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Effects of angiotensin II and C-type natriuretic peptide on the in situ perfused trunk preparation of the dogfish, *Scyliorhinus canicula*

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Abstract

The renal roles of physiologically relevant doses of angiotensin II (Ang II) and C-type natriuretic peptide (CNP) were investigated in the dogfish, *Scyliorhinus canicula*, using an in situ perfused trunk preparation. Perfusion with 10^{-9} M Ang II resulted in a glomerular antidiuresis and decreases in perfusate flow rate, transport maxima for glucose and the proportion of filtering glomeruli. In addition, the renal clearances and excretion of urea, sodium, and chloride were significantly reduced, whereas the relative clearances of these parameters remained unchanged. In contrast, perfusion of 10^{-9} M CNP caused a glomerular diuresis, an increase in transport maxima for glucose, but no significant change in the proportion of filtering glomeruli. In addition, the renal clearances of urea, sodium, and chloride were significantly increased but there was no effect on the relative clearances of urea, sodium, or chloride. Perfusion with 10^{-10} M Ang II or CNP had no significant renal effects. Our results suggest that these hormones act at the level of the glomeruli rather than at a tubular level.

Keywords: Antidiuresis; Diuresis; Seawater; *Scyliorhinus canicula*; Dogfish; Glomerular perfusion patterns

1. Introduction

The renal actions of peptide hormones in elasmobranch fish are largely unknown and investigations into the hormonal control of renal function in elasmobranch fish have concentrated on in vivo effects (Benyajati and Yokota, 1990; Brown and Green, 1987). However, the relatively low urine flow rate in seawater (SW)-acclimated elasmobranchs, coupled with the very long and convoluted urinary sinuses means that urine output tends to occur during periods of spontaneous swimming activity rather than as a continuous flow. This problem is exacerbated in *Scyliorhinus canicula*, which does not need to swim in order to irrigate the gills. In vivo measurements of urine flow therefore require long collection periods (Brown and Green, 1987), making investigation of the hormonal control of renal function difficult.

To avoid at least some of these complications and to determine the actions of individual peptides on renal function, previous studies in teleost fish have utilized an in situ renal trunk preparation (Amer and Brown, 1995; Dunne and Rankin, 1992). We have recently developed and validated a similar in situ renal trunk preparation in *S. canicula* and assessed the renal effects of arginine vasotocin (AVT) (Wells et al., 2002). In addition, this in situ renal trunk preparation has been used to confirm the presence of an intrarenal RAS in *S. canicula* (Wells et al., 2003). A major advantage of this preparation is that it allows precise control of variables, such as pressure, perfusion flow, and peptide concentration.

Historically, the renin angiotensin system (RAS) was thought to have evolved in the bony fishes (Nishimura

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and Ogawa, 1973). However, the identification and synthesis of homologous angiotensin I (Ang I) in the Japanese dogfish, *Triakis scyllia* (Takei et al., 1993) confirmed the presence of an endogenous RAS in elasmobranch fish. In addition, angiotensin-like receptors have been observed in a variety of tissues in *T. scyllia*, including the kidney (Tierney et al., 1997b). Angiotensin II (Ang II), the principal bioactive component of the RAS is a major peptide influencing body fluid osmoregulation in higher vertebrates (Kobayashi and Takei, 1996). We have recently published evidence of a tissue specific, intra-renal RAS, indicating that the RAS may play a role in renal function (Wells et al., 2003). Circulating concentrations of Ang II have previously been measured following long-term acclimation to SW (109.9 fmol/ml) (Tierney et al., 1998) and 1 h after acute transfer from 80% SW to 100% SW when Ang II concentrations in excess of 1000 fmol/ml were recorded (Anderson et al., 2002). There have been no previous studies of the renal effects of Ang II in elasmobranchs.

