The Neurohypophysis and Urophysis: Ancient Piscine Neurovascular Interfaces

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Abstract

Vertebrate homoeostasis is regulated by secretion of neurohormones from specialized neuroendocrine neurovascular interfaces such as the hypothalamic–neurohypophyseal system (HNS). Fish are shown to possess an additional caudal neurosecretory system (CNSS), which is termed urophysis, due to its anatomical location at the caudal spinal cord and its structural similarity to the hypophysis gland. The urophysis is a vascularized gland-like structure, which is interfaced by exceptionally large neurons termed Dahlgren cells. In contrast to the well-studied HNS of fish and mammals, the development and function of the urophysis/CNSS are not well understood, and related research has strongly declined in the last three decades. In this chapter, we summarize the main knowledge regarding the evolution, development and structure of the two neuroendocrine interfaces. Additionally, we describe the main knowledge regarding their regulatory and functional roles in fish homoeostasis. Where applicable, a general comparison to non-piscine vertebrates is described.

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4.1 Introduction

Neuroendocrine regulation of homoeostasis in most vertebrates is mainly orchestrated by the hypothalamus, a brain region whose neurons either affect the anterior pituitary gland by means of a vascular portal system, or directly form neurovascular interfaces with the capillary network of the posterior pituitary gland, the neurohypophysis, to release neurohormones into the circulation (Wircer et al. 2016; Biran et al. 2018). Interestingly, piscines uniquely possess an additional homoeostatic neurovascular interface known as the caudal neurosecretory system (CNSS, Fig. 4.1). In 1914, Dahlgren identified huge secretory cells residing in the spinal cord of elasmobranchs (Dahlgren 1914). A few years later, Speidel performed a systematic analysis of the caudal spinal cord of various fish species and identified the cells of Dahlgren in 26 out of 30 species he examined. Moreover, Speidel found that in more evolved fishes, Dahlgren cells innervate a vascularized glandular structure that shares high structural homology to the neurohypophysis (Speidel 1922). This glandular structure was later termed the urophysis. These discoveries initiated a great deal of research which resulted in the identification of novel

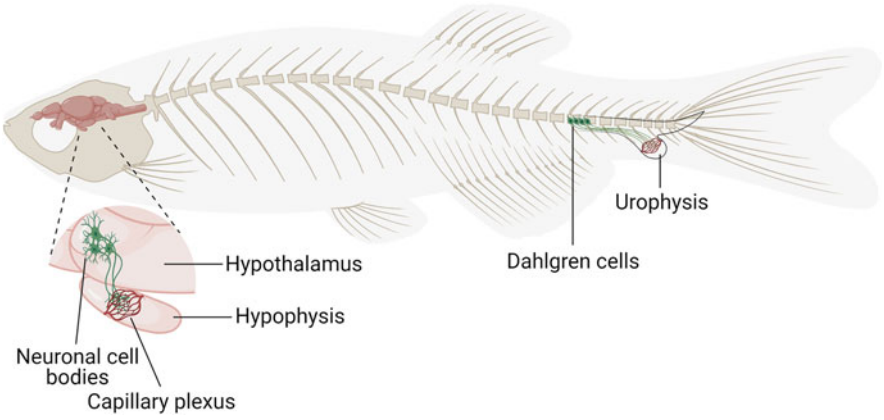

Fig. 4.1 Schematic representation of the neurohypophysis and urophysis in fish. The neurohypophysis is located in the posterior pituitary of the zebrafish brain, with axonal projections coming in from the hypothalamus. The axonal projections are interspersed within the vascular plexus of the posterior pituitary (magnified schema). The urophysis is located on the caudal region of the spinal cord with projections coming in from the Dahlgren cells located along the spine

neuropeptides affecting blood pressure—the urotensins, which were later shown to be functionally important in other vertebrates. Importantly, while the neurohypophysis releases its neuropeptides to the adenohypophysis and to the general circulation, vascular drainage of the urophysis delivers caudal neurohormones into the kidney, liver and swim bladder (Bern 1985). Despite the above findings, in the last twenty years there has hardly been any published information concerning the urophysis. This might be due to the uniqueness of the urophysis to fish species and the failure to identify a robust physiological function, which could be directly attributed to the CNSS (Bern 1985). In this chapter, we review some of the major findings regarding the piscine neurohypophysis and urophysis and their suggested neuroendocrine physiological functions in fishes.

4.2 The Hypothalamo–Neurohypophysis

The hypothalamo–neurohypophyseal system (HNS) is a neurosecretory interface, which is conserved across all vertebrate organisms. The fish HNS comprises two distinct populations of neurons that secrete the arginine-vasopressin-like (AVPL) and oxytocin-like (OXTL) neuropeptides, also known as arginine-vasotocin and isotocin, respectively, in all fish species other than zebrafish. In the interest of simplicity, we will henceforth refer to the piscine neurohypophyseal neurohormones as OXTL and AVPL. These cells reside in the piscine neurosecretory preoptic region (NPO) and posterior tuberculum (PT) of the fish diencephalon and project their axons onto the posterior pituitary, also known as the neurohypophysis, where they secrete their neurohormone cargo through a neuroendocrine–vascular interface (Biran et al. 2018). The termini of these neurons have distinctive swellings along their length which serve as synaptic release sites for their neurohormones (Tweedle et al. 1989). Upon their release, OXTL and AVPL are taken up by the fenestrated, i.e. permeable, capillary plexus of the neurohypophysis. The vasculature of this particular region is an extension of the cerebral vascular network; however, it possesses distinct qualities that allow for its selective permeability. Together, they also represent one of the key neurovascular interfaces, which will be discussed in length in a later section.

