Development of an in situ perfused kidney preparation for elasmobranch fish: action of arginine vasotocin

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Wells, Alan, W. Gary Anderson, and Neil Hazon. Development of an in situ perfused kidney preparation for elasmobranch fish: action of arginine vasotocin. Am J Physiol Regulatory Integrative Comp Physiol 282: R1636–R1642, 2002; 10.1152/ajpregu.00810.2000.—Acclimation of the European lesser-spotted dogfish Scyliorhinus canicula to reduced environmental salinity [85–70% seawater (SW)] induced a significant diuresis in addition to a significant decrease in plasma osmolality in vivo. The threshold for this diuresis was determined to be 85% SW. Therefore, S. canicula acclimated to 85% SW was selected for further study as a diuretic model in the development of an in situ perfused kidney preparation. The renal role of arginine vasotocin (AVT) in the in situ perfused trunk preparation was investigated. In SW, perfusion of 10⁻⁹ and 10⁻¹⁰ M AVT resulted in a glomerular antidiuresis and decreases in tubular transport maxima for glucose and perfusate flow. In 85% SW, 10⁻⁹ M AVT had no significant effect on these renal parameters with the exception of transport maxima for glucose and perfusate flow. Tubular parameters remained unchanged by either 10⁻⁹ or 10⁻¹⁰ M AVT. The results demonstrate that the perfused kidney preparation was a viable tool for the investigation of renal parameters in elasmobranch fish and that AVT induced a glomerular antidiuresis.

antidiuresis; seawater; Scyliorhinus canicula; dogfish; neurohypophysial hormones

MARINE ELASMOBRANCHS MAINTAIN their plasma isoosmotic or slightly hyperosmotic to that of the surrounding environment, primarily due to the retention of urea (22). Plasma sodium and chloride concentrations are generally higher than those found in marine teleosts, but the fish still face a continuous influx of NaCl across semipermeable membranes, particularly the gills (18, 20). Due to the plasma iso/hyperosmolality, some influx of water will occur and urea will be lost to the environment across permeable surfaces, along a concentration gradient. Elasmobranchs are not capable of producing a hyperosmotic urine with respect to body fluids, and the kidneys are not the major site of NaCl excretion (17). It appears that renal retention of urea may be a more important function for the kidney in elasmobranch fish (7).

Arginine vasotocin (AVT), a homologue of arginine vasopressin (AVP) in mammals, is the major neurohypophysial peptide in lower vertebrates. It has been characterized in all elasmobranchs examined to date (1, 2). In addition to AVT, the European lesser-spotted dogfish Scyliorhinus canicula also has the oxytocin-like peptides, phasvatocin and asvatocin, which occur in roughly equal molar amounts in the pituitary (13). AVT is also present in the pituitary in a proportional amount that is lower by about a factor of 20 (13). This low pituitary level was thought to reflect a permanent secretion of AVT (2). Although accurate levels of AVT have been measured in teleosts, the presence of additional neurohypophysial peptides in elasmobranchs and the potential for these to cross-react with the antiserum have meant that it has not been possible at present to provide an accurate measurement of AVT in elasmobranch plasma (25).

Glomerular antidiuretic actions of AVT in an isolated trunk preparation from the rainbow trout Oncorhynchus mykiss have previously been described (3). However, the renal actions of AVT in elasmobranch fish are largely unknown, and investigations into the hormonal control of renal function in elasmobranchs have concentrated on in vivo effects (5, 10). Whole animal data can be difficult to interpret because of the potential effects of a range of factors (e.g., paracrine and endocrine) that may increase or decrease blood pressure and thereby change glomerular filtration rate (GFR) and renal function. In addition, S. canicula possess very long and convoluted urinary sinuses, and urine output in vivo tends to occur during periods of spontaneous swimming activity rather than as a continuous flow.

To avoid at least some of these complications and to determine the actions of individual peptides on kidney function, previous studies in teleost fish used an in situ renal trunk preparation (3, 15). The present study developed such a preparation for use in S. canicula. Furthermore, glomerular and tubular effects of AVT on fish acclimated to 100% seawater (SW) and 85% SW were examined.

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METHODS

Animals. Female dogfish Scyliorhinus canicula (600–1,100 g) were caught off the Isle of Cumbrae, West Coast of Scotland, and transported to the Gatty Marine Laboratory where they were allowed to acclimatise for at least 2 wk. Dogfish stocks were maintained in aerated free-flowing SW (osmolality 938 mosmol/kgH2O; mM) 399 Na, 8.5 K, 12 Ca, 45 Mg, and 368 Cl) under a natural photoperiod at 12°C. The experimental fish were not fed for at least 2 wk before experimentation. All procedures were carried out by licensed personnel in accordance with UK Home Office regulations [Animals (Scientific Procedures) Act, 1986].

