

Review

## Update: brain and pituitary hormones of lampreys

Stacia A. Sower<sup>a,b,\*</sup>, Hiroshi Kawauchi

<sup>a</sup>Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH 03824, USA

<sup>b</sup>Laboratory of Molecular Endocrinology, School of Fisheries Sciences, Kitasato University, Sanriku, Iwate 022-0101, Japan

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### Abstract

Lampreys and hagfish of the class Agnatha are of particular importance in understanding endocrinological relationships since they represent the oldest lineages of extant vertebrates which evolved over 550 million years ago. This review briefly summarizes the latest findings on the reproductive endocrinology of the sea lampreys. Since the First International Symposium of Fish Endocrinology in 1988, when virtually little was known of the hypothalamic–pituitary–gonadal axis, substantial new biochemical, molecular, physiological and immunological evidence has now clearly shown that lamprey reproduction is controlled by the neuroendocrine axis. In addition, five brain and six pituitary hormones of lampreys have been identified mainly by Sower and Kawauchi and colleagues between 1986 and 2000. We now hypothesize that lamprey reproduction is a highly synchronized process that is initiated or mediated by a coordination of complex integration of environmental cues and hormonal mechanisms which is broadly similar to that exhibited by gnathostome vertebrates. © 2001 Elsevier Science Inc. All rights reserved.

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### 1. Introduction

At the First International Symposium of Fish Endocrinology in 1988, there was a Cyclostome Workshop that was organized by Stacia A. Sower (Sower, 1989). This was a small informal workshop with five presentations devoted to four top-

ics: endocrine control of metamorphosis by John Youson and Jean Joss; pancreatic hormones in lampreys by Erika Plisetskaya; steroids in lampreys by Stacia A. Sower; and sexual differentiation in hagfish by Aubrey Gorbman. In addition there were approximately 15 participants, including Hiroshi Kawauchi and Masumi Nozaki. At that time, there was little known of the hypothalamic–pituitary–gonadal axis. In 1988, only one pituitary and one brain hormone had been identified in lampreys: arginine vasotocin (Lane et al., 1988) and lamprey gonadotropin-releasing hormone-I (lamprey GnRH-I, Sherwood et al., 1986),

\* Corresponding author. Department of Biochemistry and Molecular Biology, Rudman Bldg., 46 College Road, University of New Hampshire, Durham, NH 03824, USA. Tel.: +1-603-862-2103; fax: +1-603-862-4013.

E-mail address: sasower@cisunix.unh.edu (S.A. Sower).

respectively. This workshop resulted in an extensive collaboration among the participants that has continued until the present. Since the workshop, the identification of five brain and six pituitary hormones of lampreys has been identified mainly by Sower and Kawauchi and colleagues between 1986 and 2000 (Table 1). Additional new information on metamorphosis (John Youson) and the localization of pituitary hormones (Masumi Nozaki) has also occurred due mainly to the collaborations that were established at the first Symposium. Based on the substantial progress that has resulted since that first workshop, we (Hiroshi Kawauchi and Stacia Sower) decided to hold a symposium entitled 'Agnathan and Chondrichthyes Reproduction and Adaptation' at the Fourth International Symposium on Fish Endocrinology to not only update the status of agnathan endocrinology but also to extend the information to the Chondrichthyes. There are several papers in this volume devoted to this symposium.

The following sections summarize the most recent findings on the structure and function of

brain and pituitary hormones. There have been several reviews on lamprey reproduction (Larsen, 1980; Larsen and Dufour, 1998; Sower and Gorbman, 1999) and brain and pituitary hormones of lampreys (Sower, 1990b, 1995, 1998). This review will briefly update the latest findings on the reproductive endocrinology and pituitary hormones of the sea lampreys. We propose that lamprey reproduction is a highly synchronized process that is initiated and mediated by a coordination of complex integration of environmental cues and hormonal mechanisms for which we have now provided substantial evidence.