In addition to these components, the neurohypophysis also contains specialized astrocyte-like cells called pituicytes (Anbalagan et al. 2018; Chen et al. 2020). The pituicytes extend processes that engulf the secretory axonal termini, likely to act as a regulatory barrier to neurosecretion (Miyata 2017), like the glia of the fish urophysis, which were named urocytes (Kriebel 1980),

4.2.1 Evolution and Ontology of the Neurohypophysis

Box 4.1 The historical tale of the neurohypophysis
The hypothalamo–neurohypophyseal system has long posed enigmatic questions regarding its existence, and later its true function. The Dutch physiologist Van Rijnberk stated in 1901 that the posterior pituitary is a functionless rudimentary organ (Described in: Hackenberg and Etminan 2003). In 1908, Herring (Herring 1908) alluded to the presence of nerve fibres and neuroglia in the posterior pituitary, and described what would later be referred to as Herring bodies. A year later, Blair-Bell described the effects of pituitary extracts on atonic uteri during labour (Bell 1909); this work was in line with that of others, suggesting the physiological effects of pituitary extracts (Von den Velden 1913). However, the concept of neurosecretion in the neurohypophysis was first suggested in 1917, by Speidel (Scharrer 1987). This idea was carried forward by the seminal work of Ernst Scharrer in 1928 who described the histology of the European Minnow nucleus magnocellularis preopticus and revealed vacuoles/vesicles in nerve-gland cells in the diencephalon that may secrete hormones into the neurohypophysis (Scharrer 1928). In 1940, Ernst and Berta Scharrer published their neurosecretion concept of the hypothalamo–neurohypophyseal system (HNS), which was shown to be conserved across multiple vertebrate species (Scharrer and Scharrer 1940). The functionality of the HNS as a pathway for the secretion of neurohormones was finally accepted unanimously in 1949 after neurosecretory material in the hypothalamic neurons and the neurohypophysis were shown to be one and the same (Bargmann and Hild 1949).

The pars nervosa of the posterior pituitary is a conserved structure across all vertebrates, including over 34,000 piscine species. The structure and morphology of the HNS vary from the most primitive fishes, Cyclostomes, through to the higher vertebrates, including humans, however, the basic components of the system seem to remain constant.

Primitive fish, or the Elasmobranchs, lack a clear demarcation between the magnocellular (i.e. larger cells) and parvocellular cells (i.e. smaller cells) of the preoptic nuclei. Within the same class, we see larger cells, indistinguishable from each other, in the subclass of Holocephali. In more evolved fishes, the presence of two distinct populations of cells, the parvocellular and the magnocellular neurons, is noted from pre-teleosts through to the advanced teleosts (Wircer et al. 2016; Perks 1969).

As in pre-teleost bony fish, primitive teleosts such as the European eel (*Anguilla anguilla*) present a neurohypophyseal structure that is a thickened extension of the infundibular stalk. In advanced teleosts, this structure is better represented as a pituitary core, surrounded by adenohypophyseal tissue. Axonal innervations pass through the hypophyseal tract into the pars nervosa, characterized by bead-like

droplets along their length, carrying neurosecretory material. Multiple studies have shown the presence of granules or elementary vesicles in these swellings, containing neurohormones (Holmes and Knowles 1960; Navone et al. 1989; Anbalagan et al. 2019). While some researchers reported that the sizes of these elementary vesicles were similar to those found in the preoptic nucleus, other works demonstrated that two distinct elementary vesicles containing two different peptides may be present in the pars nervosa (Knowles et al. 1966; Leatherland and Dodd 1967).

Within the pars nervosa, the axonal termini are distributed amongst a dense capillary network that conveys neurosecretory material into the systemic circulation (Anbalagan et al. 2019). Within the purview of vascular structures of the pars nervosa, pre-teleostean fish, such as the longnose gar (*Lepidosteus osseus*), seem to possess a vascular portal system, while the teleostean structure lacks it (Sathyanesan and Chavin 1967).

The presence of neurohypophyseal glia, the pituicytes, is consistent across teleostean species. A striking result regarding these cells was observed in a few unrelated teleosts, European eel, European conger (*Conger conger*) and goldfish (*Carrassius auratus*) (Knowles and Vollrath 1966; Leatherland 1972). These studies showed that axonal bundles carrying neurosecretory material terminated not only around the dense capillary plexus, but also on the surface of the pituicytes (Knowles and Vollrath 1965). In primitive fish like the Cyclostomes, the pituicytes were described as being derived from the ependymal cells of the ventricles which proliferated into the pars nervosa (Green and Maxwell 1959). These ependymal cells were also found to be present in the Elasmobranchs, the spiny dogfish (*Squalus acanthias*), and were then described as "parenchymatous pituicytes" (Van de Kamer and Verhagen 1955).

4.2.2 Development of the Neurohypophysis

The neurohypophysis is formed as an invagination of the diencephalon floor, deepening to form the infundibular cavity. In zebrafish (*Danio rerio*), precursor cell clusters on both sides of the diencephalon merge to form a pituitary cluster at about 28 h post-fertilization (Glasgow et al. 1997; Chapman et al. 2005). Within 36 h post-fertilization, cell bodies from the NPO generate axonal convergence along the midline at the developing neurohypophysis (Gutnick et al. 2011). Structural analysis of the HNS in the adult European eel shows that axonal fibres projecting into the neurohypophysis are separated into bundles by the radial pituicytes (Knowles and Vollrath 1965). This suggests the possibility that the pituicytes reside in the pars nervosa during, and perhaps prior to axonal enervation.

