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Osmoregulatory role of vasotocinergic and isotocinergic systems in the gilthead sea bream (*Sparus aurata* L)

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ABSTRACT

Gilthead sea bream, *Sparus aurata* L., is an important fish species for the Mediterranean aquaculture and is considered a good model for studying the osmoregulatory process, due to its capacity to cope with great changes in environmental salinity (5–60‰). Our group studied the osmoregulatory role of different endocrine systems in this species, focusing on the vasotocinergic and isotocinergic systems over several years. For this purpose, the cDNAs coding for pro-vasotocin (*pro-vt*), pro-isotocin (*pro-it*), two arginine vasotocin (AVT) receptors (*avtr v1a2*- and *v2*-types) and one IT receptor (*itr*) were cloned. Acclimation to different environmental salinities induced a direct lineal relationship between plasma AVT levels and salinity, with no changes in plasma IT values. In addition, higher values in vasotocinergic, isotocinergic and stress pathways (*pro-vt* and *pro-it* gene expression, AVT and IT storage and plasma cortisol levels) in both hypo- and/or hyper-osmotic transfers, suggest an interaction between cortisol and AVT/IT pathways. Moreover, gene expression of specific receptors, as well as the use of different *in vitro* techniques, demonstrated an important osmoregulatory orchestration in different organs. In addition, individuals intraperitoneally injected with AVT and transferred to different environmental salinities enhanced plasma cortisol levels and/or gill Na^+, K^+-ATPase activity. These effects could be related to the energy repartitioning process occurring during osmotic adaptation of *S. aurata* to extreme environmental salinities, which could be mediated not only by plasma cortisol but also by AVT. Finally, our results indicated a very important role of the vasotocinergic and/or isotocinergic systems in both osmoregulatory and non-osmoregulatory organs.

1. Introduction

Fish nonapeptides arginine vasotocin (AVT) and isotocin (IT), orthologs to mammalian arginine vasopressin (AVP) and oxytocin (OXY), are produced in the hypothalamic magnocellular and parvocellular neurons of the *nucleus preopticus*, from where they are transported to the neurohypophysis for storage and release (Bentley, 2002). After that, these neuropeptides will bind to specific receptors located in different tissues to produce the physiological action required (Fig. 1). Several studies have shown their synthesis and secretion into the circulation in response to environmental salinity (Maetz and Lahlou, 1974; Haruta et al., 1991; Hyodo and Urano, 1991; Perrott et al., 1991). The neurohypophysial hormones AVT and IT are presumed to play a role in quick and long-term adaptation of teleost fish to external salinity changes (Kulczykowska, 1997, 2001; Bond et al., 2002; Warne et al., 2005). However, data on the osmoregulatory role of AVT in fish are often contradictory, and the physiological role of these hormones in maintenance of water/ions homeostasis does not seem to be uniform among fish species (Kulczykowska, 2007). Moreover, the osmoregulatory role of IT remains unclear.

In this mini-review we will focus on the osmoregulatory role of the vasotocinergic and isotocinergic systems in the gilthead sea bream (*Sparus aurata*), an euryhaline species with a capacity to cope with demands in a wide range of environmental salinities (5–60‰; Laiz-Carrión et al., 2005a). This particularity makes the sea bream a perfect model-fish species to study the osmoregulatory response to salinity-adaptive processes and its hormonal control. In addition, the great importance of this species in the

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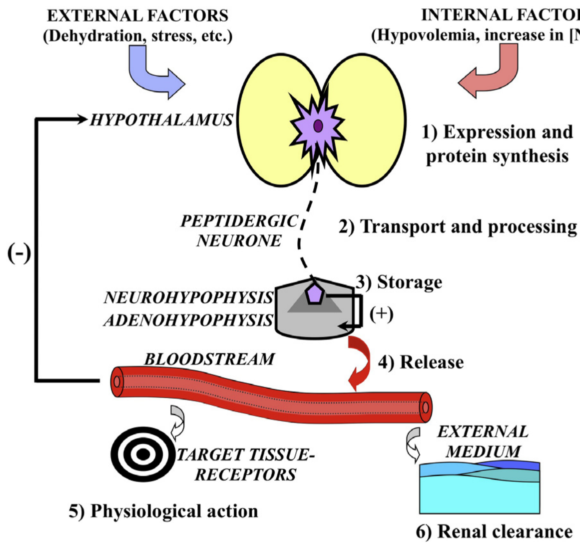

Fig. 1. Schematic representation of vasotocinergic and isotocinergic systems.

Mediterranean aquaculture activity, as well as its differential growth under different environmental salinities (Laiz-Carrión et al., 2005b), is an additional argument to improve our knowledge on the osmoregulatory system of this species.

2. Getting the molecular tools

For this purpose, cDNAs for *pro-vt*, *pro-IT*, two AVT receptors (*avtr v1a2*- and *v2*-types) and one IT receptor (*itr*) were cloned (Martos-Sitcha et al., 2013b, 2014). In short, degenerate primers were designed according to the most highly conserved sequences of cDNA between different fish species for each pro-peptide and receptor. These sets of primers where used to obtain partial cDNA sequences by classical PCR techniques by using the reverse transcription of total RNA from brain as template. After sequencing and checking the partial sequences, each one was individually radiolabeled with ^{32}P and used as a probe to obtain their corresponding full-length cDNAs after screening a brain cDNA library. The obtained clones were fully sequenced in both strands, sequence homologies for all cDNAs were confirmed by NCBI blastn, and phylogenetic analyses were performed (Fig. 2A and B) in order to ensure their correct identification and belonging to the appropriate group. Finally, the complete sequences obtained were used to design specific primers for their use in real time PCR (qPCR). Without this tool development almost all the work described here will have been impossible to attain.

