Mini-Review

Brain and Pituitary Hormones of Lampreys, Recent Findings and their Evolutionary Significance

STACIA A. SOWER
Dept. of Biochemistry and Molecular Biology, Univ. of New Hampshire, Durham, New Hampshire 03824

SYNOPSIS. Lampreys of the Class Agnathan are of particular importance in understanding endocrinological relationships since they represent one of the oldest lineages of extant vertebrates, which evolved over 550 million years ago. In this review, agnathans are considered to be paraphyletic in origin, with the modern agnathans classified into two groups, myxinoids (hagfish) and petromyzonids (lamprey). Recent paleontological analysis of modern groups suggests that the jawed vertebrates are more closely related to lampreys than either is to the hagfishes. It has been proposed that there have been two periods of gene duplication during the course of vertebrate evolution, one of which occurred close to the origin of the vertebrates. This paper will summarize the most recent information on the structure and function of newly identified hormones and genes of the brain (lamprey GnRH-I and -III, somatostatin-14; neuropeptide Y-related peptide and a lamprey tachykinin) and pituitary (ACTH, MSH-A, MSH-B, NHF-nasohypophysial factor, POM, and POC) in lampreys. In addition, these data provide further information for the prediction of gene duplication during the early development of vertebrates and increases our understanding of the molecular evolution and functional diversity of these hormones.

BRAIN PITUITARY RELATIONS

Because of its significance in the evolution of early vertebrates, lamprey endocrinology has been the subject of reviews in the past, but such reviews have been limited by the dearth of available information. This paper will summarize the most recent findings on the structure and function of newly identified hormones of the brain and pituitary in lampreys (Table 1). The morphology of the lamprey pituitary gland resembles that of gnathostome fish, consisting of an adenohypophysis and a neurohypophysis. The adenohypophysis is divided into three regions, the rostral pars distalis (RPD), the proximal pars distalis (PPD) and the pars intermedia (PI) (Gorbman, 1980). The neurohypophysis has two regions, the anterior neurohypophysis (homologue of 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). Therefore, characterization of the pituitary hormones from one of the jawed vertebrates is particularly important for understanding the molecular evolution and functional diversity of the pituitary hormones in this group of fishes.

Of all vertebrates, only agnathans and teleosts lack a portal vascular system (median eminence) for transferring regulatory peptides from the brain to the adenohypophysis. The adaptive importance of such a portal system is that it makes possible central nervous regulation of such vital processes as reproduction by external (and internal) cycling environmental conditions. The teleosts have solved this structural problem by direct innervation of the pars distalis by appropriate neurosecretory neurons from the adjacent hypothalamus (Peter, 1990). Agnathans, however, have no nervous or vascular communication between
TABLE 1. Summary of identified hormones and genes from the brain and pituitary in lampreys.*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Hormone</th>
<th>Gene/DNA</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus/Brain</td>
<td>lamprey GnRH-I</td>
<td></td>
<td>Neurohormone</td>
<td>Sherwood et al., 1986</td>
</tr>
<tr>
<td></td>
<td>lamprey GnRH-III</td>
<td></td>
<td>Neurohormone</td>
<td>Sower et al., 1993</td>
</tr>
<tr>
<td></td>
<td>somatostatin-14</td>
<td></td>
<td>Unknown</td>
<td>Sower et al., 1994</td>
</tr>
<tr>
<td></td>
<td>PMY</td>
<td></td>
<td>Unknown</td>
<td>Conlon et al., 1994</td>
</tr>
<tr>
<td></td>
<td>lamprey tachykinin</td>
<td></td>
<td>Unknown</td>
<td>Waugh et al., 1994</td>
</tr>
<tr>
<td></td>
<td>NPY</td>
<td></td>
<td>steroidogenesis</td>
<td>Soderberg et al., 1993</td>
</tr>
<tr>
<td></td>
<td>lamprey GnRH-I</td>
<td></td>
<td>melanotropic</td>
<td>Gamble et al., 1997</td>
</tr>
<tr>
<td>Pituitary</td>
<td>ACTH</td>
<td></td>
<td>melanotropic</td>
<td>Takahashi et al., 1995a</td>
</tr>
<tr>
<td></td>
<td>MSH-A</td>
<td></td>
<td>melanotropic</td>
<td>Takahashi et al., 1995a</td>
</tr>
<tr>
<td></td>
<td>MSH-B</td>
<td></td>
<td>unknown</td>
<td>Takahashi et al., 1995a</td>
</tr>
<tr>
<td></td>
<td>NHF</td>
<td></td>
<td>unknown</td>
<td>Lane et al., 1988</td>
</tr>
<tr>
<td></td>
<td>AVT</td>
<td></td>
<td>unknown</td>
<td>Takahashi et al., 1995b</td>
</tr>
<tr>
<td></td>
<td>POM</td>
<td></td>
<td>unknown</td>
<td>Heinig et al., 1995</td>
</tr>
<tr>
<td></td>
<td>POC</td>
<td></td>
<td>unknown</td>
<td>Suzuki et al., 1995</td>
</tr>
<tr>
<td></td>
<td>AVT</td>
<td></td>
<td>unknown</td>
<td></td>
</tr>
</tbody>
</table>

* GnRH, gonadotropin releasing hormone; PMY, NPY, neuropeptide Y-related peptide; ACTH, adrenocorticotropic; MSH-A, MSH-B, melanotropins; NHF, nasohypophysial factor; AVT, arginine vasotocin; POM, proopiomelanotropin, and POC, proopiocortin.

the brain and neurohypophysis (Tsuneki and Gorbman, 1975). This has led to speculation that nervous regulation of the agnathan pars distalis is by diffusion of brain peptides from the adjacent neurohypophysis, across the thin connective tissue layer that separates the neural from the glandular tissues.

Proof that diffusion is an adequate basis for brain regulation of the pars distalis has rested on such experiments as those of Nozaki et al. (1975) and Tsukahara et al. (1986). They injected substances of varying molecular size into the third ventricles of hagfish. By use of staining procedures that revealed the positions of these substances a few minutes after injection, they showed that significant amounts of test materials diffused rapidly from the third ventricle, through the neurohypophysis, to the pars distalis. In several ways, however, it was felt that experiments with hagfish might not represent the diffusion hypothesis fairly. There is some question as to whether hagfish species have an environmentally regulated reproductive cycle (Gorbman and Dickhoff, 1978). Indeed, it has not been established whether the hagfish pituitary contains tropic hormones of any kind (Matty et al., 1976).

