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NEUROENDOCRINE SYSTEMS OF THE FISH BRAIN

JOSE MIGUEL CERDÁ-REVERTER
LUIS FABIÁN CANOSA

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The study of the neuronal systems innervating the pituitary is a key point for understanding the regulation of a wide array of vital processes. This chapter offers a study of the neuroendocrine territories in fish. We first describe the anatomy of the main neuroendocrine territories of the teleost brains, making correspondences between the differently proposed nomenclatures. Teleost fish lack a canonical median eminence, and the hypothalamic neurons terminate very close to the adenohypophysial cells or make synaptoid contact upon them. This anatomical characteristic allows the study of the hypothalamo-hypophysial system by retrograde tracing experiments. Tract-tracing techniques have corroborated early studies showing the preoptic area and tuberal hypothalamus as loci for the neuronal cell bodies whose axons reach the neuro- and adenohypophysis along well-defined fiber tracts. We review the different neuronal systems that produce hypothalamic releasing or inhibitory peptides and neurotransmitters, and innervate the pituitary. Finally, the chapter describes the peptidergic innervation of the different pituitary domains and the association with the different secretory cells.

1. INTRODUCTION

The pioneering works of Ernest Scharrer suggested the presence of secretory cells in the brain of teleost fish (Scharrer, 1928). These studies suggested that the glandular nerve cells were responsible for the secretion of hormones in the pars nervosa of the pituitary. Scharrer's ideas, exclusively based on morphological criteria, met with powerful resistance and were not immediately accepted but they did establish a new discipline in neurosciences, neuroendocrinology. Subsequently staining studies identified two areas in the ventral forebrain, the preoptic area and hypothalamus, as loci for the neuronal cell bodies whose axons reach the neurohypophysis along well-defined fiber tracts, the hypophysial and the preoptico-hypophysial tracts (Palay, 1945). These pioneering studies revealed the existence of a hypothalamo-hypophysial system linking the central nervous (CNS) and endocrine systems to regulate an array of vital processes.

The hypothalamo-hypophysial system in fish is divided into three main areas: the hypothalamus, which is part of the diencephalon; the neurohypophysis, which derives from the ventral diencephalon and represents the neural compartment of the pituitary; and the adenohypophysis, which is the non-neuronal part of the gland (Figure 1.1; Pogoda and Hammerschmidt, 2007). The neurohypophysis is made up of the nerve terminals whose cell bodies are found mainly in the preoptic area and cells exhibiting glial-like

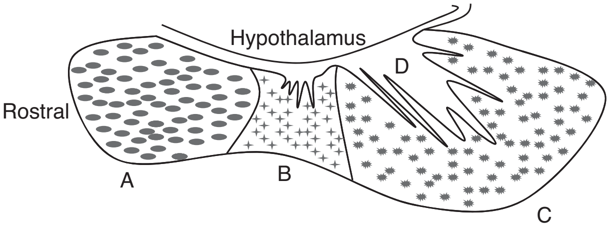

Fig. 1.1. Structure of the fish pituitary. A, Rostral pars distalis or pro-adenohypophysis; B, proximal pars distalis or meso-adenohypophysis; C, pars intermedia or meta-adenohypophysis. Regions A, B, and C are parts of the adenohypophysis. D, Pars nervosa of the neurohypophysis.

properties or pituicytes, which have a supportive function. In some fish species, like plainfin midshipman (*Porichthys notatus*), the neurohypophysis draws away from the brain and forms a stalk of nerve tissue (Sathyanesan, 1965), while in other fish there is no infundibular stalk or the gland is placed at the end of a short infundibular stalk (see Gorbman *et al.*, 1983). In elasmobranchs (van de Kamer and Zandbergen, 1981) and non-teleost bony ray-finned fishes (Lagios, 1968), the neurohypophysis is divided into the median eminence and the pars nervosa. The former includes the portal system, a blood capillary network where the hypothalamic neurons release their secretory products into the vascular system for subsequent delivery to the adenohypophysis. Agnathan species including lampreys and hagfishes have no canonical median eminence but exhibit an anterior neurohemal region through which materials can rapidly diffuse from the brain into the adenohypophysis (neuro-diffuse system, Nozaki *et al.*, 1994). On the contrary, there is no median eminence and no portal system in teleost fish, in which hypothalamic neurons terminate very close to adenohypophysial cells, reducing the diffusional distance, or make synaptoid contact with adenohypophysial cells. This means that the hypothalamic control over the adenohypophysis can be exerted through direct action upon the secretory cells. Figure 1.2 shows the possible adenohypophysial innervation patterns observed in fish. There is some controversy concerning whether or not central neurosecretions indirectly reach the adenohypophysis through the vascular plexus in the neurohypophysis, which would mean the possible existence of a dual mechanism operating by direct (neural) and indirect (vascular) pathways in teleost fish (Hill and Henderson, 1968). However, it is clear that teleost fish do not have a vascular pattern like that of tetrapod species in which a median eminence-like primary plexus leads to a portal vein which subsequently leads to a secondary capillary plexus (Gorbman *et al.*, 1983).

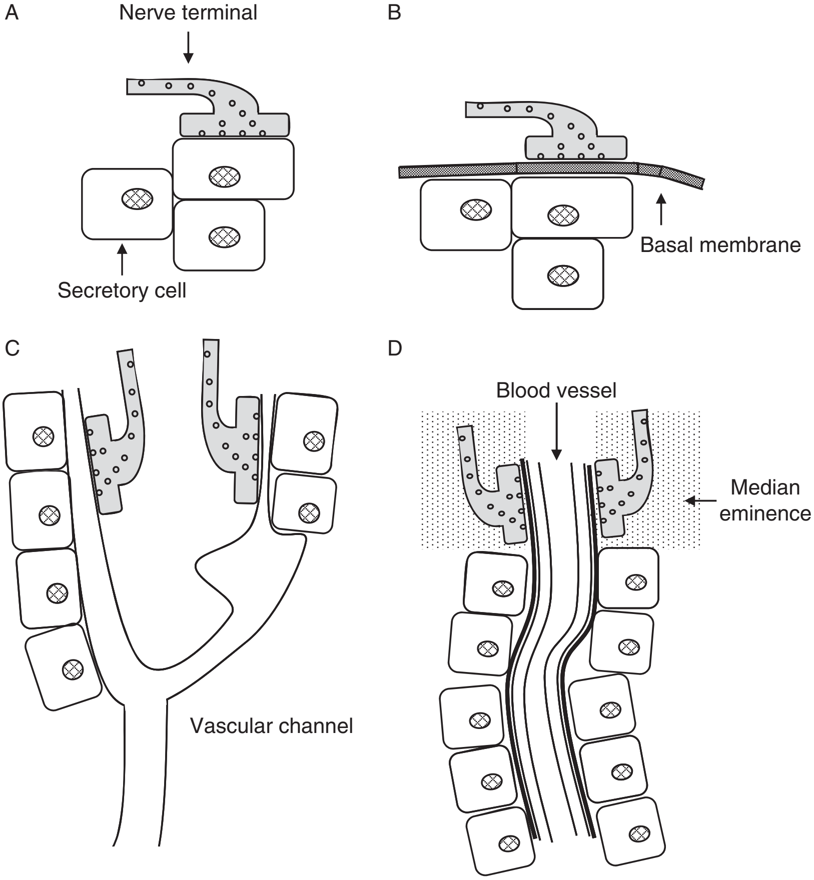

Fig. 1.2. Schematic representation of the possible association types between the central nervous system and the adenohypophysial endocrine cells in teleost fish (A–C) and non-teleost fish (D). (A) Direct synaptic contact between the neurosecretory terminals and endocrine cell in the seahorse (*Hippocampus* sp.). (B) The basement membrane is interposed between the neurosecretory ends and the endocrine cell in the tench (*Tinca* sp.). (C) In the eel, (*Anguilla anguilla*), the neurosecretory terminals lie over the vascular channels among the endocrine cells. (D) Physical separation between the neurosecretory terminals and the adenohypophysial cells. Hypothalamic neurons release their secretory products into the hypothalamic vascular system (median eminence) for subsequent delivery to the adenohypophysis. This later type of innervation is also observed in tetrapod vertebrates (Modified from Vollrath, 1967).

Adenohypophysial cells synthesize their own hormones and remain organized in discrete domains (Pogoda and Hammerschmidt, 2007). In contrast to tetrapod species, fish adenohypophysis lacks a pars tuberis. Two different nomenclatures have been proposed for the regionalization of fish adenohypophysis (Green, 1951; Pickford and Atz, 1957). Both terminologies recognize three distinct zones: the most rostral part, termed the rostral pars distalis (RPD) (Green, 1951) or pro-adenohypophysis (Pickford and Azt, 1957); the remaining pars distalis (PD), termed the proximal pars distalis (PPD) or meso-adenohypophysis; and the pars intermedia (PI) or meta-adenohyopophysis. In contrast to mammalian species, there is no strict morphological separation between the PD and the PI. The pars nervosa (PN) or the posterior part of the neurohypophysis is fully interdigitated with the PI of the adenohypophysis to form the neurointermediate lobe (NIL). The PD also is invaded by tongues of the neurohypophysis. The PN contains nerve terminals from neurons in the preoptic area which color with neurosecretory stains. The anterior part of the neurohypophysis that is interdigitated with the PD is not stained by the above techniques and contains endings of nerve fibers from the lateral tuberal nucleus and some other ventral areas of the forebrain that may be considered as hypophysiotropic areas (Gorbman *et al.*, 1983).

Fish adenohypophysis synthesizes at least eight different hormones (Figure 1.3), which can be divided into three different categories (Pogoda and Hammerschmidt, 2007). Category 1 includes hormones belonging to the growth hormone (GH)/prolactin (PRL)/somatolactin family including the growth hormone (GH) made in the somatotropes, prolactin (PRL) generated by lactotropes, and somatolactin (SL) produced in the somatolactotropes (Kawauchi and Sower, 2006). Category 2 consists of thyroid-stimulating hormone or thyrotropin (TSH), and two gonadotropins (GTH): follicle-stimulating hormone (FSH/GTH-I) and luteinizing hormone (LH/GTHII). All three hormones are heterodimeric glycoproteins, formed by a common α subunit and a hormone-specific β subunit encoded by different genes. While the α subunit gene is commonly expressed in the gonadotropes and thyrotropes, β-subunit gene expression is cell-specific (Querat *et al.*, 2000). Category 3 comprises peptides derived from a common precursor, proopiomelanocortin (POMC). It is comprised of three main domains: N-terminal pro-γ-melanocyte-stimulating hormone (MSH), central adrenocorticotropic hormone (ACTH) and C-terminal β-lipotropin. Each domain contains one MSH peptide, i.e., γ-MSH in pro-γ-MSH, α-MSH as the N-terminal sequence of ACTH and β-MSH in the β-lipotropin domain. The last domain further includes C-terminal β-endorphin peptide. Teleost fish lack γ-MSH domain but retain both α- and β-MSH regions and a β-endorphin deduced peptide. Elasmobranchs have a fourth MSH domain termed δ-MSH (Takahashi *et al.*, 2001). Following a general pattern in teleost fish (Laiz-Carrión *et al.*, 2003), PRL-producing cells (lactotropes) and ACTH-producing cells (corticotropes) are localized in the RPD, with lactotropes ventrally positioned to the corticotropes. Somatotropes (GH-producing cells) and

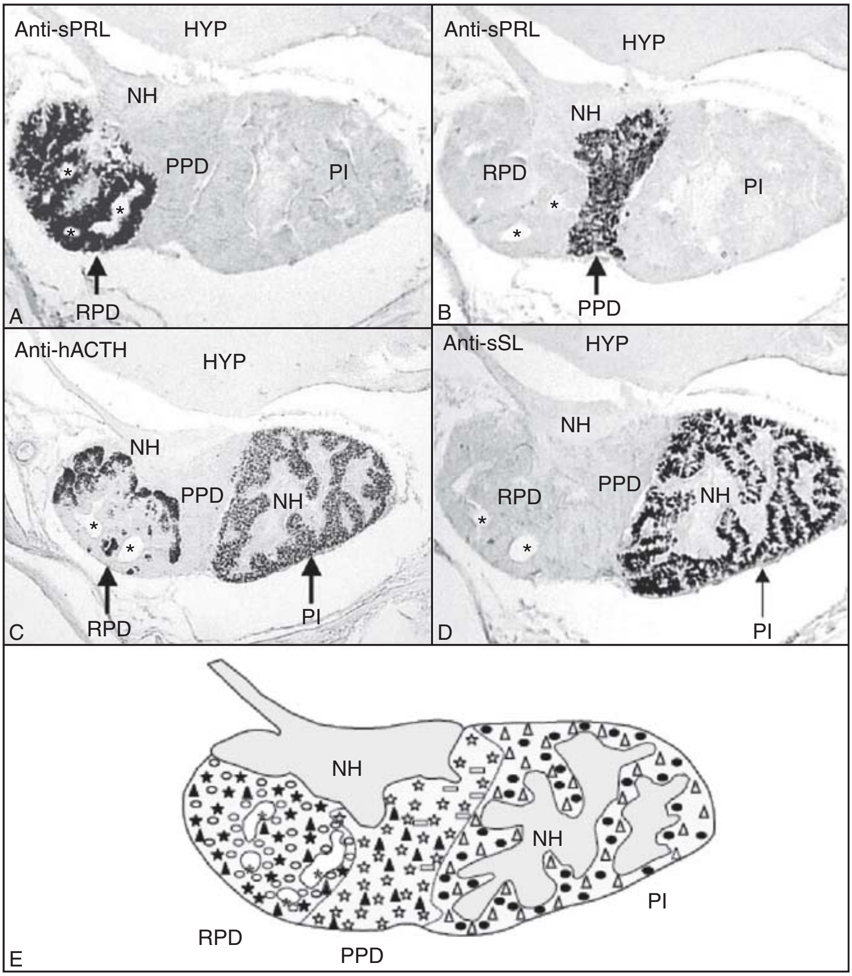

Fig. 1.3. Immunoreactive demonstration of lactotropes. (A) Anti-chum salmon prolactin (sPRL)-immunoreactive (ir) cells), somatotropes; (B) anti-seabream growth hormone (GH)-ir cells, corticotropes; (C) anti-human adrenocorticotropin (ACTH)-ir cells and somatolactotropes; (D) anti-chum salmon somatolactin (SL)-ir cells distribution in the adenohypophysis of the American shad (*Alosa sapidissima*). (E) Schematic sagittal representation of the pituitary showing the distribution of adenohypophysial cells. PRL (★), ACTH (○), GH (☆), GTH (▲), TSH (□), SL (●) and MSH (Δ). Asterisk indicates cavities; HYP, hypothalamus; NH, neurohypophysis; PI, pars intermedia; PPD proximal pars distalis; RPD, rostral pars distalis. Modified from Laiz-Carrión *et al.* (2003).

thyrotropes (TSH-producing cells) are concentrated within the following domain or PPD whereas somatolactotropes (SL producing cells) occupy the caudal-most domain, the PI, where they are intermingled with the melanotropes (α-MSH-cells). Finally, both FSHβ and LHβ are produced in different gonadotropes in the PPD, with some LHβ cells which line the external border of the PI. In the region posterior to the NIL and on the midline of the inferior hypothalamic lobules, most bony and cartilaginous fishes have the saccus vasculosus, a circumventricular organ consisting of highly vascularized neuroepithelium, which is formed by an exclusive cell type termed coronet cells, as well as by neurons contacting the cerebrospinal fluid and supporting cells (Sueiro *et al.*, 2007).

2. CYTOARCHITECTURE OF THE MAIN HYPOPHYSIOTROPIC TERRITORIES

The organization of the CNS can be studied by specific tissue-staining techniques. Nissl's staining, which uses basic aniline dyes, remains the routine technique for the investigation of the cytoarchitecture of the CNS. This technique is an indispensable first step in the analysis of the CNS and one of the most powerful tools in neuroanatomy. This method stains RNA blue, revealing the localization of the so-called "Nissl's bodies," which are granular basophilic bodies found in the cytoplasm of neurons and composed of rough endoplasmic reticulum and free polyribosomes. The staining shows exclusively neuronal perikarya while dendrites, axons and terminals are not stained. It allows clusters of neuronal somata called "nuclei" to be delimited. The nuclei can be identified according to several morphological criteria including size, shape and staining intensity of the perikarya, packing density and distribution pattern of the cell bodies, neuropil surrounding cell groups and the consistency of cell groups. In this section, we will describe the Nissl cytoarchitecture of the main hypophysiotropic territories in the fish brain. The description will focus briefly on the morphology of the teleost forebrain that includes the telencephalon and diencephalon (Figure 1.4). We use the nomenclature of Braford and Northcutt (1983) with additional elaborations by Cerdá-Reverter (2001a,b). For a more extensive comparative study, readers should consult Braford and Northcutt (1983), Nieuwenhuys *et al.* (1998) and Butler and Hodos (2005).