3. AVT/IT systems and direct osmoregulatory effects

Similarly to other euryhaline teleosts, juvenile sea breams under hypo- and hyperosmotic transfers showed two different stages in osmoregulatory and metabolic parameters: (i) an *adaptive period* during the first hours/days of acclimation (12 h – 3 days), with important changes in these parameters, and (ii) a *chronic regulatory period* (after day 3 post-transfer) where parameters reached homeostasis (Laiz-Carrión et al., 2005a; Sangiao-Alvarellos et al., 2005). Moreover, a similar situation has also been detected in both vasotocinergic and isotocinergic systems of *S. aurata* after salinity challenges (Martos-Sitcha et al., 2013b, 2014). During the *adaptive period*, *pro-vt* mRNA increased in both hypo- and hyperosmotic challenges, suggesting a relationship between *pro-vt* gene expression and the initial stress caused by the acute osmotic challenge (see Section 5). During this time, enhancement of plasma metabolites (glucose, triglycerides and protein) levels, concomitantly with plasma cortisol values as well as a consumption of energy reserves, identified by the lower liver glycogen storage, suggested the existence of a clear primary stress response with an energetic reorganization in both hypo- and hyperosmotic environments. In addition, the osmoregulatory role of AVT depends on the tissue in which the active hormone carried out its physiological function. Thus, changes in environmental salinity demonstrated that AVT acts, through its specific receptors, in important osmoregulatory (gills, caudal kidney, or intestine) and non-osmoregulatory (brain, liver, head kidney) organs. In *S. aurata*, both hypo- (5‰) and hyperosmotic (55‰) challenges induced variations in the gene expression of AVT receptors in a tissue-dependent manner, being these changes associated with different enzymes, ion transporters or channels to produce the necessary osmoregulatory or metabolic effects. Even so, most of these variations persist or are enhanced during the *chronic regulatory period* (Martos-Sitcha et al., 2014).

During the *chronic regulatory period* plasma and pituitary concentrations of AVT, and *pro-vt* gene expression in hypothalamic neurons are sensitive to osmotic challenges. Thus, a chronic decrease in AVT pituitary content together with increased AVT plasma levels has been reported in several species after transfer from hypo- to hyperosmotic environments, pointing out a clear osmoregulatory role for AVT during hyperosmotic adaptation (Perrott et al., 1991; Balment et al., 1993; Harding et al., 1997; Kulczykowska, 1997, 2001). In *S. aurata*, a steady enhancement in plasma AVT correlates with increasing water salinity (Kleszczyńska et al., 2006), pointing out a clear hypoosmoregulatory role for AVT in this euryhaline species. Moreover, AVT-treatment of seawater acclimated *S. aurata* individuals enhanced gill Na^+, K^+-ATPase (NKA) activity, a key enzyme for extruding the excess of ions (Laiz-Carrión et al., 2005a), suggesting a role of AVT for acclimation to hyperosmotic environments in this species (Sangiao-Alvarellos et al., 2006). In addition to that, NKA and other ion and water transporters, like cystic fibrosis transmembrane conductance regulator (CFTR), apical Na-K-Cl cotransporter (NKCC2), or aquaporin 1 paralogues (AQP1a and AQP1b), have been closely associated and regulated by different AVT receptors in *S. aurata* in several osmoregulatory tissues, as gills and kidney (Martos-Sitcha et al., 2014), intestine (Martos-Sitcha et al., 2013a, 2015b), and opercular epithelium (Martos-Sitcha et al., 2015a), facilitating the exchange of ions and water to keep their homeostasis.

To our knowledge, only a few studies are available concerning the putative osmoregulatory role of IT (Hiraoka et al., 1996; Kulczykowska, 1997; Warne et al., 2000; Kleszczyńska et al., 2006; Motohashi et al., 2009), where changes in plasma levels, and its control and release, appear differentially sensitive to changes in plasma osmolality. As it has been described for vasotocinergic system, previous results in *S. aurata* indicated the existence of two periods in *pro-it* expression changes, with an initial phase associated with the *adaptive period*, and a later one related to the *chronic regulatory period* (Martos-Sitcha et al., 2013b). In the first period, only transfer to high salinity water enhanced *pro-it* gene expression, suggesting a role of the isotocinergic system in the stress response linked to hyperosmotic, but not to hypoosmotic, transfer. Moreover, the observed enhancement in pituitary IT contents during this *adaptive period* after hyperosmotic transfer, just in the same direction and time post-transfer with respect to the AVT storage values, could suggest that the isotocinergic pathway is stimulated to act throughout the vasotocinergic system as an alternative pathway in the control of osmoregulation and/or stress mediated by the paracrine regulation in the AVT cells (Martos-Sitcha et al., 2013b).

Differences observed in the pituitary IT storage after hyper- and hypoosmotic environments acclimation may suggest no secretion

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