In vivo urine collection. Dogfish were anesthetized by immersion in 0.015% MS222, buffered with an equal amount of NaHCO3, and the urinary papilla was catheterized (PE 50, Portex). The cannula was held in place with a suture to the dorsal Portex). The cannula was held in place with a suture to the dorsal

In situ perfusion of the kidney. Kidney function was assessed in situ using an isolated trunk preparation adapted from Amer and Brown (3). Fish were killed with a sharp blow to the head followed by pithing to prevent subsequent movement of the trunk. The fish were immediately weighed, and the urinary papilla was catheterized by 40-I experimental tanks, containing the same salinity, and three 24-h urine samples were taken. Blood samples were collected after incubation of the preparation for 1 h. Blood was allowed to stabilize for at least 1 h before collection. The collection period immediately before addition of AVT to the perfusate was 1 h. Blood samples were collected for 1-h periods. The trunks were then placed ventral side up. A polythene cannula (tapering to a clean cut immediately behind the pectoral fins, and the trunk was placed ventral side up. A polythene cannula (tapering to

Renal actions of AVT. Kidney preparations were perfused with Ringer containing inulin (0.25 g/l; Sigma) to monitor inulin clearances as a measure of GFR. Tubular transport maxima for glucose (TmG) were determined, as a measure of functional tubular mass, by addition of glucose (4.5 g/l) to the perfusate. Urine flow was allowed to stabilize for at least 1 h to enable constant renal function and clearance of the dead space volume associated with the urinary sinus and cannula. When two 1-h urine samples were collected into preweighed microcentrifuge tubes, and urine flow rates were determined gravimetrically assuming a specific gravity of 1. Two further 1-h urine samples were collected after addition of 10−9 or 10−10 M AVT to the perfusate (n = 6 at each dose). Comparisons of renal parameters were made between the last 1-h collection period immediately before addition of AVT to the perfusate and the final 1-h collection period during administration of AVT.

Analyses and calculations. Blood samples (1 ml) from the in vivo fish were placed in tubes and immediately centrifuged at 13,000 rpm for 1 min to obtain plasma. The plasma was analyzed for plasma osmolality (Roehling Osmometer, Cambab, Cambridge, UK), plasma concentrations of sodium (Corning 480 Flame Photometer, Corning, Essex, UK), chloride (Corning Chloride Analyser 925), and urea spectrophoto- metrically (Sigma Kit No. 640). Urine was also analyzed for osmolality, sodium, chloride, and urea concentrations.

Urine and perfusate osmolalities were also determined for the in situ trunk preparations. Osmolar clearance (Cosm) = [Uosm/Posm] × urine flow rate (UFR), where Uosm is osmolality of urine and Posm is osmolality of perfusate. Free water clearance (CH2O) was calculated from C H2O = P H2O − Cosm. Urea clearance was calculated from Uarea/Parea × UFR, where Uarea is the concentration of urea in the urine and Parea is the concentration of urea in the perfusate.

Inulin was analyzed spectrophotometrically (21), and GFR was calculated from GFR = UFR × Uin/Pin, where Uin and Pin are urinary and perfusate inulin concentrations, respectively.

Glucose of perfusate and urine samples was assayed by use of a glucose oxidase/peroxidase kit (Sigma). TmG was measured as the difference between filtered and excreted glucose and was calculated from (GFR × Pj) − [UFR × Uj], where Uj and PJ were glucose concentrations of urine and perfusate, respectively.

Data and statistical analysis. All data are presented as means ± SE. One-way ANOVA followed by Tukey’s post hoc test was used to assess changes in vivo. Paired t-tests were used to assess physiological changes in the in situ perfused trunk preparation. Significance was accepted at P < 0.05.

RESULTS

In vivo urine collection. Accumulation of dogfish to reduced salinity resulted in increased UFRs (Fig. 1A). Urine flow increased in a stepwise manner from a SW control value of 0.082 ± 0.030 ml·kg−1·h−1 to a maximal value of 0.727 ± 0.094 ml·kg−1·h−1 in 70% SW. During the same periods, plasma osmolality decreased from 939 ± 1.1 mosmol/kgH2O in SW to 665 ± 1.9 mosmol/kgH2O in 70% SW (Fig. 1B). Urinary composition during acclimation to 70% SW is shown in Table 1. Plasma and urine osmolality, sodium concentration, and chloride concentration were all significantly lower than 100% acclimated fish. Plasma urea concentration was also significantly lower in fish acclimated to reduced salinity, whereas urinary urea concentration
remained unchanged. However, when coupled with the increase in UFR, this resulted in an increased clearance of urea from the fish. In vivo, 85% SW represented a salinity threshold where a significant diuresis occurred compared with 100% SW. Consequently, 85% SW was selected for further study as a diuretic model in the in situ trunk preparation alongside trunk preparations from 100% SW-acclimated fish.