In tetrapods, natriuretic peptides form a family of peptides that include A-type (ANP), B-type (BNP) and C-type (CNP). In addition, V-type natriuretic peptide (VNP) has been identified in teleost fish (Takei, 2000). To date the only circulating natriuretic peptide identified in elasmobranch fish is CNP (Schofield et al., 1991; Suzuki et al., 1991, 1992, 1994; Takano et al., 1994). Indeed, it has been suggested that CNP is the only natriuretic peptide present in elasmobranchs (Kawakoshi et al., 2001). Circulating CNP has been measured at 1.97 pmol/ml in *T. scyllia* (Suzuki et al., 1994) but to our knowledge, circulating levels of CNP have not been published for *S. canicula*. Synthetic mammalian ANP (atriopeptin II) has previously been shown to be antidiuretic and anti-natriuretic in *Squalus acanthias* (Benyajati and Yokota, 1990) but actions of homologous CNP on the kidney are as yet unclear.

The aim of this study was to investigate the glomerular and tubular effects of physiological concentrations (10^{-9} and 10^{-10} M) Ang II and CNP using the in situ perfused trunk preparation of *S. canicula*.

2. Methods

2.1. Animals

Female dogfish (600–1100 g) were caught in the waters around Aberdeen, East Coast of Scotland, and transported to the Gatty Marine Laboratory where they were allowed to acclimatize to tank conditions for at least 2 weeks. Dogfish were maintained in aerated free-flowing SW (osmolality 938 mOsm kg^{-1}; Na, 399 mM, K, 8.5 mM, Ca, 12 mM; Mg, 45 mM; Cl, 368 mM) under a natural photoperiod at 12 °C. The experimental fish were not fed for at least 2 weeks prior to experimentation. All experimental procedures were carried out by licensed personnel in accordance with UK Home Office regulations (Animals (Scientific Procedures) Act, 1986).

2.2. In situ perfusion of the kidney

Kidney function was assessed in situ using an isolated trunk preparation as described previously (Wells et al., 2002). This involved decapitation of the fish following sacrifice and insertion of a cannula into the dorsal aorta to perfuse the trunk with the kidney in situ. Other organs were removed, the in situ kidney was perfused from a constant pressure head (28 mm Hg/39 cm H_2O) and urine was collected in pre-weighed vials.

2.3. Renal actions of Ang II/CNP

Two, one-hour urine samples were collected into pre-weighed microcentrifuge tubes and urine flow rates were determined gravimetrically assuming a specific gravity of 1. Two further one-hour urine samples were collected after addition of 10^{-9} or 10^{-10} M Ang II or CNP to the perfusate ($n = 6$ at each dose). Comparisons of renal parameters were made between the last one-hour collection period immediately prior to addition of peptide to the perfusate and the final one-hour collection period during administration of peptide. [Asn^1-Pro^3-Ile^5]- Ang II and dogfish CNP-22 (both Peptide Institute, Osaka, Japan) were generously provided by Prof Yoshio Takei, and used throughout this study.

Urine and perfusate were analysed for osmolality (Roebling Osmometer, Camlab, Cambridge, UK), concentration of sodium (Corning 480 Flame Photometer, Corning Ltd., Essex, UK), chloride (Corning Chloride Analyzer 925), and urea, inulin (for measurement of GFR), and glucose (for measurement of Transport Maxima for Glucose (TmG)) were analysed spectrophotometrically (Wells et al., 2002). TmG can be used as an indication of the functional tubular mass of the whole kidney and provides an indirect measurement of the filtering population of glomeruli.

2.4. Direct measurement of the filtering population of glomeruli

The filtering population of glomeruli was measured directly, using the ferrocyanide technique (Amer and Brown, 1995; Hanssen, 1958) which allows glomeruli to be categorised according to the presence or absence of precipitated Prussian blue (see below). Individual glomeruli, complete with a section of renal tubule, were dissected free and categorised according to the presence or absence of Prussian blue: Perfused and filtering (P&F) – Prussian blue present both in the glomerulus and the neck segment of the renal tubule; Perfused, but not filtering (PNF) – Prussian blue present in the glomerulus but not in the neck segment; Non-perfused (NP) – Prussian blue absent from both the glomerulus and the neck segment.

Glomeruli were classified until the proportions of glomeruli in each classification reached constancy. This usually required the classification of between 120 and 160 glomeruli per kidney.