Lampreys, on the other hand, are clearly seasonal and temperature-responsive in the timing of their anadromous migrations and mating and breeding (Hardisty, 1979). In addition, there is anatomical evidence to support the concept of hypothalamic control of adenohypophysial function by diffusion of the neurohormones from the neurohypophysis to the pars distalis of the adenohypophysis (Nozaki et al., 1984a; King et al., 1988; Tsuneki, 1988). In the lamprey, gonadotropin-releasing hormone-like neurons identified by immunocytochemistry project their fibers primarily into the neurohypophysis from the preoptic region (Nozaki and Kobayshi, 1979; Crim et al., 1979; Nozaki et al., 1984b; King et al., 1988). In addition, Crim (1981) and King et al. (1988) using a lamprey GnRH-I antibody showed that GnRH neurons project into the third ventricle. These authors proposed an additional route of GnRH via secretion into the third ventricle and transport by tanyocytes to the adenohypophysis. To test whether diffusion can occur, further exploration of the functional anatomical relationship between the hypothalamus and adenohypophysis was done by injecting horse-radish peroxidase (HRP), a protein that can be visualized by appropriate histochemical procedures, into the third ventricle of the brain of adult lampreys (Nozaki et al., 1984a).
Within 5 to 15 minutes HRP had passed through the neurohypophysis, which forms the floor of the third ventricle, and it had diffused throughout the connective tissue separating the adenohypophysial follicles from the neurohypophysis and into intracellular spaces in the adenohypophysis. It was concluded that neurosecretory peptides like gonadotropin-releasing hormone can diffuse from the brain (neurohypophysis) to the adenohypophysis, and thus regulate its secretory activity in lampreys.

We can say that the structural features of the lamprey (and hagfish) pituitary and surrounding tissues appear to represent adaptive evolution in these early evolved vertebrates to make diffusion as efficient as possible for pituitary regulation by brain peptides. The evolution of the agnathan "diffusional median eminence" may be considered as characteristic of hagfish and lampreys as is the anatomical system of the hypothalamic neural penetration of the pars distalis of teleosts.

We can conclude that in the evolutionary sense there have been three types of regulation of the adenohypophysis developed in the vertebrates: the agnathan diffusional type, the teleostean direct innervational type, and the vascular type seen in all other vertebrates (Nozaki et al., 1994) (Fig. 1). Whether we should speak of the agnathan diffusional type as "primitive" is difficult to say. Perhaps the principal advantage of the vascular median eminence type of control of the pars distalis by the brain is that it permitted development of larger and thicker glands as vertebrates became larger and more complicated in form and the distance between the hypothalamus and pituitary increased significantly.

**PITUITARY HORMONES: INTRODUCTION**

There are four families of pituitary hormones that have been identified in verte-
brates: the growth hormone/prolactin family, the proopiomelanocortin (POMC)-related family, the glycoprotein family in the adenohypophysis; and the isotocin and vasotocin family in the neurohypophysis (Kawauchi, 1989). Members of the growth hormone and prolactin family include growth hormone, prolactin, and somatolactin (teleost fish only). 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). Members of the glycoprotein family include gonadotropins and thyrotropins. In mammals, the gonadotropins are referred to as luteinizing hormone (LH) and follicle stimulating hormone (FSH). All gonadotropins identified to date are heterodimeric glycoproteins consisting of two subunits, an α-subunit and a unique β-subunit. The α-subunit is common to all members of the gonadotropin and thyrotropin hormones. The unique β-subunit confers biological specificity to the hormone. In the proopiomelanocortin (POMC)-related family, POMC is the common precursor of adrenocorticotropin (ACTH)-related peptides, β-endorphin and melanotropins. In most vertebrates, there are generally two hormones produced in the brain that are extractable from the neurohypophysis, one basic peptide (arginine vasopressin or arginine vasotocin) and at least one neutral peptide (oxytocin).

Over the past several years there have been many studies that have successfully isolated and characterized pituitary hormones in teleosts, but these same efforts have not been applied to other groups of fish. Until recently, the only pituitary hormone that had been structurally identified in the lamprey was arginine vasotocin (Lane et al., 1988). Extensive immunohistochemical studies had tentatively identified several vertebrate peptide and protein hormones in specific areas of the brain, hypothalamus, and pituitary (Nozaki, 1985, for review). While several pituitary hormones had been proposed in the lamprey, with the exception of AVT, none has been isolated and chemically characterized. The first anterior pituitary hormones from the lamprey that have been recently sequenced include adrenocorticotropic (ACTH), melanotropins, MSH-A and MSH-B (Takahashi et al., 1995a) and a putative pituitary hormone, nasohypophysial factor (NHF) (Sower et al., 1995). In gnathostomes, MSH and ACTH are produced from a common precursor protein, POMC, in the pars intermedia and pars distalis, respectively (Dores et al., 1993). However, in lampreys, there are two precursor genes, proopiomelanotropin (POM) (Takahashi et al., 1995b) and proopiocortin (POC) (Heinig et al., 1995) that encode for these hormones. These are the first genes encoding lamprey anterior pituitary hormones that have been characterized. Based on the distribution of the melanotropin sequences within the POMC of vertebrates, Takahashi et al. (1995a) proposed that the POMC gene may have evolved by intragenic duplication of a primordial MSH gene during early vertebrate evolution.

**POMC and POC**

It has been established that a common POMC gene, encoding ACTH, MSH and β-endorphin (END), is expressed in two discrete areas in the pituitary gland of vertebrates. ACTH and MSHs are produced by corticotrophs in the pars distalis and by melanotrophs in the pars intermedia, respectively, through tissue-specific post-translational proteolytic processing (Nakanishi et al., 1979; Smith and Funder, 1988; Dores et al., 1993). In the lamprey, MSH and ACTH were found to be encoded on two distinct genes, POM and POC, which are expressed in the pars intermedia and pars distalis of the adult sea lamprey, respectively (Takahashi et al., 1995b; Heinig et al., 1995). POM encodes MSH-B, MSH-A and β-END, while POC encodes NHF, ACTH and a different β-END. MSH-A is a 19 amino acid peptide and MSH-B is a 20-amino acid peptide (Takahashi et al., 1995a). Both MSHs differ significantly from gnathostome MSHs and cannot be assigned as α-MSH, β-MSH or γ-MSH. The lamprey ACTH resembles dogfish ACTH more closely than salmon ACTH (Takahashi et al., 1995a). In several vertebrates, teleosts and amphibians, there are two POMC genes that are expressed. However, in no group...
FIG. 2. A schematic diagram of the POMC family. In all vertebrates except lampreys, there is a single precursor gene for POMC, proopiomelanocortin. In lampreys, there are two related precursor genes, POM and POC, the most ancestral genes encoding hormones from the pituitary that have been identified. Closed circles show Cys residues. SP: signal peptide. Reproduced from Takahashi el al. (1995b).

