CHAPTER

6

Arginine Vasotocin and Isotocin: Towards their Role in Fish Osmoregulation

Ewa Kulczykowska

NEUROPEPTIDES ARGININE VASOTOCIN AND ISOTOCIN—GENERAL VIEW

Neuropeptides are defined as peptides synthesized in neurons that play an important role in transmitting information in the nervous system. The mechanism of known neuropeptides biosynthesis is similar in general: the gene of the peptide precursor is first transcribed into m-RNA, which is then translated into the propeptide. After various modifications (i.e., proteolysis of the propeptide), the mature peptide packed into secretory granules is transported via axonal flow to the nerve terminal where it is stored and released in response to appropriate stimulus.

That view does fully apply to arginine vasotocin (AVT) and isotocin (IT), fish neuropeptides synthesized in the hypothalamic magnocellular

Author's address: Department of Genetics and Marine Biotechnology, Institute of Oceanology of Polish Academy of Sciences, Sopot, Poland.

E-mail: ekulczykowska@iopan.gda.pl

neurons of the NPO (nucleus preopticus) from where they are transported to the neurohypophysis for storage and release. The identification of the neuronal origin of both neurohypophysial peptides and evidence for the presence of separate hypothalamic neurosecretory neurons producing AVT and IT in fish have been provided by various methods. These procedures include immunocytochemistry either alone or combined with carbocyanine tract tracing and confocal double-color immunofluorescent microscopy (Goossens *et al.*, 1977; Van den Dungen *et al.*, 1982; Holmquist and Ekström, 1995; Saito *et al.*, 2004). The peptides, closely related to mammalian vasopressin and oxytocin, containing nine amino acids residues, have been identified in the hypothalamo-neurohypophysial system of teleosts by Acher *et al.* (1961, 1962). As is the case with other neuropeptides, both AVT and IT are produced as a part of larger precursor molecule. The vasotocin and isotocin precursor sequences consist of a signal peptide, hormone and neurophysin (Fig. 6.1). Both pro-vasotocin and pro-isotocin have elongated carboxyl-terminals with a leucin-rich segment similar to copeptine-like sequence of vasopressin precursor, but its glycosylation does not appear to be possible. The polypeptide neurophysin, cysteine rich and capable of binding the neurohypophysial hormone, has been shown for the first time in fish by Pickering (1968). Since the early nineties, the structural organization of pro-vasotocin and pro-isotocin genes has been described in several fish species: *Catostomus commersoni*, *Oncorhynchus keta*, *O. masou*, *Platichthys flesus*, *Triakis scyllium*, *Neoceratodus forsteri, Danio rerio* (Heierhorst *et al.*, 1989, 1990; Suzuki *et al.*, 1992; Hyodo *et al.*, 1997, 2004; Warne *et al.*, 2000; Unger and Glasgow, 2003). Prior to secretion, each hormone is stored in secretory granules in the form of a non-covalent complex with its associated neurophysin, which is important during formation of the mature nonapeptide hormone. Release of the complex into the blood by exocytosis leads to its spontaneous dissociation. In many teleost species, especially salmonids, a duplication of the nonapeptides genes possessing the different expression level has been presented (Hiraoka *et al.*, 1993).

The homologous neurohypophysial hormones have been identified in representatives of all vertebrates systematic groups (Acher, 1995; Bentley, 1998). In general, they are arranged in a five-amino acid ring, joined by a disulfide bridge and a side chain of three amino acids. So far, at least 12 homologous nonapeptides have been identified among the vertebrates (Fig. 6.2). Amino acid substitution occurs at the second, third, fourth, or

ARGININE VASOTOCIN
Cys - *Tyr - Ile - Gln* - Asn - Cys - Pro - *Arg* - Gly - NH_2
1 2 3 4 5 6 7 8 9

ARGININE VASOPRESSIN
Cys - *Tyr - Phe - Gln* - Asn - Cys - Pro -*Arg* - Gly - NH_2

LYSINE VASOPRESSIN
Cys - *Tyr - Phe - Gln* - Asn - Cys - Pro - *Lys* - Gly - NH_2

PHENYPRESSIN
Cys - *Phe - Phe - Gln* - Asn - Cys - Pro - *Arg* - Gly - NH_2

ISOTOCIN
Cys - *Tyr - Ile - Ser* - Asn - Cys - Pro - *Ile* - Gly - NH_2

OXYTOCIN
Cys - *Tyr - Ile - Gln* - Asn - Cys - Pro - *Leu* - Gly - NH_2

MESOTOCIN
Cys - *Tyr - Ile - Gln* - Asn - Cys - Pro - *Ile* - Gly - NH_2

VALITOCIN
Cys - *Tyr - Ile - Gln* - Asn - Cys - Pro - *Val* - Gly - NH_2

GLUMITOCIN
Cys - *Tyr - Ile - Ser* - Asn - Cys - Pro - *Gln* - Gly - NH_2

ASPARGTOCIN
Cys - *Tyr - Ile - Asn* - Asn - Cys - Pro - *Leu* - Gly - NH_2

ASVATOCIN
Cys - *Tyr - Ile - Asn* - Asn - Cys - Pro - *Val* - Gly - NH_2

PHASVATOCIN
Cys - *Tyr - Phe - Asn* - Asn - Cys - Pro - *Val* - Gly - NH_2

Fig. 6.1 Structure of pro-hormones for pro-vasotocin and pro-isotocin in fish.

eighth position in the molecule. The distribution of these natural analogs is well-defined: in mammals vasopressin and oxytocin are the main neurohypophysial hormones, in non-mammalian vertebrates arginine vasotocin (vasopressin-like peptide) is present together with usually one of the eight already identified variants of oxytocin-like peptide. There is a great deal of variability in nonapeptides variants between fish (Acher, 1993; Acher and Chauvet, 1995). Both nonapeptides vasotocin and isotocin are found in all bony fish except for lungfish possessing mesotocin instead of isotocin and chondrosteans in which glumitocin, aspargtocin, asvatocin, phasvatocin or valitocin are recognized besides vasotocin. In cyclostome fish, however, vasotocin is a sole neurohypophysial nonapeptide. Since vasotocin is present in all vertebrates, it has been considered as a precursor molecule for the neurohypophysial hormones

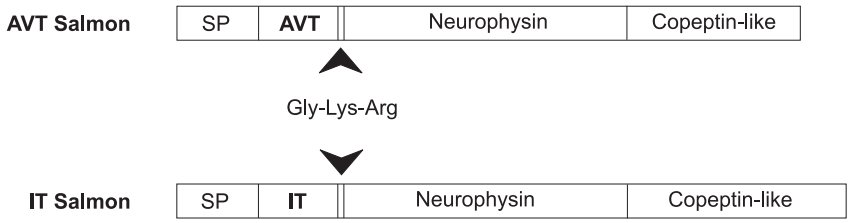

Fig. 6.2 Amino acid sequences of neurohypophysial peptides in vertebrates.

family (Bentley, 1998). It is worth emphasizing that the neurohypophysial hormones present in one species differ by two amino acid substitutions at the most, but their biological activities are considerable distinct, i.e., vasopressin—mammalian well-known antidiuretic hormone and oxytocin—hormone involved in parturition and lactation (Bentley, 1998).

Neuropeptides action is generally determined by their binding to the specific receptors in the central nervous system (CNS), or in other sites of the body. In the CNS, neuropeptides play a role of neurotransmitters and/or neuromodulators, while distributed with the circulatory system they act as hormones. Just the same, AVT and IT may act, either locally in the CNS or in the peripheral target organs (Goossens *et al.*, 1977; Van den Dungen *et al.*, 1982; Acher, 1993; Acher and Chauvet, 1995; Goodson and Bass, 2000).