Over the next 36 h, i.e. 72 h post-fertilization, the embryonic neurohypophysis undergoes vascularization, probably from angiogenic cues released by the axonal termini and astroglia in the region (Gutnick et al. 2011). The hypophyseal artery and vein grow into the developing region, conceivably from existing cerebral vasculature. Thus, endothelial vessels in the ventral diencephalon sprout towards the palatocerebral artery from 48 h post-fertilization, giving rise to the hypophyseal artery. At the same time, the primary head sinus sprouts bilaterally towards the

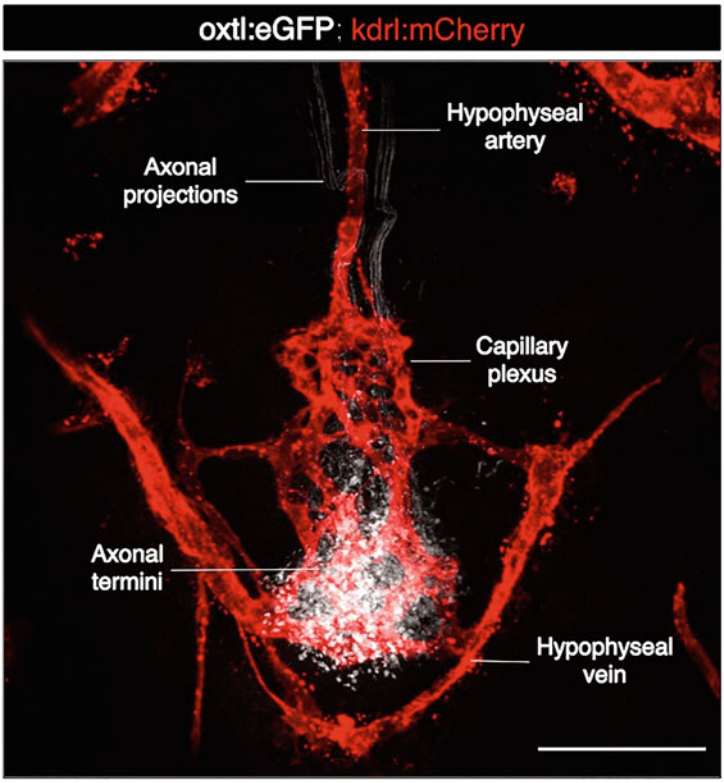

Fig. 4.2 Neurohypophysis in juvenile zebrafish. A confocal microscope image of a transgenic juvenile zebrafish (30-day old) in which both the hypothalamo–neurohypophyseal oxytocin neurons and blood vessels are genetically tagged with fluorescent proteins. The image shows the hypophyseal capillary plexus (in red) which is innervated by hypothalamic axonal projections (in grey) forming multiple neuro-vascular interfaces through which the oxytocin neurohormone is released into the peripheral blood circulation. *Oxtl* oxytocin-like, *kdrl* vascular endothelial growth factor receptor kdr-like

midline giving rise to the hypophyseal vein. By 72 h post-fertilization, these vascular branches fuse to create a loop-like structure dubbed the hypophyseal capillary (Gutnick et al. 2011). Thereafter, the hypophyseal capillary makes tight connections with the axonal termini, and along with the resident pituicytes, forms the basis of a functional neurovascular interface of the neurohypophysis (Fig. 4.2). As the animal develops further, the density of the axonal projections increases along with the complexity of the vasculature, forming an anterior and posterior capillary plexus with dense innervation of nerve fibres, and numerous pituicytes (Anbalagan et al. 2018; Gordon et al. 2019).

The adult neurohypophyseal structure bears clear differences in the structure of the pars nervosa (as outlined in the previous section) between the different classes of fish as well as in the interaction with intermediate lobes and the adenohypophysis parts of the pituitary. Non-teleostean fish, from the Elasmobranchs up to the

Holosteans, show the presence of neuronal projections from the pars nervosa projecting into the intermediate lobe of the pituitary. Remarkably, in teleosts the neural tissue invaginates into parts of the intermedia, extending all the way into the adenohypophysis (Perks 1969).

4.2.3 Neurohypophyseal Function

As in other vertebrates, the Piscine neurohypophyseal system is the primary region of secretion of two major homeostatic hormones, OXTL and AVPL. Together, these two neuropeptides regulate the homeostatic responses to various internal and external physiological challenges, ranging from water balance to social behaviour.

4.2.3.1 Osmoregulation

In mammals, AVP was first identified as an antidiuretic hormone, maintaining water balance in the organism by affecting water reabsorption rates from the kidney (Baratz and Ingraham 1959). AVP in the mammalian kidneys acts on AVP-V2 receptor to increase the expression of aquaporins in the membrane of nephron tubule cells, thereby increasing water reuptake rates. In teleost species, the V2 receptor was shown to be expressed in the nephros and the gills. For example, freshwater eels exposed to salt water had a marked increase in plasma AVPL levels. This result was also replicated when the freshwater eels were injected with saline solution intraperitoneally (Warne and Balment 1995).

Interestingly, the mRNA of avplv1 receptor was found to be expressed in the gills of the freshwater eel and the density of its expression was found to change depending on the osmolarity of the environment, salt water inducing increased expression of the receptor compared to freshwater (Balment et al. 2006). This suggests that osmoregulation in teleosts is mediated by coordination between neurohypophyseal AVPL and expression of its receptors in the gills.