has the organization of the two genes diverged as much as is seen in the lamprey. This information supports Ohno et al. (1968) evidence 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. Although the N-terminal portion of POM shows little sequence identity with NHF, the locations of β-END sequences in the preprohormones are well conserved, with 45% identity, and MSH-B can be aligned with 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 β-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 β-END segment at the C-terminus. A γ-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 β-MSH. More recent studies have shown that in the white sturgeon, a primitive ray-finned fish that is a member of the chondrosteans, that the POMC is similar to tetrapod molecules as shown by the presence of a γ-MSH-like segment identified in the N-terminal in addition to ACTH in a middle region of POMC (Amemiya el 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., 1995a).

ADRENOCORTICOTROPIN AND MELANOTRINS

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). ACTH-like immunoreactivity was found in most cells of the rostral pars distalis and in a few scattered cells of the proximal pars distalis. MSH-A-like immunoreactivity was found in most cells of the rostral pars distalis, a few scattered cells of the proximal pars distalis, and almost all cells of the pars intermedia. MSH-B-like immunoreactivity was found only in the pars intermedia, where almost all cells were stained. These studies indicate that POC and POM are not expressed in the same cell. However, to verify that POM and POC are not co-expressed in the same cell will require further studies such as in situ hybridization and immunocytochemistry using other antisera, i.e., beta-endorphin antisera.

α-MSH has been recognized as the most potent naturally occurring melanotropic peptide (Eberle, 1988). Takahashi and colleagues (Takahashi et al., 1995a) demonstrated that synthetic lamprey MSH-B is about 10 times more potent than α-MSH. Since hypophysectomy results in melanin concentration and pallor in Geotria 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 the study of Takahashi et al., lamprey ACTH was virtually inactive in the frog skin assay. In these same studies, ACTH was biologically active in stimulating steroidogenesis in the adenocortical cells located in 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.

Nasohypophysial Factor

As stated above, in lampreys, POC encodes NHF, ACTH and a different β-END. NHF is a novel homodimeric glycoprotein that was isolated and characterized from the pituitaries of adult 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 adenohypophysial 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 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 somatolactin (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 the N-terminal fragment 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 proopiomelanocortin, 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.

NHF has served as a useful cellular
marker that has revealed some interesting and unexpected developmental relationships between the lamprey pituitary and olfactory systems. NHF distribution in larval lampreys indicates that there are two possible cellular routes of migration between the olfactory system and the hypothalamo-hypophysial system. One is the neuronal pathway from the olfactory epithelium, through the telencephalon, to the hypothalamus; the other is the epithelial route via the nasohypophysial duct connection. The neuronal route, as a path of GnRH cells from the olfactory epithelium to the hypothalamus, has been studied in a number of vertebrate groups, including the mammals (Muske and Moore, 1988, 1990; Schwanzel-Fukuda and Pfaff, 1989; Norgren and Lehman, 1991; Kawamura et al., 1992). While it has not been demonstrated that GnRH follows a similar migration in lampreys (Tobet et al., 1995), its olfactory origin has been described in embryos of birds and mammals in addition to amphibians, which suggests that it is a common vertebrate phenomenon.

If NHF is not migratory, like GnRH, but is synthesized simultaneously in the olfactory organ, the nasohypophysial duct and the adenohypophysis, then it is probable that NHF synthesis is a common property of all cells descended from the original head epithelium that forms the olfactory epithelium and nasohypophysial duct in lamprey embryos (Gorbman and Tamarin, 1985). The data from Sower et al. (1995) showed that in the adult lamprey, NHF is no longer present in the olfactory organ or brain. NHF is a prominent cellular constituent of the pituitary and, in particular, the RPD. It is possible that during development, NHF presence is a common, and possibly primitive, property of all nasal and nasohypophysial tissues in early vertebrate embryos (Sower et al., 1995).

**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 the 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. (1988a). 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 (Kawauchi et al., 1991); 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).

In mammals and certain species of fish, there is only a single α subunit of the gonadotropin; in contrast, it has been determined that in chum salmon and carp there are two types of α-subunit, α1 and α2 (Suzuki et al., 1988b; Chang et al., 1988). The homologies of these two α subunits has 72% identity in chinook salmon and 96% in carp. Suzuki et al. (1988b) showed that the α2 subunit is common to both GTH-I and GTH-II and α1 subunit is unique to GTH-I. There is little information on the GTHα subunit gene structure. It has been shown that carp GTH α1 and α2 have intron-exon organization similar to the mammalian GTH hormone α subunit genes (Huang et al., 1992).

The purification of lamprey gonadotropin(s) has been very difficult due to the size of the pituitary and particular difficulties associated with purification. Evidence from physiological and immunocytochemical studies strongly suggests the presence of a gonadotropin-like molecule in lampreys indicating that a reasonably typical pituitary-gonadal relationship exists in this group (Sower, 1990; Sower and Larsen, 1991). As described above, in an attempt to identify a GTH homologue in the pituitary glands of adult sea lampreys, a novel homodimeric glycoprotein, NHF, was instead isolated and sequenced (Sower et al., 1995). While these previous attempts have isolated additional pituitary...
factors (MSHs, ACTH), a gonadotropin-like molecule has remained elusive. Recent experiments suggest the promise of molecular approaches to isolate such a molecule, and provide additional tools to study regulation.

GROWTH HORMONE AND PROLACTIN PITUITARY HORMONES

Prolactin 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 has mitogenic, growth-promoting, and metabolic actions on many cell types in mammals (Daughaday, 1989), and mammalian and teleostean growth hormone have been shown to stimulate general body growth in teleosts (Donaldson et al., 1979). In addition, both growth hormone and prolactin 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 is the latest member of the growth hormone/prolactin family to be identified in teleost fish. The function of somatolactin remains unclear, although it may also have a role in reproduction (Rand-Weaver et al., 1992; Planas et al., 1992).