In this review, agnathans are considered to be paraphyletic in origin. The modern agnathans are classified into two groups, myxinoids (hagfish) and petromyzonids (lamprey) (Forey and Janvier, 1993, 1994) while the gnathostomes constitute all the other living vertebrates, including the bony and cartilaginous fishes and the tetrapods. Forey and Janvier (Forey and Janvier, 1994), have hypothesized from their phylogenetic and paleontological analysis that modern lampreys are more closely related to gnathostomes than they are to hagfish;

Table 1  
Summary of identified hormones and genes from the brain and pituitary in lampreys

Tissue	Hormone	Gene/cDNA	Function	References
<i>Brain</i>	Lamprey GnRH-I	Lamprey GnRH-I	Neurohormone	Sherwood et al., 1986
	Lamprey GnRH-III		Neurohormone	Sower et al., 1993
	Somatostatin-14		Unknown	Sower et al., 1994
	PMY-like (NPY)		Reproduction	Conlon et al., 1994
	Lamprey tachykinin NPY		Unknown	Waugh et al., 1994 Soderberg et al., 1994 Gamble et al., 1997; Suzuki et al., 2000
<i>Pituitary</i>	ACTH	POM POC AVT GH	Steroidogenesis	Takahashi et al., 1995a
	MSH-A		Melanotropic	Takahashi et al., 1995a
	MSH-B		Melanotropic	Takahashi et al., 1995a
	NHF		Unknown	Sower et al., 1995
	AVT		Unknown	Lane et al., 1988
			Takahashi et al., 1995b	
			Heinig et al., 1995 Suzuki et al., 1995	
	IGF expression	Kawauchi, Suzuki, Yamazaki, Nozaki, Moriyama, Takahashi and Sower, unpublished		

*Abbreviations:* GnRH, gonadotropin releasing hormone; PMY, NPY, neuropeptide Y-related peptide; ACTH, adrenocorticotropin; MSH-A, MSH-B, melanotropins; NHF, nasohypophysial factor; AVT, arginine vasotocin; POM, proopiomelanotropin; POC, proopiocortin. Modified from Sower (1998).

however, the phylogenetic position of hagfish in vertebrate evolution is currently controversial (Kuraku et al., 1999; Delarbre et al., 2000).

## 2. Reproductive cycle of the lampreys

There are approximately 40 species of lampreys that are classified as parasitic or non-parasitic. Lampreys spawn only once in their lifetime, after which they die. The parasitic lampreys are generally, but not exclusively, anadromous. All larval lampreys, called ammocoetes, live in fresh water as burrowing organisms in the bottoms of streams and occasionally lakes. In the parasitic sea lamprey, *Petromyzon marinus*, as in all other lamprey species, sexual maturation is a seasonal, synchronized process. The sea lampreys begin their lives as freshwater ammocoetes, which are blind, filter-feeding larvae. After approximately two to seven years in freshwater streams, metamorphosis occurs and the ammocoetes become free swimming, sexually immature lampreys (juveniles), which migrate to the sea or lakes. During the approximately 15-month-long parasitic sea or lake phase, gametogenesis progresses. In males, spermatogonia proliferate and develop into primary and secondary spermatocytes, in females, vitellogenesis occurs. After approximately 15 months, lampreys return to freshwater streams and undergo the final maturational processes resulting in mature eggs and sperm, spawning and finally death.

## 3. Gonadotropin-releasing hormone

A key neuroendocrine function of the hypothalamus in the control of reproduction is the timed release of the decapeptide, GnRH, in response to external and internal cues. GnRH acts on the pituitary to regulate the pituitary–gonadal axis for all vertebrates. Gonadotropins, secreted in response to GnRH, are released from the pituitary gland and are the major hormones influencing steroidogenesis and gametogenesis in the gonad. Until approximately 15 years ago, there had been little evidence for brain control of reproduction in lampreys. However, Sower's laboratory has made substantial progress in this area (See reviews: Sower, 1990b, 1997, 1998). Two molecular forms of GnRH, lamprey GnRH-I and lamprey GnRH-III, have been sequenced in the sea lam-