It is a well-established fact that the neurohypophysial hormones receptors belong to the superfamily of guanine nucleotide binding protein (G-protein)-coupled receptors. The topography of the receptors is typical with seven helical transmembrane-spanning domains, four extracellular domains and three intracellular domains (Bentley, 1998) (Fig. 6.3). Vasotocin and isotocin receptor transcripts are widely distributed in different fish organs: brain, pituitary, spleen, lateral line, ovary, bladder, intestine, liver, heart, gills, kidney and skeletal and smooth muscle, suggesting a function of nonapeptides there (Mahlmann *et al.,* 1994; Hausmann *et al.,* 1995). So far, to this author's knowledge, AVT and IT receptors have been cloned in two fish species, *C. commersoni* and flounder (*Platichthys flesus*) (Mahlmann *et al.,* 1994; Hausmann *et al.,* 1995; Warne, 2001). Cloning of the vasotocin and isotocin receptor genes has revealed the presence of residues that are conserved among nonapeptide receptors and have been suggested to contribute to the ligand binding domain (Hausmann *et al.*, 1996) (Fig. 6.3). There is a growing body of evidence

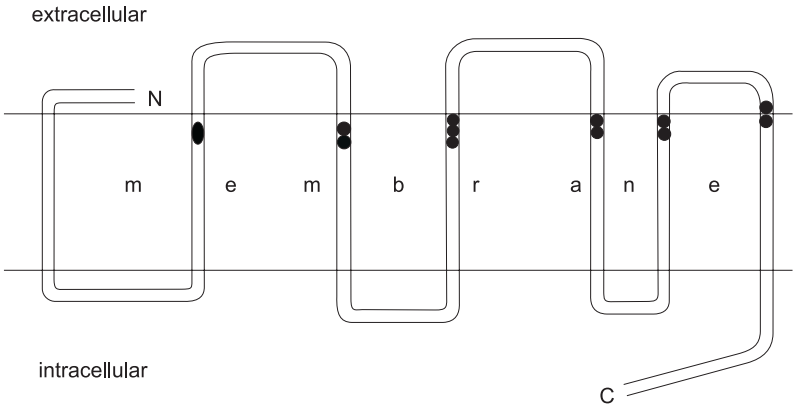

Fig. 6.3 Topography of the arginine vasopressin and isotocin receptors. The receptors are the members of the G protein-coupled receptor superfamily. The conservative regions marked in black are probably the sites of interaction with the hormone.

that AVT, which binds to the receptors on the pre-synaptic or post-synaptic membranes in the central nervous system of fish, acts as neurotransmitter and/or neuromodulator and influences reproductive physiology and related social behavior (Foran and Bass, 1998; Goodson and Bass, 2000). Recently, the target neurons for brain action of vasotocin were identified in newts (Lewis *et al.*, 2004). On the other hand, a number of studies in fish have demonstrated a role of AVT, when distributed with circulation, in maintenance of water and electrolytes balance, cardiovascular activity and regulation of endocrine secretion (Babiker and Rankin, 1978, 1979; Fryer and Leung, 1982; Acher, 1993; Conklin *et al.*, 1997).

SIGNALS FOR AVT/IT SYNTHESIS AND RELEASE

Several studies have pointed out that synthesis of teleosts nonapeptides in hypothalamus and their secretion into circulation in the neurohypophysis have changed in response to environmental salinity (Maetz and Lahlou, 1974; Haruta *et al.*, 1991; Hyodo and Urano, 1991; Perrott *et al.*, 1991). In early sixties, it was already observable that neurosecretory material in hypothalamus and pituitary was reduced after transfer of rainbow trout (*Oncorhynchus mykiss*), eel (*Anguilla anguilla*) and stickleback (*Gasterosteus aculeatus*) to a hyperosmotic medium (Fridberg and Olsson, 1959; Holmes and McBean, 1963; Sharratt *et al.*, 1964). Moreover, in

rainbow trout transferred from FW to SW, Carlson and Holmes (1962) and Elders (1964) demonstrated the transitory decrease in antidiuretic and pressor activity of pituitary, which recovered after 6 hours. Novel studies by Hyodo and Urano (1991) applying an *in situ* hybridization method have coincided well with those early results. They have shown in rainbow trout, that proAVT mRNA level is markedly decreased by transfer of fish from FW to 80% SW and remains consistently low by day 14. Just after retransfer of fish to FW, proAVT mRNA level rises to the initial FW value. A significant decrease in proIT mRNA levels in SW salmonid fish is also observed but only on day 1 after transfer. The results have suggested that the acute change of water salinity is an important factor influencing neurohypophysial hormones synthesis and/or release (Hyodo and Urano, 1991; Urano *et al.*, 1994).

The changes in the pattern of secretion from pituitary and resulting plasma concentrations of the AVT evoked by alterations in environmental tonicity was exhibited in the flounder, rainbow trout and carp (*Cyprinus carpio*) by Perrott *et al.* (1991). In the euryhaline flounder and trout, the higher pituitary AVT in FW-adapted fish was associated with a higher plasma concentration of the hormone compared with SW-acclimated fish. However, in the stenohaline carp, there was no difference in plasma AVT between fish adapted to either FW or 40% SW. On the other hand, the initial response of the euryhaline flounder to hypotonic challenge involved a decrease in plasma AVT concentration (Bond *et al.*, 2002) similarly to that observed in rainbow trout by Kulczykowska (1997). In other euryhaline fish medaka (*Oryzias latipes*), the AVT content in the pituitary temporarily decreased after transfer to SW, thus, suggesting an increase of AVT release during the first hours of SW adaptation. During readaptation to FW, however, pituitary content of AVT was elevated within the first 2 hours after transfer, again indicating the pronounced storage of the hormone (Haruta *et al.*, 1991). In dogfish (*Triakis scyllium*), marine elasmobranch utilizing a unique strategy for adaptation to elevated salinity by accumulation of urea, AVT secretory activity was also enhanced by transfer to hyperosmotic environment (Hyodo *et al.*, 2004). A teleost species *Rivulus marmoratus*, dwellers of brackish waters of tropical mangrove forests, while exposed to different salinities, reacted by a varying pattern of vasotocin immunoreactivity in the pituitary and in the preoptic nucleus (Nürnberger *et al.*, 1996). The response of circulating AVT and IT to osmotic challenge was also reported in rainbow trout transferred from

FW to brackish water (BW): the AVT and IT levels increased significantly 2 hr and 24 hr after transfer, respectively. In FW-transferred fish, both hormones decreased steadily, achieving the lowest values 2 and 24 hr after transfer. However, after 10 days of fish adaptation to BW plasma, AVT concentration decreased below FW value, whereas after 10 days of acclimatization in FW that increased above BW value (Kulczykowska, 1997). In rainbow trout subjected to acute osmotic stress, a significant increase in plasma AVT, but not in plasma IT level was observed (Kulczykowska, 2001). Although it was not clear whether plasma AVT/IT variations reflect changes in AVT/IT production or secretion rates, or both, but the hormonal responses to the acute and prolonged changes of water salinity were evident. Yet both hormones synthesis and release seemed to be differentially sensitive to plasma osmolality changes (Kulczykowska, 1997, 2001).

Taken together, it appears that in the euryhaline fish species, the synthesis, storage and secretion of AVT are sensitive to environmental stimuli such as exposure to extreme salinities. A question here arises: what is an internal signal for the neurohypophysial hormone release?

It is a well-established fact in mammals that an increase in plasma osmolality is the principal physiological stimulus for vasopressin secretion. Also, severe hypovolemia and/or hypotension are strong signals for this nonapeptide release (Szczepańska-Sadowska *et al.,* 1983). It is not clear, however, if similar signals are also engaged in vasotocin and isotocin secretion in fish. A consistent relationship between plasma AVT concentration and plasma Na^+, Cl^-, and osmolality was demonstrated in seawater-adapted flounder (Warne and Balment, 1995). In rainbow trout transferred between FW and BW, plasma vasotocin concentration also correlated with plasma osmolality (Kulczykowska, 1997). On the other hand, although an increase in plasma AVT concentration with increasing plasma osmolality or sodium concentration was apparent for SW-adapted flounder, this was not the case in FW-adapted fish (Perrott *et al.*, 1991; Balment *et al.*, 1993; Harding *et al.*, 1997). Thus, the other factor involved in controlling of vasotocin secretion, i.e., volemic status of the animal, was considered. However, the volemic expansion or hemorrhage protocols applied in chronically cannulated flounder studies failed to demonstrate that plasma AVT level may be sensitive to volemic status (Warne and Balment, 1995). Therefore, a rapid increase in plasma osmolality seems to be a major factor to control circulating AVT level in fish.