2.1. Telencephalon

In teleost fish, the topology of the telencephalon is highly distorted. During the initial steps of development in most vertebrates (lampreys, cartilaginous fish, amphibians and amniotes) the telencephalon develops through a process called evagination. During this process, the lumen of the neural tube enlarges to form the telencephalic ventricles as the telencephalon

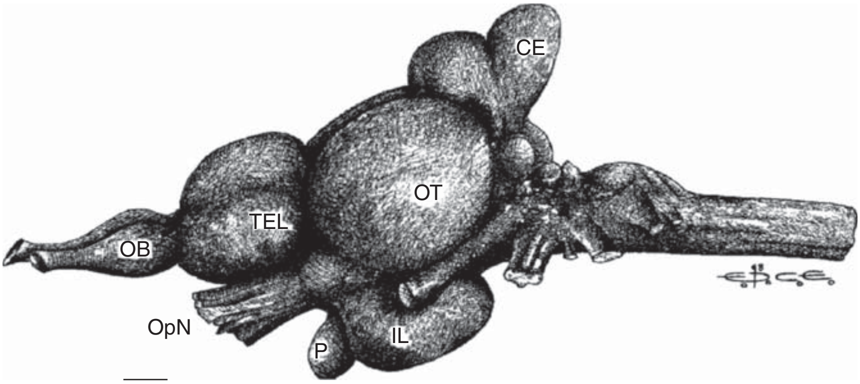

Fig. 1.4. Lateral view of the brain of the sea bass (*Dicentrarchus labrax*). CE, cerebellum; IL, inferior lobe of the hypothalamus; OB, olfactory bulb; OpN, optic nerve; OT, optic tectum; P, pituitary; TEL, telencephalon. Scale bar = 1 mm. (Modified from Cerdá-Reverter *et al.*, 2000a).

protrudes and expands. In contrast, the telencephalon of ray-finned fish undergoes a process of eversion in which the roof of the neural tube extends laterally so that the paired dorsal parts roll out lateroventrally. As a result of eversion, the telencephalon is covered by thin tela (Figure 1.5; Northcutt, 1995). This developmental difference makes it difficult to infer the homology between the structures of the telencephala of ray-finned fish and other vertebrate species, and the independent nomenclature has been proposed (Northcutt and Davis, 1983; Meek and Nieuwenhuys, 1998). Recently, however, some potential homologies have been established using neuronal circuitry patterns and distribution of chemical transmitters and peptides (Wullimann and Mueller, 2004).

The telencephalon comprises telencephalic hemispheres and olfactory bulbs (Figure 1.4). The hemispheres can be divided into two main regions: an area dorsalis telencephali (pallium) and an area ventralis telencephali (subpallium). The area dorsalis is the everted part of the telencephalon and exhibits a number of large histologically distinct zones where most of the cells lie far from the ventricle surface. In contrast, the area ventralis telencephali is organized into nuclei, most of which are in proximity to the ventricular area (Wullimann and Mueller, 2004).

The dorsal area or pallium represents the everted part and largest region of the teleost telencephalon. Using cytoarchitectonic criteria it can be divided into three periventricular, externally placed, zones – the pars medialis (Dm), pars dorsalis (Dd) and pars lateralis (Dl) – one central area, the pars centralis (Dc), and a posterior area, the pars posterior (Dp). Neurons in the dorsal telencephalon do not project to the pituitary. The ventral area is the

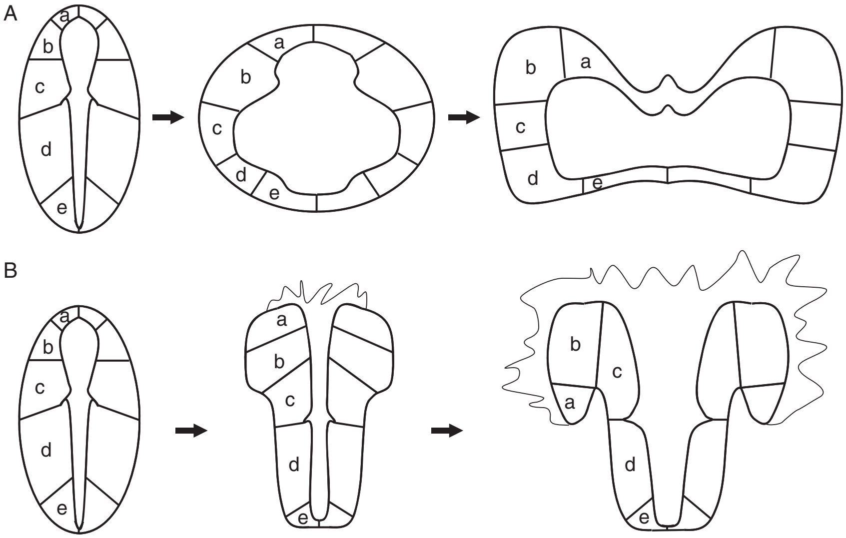

Fig. 1.5. (A) Process of evagination in the forebrain of most vertebrates during early development. (B) Process of eversion in the forebrain of ray-finned fish. Comparison of the position of lower case letters makes for easy comparisons between evagination and eversion processes. Modified from Butler and Hodos, 2005.

non-everted part of the teleost telencephalon placed rostral to the preoptic area. This area is split into precommissural and postcommissural nuclei, where the anterior commissure is the inflexion point. Dorsal (Vd), ventral (Vv) and lateral (Vl) nuclei are the main precommissural nuclei, whereas central (Vc), supracommissural (Vs), postcommissural (Vp), intermediate (Vi) and entopeduncular nuclei (E) are found in postcommissural position (Figure 1.6). From the neuroendocrine perspective, both the Vv and Vc nuclei have been reported to project to the pituitary in several teleost fish (see later). In some species such as sea bass (*Dicentrarchus labrax*; Cerdá-Reverter *et al.*, 2001a), the Vv is characterized by the presence of an ependymal column of small, densely stained packed cells lining the ventricle and associated with the dorsal-most aspect of the nucleus. The central nucleus (Vc) contains large and intensely stained perikarya intermingled with the fibers of the lateral forebrain bundle (LFB). Neuronal circuitry patterns and distribution of chemical transmitters and peptides suggest that the Vd and Vc nuclei of the area ventralis telencephali represent the mammalian striatal formation, whereas the Vl and Vv nuclei correspond to the septal formation. One of the arguments used to defend the homology between the Vl-Vv and the septal formation is the massive descending output to the midline hypothalamus, which is also a characteristic of the septal formation in amniotes (Wullimann and Mueller, 2004).

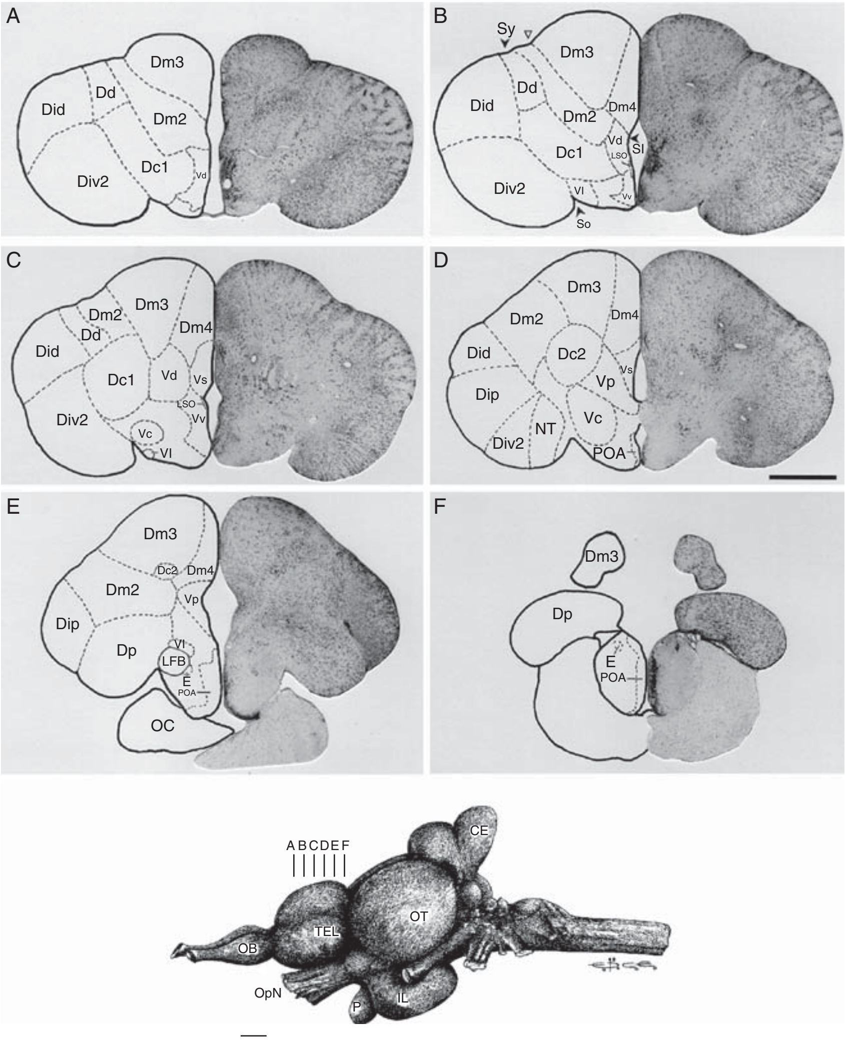

Fig. 1.6. Photomicrographs of cross-sections of sea bass (*Dicentrarchus labrax*) brain stained with cresyl violet showing different tencephalic cell masses. Dc1, dorsal telencephalic area, central part, subdivision 1; Dc2, dorsal telencephalic area, central part, subdivision 2; Dd, dorsal telencephalic area, dorsal part,; Dld, dorsal telencephalic area, lateral part,; Dlp, dorsal telencephalic area, lateral posterior part; Dlv1, dorsal telencephalic area, lateral ventral, subdivision 1; Dlv2, dorsal telencephalic area, lateral ventral part, subdivision 2; Dm2, dorsal telencephalic area, medial part, subdivision 2; Dm3, dorsal telencephalic area, medial part, subdivision 3; Dm4, dorsal telencephalic area, medial part, subdivision 4; Dp, dorsal telencephalic area, posterior part; E, entopeduncular nucleus; LSO, lateral septal organ; NT, nucleus taenia; OB, olfactory bulb; OC, optic chiasma; OpN, optic nerve; POA, preoptic area; Sy, sulcus ypsiliformis; Vc, ventral telencephalic

2.2. Preoptic Area

The preoptic area forms a structural and functional continuum with the hypothalamus. It is convenient to treat these two entities as forming a single complex and to allocate this preoptic–hypothalamic continuous to the diencephalon (Nieuwenhuys *et al.*, 1998). Endocrinologists often include the preoptic area within the hypothalamus when discussing the hypophysiotropic control of pituitary activity.

The preoptic region surrounds the preoptic periventricular recess and is located between the anterior commissure and the optic chiasm. Following Braford and Northcutt (1983) and Meek and Nieuwenhuys (1998), the preoptic area can be divided into a magnocellular (PM) and parvocellular preoptic (PP) nucleus (Figure 1.7). The magnocellular preoptic nucleus contains large neurosecretory cells and it is subdivided into the parvocellular (PMpc), magnocellular (PMmc) and gigantocellular (PMgc) parts. This nucleus is shaped, in longitudinal plane, like an inverted L with the vertical rod located rostrally and the horizontal rod extending dorsocaudally. The smaller rostroventral cells constitute the parvocellular parts of the PM and the larger dorsocaudal cells constitute the magno- and gigantocellular parts. In turn, the parvocellular preoptic nucleus can be further subdivided into anterior (PPa) and posterior pars (PPp), which are located anterior and caudoventral to the magnocellular preoptic nucleus. According to Peter's nomenclature for goldfish (*Carassius auratus*), killifish (*Fundulus heteroclitus*) and two salmonid species (Peter *et al.*, 1975; Peter and Gill, 1975; Billard and Peter, 1982; Peter *et al.*, 1991), the PPa is called the preoptic parvocellular nucleus (NPP), the magnocellular division is termed the preoptic nucleus (NPO) and the PPp is termed the periventricular nucleus and is subdivided into the anterior (NAPv) and posterior (NPPv) periventricular nucleus. However, the caudal region of the NPPv corresponds to the zona limitans diencephali (ZL) of Braford and Northcutt (1983). According to Cerdá-Reverter's nomenclature for the sea bass, the NPP (Peter and Gill, 1975) or PPa (Braford and Northcutt, 1983) is termed the parvocellular preoptic nucleus and it is subdivided into the anteroventral (NPOav) and parvocellular pars (NPOpc). The NPOav is termed the supraoptic division of the preoptic nucleus (NPOs) in the catfish (*Clarias batrachus*; Prasada Rao *et al.*, 1993). According to Maler's nomenclature for brown ghost knifefish (*Apteronotus leptorhynchus*; Maler *et al.*, 1991) the PM (Braford and

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area, central part; Vd, ventral telencephalic area, dorsal part; Vi, ventral telencephalic area, intermedial part; Vl, ventral telencephalic area, lateral part; Vp, ventral telencephalic area, postcommissural part; Vs, ventral telencephalic area, supracommissuralis part; Vv, ventral telencephalic area, ventral part. Scale bar = 100 μm. From Cerdá-Reverter *et al.* (2001b).

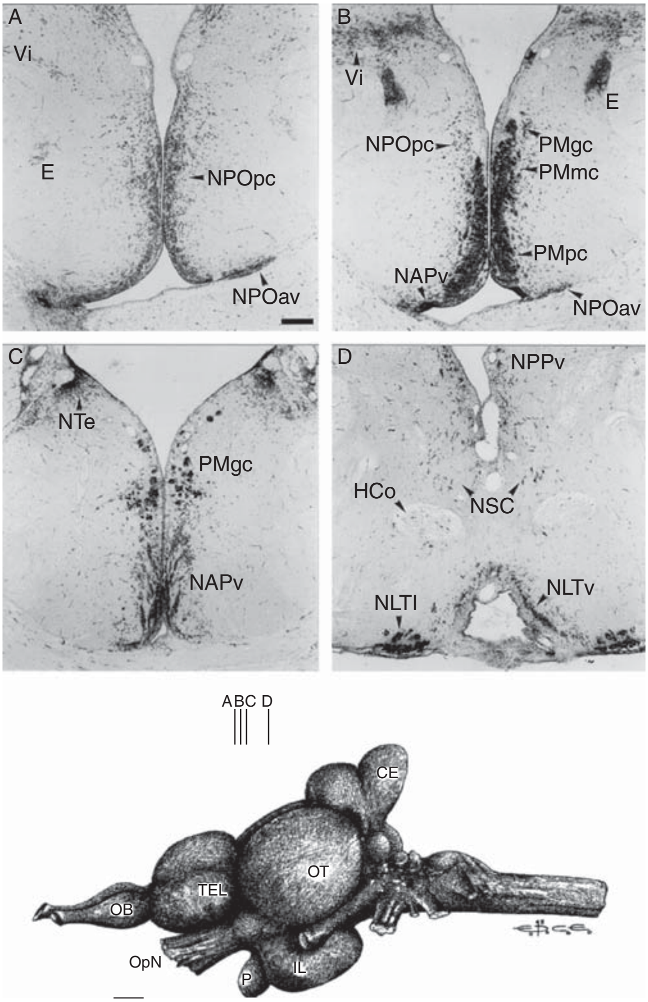

Fig. 1.7. Photomicrographs of cross-sections of sea bass (*Dicentrarchus labrax*) brain stained with cresyl violet showing different preoptic cell masses. E, entopeduncular nucleus; HCo, horizontal commissure; NAPv, anterior periventricular nucleus; NLTl, lateral part of the lateral tuberal nucleus; NLTv, ventral part of the lateral tuberal nucleus; NPOav, anteroventral part of the parvocellular preoptic nucleus; NPOpc, parvocellular part of the parvocellular preoptic nucleus; NPPv, posterior periventricular nucleus; NSC, suprachiasmatic nucleus; NTe, nucleus of the thalamic eminentia; PMgc, gigantocellular part of the magnocellular preoptic nucleus; PMmc, magnocellular part of the magnocellular preoptic nucleus; PMpc, parvocellular part of the magnocellular preoptic nucleus; Vi, intermediate nucleus of the ventral telencephalon. Scale bar = 100 μm. From Cerdá-Reverter *et al.* (2001b).

Northcutt, 1983) has been referred to as anterior hypothalamic nucleus (Ha) and the PPp (Braford and Northcutt, 1983) is subdivided into the proper PPp and the anterior periventricular nucleus (nAPv). Finally, all the nomenclatures include the suprachiasmatic nucleus (NSC) in the most caudoventral region of the preoptic area although in some species it is not recognized as a separate nucleus (Gómez-Segade and Anadón, 1988).

2.3. Hypothalamus

As the name indicates, the hypothalamus is located below the thalamus, caudal to the preoptic area. It appears abruptly caudal to the chiasmatic ridge and is easily distinguished from the preoptic area by its densely packed and stained cells surrounding the nascent infundibular recess (Figure 1.8). The hypothalamus is the largest diencephalic area and is connected to the pituitary via the pituitary stalk, which contains hypothalamic and preoptic neuroendocrine fibers (see Section 1). In several teleost species, the infundibular recess or hypothalamic portion of the third ventricle is laterally expanded to form caudally directed paired diverticula termed the lateral recess. In the caudal area, the third ventricle gives rise to the posterior recess, which also has small dorsolaterally directed diverticula (Figure 1.8; Braford and Northcutt, 1983). Cytoarchitectonic studies of the teleostean hypothalamus point to considerable variation (Peter *et al.*, 1975; Braford and Northcutt, 1983; Gómez-Segade and Anadón, 1988; Striedter, 1990; Maler *et al.*, 1991; Wullimann *et al.*, 1996; Cerdá-Reverter *et al.*, 2001b), however it is uncertain whether the same subdivisions in different teleost species are comparable or whether subdivisions merely reflect different criteria for subdivision (Meek and Nieuwenhuys, 1998).