In situ perfusion of the kidney. In the absence of AVT, all renal parameters remained stable over 6 h of perfusion (Fig. 2A). The UFRs for in situ preparations of 85% SW-acclimated fish were significantly greater than 100% SW-acclimated fish (P < 0.005). Therefore, the diuresis observed in vivo with acclimation of fish to 85% SW (Fig. 1A) was maintained in the in situ preparations.

Perfuse flow rates. There was a significant decrease in perfusate flow rate during AVT infusion in both SW and 85% SW at both concentrations of AVT (Table 2).

Renal effects of AVT. Addition of 10\(^{-9}\) M AVT to the perfusate resulted in a decrease in UFR and GFR that was detected in the final urine collection (Fig. 2B). Addition of 10\(^{-9}\) M AVT caused a significant antidiuresis in both SW and 85% SW-acclimated preparations (see Fig. 4, A and B). However, addition of 10\(^{-10}\) M AVT to the perfusate resulted in a significant antidiuresis in SW but not in 85% SW (Fig. 3, A and B).

Glomerular effects. Addition of 10\(^{-9}\) M AVT to the perfusate resulted in a decrease in GFR in both SW and 85% SW preparations (Fig. 4, A and B). There was a significant decrease in GFR in SW when the preparations were perfused with 10\(^{-10}\) M AVT but no effect in 85% SW (Fig. 3, A and B).

Tubular effects. Tubular function is summarized in Table 3. The mean urine/plasma inulin concentration ratio (U/P ratio) remained unchanged during perfusion of AVT in either SW or 85% SW. Urine was slightly hypotonic relative to plasma with a mean U/P osmolality ratio of 0.99. Hence there was a small relative free water clearance of <1%, whereas ~30% of filtered osmolytes were excreted. Perfusion of AVT had no

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Table 1. Osmolality, sodium, chloride, and urea concentrations of plasma and urine and urine urea clearance during in vivo urine collection

<table>
<thead>
<tr>
<th></th>
<th>Osmolality, mosmol/kg H(_2)O</th>
<th>Sodium, mmol/l</th>
<th>Chloride, mmol/l</th>
<th>Urea, mmol/l</th>
<th>Urea Clearance, ml·kg(^{-1})·h(^{-1})</th>
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<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>939 ± 1</td>
<td>263 ± 6</td>
<td>266 ± 3</td>
<td>327 ± 5</td>
<td></td>
</tr>
<tr>
<td>90% SW</td>
<td>853 ± 5‡</td>
<td>247 ± 5(^*)</td>
<td>249 ± 2(^*)</td>
<td>296 ± 4(^*)</td>
<td></td>
</tr>
<tr>
<td>85% SW</td>
<td>809 ± 10‡</td>
<td>238 ± 5(^*)</td>
<td>237 ± 4(^*)</td>
<td>280 ± 3(^*)</td>
<td></td>
</tr>
<tr>
<td>80% SW</td>
<td>764 ± 10‡</td>
<td>233 ± 5(^*)</td>
<td>227 ± 4(^*)</td>
<td>263 ± 2(^*)</td>
<td></td>
</tr>
<tr>
<td>75% SW</td>
<td>718 ± 4§</td>
<td>225 ± 4</td>
<td>218 ± 4</td>
<td>243 ± 3</td>
<td></td>
</tr>
<tr>
<td>70% SW</td>
<td>665 ± 2§</td>
<td>219 ± 2</td>
<td>211 ± 3</td>
<td>226 ± 3</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>921 ± 2</td>
<td>221 ± 9</td>
<td>218 ± 15</td>
<td>78 ± 14</td>
<td>0.019 ± 0.009(^*)</td>
</tr>
<tr>
<td>90% SW</td>
<td>842 ± 8(^*)</td>
<td>202 ± 8</td>
<td>196 ± 11</td>
<td>79 ± 6</td>
<td>0.054 ± 0.015(^*)</td>
</tr>
<tr>
<td>85% SW</td>
<td>791 ± 6(^*)</td>
<td>180 ± 10(^†)</td>
<td>183 ± 9(^*)</td>
<td>83 ± 5</td>
<td>0.185 ± 0.032(^*)</td>
</tr>
<tr>
<td>80% SW</td>
<td>734 ± 2(^*)</td>
<td>173 ± 6</td>
<td>171 ± 11(^†)</td>
<td>79 ± 15</td>
<td>0.155 ± 0.021(^*)</td>
</tr>
<tr>
<td>75% SW</td>
<td>704 ± 13‡</td>
<td>160 ± 11(^†)</td>
<td>155 ± 15(^†)</td>
<td>67 ± 18</td>
<td>0.191 ± 0.029(^*)</td>
</tr>
<tr>
<td>70% SW</td>
<td>641 ± 17‡</td>
<td>152 ± 12(^†)</td>
<td>153 ± 25(^†)</td>
<td>70 ± 12</td>
<td>0.226 ± 0.041(^†)</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 4 in each group. \(^*\)P < 0.05, \(^†\)P < 0.01, and \(^‡\)P < 0.005 indicate significant difference from sea water (SW) values.

