the adenohypophysis in the larval anadromous sea lamprey, *Petromyzon marinus* L. J Morphol 176:325–339

Wright GM (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

Yui R (1983) Immunohistochemical studies on peptide neurons in the hypothalamus of the bullfrog *Rana catesbiana*. Gen Comp Endocrinol 49:195–209

Accepted March 4, 1986