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Altered plasma and pituitary arginine vasotocin and hypothalamic provasotocin expression in flounder (*Platichthys flesus*) following hypertonic challenge and distribution of vasotocin receptors within the kidney

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Abstract

Plasma AVT concentration, pituitary AVT content, hypothalamic provasotocin mRNA expression and other osmoregulatory parameters were measured in euryhaline flounder 4, 8, and 24 h after the hypertonic challenge of transfer from fresh water (FW) to seawater (SW). Osmolality and the concentration of major plasma ions, sodium and chloride, were significantly higher in fish transferred to SW by comparison with time matched controls, an effect evident within 4 h. By comparison with time matched controls, pituitary store of AVT was lower while plasma AVT concentration was higher 8 and 24 h after transfer to SW. Higher provasotocin mRNA expression in the hypothalamus was also seen at 4 and 8 h in flounder transferred from FW to SW compared with time matched controls. The lower pituitary store and higher circulating levels imply substantial AVT secretion occurs in the early phase response to this hypertonic challenge. Changes in the regulation of AVT synthesis and secretion appeared quickly following movement to SW, consistent with the rapid osmoregulatory response, including reduced urine production that fish require to accommodate the dehydrative water losses and salt loading on exposure to the new hyperosmotic environment. qPCR measures of whole kidney vasotocin receptor mRNA expression indicated similar levels in SW and FW. Immunohistochemistry for the vasotocin receptor in flounder kidney showed localisation on the afferent and efferent arterioles of the glomerulus and on the capillary bed that extends from the efferent arteriole to the smooth muscle surrounding the collecting duct. Localisation of the vasotocin receptor was comparable in SW and FW fish.

Keywords: Arginine vasotocin; Provasotocin; Teleost; Osmotic challenge; Kidney; Glomerular intermittency; Flounder (*Platichthys flesus*); Vasotocin receptor; Euryhaline; Pituitary; Hypothalamus

1. Introduction

Arginine vasotocin (AVT) is a neurohypophysial peptide present in all non-mammalian vertebrates studied. In mammals it is replaced by arginine vasopressin (AVP) which differs by a single amino acid at position 3 (Acher, 1996). Many of the physiological actions of AVT and AVP are common through the vertebrate series, inducing blood pressure and osmoregulatory effects. In relation to osmoregulation the actions of AVT and AVP are well understood in terrestrial vertebrates. These peptides promote renal water conservation primarily through a combination of modulating the numbers of functional filtering nephrons and altering the water permeability of the distal tubule and collecting duct. The latter effects are the primary mechanism of AVP action in mammals (Warne et al., 2002).

It is apparent from studies of euryhaline fish that AVT may similarly also play a key role in renal water

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conservation. In fresh water (FW) adapted fish AVT administration results in a decrease in the number of nephrons filtering and urine production both in in vivo and in situ kidney preparations (Amer and Brown, 1995; Henderson and Wales, 1974). The mechanism by which AVT alters the numbers of nephrons filtering in fish and other non-mammalian vertebrates remains unclear. As in mammals, there is also evidence to suggest that AVT influences salt balance, modulating extrarenal activity such as chloride transport across the gills (Avella et al., 1999; Guibbolini and Avella, 2003).

Accordingly, in fish there is strong evidence that AVT acts to promote body water conservation (Warne, 2002), however, there is less information to support the notion that AVT secretion is appropriately regulated to serve such a homeostatic function. Osmotic challenge does result in significant changes in the AVT system in teleost fish. A transient drop in the pituitary store of AVT was observed in euryhaline trout, medaka and flounder following transfer from FW to seawater (SW) (Carlson and Holmes, 1962; Haruta et al., 1991; Perrott et al., 1993), which suggests that AVT secretion rises after exposure to dehydrating and salt loading hyperosmotic media. Changes in the hypothalamic levels of provasotocin (proVT) expression in trout have also been shown to be sensitive to osmotic challenge, though in this study decreased expression occurred after exposure to hypertonic medium (Hyodo and Urano, 1991). We have previously shown that acute hyperosmotic challenge in flounder, induced by injections of hypertonic saline, does result in increased plasma AVT concentrations (Warne and Balment, 1995). We have also shown that the haemodilution associated with transfer of fish from SW to hypotonic FW is associated with a transient fall in circulating AVT levels (Bond et al., 2002).

These earlier data are difficult to fully integrate as they reflect measures in different species and also importantly different time points after osmotic challenge. Nonetheless, a model is emerging in which AVT secretion may be triggered by dehydration (raised blood osmolality) and that the actions of AVT result in water conservation. Previously, we reported no change in the AVT neuroendocrine system 72 h after exposure to hyperosmotic challenge (Warne et al., 2000). In the current work, we now test the hypothesis that the AVT neuroendocrine system is activated to play a significant role in the initial hours following movement from hypo- to hypertonic media. Accordingly, we have made coincident measures of plasma AVT concentration, pituitary AVT content and hypothalamic proVT mRNA expression over the first 24 h following the movement of FW adapted flounder to SW. While it is apparent that AVT secretion may be adapted in response to acute challenge it is also clear in fully adapted SW and FW euryhaline fish that circulating AVT levels are comparable (Warne, 2002). It is thus likely that changes in AVT receptor expression, both location and quantity, may contribute to the contrasting roles of AVT in target tissues such as the kidney in these chronic conditions. We have, therefore, examined the location of AVT V_1-type receptor (Warne, 2001) protein expression along the nephron of FW and SW adapted flounder and using Semi-quantitative realtimePCR (qPCR) have compared whole kidney V_1-type receptor mRNA expression in FW and SW adapted animals.

2. Materials and methods

2.1. Fish

Flounder (*Platichthys flesus*) were caught by local fishermen in Morcambe bay, Cumbria, and maintained in tanks with constantly recirculating water at 8–10 °C under 12 h light/12 h dark lighting at the University of Manchester for at least 14 days before experimentation. Fish were both male and female and weighed between 300 and 900 g. Animals were held in FW or SW and left for a minimum of two weeks to fully adapt to the medium prior to experimentation. All experiments were performed with local ethical committee and Home Office licence approval. The acute hypertonic challenge experiment was carried out during November.

2.2. Acute hypertonic challenge experimental protocol

FW adapted flounder were randomly assigned to two groups, the first group was transferred directly to SW tanks ($n = 22$). The second group was transferred to a new FW tank following exactly the same handling procedure for the SW transfer group to act as controls ($n = 20$). For both FW to SW and control FW to FW groups at the time points, 4, 8, and 24 h, after transfer, 6–8 fish were sampled and tissues collected.

2.3. Blood sampling

Blood was collected into heparinised syringes by direct needle puncture of the caudal artery and vein of unanaesthetised fish and completed within 2 min of fish removal from a tank. An aliquot of collected blood was taken into a capillary tube for determination of haematocrit. Plasma was separated by centrifugation at 16,000*g* for 3 min. An aliquot of plasma was taken for osmolality and ion determination and stored at 4 °C until assay, the remaining plasma was snap frozen and stored at −80 °C for later hormone assay.

2.4. Brain and pituitary collection

After blood collection fish were stunned by a blow to the head before the top of the skull was removed to