These effects, while predominantly studied in the context of AVPL, were also observed in the case of OXTL. Studies in banded houndshark (*Triakis scyllium*) identified an increase in plasma OXTL after exposure of freshwater fish to salt water (Hyodo et al. 2004). OXTL has also been implicated in regulating ionocyte differentiation in zebrafish, thus affecting ion exchange to maintain optimal internal salt balance (Chou et al. 2011).

In the elasmobranch dogfish (*Scyliorhinus canicular),* perfusion of AVPL into *in situ* preparations of the kidney showed a marked antidiuretic effect. This study also implicated the addition of AVPL in decreased glomerular filtration rates, a possible mechanism by which the dogfish acclimatizes to reduced salinity (Wells et al. 2002).

4.2.3.2 Reproduction

OXT has been widely studied in mammalian models in the context of pregnancy, childbirth and lactation (Russell et al. 2003). In mammals, oxytocin receptors are widely found in both female and male reproductive tissues, affecting uterine contractions during labour, menstrual cycles and ovulation, milk ejection reflex

during lactation, sperm shedding from the testis and ejaculation (Burbach et al. 2006). In piscine species, however, OXTL has been implicated to play a major role in courting behaviour, egg-laying and sexual health of teleosts (Altmieme et al. 2019; Piccinno et al. 2014; Viveiros et al. 2003).

Male zebrafish subjected to female pheromones demonstrated increased courtship behaviour, which was inhibited following administration of OXTL and AVPL antagonists; it was, therefore, suggested that triggering the release of these neurohormones stimulates the central behavioural pathways, thereby increasing the possibility of reproductive success (Altmieme et al. 2019).

The two neurohypophyseal peptides also play a key role in the release of oocytes from fish females through their action on the smooth muscle cells of the ovarian wall. Ovarian wall contractility of gilthead seabream (*Sparus aurata* L.) was shown to be induced by *in vitro* administration of OXTL in vitellogenic non-spawning females (Piccinno et al. 2014). Similarly, when exposed to OXTL *in vitro*, testes slices of the African catfish (*Clarias gariepinus*) increased semen release to the media (Viveiros et al. 2003). These data demonstrate the involvement of OXTL and AVPL peptides in the regulation of reproductive functions at both central and gonadal levels in both mammals and fishes.

4.2.3.3 Behaviour

AVPL and OXTL exert major effects on mammalian behaviour, specifically in the context of social behaviour and dysfunction. In non-human mammalian models, these peptides allowed for better social recognition (Ross et al. 2009; Veenema et al. 2012), maternal behaviour (Bosch and Neumann 2012), and conversely played a role in cognitive impairments (Abramova et al. 2020).

Some of the mammalian phenotypes are recapitulated in fish shoaling and mating behaviours. In the case of the goldfish, these two peptides were shown to act in a conflicting fashion. While OXTL increased the tendency to social approaching, AVPL inhibited it (Thompson and Walton 2004). Additional complexity is added by the demonstration that HNS hormones also alter sex-specific social tendencies, as seen in the case of *Porichthys notatus* where the acoustic behavioural responses decreased in males and females only on exposure to AVPL and OXTL, respectively (Goodson and Bass 2000a, 2000b). Within the purview of hierarchical behaviour, exposing shoaling *Neolamprologus pulcher* cichlids to OXTL increased their awareness towards the dominant individuals (Reddon et al. 2012; Balshine et al. 2014).

Zebrafish are a social species in that they display collective behaviour in the formation of small, loose groups, known as shoals (Robinson et al. 2019; Miller and Gerlai 2012; Suriyampola et al. 2016). The absence of OXTL-mediated signalling was shown to reduce their shoaling tendencies, the converse of which was true when they were exposed to the peptide (Landin et al. 2020). Zebrafish OXTL receptor regulates memory recognition of familiar vs novel conspecifics (Ribeiro et al. 2020a, 2020b). The zebrafish receptor is also involved in the perception of biological motion, but not conspecific shape—two specific visual features that zebrafish use to appraise and react to social cues (Nunes et al. 2020).

It can be proposed that the effect of OXTL on fish behaviour is context dependent, as put forward in Ramsey's analysis of the social salience hypothesis that oxytocin expression allows improved cognitive processing in social contexts (Ramsey et al. 2019). The same can also be said in the case of AVPL expression in teleosts. Much like OXTL, the effects of AVPL on their behaviour seem to be context dependent. For example, intraperitoneal injection of AVPL into bluehead wrasse (*Thalassoma bifasciatum*) was shown to decrease aggression in territorial males while increasing it in non-territorial males (Semsar et al. 2001). Cohesively, the administration of Manning compound (AVPL receptor antagonist) was seen to inhibit these behavioural effects.

A key feature that should be noted is the differential effect of centrally and peripherally released hormones. In white perch (*Morone Americana*), intracerebroventricular administration of AVPL peptide showed strong activation of circuits involved in mating behaviour while circulating intraperitoneal AVPL injection had negligible effects on this behaviour (Salek et al. 2002).

Finally, although the classical effects of hormones, including OXT, is to activate or facilitate specific behavioural responses in an acute manner, OXT can have organizational effects on the developing social brain as well. Thus, pharmacological treatment of neonatal rats with OXT had long-term effects on behaviour in the adult (Noonan et al. 1989). Recently, it has been shown that OXTL can shape the structure of the developing forebrain as well as the functional connectivity of the so called social decision making network (SDMN) in zebrafish (Nunes et al. 2021). Thus, perturbation of zebrafish OXTL neurons during early but not late development disrupts the behavioural display of social drive in the adult, affecting the neurodevelopment of specific dopaminergic clusters associated with visual processing and reward (Nunes et al. 2021). Taken together, these data suggest that OXTL in fish regulates complex social behaviours including the ability to assimilate and process more social cues and information.