prey (Sherwood et al., 1986; Sower et al., 1993). In addition, a cDNA of lamprey GnRH-I has been cloned (Suzuki et al., 2000). Lampreys are the most primitive vertebrates for which there are demonstrated functional roles for multiple GnRH neurohormones involved in pituitary-reproductive activity. Both lamprey GnRH-I and -III have been shown to induce steroidogenesis and spermiation/ovulation in adult sea lampreys (Sower, 1990a; Sower et al., 1993; Deragon and Sower, 1994; Gazourian et al., 1997). In lampreys undergoing metamorphosis, there is an increase of brain lamprey GnRH-I and -III that coincides with the acceleration of gonadal maturation (Youson and Sower, 1991; Youson et al., 1995). In immunocytochemical studies, both ir-lamprey GnRH-I and -III can be found in the cell bodies of the rostral hypothalamus and preoptic area in larval and adult sea lamprey (King et al., 1988; Wright et al., 1994; Tobet et al., 1995; Nozaki et al., 2000). Most of the ir-GnRH in the brain of larval lampreys has been shown to be lamprey GnRH-III. Thus, lamprey GnRH-III may be the more active form during gonadal maturation. Thus these biochemical, molecular, immunocytochemical and functional studies on the structure and function of the GnRHs in lampreys support the notion that the GnRHs in vertebrates are highly conserved throughout vertebrate evolution.

Sower's laboratory has isolated and sequenced the cDNA of a gene encoding lamprey pregonadotropin releasing hormone-I (lamprey GnRH-I) in the sea lamprey (Gamble et al., 1997; Suzuki et al., 2000). The lamprey GnRH-I precursor is the first identified in an ancient lineage of vertebrates and has the same overall tripartite structure as other gnathostome GnRH precursors. The amino acid sequence of lamprey GnRH-I and the processing site (Gly-Lys-Arg) have been highly conserved during 500 million years of evolution with 60–70% identity compared to those of tetrapod and teleost GnRH precursors. In contrast, the GnRH-associated peptide (GAP) regions are markedly divergent with less than 20% identity compared to all identified vertebrate precursors. Unlike all other known vertebrate GnRH precursors, which typically have one and in a single case two transcripts, three distinct transcripts were isolated and sequenced in lampreys. The lamprey GnRH-I transcripts, termed GAP49, GAP50 and GAP58, differed in the length of the GAP coding sequence and were demonstrated to be the

products of a single gene. Analysis of the lamprey GnRH-I gene intron-2 splice junction demonstrated that alternate splicing produces the different lamprey GnRH-I transcripts. Lamprey GnRH-I is the first GnRH gene demonstrated to utilize splice sequence variants to produce multiple transcripts which may reflect an ancestral gene regulatory mechanism.

Most of the studies in lampreys have been done using the sea lamprey. In another study, gonadotropin-releasing hormone (GnRH)-like molecules were characterized in the brains of representatives of the two Southern Hemisphere families of lampreys, Geotriidae and Mordaciidae (Sower et al., 2001). Chromatographic and immunocytochemical evidence showed that the brains of *Geotria australis* and *Mordacia mordax* contain two forms of GnRH-like molecules. These two forms corresponded to lamprey GnRH-I and -III which were first sequenced from the brain of the anadromous sea lamprey, *P. marinus*, a representative of the family Petromyzontidae that is found only in the Northern Hemisphere. In chromatographic studies (HPLC) using lamprey GnRH-I and -III antiserum, two early eluting GnRH forms co-eluted with synthetic lamprey GnRH-I and -III standards in the brains of *G. australis* and *M. mordax*. These studies thus indicated that, despite their apparently long period of separation, the three families of extant lampreys have each retained both of the lamprey GnRH (-I and -III forms) molecules. Moreover, immunocytochemical localization of lamprey GnRH indicated that the pattern of its distribution in the adult brain of at least one of these Southern Hemisphere lampreys (*G. australis*) is similar to that previously described for *P. marinus*. Distribution of GnRH in the brain of larval *G. australis* was not as extensive as in larval *P. marinus*, which may account for the later gonadal development in the former species. The fact that lamprey GnRH-I and -III are the dominant GnRH forms in all three families of lampreys implies that these neurohormones have an ancient origin.