None of the members of the growth hormone/prolactin family has been isolated from agnathan pituitary glands. Although Wright (1984) localized positive immunoreactivity using antisera to mammalian growth hormone and prolactin in the lamprey adenohypophysis using immunoperoxidase methods, Aler et al. (1971) were not able to detect any immunoreaction in the pituitaries of lampreys or hagfish using rabbit antiserum to ovine prolactin. In addition, in developing an enzyme-linked immunosorbent assay (ELISA) recognizing salmon growth hormone, Farbridge and Leatherland (1991) did not detect any immunoreaction in sea lamprey plasma. Therefore, if the sea lamprey does possess prolactin, growth hormone, or somatolactin, the primary structures probably differ significantly from that of gnathostomic vertebrates. Further studies on the role of pituitary hormones in cyclostomes are necessary to understand the evolution of these hormones in the vertebrates.

ARGININE VASOTOCIN

Only one basic peptide has been identified in agnathans, arginine vasotocin. Arginine vasotocin (AVT) was the first pituitary hormone to be isolated from lampreys (Lane et al., 1988). Arginine vasotocin 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 (Goosens et al., 1977). A survey of major neutral peptides in lamprey extracts produced no evidence for peptides with homology to the neurohypophyseal nonapeptide family (Lane et al., 1988). This tends to support previous studies which have found no immunoreactivity with antisera generated against oxytocin, isotocin, or their homologs.

The common interpretation of these data is that lampreys and hagfish represent two groups of modern agnathans that diverged before the gene duplication event which gave rise to multiple neurohypophyseal nonapeptides (reviewed by Acher, 1974). However, none of these studies can address the distinct possibility that another neurohypophyseal nonapeptide once existed, or currently exists in very low concentrations, or exists in an unrecognized state, in the lamprey lineage. The gene for such a peptide may have been lost or undergone extensive modification over the 500 million years since the divergence of the two lineages of modern agnathans.

In more recent studies, the nucleotide sequences of cDNAs encoding precursors of arginine vasotocin were determined in the lamprey, Lampeatra japonica and the hagfish, Epipterygus burgeri (Suzuki et al., 1995). In these studies, the predicted va-
**Endocrinology of Lampreys**

**Bovine VP**
**Toad VT**
**White Sucker VT-I**
**Masu Salmon VT-I**
**Chum Salmon VT-I**
**Chum Salmon VT-II**
**White Sucker VT-II**
**Japanese Hagfish VT**
**American Hagfish VT**
**Lamprey VT**
**Pond Snail CP**

**FIG. 3.** A phylogenetic tree of precursors of the vasopressin (VP) family hormones from the lamprey, Japanese and American hagfish, cattle, toad, masu salmon, chum salmon, white sucker and pond snail obtained by using the maximum likelihood method. The length of each branch is proportional to the estimated number of amino acid substitutions. The lamprey vasotocin (VT) precursors forms the monophyletic association with the gnathostome vasotocin and vasopressin precursors. CP, conopressin. Reproduced with permission from Suzuki et al., 1995.

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 those 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. (1995) estimated the evolutionary relationships of the precursors of the vasopressin family among the lamprey, hagfish, gnathostomes and a mollusc (Fig. 3). This analysis showed that the lamprey vasotocin precursor is more closely related to the gnathostome vasotocin and vasopressin precursors than to the hagfish vasotocin precursors.

**Gonadotropin-Releasing Hormone**

Research during the past several years has now established that there is considerable diversity in the molecular structure of GnRHs among protochordates and vertebrates. Currently, nine structures of GnRH have been determined in various vertebrate species and two in invertebrates. Included in this family are the structures of GnRHs of three fish species of ancient origin, an agnathan, the sea lamprey, Petromyzon marinus (lamprey GnRH-I and III) (Sherwood et al., 1986; Sower et al., 1993); an elasmobranch, the spiny dogfish shark, Squalus acanthias, (dogfish GnRH and chicken GnRH-II) (Lovejoy et al., 1992); and a holocephalan, the ratfish, Hydrolagus colliei, (chicken GnRH-II) (Lovejoy et al., 1991). Recently, two GnRH structures (tunicate GnRH-I and -II) have been isolated from the invertebrate, the sea squirt (Protochordata: Tunicata; Chelyosoma productum) (Powell et al., 1996). The primary structures are shown below (Sower et al., 1993; Powell et al., 1996). The underlined amino acids indicate those amino acids that are different compared to the amino acids of the mammalian GnRH structure.
By 1986, two molecular forms of gonadotropin-releasing hormone (GnRH-I and II) were known from the brain of the sea lamprey. Analysis of these two forms yielded the primary structure of GnRH-I and the amino acid composition of GnRH-II (Sherwood et al., 1986). A third molecular form of GnRH (lamprey GnRH-III) was isolated from the brains of the sea lamprey that is different from GnRH-I and -II (Sower et al., 1993). The primary structure of lamprey GnRH-III differs by three amino acids, compared with lamprey GnRH-I. Lamprey GnRH-III is more closely related to the other members of the GnRH family than is lamprey GnRH-I. Lamprey GnRH-III has 80% sequence identity with chicken GnRH-II and dogfish GnRH; 70% identity with catfish GnRH-I, lamprey GnRH-I, and salmon GnRH; and 60% identity with mammal GnRH and chicken GnRH-I (Sower et al., 1993). Tunicate GnRH-I more closely resembles lamprey GnRH-III because of the absence of Gly<sup>6</sup> and the presence of Lys<sup>8</sup>. Variations in the structure of GnRH occur most frequently at position 8, followed by positions 5 and 7, and then position 6. In all GnRH peptides, certain regions of the molecule have been highly conserved including the NH<sub>2</sub>-terminal, pGlu<sup>1</sup>-His<sup>2</sup> and Ser<sup>4</sup>, and the COOH-terminal. These regions and the length of the molecule have remained unchanged during the 500 million years of evolution of the chordates. The conservation of the NH<sub>2</sub> and COOH-termini suggests these regions are significant for conformation, receptor binding, resistance to enzymatic degradation and in receptor-mediated events required for gonadotropin release (Millar and King, 1987).

