

Drinking and Alimentary Transport in Teleost Osmoregulation

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Summary

Alimentary uptake of salts and of water in teleost fishes depends on the interplay of drinking, food ingestion and several intestinal transport processes, and on the complex regulation of these components by the nervous and endocrine systems. Recent studies have elucidated the comparative physiology of thirst and dipsogenesis in the fishes, and have implicated the renin-angiotensin (dipsogenic) and natriuretic peptide (anti-dipsogenic) systems in their endocrine regulation. Since drinking and intestinal processing are sequentially related with regard to their physiological functions, dipsogenic regulation may have profound and overriding effects on alimentary contributions to water balance. Moreover, intestinal lumen status may influence drinking rate through endocrine mediation. Emerging knowledge about the expression of aquaporin water channels in teleost tissues will likely stimulate a reevaluation of alimentary water transport.

Introduction

Successful salinity adaptation by teleost fishes requires the coordinated regulation of drinking, the active absorptive transport of salts and fluid from the alimentary tract, and the transport (uptake or extrusion) of salts at renal and extrarenal sites. Homer Smith, earlier

in this century, identified the specific features of teleostean osmoregulation in the hyperosmotic marine environment (Smith, 1930). In contrast to freshwater fishes that drink little, eliminate copious amounts of dilute urine and accumulate needed salts from their diet, marine fishes actively drink large amounts of seawater, absorb salts and water from the intestinal lumen and excrete a modest amount of near-isoosmotic urine. The alimentary uptake of salts and of water depends on the interplay of drinking, food ingestion and several alimentary transport processes, and on the complex regulation of these components by the nervous and endocrine systems.

Discussion

Background

Traditionally, drinking and intestinal ion and water transport processes have been separately studied using quite different techniques, and from these independent approaches have derived some general findings regarding physiological and endocrine regulation relative to salinity adaptation. Drinking in the Japanese eel (*Anguilla japonica*), for example, is stimulated almost immediately by exposure to chloride ions in the external saline medium, by hemorrhage-induced hypovolemia, and by the dipsogenic hormone angiotensin II (ANG II; Hirano, 1974; Takei, 2000). Regulation of intestinal fluid transport in several euryhaline species is consistent with the generally accepted involvement of major systemic hormonal factors in salinity adaptation. Specifically, prolactin, the so-called “freshwater-adapting hormone” of teleosts, reduces NaCl and water absorption by the intestine (Loretz and Bern, 1982); the apparently redundant action of prolactin to inhibit fluid absorption when drinking is already reduced in freshwater-adapted fishes may be protective to limit the uptake of incidentally swallowed water or preformed water in the diet. As seawater-adapting hormones, the interrenal steroid cortisol and pituitary protein growth hormone (perhaps involving mediation by insulin-like growth factors) stimulate NaCl transport via elevated Na⁺,K⁺-AT-Pase expression, and promote coupled water uptake (Hazon and Balment, 1998). The caudal neurosecretory peptide urotensin II (UII), delivered to the intestine from the urophysis via the caudal vein, stimulates NaCl uptake even further in the seawater-adapted goby (Loretz, 1995); the secretory source of UII in the caudal neurosecretory sys-

tem suggests control by higher centers and a centrally located sensor mechanism. Recent work by our laboratory and by others suggests that the patterns and control of drinking and intestinal absorption are far more intricate than originally thought, and that a substantial degree of interdependence and coordination exists.

Drinking

In some ways, the regulation of drinking in fishes is similar to that in tetrapods (including the mammals), but there are notable differences that may likely relate to the aquatic habitat of fishes. ANG II (as the terminal messenger in the renin-angiotensin system, RAS) is a potent and important dipsogenic factor in fishes, as it is in mammals; whereas in the mammals ANG II acts on forebrain centers to stimulate thirst, in fishes it stimulates medullary reflex swallowing centers (Takei, 2000). ANG II administration evokes drinking responses in euryhaline, stenohaline marine and freshwater teleost species; moreover, the high drinking rates of fish in seawater are promoted by an endogenous RAS (Perrott *et al.*, 1992). From experiments designed to probe the coupling of drinking to circulating ANG II concentrations, it appears that other chemical mediators may play determining roles in the control of drinking (Takei and Tsuchida, 2000). Although the angiotensin-converting enzyme (ACE) inhibitor SQ-14225 reduced drinking rate as expected in these studies, drinking rate was not restored following withdrawal of the drug, despite rebound of circulating ANG II levels; similarly, infusion of anti-ANG II serum reduced plasma ANG II levels without effect on drinking rate. Since ACE also converts bradykinin, an antidipsogen in fishes, to its inactive metabolites, these workers suggest that the antidipsogenic effects of SQ-14225 may result from activation of the kallikrein-kinin system and that bradykinin may be a regulator of drinking. Ando *et al.* (2000) have independently demonstrated altered drinking in the eel by a number of chemical messengers, including mammalian bradykinin, which was antidipsogenic. In eels, as in mammals, hemorrhage-induced hypovolemia stimulates drinking (Hirano, 1974; Tsuchida and Takei, 1998; Takei, 2000). Paradoxically, osmotic stimulation (specifically, cellular dehydration) induced by hypertonic saline injection is antidipsogenic in eels, in contrast to its strong dipsogenic action in mammals (Takei, 2000). Hirano (1974), and subsequently Takei *et al.* (1998) and Birrell *et al.* (2000), demonstrated that in eels anticipatory drinking begins almost immediately after transfer to seawater, and well before elevations in plasma