According to Meek and Nieuwenhuys (1998), the teleostean hypothalamus can be divided into three main regions, i.e., the periventricular region, the medially located tuberal region and the paired inferior lobes, which are separated from the tuberal hypothalamus by a deep ventral sulcus. However, other authors subdivide the hypothalamus into dorsal (Hd), ventral (Hv) and caudal (Hc) regions (Braford and Northcutt, 1983; Striedter, 1990; Wullimann *et al.*, 1996). Ventral and caudal zones constitute most of the median tuberal portion of the hypothalamus. All three subdivisions display periventricular cell populations bordered by laterally migrated nuclei. According to Braford and Northcutt (1983), the rostral area of the hypothalamus is occupied by two periventricular cell populations, the dorsal (Hd) and ventral (Hv) periventricular hypothalamus, and two laterally migrated populations, the anterior tuberal nucleus (NAT) and the lateral hypothalamic nucleus (LH). The border between the two periventricular subdivisions is very diffuse. The ventral aspect of the third ventricle is bound by cells of the

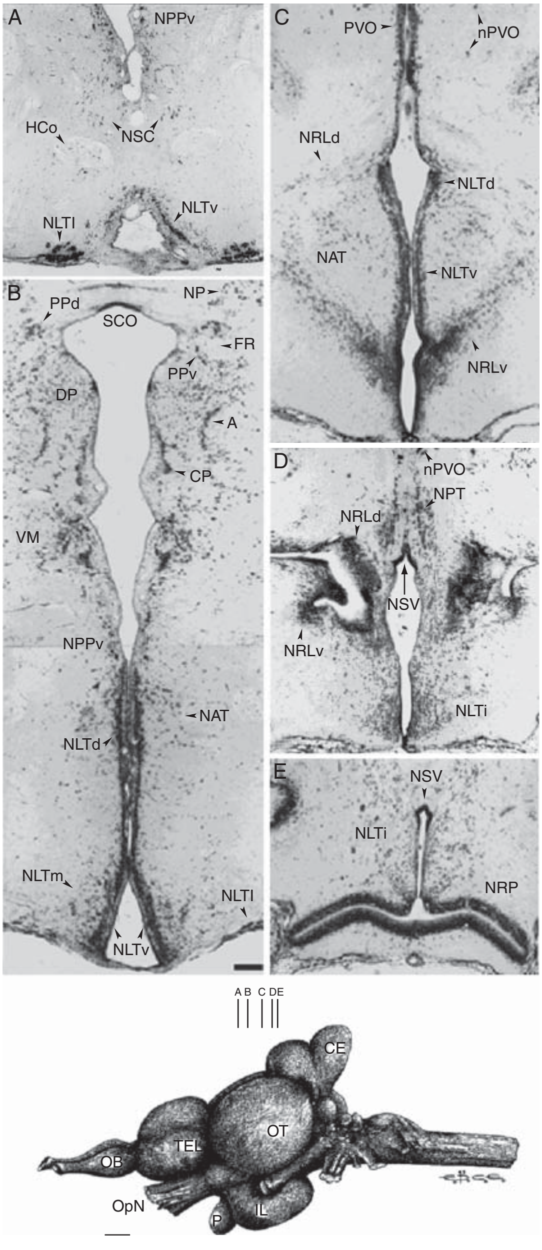

Fig. 1.8. Photomicrographs of cross-sections of sea bass (*Dicentrarchus labrax*) brain stained with cresyl violet showing different diencephalic cell masses. A, Anterior thalamic nucleus; CP, central posterior thalamic nucleus; DP, dorsal posterior thalamic nucleus; FR, fasciculus

Hv, which are densely packed along the ventricular wall, forming a 1–10 cell-thick lamina. Cells of the Hd are slightly bigger, less densely packed and form a 1–4 cell-thick layer (Cerdá-Reverter *et al.*, 2001b). The anterior tuberal nucleus (NAT) is formed by scattered and large fusiform cells located lateral to the Hd. All nomenclatures are coincident with the designation of a NAT but its location is variable (Peter and Gill, 1975; Braford and Northcutt, 1983; Gómez-Segade and Anadón, 1988; Striedter, 1990; Maler *et al.*, 1991; Wullimann *et al.*, 1996). Some authors consider that the NAT extends to the periventricular wall (Peter and Gill, 1975; Striedter, 1990; Maler *et al.*, 1991), while others further subdivide the nucleus into rostral and ventral regions (Striedter, 1990). In the zebrafish (*Danio rerio*) brain atlas, Wullimann *et al.* (1996) considered that the Hv extends farther rostrally than the Hd and therefore the rostral part of the Hd is designated Hv. Consequently, the NAT lies laterally to the Hv.

The lateral aperture of the third ventricle delimits the rostrocaudal level where the Hd emerges. Just before the lateral opening of the hypothalamic ventricle, the small cells of the LH extend dorsolaterally. This nucleus is located lateral to the caudal region of the Hv and its cells seem to coat the ventral aspect of the lateral recess (LR). In fact, this lateral aperture of the third ventricle could be considered as a transitional zone leading to the Hc. At this level, the cells of the Hd coat the LR, whereas the dorsal area of the third ventricle is massively occupied by the cells of the posterior tuberal nucleus (NPT). Slightly caudal and approximately coinciding with the complete separation of the LR from the medial hypothalamic ventricle, the Hc emerges.This is a small area bordering the caudal aspect of the posterior recess dorsally limited by the NPT. The Hc arises as a thickening of the ependymal cell layer and more caudally the nucleus expands laterally to adopt a columnar disposition along the laterally directed diverticula of the posterior recess. The Hc contains a small cell population termed the nucleus of the saccus vasculosus (NSV) as well as the posterior recess nucleus (NRP) of Peter and Gill (1975).

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retroflexus; NAT, anterior tuberal nucleus; NLTd, dorsal part of the lateral tuberal nucleus; NLTi, inferior part of the lateral tuberal nucleus; NLTm, medial part of the lateral tuberal nucleus; NLTl, lateral part of the lateral tuberal nucleus; NLTv, ventral part of the lateral tuberal nucleus; NP, paracommissural nucleus; NPPv, posterior periventricular nucleus; NPT, posterior tuberal nucleus; nPVO, nucleus of the paraventricular organ; NRLd, dorsal part of the nucleus of the lateral recess; NRLv, ventral part of the nucleus of the lateral recess; NRP, nucleus of the posterior recess; NSC, suprachiasmatic nucleus; NSV, nucleus of the saccus vasculosus; PPd, dorsal periventricular pretectal nucleus; PPv, ventral periventricular pretectal nucleus; PVO, paraventricular organ; SCO, subcommissural organ; VM, ventromedial thalamic nucleus. Scale bar = 100 μm. From Cerdá-Reverter *et al.* (2001b).

Following the nomenclature of Cerdá-Reverter *et al.* (2001b), which is partially based on Peter and Gill (1975), the rostral periventricular region is collectively termed the lateral tuberal nucleus (NLT). In the transversal plane, this nucleus presents two differentiable subdivisions, i.e., dorsal (NLTd) and ventral (NLTv), which correspond to the rostral region of the Hd and Hv of Braford and Northcutt (1983), respectively. Within this rostral hypothalamic area, two additional laterally migrated populations, the lateral (NLTl) and medial (NLTm) part of the NLT, have been described in the sea bass (Cerdá-Reverter *et al.*, 2001b). Cells of the NLTl are the first cells of the hypothalamus to appear in a cross-section. In the sea bass, the NLTl consists of very large and darkly stained cells lying ventrolateral to the horizontal commissure and lateral to the incipient infundibular recess (Cerdá-Reverter *et al.*, 2001b). Caudally, these neurons are smaller and located on the ventrolateral surface of the brain. The large neurons of the rostral portion were also recognized by Braford and Northcutt (1983) and designated ventral tuberal nucleus (TV), whereas the smaller caudal neurons were named LH. Neurons of the NLTl are chemically differentiable because they synthesize melanin-concentrating hormone (MCH), a neurohormone of the teleost brain (Baker and Bird, 2002). The NLTm exhibits scattered cells located between the ventral and lateral parts of the NLT. This subdivision was previously reported in the African cichlid fish (*Haplochromis burtoni*; Fernald and Shelton, 1985). The NLTm of the sea bass corresponds partially to the anterior and posterior parts of the NLT of Peter and Gill (1975) and to the ventral hypothalamus of Braford and Northcutt (1983).

Two cell masses that will later surround the LR, i.e., the dorsal and ventral parts of the lateral recess nucleus (NRLd and NRLv, respectively), are visible immediately before the lateral opening of the ventricle. The NRLv is located ventral to the NLTv and both nuclei coexist as far as the ventral periventricular zone is caudally occupied by both the nucleus of the posterior recess and the laterally placed inferior part of the lateral tuberal nucleus (NLTi). The NRLv corresponds to the LH of Braford and Northcutt (1983), Striedter (1990) and Wullimann *et al.* (1996) and also contains the ventricular cells of the caudal Hv. Cells of the NRLd and the paraventricular organ (PVO) presumably migrate laterally, coating the dorsal aspect of the nascent lateral recess whereas the ventral aspect is coated by cells of the NRLv. According to Braford and Northcutt (1983), the NRLd matches the caudal region of Hd.

The inferior lobes are by far the largest part of the teleost hypothalamus and are clearly visible on the ventral surface of the brain. Cells, presumably migrated from the dorsal periventricular populations, "fill up" the inferior lobes and thus these could be considered as a part of the Hd which expands laterally (Wullimann *et al.*, 1996). According to Braford and Northcutt (1983) and Wullimann *et al.* (1996), three populations can clearly be distinguished: the Hd, the diffuse nucleus of the inferior lobe (NDLI) and the central nucleus of the inferior lobe (NCLI). The Hd coats the LR which occupies a central position within the inferior lobe. The NDLI is comprised of very small, lightly stained scattered cells, whereas the NCLI arises more caudally in a ventromedial position to the lateral torus which is made up of large fusiform and ovoid cells. In the sea bass, the NDLI is further subdivided into medial (NDLIm) and lateral (NDLIl) parts (Cerdá-Reverter *et al.*, 2001b), whereas the population coating the LR is called lateral part of the lateral recess nucleus (NRLl). In addition, based on cytoarchitectonic criteria, we described the medial nucleus of the inferior lobe (NMLI), located in the vicinity of the NRLl, ventromedial to the nucleus glomerulosus and lateral to the corpus mamillare. This nucleus was later confirmed by study of neuronal circuitry in other teleosts (Ahrens and Wullimann, 2002).

3. HYPOPHYSIOTROPIC TERRITORIES IN THE FISH BRAIN: TRACT-TRACING STUDIES

The lack of median eminence and portal system in teleost fish has facilitated the study of hypophysiotropic neurons in this vertebrate group using tract-tracing techniques. These studies are based on the retrograde transport of marker substances, mainly lectins or dextran amines. Carbocyanine fluorescent dyes such as DiI (1-1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanin) offer several advantages over other techniques such as autoradiography, horseradish peroxidase (HRP) or cobalt tracing. These advantages include the possibility of using the carbocyanine tracers in paraformaldehyde-fixed tissue, and their compatibility with other fluorescent tracers as well as with immunohistochemical techniques (Holmqvist *et al.*, 1992). The technique is based on the implantation of small crystals of dye into the structure by using insect pins or glass electrodes and subsequent sectioning after incubation. Using tracing techniques, the hypophysiotropic neurons of the catfish (*Clarias batrachus*; Rama Krishna and Subhedar, 1989; Prasada Rao *et al.*, 1993), goldfish (Fryer and Maler, 1981; Anglade *et al.*, 1993), brown ghost knifefish (Johnston and Maler, 1992; Zupanc *et al.*, 1999; Corrêa, 2004) and Atlantic salmon (*Salmo salar*; Holmqvist *et al.*, 1992; Holmqvist and Ekström, 1995) have been studied. As mentioned above (see Section 1), tract-tracing studies have revealed two main areas where the neuronal bodies project toward the pituitary: the preoptic area and the tuberal hypothalamus. However, the alternative nomenclatures used to describe the different nuclei of fish brain sometimes make it difficult to compare species. Table 1.1 summarizes the distribution of the hypophysiotropic structures revealed by tract-tracing techniques in teleost fish.

Table 1.1

Main hypophysiotropic territories of the teleost brain

	Apteronotus leptorhynchus	*Salmo salar*	*Carassius auratus*	*Clarias batrachus*	Bradford and Northcutt (1983)
Technique	DiI Dextran Biocytin	DiI	HRP DiI	HRP Cobaltous-lysine	
Telencephalon					
Olfactory bulb (OB)			X		OB
Ventral part of the ventral telencephalon (Vv)	X	X	X		Vv
Supracommissural part of the ventral telencephalon (Vs)			X		Vs
Central part of the ventral telencephalon (Vc)		X	X		Vc
Preoptic area					
Periventricular preoptic nucleus, anterior part (PPa)	X	X			PPa
Periventricular preoptic nucleus, posterior part (PPp)	X	X			PPp
Suprachiasmatic nucleus (NSC)	X	X	X		NSC
Periventricular preoptic nucleus (NPP)			X		PPa
Preoptic nucleus, supraoptic division (NPOs or SO)				X	PPa
Preoptic nucleus paraventricular division (NPOp or PV)				X	PM
Preoptic nucleus parvocellular part (NPOpc)		X			PMpc
			X		PPa
Preoptic nucleus magnocellular part (NPOmc)		X			PMmc
			X		PMmc-PMgc
Preoptic nucleus gigantocellular part (NPOgc)		X			PMgc
Anterior periventricular nucleus (NAPv)		X	X		PPp
Posterior periventricular nucleus (NPPv)			X		PPp
Anterior hypothalamus (Ha)	X				PM
Tuberal hypothalamus					
Dorsal hypothalamus (Hd)	X				Hd
Ventral hypothalamus (Hv)	X				Hv
Caudal hypothalamus (Hc)	X				Hc

Lateral hypothalamus (Hl)	X				LH, partially
Anterior tuberal nucleus (NAT)	X		X		NAT
Lateral tuberal nucleus (NLT)		X		X	Hd/Hv/LH
Lateral tuberal nucleus, anterior part (NLTa)	X				Hv and Hc
Lateral tuberal nucleus, posterior part (NLTp)	X				Hc
Lateral tuberal nucleus, lateral part (NLTl)			X		LH
Lateral tuberal nucleus, anterior part (NLTa)			X		Hv
Lateral tuberal nucleus, posterior part (NLTp)			X		Hd/Hv
Lateral tuberal nucleus, inferior part (NLTi)			X		Hv
Lateral recess nucleus (NRL)		X	X	X	Hd
Posterior recess nucleus (NRP)		X	X	X	Hc
Nucleus of the saccus vasculosus (NSV)		X			PTN
Central nucleus of the inferior lobe (NCLI)	X				NCLI
Posterior tubercle					
Paraventricular organ (PVO)		X		X	PVO
Thalamus					
Central posterior nucleus (CP)	X				CP
Dorsolateral thalamic nucleus (DL)			X		Vm/DP/I/A
Reticular formation	X				R

In the telencephalon several studies have demonstrated the presence of retrograde labeled cells and fibers have been consistently found within the ventral (Vv) and central areas (Vc) of several teleost species. In addition, some hypophysiotropic cells were also placed within the olfactory bulbs and supracommissural part of the ventral telencephalon (Vs) of the goldfish.

The preoptic area is the major field innervating the teleost pituitary. Studies in several species have demonstrated that all three anterior (PPa), posterior (PPp) periventricular and suprachiasmatic nuclei (NSC) project towards the pituitary (see Table 1.1). In addition, retrograde cell bodies were found in the all three subdivisions of the preoptic nucleus, i.e., NPOpc, NPOgc and NPOmc. Studies in Atlantic salmon also reported DiI-labeled cells in the most rostral preoptic region, which were similar in size and morphology to those of the NPOgc but clearly separated from the main cell masses of the NPO. They suggested naming this cell group the rostral preoptic area (NPOr).

The tuberal hypothalamus is the second major area innervating the teleost pituitary. Studies in several species have reported consistently the presence of labeled cells of the tuberal hypothalamus within the dorsal, ventral lateral and caudal hypothalamus. The presence of hypophysiotropic cells within the anterior tuberal nucleus (NAT) seems to be species dependent (see Table 1.1) and only one study has reported the presence of retrogradely labeled cells in the inferior lobe (Corrêa, 2004). In addition, studies in goldfish using DiI showed labeled cells within the ventral tuberal nucleus (TV).

4. CENTRAL NEUROHORMONES

4.1. Arginine-Vasotocin (AVT)

Arginine-vasotocin (AVT) peptide belongs to a family of neuro-nonapeptides that includes isotocin (IST) as well as the mammalian homologues, arginine-vasopressin (AVP) and oxytocin (Acher, 1996). AVT has a key role in the endocrine control of the salt/water homeostasis and vascular function (McCormick and Bradshaw, 2006) as well as in a wide range of physiological processes (Balment *et al.*, 2006) and social behavior (Goodson and Bass, 2001). AVT is one of the main neurohypophysial hormones in lower vertebrates; it is synthesized by preoptic neurons, travels through the preoptico-hypophysial tract and is released into the vascular system via neurohypophysial axon terminals. Studies in several teleost fish have consistently demonstrated that AVT-immunoreactive (ir) neurons are exclusively localized in the parvocellular and magnocellular neurons (see Table 1.2) of

Table 1.2
Main hypophysiotropic territories containing neuropeptides

Peptide	Telencephalon	Preoptic area	Hypothalamus
AgRP			Hv
AVT	–	PPa, PPp PMmc ^{1}	–
CART	Ventral telencephalon	PMmc	Hv, Hc, NCLI
CCK	Ventral telencephalon	PPa, PMpc PMmc	Hv, Hc, Hd, PVO
CRF	OB, ventral telencephalon	PPa, PPp, PM, NSC	Hv, Hc
Galanin	OB, Vs	PPa, PM	Hv, LH, Hc
GHRH	–	PPa, PPp, PMpc, PMmc	Hv, LH, DF(NDLI)
GnRH	OB, Vv	PPa	LH
GRP	Ventral TEL	PPa, PM, PPp	Hv, NAT, Hd, PVO
IST	–	PPa, PMmc	–
MCH	–	–	LH, PVO, Hv
NPY	OB, Vd, Vv, Vs, Vl, Vc	PPa, PPp PMgc,	Hv, LH, NAT, Hc
Orexin	–	PMmc, PMgc, PPp, NSC	PVO, Hc
PACAP	–	PPa, PPp, PMgc	Hv,
RF-amide	OB	-	Hv (PrRP)
SS	OB, Vl, NE	PPa, PMpc, PMmc, PPp, NSC	Hv, Hd, Hc PVO
TRH	OB, Ventral TEL,	PPa, PMmc, PPp, NSC	Hv, NAT, NPT, Hc
UI	Vv, Vd, Vp,	PPa, PPp	Hv, Hc
α–MSH	–	Hv, LH	

Nomenclature of nuclei was adapted following Bradford and Northcutt (1983).