ARGININE VASOTOCIN/ISOTOCIN: TARGETS OF ACTION AND MECHANISMS

Overview

To have a clearer view of the role of neurohormones in osmoregulation in fish, it is essential herein to remind the general rules of that process. The biological structures and regulatory mechanisms participating in osmoregulation in aquatic and terrestrial vertebrates are different, but they have also many common features, i.e., neurohypophysial hormones analogs playing similar regulatory roles (Bentley, 1998). Terrestrial vertebrates are equipped with kidneys, which are highly specialized in preserving water and ions. Fish, in general, posses less complicated mechanism for regulation of ions and water balance, because of easier access to these elements in water. However, water and ions movements in freshwater and seawater fish require the regulation in the opposite direction: marine fish must secrete NaCl and conserve water and freshwater fish must accumulate salt from environment and eliminate excess water. Many species use both strategies to maintain electrolytes balance during a lifetime, i.e., migrating salmon, eel, etc. (Bentley, 1971).

There are three main organs engaged in osmoregulation in fish (in order of importance): gill, gastrointestinal tract (GIT) and kidney. The responses of fish to osmotic perturbation are summarized in Fig. 6.4.

Osmoregulatory tissues possess a high level of membrane-bound enzyme sodium ion-potassium ion-adenosinetriphosphatase activity, i.e., Na^+- K^+- ATPase, 'sodium pump'. The gill is the primary site of active ion transport responsible for body electrolyte homeostasis in teleosts. In freshwater teleosts, the gills actively absorb salt and passively intake water due to the osmotic influx, whereas seawater teleosts actively excrete NaCl. The branchial chloride cells play an essential role in the seawater acclimation of euryhaline teleost being responsible for Cl^- secretion (Bentley, 1971). The second important organ for water and salt exchange in fish is the gastrointestinal tract. The GIT's role differs in various fish species. Fish living in fresh water scarcely drink, but the intestine actively transports sodium from the lumen to the blood. Conversely, drinking and subsequent absorption of water by the intestine is essential for seawater fish (Bentley, 1971). The kidney does not have a pivotal role in fish osmoregulation, but its significance should not be underestimated. Fish are the only vertebrates with kidneys able to produce urine by glomerular and

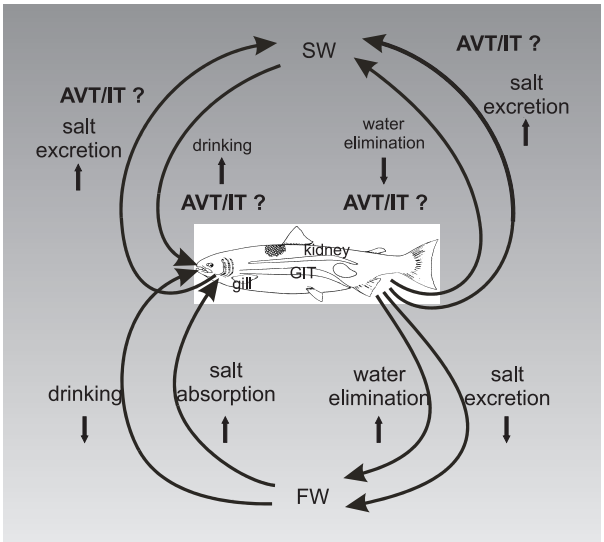

Fig. 6.4 Physiological responses of fish to different water salinities: potential involvement of the neurohypophysial neuropeptides. Modified from: Kulczykowska, E. (2002). A review of multifunctional hormone melatonin and a new hypothesis involving osmoregulation. *Reviews in Fish Biology and Fisheries* 11, 321-330.

aglomerular mechanisms defined by Beyenbach (1995) and Renfro (1999). Glomeruli appear to be particularly suited to the excretion of the large volumes of water accumulated by fish living in fresh water. The renal tubules of fish are differentiated from one or two tubular segments to the complement structure of the vertebrate nephron. Thus, the contribution of the kidney to extracellular fluid homeostasis is not universal in fish, and renal function spans the whole spectrum from glomerular filtration to tubular secretion (for review see: Beyenbach, 1995; Renfro, 1999; Dantzler, 2003; Nishimura and Fan, 2003). The main function of the proximal tubule of glomerular kidney in higher vertebrates is to reabsorb fluid. Surprisingly, the renal proximal tubules of glomerular kidneys in marine and euryhaline fish, e.g., winter flounder (*Pseudopleuronectes americanus*), dogfish shark (*Squalus acanthias*) and killifish (*Fundulus heteroclitus*)) appear to secrete fluids containing Na^+ and Cl^-. Interestingly, the same phenomenon is observed in the kidney of aglomerular toadfish (*Opsanus tau*). In elasmobranchs, the kidney is a major site of urea retention.

It is a well-established fact in mammals that neurohypophysial hormone arginine vasopressin regulates water and ions transport by epithelia (kidney tubules, skin, bladder) by stimulating adenylate cyclase via V2-type receptors. Other effects of that hormone (vasoconstriction, glycogenolysis, platelet aggregation) are mediated by stimulation of phosphoinositide breakdown and/or calcium mobilization via V1-type receptors (Bentley, 1998). In non-mammalian vertebrates—among them fish—arginine vasopressin is replaced by arginine vasotocin. If vasotocin triggers both signaling pathways and fulfils essentially similar functions in fish as vasopressin does so in mammals is not clear and requires elucidation. A role of the second neurohypophysial hormone in teleost, i.e., oxytocin-like isotocin is even more enigmatic.

Are osmoregulatory organs: gill, GIT and kidney the physiological goals for AVT/IT action? This question will be addressed below.

Gill

The first suggestions that the gill may be a target organ for AVT/IT action appeared as early as in the sixties. The gills of fish living in seawater are the site of considerable sodium exchange, with the outflux exceeding the influx by an amount which is about equivalent to that gained through the gut (urinary loss is insignificant). In seawater fish, chloride cells secrete chloride actively into the external water. It was proved that injections of neurohypophysial peptides—vasotocin and oxytocin—facilitated the branchial outflux of sodium in flounder transferred from fresh water to seawater (Motais and Maetz, 1967). In fish living in fresh water, however, sodium and chloride depletion stimulates the accumulation of sodium by the gills. It was demonstrated in goldfish that isotocin and, to a lesser extent vasotocin, while administered, increased the rate of sodium uptake across the gills (Maetz *et al.*, 1964).

A more updated data will evidently broaden this view. There is no doubt that an adenylate cyclase in plasma membranes from the teleost gill is sensitive to fish neurohypophysial peptides. In rainbow trout, AVT and IT, when administered in concentrations between 1 and 100 pM, have inhibited both basal and glucagon stimulated activity of the enzyme (Guibbolini and Lahlou, 1987a). The ability of the inhibition appeared to be sensitive to the environmental salinity, being especially expressed in high-salt media (Guibbolini and Lahlou, 1987a). The first evidence for a saturable, reversible and high-affinity specific binding of AVT by cells

isolated from the gills of eels adapted to freshwater (FW) and seawater (SW) have been provided by means of ^{125}I-labelled AVT binding (Guibbolini *et al.*, 1988). Further studies conducted on rainbow trout gill epithelium, using specific neurohypophysial analogues, strongly suggested the presence of a new type of vasotocin and isotocin receptors designated as NH_f (Guibbolini and Lahlou, 1990), closer to the V1 than to the V2-type, with reference to the mammalian model. In experiments with the V1 receptor agonists in rainbow trout gill epithelium, reduction of adenylate cyclase activity have been more noticeable in SW rather than in FW (Guibbolini and Lahlou, 1990). Then it has been confirmed that the effects of fish nonapeptides are mediated by a G_i protein sensitive to pertussis toxin and guanine nucleotides (Guibbolini and Lahlou, 1992). Shortly afterwards, the molecular structures of AVT and IT receptors has been established by Mahlmann *et al.* (1994) and Hausmann *et al.* (1995) in teleost fish *C. commersoni*. The sequence of AVT and IT receptor has displayed the similarity to the mammalian V1-type receptor and the oxytocin receptor, respectively. Mutational analysis has shown that the different regions of the vasotocin receptor participate in hormone binding (Hausmann *et al.*, 1996) (Fig. 6.3). Subsequently, an AVT receptor from the euryhaline flounder has been cloned and its specificity for AVT has been documented (Warne, 2001). It has been demonstrated that activation of the receptor is coupled to phospholipase C and the inositolphosphate/calcium pathway, similarly to that presented in *C. commersoni*. Moreover, the flounder receptor has been shown to be more similar to the mammalian V1 than to the V2-type, in terms of its potent activation by AVP agonists for V1 receptor subtype and its higher homology with V1-type receptors (Warne, 2001). The AVT receptor mRNA expression studies confirm gills as a site of the hormone action in bony fish (Mahlmann *et al.*, 1994; Warne, 2001).