osmolality; this response is likely mediated centrally in response to salinity sensing by external chloride ion receptors.

Despite their otherwise seawater-adapting roles in teleosts, natriuretic peptides [NP; specifically atrial (ANP) and ventricular natriuretic peptide (VNP), the release of which are stimulated by elevations in plasma osmotic pressure] are antidipsogenic in the eel (*cf.* Loretz and Pollina, 2000). Since cardiac NP secretion parallels the transient elevation of plasma osmotic pressure following seawater transfer (Kaiya and Takei, 1996), NP-induced depression of drinking might limit further drinking-coupled accumulation of salt in a system already challenged by hypernatremia. The ANP-induced inhibition may be indirect via effects on the RAS since infusion of ANP into freshwater- and seawater-adapted eels caused both inhibition of drinking and parallel reductions in plasma ANG II concentrations (Tsuchida and Takei, 1998).

Whereas generalizations regarding depressed drinking in fresh water and elevated drinking in seawater are inviting and historically embedded in textbooks, drinking rates in teleosts are nevertheless variable and appear to depend on a number of factors. Freshwater rainbow trout (*Oncorhynchus mykiss*) fed dry pellet food ingest substantially more water than either trout fed moist food or unfed trout, suggesting a substantial dynamic range in the drinking rates of fishes (Ruohonen *et al.*, 1997). Drinking rates also vary with developmental state. The expression of drinking control can appear well before the full development and maturation of osmoregulatory organs and systems. European eels (*Anguilla anguilla*) increased their drinking rate ten-fold over a five-month period that encompassed the glass eel-to-elver transition (Birrell *et al.*, 2000), and tilapia (*Oreochromis mossambicus*) larvae increased their drinking rate steadily during days 2-10 post hatching (Miyazaki *et al.*, 1998). In larvae of both eel and tilapia, drinking rates in seawater exceeded those in fresh water at all times, consistent with the primary determining influence of salinity. Developmental state also influences the drinking rates of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*); compared with pre-smolt fish, salmon and trout smolts drank at significantly higher rates following transfer to seawater (Fuentes and Eddy, 1997; Nielsen *et al.*, 1999).

Intestinal Transport

Drinking-coupled ion and water absorption in marine teleosts is

essential for survival. Along its passage through the gut, as much as 80% of the ingested fluid is transferred across the intestinal wall. Water absorption is osmotically driven in response to a gradient established by the combined activity of the basolateral membrane $\text{Na}^+\text{-K}^+\text{-ATPase}$ and an apical membrane $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter (Loretz, 1995; Cutler *et al.*, 1996). Primary control of intestinal absorption is at the level of drinking, which determines the delivery of transportable substrate to the lumen; large-scale, salinity-appropriate regulation of membrane transport activity is mediated by prolactin, cortisol and growth hormone (see above). Whereas the absorptive transport of salts accomplishes appropriate physiological goals in all teleosts (replacement of lost salts in freshwater teleosts, and the establishment of an osmotic gradient driving water absorption in seawater teleosts), the uptake of water is physiologically adaptive only to teleosts in the dehydrating marine environment. It is probably significant that there exists redundant control over alimentary water uptake in freshwater teleosts, *viz.*, suppressed drinking and prolactin-induced reduction in water permeability. Similar to the developmental influences on drinking mentioned above, intestinal fluid transport also depends on developmental state. Net water uptake across the posterior intestine of freshwater yearling coho salmon (*Oncorhynchus kisutch*) and brown trout increases during spring smoltification to a level typical of seawater-adapted fish (Collie and Bern, 1982; Nielsen *et al.*, 1999).