See Table 1.1 for abbreviations of hypophysiotropic territories.

AgRP, agouti-related peptide; AVT, arginine-vasotocin; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CRF, corticotropin-releasing factor; GHRH, growth hormone releasing hormone; GnRH, gonadotropin-releasing hormone; GRP, gastrin-releasing peptide; IST, isotocin; MCH, melanin-concentrating hormone; MSH, melanin-stimulating hormone; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating polypeptide; SS, somatostatin; TRH, thyrotropin-releasing hormone; UI, urotensin I.

the preoptic nucleus (Goossens *et al.*, 1977; Batten *et al.*, 1990a; Duarte *et al.*, 2001; Lema and Nevitt, 2004; Saito *et al.*, 2004; Mukuda *et al.*, 2005; Bond *et al.*, 2007; Maruska *et al.*, 2007). However, immunostaining studies in plainfin midshipman showed an additional AVT population in the ventral hypothalamus. This second neuronal group is composed of a few small and round AVT-ir neurons often embedded in the preoptico-hypophysial tract (Goodson and Bass, 2001). In this species, most of the AVT neurons are placed in the PPa and only some weakly labeled cells are localized in the PPp. Within the PM, most AVT cells are placed in the magnocellular subdivision and these neurons are more intensely labeled than those in the PPa and PPp. Such a distribution of AVT-ir cells in the preoptic area is conserved in most teleost species studied although some divergences have been reported. Studies in valley pupfish (*Cyprinodon nevadensis*) reported a similar pattern but no AVT-ir cells were found in the PPp whereas peptide-ir in the PM was found in all three subdivisions, i.e., PMpc, PMmc and PMgc (Lema and Nevitt, 2004). Similar results were reported in rainbow trout (*Oncorhynchus mykiss*) where AVT neurons occupy almost 50% of neurosecretory cells in the gigantocellular part of the PM but only 15% in the PPa. Neuronal tract tracing combined with AVT immunohistochemical studies in Atlantic salmon showed that most DiI-labelled neurons in the PM are AVT-ir. However, not all AVT-ir neurons are hypophysiotropic since some cell bodies, especially in the PMgc, were not DiI labeled. The AVT neurons in the PPa did not show retrograde labeling and therefore were suggested to be AVT-ir non-hypophysiotropic preoptic neurons. This study reported hypothalamic and extrahypothalamic projections of the preoptic neurosecretory AVT-ir cells (Holmqvist and Ekström, 1995) as also reported in the plainfin midshipman (Goodson and Bass, 2001). Detailed work in rainbow trout using single-cell staining techniques supported these studies and further showed that in all studied cases, AVT-ir neurons simultaneously project toward the pituitary and the extrahypothalamic regions, including the ventral telencephalon, thalamus and mesencephalon. Multiple projections of single neurosecretory cells are not known in other vertebrates. These projections are suitable for the coordinated control of peripheral and central outputs through the synchronization of the electrical activity under different physiological challenges (Saito *et al.*, 2004). In the PM of goldfish, rainbow trout and eel (*Anguilla* sp.), AVT neurons frequently cluster together by means of soma–somatic apposition near the ventricular wall (Cumming *et al.*, 1982; Saito *et al.*, 2004). The function of this anatomical apposition is unknown but it could be involved in communication by changing local field potentials and/or the somatodendritic release of peptides (Saito *et al.*, 2004). A significant feature of AVT neurons in the PM is the frequent contacts between their proximal processes, originated in the same or different clusters, which may also be involved in the communication among AVT neurons (Saito *et al.*, 2004). Ultrastructural studies in the goldfish demonstrated axo-dendritic synapses with AVT-ir axon terminals in the PM. It suggests the existence of synaptic contacts between AVT cells and the presence of a local neuronal circuitry in the PM (Cumming *et al.*, 1982), probably acting in the synchronization of the neurosecretory cells to fit the secretory activity to physiological requirements (Saito and Urano, 2001; Saito *et al.*, 2004). The AVT neurons in the PM have been further demonstrated to contact directly with the systemic circulation in the eel (Mukuda *et al.*, 2005), receiving chemical information from the periphery. In fact, AVT neurons in all three subdivisions of the PM express glucocorticoid receptor in the flounder

(*Platichthys flesus*) and are modulated by confinement stress (Bond *et al.*, 2007). AVT neurons have also been shown to colocalize with CRF and galanin in the PM and PPa, respectively, in several species (see later). A second notable characteristic of the AVT neuronal system is sexual dimorphism observed in several species (Grober and Sunobe, 1996; Maruska *et al.*, 2007), as well as the differences observed between males exhibiting different reproductive tactics (Foran and Bass, 1998; Goodson and Bass, 2001). In addition, sex reversal in the marine goby (*Trimma okinawae*) is correlated with a variation in the AVT-ir cell size, but no differences in the number of cells were observed (Grober and Sunobe, 1996).

As stated above, preoptic AVT neurons mainly project to the neurohypophysis. However, in the deep interdigitations of the neurohypophysis into the RPD a close association between AVT terminal and corticotropic cells was observed in the sea bass pituitary (see Table 1.3). Within the proximal pars distalis, AVT fibers were intermingled with TSH and GH cells and occasionally with gonadotropic cells. In the PI, numerous AVT fibers were in contact with both MSH and somatolactin cells (Moon *et al.*, 1989). Ultrastructural studies in green molly (*Poecilia latipina)* demonstrated that AVT fibers were mainly restricted to the pericapillary areas of the neurohypophysis but some immunoreactive profiles abutted the basement lamina and occasionally discontinuities in the lamina allowed direct contact between AVT fibers and ACTH cells and rarely between AVT fibers and prolactin cells. In the central pituitary, discontinuities of the basal lamina allowed intermingling of AVT fibers and GH and TSH cells and rarely with gonadotropic cells. Similar to the sea bass, the AVT fibers in the PI contact MSH and somatolactin cells (Batten, 1986).

4.2. Isotocin (IST)

Isotocin (IST) is the peptide homologue of mammalian oxytocin in bony fish (Acher, 1996). As in the AVT neuronal system, the distribution of IST-ir in the teleost brain seems to be quite similar in several species examined, i.e., goldfish, European plaice (*Pleuronectes platessa*; Goossens *et al.*, 1977), green molly (Batten *et al.*, 1990a), plainfin midshipman, gulf toadfish (*Opsanus beta;* Goodson *et al.*, 2003), rainbow trout (Saito *et al.*, 2004) and white sea bream (*Diplodus sargus*; Duarte *et al.*, 2001). In all fish species, IST is produced in the PPa and PM (Table 1.2). However, studies in plainfin midshipman (Goodson *et al.*, 2003) also reported a few IST-ir perikarya in the PPp. IST projections are widely spread through the teleost brain suggesting that IST exerts very broad modulatory action on physiological,

Table 1.3
Peptidergic innervation of fish pituitary and phenotype of the target cells

Peptide	Target cell type	Observations	References
AVT	MSH, ACTH, SL, GH, TSH	Occasionally GTH and PRL. Most AVT-ir terminals surround capillaries	Batten (1986); Moons *et al.* (1989); Batten *et al.* (1999).
CART	GH, TSH	Only some fibers in the PI	Singru *et al.* (2007)
CCK	GH, non-specified RPD cells	CCK innervation was rarely observed in goldfish	Moons *et al.* (1989); Himick *et al.* (1993); Batten *et al.* (1999)
CRF	MSH, TSH, ACTH	No contacts with GH and GTH cells	Moons *et al.* (1989); Batten *et al.* (1999); Matz and Hofeldt (1999); Duarte *et al.* (2001); Pepels *et al.* (2002)
Galanin	SL, GH, TSH, GTH, ACTH, PRL	Innervation of SL was shown in platyfish and trout, but not observed in four-eyed fish	Moons *et al.* (1989); Magliulo-Cepriano *et al.* (1993); Anglade *et al.* (1994); Power *et al.* (1996); Jadhao and Pinelli (2001)
GHRH	MSH, LS, GH, less extensive GTH, ACTH (?)	Innervation to PI was shown in goldfish, sea bass, sculpin, and pejerrey, but was not observed in cod, eel, and trout. Innervation to RPD was shown in the sea bass, grey mullet, sculpin, but not in eel, carp, goldfish, cod and salmonids	Marivoet *et al.* (1988); Moons *et al.* (1988, 1989); Pan *et al.* (1985)>; Olivereau *et al.* (1990); Miranda *et al.* (2002)
GnRH	SL, GTH, GH, PRL (?)	Innervation to RPD was shown in pejerrey and Nile perch but not in other teleost	Parhar and Iwata (1994); Parhar *et al.* (1995); Parhar (1997); Batten *et al.* (1999); Vissio *et al.* (1999); Mousa and Mousa (2003); Pandolfi *et al.* (2005)
GRP	GH (?), non-specified PI cells	Not direct innervation to PPD; it may diffuse from PI.	Himick and Peter (1995)

(*continued*)

Table 1.3 (*continued*)

Peptide	Target cell type	Observations	References
IST	MSH, ACTH, SL, GH, TSH,	Occasionally GTH and PRL, practically overlapping AVT innervation. Most IST-ir terminals surround capillaries	Batten (1986); Moons *et al.* (1989); Batten *et al.* (1999).
MCH	Blood capillaries, MSH, SL, ACTH	MCH-ir terminals surround capillaries	Batten and Baker (1988); Powell and Baker (1988); Batten *et al.* (1999); Amano *et al.* (2003); Pandolfi *et al.* (2003)
NPY	MSH, GH, GTH	Innervation species dependent, see Section 5.9 for details	Moons *et al.* (1989); Pontet *et al.* (1989); Zandbergen *et al.* (1994); Marchetti *et al.* (2000); Gaidwad *et al.* (2004)
PACAP	MSH, SL, β-endorphins, GH, PRL, ACTH		Montero *et al.* (1998); Wong *et al.* (1998); Matsuda *et al.* (2005a,b)
RF-amide	Non-specified PPD cells, PRL (PrRP)	Local production in RPD (potential paracrine effect). PrRP innervation	Rama Krishna *et al.* (1992); Magliuo-Cepriano *et al.* (1993); Amano *et al.* (2007)
SS	MSH, SL, GH, less extensive GTH, PRL, ACTH	Innervation of RPD was shown in tilapia, sea bass, catfish but not in killifish, mudsucker and sea bream	Olivereau *et al.* (1984a); Batten *et al.* (1985); Grau et al, (1985); Moons *et al.* (1989); Power *et al.* (1996); Batten *et al.* (1999)
TRH	MSH, GH, TSH (?)	TSH cells were not positively identified	Batten *et al.* (1999); Díaz *et al.* (2001, 2002)

ACTH, adrenocorticotropin-releasing hormone; AVT, arginine-vasotocin; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CRF, corticotropin-releasing factor; GH, growth hormone; GHRH, growth hormone releasing hormone; GnRH, gonadotropin-releasing hormone; GRP, gastrin-releasing peptide; GTH, gonadotrophin; IST, isotocin; MCH, melanin-concentrating hormone; MSH, melanin-stimulating hormone; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating polypeptide; PI, pars intermedia; PPD, proximal pars distalis; PRL, prolactin; PrRP, prolactin-releasing peptide; RPD, rostral pars distalis; SL, somatolactin; SS, somatostatin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

behavioral and sensorimotor processes (Goodson *et al.*, 2003). In the rainbow trout IST-ir neurons are placed more laterally in the PM, whereas AVT neurons occupy a medial position (Saito *et al.*, 2004). AVT neurons extended more caudally than IST neurons in the white sea bream (Duarte *et al.*, 2001) but neither of the AVT-/IST-ir perikarya showed a preferential position in goldfish, European plaice or green molly (Goossens *et al.*, 1977; Batten *et al.*, 1990a). Similar to AVT neurons, IST neurons cluster together and display contact between their processes in the PM regions of the rainbow trout. However, intracellular staining demonstrated the incidence of dye coupling among the IST neurons, but not AVT, of the same or different clusters in the PM of rainbow trout. This suggests the involvement of the electrical coupling in the communication among IST neurons. IST neurons can use electrical and/or chemical synapses for local neuronal circuitry. Taken together, the evidence suggests that AVT and IST neurons in the PM form cell-type specific neurons, from which neurohypophysial and extrahypothalamic projections arise (Saito *et al.*, 2004).

Ultrastructural studies in green molly demonstrated that IST fibers outnumber AVT fibers (2:1) in the posterior neurohypophysis but both types occurred in similar number in the rostral area (Batten, 1986). Few IST fibers contact the basement lamina of the RPD with similar relationship to ACTH and PRL cells as AVT fibers but most of them were centrally located surrounding the capillaries (Table 1.3). At the interface between neuro- and adenohypophysis IST fibers are placed around all cell types in the PPD and PI, particularly TSH, GH and MSH cells. A similar fiber distribution was also observed in the sea bass pituitary (Moons *et al.*, 1989).

4.3. Melanin-concentrating Hormone (MCH)

From an evolutionary perspective, MCH is probably one of the most fascinating peptides in the vertebrate CNS. It was originally purified from chum salmon (*Oncorhynchus keta*) pituitaries and characterized as a circulating-cyclic heptadecapeptide mediating color change in teleost fish (Kawauchi *et al.*, 1983), where it induces reversible centripetal aggregation of melanosomes through the dendritic processes of the melanophores. This central accumulation of the pigmentary organelles results in a change in the refractive index, which makes the scales appear paler, thus allowing cryptic camouflage (reviewed by Kawauchi and Baker, 2004). In teleost fish, MCH works as a neurohypophysial hormone that is released to the blood stream when fish move onto a pale-colored background. Most of the MCH axons entering the teleost pituitary terminate in the posterior area of the neural lobe. In addition, positive fibers are commonly distributed in the neurohypophysial digitations of the PI, where MSH and somatolactin cells are localized

(Table 1.3). Some fibers also penetrate the pars distalis, thus suggesting its involvement in the control of synthesis/release of adenohypophysial hormones (Batten and Baker, 1988; Powell and Baker, 1988; Gröneveld *et al.*, 1995; Baker and Bird, 2002; Amano *et al.*, 2003; Pandolfi *et al.*, 2003). Electron microscopy studies in eel (Powell and Baker, 1988) and molly (Batten and Baker, 1988) showed that MCH-like terminals are placed close to the thick basement membrane which separates the PI from the neural tissue. MCH terminals are located mainly opposite the α-MSH cells and less commonly terminate near the blood capillaries. On the contrary, contact between the MCH fibers and the basement membrane surrounding the capillaries is prominent within the rostral and central region of the neurohypophysis. Therefore, MCH terminals are well sited for the released hormone to diffuse along this membrane into the vascular system and also to diffuse into the PI (Powell and Baker, 1988). The ultrastructural studies in green molly further showed that some MCH fibers can come into direct contact with the endocrine cells of the PI, most often MSH cells, through the discontinuities in the basement membrane. Both studies also reported synaptoid contacts between the MCH fibers and pituicytes of the neurohypophysis but the functional significance of these contacts remains unknown. Suggestively, Gröneveld *et al.* (1995) reported the expression of MCH by *in situ* hybridization in the neurohypophysis of tilapia (*Oreochromis mossambicus*) and suggest that probably subpopulations of pituicytes could produce MCH mRNA.

The neuronal cell bodies producing MCH are commonly localized within the LH or NLTl of fish (Table 1.2). However, the number of MCH-producing cell populations, the cellular morphology and the position with respect to the ventricular surface is variable (Baker and Bird, 2002). In polypteriform and chondrostean species, the MCH neurons are placed mainly on the hypothalamic periventricular surface. A first group of neurons is associated to the PVO over the dorsal surface of the third ventricle, whereas a second group of neurons occurs around the lateral surface of the third ventricle. A migrated MCH-producing group placed in the LH was first seen in holostean species which share the periventricular populations observed in polypteriformes and chondrostean species. In teleosts, most MCH neurons migrate away from the periventricular areas and it is within euteleosts that hypothalamic hypophysial magnocellular neurons of the LH represent the most prominent MCH-producing area (Baker and Bird, 2002). These neurons in the lateral hypothalamus are the main neurons responsible for the pituitary innervation and are present in all the euteleost species studied. More caudally, coinciding with the lateral aperture of the hypothalamic region of the third ventricle, a second group of small perikarya is associated dorsally to the PVO. At the same level, some scattered cell bodies producing

MCH are also placed in the Hv, close to the ventral surface of the lateral aperture of the third ventricle. The function of these caudal hypothalamic MCH neurons is unknown, but a specific increase of MCH mRNA levels has been observed in repeatedly disturbed tilapia. MCH gene expression levels in these caudal hypothalamic cells were also modified by salt water challenge (Gröneveld *et al.*, 1995).

5. HYPOPHYSIOTROPIC PEPTIDES

5.1. Cholecystokinin (CCK)

Cholecystokinin (CCK) and gastrin constitute a family of peptides characterized by the common C-terminus of Trp-Met-Asp-Phe-NH2 (for review see Chandra and Liddle, 2007; Rehfeld *et al.*, 2007). The structure of the C-terminus octapeptide of CCK has been well conserved during evolution and is identical in mammals, chicken, turtle and frog, with only one amino acid substitution in fish (Johnsen, 1998; Peyon *et al.*, 1998). This octapeptide is the main CCK peptide produced in the nervous system, while longer peptides such as CCK 58, 33 and 22 are also found in peripheral tissues and circulation (Rehfeld *et al.*, 2007).

