

WATER TRANSPORT AND AQUAPORIN EXPRESSION IN FISH

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I. INTRODUCTION

One of the major differences between terrestrial and aquatic vertebrates is that the latter group have a potentially greater difficulty in maintaining osmotic homeostasis because they are surrounded by an external environment which is almost always in osmotic dis-equilibrium with their body fluids. In comparison to terrestrial animal species, aquatic organisms such as teleost fish may well possess a distinct set of mechanisms in order to adapt and survive the osmotic challenges posed by the seawater (SW: hyper-osmotic) or freshwater (FW: hypo-osmotic) environments. However, to date, little is known about how these mechanisms manifest themselves at the molecular level. In order to begin an evaluation of which proteins may be involved in osmoregulation in both the freshwater and marine environments, investigations have been initiated using the euryhaline teleost the European eel (*anguilla anguilla*) which naturally inhabits both environments.

II. WATER TRANSPORT

A. Water Transport Across The Gills Of Teleost Fish

The gills are the major site of water exchange between the internal milieu and the external aquatic environment inhabited by teleost fish, with for example in FW, the branchial epithelium (gill) responsible for over 90% of the total body water influx (Motais *et al* 1969; Haywood *et al* 1977). In FW the diffusional component of water fluxes across the gill occurs primarily across the secondary lamellae, through so-called 'respiratory cells', due in part to the large percentage of the exterior surface area of the gills these cells occupy (Isaia 1982). Consequently, the diffusional permeability of the gill is not radically different, although it is slightly higher, in FW compared to SW adapted euryhaline teleosts (Isaia 1984).

However, the situation regarding osmotic water permeability of the gills is some what different. Measurements in euryhaline teleosts demonstrate that osmotic water permeability is generally higher in FW than in SW adapted

fish, and in particular in the European eel permeability is 6 times greater (Isaia 1984).

It has been suggested that water fluxes through so-called gill 'chloride cells' (mitochondrial-rich ion transporting cells) represent the principal osmotic water flux pathway across the gill. This process has been associated with a 'bulk flow' water and ion transporting mechanism through a specialised membrane structure (the baso-lateral tubular network) of chloride cells, resulting in water efflux through "leaky" tight junctions into the external environment (Isaia 1984). While this may explain a portion of gill water permeability in marine fish, fluxes through the tubular network of chloride cells in FW teleosts are likely to be lower, as there are fewer chloride cells (which also have more extensive tight junctions) in FW adapted fish (Potts 1984). This suggests that the bulk of osmotic water fluxes (particularly in FW fish) must occur through or between other surface epithelial cells such as secondary lamellar respiratory cells (Rankin and Bolis 1984). While it is not clear why osmotic water permeability should be so much greater in FW compared to SW acclimated eels, it does suggest that a regulated pathway for water movement in the gill may exist, the components of which would be expected to be more abundant in FW than SW fish.

B. Hormonal Control of Water Transport Across the Gills

If a regulated pathway for water transport did exist within gill surface epithelial cells it is likely that it would be hormonally regulated. Evidence for the action of several hormones on gill water fluxes does exist and the most convincing evidence comes from experiments investigating the effect of catecholamines and their associated agonist and antagonist drugs. Adrenaline significantly increases the level of cAMP in the branchial epithelium and also increases the diffusion component of the water flux (Isaia 1979) and this effect is 100% greater in FW- rather than SW-acclimated fish. The increase in water flux was also shown to result from a direct effect of adrenaline on membrane permeability rather than on changes in perfusion caused by changes in vascular resistance (Haywood *et al* 1977). The β -adrenergic agonist, isoproterenol, has also been demonstrated to acutely increase water movement across isolated Killifish opercular membranes, which are often used as analogues of the gill epithelium (Zadunaisky 1984).

A number of other hormones may regulate water fluxes across the branchial epithelium although the evidence is somewhat less clear cut. Both prolactin and cortisol have been implicated in the regulation of water

fluxes/permeability in the gill (Ogawa, 1975 Ogasawara and Hirano 1983 Rankin and Bolis 1984).

C. Water Transport Across The Gut

Marine teleosts have a major osmoregulatory problem with their aquatic environment in that they are constantly losing water across permeable body surfaces such as the gills. In order to prevent dehydration, fish have a regulated drinking response. Imbibed SW is first partially desalinated in the oesophagus before passing through the stomach and entering the intestine. The absorption of water across the intestinal epithelium takes place with the concomitant uptake of salts.