Although the results pointed out the fish gill as a physiological target organ for neurohypophysial hormones, the heterogeneity of the branchial epithelium comprising gill chloride, respiratory, pillar and mucous cells made it difficult to identify the exact target cells. Only when a detailed study on primary cultures of gill cells from a marine fish sea bass has been performed, the respiratory like cells have been shown to be an effective goals for both neurohypophysial peptides (Guibbolini and Avella, 2003). It has been demonstrated that both AVT and IT induce a dose-dependent stimulation of Cl^- secretion through the epithelium. It is consistent with

the phenomenon of ions excretion through the gills observed in fish living in seawater. It has been suggested that the physiological effects of AVT and IT are mediated via two pharmacologically similar, V1-like receptors, located in gill respiratory-like cells. Probably V1-type receptors are also present in the chloride cells. Early experiments with isolated gill cells from SW-adapted eels, showed that AVT is a potent regulator of Cl^-secretion in the chloride cells (Guibbolini *et al.*, 1988). Moreover, in rainbow trout adapted to SW, an increase of gill chloride cells number and a more pronounced inhibition of adenylate cyclase activity by AVT and IT was observed, which may indicate the multiplication of the AVT/IT receptors sites located on the chloride cells during SW adaptation of euryhaline fish (Guibbolini and Lahlou, 1987a, b). Further, the morphological changes in the chloride cells of the epithelium in respect to the external salinity, which correlate strikingly with the AVT-binding parameters, point out to these cells as the site of AVT action (Guibbolini *et al.*, 1988). Also, recent studies of the estuarine teleost killifish (*Fundulus heteroclitus*) suggest that sodium chloride secretion upregulated on return of the fish to full seawater, is mediated via arginine vasotocin receptors present in basolateral membrane of mitochondria-rich cell in gill epithelium (Marshall, 2003).

Considering the fish gill as a target organ for neurohypophysial hormones, also the branchial blood vessels should be taken into account as potential effectors of the hormone action. AVT is known to be a vasopressor in all major vertebrate groups, among them in fish (Le Mevel *et al.*, 1993; Bentley, 1998). The vascular action of both neurohypophysial hormones in the branchial vasculature has been shown in many experiments (Bennett and Rankin, 1986; Oudit and Butler, 1995; Conklin *et al.*, 1996, 1997, 1999). In the isolated perfused gills of the European eel, Bennett and Rankin (1986) demonstrated a vasoconstriction of the arterio-arterial pathway and a decrease in arterio-arterial flow with no effect on the arterio-venous component of branchial flow. More recently, Oudit and Butler (1995) have postulated that AVT-induced vasoconstriction of arterio-arterial pathway is responsible for increased branchial shunting in freshwater eel. AVT in physiological concentrations produced contraction in afferent branchial arteries in holostean gar (*Lepisosteous* spp.) (Conklin *et al.*, 1996). In rainbow trout, AVT injection had a greater impact on branchial resistance than it did on systemic resistance, but in the isolated perfused gill, the hormone affected both the

arterio-arterial and arterio-venous pathways (Conklin *et al.*, 1997). In the same study, the AVT at low concentrations stimulated constriction in the arterio-venous pathway and thus increased flow through the alternative arterio-arterial pathway. On the contrary, the AVT at elevated levels decreased arterio-arterial flow while increased arterio-venous flow (Conklin *et al.*, 1997). The alterations in redistribution of blood in gills may change AVT and IT delivery to the sites of ion exchange in gill epithelium and influence the ions and water transport via epithelium, as it was suggested by Maetz and Lahlou (1974).

The magnitude of AVT-stimulated constriction of the isolated efferent branchial artery in rainbow trout—high above that of other well-known vasoconstrictors—indicates a significance of AVT in regulation of gill vascular resistance, at least in this species. Studies in rainbow trout and eel have shown that the gill vasculature is far less sensitive to IT than to AVT (Bennett and Rankin, 1986; Conklin *et al.*, 1999). Vascular actions of neurohypophysial peptides have also been studied in free-swimming, chronically cannulated flounder by Warne and Balment (1997a, b). The initial fall in dorsal aortic blood pressure observed immediately after AVT and IT injections have been proposed to be due to AVT/IT dependent branchial vasoconstriction. It has been suggested that the AVT primary effect is a constriction of the arterio-arterial pathway (Warne and Balment, 1997a, b), as it has been reported in the *in vitro* perfused gill preparation by Bennett and Rankin (1986). A subsequent rise in the post-branchial blood pressure in response to AVT and lack of this effect following IT injection would suggest the presence of different types of hormones receptors (Warne and Balment, 1997a, b). However, it should be stressed here that the most of vascular effects in gill have been observed at hormones doses high above those considered as physiologically relevant. Also in the study of conscious, chronically cannulated Atlantic cod (*Gadus morhua*), the biphasic vascular reaction to administered AVT was demonstrated, but the dose of the hormone can be considered evidently as pharmacological. The absence of any response to IT injection was obvious in this study (Kulczykowska, 1998).

Taking together, a physiological action of both peptides in the regional blood flow distribution in fish gill and significance of this mechanism in osmoregulatory process needs to be reconsidered.

Gastrointestinal Tract (GIT)

It is well known that fish living in freshwater drink little water, but teleosts in the sea must drink salt water permanently in order to prevent dehydration. The intestine of freshwater fish actively absorbs sodium from the food, while fasting the fish gain little sodium from the water they drink. In seawater, a salt absorption and coupled to this water absorption take place in teleost fish, but the cartilaginous fish have developed an original urea-based osmoregulation model and use a rectal salt gland for sodium chloride excretion (Bentley, 1971).

There are no existing data—to this author's knowledge—on the potential physiological action of neurohypophysial hormones in GIT of fish, although the IT receptor transcripts in the intestine of *C. commersoni* (Hausmann *et al.*, 1995) and AVT receptors in the GIT's smooth muscle of rainbow trout (Conklin *et al.*, 1999) were reported. AVT has been shown to contract gastrointestinal tissue, but at concentrations 10-100 times higher than those that contract blood vessels (Conklin *et al.*, 1999). It may mean that GIT is not a natural site of AVT action or else a local supplementing production of AVT takes place here, as has been shown for mammalian AVP (Friedmann *et al.*, 1993). An immuno-histochemical localization of AVP in cells of mucosal epithelium and in fibers near the capillaries situated along the basal side of the epithelium cells suggests an action of this nonapeptide in mammalian GIT (Sanchez-Franco *et al.*, 1986). Whether there is the case of AVT in fish, is a matter of speculation. In marine cartilaginous fish, the rectal salt gland devoted to sodium chloride excretion is considered as a potential site of AVT and oxytocin-like peptides action (Acher *et al.*, 1999), but so far, to the author's knowledge, no investigations have been carried out.

Kidney

A renal function in fish depends on the external environment and can exemplify hyperosmoregulation in FW medium or hypoosmoregulation in SW medium. In freshwater teleosts, the major role of the kidney is to eliminate excess water and to retain electrolytes. In the distal tubule, NaCl is actively reabsorbed without the accompaniment of water. In marine teleost, however, the role of the kidney is different, i.e., to conserve water. The proximal tubules reabsorb NaCl to restore water, but the distal tubules are degenerated and a considerable volume of water is lost (Bentley, 1971).

Adaptations of euryhaline teleosts to both hypo- and hyperosmotic media probably include changes of both: the glomerular filtration rate and the permeability of the renal tubules. However, in contrast to well-documented AVP renal effects in mammals, our current knowledge of AVT actions in fish kidney are far behind.

In the early studies in chronically cannulated eels, AVT administration was shown to induce dose dependent changes in urine production (Chester Jones *et al.*, 1969; Henderson and Wales, 1974; Babiker and Rankin, 1978). In the freshwater eel, doses less than 0.1 ng/kg body weight were antidiuretic while doses greater than 1 ng/kg body weight were diuretic (Henderson and Wales, 1974). Similar results were obtained by Babiker and Rankin (1978) with low doses of AVT and IT (1 pg - 1 ng/kg body weight) reducing urine production in eels adapted to FW (but not in SW fish), and high doses (more than 10 ng/kg body weight) resulting always in diuresis. In the light of more recently developed AVT/IT assays (Warne *et al.*, 1994; Pierson *et al.*, 1995; Gozdowska and Kulczykowska, 2004), sensitive enough to measure the circulating hormones levels, it appears that the diuretic actions reported in earlier studies were present only at high, pharmacological hormones concentrations. Smaller doses of the hormones, which gave plasma AVT/IT concentrations within physiological range, were antidiuretic.

