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Neuroendocrine control of ionic balance in zebrafish

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ABSTRACT

Zebrafish (*Danio rerio*) is an emerging model for integrative physiological research. In this mini-review, we discuss recent advances in the neuroendocrine control of ionic balance in this species, and identify current knowledge gaps and issues that would benefit from further investigation. Zebrafish inhabit a hypo-ionic environment and therefore are challenged by a continual loss of ions to the water. To maintain ionic homeostasis, they must actively take up ions from the water and reduce passive ion loss. The adult gill or the skin of larvae are the primary sites of ionic regulation. Current models for the uptake of major ions in zebrafish incorporate at least three types of ion transporting cells (also called ionocytes); H^+-ATPase-rich cells for Na^+ uptake, Na^+/K^+-ATPase-rich cells for Ca^{2+} uptake, and Na^+/Cl^--cotransporter expressing cells for both Na^+ and Cl^- uptake. The precise molecular mechanisms regulating the paracellular loss of ions remain largely unknown. However, epithelial tight junction proteins, including claudins, are thought to play a critical role in reducing ion losses to the surrounding water. Using the zebrafish model, several key neuroendocrine factors were identified as regulators of epithelial ion movement, including the catecholamines (adrenaline and noradrenaline), cortisol, the renin-angiotensin system, parathyroid hormone and prolactin. Increasing evidence also suggests that gasotransmitters, such as H_2S, are involved in regulating ion uptake.

1. Introduction

Previous studies using traditional models [e.g. rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*), killifish (*Fundulus heteroclitus*) and American eel (*Anguilla rostrata*)] have provided important information on the fundamental mechanisms of osmoregulation in fish. However, limitations in the use of molecular physiological approaches in these species have hindered precise characterization of the underlying molecular pathways and mechanisms. The zebrafish (*Danio rerio*) has emerged as an important model for integrative physiological research, owing to the availability of genetic databases, applicability of genetic editing and manipulation, and transparency of the embryos allowing direct cellular/tissue observations. The ion transport pathways in zebrafish are thought to resemble those occurring in different segments of the mammalian kidney (Hwang and Chou, 2013). For example, the expression of different transporters for the absorption of Na^+, Ca^{2+} and Cl^-, and their regulatory functions, appear to be conserved between zebrafish gills/skin and mammalian kidney. Thus, the zebrafish is a useful model system for advancing our understanding of the ion-regulatory functions in the mammalian kidney. Several previous studies using zebrafish have identified key neuroendocrine factors which are important in regulating ionic homeostasis and transepithelial ion movements. The regulation of ion movement in zebrafish was described in several previous reviews (Hwang, 2009; Hwang and Perry, 2010; Hwang and Chou, 2013; Kwong et al., 2014a). The present review provides a brief overview of the mechanisms of ionic regulation in zebrafish and discusses some of the recent advances surrounding their neuroendocrine control. More specifically, we focus on the adrenergic and glucocorticoid systems, angiotensin II, parathyroid hormone (PTH), prolactin and gasotransmitters. Finally, this review reveals current knowledge gaps and identifies issues that could be addressed in future studies.

2. Molecular physiology of ionic regulation in zebrafish

Zebrafish, a freshwater teleost, are hyper-ionic to their environment and thus are challenged constantly by diffusive ion losses. To maintain whole body ionic homeostasis, they need to actively absorb ions from, and reduce passive ion losses to, the environment. In adults, the gills are the predominant site for active ion uptake through specific ion-transporting cells termed ionocytes. During larval stages before the gills are fully

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developed, regulation of epithelial ion transport is primarily mediated by the ionocytes found on the skin of the yolk sac. Although ionocytes are recognized as the universal site of ionic uptake in fish, there is no common nomenclature that yet has been developed to designate their structure and function. Indeed, the development of a common nomenclature is challenging because of interspecific differences in ionocyte subtype that are known to exist across species (for a review, see Dymowska et al., 2012). Current models for the absorption of the major ions (i.e. Na^+, Ca^{2+} and Cl^-) in zebrafish incorporate at least 3 types of ionocytes; H^+-ATPase-rich cells (HRCs), Na^+/K^+-ATPase-rich cells (NaRCs) and Na^+/Cl^--cotransporter-expressing cells (NCCCs) (Fig. 1). HRCs express H^+-ATPase and Na^+/H^+-exchanger at the apical membrane (i.e. NHE3b) for H^+ secretion and Na^+ uptake, respectively; NCCCs express Na^+/Cl^--cotransporter for both Na^+ and Cl^- uptake; a subset of NaRCs express epithelial Ca^{2+} channel (ECaC) at the apical membrane for Ca^{2+} uptake, and Na^+/Ca^{2+}-exchanger and Ca^{2+}-ATPase at the basolateral membrane for Ca^{2+} extrusion. Different paralogues of Na^+/K^+-ATPase are expressed at the basolateral membrane in all these 3 types of ionocytes (Horng et al., 2007; Kumai and Perry, 2011; Liao et al., 2009; Pan et al., 2005; Shih et al., 2012; Wang et al., 2009). Recently, the acid-sensing ion channels (ASICs), the closest relatives of the mammalian epithelial Na^+ channels (ENaC), were identified at the apical membrane of HRCs in adult zebrafish gills (Dymowska et al., 2015).

