Renal Morphology of the Euryhaline Flounder (*Platichthys flesus*)

Distribution of Arginine Vasotocin Receptor

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ABSTRACT: The current study characterized tubular segmentation of the European flounder nephron and localized the vasotocin receptor expression by immunohistochemistry. Flounder nephron was shown to comprise a prominent renal corpuscle, short neck segment, proximal tubule I, proximal tubule II, collecting tubule, and collecting duct. Using specific antibodies raised against flounder vasotocin receptor, specific V_1 receptor staining was detected within the glomeruli, the endothelial surface of the afferent and efferent arterioles, and the capillaries surrounding the collecting duct system. Immunostaining for the receptor was exclusively vascular and there did not appear to be a tubular component.

KEYWORDS: AVT; flounder; kidney

INTRODUCTION

In teleosts, nephrons vary considerably among species. Multisegmental nephrons prevail in less advanced teleosts such as the anguilliforms and salmonids; in contrast, in more advanced marine teleosts such as sticklebacks, the distal segment is generally lacking.[1,2] It is important, therefore, to describe in detail the nephron for species being used to examine renal function. The neurohypophysial hormone, arginine vasotocin (AVT), is a key regulator of kidney function in teleost fish, having a role in water conservation through an antidiuretic action.[3] In this study, we describe the structure of the nephron and distribution of AVT receptor in the European flounder kidney.

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MATERIALS AND METHODS

The kidneys of flounder (*Platichthys flesus*) adapted to seawater were fixed *in situ* by perfusion with 4% paraformaldehyde/Bouin's solution (Sigma, U.K.). The kidney was dissected out, placed into fresh fixative, and stored overnight at 4°C. Tissue was dehydrated in ethanol and embedded in paraffin wax, and serial (5 μm) sections were cut and stained with hematoxylin and eosin to identify the components of the nephron.

Immunohistochemical localization of the vasotocin receptor[4] was carried out using affinity-purified, specific polyclonal rabbit antibody raised to a section of the third intracellular loop of the receptor. Nonspecific binding was blocked with 1% BSA, 0.2% gelatin, and 0.05% saponin in PBS, before incubation (overnight at 4°C) with the primary antibody. Antibody localization was detected by the use of a secondary antibody (goat antirabbit conjugated to horseradish peroxidase, Sigma, U.K.). Immunoreactive sites were visualized using a freshly prepared solution of 3,3′-diaminobenzidine tetrahydrochloride (DAB) and counterstained with Mayer's hematoxylin.

RESULTS

Six distinct nephron regions were identified—the renal corpuscle, neck segment, proximal tubule I (PI), proximal tubule II (PII), collecting tubule (CT), and collecting duct (CD)—typically as seen in other advanced teleosts. PI was characterized by basally located nuclei, microvilli brush border, and large apical vacuoles, absent from PII. PII was identifiable by the presence of a dense brush border and centrally located uniform nuclei, leading to the CT distinguishable by apically located nuclei, small vesicles, and the absence of the brush border so distinctive of the proximal tubule. The CT finally leads into the CD with its highly characteristic smooth muscle surround and large size. Immunostaining specific for the vasotocin receptor was observed on the efferent glomerular arteriole and to a lesser extent on the afferent glomerular arteriole. The vasotocin receptor was also localized to the capillaries of the glomerulus within the tuft associated with the efferent arteriole. Staining for the receptor was also present on the capillary network that extended from the efferent arteriole to surround the collecting duct system.

DISCUSSION

This study has shown that the flounder nephrons consist of neck, proximal, and collecting tubules; no distal or intermediate segments were present. This finding is similar to previous studies in two other species of marine flatfish,[5,6] but different to that found in the southern flounder[2] where a distal segment was found to be present. AVT has been shown to have a potent action on the kidney, acting to reduce the number of filtering glomeruli,[3,7] although the precise mechanism is unclear. Using antibody raised against the vasotocin receptor, it appeared that receptor localization was restricted to the vasculature of the glomerulus and the small vessels surrounding the collecting ducts (see FIG. 1). The actions of AVT mediated by this receptor would

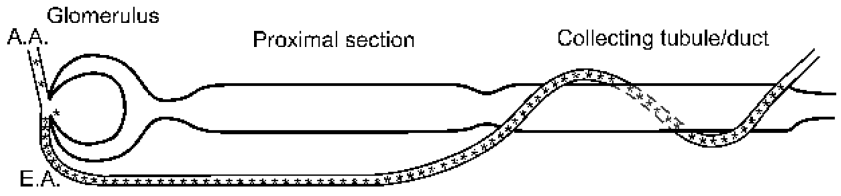

FIGURE 1. Schematic diagram of the flounder nephron, highlighting the arginine vasotocin receptor (*) localization on the afferent arteriole (A.A.) and efferent arteriole (E.A.) and vessels extending to the collecting tubule/duct system.

not appear to have a tubular transport component. It is likely that the antidiuretic effect of AVT is brought about by actions at the vascular receptors to reduce the number of filtering nephrons.

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