#### 4. Neuropeptide Y-related peptide and GABA

Many factors have been identified in vertebrates which are able to modulate reproductive events through their influence on the hypothalamic–pituitary–gonadal axis. Two such modula-

tors have been found to establish relationships with GnRH neurons from the earliest stages of their development. Thus neuropeptide Y (NPY) and  $\gamma$ -aminobutyric acid (GABA) have been shown to influence reproductive processes in vertebrates, and have been found in and around GnRH neurons during their migration in several different species. In sea lampreys, we have determined that both peptide methionine-tyrosine (PMY, a neuropeptide Y-like hormone) and  $\gamma$ -aminobutyric acid (GABA) interact with the reproductive neuroendocrine axis (MacIntyre et al., 1997). NPY is a 36 amino acid peptide that has been shown to act at the level of the hypothalamus and pituitary to alter GnRH and gonadotropin (GTH) release, respectively, (reviewed in Larhammar, 1996). Immunocytochemical studies have determined, that in both teleosts and mammals, NPY containing cells can be identified in close proximity to GnRH containing cells (Larhammar, 1996). Whether NPY exerts a stimulatory or inhibitory effect at either of these levels has proven to be highly dependent on the hormonal milieu. In teleosts, NPY is able to stimulate GnRH and GTH release from the hypothalamus and pituitary and potentiate GnRH induced GTH release when conducive steroidal conditions exist (Larhammar, 1996).

A NPY-like peptide, known as peptide methionine-tyrosine (PMY) was isolated first from the intestine and then from the brain of the sea lamprey (Conlon et al., 1991, 1994). PMY is structurally more similar to NPY than other NPY-family members as it has the same amino acid residues at key positions identified in all other vertebrate forms of NPY (Conlon et al., 1991). Our studies have shown that PMY was able to suppress estradiol levels in female sea lamprey (MacIntyre et al., 1997). We further demonstrated that PMY elevated brain lamprey GnRH-I and -III content which is consistent with the function of NPY observed in other vertebrates (MacIntyre et al., 1997). At this time, it is undetermined whether PMY altered estradiol concentration through direct action at the ovaries or if PMY affected pituitary function.

#### 5. Tachykinin: substance P-like molecule, and somatostatin-14

The tachykinins consist of a large family of

peptides that have a common amino acid sequence at the C-terminus (Jensen and Conlon, 1992). These peptides are widely distributed in the nervous system and endocrine cells of vertebrates and are proposed to be involved in the physiological regulation of the cardiovascular and gastrointestinal functions in vertebrates (Jensen and Conlon, 1992). The first tachykinin-related peptide amino acid sequence was determined from the brains of adult sea lamprey as Arg-Lys-Pro-His-Pro-Lys-Glu-Phe-Val-Gly-Leu-Met-NH<sub>2</sub> (Waugh et al., 1994). The lamprey tachykinin has structural features that are similar both to neurokinin A and substance P. However, the N-terminal region of the lamprey peptide molecule was more similar to mammalian substance P. In common with all other substance P-related peptides characterized, the lamprey tachykinin contains the motif, Lys/Arg-Pro-Xaa-Pro. Thus, on the basis of structural features, the lamprey peptide is classified with substance P rather than with neurokinin A. The function of the lamprey tachykinin is unknown at this time.