The taxonomic distribution of the primary structures of vertebrate GnRHs is shown in Fig. 4. These studies have shown by direct methods that in five classes of vertebrates, two GnRHs have been isolated from a representative species and sequenced. Using indirect methods, two GnRH-like peptides have been identified in representative species of every class of vertebrates and two classes of invertebrates (King and Millar, 1995; Sower et al., 1993; Sherwood et al., 1994). Based on this information as well as the recent information on cDNAs, several evolutionary models for GnRH have been proposed (Sherwood et al., 1983; Millar and King, 1987; King and Millar, 1991, 1995; King et al., 1994; Lovejoy et al., 1991; Andersen and Klungland, 1993; Grober et al., 1995; Sherwood et al., 1994; Sower et al., 1993). However, before a complete analysis of phylogenetic trees of GnRH can be done, there needs to be more information on the identification and function of GnRHs in representative species; there needs to be full identification of novel GnRH forms in vertebrates and protochordates; and the cDNAs need to be sequenced.
ENDOCRINOLOGY OF LAMPERYS

PHYLOGENETIC DISTRIBUTION OF GNRRS IN VERTEBRATES

GnRH: M CI SB C CII D LI LII

Agnatha
- Lamprey
- Chondrichthyes
- Osteichthyes
- Salmon
- Catfish
- Seabream/Cichlid
- Amphibians
- Frog
- Reptile
- Alligator
- Bird
- Chicken
- Mammal
- Pig
- Sheep

Fig. 4. The taxonomic distribution of the primary structures of vertebrate GnRH. The primary structures of GnRH have been determined in mammal (Matsuo et al., 1971; Burgus et al., 1971, 1972; salmon (Sherwood et al., 1983); two forms in chicken (chicken GnRH-I and -II) (Miyamoto et al., 1983, 1984; King and Millar, 1982); two forms in catfish (catfish-I and chicken GnRH-II) (Ngamvonghon et al., 1992); three forms in seabream/cichlid (seabream, salmon, and chicken GnRH-II) (Gothilf et al., 1996; White et al., 1995; Powell et al., 1994); one form in ratfish (chicken GnRH-II) (Lovejoy et al., 1991); two forms in alligator (chicken GnRH-I and -II) (Lovejoy et al., 1991); two forms in dogfish (dogfish GnRH and chicken GnRH-II) (Lovejoy et al., 1992); and two forms in lamprey (lamprey GnRH-I and -III) (Sherwood et al., 1986; Sower et al., 1993).

in more species, particularly the early evolved vertebrates and the protochordates.

Similar to other neurohormones, GnRH is first synthesized as a precursor protein called prepro-GnRH, and is then processed to its final decapeptide form (Klungland et al., 1992a). Although multiple forms of GnRH have been isolated, until 1994 (White et al., 1994) only one gene encoding GnRH in an organism had been identified. White and colleagues (1994) isolated the prepro-chicken GnRH-II gene in the African cichlid; whereas the salmon GnRH gene had already been isolated in the cichlid (Bond et al., 1991). Bogerd et al. (1994) isolated the cDNAs for both catfish GnRH and chicken GnRH-II in the African catfish. More recently, the cDNAs for three GnRH precursors (salmon GnRH, chicken GnRH-II, and seabream) have been isolated from the African cichlid (White et al., 1995) and seabream (Gothilf et al., 1995a).

Currently, the nucleotide sequence for the precursor molecule to mammalian GnRH has been determined in human (Seeburg and Adelman, 1984; Adelman et al., 1986), rat (Goubau et al., 1992), mouse (Mason et al., 1986), and frog (Hayes et al., 1994). Prepro-salmon GnRH has been identified in multiple salmonid species (Klungland et al., 1992a, b; Suzuki et al., 1992; Ashihara et al., 1995; Coe et al., 1992), African cichlid (Bond et al., 1991), red seabream (Okuzawa et al., 1994), plainfin midshipman (Grober et al., 1995), and striped bass (Gothilf et al., 1995a). The precursor to chicken GnRH-II has been sequenced in the African cichlid (White et al., 1994) and catfish (Bogerd et al., 1994). Prepro-seabream GnRH has been sequenced in the seabream, striped bass (Gothilf et al., 1995a, b), and African cichlid (White et al., 1995). Prepro-chicken GnRH-I and the catfish precursor have been identified in the chicken (Dunn et al., 1993) and catfish (Bogerd et al., 1994) respectively. Although
the molecular organization of the precursors appear to be similar throughout these species, the amino acid composition is highly divergent.

The sequence encoding the GnRH peptide and the following cleavage site are highly conserved among all the vertebrates, while the signal peptide and GAP remain quite divergent (Andersen and Klungland, 1993). In masu salmon and mammalian prohormones there is 82–85% amino acid sequence identity in the GnRH and following cleavage site while there is only 8.3–15% sequence identity in the GAP region (Suzuki et al., 1992). There is considerable similarity among the species specific forms of the different prepro-GnRHs. There is 72–89% similarity between mammalian forms (although the frog prepro-mGnRH is only 42% identical to the other mGnRHs [Hayes et al., 1994]); 67–84% nucleotide sequence identity between the prepro-salmon GnRHs; 71% between prepro-chicken GnRH-II sequences; and 98% between catfish GnRH cDNAs (Okuzawa et al., 1994; King and Millar, 1995). Recently, a cDNA encoding the precursor for lamprey GnRH-I and -III was isolated and characterized in the sea lamprey (Gamble et al., 1997). The cDNA consisted of 641 bp which included an open reading frame of 264 bp encoding the 88 amino acid sequence of prepro-lamprey GnRH-I. The lamprey GnRH-I precursor had the same tripartite structure as the other known GnRH precursors consisting of a 24 residue signal peptide followed by the lamprey GnRH-I decapeptide ([Tyr³, Leu⁵, Glu⁶, Trp⁷, Lys⁸] GnRH) and a Gly-Lys-Arg processing and cleavage site connecting a 50 amino acid long GnRH-associated peptide (GAP). GnRH-I did not show any significant identity to other known vertebrate cDNA forms based on the low sequence similarity at the protein level of both the deduced amino acid and nucleotide sequences of the precursor to lamprey GnRH-I.

As stated earlier, to determine the evolution of GnRH and its phylogenetic relationships will require the analysis of the precursors of GnRH along with the analysis of other hormone precursors and the identity and presence of the GnRHs among the protochordates and vertebrates. It is clear that not all GnRHs have been identified in representative species of chordates and it is also clear that there are likely other novel forms of GnRH. One could speculate on the information to date that an ancestral GnRH gene duplicated to give rise to two genes that subsequently diverged and that additional duplication occurred early in vertebrate evolution.