Paracellular movement of ions and fluid is regulated predominantly by the tight junction proteins. Occludin, claudins, tricellulins and junctional adhesion molecule are major components of the tight junctions. However, claudins are thought to be the primary determinant of the epithelial barrier functions in vertebrates (Turksen and Troy, 2004). To date, there are over 50 different isoforms of claudins that have been identified in zebrafish, and most of them are expressed in a cell- or tissue-specific manner (Baltzegar et al., 2013; Clelland and Kelly, 2010; Kumai et al., 2011; Kwong and Perry, 2013b). The functional characteristics of most of the claudins remain largely unknown. Nonetheless, certain claudin isoforms appear to form selective pores or barriers to regulate the paracellular movement of ions or fluid (Bagnat et al., 2007; Kwong et al., 2013; Kwong and Perry, 2013b; Zhang et al., 2010), and are critical for development and osmoregulation (for a review, see Kolosov et al., 2013).

3. Neuroendocrine control of ionic regulation in zebrafish

3.1. Catecholamines

In vertebrates, the catecholamines, adrenaline and noradrenaline, are essential for regulating cardiovascular and respiratory functions. It is also documented that the adrenergic system is involved in osmoregulation and acid-base balance in fish (Donald, 1989; Kumai et al., 2012b; Marshall et al., 1993; McDonald et al., 1989; Perry et al., 1984, 1996; Perry and Vermette, 1987; Reid et al., 1998; Vermette and Perry, 1987). Catecholamines either can interact with target cells after their secretion as neurotransmitters from nearby nerve endings, or after their release from chromaffin cells for more widespread distribution via the circulatory system. Using high-performance liquid chromatography (HPLC), catecholamines were detectable in whole embryos at 1 h post fertilization (hpf), presumably derived from maternal transfer (Steele et al., 2011). At 2 days post fertilization (dpf), chromaffin cells were found to be dispersed as clusters in the inter-renal organ, which then converged to midline by 3 dpf; the chromaffin cells stay in intimate contact with steroidogenic cells throughout the subsequent development (Liu, 2007; To et al., 2007). In zebrafish, both HRCs and NaRCs are innervated (Jonz and Nurse, 2006; Kumai et al., 2012b). Using fluorescently labelled propranolol (non-selective β-adrenergic receptor antagonist), β receptors were found to be distributed in HRCs, as well as in mitochondrion-rich ionocytes (likely NaRCs). The *ecac*-expressing NaRCs also are closely associated with an extensive network of nerves (Fig. 2). These observations suggest the possible direct control of ionocyte functions by the neuronal systems in zebrafish.

Using pharmacological approaches, it was demonstrated that blockade of β-adrenergic receptors decreased Na^+ uptake in larval zebrafish, whereas blockade of α-adrenergic receptors increased Na^+ uptake (Kumai et al., 2012b). Similarly, pharmacological inhibition of β- and α-adrenergic receptors decreased and increased Ca^{2+} uptake, respectively (R.W.M. Kwong and S.F. Perry; unpublished results). Therefore, it appears that β- and α-adrenergic systems play opposing roles in regulating Na^+ and Ca^{2+} uptake. More importantly, knockdown of specific β-adrenergic receptors was shown to inhibit Na^+ uptake in larval zebrafish exposed to acidic or ion-poor water, conditions known to increase Na^+ transport

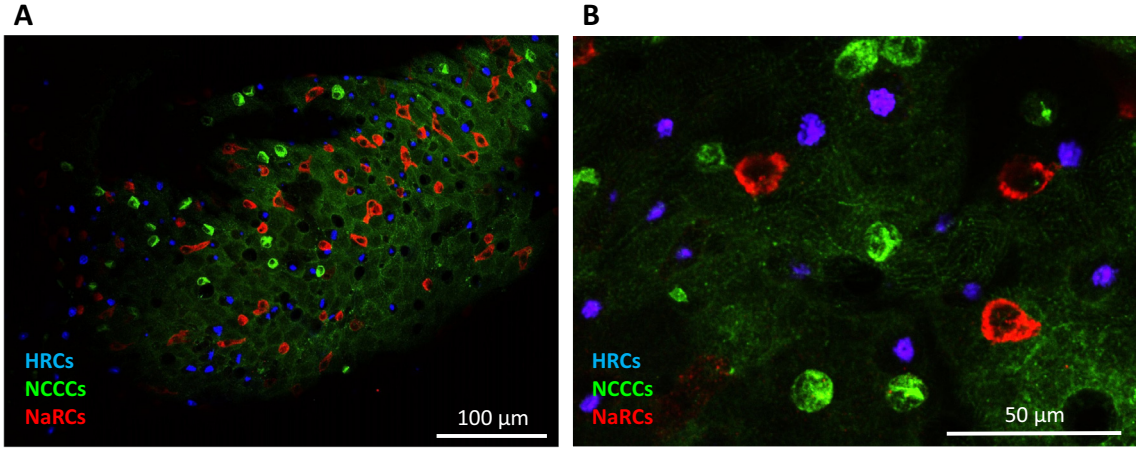

Fig. 1. (A) Fluorescent immunohistochemistry and confocal microscopy showing the H^+-ATPase-rich cells (HRCs; blue), Na^+/Cl^--cotransporter expressing cells (NCCCs; green) and Na^+/K^+-ATPase-rich cells (NaRCs; red) on the skin of yolk sac in larval zebrafish at 4 days post fertilization (dpf). Image (B) shows a higher magnification of the ionocytes. HRCs were labelled with concanavalin A. NCCCs and NaRCs were labelled with a polyclonal antibody raised against the zebrafish NCC and a monoclonal antibody against the α-subunit of avian Na^+-K^+-ATPase, respectively.