By analogy with mammalian AVP, two components of AVT/IT action in fish kidney, i.e., vascular and tubular should be considered. The diuresis observed after pharmacological AVT doses (100 and more times AVT physiological levels) was accompanied by a significant increase in systemic blood pressure (Henderson and Wales, 1974). In many teleost fish—in contrast to mammals—glomerulotubular balance seems to be poorly developed, and GFR is readily increased by an increase in renal perfusion pressure (Nishimura and Bailey, 1982). Therefore, the changes in systemic blood pressure affect glomerular filtration in fish kidney. Thus, AVT and IT inducing hypertension may be responsible for an increase in GFR, and in this way affect the water/ions balance in fish. The model of *in situ* trout kidney perfused under constant pressure was employed by Amer and Brown (1995) to evaluate the effect of 10^{-9} and 10^{-11} M AVT on glomerular function. The investigations have clearly demonstrated that both concentrations of AVT have a potent glomerular antidiuretic action resulting from the decrease in the filtering population of glomeruli. The AVT induced antidiuresis accompanied by a fall in the number of filtering

nephrons has also been shown in freshwater eel (Henderson and Wales, 1974). Moreover, in a model of trout trunk preparation, the renal response to AVT would appear to be a result of glomerular action of the hormone rather than tubular one (Warne *et al.*, 2002). It has been proposed by Warne *et al.* (2002) that the mechanism of that involved smooth muscle constriction of the renal arterioles regulating blood flow to the glomeruli. Whether it would be V1 type AVT receptor, as suggested by Pang *et al.* (1983) using mammalian V1 receptor antagonists in the trout trunk model, still remains to be elucidated. A decrease in GFR, population of filtering nephrons and urine flow rate induced by AVT has also been shown in a perfused trunk preparation of *Scyliorhinus canicula* by Wells *et al.* (2002).

In addition to the glomerular effect of the hormones, their tubular action has also been taken into account. In isolated nephron of the trout, a dose-dependent rise in cAMP production as a result of the administration of 10^{-5} - 10^{-11} M AVT was demonstrated by Perrott *et al.* (1993) and the presence of a receptor similar to mammalian V2-type receptor was suggested (Balment *et al.*, 1993). More recently, significant stimulation of intracellular accumulation of cAMP by AVT at lower concentrations (10^{-12} M) has been observed in the case of *in vitro* preparation of trout kidney tubules (Warne *et al.*, 2002). Amer and Brown (1995) in their trout trunk studies have presented an increase of tubular reabsorption of water after small dose of AVT (10^{-11} M) and a decrease of that after the higher dose of the hormone (10^{-9} M). On the other hand, Nishimura *et al.* (1983) in early studies of the isolated renal tubules in freshwater trout suggested that in the distal tubule water flux and osmotic water permeability were not affected by neurohypophysial peptides. As yet, there has been no clear picture of either a site of AVT/IT action or a presence of well-defined type AVT/IT receptors in fish renal tubules. The fish nephron lacks the loop of Henle and cannot produce concentrated urine by way of water withdrawal in the collecting duct. Thus, the tubular action of AVT, if any, is probably different from that of AVP in mammalian kidney and any analogies between both nonapeptides should be drawn carefully.

The fluid produced by the kidney passes to a urinary bladder, organ of a high permeability to water and solute, where the composition of renal output can be modified probably by the hormones. In the 1990s, the AVT and IT receptors have been identified in urinary bladder in *C. commersoni*

by Mahlmann *et al.* (1994) and Hausmann *et al.* (1995), but a direct role of nonapeptides remains to be determined.

INTERACTIONS WITH OTHER HORMONES

The hormonal regulation of water and ion homeostasis requires participation and interaction of many endocrine systems at many functional levels in the organism. Hence, the role of potential relationships between AVT/IT and other hormones systems contributing to osmoregulation in fish is considered. However, such interactions, with few exceptions, have not been studied to date in fish and, therefore, data presented herein are scarce.

Cortisol, prolactin and growth hormone (GH) have been well documented to be involved in osmoregulation in fish since the sixties (Bentley, 1971). The investigations undertaken in goldfish demonstrated that both AVT and IT can stimulate cortisol secretion in this species and suggested the corticotropin-releasing factor (CRF) activity of these hormones (Fryer and Leung, 1982). Moreover, Pierson *et al.* (1995) showed that both neurohypophysial hormones induced in a dose-dependent way the corticotropin (ACTH) release from trout pituitary. On the other hand, in view of papers by Fryer *et al.* (1985) and Olivereau and Olivereau (1990), the participation of AVT in stimulation of ACTH release in teleosts seems to be less clear, but can not be excluded. In terms of other osmoregulatory hormones, i.e., GH and prolactin, there is a lack of satisfactory data on the relationship with neurohypophysial nonapeptides, although the binding sites for AVT found in GH-producing cells in the pituitary may suggest an involvement of AVT in GH release (Moons *et al.*, 1989).

The renin-angiotensin system (RAS) appears to be an important factor in the regulation of blood pressure and drinking, and thus in the control of body fluid homeostasis in teleost fish (Russell *et al.*, 2001). Hence, a possible interaction between angiotensin II, a potent dipsogenic hormone, and neurohypophysial hormones in fish has also been considered. In mammals, administration of angiotensin II stimulates vasopressin secretion from pituitary (Yamaguchi *et al.*, 1985). In the chronically cannulated flounder model, however, an inhibitory effect of angiotensin II on AVT release has been observed (Balment *et al.*, 2003). It is not yet clear whether angiotensin II is a factor engaged in physiological control of AVT release in fish, but a potential relationship between

hormones would be of special significance in species migrating from fresh- to seawater, when angiotensin II stimulate drinking (Takei, 2000).

ANALOGY BETWEEN NEUROHYPOPHYSIAL HORMONES IN FISH AND MAMMALS: A USEFUL PARADIGM?

The advanced knowledge of AVP engagement in mammalian osmoregulation and the absence of equivalent data on AVT and IT in fish are templates for drawing analogies. Is that legitimate?

In mammals, the kidney is a sole important osmoregulatory organ and vasopressin is a main antidiuretic hormone acting here. On the basis of functional and pharmacological criteria, two types of renal vasopressin receptors have been distinguished: V1 receptors which activation is associated with mobilizing intracellular calcium or stimulating phosphoinositide breakdown and V2 receptors leading to the activation of adenylate cyclase. Generally, V1 type receptor is associated with the vascular action of AVP and V2 type receptor is linked to the tubular antidiuretic action of the hormone in the distal parts of nephron.

In fish, on the other hand, the many structures such as gill, GIT, salt gland and urinary bladder act in concert with the kidney so as to maintain homeostasis of body fluids and electrolytes. Moreover, the kidney seems to be less important organ in fish osmoregulation process. The precise characterization of AVT and IT receptors and their distribution in osmoregulatory organs is far from being established. In fish species studied to date, i.e., flounder and *C. commersoni*, AVT receptors have a higher homology with vasopressin V1 type than with the V2 type and are linked to the phospholipase C-phosphatidylinositol signaling pathway. On the other hand, the isotocin receptors are closely related to mammalian oxytocin receptors and vasopressin V1 type. Also, V2 type receptors linked to adenylate cyclase seem to be present in fish kidney. A dose-dependent increase of cAMP production after AVT administration was shown in trout renal tubules *in vitro* (Perrott *et al.*, 1993). Moreover, a significant accumulation of cAMP by AVT within the range of physiological concentration was presented in trout nephron suspension (Warne *et al.*, 2002). The occurrence of V2 type receptors was also suggested in other transport epithelia in fish. Urea excretion in marine toadfish (*Opsanus beta*) gill was proposed to involve a specific transport mechanism analogous to the vasopressin-sensitive renal urea transporter of mammals linked to V2 type receptors (Walsh, 1997). It was shown in this

aglomerular fish, that pulsatile branchial urea excretion was under the control of arginine vasotocin (Perry *et al.,* 1998). However, the recent experimental data by Wood *et al.* (2001) do not confirm this hypothesis. Therefore, further studies are essential to examine whether in fish nephron the AVT receptors related to mammalian V2 type are present. The character of the tubular action of AVT in fish, if any, would be probably different from that of AVP in mammals, because the fish nephron lacks the loop of Henle and cannot produce concentrated urine by water withdrawal in the collecting duct.