When eels are transferred to the marine environment they immediately drink the SW which enters the oesophagus at a slow but steady rate (Parmelee and Renfro 1983). Here salts are thought to be absorbed by both secondary active and passive processes and, as water permeability of the oesophagus is extremely low, only small net effluxes of water occur due to osmosis, leading to an overall dilution of the oesophageal fluid concentration to around one half to one third of the initial osmolality (Hirano and Mayer-Gostan 1976 Parmelee and Renfro 1983 Nagashima and Ando 1993). Very little further ion uptake or water secretion occurs as the luminal fluid passes through the stomach (Hirano and Mayer-Gostan 1976). When the luminal fluid reaches the intestine most of the remainder of the salts and water are absorbed (Skadhauge 1969 Hirano and Mayer-Gostan 1976). The oesophageal desalination appears to be an essential process as, if the intestinal lumen is artificially exposed to strongly hyper-osmotic fluids such as SW, water is secreted into the lumen rather than being absorbed (Skadhauge 1969). Salts are actively absorbed in the intestine and as the osmolality of the luminal fluids is reduced to a point where no net water flux occurs (known as the turning point osmolality: TP osm). The osmolality at which this occurs is above that of the eel's plasma. Subsequently, as further salts are absorbed, net water is absorbed against the opposing osmotic gradient. Once the osmolality of the luminal contents are reduced to that of the plasma, fluid uptake continues until the Na and Cl concentrations are reduced to around 30-90 meq/l, (Skadhauge 1969 Parmelee and Renfro 1983). As the net uptake of divalent ions is very low and other solutes are actively secreted, the final osmolality of the luminal fluid still remains iso-osmotic to that of the body fluids (Parmelee and Renfro 1983). The model proposed to explain these phenomena suggests that water transport occurs by two processes, 1) secondary-active water transport (which can transport water against the osmotic gradient) thought to occur through a trans-/para-cellular shunt pathway and 2) passive water fluxes which follow the osmotic

gradients and which are entirely transcellularly based (Skadhauge 1969). The secondary-active water transport component has been investigated in a number of studies principally using iso-osmotic conditions (to eradicate the normal passive osmotic water fluxes) and is tightly coupled to the transport of chloride and sodium and/or potassium ions (Skadhauge 1969 Skadhauge 1974 Ando 1975 Ando 1980 Ando 1981 Ando 1983 Ando 1985). Hence it has been described as solute-coupled water flux. The osmolality at which the TP osm occurs (a measure of the solute-linked secondary-active salt/water transport) is directly proportional to the salinity of the external environment in which the eels are acclimated. This suggests that the capacity of the solute-linked water flux increases with acclimation to increasing salinities (Skadhauge 1969 Skadhauge 1974) with this component increasing 3-3.4 fold following FW/SW acclimation (Utida *et al* 1972 Ando 1975). Furthermore, the passive (or general) osmotic water permeability (assessed using osmotic gradients produced without additional NaCl) also increases by 2-6 fold following SW acclimation (Skadhauge 1969 Ando 1975). The magnitude of water fluxes across various parts of the intestine have also been determined, with highest levels occurring in the mid region followed in descending order by the posterior, anterior intestine and rectum (Ando and Kobayashi 1978 Ando 1980). This evidence taken together also suggests that there may be a role for a regulated cellular pathway for water movement in the intestine, the components of which would be expected to be more abundant in SW than FW eels.

D. Hormonal Regulation Of Intestinal Water Transport

Both cortisol and ACTH have been shown to significantly increase the level of water flux across the intestine in FW-acclimated eels. Increases in water flux were observed after a latent period of about ten hours and lasted for at least a week (Utida *et al* 1972). The hormone prolactin has also been reported to act antagonistically to cortisol and has been demonstrated to significantly decrease water fluxes across the intestine in SW acclimated eels (Utida *et al* 1972).

Various other hormones have also been implicated in the acute regulation of intestinal water fluxes, the most potent of these being atrial natriuretic peptide (ANP) which inhibited net absorption by 50-60% after approximately one hour exposure to the hormone (Ando *et al* 1992 Ando and Kondo 1993). Serotonin (via a 5-HT₃-like receptor), and the acetylcholine muscarinic agonist methacholine, both inhibited water fluxes by around one third, and these actions were antagonised by noradrenaline (Mori and Ando 1991 Ando and Kondo 1993) However noradrenaline failed

to antagonise the ANP induced inhibition of water flux (Ando and Kondo 1993).

E. Water Transport In The Kidney

In mammalian kidney the physiological and molecular mechanisms of water transport have been widely studied and well documented, however, much less information is available for teleost fish. Freshwater teleost fish excrete relatively large volumes of dilute urine (around 35 mOsmol/l) due to a high glomerular filtration rate (GFR) and an almost complete reabsorption of NaCl. Ions remaining in the tubular are further absorbed by the urinary bladder. Marine teleosts generally only produce low volumes of urine due to a low GFR, the kidney of the marine teleost is unable to concentrate salts in the urine and the fluid extracted is still hypo-osmotic compared to plasma and contains relatively low concentrations of NaCl. These processes suggest that water transport mechanisms might be expected to be of less importance to teleost kidney function in comparison to the significance of those found in mammalian kidney.