The neurohormone, somatostatin-14 (SS-14) was first isolated from the ovine hypothalamus and was shown to inhibit release of pituitary growth hormone (Brazeau et al., 1973). Subsequent studies have shown that somatostatin is widely distributed in vertebrate neuroendocrine tissues (Conlon, 1990b; Sheridan et al., 2000). Previous studies have led to the identification of three biosynthetically related molecular forms of somatostatin (somatostatin-14, -34 and -37) from the pancreas of the sea lamprey (Andrews et al., 1988). Since that time, another form was isolated from the brain of the lamprey and shown to be a second form of somatostatin-14 (SS-14), identical to mammalian somatostatin-14 and differing from lamprey pancreatic somatostatin-14 by the substitution Ser<sup>12</sup> to Thr (Sower et al., 1994). These data support the conclusion that the complete primary structure of SS-14-I or invariant SS-14 has been very highly conserved during vertebrate evolution. The amino acid sequence of the peptide is the same in species from all classes of vertebrates that have been studied to date with the exception of the holocephalan fish, *Hydrolagus colliei* (Pacific ratfish) (Conlon, 1990a). The isolation of invariant SS-14 from lamprey brain provides good evidence for the expression of more than one somatostatin gene in an agnathan. Invariant SS-14 or SS-14-I was not detected in the

lamprey pancreas suggesting that expression of the different somatostatin genes may be tissue-specific (Andrews et al., 1988). The antiserum to SS-14 used in the Sower et al. (1994) study does not detect peptides with [Tyr<sup>7</sup>,Gly<sup>10</sup>]SS-14 at their COOH-termini leaving the question open to whether the prosomatostatin-II gene is expressed in Agnatha.

## 6. Pituitary hormones

### 6.1. Arginine vasotocin

Arginine vasotocin (AVT) was the first pituitary hormone to be isolated from lampreys (Lane et al., 1988). AVT appears to be the most primitive of the neurohypophyseal peptides, since it is found in representative species of all vertebrates (Gorbman et al., 1983). Although arginine vasotocin is replaced by the vasopressins in adult mammals, it is present in the fetal neurohypophysis of some mammals. Oxytocin-related peptides generally have not been identified in lamprey pituitary extracts by bioassay or immunohistochemistry (Goossens et al., 1977). A survey of major neutral peptides by Lane et al. (1988) produced no evidence for peptides with homology to this neurohypophyseal nonapeptide family. This tends to support previous studies which have found no immunoreactivity with antisera generated against oxytocin, isotocin, or their homologs.

In more recent studies, the nucleotide sequences of cDNAs encoding precursors of arginine vasotocin were determined in the lamprey, *Lampetra japonica* and the hagfish, *Eptatretus burgeri* (Suzuki et al., 1993). In these studies, the predicted vasotocin precursors were both composed of a signal peptide, arginine vasotocin, Gly-Lys-Arg and a neurophysin, similar to that shown for precursors of the vasopressin family of hormones. The central region of the lamprey neurophysin was similar to that of previously characterized gnathostome neurophysins. In contrast, the hagfish neurophysin showed at least two insertions and one deletion in the conserved central region. From these data, Suzuki et al. (1993) estimated the evolutionary relationships of the precursors of the vasopressin family among the lamprey, hagfish, gnathostomes and a mollusc. This analysis showed that the lamprey vasotocin precursor is more closely related to the gnathos-

tome vasotocin and vasopressin precursors than to the hagfish vasotocin precursors.

## 6.2. Proopiomelanocortin-related hormones

The first anterior pituitary hormones from the lamprey that have been recently sequenced include adrenocorticotropin (ACTH), melanotropins, MSH-A and MSH-B (Takahashi et al., 1995a) and a putative pituitary hormone, nasohypophysial factor (NHF) (Sower et al., 1995).