CCK/gastrin-ir perikarya and fibers are widely distributed in the forebrain, midbrain and hindbrain of goldfish (Himick and Peter, 1994). A highly concentrated and extensive CCK/gastrin-ir perikarya and fiber system is prominent in the posterior ventromedial and ventrolateral hypothalamus, and the inferior hypothalamic lobes of goldfish (Himick and Peter, 1994). Thus, CCK/gastrin-ir cell bodies were found scattered in the preoptic area, periventricular and lateral Hv and Hc and the dorsal and ventral thalamus (Table 1.2). A similar brain distribution of CCK-ir has been described in the green molly and rainbow trout (Notenboom *et al.*, 1981; Batten *et al.*, 1990a). In rainbow trout, CCK/gastrin-like cell bodies were located in the ventromedial hypothalamus in the caudal region of the Hv, from where the pituitary innervation originates. In the guppy, CCK-ir was found throughout the brain. Notably, CCK-ir cell bodies were detected in the ventral telencephalon, the preoptic area and tuberal and periventricular hypothalamus (Batten *et al.*, 1990a). Furthermore, studies in goldfish have revealed that CCK mRNA, similar to CCK-ir, is expressed widely in the brain of goldfish, the hypothalamus being the main brain area expressing CCK (Peyon *et al.*, 1999). *In situ* hybridization analysis has shown CCK expression in the ventroposterior hypothalamus (Peyon *et al.*, 1999). In rainbow trout, three different mRNA for CCK (CCK-N, CCK-L CCK-T) have been isolated, which differ from each other in one amino acid substitution (Jensen *et al.*, 2001). Each of these variants shows a specific expression pattern in the brain, as well as in the peripheral tissue. CCK-N and CCK-L were found in the preoptic area and ventroposterior hypothalamus, respectively (Jensen *et al.*, 2001).

CCK/gastin-ir fibers, originating in the ventral hypothalamus, enter the hypophysis as a large bundle towards the neurohypophysis (Table 1.3). This bundle branches into smaller bundles and even into single fibers at the level of the PPD. These immunoreactive fibers appear to terminate on the basal lamina separating the neuro- from the adenohypophysis (Notenboom *et al.*, 1981; Batten *et al.*, 1999). Studies have shown CCK-ir fibers in close relationship with GH cells (Moons *et al.*, 1989; Himick *et al.*, 1993; Batten *et al.*, 1999), strongly supporting a role for CCK in the neuroendocrine regulation of GH release (Canosa *et al.*, 2007). CCK-ir fibers were not observed to innervate the teleost PI (Batten *et al.*, 1999; Himick *et al.*, 1993), neither were they found in the RPD of the green molly, African catfish or sea bass (Batten *et al.*, 1999), but were only rarely seen in goldfish RPD (Himick *et al.*, 1993).

5.2. Cocaine- and Amphetamine-regulated Transcript (CART)

Cocaine- and amphetamine-regulated transcript (CART) peptide was originally isolated from ovine hypothalamus and suggested to be an SS-like peptide (Spiess *et al.*, 1981). Subsequently, CART mRNA expression was found to increase in the rat striatum after the administration of psychostimulant drugs such as cocaine and amphetamine (Douglas *et al.*, 1995). In rat, CART gene is alternatively spliced to produce a peptide of either 129 or 116 amino acids containing a 27 amino acid signal peptide thus resulting in a pro-peptide of either 102 (long form) or 89 (short form) residues (Douglas *et al.*, 1995). These pro-peptides are further processed to release the same main product, referred to as rat long CART [55–102] or rat short CART [42–89] depending on the length of the precursor (Dylag *et al.*, 2006).

In fish, CART mRNA has been characterized in goldfish (Volkoff and Peter, 2001) and cod (*Gadus morhua*), but DNA sequences are also available for zebrafish and pufferfish (*Takifugu rubripes*; Kehoe and Volkoff, 2006). Immunohistochemical localization of the CART peptide in fish brain has only been studied in the catfish by using antibodies against rat CART [55–102] (Singru *et al.*, 2007). The results demonstrated that CART immunoreactivity is widely distributed within the catfish brain, where it is localized within the main neuroendocrine territories (Table 1.2). CART cells were reported within the ventral telencephalon including Vv V, Vc and Vs nuclei. Intense immunoreactivity was detected in the PMgc. CART-ir terminals were seen in the neurohypophysis and several long fibers were detected throughout the PPD, where somatotropes and thyrotropes are concentrated

(Table 1.3). Only a few fibers were seen in the PI of the catfish pituitary. These hypophysial CART terminals could also originate in the tuberal hypothalamus, where few neurons were immunoreactive to CART peptide. The authors termed this area the arcuate nucleus, which corresponds to the Hv. Additionally, some authors have reported CART-ir neurons in the Hc or NRP and NCLI, both shown to be hypophysiotropic areas in catfish and brown ghost knifefish brain, respectively.

5.3. Corticotropin-releasing Factor (CRF) and Related Peptides

In fish, the corticotropin-releasing factor (CRF) system comprises four neuropeptides, i.e., CRF, urotensin I (UI) and the orthologues of the mammalian urocortin 2 and 3, two G-protein-coupled receptors (CRF_1 and CRF_2) and a binding protein (CRF-BP, Bernier, 2006). The CRF system is pivotal in the coordination of the stress response. This system regulates the pituitary–adrenal axis by modulating the activity of the adenohypophysial ACTH and MSH cells. However, it has also been involved in the regulation of additional physiological processes (Flik *et al.*, 2006; see Chapter 6, this volume).

The distribution of the CRF-expressing neurons has been studied in the white sucker (*Catostomus commersoni*; Okawara *et al.*, 1992) and recently in the zebrafish (Alderman and Bernier, 2007) by *in situ* hybridization. In the latter species, CRF is widely expressed in several hypophysiotropic areas of the brain including the ventral olfactory bulb, telencephalon, preoptic area and tuberal hypothalamus (Table 1.2). In addition, some expression levels were also found within the dorsal telencephalon, dorsal and ventral thalamus, optic tectum (OT) and tegmentum. Within the ventral telencephalon, CRF is expressed in theVv and Vc (Table 1.2) both projecting towards the pituitary gland in salmon and goldfish (Table 1.1). Immunostaining studies in tilapia (Pepels *et al.*, 2002) localized CRF-ir in the Vl and Vc whereas only the CRF population in the lateral region was reported in green molly (Batten *et al.*, 1990a). However, no immunoreactive cell bodies were reported in the telencephalon of some species (Olivereau *et al.*, 1984b; Yulis and Lederis, 1987; Olivereau and Olivereau, 1988; Mancera and Fernández-Llébrez, 1995; Matz and Hofeldt, 1999; Zupanc *et al.*, 1999; Duarte *et al.*, 2001).

The preoptic area is a major CRF-producing region. There are four populations of CRF-expressing neurons in the preoptic area of the zebrafish localized in the PPa, PPp, PM and NSC (Alderman and Bernier, 2007). Expression in the PPa and PM was previously reported in white sucker (Yulis and Lederis, 1987; Okawara *et al.*, 1992). In addition, the confinement-induced stress was shown to stimulate CRF synthesis in the preoptic area of the rainbow trout (Ando *et al.*, 1999). CRF-like-ir in the preoptic area has been demonstrated in almost all studies reporting the CRF distribution in the fish brain although the neuronal CRF circuits differ depending on the species. Only a few cells were seen in the PM of green molly (Batten *et al.*, 1990a). Both populations (PM and PPa) exhibited CRF-ir in salmonids, eel, brown ghost knifefish and cyprinid species (Olivereau *et al.*, 1984b; Olivereau and Olivereau, 1988; Zupanc *et al.*, 1999). Studies carried out in sparid species, white (Duarte *et al.*, 2001) and gilthead sea bream (*Sparus aurata*; Mancera and Fernández-Llébrez, 1995), found no CRF neurons in the entire rostral preoptic area and only some scattered perikarya in the PPp, just rostral to the lateral aperture of the tuberal recess (ZL). Some CRF neurons in the PM have been shown to contain AVT in the eel (Olivereau *et al.*, 1988), green molly (Batten *et al.*, 1990a), carp (Huising *et al.*, 2004) and tilapia (Pepels *et al.*, 2002), whereas all CRF neurons in the PM of white sucker displayed AVT-ir (Yulis and Lederis, 1988). This colocalization was also found in fibers forming the preoptico-hypophysial tract and in the neurohypophysial interdigitations projecting to the neurointermediate lobe. In fact, CRF cells in the PM seem to innervate the neurointermediate lobe whereas cells in the ventral hypothalamus project to the RPD of the adenohypophysis in the white sucker (Table 1.3). Peptide colocalization was also suggested in the preoptic area of rainbow trout (Ando *et al.*, 1999). Neuronal tract tracing combined with CRF immunohistochemical studies in brown ghost knifefish have demonstrated that approximately 6% of the retrogradely traced cells in the PPa and PM exhibited CRF-like-ir.

Within the tuberal hypothalamus, CRF expression has been localized in the Hv and Hc of the zebrafish. In addition, some CRF-mRNA expressing neurons were also detected in the PTN (Alderman and Bernier, 2007). However, no hybridization signal was reported in the tuberal hypothalamus of the white sucker (Okawara *et al.*, 1992) but peptide-ir was detected in the Hv (Yulis and Lederis, 1987). Similarly, CRF-ir in the Hv has been reported in sparid species (Mancera and Fernández-Llébrez, 1995; Duarte *et al.*, 2001), tilapia (Pepels *et al.*, 2002) and green molly (Batten *et al.*, 1990a). In the latter species, CRF neuronal bodies are located close to the pituitary stalk suggesting the hypophysial innervation. In several teleost fish including brown ghost knifefish, salmonid species and eel, CRF-ir in the tuberal hypothalamus was absent (Olivereau and Olivereau, 1988; Zupanc *et al.*, 1999) but was the only CRF system projecting to the pituitary in the gilthead and white sea bream (Mancera and Fernández-Llébrez, 1995; Duarte *et al.*, 2001).

The distribution of CRF fibers in the pituitary has also been studied in several species (Table 1.3). Early studies in the sea bass demonstrated that the great mass of CRF fibers run into the posterior neurohypophysis and the PI of the sea bass (Moons *et al.*, 1989). However, a portion of CRF-positive fibers reached RPD and PPD. A similar scenario has been shown in some teleost species (Yulis and Lederis, 1987; Matz and Hofeldt, 1999), but no CRF-ir was reported in the PPD of the eel, salmon (Olivereau and Olivereau, 1988), white sea bream (Duarte *et al.*, 2001), tilapia (Pepels *et al.*, 2002) and carp (Huising *et al.*, 2004). The RPD, where ACTH cells are placed, seems to be the main destination of the CRF fibers in some species (Olivereau *et al.*, 1984b) but in others this area receives no CRF-ir fibers (Mancera and Fernández-Llébrez, 1995). In some species, it has been suggested that CRF may reach ACTH cells via blood vessels or in a paracrine fashion. In the RPD of the sea bass, CFR fibers are placed in the vicinity of the ACTH cells, in line with the well-defined action of ACTH-releasing factor, whereas in the PPD they are in close apposition to the TSH cells but no contacts with somatotropes and gonadotropes are seen. Similar results were reported in Chinook salmon (*Oncorhynchus tshawytscha*) in which immunostaining of adjacent sections with CRF and TSH antibodies demonstrated partial overlapping (Matz and Hofeldt, 1999). *In vitro* studies previously demonstrated the thyrotropin-releasing activity of CRF and UI in the latter species (Larsen *et al.*, 1998). Double immunostaining methods showed CRF fibers running close to the ACTH cells whereas immunoreactive fibers in the neurohypophysis ended close to MSH cells in the salmonids, eel (Olivereau and Olivereau, 1988) and cyprinid (Huising *et al.*, 2004) adenohypophysis. A similar situation has also been reported in white sea bream, thus suggesting CRF control of the pituitary MSH system (Duarte *et al.*, 2001). In fact, CRF is known to stimulate MSH secretion in teleost fish (see Chapter 6, this volume).

Early studies on the fish brain demonstrated the presence of UI-like-ir in the diencephalon of white sucker (Yulis *et al.*, 1986). Immunoreactivity was located within the Vp, Hv and Hc. Additional studies in white sucker further reported UI-ir in the Vv and pretectal area (Yulis and Lederis, 1986). UI-like fibers in the pituitary were restricted to the PDP in close proximity to the adenohypophysial cells (Table 1.3). Similarly, studies in eel reported the presence of UI transcript in the dorsal and ventral subdivisions of the PVO (Kawauchi *et al.*, 2003). The latter areas probably correspond to the caudal region of the dorsal and ventral hypothalamus, respectively, according to Braford and Northcutt (1983). Recent expression studies have shown a wider expression of UI mRNA in the brain of zebrafish (Alderman and Bernier, 2007). UI transcripts were localized within the dorsal and ventral telencephalon, preoptic area and tuberal hypothalamus, mesencephalic tectal and tegmental areas. In the telencephalon, UI-mRNA-expressing cell bodies were located within the Vd as well as in the caudal region, probably the Vp. The expression in the preoptic region is restricted to the parvocellular region with stronger labeling in the PPa than in the PPp. Within the tuberal hypothalamus, some expression was detected in the Hv and Hc.

To our knowledge, the distribution of urocortin 2 and 3, and the participation of these peptides in the neuroendocrine system of fish, are unknown.

5.4. Galanin

Galanin is a 29-amino acid N-terminal peptide originally isolated from the porcine intestine. It is proteolytically processed from the prepropeptide along with a peptide known as galanin message-associated peptide. This neuropeptide binds to three different G-protein coupled receptors which exhibit substantial differences in their functional coupling and subsequent signaling activities. Galanin has been shown to have a wide distribution in the central and peripheral nervous system of many mammalian species and multiple biological effects, including feeding and metabolism, osmotic regulation and water intake, learning and memory consolidation, anxiety and related behaviors, arousal and sleep regulation, reproduction and nociception (reviewed by Lang *et al.*, 2007). Galaninergic fibers have been reported in the pituitary of several vertebrate groups including fish, suggesting that this peptide could be involved in the regulation of the secretion of this gland (Moons *et al.*, 1989; Batten *et al.*, 1990a; Olivereau and Olivereau, 1991; Magliulo-Cepriano *et al.*, 1993; Anglade *et al.*, 1994; Rodríguez-Gómez *et al.*, 2000a; Jadhao and Pinelli, 2001). Ultrastructural studies in fish have further demonstrated the presence of galanin-immunoreactive terminals in the central region of the neurohypophysis where some fibers follow the interdigitations of neural tissue into the proximal par distalis ending in the basal lamina opposite to PRL, ACTH, GH, TSH and GTH cells (Table 3) but did not show particular association with any one endocrine cell type of the PPD (Batten *et al.*, 1990c, 1999). Immunocytochemical studies in the sea bass suggested that galanin fibers directly innervate ACTH and PRL adenohypophysial cells. Similarly, studies in platyfish (*Xhiphophorus maculates*) demonstrated that galanin immunoreactive fibers colocalize with SL, PRL and GH cells. Accordingly, binding studies in the latter species showed galanin receptors to be confined within the area occupied by the prolactin cells in the rostral part of the adenohypophysis (Moons *et al.*, 1991).

Galanin-ir cell populations are mainly located within the preoptic area and tuberal hypothalamus (Table 1.2) but sexual dimorphism has been reported in several species (Prasada Rao *et al.*, 1996; Jadhao and Meyer, 2000; Rodríguez *et al.*, 2003). However, inter-sex dimorphism of the central galaninergic system was not found in "four eyed" fish (*Anableps anableps*; Jadhao and Pinelli, 2001). In addition, galanin-ir neuronal populations have also been reported within the olfactory bulb of the brown trout (*Salmo trutta fario*; Rodríguez *et al.*, 2003) and in the Vs in the goldfish (Prasada Rao *et al.*, 1996) and rainbow trout brain (Anglade *et al.*, 1994). In most teleost species, galanin-ir and/or gene expressing cells have been commonly localized within the PPa. The number of immunoreactive cells in the PPa was consistently greater in goldfish (Prasada Rao *et al.*, 1996) and brown trout males than in females (Rodríguez *et al.*, 2003), but no sex differences were reported in the PPa of the sockeye salmon (*Oncorhynchus nerka*; Jadhao and Meyer, 2000) or green molly (Cornbrooks and Parsons, 1991). A second population of galanin-ir neurons has been reported in the PM of several teleost species (Batten *et al.*, 1990c; Magliulo-Cepriano *et al.*, 1993; Prasada Rao *et al.*, 1996; Jadhao and Meyer, 2000; Jadhao and Pinelli, 2001; Rodríguez *et al.*, 2003; Adrio *et al.*, 2005). This neuronal population was absent in goldfish and molly females but conspicuous in males (Cornbrooks and Parsons, 1991; Prasada Rao *et al.*, 1996). Opposite results were reported for sockeye salmon (Jadhao and Meyer, 2000). Our expression studies in the goldfish reported the presence of galanin mRNA in the PPa but not in the PM (Unniappan *et al.*, 2004). Double labeling studies suggested the coexistence of galanin- and CRH-ir in some neurons of the PM of molly and killfish (Batten *et al.*, 1990c). Galanin-ir cells were not found in the PM of rainbow trout (Anglade *et al.*, 1994) and Senegalese sole (*Solea senegalensis*; Rodríguez-Gómez *et al.*, 2000a).