4.2.4 Neurovascular Interface

The teleost HNS neuronal populations are mostly investigated for their roles in the central regulation of homeostatic processes. However, the role of HNS vasculature and non-neuronal cells in this regulation is less clear. Importantly, understanding the mechanism through which the HNS exerts its systemic influence requires us to elaborate on a crucial topic—the neurovascular interface.

As described earlier, the axonal fibres in the neurohypophyseal tissue form direct contact with the capillary plexus. This capillary plexus in adult zebrafish arises from a simple loop-like structure of the embryonic HNS (Gutnick et al. 2011). While this capillary is an extension of the cerebral vasculature, it lacks a blood–brain barrier and instead, the vasculature of this region is highly fenestrated, i.e. permeable, allowing the exchange of blood-borne proteins and hormones between the brain and the peripheral circulation (Gordon et al. 2019; Anbalagan et al. 2018).

Functionally, the presence of fenestrated capillaries in the neurohypophysis is of great significance as it allows for the HNS to respond to peripheral stimuli and

allows the direct release of neurohormones into the blood circulation. This versatile structure is maintained by factors released by the resident pituicytes. We have recently shown that several angioactive ligands released by the pituicytes inhibit the formation of tight junctions between the vascular endothelia while maintaining the endothelial cell fenestrations (Anbalagan et al. 2018). Electron microscopy images of this region in adult zebrafish demonstrated the presence of neurosecretory termini seated near the basement membrane of the vasculature with pituicyte processes ensheathing them (Anbalagan et al. 2018, 2019).

4.3 Urophysis

Box 4.2 The urophysis—an underexplored neuroendocrine interface
The first indication of a caudal secretory system came from Weber in 1827 (Weber 1826) when dissection of the carp spinal cord indicated a caudal structure at the termini. This was further investigated almost a century later when Dahlgren described large cells along the spine of skates, which secreted granules into the blood (Dahlgren 1914). In 1925, Favaro (1925) showed the morphological similarities between a caudal bulge of teleosts and that of the neurohypophysis. Enami and Imai (1955) showed the conserved anatomical organization of this structure across fish species. By this point, it had become evident that this caudal neurosecretory system existed only in fishes, and it was suggested that it could serve as a neuroendocrine interface. A seminal study concerning the caudal neurosecretory system (CNSS) was the 1959 description of Dahlgren cells and their axonal projections into the vasculature of the urophysis (Enami 1959). The presence of neurosecretory products released in the urophysis was elucidated in 1969 where urophyseal extracts were shown to be functionally significant in blood pressure (Bern and Lederis 1969) and later in maintaining osmolality (Loretz and Bern 1981). By the 1990s (Conlon et al. 1996), urotensin II had been identified in species that lacked a caudal neurosecretory system, indicating a conserved role for the ancient hormone.

Fishes are unique amongst vertebrates, due to the presence of an additional neurosecretory organ associated with the spinal cord at its caudal end (Fig. 4.1). Importantly, the posterior pituitary interface bears a strong resemblance to the previously described caudal neurosecretory system in fish (Kriebel 1980). As demonstrated in the *Pomolobus aestivalis*, the urophysis consists of an axonal-vascular entanglement termed the neurohaemal zone/urophysis, which is roughly comparable to the hypophyseal neurovascular interface. Axonal fibres terminating in the urophysis are surrounded by fenestrated capillaries, with a predominant perivascular space. The ultrastructure of this region also shows the presence of neurosecretory granules contained in axonal swellings, dubbed Herring bodies, similar to what is observed in the pars nervosa (Kriebel et al. 1979). In 1914, Dahlgren identified giant

neurosecretory cells located at the distal end of the spinal cord of skates (*Rajidae*) (Dahlgren 1914) and in 1927 Weber found these localized swellings in the posterior end of the spinal cord (Weber 1927). This was later shown to generate a neurovascular structure, which was designated urophysis/urohaemal-organ, and the giant neural secretory cells were later termed Dahlgren cells (Enami 1959). Dahlgren cells are large magnocellular neurons projecting into the urophysis through thick non-myelinated axon endings. The axon endings are rich in secretory granules and have an intimate relationship with endothelial cells for the transfer of neurosecretory products (Holmgren 1964). The structural anatomical assembly of Dahlgren cells with the urophysis is referred to as the CNSS. The simplest organized form of the urophysis was commonly found in elasmobranchs (*Chondrichthyes*) whereas the highest organized form was found to be present in all bony fishes (*Osteichthyes*). Teleosts develop a discrete CNSS which shows a structural analogy to the cranial HNS (Bern 1985; Winter et al. 2000). The piscine CNSS is located at the distal end of the spinal cord, and in teleosts it spans the last three vertebrae (Holmgren 1964). The urophysis resides at the end of the spinal cord, posterior to the last spinal nerve (Fig. 4.1). In some species, the urophysis is innervated by means of a stalk through which the nerves and ependymal fibres enter, while in other species innervation is more diffused (Bern and Takasugi 1962). The urophysial outpouching structure is covered by meninges that arise from the end of the spinal cord and was shown to be populated by glial cells. Furthermore, ependymal and glial fibres from the spinal cord and vasculature generate an anatomical network (Amin et al. 1992; Fridberg 1962).