Recent studies demonstrate a remarkable degree of local control over transport by paracrine factors. Local enteric production of NP in response to luminal distension or to nutrient secretagogues inhibits NaCl absorption through an A-type guanylyl cyclase receptor (GC-A); this inhibition may shift the balance between $\text{Na}^+\text{-Cl}^-$ and $\text{Na}^+\text{-nutrient}$ cotransport systems according to the feeding status of the organism and yet not interrupt the transfer of osmotically-active substances from the lumen that is essential for water transport (Loretz *et al.*, 1997; Loretz and Takei, 1997a). Whereas cardiac secretion of ANP and VNP following abrupt transfer of eels to seawater (Kaiya and Takei, 1996) might be expected to inhibit salt absorption, the circulating concentrations of cardiac-derived NP are well below the effective doses for inhibition (Loretz and Takei, 1997a, b). Guanylin, a ligand of the C-type GC receptor located on the luminal membrane of enterocytes, has recently been cloned from the eel; expression of eel guanylin was highest in the intestine out of several tissues examined, and was elevated following seawater acclimation (Comrie *et*

al., 2001). Comrie *et al.* (2001) propose two osmoregulatory roles for guanylin: (1) guanylin, following translocation to the luminal space and binding to the GC-C receptor, might influence intestinal epithelial transport (with an effect similar to that of NP); and (2) guanylin, after filtration at the glomerulus, might trigger GC-coupled changes in the renal transport of salts and water similar to uroguanylin signalling in the mammalian intestine-kidney axis.

Regulatory Interactions Between Drinking and Intestinal Transport

Although it is clear that drinking rate influences the physiological impact of alimentary fluid transport through substrate delivery, the intestine may also regulate drinking behavior. Hirano (1974) demonstrated that stomach distension strongly inhibits drinking in the eel; following abrupt transfer to seawater, an initial bout of intense drinking is soon followed by a more modest, cyclical pattern of drinking that may reflect filling and emptying of the stomach (Takei *et al.*, 1998). Ando and Nagashima (1996) examined the influence of luminal fluid composition on drinking using an intestinal perfusion technique; they noted that luminal Cl⁻ inhibited drinking. They proposed based on the time course of changes in luminal composition, drinking rate and urine flow that the observed inhibition was direct rather than mediated by changes in blood volume. Since esophageal desalinization efficiency is inversely related to drinking rate, this regulatory pathway may optimize fluid delivery to the gut (Ando and Nagashima, 1996). Although the nature of the inhibitory pathway (neural or endocrine) is not known, an endocrine candidate might be NP, which is antidipsogenic and secreted by the intestine in response to distension (Loretz and Takei, 1997b; Loretz *et al.*, 1997; Takei, 2000). The notions of Cl⁻ sensitivity by the intestine and the release of a chemical mediator to inhibit drinking are consistent with earlier reports of salt "tasting" by the intestinal tract; salt loads delivered to the intestinal lumen of mammals were more effective in causing natriuresis than equivalent intravenously-delivered loads (Lennane *et al.*, 1975a, b).

Water Channels

Only in the last decade have the molecular identity, expression and functional features of water channels, or aquaporins (AQP), been

characterized (Agre *et al.*, 1998). The high water permeability of the intestine is integral to its role in fluid absorption, and the expression of several AQP isoforms (AQP1, AQP3, AQP4, AQP7 and AQP8) in the intestinal epithelium of mammals is therefore notable and might infer several avenues of regulation (Ma and Verkman, 1999). Interestingly, this inventory includes members of the aquaporin (AQP1, AQP4, AQP8; selective for water) and aquaglyceroporin groupings (AQP3, AQP7; permeable to water, glycerol and small solutes; Agre *et al.*, 1998), suggesting multifunctionality of these channels in gastrointestinal physiology. Little is known yet about aquaporins in the fishes. Homologues of AQP1 and AQP3 are expressed in the intestinal epithelium of both yellow and migratory silver eels; expression of the AQP1 mRNA is markedly increased following transfer from fresh water to seawater (C.P. Cutler and G. Cramb, personal communication). Water transport may also be accomplished through direct coupling to Na⁺-nutrient cotransport systems (Meinild *et al.*, 1998). This is a fertile area for exploration and discovery, and it will complement earlier studies on the endocrine regulation of water permeability.

Conclusion and Perspectives

Recent work by our laboratory and by others suggests that the patterns and control of drinking and intestinal absorption are far more intricate than originally thought, and that a substantial degree of interdependence and coordination exists. Whereas primary control over both systems may result from global endocrine signalling related to salinity adaptation, local sensing and paracrine communication are important components in successful osmoregulation.

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