The third population of galanin-producing cells is located in the tuberal hypothalamus, within the posterior and caudal areas. *In situ* hybridization studies in the goldfish localized galanin mRNA within the LH as well as in the Hv (Unniappan *et al.*, 2004). The cell population of the Hv has also been reported in several species (Power *et al.*, 1996; Rodríguez-Gómez *et al.*, 2000a) but this locus is sometimes called the posterior part of the lateral tuberal nucleus (Batten *et al.*, 1990c; Magliulo-Cepriano *et al.*, 1993; Anglade *et al.*, 1994; Prasada Rao *et al.*, 1996; Jadhao and Meyer, 2000; Jadhao and Pinelli, 2001) or NAT (Rodríguez *et al.*, 2003). This tuberal population seems to be continuous with that of the Hc and PTN described in some species (Anglade *et al.*, 1994; Power *et al.*, 1996; Prasada Rao *et al.*, 1996; Jadhao and Pinelli, 2001; Rodríguez *et al.*, 2003). Sexual dimorphism within the tuberal hypothalamus has only been reported in the brown trout, in which the number of galanin-ir cells was greater in males than in females.

5.5. Gastrin-releasing Peptide (GRP)

Gastrin-releasing peptide (GRP), a 27-amino acid peptide, is part of the bombesin (BBS) and neuromedin B family of peptides (Martínez and Taché, 2000). This peptide has been isolated from several mammalian species and is also found in other vertebrates including fish (McDonald *et al.*, 1979; Holmgren and Jensen, 1994). This group of peptides is characterized by a highly conserved C-terminus, which is important for biological actions

(Martínez and Taché, 2000). Due to the structural similarity between BBS and GRP, exogenous BBS effects most likely reflect the function of endogenous GRP. BBS/GRP peptides are widely distributed in the gastrointestinal tract and CNS (McCoy and Avery, 1990), and have been shown to be potent anorexigenic substances when administered intraperitoneally or centrally in mammals and fish (Gibbs *et al.*, 1979; Flynn, 1991; Himick and Peter, 1995; Volkoff *et al.*, 2005). In addition, a BBS-like peptide has also been shown to be involved in the regulation of gut motility and visceral activity in fish species (Holmgren and Jonsson, 1988; see Chapter 10, this volume). In goldfish, BBS-ir fibers were present in the ventral telencephalon, the preoptic area, the tuberal hypothalamus, the posterior hypothalamus, areas associated with the putative feeding center in fish and some thalamic nuclei (Himick and Peter, 1995). In addition, BBS-ir perikarya were found in the preoptic area and the periventricular regions including NAT, Hv (cell population coating the lateral recess) and Hc (Himick and Peter, 1995). In rainbow trout, BBS-ir cell bodies were found in the PPp, Hv (coating the lateral recess) and Hc (Cuadrado *et al.*, 1994). BBS-ir fibers were widely distributed in the diencephalon, midbrain and hindbrain, the hypothalamus being the most densely labeled (Cuadrado *et al.*, 1994). The mRNA encoding for GRP has been identified in goldfish (Volkoff *et al.*, 2000). RT-PCR analysis shows that GRP mRNA is widely expressed in the brain of goldfish, as well as in the ovary, gill, skin, gut and pituitary (Volkoff *et al.*, 2000). Within the pituitary gland, BBS/GRP fibers were observed mainly in the PI.

5.6. Gonadotropin-releasing Hormone (GnRH)

In vertebrates, gonadotropin-releasing hormone (GnRH) is a decapeptide that has a cyclic structure with a pyro-glutamate modification in the amino terminus and an amide function at the carboxyl terminus. As many as 24 molecular isoforms of GnRH have been characterized so far and eight variants have been found in the teleost brain, six of them being exclusive to teleost species (Kah *et al.*, 2007). White and colleagues (1998) have proposed grouping the GnRH diversity into three types. As such, GnRH type 1 represents hypophysiotropic GnRH variants such as mGnRH, cfGnRH, pjGnRH and sbGnRH. Type 2 includes the cGnRH-II from all species studied which are localized in the midbrain, whereas type 3 includes sGnRH from several fish species (Lethimonier *et al.*, 2004). While it seems clear that type 1 has hypophysiotropic functions, the role for type 2 and 3 has not been established. There is some evidence for type 2 being involved in sexual behavior (Volkoff and Peter, 1999; Canosa *et al.*, 2008); however, the case of type 3 is more elusive. In several teleost species in which both type 1 and 3 coexist in the ventral forebrain, fibers of type 3 GnRH also innervate the pituitary (Kah *et al.*, 2007; González-Martínez *et al.*, 2002; Pandolfi *et al.*, 2005) suggesting that the type 3 isoform retains some hypophysiotropic activity.

The forebrain distribution of GnRH peptides expands from the olfactory bulb, ganglion cells of the terminal nerve (TNgc), along the ventral telencephalon, the preoptic area, and in some cases, the anteroventral hypothalamus (Lepretre *et al.*, 1993; Montero *et al.*, 1994; González-Martínez *et al.*, 2002; Mohamed *et al.*, 2005; Pandolfi *et al.*, 2005). However, the GnRH hypophysiotropic areas in teleost fish are mainly confined to the parvocellular preoptic area with a few cells in the mediobasal hypothalamus (Lepretre *et al.*, 1993; Montero *et al.*, 1994; Yamamoto *et al.*, 1998; González-Martínez *et al.*, 2002; Mousa and Mousa, 2003). In species with only two GnRH systems (type 1 or 3 in the forebrain and type 2 in the midbrain) the same peptide is found all along the forebrain distribution area. On the other hand, for those species which carry three GnRH systems, types 1 and 3 greatly overlap in the forebrain distribution area and pituitary innervation (González-Martínez *et al.*, 2002; Pandolfi *et al.*, 2005). In general, though, type 1 (sGnRH) is principally found in the olfactory region and TNgc, while type 3 (hgGnRH, sbGnRH, pjGnRH) is mainly expressed in preoptic area neuronal cells that innervate the pituitary (González-Martínez *et al.*, 2002; Pandolfi *et al.*, 2005).

In terms of GnRH brain distribution, goldfish are unique in being the only species that expresses cGnRH-II in the forebrain (Yu *et al.*, 1988). An immunohistochemical study has shown sGnRH-ir cell bodies localized in the TNgc, the ventral telencephalon, preoptic area and hypothalamus (Kim *et al.*, 1995). Within the forebrain, cGnRH-II-expressing neurons were found in lower numbers but in the same areas as sGnRH. Moreover, sGnRH-ir and cGnRH-II-ir fibers were distributed in the hypothalamus as well as in the pituitary gland (Kim *et al.*, 1995). Recent studies correlating GnRH mRNA levels with serum LH levels during ovulation and spawning in goldfish suggest that forebrain cGnRH-II in this species does not initiate the ovulatory LH surge (Canosa *et al.*, 2008).

The presence of GnRH fibers in the PI has been documented in salmonid and Nile perch (*Lates niloticus*) in close proximity with SL cells (Parhar and Iwata, 1994; Parhar *et al.*, 1995) and SL and MSH cells (Mousa and Mousa, 2003), respectively. In addition, South American cichlid (*Cichlasoma dimerus*) shows a strong presence of sbGnRH fibers in the PI running along the border and probably interacting with PI endocrine cells (Pandolfi *et al.*, 2005). In pejerrey fish, double immunostaining for GnRH and SL showed a close apposition betweeen pjGnRH fibers and SL cells in the PI (Vissio *et al.*, 1999). Furthermore, GnRH binding sites have been detected on dispersed SL cells from pejerrey fish pituitaries (Stefano *et al.*, 1999).

GnRH-ir was found in tissue penetrating the GTH zone in the PPD of green molly, sea bass and Indian catfish (Moons *et al.*, 1989; Batten *et al.*, 1999). In tilapia, GnRH fibers were also found in the PPD innervating GTH and GH cells (Melamed *et al.*, 1995; Parhar, 1997). In European eel, an extensive mGnRH innervation of the pituitary was observed, while only a few cGnRH-II-ir fibers were detected (Montero *et al.*, 1994). Thus, the hypophysiotropic form of GnRH for different species has been shown to innervate the PPD in close proximity with GH- and GTHs-producing cells (Vissio *et al.*, 1999; Mousa and Mousa, 2003; Pandolfi *et al.*, 2005).

The presence of GnRH fibers in RPD and its function on RPD hormonal secretion is scarce and limited to a few teleost species. In tilapia, GnRH stimulated the release of PRL *in vitro* (Weber *et al.*, 1997). Furthermore, GnRH binding sites have been shown on dispersed PRL cells from pejerrey fish (Stefano *et al.*, 1999). In this species, pjGnRH-ir fibers were found to penetrate the RPD (Vissio *et al.*, 1999); however, no GnRH fibers were detected in RPD of a South American cichlid (Pandolfi *et al.*, 2005).

5.7. Growth Hormone-releasing Hormone (GHRH)/Pituitary Adenyl Cyclase Activating Polypeptide (PACAP)

Growth hormone-releasing hormone (GHRH) and pituitary adenyl cyclase activating polypeptide (PACAP) belong to the glucagon/vasoactive intestinal peptide (VIP)/secretin superfamily of peptides and have a number of structural and functional similarities (for review, see Sherwood *et al.*, 2000; Vaudry *et al.*, 2000). Apparently, PACAP is the most conserved member of the family and represents the ancestral molecule from which other members have arisen by in-tandem exon duplication and subsequent gene duplication (Sherwood *et al.*, 2000). PACAP shows that amino acid sequence identity varies by between 88 and 97% across vertebrates, whereas GHRH sequences have only 32–45% identity between human and non-mammalian vertebrates (Sherwood *et al.*, 2000; Vaudry *et al.*, 2000). In mammals, separate genes encode for each peptide. In addition, a C-peptide, with no known function, is encoded along with GHRH whereas PACAP-related peptide (PRP) is present in the same transcript as PACAP (Mayo *et al.*, 1985; Hosoya *et al.*, 1992). It was thought that in tunicates, teleost fish, amphibians and birds, the PACAP gene included an upstream exon that encoded for GHRH (see for review Sherwood *et al.*, 2000; Vaudry *et al.*, 2000); however, the existence of a different set of genes coding for GHRH and its specific receptors was recently proved in fish (Lee *et al.*, 2007). This GHRH gene would be homologous with the mammalian counterpart since the derived peptide is more potent in eliciting GH release than the previous GHRH peptides described in fish (Lee *et al.*, 2007). Therefore, the previous GHRH peptides would represent the homologue of mammalian PACAP-related peptide (PRP) rather than GHRH. This finding would also explain why these previous peptides have shown low potency in GH stimulation compared to PACAP (see Canosa *et al.*, 2007, and Chapter 4, this volume, for further discussion). Nevertheless, PACAP seems to have clear hypophysiotropic effects in fish (Montero *et al.*, 1998; Wong *et al.*, 1998; Kong *et al.*, 2007; Canosa *et al.*, 2008; Mitchell *et al.*, 2008).

The immunohistological distribution of GHRH has been studied in the brains of several teleost species (Pan *et al.*, 1985; Marivoet *et al.*, 1988; Luo and McKeown, 1989; Batten *et al.*, 1990a; Olivereau *et al.*, 1990; Rao *et al.*, 1996; Miranda *et al.*, 2002). Early studies in cod, using antiserum against the C-terminus and middle portions of human GHRH forms, identified two main groups of immunoreactive cell bodies in the PM, LH and Hv. In the PM, both parvocellular and magnocellular neuronal perikarya were GHRH positive while in the Hv the cells were of the magnocellular type (Pan *et al.*, 1985). In the sea bass, positively stained neurons were located in the PMpc and PMmc and Hv (Marivoet *et al.*, 1988). In rainbow trout, GHRH-ir perikarya were located mainly in the LH and Hv. A small group of GHRH-ir cell bodies were also found in the caudal region of the PM (Luo and McKeown, 1989). Several species of fish including goldfish, carp, eel, salmonids and sculpin (*Myoxocephalus octodecimspinosus)* present GHRH-ir perikarya in PMpc and PMmc and occasionally in the Hv (Olivereau *et al.*, 1990). In the green molly, GHRH-ir cell bodies were only found in the PMgc and PMpc, and these cells also expressed AVT (Batten *et al.*, 1990a). More recently, in goldfish, GHRH-ir perikarya were identified in the preoptic region, Hv, the pineal nucleus and the lateral lemniscus in the midbrain tegmentum (Rao *et al.*, 1996). GHRH-ir fibers were found in the ventral telencephalon, preoptic region, pituitary, mesencephalic tegmentum and the hypothalamic inferior lobes of goldfish. The ontogeny of GHRH-ir has been examined in pejerrey (Miranda *et al.*, 2002).

The brain distribution of PACAP has been studied in European eel (*Anguilla anguilla*; Montero *et al.*, 1998) and the stargazer, (*Uranoscopus japonicus*; Matsuda *et al.*, 2005a). PACAP has a similar distribution to GHRH, being found in the preoptic and hypothalamic regions, and is also present in some neurons in the hindbrain (Matsuda *et al.*, 2005a). In the European eel, PACAP-ir perikarya were observed in the preoptic area, in the PPa and PPp and in the PM. In addition, groups of immunoreactive perikarya were also found in the thalamus within the ventral and dorsal thalamic nuclei (Montero *et al.*, 1998). No other brain regions were positive for PACAP. Immunoreactive fibers were widespread in the brain of the European eel (Montero *et al.*, 1998). In the stargazer, PACAP-ir cell bodies were found in the PMpc and PMgc. In addition, some PACAP-ir cell bodies were also observed in the Hv, corpus cerebelli and in the ventral horn of the spinal cord (Matsuda *et al.*, 2005a).

Both GHRH and PACAP fibers innervate the adenohypophysis and intermingle among the endocrine cells in all three PD subdivisions (Table 1.3). GHRH-ir fibers were shown running along blood vessels in the interdigitations of PN and penetrating into the PI in sea bass, goldfish, carp, sculpin and pejerrey (Marivoet *et al.*, 1988; Moons *et al.*, 1989; Olivereau *et al.*, 1990; Miranda *et al.*, 2002). Within this pituitary zone GHRH fibers contact both the MSH and the SL (or PAS-positive) cells (Marivoet *et al.*, 1988; Moons *et al.*, 1989). Conversely, no GHRH-ir fibers were found in cod, eel and trout (Pan *et al.*, 1985; Olivereau *et al.*, 1990). GHRH-ir fibers originating in the hypothalamus project to the PPD in a variable number depending on the species (Pan *et al.*, 1985; Olivereau *et al.*, 1990). These fibers run among GH cells showing terminals in close relationship with GH cells and, less obviously, with GTH cells (Marivoet *et al.*, 1988; Moons *et al.*, 1988, 1989). In addition, a close relationship between GHRH fibers and TSH cells was documented in sea bass (Moons *et al.*, 1989). Recently, an ontogenetic study demonstrated the appearance of GHRH immunohistological perikarya and fibers in the preoptic region and PPD of the pituitary about 1 week after hatching of the pejerrey (Miranda *et al.*, 2002), indicating a possible early involvement of GHRH in the regulation of GH release. Whereas in the sea bass, sculpin and grey mullet (*Mugil cephalus*) GHRH-ir nerve fibers, originating in the hypothalamus, projected to the RPD (Marivoet *et al.*, 1988; Moons *et al.*, 1989; Olivereau *et al.*, 1990), in eel, carp, goldfish, salmonids and cod, GHRH-ir fibers did not enter the RPD (Pan *et al.*, 1985; Olivereau *et al.*, 1990). Double staining studies showed a close relationship between GHRH-ir nerve fibers and ACTH cells (Marivoet *et al.*, 1988; Moons *et al.*, 1989).

PACAP-ir fibers were seen going through the PN and in close proximity to SL-, POMC (MSH)- and endorphin-ir endocrine cells in the PI of the stargazer (Matsuda *et al.*, 2005a,b) and European eel (Montero *et al.*, 1998). In addition, PACAP-ir fibers were found in the PPD of European eel (Montero *et al.*, 1998), goldfish (Wong *et al.*, 1998) and stargazer (Matsuda *et al.*, 2005b). PACAP-ir fibers were observed in the proximity of somatotrophs in goldfish (Wong *et al.*, 1998) as well as young stargazer but not in adults (Matsuda *et al.*, 2005b). In contrast, no PACAP-ir fibers were found innervating GTH cells. In the RPD, PACAP-ir fibers were found ending in close proximity to PRL and ACTH cells (Montero *et al.*, 1998; Matsuda *et al.*, 2005a,b).

5.8. Melanocortin System

The central melanocortin system is composed of POMC-expressing neurons, neurons expressing the endogenous melanocortin antagonist, agouti-related protein (AgRP), and downstream targets of these neurons expressing central melanocortin receptors. The POMC gene encodes a complex precursor exhibiting three main domains, each containing an MSH peptide (see Section 1). α-MSH is found within the second domain as the N-terminal sequence of ACTH (Cerdá-Reverter *et al.*, 2003a). POMC is mainly produced in the pituitary and its post-transcriptional processing occurs in a tissue-specific manner. The proteolytic cleavage by proconvertase 1 (PC1) generates ACTH and β-lipotropin in the corticotropes in the RPD, whereas cleavage by PC1 and PC2 produces α-MSH and β-endorphin in the melanotropes in the PI. POMC is also expressed in some neuronal populations of the CNS, where it is mainly processed to α-MSH and β-endorphin (reviewed by Castro and Morrison, 1997).

The melanocortins ACTH and MSH are involved in a wide range of physiological functions and exert their physiological role by binding to a family of G-protein coupled receptors. In tetrapods, five melanocortin receptor subtypes (MC1R–MC5R) have been cloned (reviewed by Schiöth *et al.*, 2005). The zebrafish genome contains six MCR subtypes, since MC5R is duplicated, whereas only four subtypes were found in the genome of *Fugu rubripes* (MC1R, MC2R, MC4R and MC5Rl; Logan *et al.*, 2003).