Thus, the mammalian paradigm offered to elucidate the mode of involvement of AVT/IT in osmoregulation in fish, although essential considering a lack of satisfactory fish data, can be applied only within strict limit.

SUMMARY. ARE THERE CONVINCING EVIDENCES ON A ROLE OF AVT/IT IN FISH OSMOREGULATION?

In this chapter, the current state of the knowledge of the role of neurohypophysial hormones arginine vasotocin and isotocin in fish osmoregulation has been summarized.

It has been shown that the synthesis of nonapeptides in hypothalamus and their secretion into circulation from the neurohypophysis are sensitive to an important environmental factor: salinity. Rapid transfer of teleost fish between fresh and seawaters results in altered mRNA expression of AVT precursors in hypothalamic neurons and consequently in altered content of AVT in pituitary. The patterns of plasma AVT and IT concentrations in response to external salinity changes suggest the role of the hormones in the mechanism of fast adaptation. AVT and IT releases appear to be controlled independently. There are three main osmoregulatory organs in fish: gill, GIT and kidney, which are considered as potential goals for neurohypophysial nonapetides action. Physiological responses of fish to different water salinities and suggested involvement of AVT/IT are summarized in Fig. 6.4.

There are clearly many unresolved questions to be explored. Data on the osmoregulatory role of AVT in fish still remains fragmentary. Implication of isotocin in this process is even less clear. Moreover, the physiological role of neurohypophysial hormones in the maintenance of water/ions homeostasis in fish does not seem to be uniform among species, in contrast to their antidiuretic and sodium-retaining function in

tetrapods. A mammalian paradigm, although helpful in interpretation of fish data, needs to be verified by studies in fish. The new experimental approaches and modern techniques addressed to arginine vasotocin and isotocin gives a promise to elucidate a role of both nonapeptides in fish osmoregulation.

References

Acher, R. 1993. Neurohypophysial peptide systems: processing machinery, hydro-osmotic regulation, adaptation and evolution. *Regulatory Peptides* 45: 1–13.

Acher, R. 1995. Evolution of neurohypophysial control of water homeostasis: Integrative biology of molecular, cellular and organismal aspects. In: *Recent Progress of Vasopressin and Oxytocin Research*, T. Saito, K. Kurosawa and S. Yoshida (eds.) Neurohypophysis, Elsevier Science, Amsterdam, pp. 39–54.

Acher, R. and J. Chauvet. 1995. The neurohypophysial endocrine regulatory cascade: Precursors, mediators, receptors, and effectors. *Frontiers in Neuroendocrinology* 16, 237–289.

Acher, R., J. Chauvet, M.T. Chauvet and D. Crepy. 1961. Les hormones neurohypophysaires des poissons: Isolement d'une vasotocine du tacaud (*Gadus luscus* L.). *Biochimica et Biophysica Acta* 51: 419–420.

Acher, R., J. Chauvet, M.T. Chauvet and D. Crepy. 1962. Isolement d'une nouvelle hormone neurohypophysaire, l'isotocine presente chez les poissons osseux. *Biochimica et Biophysica Acta* 58: 624–625.

Acher, R., J. Chauvet, M.T. Chauvet and Y. Rouille. 1999. Unique evolution of neurohypophysial hormones in cartilaginous fishes: Possible implication for urea-based osmoregulation. *Journal of Experimental Zoology* 284: 475–484.

Amer, S. and A. Brown. 1995. Glomerular actions of arginine vasotocin in the *in situ* perfused trout kidney. *American Journal of Physiology* 269: R775–R780.

Babiker, M.M. and J.C. Rankin. 1978. Neurohypophysial hormonal control of kidney function in the European eel (*Anguilla anguilla* L.) adapted to sea-water or fresh water. *Journal of Endocrinology* 76: 347–358.

Babiker, M.M. and J.C. Rankin. 1979. Renal and vascular effects of neurohypophysial hormones in the African lungfish *Protopterus annectens* (Owen). *General and Comparative Endocrinology* 37: 26–34.

Balment, R.J., J.M. Warne, M. Tierney and N. Hazon. 1993. Arginine vasotocin (AVT) and fish osmoregulation. *Fish Physiology and Biochemistry* 11: 189–194.

Balment, R.J., J.M. Warne and Y. Takei. 2003. Isolation, synthesis, and biological activity of flounder [Asn^{1}, Ile^{5}, Thr^{9}] angiotensin I. *General and Comparative Endocrinology* 130: 92–98.

Bennett, M.B. and J.C. Rankin. 1986. The effect of neurohypophysial hormones on the vascular resistance of the isolated perfused gill of the European eel *Anguilla anguilla* L. *General and Comparative Endocrinology* 64: 60–66.

Bentley, P.J. 1971. Endocrines and osmoregulation. A comparative account of the regulation of water and salt in vertebrates. In: *Zoophysiology and Ecology*. W.S. Hoar,

J. Jacobs, H. Langer and M. Lindauer (eds.). Springer-Verlag, New York: Vol. 1. pp. 1–37, 199–254.

Bentley, P.J. 1998. *Comparative Vertebrates Endocrinology*. 3rd Edition. Cambridge University Press, Cambridge, pp. 67–176.

Beyenbach, K. 1995. Secretory electrolyte transport in renal proximal tubules of fish. In: *Cellular and Molecular Approaches to Fish Ionic Regulation*, C.M. Wood and T.J. Shuttleworth (eds.). Academic Press, San Diego, pp. 85–105.

Bond, H., M.J. Winter, J.M. Warne, C.R. McCrohan and R.J. Balment. 2002. Plasma concentrations of arginine vasotocin and urotensin II are reduced following transfer of the euryhaline flounder (*Platichthys flesus*) from seawater to fresh water. *General and Comparative Endocrinology* 125: 113–120.

Carlson, I.H. and W.N. Holmes. 1962. Changes in the hormone content of the hypothalamo-hypophysial system of the rainbow trout, *Salmo gairdneri*. *Journal of Endocrinology* 24: 23–32.

Chester Jones, I., D.K.O. Chan and J.C. Rankin. 1969. Renal function in the European eel (*Anguilla anguilla* L.): Effects of the caudal neurosecretory system, corpuscles of Stannius, neurohypophysial peptides and vasoactive substances. *Journal of Endocrinology* 43: 21–31.

Conklin, D.J., N.W. Mick and K.R. Olson. 1996. Arginine vasotocin relaxation of gar (*Lepisosteous* spp.) hepatic vein *in vitro*. *General and Comparative Endocrinology* 104: 52–60.

Conklin, D.J., A. Chavas, D.W. Duff, L. Weaver, Y. Zhang and K.R. Olson. 1997. Cardiovascular effects of arginine vasotocin in the rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* 200: 2821–2832.

Conklin, D.J., M.P. Smith and K.R. Olson. 1999. Pharmacological characterization of arginine vasotocin vascular smooth muscle receptors in the trout (*Oncorhynchus mykiss*) *in vitro*. *General and Comparative Endocrinology* 114: 36–46.

Danzler, W.H. (2003). Regulation of renal proximal and distal tubule transport: sodium, chloride and organic anions. *Comparative Biochemistry and Physiology* A 136: 453–478.

Foran, C.M. and A.H. Bass. 1998. Preoptic AVT immunoreactive neuron of a teleost fish with alternative reproductive tactics. *General and Comparative Endocrinology* 111: 271–282.

Friedmann, A.S., V.A. Memoli, X.M. Yu and W.G. North. 1993. Biosynthesis of vasopressin by gastrointestinal cells of Brattleboro and Long-Evans rats. *Peptides* 14: 607–612.

Fridberg, G., and R. Olsson. 1959. The preopticohypophysial system, nucleus tuberis lateralis and subcommisural organ of *Gasterosteus aculeatus* after changes in osmotic stimuli. *Zeitschrift fur Zellforschung und Mikroscopist Anatomie* 49: 531–540.