III. AQUAPORINS

A. Expression Of Aquaporins In Teleost Fish

The purpose of the molecular investigations undertaken in this study has been to examine the role that fish aquaporin water channel homologues play in the physiological processes mentioned above. As there was no published evidence for the presence of aquaporins in fish, initial studies focused on the cloning and identification of aquaporin homologues using degenerate RT-PCR.

Experiments concentrated on identifying aquaporins in target tissues such as the gill, intestine and kidney. The first aquaporin homologue identified from the gill, shared highest levels of nucleotide and amino acid homology with mammalian AQP 3 (Ma *et al* 1994) and subsequent Northern blotting showed that it is expressed as a 2.4 kb mRNA in the eye, oesophagus, intestine and gill with a minor amount of a 7 kb mRNA species also present. Quantitative studies revealed that the major site of expression was in the gill of FW eels and that levels decreased to 3% of FW values, three weeks after transfer of fish to SW. Further studies revealed that the down-regulation of mRNA coding for this AQP3 homologue in SW occurred rapidly with a half-time of around 10 hours. The levels of mRNA expression in the intestine were relatively low in both FW or SW fish. This data suggests that the AQP3 homologue may be, at least in part, responsible for the increased

osmotic water permeability documented for the gills of FW eels (Isaia, 1984). As suggested above, the most likely cellular location of functional expression of the AQP 3 homologue within the gill is therefore probably within the basolateral membranes of surface epithelial cells, where it would serve to release water entering apically from the hypo-osmotic FW environment. This process would prevent cell swelling and eventual bursting and is presumably present because cell volume regulation, in the face of continuous water uptake, could not be achieved by the secretion of ions or other osmolytes.

Although the AQP 3 homologue was shown to be present in the intestine, the low levels of expression suggested the presence of other AQP homologues in this tissue. Consequently, the existence of further intestinal aquaporins was investigated and a homologue of AQP 1 was identified (Preston and Agre 1991). This homologue had a wider tissue distribution than that of the AQP 3 homologue and expression of a 1.4 kb mRNA was found in brain, eye, heart, pancreas, oesophagus, stomach, and intestine, with much lower levels in skeletal muscle, gill and kidney. A minor 3.1 kb mRNA component was also present in some tissues. Quantitative studies revealed that the level of mRNA expression in the intestine increased 10-25x following the transfer of FW eels to SW. Messenger RNA abundance was also significantly decreased to 28% of FW values in the kidneys of SW adapted fish, although overall levels in the kidney were much lower than the intestine. The results in the intestine strongly suggest that the AQP 1 homologue may have a role to play in the mechanisms associated with the absorption of water in this tissue, however the exact nature of this role remains unclear. It seems unlikely that an AQP 1 homologue could play a direct role in the secondary-active water transport taking place in the intestine, however, it could be involved indirectly, possibly by providing a basolateral water efflux pathway for luminal epithelial cells, and hence the expression of an AQP 1 homologue may at least be part of the explanation for the increases in water permeability also found.

As only limited expression of the AQP 1 homologue was found in eel kidney, this tissue is a major site of mammalian aquaporin expression and therefore a further search was recently undertaken to identify other aquaporin homologues in this tissue. These studies resulted in the discovery of a third aquaporin which, initial indications suggest, is a duplicate isoform of the AQP 1 homologue, with which, the cDNA fragment isolated, shares 69.7% derived amino acid homology. Initial studies have indicated that mRNA for this isoform is only has detectable in the oesophagus and kidney, and that the high level of mRNA expression in the kidney is dramatically down-regulated when eels are transferred from FW to SW, in a similar fashion to the expression of AQP1 homologue in kidney. The physiological

role of these changes in expression are difficult to reconcile with the current models of kidney function in teleosts, especially when high levels of aquaporin expression are present during physiological conditions which induce the production of dilute urine associated with a minimal level of water re-absorption. The presence of high levels of expression of the AQP 1 homologue and its duplicate in the oesophagus is also puzzling when this tissue is thought to represent a particularly tight epithelium with very low water permeability. It suggests that the aquaporins expressed in these tissues are concerned primarily with cell volume control rather than vectoral water transport.

IV. CONCLUSION

The considerable changes in aquaporin homologue expression that occur in various tissues, following salinity acclimation in teleost fish, suggest that there are a significant number of interesting avenues for further research to elucidate the role of water channels in aquatic species.

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VI. REFERENCES

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