Expression studies using *in situ* hybridization techniques have reported that POMC is exclusively produced in the lateral tuberal nucleus or Hv in salmon and goldfish brains (Salbert *et al.*, 1992; Cerdá-Reverter *et al.*, 2003a). Similarly, immunostaining studies using antibodies against either α-MSH or ACTH (1-39) commonly reported that melanocortin-ir is exclusively localized in the tuberal hypothalamus of several species (Kishida *et al.*, 1988; Bird *et al.*, 1989; Olivereau and Olivereau, 1990; Amano *et al.*, 2005; Forlano and Cone, 2007; Table 2). Generally, α-MSH-ir perikarya are located immediately adjacent to the hypothalamic floor in the posterior part of the ventral hypothalamus. In carp, MSH-ir has been observed also in the LH coinciding with the MCH neurons (Kishida *et al.*, 1988). Only one study reported the presence of ACTH-ir in the fish brain using an antibody directed against the α-MSH-corticotropin-like intermediate peptide (CLIP) transition of carp ACTH (10Gly–23Tyr) that did not cross-react against α-MSH or CLIP. ACTH-ir cell bodies were localized in the PMgc. ACTH-containing cell bodies were negative for AVT and no ACTH fibers were observed in the pituitary. The function of ACTH in the fish brain is unknown but POMC expression in the PM, as determined by PCR, is up-regulated during stress (Metz *et al.*, 2004). However, two previous studies in carp could not find melanocortin immunoreactivity in the PM after immunostaining with antiserum against synthetic α-MSH, synthetic ACTH (11-24) or salmon N-terminal POMC peptide (Kishida *et al.*, 1988; Bird *et al.*, 1989). α-MSH fibers are widespread in the teleost brain but pituitary innervation by melanocortinic axon terminals does not seem prominent (Table 1.3). Studies in goldfish reported that MSH fibers do not penetrate deep into the neurohypophysis. No MSH projections from hypothalamic neurons to the pituitary were seen in the barfin flounder (Amano *et al.*, 2005) but a dense tract was observed coursing ventrally through the pituitary stalk and terminating in the neurohypophysis of the rainbow trout. Some fibers were also intermingled in the pars distalis, suggesting a neuroendocrine role of α-MSH (Vallarino *et al.*, 1989). In addition, MSH cells in the LH in carp seem to project to the pituitary gland (Kishida *et al.*, 1988).

Atypically, melanocortin signaling is not exclusively regulated by the binding of endogenous agonists, since naturally occurring antagonists, agouti and agouti-related protein (AgRP), compete with melanocortin peptides by binding to MCRs. In mammalian species, AgRP is mainly produced within the hypothalamic arcuate nucleus, in the same neurons where NPY is expressed, and it is potent in inhibiting melanocortin signaling at MC3R and MC4R (Cerdá-Reverter and Peter, 2003). Binding studies with zebrafish receptors demonstrated that AgRP acts as a competitive antagonist at MC3R, MC4R and MC5R, all three of which are expressed in the brain (Song and Cone, 2007). Only one study to date has precisely localized AgRP mRNA in brain regions in teleost fish (Cerdá-Reverter and Peter, 2003) whereas one other has reported AgRP-ir in the fish brain (Forlano and Cone, 2007). Both studies identified AgRP production within the posterior region of the ventral hypothalamus of the sea bass and zebrafish, respectively. However, there is no information about the pituitary innervation by AgRP fibers. In the zebrafish, AgRP and α-MSH projections strikingly match the nuclei that express MC4R (Cerdá-Reverter *et al.*, 2003b) and MC5R mRNA (Cerdá-Reverter *et al.*, 2003c) in the goldfish.

5.9. Neuropeptide Y (NPY) Family of Peptides

The neuropeptide tyrosine (NPY) family of peptides consists of 36-amino acid peptides exhibiting carboxy terminal (C-terminal) amidation (Cerdá-Reverter and Larhammar, 2000). The family comprises three different peptides: the NPY, tyrosine–tyrosine peptide (PYY) and the pancreatic polypeptide (PP). Tetrapod species produce all three peptides, whereas non-tetrapod vertebrates have only two types, i.e., NPY and PYY. Teleost fish, which have undergone a third round of genome duplication, synthesize two different versions of each peptide, i.e., NPY and PYY but no PP (Sundström *et al.*, 2008). Several studies in fish have reported the involvement of NPY in the control of the endocrine secretion of adenohypophysial cells (see Chapters 4, 5 and 6, this volume) including GH and LH (Kah *et al.*, 1989; Peng *et al.*, 1993; Cerdá-Reverter *et al.*, 1999).

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Studies in ayu (*Plecoglossus altivelis*) demonstrated that neurohypophysial NPY innervation exhibits seasonal variations, supporting an involvement in the regulation of the reproductive axis. Double immunostaining has occasionally demonstrated close apposition of NPY terminals to the GnRH cells in the preoptic area. However, NPY and GnRH terminals were distinct though intermingled in the neurohypophysis (Chiba *et al.*, 1996). Similar apposition was found in the median eminence of the gar (*Lepisosteus oculatus*, Chiba, 2005) and catfish (Gaikwad *et al.*, 2005). Ultrathin sectioning in the latter species demonstrated that neurosecretory axons carrying NPY-ir gold particles were occasionally seen in close association with GnRH-containing cells in the PPD, suggesting the involvement of NPY in the regulation of GnRH secretion. In fact, the central administration of NPY induced increases of the GnRH contents in the olfactory bulb, medial olfactory tract, telencephalon/preoptic area and pituitary (Gaikwad *et al.*, 2005).

Immunochemical and *in situ* hybridization studies have localized NPY neuronal cell bodies in several hypophysiotropic areas in the teleost brain. The rostral-most NPY immunoreactivity in the teleost brain is localized in the olfactory bulb. In the sea bass, there is a conspicuous NPY-mRNA expressing population within the internal cell layer (ICL; Cerdá-Reverter *et al.*, 2000). Some controversy exists about NPY production in the nucleus olfactoretinalis or TNgc. Immunostaining studies reported the presence on NPY-ir within the TNgc of catfish (Gaikwad *et al.*, 2004), ayu (Chiba *et al.*, 1996), gar (Chiba, 2005), tilapia (Sakharkar *et al.*, 2005), zebrafish (Mathieu *et al.*, 2002), paltyfish (Cepriano and Schreibman, 1993) and killifish (Subhedar *et al.*, 1996) but no immunoreactivity was detected in the same structure of rainbow trout (Danger *et al.*, 1991), carp (Marchetti *et al.*, 2000), Senegalese sole (Rodríguez-Gómez *et al.*, 2001), goldfish (Pontet *et al.*, 1989) or catfish (*Clarias garepimnus*; Zandbergen *et al.*, 1994). Expression studies in the sea bass (Cerdá-Reverter *et al.*, 2000), goldfish (Peng *et al.*, 1994; Vecino *et al.*, 1994) and salmon (Silverstein *et al.*, 1998) have never reported expression of NPY gene in the TNgc. The ventral telencephalon is considered as a major component of the NPYergic system in teleost and non-teleost fish (Cerdá-Reverter and Larhammar, 2000). Gene expression or immunoreactivity of NPY has been reported in the Vd, Vv, Vl, Vs, Vc (or NE) nuclei of the ventral telencephalon. The Vc probably exhibits the highest levels of NPY production in the teleost brain and has been reported as the NPYergic nucleus in all studied fish species (see references above). Neurons in the Vs, Vv and Vc may participate in the control of pituitary since they project to the pituitary in the goldfish (Table 1.1). Studies in male tilapia and goldfish suggest that NPY neurons in the Vc may act as an important center for processing the sex-steroid signaling (Peng *et al.*, 1994; Sakharkar *et al.*, 2005). Actually, castration dramatically increases NPY-ir in the Vc whereas testosterone reverses the effects (Sakharkar *et al.*, 2005).

Studies have also reported the presence of NPY-ir or mRNA transcripts in the preoptic area of teleost fish but cell numbers are much lower than those observed in the telencephalon. However, studies in catfish (Zandbergen *et al.*, 1994) and carp (Marchetti *et al.*, 2000) failed to demonstrate NPY-ir in the entire preoptic area, whereas studies in goldfish (Pontet *et al.*, 1989) and Senegalese sole (Rodríguez-Gómez *et al.*, 2001) found NPY-ir to be confined to the most caudal area of the PPp. The precise location of the NPY cell bodies within the preoptic area differs among species. The most rostral NPY population has been reported in the PPa of the sea bass (Cerdá-Reverter *et al.*, 2000), ayu (Chiba *et al.*, 1996), killifish (Subhedar *et al.*, 1996), male tilapia (Sakharkar *et al.*, 2005), zebrafish (Mathieu *et al.*, 2002) and catfish (Gaikwad *et al.*, 2004). In the two last species, the PPa is the only NPY-producing nucleus in the preoptic area. NPY-ir or gene expression has also been reported in the PM of the goldfish (Peng *et al.*, 1994), killifish (Subhedar *et al.*, 1996) tilapia (Sakharkar *et al.*, 2005) and salmonids (Danger *et al.*, 1991; Silverstein *et al.*, 1998). Expression in the PM is up-regulated by treatment with sex steroids in goldfish (Peng *et al.*, 1994) but not in male tilapia (Sakharkar *et al.*, 2005) and no seasonality of NPY-ir was reported in the preoptic area of the ayu (Chiba *et al.*, 1996). Finally, NPY-ir and/or expression has also been reported in the tuberal hypothalamus of several species, including ayu (Chiba *et al.*, 1996), sea bass (Cerdá-Reverter *et al.*, 2000), Senegalese sole (Rodríguez-Gómez *et al.*, 2001), zebrafish (Mathieu *et al.*, 2002) and male tilapia (Sakharhar *et al.*, 2005). In the sea bass, NPY-expressing cells are located in the Hv, NAT and Hc. Immunoreactive levels are not affected by castration and subsequent testosterone treatment in male tilapia (Sakharkar *et al.*, 2005) but undergo a seasonal variation in ayu (Chiba *et al.*, 1996). This seasonality of NPY immunoreactivity is concomitant with that observed in the NPYergic neurohypophysial fibers, thus suggesting its participation in the control of pituitary secretion.

5.10. Orexins

Orexins, also called hypocretins, are excitatory neuromodulatory peptides produced from a common precursor of the incretin family (de Lecea *et al.*, 1998). Hypocretin 1 and 2 exert their biological function by binding to two G-protein coupled receptors which exhibit different affinity for the hypocretins as well as different distribution in the CNS (Sutcliffe and de Lecea, 2000).

In fish, hypocretins were initially characterized in pufferfish, by searching in the public DNA sequence database (Alvarez and Sutcliffe, 2002). Unlike mammals, only one receptor has been characterized in zebrafish, pufferfish, medaka and three-spined stickleback (Yokogawa *et al.*, 2007). Studies in several species have demonstrated that hypocretins stimulate food intake (Nakamachi *et al.*, 2006), whereas fasting raises orexin mRNA levels in the hypothalamus (Novak *et al.*, 2005; Nakamachi *et al.*, 2006; see Chapter 9, this volume). In addition, orexin has been shown to increase locomotor activity in goldfish (Nakamachi *et al.*, 2006). Orexin overexpression induces an insomnia-like phenotype in zebrafish (Prober *et al.*, 2006). As in mammals, zebrafish lacking a functional orexin receptor exhibit disruptions in the consolidation of sleep/wake behavior but the phenotype of zebrafish orexin receptor mutant is only sleep fragmentation and decreased sleep in darkness (Yokogawa *et al.*, 2007; see Chapter 9, this volume). Studies in goldfish demonstrated that orexin-containing neurons are found in the same areas where the MCH hormone neurons are localized (Huesa *et al.*, 2005; Nakamachi *et al.*, 2006), i.e., LH, ZL and PTN. Neurons in the ZL correspond to the PVO-associated neuronal population expressing MCH (see above). Only the latter orexin neuronal population was described in medaka (Amiya *et al.*, 2007). Studies in zebrafish have identified two prominent orexin-ir neuronal groups in the preoptic area and tuberal hypothalamus (Table 1.2). Within the preoptic area, orexin neurons were localized in the PMmc and PMgc, PPp and NSC. The second cluster was found dorsally above the lateral recess, coinciding with the cell population described in goldfish and medaka. Only the tuberal hypothalamic population could be considered to be synthesizing hypocretin neurons since both orexin-mRNA and processed peptide were found. On the contrary, the preoptic neuronal population lacks preprohypocretin mRNA (Kaslin *et al.*, 2004). The vast majority of the orexin fibers from the preoptic area were directed to the pituitary through the preoptico-hypophysial tract, turning ventrally behind the optic chiasm and running the ventral surface of the hypothalamus to the pituitary. No orexin-immunoreactive fibers were found in the pituitary of medaka (Amiya *et al.*, 2007) but fibrous staining profiles were found in the sea perch (*Lateolabrax japonicus*) neurohypophysis (Suzuki *et al.*, 2007). Orexin-ir cells were reported in the pituitary of medaka (Amiya *et al.*, 2007) and sea perch (Suzuki *et al.*, 2007). In sea perch, orexin-ir was located in the GH cells, both peptides coexisting in the secretory granules.

5.11. RF-amide Peptides

The first member of this class of peptides was discovered in molluscs by Price and Greenberg (1977). Since then, increasing immunohistochemical evidence has shown that peptides similar to molluscan FMRF-amide exist in the brain of vertebrates. For instance, FMRF-amide-ir was found in the TNgc in the goldfish co-expressed with GnRH (Stell *et al.*, 1984). Similarly, in swordtail (*Xyphophorus helleii*), FMRF-amide-ir was localized in cell bodies of the TNgc (Magliulo-Cepriano *et al.*, 1993), sometimes colocalizing with GnRH-ir (Table 1.2). Projections of these cells could be traced to the rostral area of the tuberal hypothalamus through the preoptic area. However, fibers containing FMRF-amide-ir had a broader distribution than GnRH fibers, being also found in the dorsal forebrain, OT and Hc (Magliulo-Cepriano *et al.*, 1993). Of particular note was the fact that fiber tracts that reached the tuberal hypothalamus had completed their development by the time sexual maturation was achieved. Additionally, in both catfish and swordtail, FMRF-amide-ir fibers that originate in the TNgc innervate all subdivisions of the pituitary gland (Rama Krishna *et al.*, 1992; Magliulo-Cepriano *et al.*, 1993). However, the endogenous peptide responsible for this immunoreaction still remains unknown (Table 1.3). In this context, several neuropeptides with an RF-amide motif have been identified in fish such as prolactin-releasing peptide (PrRP; Moriyama *et al.*, 2002; Seale *et al.*, 2002; Sakamoto *et al.*, 2003b; Montefusco-Siegmund *et al.*, 2006; Amano *et al.*, 2007), RF-amide-related peptide (RFRP; Hinuma *et al.*, 2000; Fukusumi *et al.*, 2001; Ukena *et al.*, 2002), other LPXRF-amides (Tsutsui and Ukena, 2006) and kisspeptin (van Aerle *et al.*, 2008).

PrRP was discovered from bovine hypothalamic extracts as a ligand for the anterior pituitary orphan G-protein coupled receptor (hGR3; Hinuma *et al.*, 1998). This peptide showed a capacity to release PRL (Hinuma *et al.*, 1998; Fujimoto *et al.*, 2006). PrRP homologues have been isolated from brain extracts of Japanese crucian carp (*Carassius cuvieri*; Fujimoto *et al.*, 1998), goldfish (Kelly and Peter, 2006), tilapia (Seale *et al.*, 2002), chum salmon (Moriyama *et al.*, 2002), and Atlantic salmon (Montefusco-Siegmund *et al.*, 2006). In teleost, PrRP facilitates *in vitro* and *in vivo* PRL secretion (Moriyama *et al.*, 2002; Seale *et al.*, 2002; Sakamoto *et al.*, 2003a,b), elevates PRL gene expression (Sakamoto *et al.*, 2003a) and regulates osmotic balance and food intake in goldfish (Fujimoto *et al.*, 2006; Kelly and Peter, 2006). Immunocytochemical staining in rainbow trout revealed that PrRP-ir cell bodies were located in the posterior part of hypothalamus (Moriyama *et al.*, 2002). In addition, in adult guppies, PrRP-ir cell bodies were detected in the Hv (Amano *et al.*, 2007). However, in the Atlantic salmon, immunoreactivity to the synthetic putative PrRP peptide was found mainly in the cerebellum and few weakly stained cell bodies in the Hc, and almost no positive fibers were seen in the pituitary stalk (Montefusco-Siegmund *et al.*, 2006). In the lamprey, two RF-amide peptides (A and B) homologous to teleost PrRP were identified (Moriyama *et al.*, 2007). In this study, using an anti-salmon PrRP antiserum, PrRP-ir cell bodies were observed in the ventral part of the periventricular arcuate nucleus of the hypothalamus (most probably corresponding to Hv). Within the pituitary, a small number of PrRP-ir fibers were observed adjacent to the PRL cell in the guppy (Amano *et al.*, 2007).

Kisspeptins, endogenous peptides displaying agonist activity on the orphan G-protein coupled receptor 54 (GPCR54), were isolated from human placenta (Ohtaki *et al.*, 2001). These 54-, 14-, 13-, and 10-amino acid peptides, with a common RF-amide C-terminus, are derived from the product of KISS-1, a metastasis suppressor gene for melanoma cells. It has recently been demonstrated in mammals that kisspeptins are critical factors for the onset of puberty (Tena-Sempere, 2006). GPCR54 has been studied in very few teleost species but was found to be expressed in GnRH neurons, while the number of neurons expressing GPCR54, as well as the level of GPCR54 expression, increased with gonadal maturation (Parhar *et al.*, 2004). A kisspeptin counterpart has been recently identified in fish by computational genome analysis (van Aerle *et al.*, 2008), but no brain and/or pituitary mapping specific for kisspeptins has been carried out yet. Of note, the FMRF-ir material studied in platyfish and swordtail (Magliulo-Cipriano *et al.*, 1993) may represent kisspeptin since, as was described above, it was shown to reach its maximum development with adulthood and sexual maturation innervating nucleus in the basal hypothalamus.