Fryer, J.N., and E. Leung. 1982. Neurohypophysial hormonal control of cortisol secretion in the teleost *Carassius auratus*. *General and Comparative Endocrinology* 48: 425–431.

Fryer, J., K. Lederis and J. Rivier. 1985. ACTH-releasing activity of urotensin I and ovine CRF: Interactions with arginine vasotocin, isotocin and arginine vasopressin. *Regulatory Peptides* 11: 11–15.

Goodson, J.L. and A.H. Bass. 2000. Forebrain peptides modulate sexually polymorphic vocal circuitry. *Nature (Lond.)* 403: 769–772.

Goossens, N., K. Dierickx and F. Vandesande. 1977. Immunocytochemical localization of vasotocin and isotocin in the preopticohypophysial neurosecretory system of teleosts. *General and Comparative Endocrinology* 32: 371–375.

Gozdowska, M. and E. Kulczykowska. 2004. Determination of arginine-vasotocin and isotocin in fish plasma with solid-phase extraction and fluorescence derivatization followed by high-performance liquid chromatography. *Journal of Chromatography* B 807: 229–233.

Guibbolini, M.E. and M. Avella. 2003. Neurohypophysial hormone regulation of Cl- secretion: Physiological evidence for V1-type receptors in sea bass gill respiratory cells in culture. *Journal of Endocrinology* 176: 111–119.

Guibbolini, M.E. and B. Lahlou. 1987a. Neurohypophysial peptide inhibition of adenylate cyclase activity in fish gills. The effect of environmental salinity. *FEBS Letters* 220: 98–102.

Guibbolini, M.E. and B. Lahlou. 1987b. Adenylate cyclase activity in fish gills in relation to salt adaptation. *Life Sciences* 41: 71–78.

Guibbolini, M.E. and B. Lahlou. 1990. Evidence for presence of a new type of neurohypophysial hormone receptor in fish gill epithelium. *American Journal of Physiology* 258: R3–R9.

Guibbolini, M.E. and B. Lahlou. 1992. G_i protein mediates adenylate cyclase inhibition by neurohypophyseal hormones in fish gill. *Peptides* 13: 865–871.

Guibbolini, M.E., I.W. Henderson, W. Mosley and B. Lahlou. 1988. Arginine vasotocin binding to isolated branchial cells of the eel: effect of salinity. *Journal of Molecular Endocrinology* 1: 125–130.

Harding, K.E., J.M. Warne, S. Hyodo and R.J. Balment. 1997. Pituitary and plasma AVT content in the flounder (*Platichthys flesus*). *Fish Physiology and Biochemistry* 17: 357–362.

Haruta, K., T. Yamashita and S. Kawashima. 1991. Changes in arginine vasotocin content in the pituitary of the medaka (*Oryzias latipes*) during osmotic stress. *General and Comparative Endocrinology* 83: 327–336.

Hausmann, H., W. Meyerhof, H. Zwiers, K. Lederis and D. Richter. 1995. Teleost isotocin receptor: Structure, functional expression, mRNA distribution and phylogeny. *FEBS Letters* 370: 227–230.

Hausmann, H., A. Richters, H.-J. Kreienkamp, W. Meyerhof, H. Mattes, K. Lederis, H. Zwiers and D. Richter. 1996. Mutational analysis and molecular modeling of the nonapeptide hormone binding domains of the [Arg^8] vasotocin receptor. *Proceedings of the National Academy of Sciences of the United States of America* 93: 6907–6912.

Heierhorst, J., S.D. Morley, J. Figureoa, C. Krentler, K. Lederis and D. Richter. 1989. Vasotocin and isotocin precursors from the white sucker, *Catostomus commersoni*: Cloning and sequence analysis of the cDNAs. *Proceedings of the National Academy of Sciences of the United States of America* 86: 5242–5246.

Heierhorst, J., S. Mahlmann, S.D. Morley, I.R. Coe, N.M. Sherwood and D. Richter. 1990. Molecular cloning of two distinct vasotocin precursor cDNAs from chum salmon, *Oncorhynchus keta* suggests an ancient gene duplication. *FEBS Letters* 260: 301–304.

Henderson, I.W. and N.A.M. Wales. 1974. Renal diuresis and antidiuresis after injections of arginine vasotocin in the freshwater eel (*Anguilla anguilla* L.). *Journal of Endocrinology* 61: 487–500.

Hiraoka, S., M. Suzuki, T. Yanagisawa, M. Iwata and A. Urano. 1993. Divergence of gene expression in neurohypophysial hormone precursors among salmonids. *General and Comparative Endocrinology* 92: 292–301.

Holmes, W.N. and R.L. McBean. 1963. Studies on the glomerular filtration rate of rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* 40: 335–341.

Holmqvist, B.I. and P. Ekström. 1995. Hypophysiotrophic systems in the brain of the Atlantic salmon. Neuronal innervation of the pituitary and the origin of pituitary dopamine and nonapeptides identified by means of combined carbocyanine tract tracing and immunocytochemistry. *Journal of Chemical Neuroanatomy* 8: 125–145.

Hyodo, S., Y. Kato, M. Ono and A. Urano. 1991. Cloning and sequence analyses of cDNA encoding vasotocin and isotocin precursors of chum salmon, *Oncorhynchus keta*: Evolutionary relationships of neurohypophysial hormone precursors. *Journal of Comparative Physiology* B 160: 601–608.

Hyodo, S. and A. Urano. 1991. Changes in expression of provasotocin and proisotocin genes during adaptation to hyper- and hypo-osmotic environments in rainbow trout. *Journal of Comparative Physiology* B 161: 549–556.

Hyodo, S., S. Ishii and J.M.P. Joss. 1997. Australian lungfish neurohypophysial hormone genes encode vasotocin and [Phe^2]mesotocin precursors homologous to tetrapod-type precursors. *Proceedings of the National Academy of Sciences of the United States of America* 94: 13339–13344.

Hyodo, S., T. Tsukada and Y. Takei 2004. Neurohypophysial hormones of dogfish, *Triakis scyllium*: structures and salinity-dependent secretion. *General and Comparative Endocrinology* 138: 976–104.

Kulczykowska, E. 1997. Response of circulating arginine vasotocin and isotocin to rapid osmotic challenge in rainbow trout. *Comparative Biochemistry and Physiology* A118: 772–778.

Kulczykowska, E. 1998. Effects of arginine vasotocin, isotocin and melatonin on blood pressure in the conscious Atlantic cod (*Gadus morhua*): hormonal interactions? *Experimental Physiology* 83: 809–820.

Kulczykowska, E. 2001. Responses of circulating arginine vasotocin, isotocin, and melatonin to osmotic and disturbance stress in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry* 24: 201–206.

Kulczykowska, E. 2002. A review of the multifunctional hormone melatonin and a new hypothesis involving osmoregulation. *Reviews in Fish Biology and Fisheries* 11: 321–330.

Lederis, K. 1964. Fine structure and hormone content of the hypothalamo-neurohypophysial system of the rainbow trout (*Salmo irideus*) exposed to seawater. *General and Comparative Endocrinology* 4: 638–661.

Le Mevel, J.C., T.-F. Pomantung, D. Mabin and H. Vaudry. 1993. Effects of central and peripheral administration of arginine vasotocin and related neuropeptides on blood pressure and heart rate in the conscious trout. *Brain Research* 610: 82–89.

Lewis, C.M., E.K. Dolence, Z. Zhang and J.D. Rose. 2004. Fluorescent vasotocin conjugate for identification of the target cells for brain actions of vasotocin. *Bioconjugate Chemistry* 15: 909–914.

Maetz, J. and B. Lahlou. 1974. Actions of neurohypophysial hormones in fishes. In: *Handbook of Physiology-Endocrinology*, R.O. Greep and E.B. Astwood (eds.). American Physiological Society, Baltimore, Vol. 4, Part 1, pp. 521–544.

Maetz, J., J. Bourguet, B. Lahlou and J. Hourdry. 1964. Peptides neurohypophysaires et osmoregulation chez *Carassius auratus*. *General and Comparative Endocrinology* 4: 508–522.

Mahlmann, S., W. Meyerhof, H. Hausmann, J. Heierhorst, C. Schönrock, H. Zwiers, K. Lederis and D. Richter. 1994. Structure, function, and phylogeny of [Arg^8]vasotocin receptors from teleost fish and toad. *Proceedings of the National Academy of Sciences of the United States of America* 91: 1342–1345.