**FUNCTION OF GONADOTROPIN-RELEASING HORMONE**

A key neuroendocrine function of the hypothalamus is the release of the decapeptide, gonadotropin-releasing hormone (GnRH), which in turn acts on the pituitary regulating the pituitary-gonadal axis for all vertebrates. Lampreys are the earliest evolved vertebrates for which there are demonstrated functional roles for multiple gonadotropin-releasing hormone (GnRH) molecules, neurohormones involved in reproductive activity (Sower et al., 1993). Both lamprey GnRH-I and -III have been shown to induce steroidogenesis and spermatiation and/or ovulation in adult sea lampreys (Sower, 1990; Sower et al., 1993; Deragon and Sower, 1994). In lampreys undergoing metamorphosis, there is an increase of brain lamprey GnRH-I and -III which coincides with the acceleration of gonadal maturation (Youson and Sower, 1991). In immunocytochemical studies, both lamprey GnRH-I and -III immunoreactivities are found in cell bodies in the rostral hypothalamus and preoptic area in larval and adult sea lamprey (Tobet et al., 1995a; Wright et al., 1994; Nozaki, Gorbman and Sower, unpublished). We have suggested that in the larval stage, most of the irGnRH is lamprey GnRH-III, indicating that GnRH-III may be the more active form during gonadal maturation.

Evidence from physiological and immunocytochemical studies strongly suggest the presence of a gonadotropin-like molecule in lampreys indicating that a reasonably typical pituitary-gonadal relationship exists in this group. Until 1986, there was little evidence for a regulatory influence of the hypothalamus on the pituitary-gonadal axis in agnathan. Using synthetic lamprey GnRH-
I and its analogs in earlier studies, the first evidence of neuroendocrine control of reproduction in lampreys was obtained (Sower et al., 1987; Sower, 1990). Investigations of the role of GnRH in reproductive process have been impeded by the lack of a purified gonadotropin that can be used in assays to measure pituitary function. However, the biological activity of lamprey GnRH-I or -III has been assessed by steroidogenesis or gametogenesis in in vitro and in vivo studies (Sower, 1987, 1989, 1990; Sower et al., 1987; Sower and Larsen, 1991; Sower et al., 1993; Deragon and Sower, 1994). Other studies have shown that there are seasonal correlations between changes in brain GnRH and gametogenic and steroidogenic activity of the gonads in adult male and female sea lampreys (Fahien and Sower, 1990; Bolduc and Sower, 1992). More recent studies indicate that lamprey GnRH-III is also a neurohormone involved in reproduction based on its ability to stimulate steroidogenesis and gametogenesis in adult sea lampreys (Sower et al., 1993; Deragon and Sower, 1994) and of the occurrence of this peptide in lampreys undergoing different stages of metamorphosis coinciding with the acceleration of gonad maturation (Youson and Sower, 1991).

Sower (1989) demonstrated that lamprey GnRH-I stimulated levels of progesterone and estradiol in adult male sea lampreys after single and two successive injections of lamprey GnRH-I. In this same study, lamprey GnRH-I was determined to induce spermiation in adult male sea lampreys compared to controls after four successive injections of lamprey GnRH-I. Lamprey GnRH-III was also shown to stimulate both progesterone and estradiol concentrations in the adult male lamprey after a single injection of lamprey GnRH-III, and induce spermiation after four successive injections of lamprey GnRH-III (Deragon and Sower, 1994). In both studies, neither lamprey GnRH-III nor lamprey GnRH-I appeared to produce a dose-related response in levels of estradiol and progesterone. The percent spermiation data demonstrate that the injection of adult male sea lampreys with lamprey GnRH-III induced a higher percent spermiation after days 16 and 21, indicating that lamprey GnRH-III may be more potent as a neurohormone than lamprey GnRH-I in the adult male sea lamprey. This is supported by the fact that lamprey GnRH-III brain content concentration was determined to be three times greater than that of lamprey GnRH-I (Sower et al., 1993). However, until the release rates of lamprey GnRH-I and lamprey GnRH-III are known, and gonadotropins can be directly measured, the differences in potency of lamprey GnRH-I and -III can only be inferred.

In lampreys, studies have shown two high affinity, specific classes of binding sites in a single vertebrate pituitary, which is in contrast to all other vertebrates in which only a single class of high affinity binding has been demonstrated (Knox et al., 1994). The proximal pars distalis region of the anterior pituitary contained most of the GnRH binding sites. It is hypothesized that lamprey GnRH-I and -III each has a different binding site or receptor, suggesting differential control of the pituitary gonadotropin-like molecules.

**Origin of GnRH During Development**

Chromatographic and immunological studies of vertebrate brain extracts have shown that there are two or more GnRH-like peptides in representative species of all vertebrate classes (Muske, 1993). The functional significance of multiple forms of GnRH within the brain and in extrahypothalamic locations within the same species, has not been elucidated with the possible exception of lampreys. The GnRHs have apparently multiple actions on phases of reproductive physiology and behavior either through pituitary or non-pituitary agents, depending on the origin of the GnRH system during development. Muske (1993) has proposed that gnathostome vertebrates have two principle GnRH systems, each with different embryonic origins, expressing different molecular forms of GnRH affecting different targets. In the vertebrates examined, neurons which contain forms of GnRH which are considered to regulate pituitary-gonadal functions are thought to be derived from progenitor cells that originate in the olfactory placode, and which migrate to their definitive adult positions in the preop-
Evolution of GnRH Systems and Functions

Agnatha

- Non-Placodal Origin
  - Pituitary regulation via diffusion from the NH
  - No Evidence

Chondrichythes, Osteichythes, Amphibians

- Non-Placodal Origin
  - Non-Pituitary and Pituitary regulatory functions
  - Olfactory Placodal Origin
    - Pituitary regulation via
      1) systematic circulatory system
      2) direct innervation
      3) hypophyseal portal system

Mammals

- Non-Placodal Origin
  - Minimal evidence
  - Olfactory Placodal Origin
    - Pituitary regulation via hypophyseal
      1) portal system

Fig. 5. A proposed diagram on the evolution of GnRH systems and functions in vertebrates. It is proposed that in contrast to all other vertebrates, that GnRH neurons in developing lampreys originate within proliferative zones of the diencephalon and not in the olfactory system. In the vertebrates examined, neurons which contain forms of GnRH which are considered to regulate pituitary-gonadal functions are thought to be derived from progenitor cells that originate in the olfactory placode, and which migrate to their definitive adult positions in the preoptic/hypothalamic areas. With some exceptions, the other GnRH system probably arises from a non-placodal origin and is involved in non-pituitary-gonadal function. Modified from Sower, 1995.