$\alpha$ -MSH has been recognized as the most potent naturally occurring melanotropic peptide in vertebrates (Eberle, 1988). Takahashi et al. (1995a) demonstrated that synthetic lamprey MSH-B is approximately 10 times more potent than  $\alpha$ -MSH in the frog skin bioassay. Since hypophysectomy results in melanin concentration and pallor in *G. australis* and *Lampetra fluviatilis* (Larsen, 1965; Eddy and Strahan, 1968), it is reasonable to speculate that MSHs are physiologically functioning as melanotropic hormones in lampreys (Takahashi et al., 1995a). In this latter study, lamprey ACTH was virtually inactive in the frog skin assay but ACTH was biologically active in stimulating in vitro steroidogenesis of the adrenocortical cells from the pronephric kidney and less so in the mesonephric kidney of the male sea lamprey. Further studies will be needed to fully confirm the corticotropic activity of lamprey ACTH.

POMC is the common precursor of adrenocorticotropin (ACTH)-related peptides,  $\beta$ -endorphin (END) and melanotropins (MSH). In gnathostomes, a common POMC gene is expressed in two discrete areas in the pituitary gland of vertebrates. ACTH are produced by corticotrophs in the pars distalis and MSHs by melanotrophs in the pars intermedia through tissue-specific post-translational proteolytic processing (Nakanishi et al., 1979; Smith and Funder, 1988; Dores et al., 1993). Immunocytochemical analysis showed that topographic distributions of MSH and ACTH in the adult sea lamprey pituitary are similar to those in other vertebrates: MSH is produced in the pars intermedia and ACTH in the pars distalis (Nozaki et al., 1995).

However, in the lamprey, MSH and ACTH were found to be encoded on two distinct genes, POM and POC, respectively; the former is expressed in the pars intermedia and the latter in the pars distalis of the adult sea lamprey (Heinig

et al., 1995; Takahashi et al., 1995b). POM encodes MSH-B, MSH-A and  $\beta$ -END, while POC encodes NHF, ACTH and a different  $\beta$ -END. The tissue specific expression of two genes has been demonstrated by in situ hybridization (Ficele et al., 1998; Heinig et al., 1998). In several vertebrates, teleosts and amphibians, there are two POMC genes that are expressed, however, in no group has the organization of the two genes diverged as much as is seen in the lamprey. This information supports the view (Ohno et al., 1968) that there have been at least two periods of gene duplication during vertebrate evolution, early in the origin of vertebrates and later in the early-evolved teleosts.

Amino acid sequence of POM has 32% sequence identity with that of POC. A striking difference between POM and POC is that the former has two repeats of the MSH sequence, MSH-B and MSH-A, while the latter contains a single MSH sequence at the N-terminal part of ACTH. It is, therefore, plausible that POM and POC originated from a common ancestral gene by duplication and subsequent divergence under powerful selective pressure for specialization of function. In this context, the conserved  $\beta$ -END in both genes may have a different physiological function in the lamprey.

POM and POC amino acid sequences can be schematically compared with those of bovine POMC (Nakanishi et al., 1979) and trout POMC (Salbert et al., 1992, fig. 2). These preprohormones share common structural features: the Cys-rich segment at the N-terminus; the ACTH-MSH middle segment; the  $\beta$ -END segment at the C-terminus. A  $\gamma$ -MSH is present in the N-terminal segment of tetrapod POMCs and not in teleost POMCs, and the MSH-A counterpart in the middle segment is absent in POC, as described above, the location of MSH-B in POM or ACTH in POC corresponds to that of ACTH in other vertebrate POMCs. Consequently, MSH-A in POM appears to be homologous to  $\beta$ -MSH. Recent studies have shown that POMC of the white sturgeon, a chondrosteian, is similar to tetrapod molecules as shown by the presence of a  $\gamma$ -MSH-like segment (Amemiya et al., 1997). Thus, it is likely that POMC has diverged by duplication of an ancestral molecule which has included the insertion, deletion and subsequent modifications of MSH segments including the possibility that the MSH-A

homologue in lamprey POC disappeared following gene duplication during the course of lamprey evolution (Takahashi et al., 1995b).