Marshall, W.S. 2003. Rapid regulation of NaCl secretion by estuarine teleost fish: coping strategies for short-duration freshwater exposures. *Biochimica et Biophysica Acta* 1618: 95–105.

Moons, L., M. Cambre, T.F.C. Batten and F. Vandesande. 1989. Autoradiographic localization of binding sites for vasotocin in the brain and pituitary of the sea bass (*Dicentrarchus labrax*). *Neuroscience Letters* 100: 11–16.

Motais, R. and J. Maetz. 1967. Arginine vasotocine et evolution de la permeabilite branchiale au sodium au cours du passage d'eau douce en eau de mer chez le Flet. *Journal of Physiology* (Paris) 59: 271.

Nishimura, H. and J.R. Bailey. 1982. Intrarenal renin-angiotensin system in primitive vertebrates. *Kidney International* 12: S185–192.

Nishimura, H. and Z. Fan. 2003. Regulation of water movement across vertebrate renal tubules. Comparative Biochemistry and Physiology A 136: 479–498.

Nishimura, H., M. Imai and M. Ogawa. 1983. Sodium chloride and water transport in the renal distal tubule of the rainbow trout. *American Journal of Physiology* 244: F247–F254.

Nürnberger, F., H.-W. Korf, M.A. Ali and D.L.G. Nokes. 1996. Salinity and vasotocin immunoreactivity in the brain of *Rivulus marmoratus* (Teleostei). *Naturwissenchaften* 83: 326–328.

Olivereau, M. and J. Olivereau 1990. Effect of pharmacological adrenalectomy on corticotropin-releasing factor-like and arginine vasotocin immunoreactivities in the brain and pituitary of the eel: immunocytochemical study. *General and Comparative Endocrinology* 80: 199–215.

Oudit, G.Y. and D.G. Butler. 1995. Cardiovascular effects of arginine vasotocin, atrial natriuretic peptide, and epinephrine in freshwater eels. *American Journal of Physiology* 268: R1273–R1280.

Pang, P.K.T., P.B. Furspan and W.H. Sawyer. 1983. Evolution of neurohypophyseal hormone actions in vertebrates. *American Zoologist* 23: 655–662.

Perrott, M.N., S. Carrick and R.J. Balment. 1991. Pituitary and plasma arginine vasotocin levels in teleost fish. *General and Comparative Endocrinology* 83: 68–74.

Perrott, M.N., R.J. Sainsbury and R.J. Balment. 1993. Peptide hormone-stimulated second messenger production in the teleostean nephron. *General and Comparative Endocrinology* 89: 387–395.

Perry, S.F., K.M. Gilmour, C.M. Wood, P. Part, P. Laurent and P.J. Walsh. 1998. The effect of arginine vasotocin and catecholamines on nitrogen excretion and the cardio-respiratory physiology of the gulf toadfish, *Opsanus beta*. *Journal of Comparative Physiology*B 168: 461–472.

Pickering, B.T. 1968. A neurophysin from cod (*Gadus morhua*) pituitary glands: isolation and properties. *Journal of Endocrinology* 42: 143–152.

Pierson, P.M., M.E. Guibbolini, N. Mayer-Gostan and B. Lahlou. 1995. ELISA measurements of vasotocin and isotocin in plasma and pituitary of the rainbow trout: Effect of salinity. *Peptides* 16: 859–865.

Renfro, J.L. 1999. Recent developments in teleost renal transport. *Journal of Experimental Zoology* 283: 653–661.

Russell, M.J., A.M. Klemmer and K.R. Olson. 2001. Angiotensin signaling and receptor types in teleost fish. *Comparative Biochemistry and Physiology* A 128: 41–51.

Saito, D., M. Komatsuda and A. Urano. 2004. Functional organization of preoptic vasotocin and isotocin neurons in the brain of rainbow trout: central and neurohypophysial projections of single neurons. *Neuroscience* 124: 973–984.

Sanchez-Franco, F., L. Cacicedo, J.L. Vasallo, J.L. Blazquez and L. Munoz Baragan. 1986. Arginine-vasopressin immunoreactive material in the gastrointestinal tract. *Histochemistry* 85: 419–422.

Sharratt, B.M., D. Bellamy and I. Chester Jones. 1964 Adaptation of the silver eel (*Anguilla anguilla*) to seawater and to artificial media together with observations on the role of the gut. *Comparative Biochemistry and Physiology* 11: 19–30.

Suzuki, M., S. Hyodo, A. Urano. 1992. Cloning and sequence analyses of vasotocin and isotocin precursor cDNA in the masu salmon, *Oncorhynchus masou*: evolution of neurohypophysial hormone precursors. *Zoological Science* 9: 157–167.

Szczepañska-Sadowska, E., D. Gray and C. Simon-Oppermann. 1983. Vasopressin in blood and third ventricle CSF during dehydration, thirst, and hemorrhage. *American Journal of Physiology* 245: R549–R555.

Takei, Y. 2000. Comparative physiology of body fluid regulation in vertebrates with special reference to thirst regulation. *Japanese Journal of Physiology* 50: 171–186.

Unger, J.L. and E. Glasgow. 2003. Expression of isotocin-neurophysin mRNA in developing zebrafish. *Gene Expression Patterns* 3: 105–108.

Urano, A., K. Kubokawa and S. Hiraoka. 1994. Expression of the vasotocin and isotocin gene family in fish. In: *Fish Physiology,* N.M. Sherwood and C.L. Hew (eds.). Academic Press, New York, Vol. 13, pp. 101–132.

Van den Dungen, H.M., R.M. Buijs, C.W. Pool and M. Terlou. 1982. The distribution of vasotocin and isotocin in the brain of the rainbow trout. *Journal of Comparative Neurology* 212: 146–157.

Walsh, P.J. 1997. Evolution and regulation of urea synthesis and ureotely in (batrachoidid) fishes. *Annual Review of Physiology* 59: 299–323.

Warne, J.M. 2001. Cloning and characterization of an arginine vasotocin receptor from the euryhaline flounder *Platichthys flesus*. *General and Comparative Endocrinology* 122: 312–319.

Warne, J.M. and R.J. Balment. 1995. Effect of acute manipulation of blood volume and osmolality on plasma [AVT] in seawater flounder. *American Journal of Physiology* 269: R1107–R1112.

Warne, J.M. and R.J. Balment. 1997a. Changes in plasma arginine vasotocin (AVT) concentration and dorsal aortic blood pressure following AVT injection in the teleost *Platichthys flesus*. *General and Comparative Endocrinology* 105: 358–364.

Warne, J.M., and R.J. Balment. 1997b. Vascular actions of neurohypophysial peptides in the flounder. *Fish Physiology and Biochemistry* 17: 313–318.

Warne, J.M., N. Hazon, J.C. Rankin and R.J. Balment. 1994. A radioimmunoassay for the determination of arginine vasotocin (AVT): plasma and pituitary concentrations in fresh- and seawater fish. *General and Comparative Endocrinology* 96: 438–444.

Warne, J.M., S. Hyodo, K. Harding and R.J. Balment. 2000. Cloning of pro-vasotocin and pro-isotocin cDNAs from the flounder *Platichthys flesus*; levels of hypothalamic mRNA following acute osmotic challenge. *General and Comparative Endocrinology* 119: 77–84.

Warne, J.M., K.E. Harding and R.J. Balment. 2002. Neurohypophysial hormones and renal function in fish and mammals. *Comparative Biochemistry and Physiology* B 132: 231–237.

Wells, A., W.G. Anderson and N. Hazon. 2002. Development of an *in situ* perfused kidney preparation for elasmobranch fish: action of arginine vasotocin. *American Journal of Physiology* 282: R1636–R1642.

Wood, C.M., J.M. Warne, Y. Wang, M.D. McDonald, R.J. Balment, P. Laurent and P.J. Walsh. 2001. Do circulating plasma AVT and/or cortisol levels control pulsatile urea excretion in the gulf toadfish (*Opsanus beta*)? *Comparative Biochemistry and Physiology* A 129: 859–872.

Yamaguchi, K., M. Kioke and H. Hama. 1985. Plasma vasopressin response to peripheral administration of angiotensin in conscious rats. *American Journal of Physiology* 248: R249–R256.