5.12. Somatostatin

Somatostatin (SS) is a potent inhibitor of basal and stimulated GH secretion in teleosts (Canosa *et al.*, 2007). This peptide belongs to a multifunctional family of peptides which includes up to three different SS precursors encoded by different genes in teleosts (for reviews, see Nelson and Sheridan, 2005; Canosa *et al.*, 2007). The most conserved form, PSS-I, encodes for SS-14 at its C-terminus and has an identical amino acid sequence in all vertebrate species studied. A second cDNA only isolated from teleosts, named PSS-II, encodes for SS peptides of variable length between 22 and 28 amino acids. These peptides are characterized by [Tyr7,Gly10]SS-14 at their C-terminus. PSS-III has been isolated from several species of fish and other vertebrates (Nelson and Sheridan, 2005; Canosa *et al.*, 2007). The peptides encoded by PSS-III characteristically bear proline in the second position of the 14-amino acid C-terminus peptide. The biology of these alternative peptides is not fully understood and most of the information on brain localization and pituitary innervation is based on immunostaining to SS-14. Although anti-SS-14 antibodies may cross-react against other SS peptides (Canosa *et al.*, 2004), the data obtained by immunostaining is considered as SS-14.

Early work in goldfish showed SS-ir in the PPa, PM and PPp in the preoptic area, the Hv in the tuberal hypothalamus and the ventral thalamus (Kah *et al.*, 1982) (Table 2). The distribution of SS in the brains of several teleosts was studied by Olivereau *et al.* (1984b), who found the SS-ir present in the Vc, PPa, PPp, Hv and Hc. In tilapia, SS-ir neurons were located in the anterior part of the Dl and Vc in the telencephalon, the preoptic area and the tuberal hypothalamus (Grau *et al.*, 1985). Brain localization of SS perikarya in the green molly showed SS cells in the Vl and Vc in the telencephalon, PPa, PM and rostral area of the PPp of the preoptic area, Hv and NAT and Hc of the tuberal hypothalamus (Batten *et al.*, 1985). A similar distribution pattern of SS-ir cell bodies was shown in the European minnow and the European eel (Vigh-Teichmann *et al.*, 1983). Many of these SS-ir cell bodies are CSF-contacting neurons, particularly those in the anterior, lateral and posterior recesses such as Hv and Hc. Furthermore, the brain distribution of SS-ir was studied in brown ghost knifefish, goldfish and sea bream (Sas and Maler, 1991; Pickavance *et al.*, 1992; Power *et al.*, 1996) and pointed to a similar distribution pattern to that mentioned in previous reports. In the hypothalamus of sea bream, SS-ir neurons were found in the PM, Hv and Hc and a network of varicose fibers projecting toward the pituitary through the preoptico-hypophysial tract (Power *et al.*, 1996).

Goldfish is the only teleost species in which three SS genes have been described (Lin *et al.*, 1999). *In situ* hybridization studies show that, although there is an overlapping brain distribution of these three mRNAs, co-expression is unlikely to occur in the same cell (Canosa *et al.*, 2004). The overall distribution of SS-positive cell bodies is in agreement with previous reports, as mentioned above, in other species of fish as well as in other vertebrates. PSS-I and PSS-III overlap in the ventral telencephalon, tuberal hypothalamus, ventral and dorsal thalamus and most areas of the ventroposterior hypothalamus, the mesencephalon and hindbrain. On the other hand, PSS-II was restricted to some nuclei in the hypothalamus and ventral thalamus: Hv, NCLI, VM and NTP (Canosa *et al.*, 2004). One of the most striking differences in the expression pattern of PSS-I and PSS-III is found in the preoptic area, where the former is expressed in the PM but not in the anterior part of the PPp while the latter shows the opposite distribution (Canosa *et al.*, 2004).

All three PD zones are innervated by SS-ir fibers (Table 1.3). In sculpin, the green molly and the catfish, SS-ir fibers in the caudal PN were found to approach the PI and to end among the MSH and SL cells (Olivereau *et al.*, 1984a; Batten *et al.*, 1999). Double immunostaining for both SS and MSH in the molly suggested that SS fibers innervate groups of MSH cells in the PI (Batten *et al.*, 1985). SS-ir fibers were also found to penetrate into the PPD in several species of fish (Kah *et al.*, 1982; Olivereau *et al.*, 1984a,b; Batten *et al.*, 1985; Grau *et al.*, 1985; Moons *et al.*, 1989; Sas and Maler, 1991; Power *et al.*, 1996). SS-ir fibers were found in contact with GH cells (Moons *et al.*, 1989; Power *et al.*, 1996) or in contact to the basal lamina GH and GTH islets where the GH cells are located peripherally (Olivereau *et al.*, 1984a,b; Batten *et al.*, 1999). In tilapia, SS-ir fibers were clearly visualized within the RPD where they innervate the PRL cells (Grau *et al.*, 1985). This pattern of SS distribution seems to be specific to tilapia since neither killifish nor mudsucker (*Gillichthys mirabilis*) showed SS-ir in the RPD (Grau *et al.*, 1985). Additionally, SS fibers were scarce or absent and did not innervate PRL or ACTH cells in the sea bream (Power *et al.*, 1996) and were never seen to penetrate the RPD of salmonid species (Olivereau *et al.*, 1984a). However, in sea bass, double staining showed SS fibers in close contact with ACTH cells (Moons *et al.*, 1989). Furthermore, in the sea bass and, to some extent, the Indian catfish, SS-ir terminals were seen to make synaptoid contact with the basal membrane in the RPD (Batten *et al.*, 1999).

5.13. Thyrotropin-releasing Hormone (TRH)

Thyrotropin-releasing hormone (TRH) was the first hypothalamic releasing factor to be chemically characterized from pig and sheep hypothalami (Schally *et al.*, 1969; Guillemin, 1970). In tetrapods but not amphibians, its role in controlling pituitary TSH release is well established (Fliers *et al.*, 2006). In teleost fish, TRH stimulates several pituitary hormones such as PRL (Batten and Wigham, 1984; Wigham and Batten, 1984; Barry and Grau, 1986), GH (Canosa *et al.*, 2007) and MSH (Tran *et al.*, 1989; Lamers *et al.*, 1991), but its actions on the pituitary–thyroid gland axis are more controversial (see Chapter 6, this volume) since contradictory results have been published, showing either stimulatory (Tsuneki and Fernholm, 1975; Eales and Himick, 1988), no (Peter and McKeown, 1975) or even inhibitory effects (Bromage, 1975). There is only one report showing TRH innervation in the PPD of catfish (Batten *et al.*, 1999) (Table 3). In sea bass, small TRH-ir cell bodies were only found in NAT and the region of the Hv coating the lateral recess (Batten *et al.*, 1990b) and in the PPa in the green molly (Batten *et al.*, 1990a) (Table 2). Similar results have been observed in carp (Hamano *et al.*, 1990). In salmonid fish, on the other hand, the distribution of TRH cell bodies seems much wider. In chinook salmon, TRH-positive cell bodies are observed in the internal cellular layer of the olfactory bulb, the supracommissural nucleus of the ventral telencephalon (Vs) and the preoptic region. In the latter area, the TRH-ir cell bodies were observed in the PMmc (Matz and Takahashi, 1994). Similar results have been shown in sockeye salmon using *in situ* hybridization (Ando *et al.*, 1998). More recently, in trout and zebrafish, TRH-ir perikarya were observed in the OB and several areas of the ventral telencephalon, in the preoptic and suprachiasmatic region, the ventromedial hypothalamus, and some thalamic nuclei (Díaz *et al.*, 2001, 2002) (Table 2).

6. HYPOTHALAMIC NEUROTRANSMITTERS

6.1. Amino Acid Neurotransmitters: Glutamate and Gamma-amino Butyric Acid (GABA)

Glutamate is considered the major excitatory neurotransmitter in the vertebrate CNS (Nakanishi, 1992; Trudeau *et al.*, 2000b). Glutamate is also an important hypophysiotropic regulator and is involved in the control of LH, GH, PRL (Trudeau *et al.*, 1996; Holloway and Leatherland, 1997; Trudeau *et al.*, 2000b; Bellinger *et al.*, 2006) and probably MSH or SL since glutamate-ir fibers were found in the NIL (Trudeau *et al.*, 1996). Moreover, glutamatergic fibers have been revealed by immunohistochemistry in goldfish pituitary (Trudeau *et al.*, 1996). However, in teleost fish, there is no report showing the central origin of this glutamatergic innervation (Table 1.3).

Gamma-amino butyric acid (GABA) represents the major inhibitory neurotransmitter in the CNS, although excitatory responses to GABA have been reported (Wagner *et al.*, 1997). In addition, GABA has an important role in the control of pituitary hormone secretion (Khan and Thomas, 1999; Mañanos *et al.*, 1999; Trudeau *et al.*, 2000a,b; Martyniuk *et al.*, 2007b). GABA is synthesized principally from glutamate in a single enzymatic step catabolized by the enzyme glutamic acid decarboxylase (GAD). This enzyme is therefore used as a marker for GABAergic cell bodies and fibers (Kah *et al.*, 1987; Martinoli *et al.*, 1990; Medina *et al.*, 1994; Anglade *et al.*, 1999). Thus, in goldfish, GAD-ir cell bodies were detected in the olfactory bulbs and telencephalon. In the diencephalon, GABA-containing cell bodies were found in the hypothalamus, particularly in the preoptic and tuberal regions. Furthermore, the ventroposterior hypothalamus, dorsal and ventromedial thalamus and pretectal area exhibited numerous GABA-positive perikarya (Martinoli *et al.*, 1990). A similar distribution of GAD-ir cell bodies was revealed in the forebrain of the silver eel (Medina *et al.*, 1994) and rainbow trout (Anglade *et al.*, 1999). Employing non-radioactive *in situ* hybridization, the distribution of GABAergic cell bodies was analyzed in goldfish brain (Martyniuk *et al.*, 2007a). In this report, transcripts for GAD65, GAD67, along with the GABA-metabolizing enzyme GABA-T, were mainly detected in the medial and ventral regions of the telencephalon, the PM in the preoptic area and the lateral aspect of the Hv, Hc. Several reports have shown that GABA plays a role in regulating LH secretion (Kah *et al.*, 1992; Sloley *et al.*, 1992; Trudeau *et al.*, 1993; Anglade *et al.*, 1998; Trudeau *et al.*, 2000b). Moreover, GABAergic fibers have been found innervating all pituitary lobes in goldfish (Kah *et al.*, 1987).

6.2. Dopamine

Dopamine (DA) is an important neurotransmitter in the CNS of vertebrates and possesses key hypophysiotropic functions. Using antibodies or riboprobes to detect tyrosine hydroxylase, an enzyme involved in DA synthesis, the dopaminergic system in the fish nervous system has been widely studied (Ma, 1997; Smeets and González, 2000; Rink and Wullimann, 2001). In addition, studies using antisera toward DA itself have also been performed (Meek *et al.*, 1989; Roberts *et al.*, 1989; Pierre *et al.*, 1997).

In fish, the highest concentration of dopaminergic neurons is localized in the posterior tuberculum and adjacent hypothalamic regions (see Ma, 2003; Ma and Lopez, 2003, and references therein). In particular, large numbers of dopamine-containing neurons are found in nuclei closely associated with the ventricle and its recesses (Table 1.4). Besides, dopaminergic neurons are located in the olfactory bulbs, the ventral regions of the preoptic area and

Table 1.4
Main hypophysiotropic territories containing neurotransmitters

Neurotransmitters	Telencephalon	Preoptic area	Hypothalamus
DA	OB	PPa, PMmc, PMgc, PPp, NSC	Hv, Hc, PVO
GABA	OB, ventral TEL	PPa, PMpc, PMmc, PPp NSC	Hv, LH, Hc, NAT, PVO, NDLI
Serotonin	OB, Vv	PMpc, PPp, NSC	Hv,Hd, Hc, PVO

Nomenclature of nuclei was adapted following Bradford and Northcutt (1983).
See Table 1.1 for abbreviations of hypophysiotropic territories.

tuberal hypothalamus (Meek *et al.*, 1989; Roberts *et al.*, 1989; Pierre *et al.*, 1997). Early studies have shown that the preoptic area and the posterior hypothalamus in goldfish contain dopaminergic neurones that project toward the pituitary gland innervating all three zones of the PD (Kah *et al.*, 1984; Fryer *et al.*, 1985; Kah *et al.*, 1986). In Atlantic salmon, major dopaminergic innervation of the pituitary originates from a subset of the anterior region of the PPp and the suprachiasmatic nucleus (Holmqvist and Ekström, 1995). In zebrafish, dopaminergic neurons have mainly been found in the preoptic area in the PPa, PM, NSC, the posterior tuberculum, and the ventroposterior hypothalamus (Ma, 2003). More recently, in the European eel, it has been shown that dopaminergic neurons innervating the gonadotropes originate in the rostral region of the PPa and these cells are responsive to steroid hormones (Weltzien *et al.*, 2006).

6.3. Serotonin

Serotonin or 5-hydroxytryptamine (5-HT) is an indoleamine neurotransmitter that constitutes the monoamine group along with the catecholamine neurotransmitters DA and NA. It has been shown that 5-HT has both behavioral (De Pedro *et al.*, 1998; Lin *et al.*, 2000; Johansson *et al.*, 2004) and neuroendocrine functions (Khan and Thomas, 1992; Trudeau, 1997; Winberg *et al.*, 1997; Canosa *et al.*, 2007). The distribution of serotoninergic cells and fibers has been studied in several vertebrate species, including fish (Kah and Chambolle, 1983; Margolis-Kazan *et al.*, 1985; Ekström and Ebbesson, 1989; Meek and Joosten, 1989; Johnston *et al.*, 1990; Khan and Thomas, 1993; Rodríguez-Gómez *et al.*, 2000b). In summary, there are two main localizations of the 5-HT system in the teleost brain: one anterior, which covers the nuclei associated with the ventricle and its recess in the caudal hypothalamus, and one posterior in the brainstem (Table 1.4).

The rostral 5-HT system mainly consists of CSF-contacting cell bodies in the PVO, where it extends to the ventromedial hypothalamus and the preoptic area (Johnston *et al.*, 1990; Khan and Thomas, 1993). Although a direct effect of 5-HT on pituitary LH secretion has been demonstrated (Somoza and Peter, 1991; Khan and Thomas, 1992), several studies in teleosts have shown very low or even undetectable levels of 5-HT or its major metabolite, 5- hydroxyindoleacetic acid (5-HIAA), in the pituitary (Sloley *et al.*, 1992; Hernández-Rauda *et al.*, 1996). Hernández-Rauda and Aldegunde (2002) analyzed the role of neurotransmitters during gonadal development in yellow snapper (*Lutjanus argentiventris*) and found that neither 5-HT nor 5-HIAA was detectable in the pituitary at any gonadal stage studied. Similarly, serotoninergic innervation of the pituitary in sea bass is scant (Batten *et al.*, 1993). In the Senegalese sole, serotoninergic-ir fibers were absent in the pituitary (Rodríguez-Gómez *et al.*, 2000b). However, 5-HT-ir has been located in the PPD of goldfish (Kah and Chambolle, 1983), and serotoninergic fibers were observed in the pituitary stalk and in all regions of the pituitary gland of the catfish (Corio *et al.*, 1991). Furthermore, serotoninergic fibers were found in PPD and PI of the platyfish (Margolis-Kazan *et al.*, 1985) and the Atlantic croaker (Khan and Thomas, 1993). In the pituitary of the Altantic croaker (*Micropogonias undulatus*), the strongest 5-HT-ir was observed in the PPD while a few scattered cells were bordering the PI and their fibers were contacting the endocrine cells in the PPD (Khan and Thomas, 1993) (Table 3).

7. CONCLUDING REMARKS

The hypothalamus–pituitary complex in teleost species shows a particular specialization in which the median eminence is greatly reduced or absent. As a consequence, the hypothalamic control of the pituitary is exerted by an important pituitary innervation that penetrates to the adenohypophysis as interdigitation of neuronal tissue. This neuronal circuitry integrates incoming information from both external and internal environments by expressing the appropriate set of hormonal receptors (Chapter 2, this volume), as well as through interneuronal communication. There is some information about how these neuronal circuits are regulated by internal factors and how sensory information is conveyed to the "regulatory brain." However, little is known about how these hypothalamic regulatory systems integrate both internal and external information and how they elaborate a coordinated final response. How do these neuronal regulatory systems interplay to coordinate different physiological functions, i.e., nutrition and reproduction?

Recent retrograde tract-tracing studies have corroborated earlier studies showing the preoptic area and tuberal hypothalamus as loci for the neuronal cell bodies whose axons reach the neuro- and adenohypophysis along well-defined fiber tracts (see Section 3). These neurons release hormones to the vascular system (see Section 4) or use different releasing peptides (see Section 5) and neurotransmitters (see Section 6) to control a wide array of physiological functions through their anatomical and functional links with the pituitary gland. Studies using double-labeling, tract-tracing, and pituitary cell culture have led to the localization of the neurons that regulate the different secretory cells of the pituitary gland, as well as the identification of the releasing peptides and/or neurotransmitters used by these neuronal systems. Direct innervation within the RPD, in proximity of either ACTH or PRL cells, is exerted by neurons that contain AVT, IST, MCH, GHRH, PACAP, SS, CRF, galanin, CCK and PrRP. The endocrine cells of the PPD (GH, LH, FSH and TSH) are innervated by fibers containing AVT, IST, NPY, GHRH, PACAP, SS, CRF, galanin, GnRH and CCK, and to a lesser extent by TRH-containing neurons. Finally, the PI is interdigitated by neuronal fibers containing AVT, IST, MCH, NPY, GHRH, PACAP, SS, CRF, galanin, GnRH, CCK, BBS and TRH, in close relationship with either MSH or SL cells. Neurosecretion within the PN mainly involves AVT, IST and MCH neurons.

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