4.3.1 Evolutionary Aspects of Urophysis

Understanding the evolutionary aspects of Dahlgren cells and urophysis can give additional insights regarding piscine evolution (Fig. 4.3). More evolved fish display elongation or extension of Dahlgren cell terminals innervating a distinct neurohaemal organ which reflects a well-developed urophysis. Elasmobranchs are known as primitive ancestral fish, and exhibit more dispersed Dahlgren cells with shorter axons and a less anatomically discrete urophysis (Fridberg and Bern 1968; Qureshi et al. 1978). Accordingly, the neurosecretory Dahlgren-like cells of less evolved fish are widely distributed and form a diffuse neurohaemal zone. For example, in the neurohaemal zone of elasmobranchs small terminals of the Dahlgren cells are connected to the ventral part of the spinal cord and directly contact the capillary bed (Bern and Hagadorn 1959). Traces of such arrangement of Dahlgren cells were also noticed in some cyprinids, where the processes of Dahlgren cells terminate at the ventral part of the spinal cord and come close to the meningeal sheath to contact the blood vessels (Fridberg 1962). The anatomical isolation of Dahlgren cell terminals from the spinal cord occurs in the course of evolution. It was proposed that the isolation of Dahlgren cell terminals occurs in three stages: (i) In elasmobranchs and the early developmental stage of CNSS in some teleosts the terminals of Dahlgren cells are present within the spinal cord. (ii) In elasmobranchs

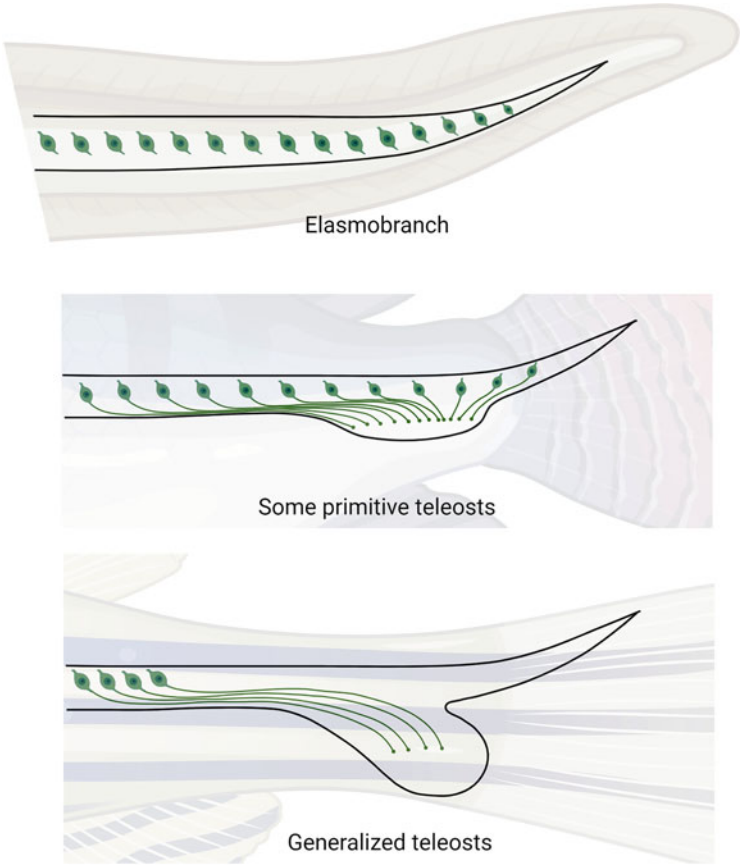

Fig. 4.3 Evolution of the piscine urophysis. Schematic representation of the urophyseal structure in fish showing the evolution of the caudal neurosecretory system from primitive fish (Elasmobranch) to the teleosts. Dahlgren cells evolve through the piscine phylogenetic tree to send projections from the caudal spinal cord into the neurohaemal interface of the urophysis

as well as in the intermediate developmental stage of CNSS in teleosts the nerve terminals penetrate the meningeal sheath. (iii) In more evolved teleost species, Dahlgren cell terminals move out from the spinal cord to penetrate the urophysis and terminate at the capillary bed, resulting in a lobular structure of the urophysis (Fig. 4.3) (Saenko 1978).

An evolutionarily related change in Dahlgren cell morphology was suggested for the evolution of teleosts from primitive fish (Speidel 1922). In early evolved piscines, the cells are small and similar to other nerve cells, without any morphological resemblance to Dahlgren cells. The second group of fish species, which are more evolved, possess small to moderate-sized cells, with limited resemblance to Dahlgren cells. The third and most evolved species show large-sized Dahlgren cells with modified morphology and are commonly seen in most teleost species

(Speidel 1922). Notably, in the early developmental stages of teleosts, moderately sized cells are present which later develop into large-sized Dahlgren cells in the CNSS of mature fish (Cioni et al. 2000). This developmental differentiation of Dahlgren cells from neuronal populations of teleost embryos further supports an evolutionary speciation process. In this view, small cells of the spinal cord initially served as specialized nerve cells in primitive piscines and later evolved into large glandular cells in teleosts.