Recent experiments in lampreys have characterized the earliest development of lamprey GnRH neurons and indicate the probable pathway of their migration (Tobet et al., 1995). Mature eggs from adult sea lampreys were fertilized and embryos and developing larvae were maintained for up to 100 days. GnRH neurons were first visualized immunocytochemically at day 22 after fertilization in the preoptic area and hypothalamus. The number of reactive neurons steadily increased with age through day 100. GnRH neurons were not seen within the olfactory system. In contrast to all other vertebrates, we propose that GnRH neurons in developing lampreys originate within proliferative zones of the diencephalon and not in the olfactory system (Fig. 5).

Neuropeptide Y-related Peptide

Neuropeptide Y, NPY, is a member of the pancreatic polypeptide (PP) family. Other members of this family include pancreatic polypeptide (PP), peptide tyrosine-tyrosine (PYY), fish pancreatic peptide Y (PY), and peptide methionine-tyrosine (PMY), isolated from the sea lamprey (Larhammar et al., 1993; Conlon et al., 1991; Larhammar, 1996). The name of this family was derived from the first member of the family to be discovered, pancreatic polypeptide. The latter was originally isolated from the chicken pancreas and was actually a byproduct from the purification of insulin (Larhammar et al., 1993). The members of the PP family have a wide anatomical distribution within an organism and accordingly, a diverse range of physiological functions.

All members of the PP family are composed of 36 amino acid residues and have a carboxyterminal amide (Larhammar et al., 1993, Larhammar, 1996). There is considerable sequence homology conserved in this peptide family. Thus far, NPY is one of the most highly conserved peptides of this length studied in vertebrates systems (Pieribone et al., 1992). NPY isolated from...
Single ancestral NPY/PYY gene

Duplication generating NPY and PYY

PP

600 500 400 300 200 100 Million years before present

Amphioxus

Hagfishes

Lampreys

Cartilaginous fishes

Bony fishes

Acanthomorph fishes

Amphibians

Reptiles and birds

Mammals

Fig. 6. A schematic chordate evolutionary tree showing probable gene duplication events in the NPY family. Both NPY and PYY were present in the ancestral vertebrate as both peptides have been found in lamprey. PP probably arose early in tetrapod evolution by duplication of the PYY gene. PY is probably a diverged form of PYY and has been found only in acanthomorph fishes which are a subgroup among the bony fishes. Reproduced with permission from Larhammar (1996).

Conlon and colleagues (1991, 1994) isolated peptide methionine tyrosine (PMY), a NPY and PYY related peptide, first from the intestine and then from the brain of the sea lamprey. The name indicates the similarity in the location of PMY and PYY in the endocrine cells of the intestine and not structural similarity (Conlon et al., 1991). This same study found PMY to be structurally more similar to NPY than PYY. The primary structure was analyzed and the amino acid sequence of PMY was determined to be Met-Pro-Pro-Lys-Pro-Asp-Asn-Pro-Ser-Pro-Glu-Glu-Leu-Ser-Lys-Tyr-Leu-Ala-Val-Arg-Asn-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-CONH₂ (Conlon et al., 1991). Proline residues were located at positions 2, 5, and 8, Tyr residues at positions 20 and 27 and there was a carboxyterminal amide. Lamprey PMY has the same amino acid residues at key positions that have been identified and conserved in all other vertebrate forms of NPY studied. The most notable substitution in the lamprey PMY was a Ser residue at position 9 (Conlon et al., 1991).
A Gly residue has been identified at position 9 in all other PP family peptides and this position has been determined to be in a sterically hindered region of the molecule (Conlon et al., 1991). The insertion of a Ser residue, with its larger side chain, in lamprey PMY may cause some steric strain in this region and make it difficult for the molecule to maintain the same structural conformation as described for NPY in this region (Conlon et al., 1991).

NPY is a neuropeptide known to play a regulatory role in reproduction of vertebrates through its influence on the hypothalamic-pituitary-gonadal axis. NPY has been shown to act at both the level of the hypothalamus and the pituitary. Whether NPY exerts a stimulatory or inhibitory effect at either of these levels has proven to be highly dependent on the hormonal milieu. Neuropeptide Y in the brains of teleost fish, and in nerve terminals, has been closely associated with the gonadotropin cells of the pituitary by radioimmunoassay and immunohistochemistry (Kah et al., 1989). There is strong evidence that NPY plays a role in reproduction in teleost fish; however, these NPY actions are complex and dependent on the steroidogenic environment and reproductive status (Breton et al., 1989, 1990, 1991; Peng et al., 1990). The physiological role of brain PMY in lampreys is essentially unknown. The biological activity of PMY was tested in a preliminary study in lampreys (Conlon et al., 1994). Intraperitoneal injection of PMY into female adult sea lampreys undergoing final maturation before spawning produced a significant decrease in plasma concentrations of estradiol compared with control lampreys. Further studies are required to determine the location of PMY in the brain of lampreys. Whether PMY plays a role in lamprey reproduction, as it does in teleost fish, remains to be shown.

TACHYKININ: SUBSTANCE P-LIKE MOLECULE

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). In recent years there have been considerable advances in the structural characterization of tachykinins from non-mammalian vertebrates. 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$_2$ (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 biosynthetic relationship between the different tachykinins in non-mammalian vertebrates is unknown. In mammals, nucleotide sequence analysis of cloned c-DNAs has shown that substance P and neurokinin A are products of the posttranslational processing of the same biosynthetic precursor (Nakanishi, 1986). An additional arginine residue at position 1 in lamprey tachykinin suggested that the lamprey is utilizing a site of posttranslation processing in the tachykinin precursor that is different from the equivalent site in mammalian and other non-mammalian preprotachykinin(s) (Waugh et al., 1994).