As stated above, in lampreys, POC encodes NHF, ACTH and a different  $\beta$ -END. NHF is a novel homodimeric glycoprotein that was isolated and characterized from the pituitaries of adult sea lampreys. The monomer consists of 121 amino-acid residues in a sequence that has no resemblance to any known pituitary hormone. While this protein is localized in most cells of the rostral pars distalis of adult lampreys, we chose to name it, nasohypophysial factor (NHF), because it is first expressed in the olfactory system of developing larval lampreys (Sower et al., 1995). Not only may NHF be a new pituitary hormone but a useful probe for examining the ontogenetic and phylogenetic relationships of the pituitary and olfactory systems in vertebrates.

The strong presence of a POC expressed protein, peptide nasohypophysial factor -NHF, in the developing olfactory and adeno-hypophysial tissues, as well as in the pituitary and blood of adult lampreys, argues for an important function for this substance. The fact that its 121 amino acid sequence bears no resemblance to that of any known pituitary hormone raises additional important questions. One unanswered question concerns the presence of NHF within the olfactory and pituitary systems of vertebrates other than the lampreys. It is difficult to conceive at this point that a pituitary protein with this developmental history, and abundance, in the RPD, has no significant functional role. The function of NHF in the sea lamprey, however, has yet to be determined. In experiments designed to identify NHF with known gonadotropin function, there was no stimulation of steroidogenesis *in vitro* in lamprey testis or ovary (Sower et al., 1995). In addition, NHF showed no immunoblot reactivity with antisera against other vertebrate gonadotropins, growth hormone, prolactin, or somatotactin (Sower et al., 1995). The function of the N-terminal fragment of POMC in gnathostomes has not yet been determined, although preliminary studies indicate that it may act as a growth-promoting factor. Tilemans et al. (1994) demonstrated that the N-terminal fragment of POMC acted as a growth factor by stimulating the development of lactotrophs in the rat pituitary. In addition, Takahashi et al. (1995b) suggested that the N-terminal fragment of salmon proopiome-

lanocortin, NPP-I, may induce effective interrenal growth after observing interrenal cell hypertrophy following injections of salmon NPP-I in trout. Therefore, NHF may be acting as a growth factor in the sea lamprey.

### 6.3. Gonadotropin

Gonadotropins, in response to GnRH, are released from the pituitary gland and are the major hormones regulating steroidogenesis and gametogenesis. Gonadotropins have not been identified from either lampreys or hagfish. Prior to the late 1980s, it was considered by many researchers that fish only had one gonadotropin, although it had been suggested that there were two. Two gonadotropins, GTH-I and GTH-II, were first identified in chum salmon by Suzuki et al. (1988). Subsequently, the duality of the gonadotropins has been shown in a number of other teleost fish; coho salmon (Swanson et al., 1991); carp (Van der Kraak et al., 1992); bonito (Koide et al., 1993) and killifish (Lin et al., 1992). It is now generally accepted that teleosts have two gonadotropins, GTH-I (which is FSH-like) and GTH-II (which is LH-like). It is suggested that GTH-I is involved in regulating gonadal steroidogenesis in the regulation of puberty and early gonadal development and GTH-II is involved in regulating the final stages of reproductive maturation and spawning in salmon (Swanson et al., 1991).

Evidence from physiological and immunocytochemical studies strongly support the presence of a gonadotropin-like molecule in lampreys indicating that a reasonably typical pituitary-gonadal relationship exists in this group (Larsen and Rothwell, 1972; Hardisty and Baker, 1982; Sower, 1990b, 1998). In river lampreys (*L. fluviatilis*), hypophysectomy followed by substitution therapy with pituitary extracts or mammalian GTHs, indicated pituitary regulation of the gonads (see Larsen, 1980). Moreover, injection of a salmon gonadotropin preparation into adult spawning sea lampreys advanced ovulation by several weeks and elevated plasma estradiol levels (Sower et al., 1983). In addition, previous studies had demonstrated that there are two distinct high affinity binding sites in the pituitary for lamprey GnRH-I and -III and that these hormones differentially regulate lamprey pituitary function (Knox et al., 1994; Materne et al., 1997; Sower, 1997, 1998). A mammalian-like immunoreactive luteinizing hor-

mone was shown to be present by immunocytochemistry in the pituitary of the sea lamprey (Wright, 1983).