4.3.2 Ontogenesis of CNSS

Embryonic development of Dahlgren cells and the urophysis was studied through various immunoreactive, histological and microscopy studies. Histological studies demonstrated that morphogenesis of the urophysis is initiated in the early larval stages, however, its mature organ form is finalized only after several months from hatching (Cioni et al. 2000; Fridberg 1962; Imai 1965; Sano and Kawamoto 1959). In chum salmon (*Oncorrhynchus keta*), immunohistochemistry of Urotensin1 (UI) and Urotensin2 (UII) localized in immature Dahlgren cells (i.e. appearing as agranular ovoidal cells) and fibres near the caudal region of the neural tube of forty-day-old embryos before hatching. Two weeks from hatching, the UI- and UII-positive cells and fibres increase in number, however, pronounced capillary formation is only detected in 3-month-old larvae and the maturation of the CNSS is finalized 5 months after hatching (Oka et al. 1993). It is interesting to note that although the HNS develops earlier than the CNSS, synthesis of UI and UII is identified in the embryonal CNSS before its appearance in the HNS (Oka et al. 1993). It was suggested that the urophysis differentiates from the meningeal tissue of the spinal cord and that Dahlgren cells originate from embryonic neuroectodermal cells, which differentiate first at the anterior region, gain secretory properties and migrate to the caudal region (Fridberg 1962; Fridberg and Bern 1968). A later study in chum salmon demonstrated that Dahlgren cells originate from neuroblasts and differentiate in the lateral plate of the caudal neural tube (Oka et al. 1993).

In Nile tilapia (*Oreochromis niloticus*), UI and UII immunoreactive perikarya and fibres were identified for the first time only in four days post-hatching larvae. At this stage, two bundles of neurosecretory fibres were observed at the future site of the urophysis. The initial differentiation of the tilapia urophysis is observed near the caudal region at 24 days post-hatching. The budding urophysis comprises a ventral swelling of the spinal cord in association with protruding dilated vessels. Further development occurs through increasing the number of neurosecretory terminals and branching of blood vessels. Meanwhile, neurosecretory cells rise in number and start to differentiate morphologically. The mature or fully formed urophysis is observed in four-month-old juveniles (Cioni et al. 2000). Obviously, additional work is needed with transgenic marker lines that will help to clearly uncover the embryonal origins of the CNSS. Nonetheless, it seems that functional speciation of Dahlgren cells begins at the initiation of hatching and free swimming and requires several months to reach the mature CNSS organ formation.

4.3.3 Physiology

The CNSS serves as the main neuroendocrine site for the synthesis of several neuropeptides with key importance in the homeostatic regulation of physiological functions. Nonetheless, although it has been recognized for more than a century, an exclusive critical role of the CNSS in physiological homoeostasis has yet to be elucidated. The CNSS is known as the major site for synthesis and release of urotensins (Ichikawa et al. 1982; Pearson et al. 1980). These neuropeptides show close similarities with other cortistatin and somatostatin peptides expressed by the central nervous system and other tissues of higher vertebrates, from reptiles and birds to mammals and humans (Lu et al. 2008; Vaudry et al. 2010). From an evolutionary perspective, this signifies the functional importance of these urotensins. The CNSS also produces and secretes additional neuropeptides such as corticotrophin-releasing factor (CRF), parathyroid hormone-related protein (PTHrP), OXTL and AVPL (Gozdowska et al. 2013; Ingleton et al. 2002; Lederis et al. 1982). Little is known regarding the functional and physiological importance of their secretion from the CNSS, however, they were found to be involved mainly in osmoregulation, reproduction and blood circulation.

4.3.3.1 Osmoregulation

UI and UII exert a direct effect on ion transport through epithelial cells in the kidney, which support their involvement in osmoregulation (Loretz et al. 1983; Marshall and Bern 1979; Ashton 2006). The importance of CNSS as an osmoregulatory structure is supported by: (i) the urophysis displays structural modifications with respect to the osmotic stress, (ii) urophysectomy affects the osmotic balance and (iii) urotensins secretion from the urophysis result in altered renal function of fish (Berlind 1973; Chan 1975). Several studies demonstrated that the urophysis undergoes structural and secretory modifications in response to altered salinity. Bonefish (*Albula vulpus*) raised in ponds with fluctuating salinity display increased intracellular cytoplasmic invagination and a higher level of secretory product was measured in their urophysis than in bonefish collected from open sea (Fridberg et al. 1966a). Cytological variations and altered urophyseal secretion were also detected in euryhaline brook trout (*Salvelinus fontinalis*) exposed to variable ion concentrations. Brook trout raised in a freshwater environment have an irregular shape of nucleus, elongated endoplasmic reticulum and Golgi bodies with reduced secretory granules. When maintained for a few days in deionized water, the cell organelles were shown to be highly developed, with increased numbers of secretory granules. Nonetheless, prolonged exposure to deionized water does not lead to increased neurosecretory activity, including changes in secretory granules. When exposed to 25% sea water for 24 h, brook trout exhibited increased secretory activity in the cells while prolonged exposure to increased salinity reduced the secretory activity in the urophysis (Chevalier 1976). These findings support the involvement of the CNSS in the homeostatic regulation of osmotic stress, mainly in response to acute environmental fluctuations. The Mozambique tilapia (*Oreochromis mossambicus*) is a hardy euryhaline fish that can grow in variable salinities from freshwater to sea water