SOMATOSTATIN-14

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 (reviewed in Conlon, 1990a). Only one gene encoding the precursor of SS has been identified in the human (Shen and Rutter, 1984) and rat (Tavianini et al., 1984) whose gene product is post-translationally pro-
cessed to SS-14 and SS-28 in a tissue-specific manner (Conlon, 1990b). The principal islets or Brockman bodies of the anglerfish, *Lophius americanus* (Hobart et al., 1980) and other teleost fish (reviewed in Conlon et al., 1988) express a second somatostatin gene encoding a biosynthetic precursor (prosomatostatin-II) that contains the sequence of [Tyr6Gly10]SS-14 at its COOH-terminus. In contrast, prosomatostatin II is processed to SS-28 or related forms (SS-22 and SS-25) in all species of teleosts studied to date (Conlon 1990a; Conlon et al., 1988). More recently, isolation of SS-14 and [Pro2,Met13]SS-14 from the brain of the frog, *Rana ridibunda*, provides evidence of polygenic expression in amphibian (Vaudry et al., 1992).

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). More recently, another form was isolated from the brain of the lamprey which was a second form of somatostatin-14 (SS-14) that is identical to mammalian somatostatin-14 and differs from lamprey pancreatic somatostatin-14 by the substitution Ser12 to Thr (Sower et al., 1994). These data support the conclusion that the complete primary structure of 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, 1990b). The isolation of SS-14 from lamprey brain which is identical in amino acid sequence to the peptide from other vertebrates, provides good evidence for the expression of more than one somatostatin gene in an agnathan. SS-14 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 [Tyr6,Gly10]SS-14 at their COOH-terminals leaving the question open to whether the prosomatostatin-II gene is expressed in agnatha. At the present time, however, there is no evidence for the expression of the prosomatostatin II gene in species other than teleost fish.

Earlier immunocytochemical studies of lamprey brain revealed that somatostatin perikarya are present in various brain regions including the ventral hypothalamus, dorsal thalamus, interpeduncular nucleus, and gray area (Nozaki et al., 1984a). In addition, somatostatin fibers were also found in nearly every part of the brain with particular abundance in the preoptic nucleus and ventral hypothalamus. However, in these same studies, there were no somatostatin fibers seen in the neurohypophysis. This would suggest that somatostatin may not act as a neurohormone involved in the regulation of pituitary function, but rather as a brain neurotransmitter and/or neuromodulator.

**SUMMARY**

In this review, agnathans are considered to be paraphyletic in origin with the modern agnathans classified into two groups, myxinoids (hagfish) and petromyzonids (lamprey) (Forey and Janvier, 1993, 1994). Recent paleontological analysis of modern groups have also suggested that the jawed vertebrates are more closely related to lampreys than either is to the hagfishes (Forey and Janvier, 1993). In support of these findings, Suzuki et al. (1995) proposed that the lamprey vasotocin precursor is more closely related to the gnathostome vasotocin and vasopressin precursors than to the hagfish vasotocin precursors. To obtain a better insight into the evolution of lamprey brain/pituitary hormones and their genes, it will be necessary to characterize these genes from the lamprey and hagfish in addition to the protochordates (amphioxus and ascidians) as possible extant relatives of the invertebrate progenitor from which the vertebrates evolved. Interrelationships of the extant and extinct early evolved vertebrates has been the subject of extensive review, particularly in light of more recent information and discoveries of extinct agnathans (reviewed in Pough et al., 1996; Forey and Janvier, 1993). More extensive determination of hormonal and developmental genes may help in resolving the phylogenetic re-
relationships among hagfish, lampreys, and jawed vertebrates.

It has also been proposed that there have been at least two periods of gene duplication during the course of vertebrate evolution, one of which occurred close to the origin of the vertebrates and one later in the early evolved teleosts (Ohno et al., 1968). There are several examples of hormones and developmental genes that supports this proposal, some of which have been discussed in this review. During development, there are a group of genes, called Hox genes, that are implicated in the control of axial patterning during embryonic development of animals (Review of Holland and Garcia-Fernandez, 1996). These authors reviewed the recent data on Hox gene diversity, genomic organization, and embryonic expression in chordates and suggested that comparative Hox gene data may help to resolve some of the outstanding controversies in chordate phylogeny. Based on the current data, Holland and Garcia-Fernandez proposed phylogenetic relationships between chordate taxa and hemichordates and echinoderms (Fig. 7). Similar to the POMC precursor, NPY family, insulin/IGF superfamily and arginine vasotocin precursor, the data on Hox genes suggest that gene duplication occurred close to the origin of the vertebrates. The insulin/IGF superfamily, another very important group of hormones, was not discussed in this review since this review focused on brain and pituitary hormones. In this family, a cDNA encoding an insulin-like peptide (ILP) has been cloned and sequenced from amphioxus, a protochordate that occupies a key position in chordate development (Chan et al., 1990). Chan and colleagues have suggested that ILP may represent an intermediate form linking the IGF genes with an ancestral insulin gene. Based on their detailed findings, these authors have proposed that insulin and IGFs may have evolved from a common ancestral form and that IGF emerged at a very early stage in vertebrate evolution from an ancestral insulin-type gene by gene duplication and divergence. In summary, characterization of brain and pituitary hormones from an extant representative species of one of the oldest lineages of vertebrates is particularly important for understanding the molecular evolution and functional diversity of these hormones and may help in resolving the phylogenetic relationships among hagfish, lampreys, and jawed vertebrates.

ACKNOWLEDGMENTS

This manuscript is dedicated to Professor Aubrey Gorbman who has been an inspiring mentor and example for me in agnathan comparative endocrinology. I would also like to thank him for his critical comments of this manuscript. In addition, I want to acknowledge my good friend and collabo-
rator, Professor Kawamura, and his colleague, Akihoshi Takahashi, who were mainly responsible for the identification of the lamprey pituitary hormones. I also want to thank many of my students and collaborators who were involved in various aspects of this research including Cindy Chase, Janet MacIntyre, Kelly Dergen, Christopher Knox, Lee Gazourian, Olivier Materne, Dr. Erika Pliatskaya, Dr. John H. Youson, Dr. Jean Joss, Dr. Stuart A. Tobet, Dr. Masumi Nozaki and Dr. Michael P. Conlon. In addition, I want to particularly thank ad-hoc reviewer #3 for the very helpful constructive critical comments of this manuscript. This research has been supported by the National Science Foundation and the Great Lakes Fisheries Commission. Scientific Contribution No. 1980 from the New Hampshire Agricultural Experiment Station.

REFERENCES