Based on this evidence of a GTH-like molecule in lampreys, we have made a concerted effort to identify GTH in sea lampreys. More recently, using immunocytochemistry, Nozaki et al. (1999) detected immunoreactive (ir) GTH in the sea lamprey pituitary using two different cytochemical approaches: (1) lectin histochemistry; and (2) immunohistochemistry. Based on these recent results, we have been performing molecular and protein isolation procedures for lamprey GTH using probes and antibodies to ovine luteinizing hormone (oLH). We now have a partial sequence of a lamprey GTH-like protein and a partial cDNA clone of a putative GTH sequence (A. Takahashi, K. Tanimizu, H. Kawauchi, and S.A. Sower, unpublished).

#### 6.4. Growth hormone

Members of the growth hormone and prolactin family include growth hormone, prolactin, and somatolactin. These three hormones have been grouped together based on structural similarities and are thought to have evolved from a common ancestral origin by gene duplication (Kawauchi, 1989). Prolactin (PRL) has diverse roles across the vertebrate classes including involvement in osmoregulation in teleost fish, growth and development of the mammary gland in mammals, and in reproduction. Growth hormone (GH) has mitogenic, growth-promoting, and metabolic actions on many cell types in mammals (Daughaday, 1989), and mammalian and teleostean GH have been shown to stimulate general body growth in teleosts (Donaldson et al., 1979). In addition, both GH and PRL have been shown to affect reproductive processes in teleosts, particularly gonadal steroidogenesis. Whether these effects are exerted directly on the gonads (Singh et al., 1988; Tan et al., 1988; Le Gac et al., 1992) or indirectly via the enhancement of gonadotropin function (Van der Kraak et al., 1990; Rubin and Specker, 1992) remains unclear. Somatolactin (SL) is the latest member of the GH/PRL family to be identified in bony fish. The function of SL remains unclear, although it may also have a role in reproduction (Planas et al., 1992; Rand-Weaver et al., 1992).

Until this past year, none of the members of

the GH/PRL family have been isolated from agnathan pituitary glands. As reported at the Fourth International Symposium on Fish Endocrinology, a GH cDNA has been cloned from the sea lamprey (Kawauchi, Suzuki, Yamazaki, Nozaki, Moriyama, Takahashi and Sower, unpublished). The GH cDNA was 1992 base pairs. Based on the deduced amino-acid sequence, we synthesized a 16-amino acid peptide corresponding to the N-terminal portion of lamprey growth hormone and prepared a rabbit antiserum against this synthetic peptide. Using this antiserum, we isolated a protein by monitoring immunoreactivity during purification of the sea lamprey pituitaries by extraction and chromatography. We then demonstrated that lamprey GH, the mature protein, consisted of 181 amino acid residues after N-terminal sequence analysis of the purified protein. The isolated protein was shown to be GH by demonstrating an increase of expression of lamprey IGF gene in the liver. The lamprey GH shows approximately 22% sequence identity with GHs of gnathostomes, 16–20% identity with SLs and 15–17% with PRLs. Phylogenetic analysis showed that lamprey GH was far removed from the other known GHs. Immunocytochemistry using the anti-lamprey GH peptide showed that GH-like protein was found in the dorsal half of the proximal pars distalis (M. Nozaki, K. Ominato, Y. Oshima, A. Takahashi, H. Kawauchi, K. Suzuki and S.A. Sower, unpublished).

#### 7. Summary

In summary, since 1988, Sower and Kawauchi and collaborators have made substantial progress on our understanding of the brain and pituitary hormones in lampreys. Characterization of brain and pituitary hormones from an extant representative species of one of the oldest lineage of vertebrates is particularly important for understanding the molecular evolution and functional diversity of these hormones and may help in resolving the phylogenetic relationships between hagfish, lampreys and jawed vertebrates.

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