(Chourasia et al. 2018). Freshwater-adapted tilapia that were urophysectomized and exposed to brackish water displayed significantly increased Na^+, K^+ and Ca^{++} in their blood than sham-operated controls. Contrastingly, sea water-adapted urophysectomized fish exhibited decreased Na^+ and K^+ in the bloodstream compared to sham-operated control fish. These results indicate that the urophysis has a role in maintaining osmotic balance in the fish (Baldisserotto et al. 1994). Similar results were obtained in urophysectomized Mozambique tilapia exposed to water containing 1.7% NaCl (Takasugi and Bern 1962). However, as treated fish exhibited increased mortality and weight loss with high level of serum chloride that was not identified in the later experiment, it was suggested that the lack of calcium in NaCl salinated water increased the osmotic stress (Baldisserotto et al. 1994; Takasugi and Bern 1962). Molecular analysis of urotensin expression in the euryhaline flounder (*Platichthys flesus*) suggested that UII is highly important for water and electrolyte homoeostasis and has an active role in preventing dehydration and salt deposition in high salinity conditions such as haemodilution in freshwater conditions (Lu et al. 2006). Urotensins were found to affect ion absorption in the urinary bladder of fish. Urinary bladders of longjaw mudsuckers (*Gillichthys mirabilis*) were exposed in vitro to physiological doses of UII, which directly altered ion transport in surface epithelia, a known component of osmoregulation. Moreover, India ink injection into the caudal vein demonstrated a direct but separate connectivity of the urophysis to the kidney and urinary bladder, further supporting a direct effect UII on the urinary bladder (Loretz and Bern 1981). It was also demonstrated by similar means that UII stimulates the absorption of Na^+ and Cl^- ions in the posterior intestine in 5% sea water-adapted longjaw mudsuckers (Loretz et al. 1983). These studies suggest that the CNSS directly modulates the main tissues known to be involved in water and electrolyte homoeostasis in fish both under baseline and osmotic stress conditions.

4.3.3.2 Reproduction

Analysis of urophysis protein extracts and molecular gene expression analysis of piscine CNSS during reproductive cycle and spawning period has demonstrated a role for the CNSS in fish reproduction. UII was found to be increased in the blood of white suckers (*Catostomus commersoni*) three months prior to the spawning period and it declined by half during and after spawning (Lederis 1973). Analysis of CNSS structure during the goldfish reproductive cycle demonstrated that the size of Dahlgren cells is altered with respect to ovarian development. Dahlgren cell size increases towards spawning initiation and decreases at the end of spawning season (Chen and Mu 2008). Importantly, while several studies demonstrated that urophysial extracts can modulate the contraction of ovary, oviduct and sperm ducts in some bony fishes (Berlind 1972; Lederis 1970), only one report demonstrated the direct effect of UII on ovarian smooth muscle contraction (Leonard et al. 1993). Urophysial extracts were found to be inefficient for spawning induction in several teleost species, further supporting their role in gonadal contraction and not as gonadal maturation factors (Behr et al. 2000). Gonad-localized and follicular-stage dependent UI levels were identified in the ovary of olive flounders (*Paralichthys olivaceus*), supporting the involvement of urotensins in piscine

ovarian development (Zhou et al. 2019), however, the possible connection and interaction between gonadal and CNSS urotensins remains to be determined.

4.3.3.3 Other Physiological Roles

Urotensins were reported to play a role in the stress regulation and muscle contraction of fishes. Dahlgren cell structure and its peptide secretion varied with temperatures. The firing frequency of Dahlgren cells was shown to increase with temperature, suggesting the role of the urophysis in thermoregulation. The response to thermal stress was suggested to be mediated through the transient receptor potential cation channel family (TRPs) (Yuan et al. 2020b). CNSS expression of UI, UII and corticotropin-releasing hormone (CRH) as well as plasma cortisol, CRH but not UII were shown to increase in olive flounders on exposure to acute hypothermal stress, returning to baseline levels following 8 days of adaptation (Yuan et al. 2020a). Chronic but not acute hyperthermal challenge led to increased expression of CNSS CRH and UI but not UII (Yuan et al. 2020a). In addition, the urophysis was suggested to be involved in the regulation of blood circulation, vascular smooth muscle contraction and the digestive system of fish (Fridberg 1962; Lederis 1977). Overall, current literature suggest pleiotropic functions of CNSS, which is not surprising considering the expression of multiple neurohormones in this structure. Further research regarding urophysial functions in homeostatic fish physiology is needed.

4.4 Conclusions and Outlook

The importance of the HNS and its related neurohormones in the regulation of homoeostatic and physiological functions is obvious given its structural and functional evolutionary conservation. Nonetheless, the existence of the CNSS in fishes as well as its evolvement in piscine species support an unidentified but highly important urophysiological role(s).

Some of the failures in underpinning major CNSS functions may be explained by the 2–3 weeks required for full regeneration of this system following complete removal of all CNSS neurohemal components (Fridberg et al. 1966b). Paradoxically, this very rapid regeneration further supports the high importance of the CNSS in fish physiology. Importantly, new and relevant pharmacological and genetic tools have been developed for the urotensin system (Lescot et al. 2008; Zhang et al. 2018) and some were also developed for non-neuronal components of the HNS (Anbalagan et al. 2018; Gordon et al. 2019). These tools may prove valid for studying both neural and non-neural components of the CNSS aiming to identify specific physiological functions of this system.

While the HNS is fully functional during early embryonal stages, CNSS components begin to differentiate at later developmental stages and its structural establishment occurs only several months later. This suggests that the CNSS functions are of importance to adult fish physiology and possibly connected to sexual maturation. As described above, euryhaline fish exhibit more developed

CNSS anatomy, which further supports this concept. Nonetheless, the ability of urophysial extracts and hormones to modulate water and electrolyte homoeostasis, as well as the CNSS anatomy, has led to an inherent bias as most fish species used to study this system were euryhaline, making at least some of the findings questionable with regard to stenohaline piscines.

Finally, much effort has been invested in recent years in understanding the regulatory mechanisms of HNS neuropeptide secretion. However, the anatomical location and complex connectivity of the HNS with additional brain centres hinder these efforts. The close morphological, cellular and structural similarities between HNS and CNSS and the ability to analyze CNSS ex vivo make the CNSS a potentially unique model for the study of neurohormone secretion.

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