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Fig. 1. Urine flow rate (A) and plasma osmolality (B) of in vivo catheterized dogfish on stepwise acclimation to dilute seawater (SW). Values are means ± SE (n = 4). \(^*\)P < 0.05; \(^‡\)P < 0.01, and \(***\)P < 0.005 indicate statistically significant difference from value in SW-acclimated fish.
significant effect on $C_{osm}$, relative free water clearance, or U/P ratio.

Functional tubular mass. Addition of $10^{-9}$ and $10^{-10}$ M AVT to the perfusate caused a significant reduction in $TmG$ values in both experimental salinities (Figs. 3 and 4).

**DISCUSSION**

Although most species of elasmobranch are regarded as marine stenohaline fish, many species do have the ability to enter brackish water, and it is clear that some species migrate between freshwater and SW as part of their natural life cycle (23). Furthermore, *Scyllorhinus canicula* has previously been shown to successfully acclimate to dilute SW under laboratory conditions (16, 24). Integral to this process is the necessity to vary urine output to control extracellular fluid volume (10), and the present study demonstrated a significant diuresis in *S. canicula* acclimated to reduced salinity. Coupled with this diuresis was a significant decrease in plasma osmolality, which was primarily due to decreases in sodium chloride and urea, in agreement with previous studies (16, 24). Urine osmolality and sodium chloride concentrations also decreased in line with plasma concentrations, although urine urea concentration remained unchanged in all salinities examined. However, when the increased UFRs are taken into account...
The present study, it is clear that the diuresis observed on transfer from SW to 85% SW in vivo was also evident in the in situ perfused kidney preparation. After the stability of the perfused trunk preparation was established, it was used to investigate renal function in *S. canicula* and to examine the potentially important role of AVT in regulating UFR and GFR.

In lower vertebrates, the vasoconstrictor effects of AVT have been reported to be entirely responsible for AVT-induced renal effects (19). Indeed, the vasoconstrictor action of AVT in *O. mykiss* (14) was suggested as the mediator of a decrease in perfusate flow rate in the perfused trunk preparation of *O. mykiss* (3). In the present study, both 10⁻⁹ and 10⁻¹⁰ M AVT induced a significant decrease in perfusate flow rate that may have been due to the vasoconstrictive actions of AVT. Clearly, additional investigation into the vasoconstrictor actions of AVT on renal vasculature is warranted.

Addition of 10⁻⁹ M AVT was found to have a profound, glomerular antidiuretic effect in the recent study, which has also been described in the isolated trunk preparation of freshwater-acclimated *O. mykiss* (3). An antidiuresis and reduction in GFR were observed in the first hour following administration of AVT that did not become significant until the second hour of collection after AVT administration (Fig. 2B). During the initial protocol development, urine was collected for a period of 30 min, and a significant antidiuresis and reduction in GFR were observed 30 min after AVT administration at 10⁻⁹ M. However, this 30-min urine collection did not routinely produce a sufficient volume of urine to complete all the required urine analysis. It was therefore necessary to adopt 1-h collection times in all subsequent studies. This increased collection period may explain the apparent extended delay of 1 h on the observation of a significant effect of AVT on UFR. It may be pertinent to note that a similar delay in renal action of at least 1 h was observed in vivo in *S. acanthias* (5) on infusion of atriopeptin.

<table>
<thead>
<tr>
<th>Tubular function in the in situ perfused trunk preparation</th>
<th>U/P&lt;sub&gt;in&lt;/sub&gt;</th>
<th>U/P&lt;sub&gt;osm&lt;/sub&gt;</th>
<th>C&lt;sub&gt;osm&lt;/sub&gt;/GFR, %</th>
<th>C&lt;sub&gt;CH2O&lt;/sub&gt;/GFR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (SW)</td>
<td>1.5 ± 0.1</td>
<td>0.99 ± 0.002</td>
<td>67 ± 3.9</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>10⁻¹⁰ M AVT</td>
<td>1.5 ± 0.1</td>
<td>1.00 ± 0.002</td>
<td>68.9 ± 3.9</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Control (SW)</td>
<td>2.2 ± 0.1</td>
<td>1.00 ± 0.008</td>
<td>44.5 ± 2.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>10⁻⁹ M AVT</td>
<td>2.0 ± 0.1</td>
<td>1.00 ± 0.006</td>
<td>50.1 ± 1.9</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Control (85% SW)</td>
<td>1.6 ± 0.4</td>
<td>0.99 ± 0.008</td>
<td>78.3 ± 13.8</td>
<td>0.7 ± 0.6</td>
</tr>
<tr>
<td>10⁻¹⁰ M AVT</td>
<td>1.7 ± 0.3</td>
<td>0.99 ± 0.007</td>
<td>68.2 ± 11.1</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>Control (85% SW)</td>
<td>1.9 ± 0.4</td>
<td>1.0 ± 0.004</td>
<td>51.5 ± 10.6</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>10⁻⁹ M AVT</td>
<td>2.3 ± 0.2</td>
<td>1.0 ± 0.003</td>
<td>45.7 ± 4.7</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE from 6 fish in each group. U/P<sub>in</sub>, urine/perfusate inulin concentration ratio; U/P<sub>osm</sub>, urine/perfusate osmolality ratio; C<sub>osm</sub>/glomerular filtration rate (GFR), relative clearance of osmolytes; C<sub>CH2O</sub>/GFR, relative free water clearance. Control values were obtained during the final 1-h renal clearance collection before AVT addition to the perfusate; AVT values were obtained in the final 1-h collection during administration of AVT.
The use of inulin to measure GFR depends on the assumption that inulin is freely filtered at the glomerulus and neither reabsorbed nor secreted by the renal tubule (10). However, slight tubular reabsorption has been reported in the urinary bladder and tubules of some teleost fish (6). Although there have been no detailed studies of the handling of inulin by elasmobranch renal tubules or the urinary sinus, the present measurements may slightly underestimate GFR. The present study suggests that AVT may play a role in the regulation of urine production in elasmobranch fish, primarily through a glomerular antidiuresis.

Filtering populations of nephrons were assessed indirectly by measurement of TmG. Interpretation of TmG data must be made with care as there is growing evidence that TmG may also result from an alteration in single nephron GFR coupled with altered tubular handling of sodium (12). Addition of $10^{-9}$ and $10^{-10}$ M AVT to the perfusate in both salinities resulted in a significant decrease in TmG. TmG in control preparations was stable so changes in TmG induced by addition of AVT to the perfusate are indicative of changes in the population of functional glomeruli. Recently, the presence of glomerular bypass shunts was confirmed in the kidney of S. canicula (9). This shunt was shown to arise from the afferent arteriole to join a peritubular network of capillaries and thereby offer the potential to vary the degree of glomerular perfusion and control the proportion of active glomeruli. It is possible that AVT may act on these shunts to decrease the population of filtering nephrons. However, additional work is required to elucidate this.

In summary, an in situ perfused dogfish trunk preparation has been developed to investigate renal function in elasmobranch fish. This preparation has been verified with respect to renal function in vivo, and the diuresis observed with acclimation of fish from 100% SW to 85% SW in vivo was maintained in the in situ perfused preparations. Addition of $10^{-9}$ M AVT to the perfusate resulted in a decrease in UFR, GFRs, and TmG in both SW and 85% SW-acclimated preparations. These data suggest that AVT induced a glomerular antidiuresis in S. canicula as previously reported for the trout.

**Perspectives**

To date, studies of the hormonal control of renal function in elasmobranch fish centered on investigations in vivo. This study provides a new perspective by establishing an isolated perfused trunk preparation to investigate the hormonal control of renal function. This will allow in the future the study of single hormones, or combinations of hormones, in controlling renal function. Furthermore, this preparation will allow the investigation of tubular transport of ions and, in particular, urea, which are essential for understanding the overall osmoregulatory mechanisms in elasmobranch fish. It would be particularly useful to couple renal studies using the perfused trunk preparation with accurate measurement of the circulating concentration of the hormones in question. This would elucidate fully the osmoregulatory role of the hormone in controlling renal function in elasmobranch fish.

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