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RENAL ELECTROLYTE AND FLUID EXCRETION IN THE ATLANTIC HAGFISH *MYXINE GLUTINOSA*

BY JEANNETTE M. ALT,* HILMAR STOLTE,*† GEORG M. EISENBACH,*† AND FINN WALVIG‡

* *Medical School Hannover, Departments of Medicine and Physiology, Hannover, Germany*

† *Mount Desert Island Biological Laboratory, Maine, U.S.A.*

‡ *University of Oslo, Biological Station, Drøbak, Norway*

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SUMMARY

Hagfishes (*Myxine glutinosa*, Cyclostomata) are the only vertebrates whose body fluids are isoosmotic to seawater due to high sodium and chloride concentrations in the plasma. The unique mode of fluid and electrolyte handling of *Myxine* has been studied by micropuncture methods at the single nephron level. In the archinephric duct, which assumes the function of the tubular apparatus of higher vertebrates, no fluid or sodium is reabsorbed from the glomerular filtrate. Calcium, glucose and magnesium are reabsorbed whereas potassium and phosphate are excreted. The intravascular volume of *M. glutinosa* is mainly regulated by the filtration rate which greatly increases with increasing arterial as well as venous pressure.

INTRODUCTION

Myxinoids keep their extracellular fluids almost in osmotic equilibrium with the environment (Robertson, 1963), not by retention of urea or trimethylamine oxide as in elasmobranchs, but by keeping the ionic concentration at seawater level. They are in many ways unique and even differ markedly from their closest relatives, the lampreys.

Urine is formed by the opisthonephros, which corresponds to the mesonephros in the embryo of other vertebrates (Fänge, 1963). Function has been studied only at the whole organ level (Munz & McFarland, 1964; Morris, 1965; Rall & Burger, 1967). However, the segmentally arranged large glomeruli and single archinephric ducts lend themselves to a study of single nephron function at the glomerular level and a study of the regulation of water and electrolyte excretion by the archinephric duct including the neck segments.

The aims of the present study were (1) to measure the single nephron glomerular filtration rate by means of micropuncture methods and (2) to study selective reabsorption or secretion mechanisms which also play a role in adaptation to environmental changes. Since no net fluid reabsorption is made by the hagfish kidney it is safe to say that the urine flow equals the filtration rate (Stolte & Eisenbach, 1973; Munz & McFarland, 1964). This means that if the urinary concentration of any substance is

higher than in the plasma a secretory mechanism is likely. This physiological peculiarity of the hagfish simplifies measurements of filtration, reabsorption and secretion.

METHODS

1. *Animals.* The animals were caught by means of baited traps either in Frenchman's Bay (Maine, U.S.A.) or in the Oslo Fjord (Drøbak Biological Station, Norway) at a depth of about 100 feet. If the hagfish were not used immediately after removal from the sea they were kept in seawater tanks at temperatures of 4–6 °C. Anaesthesia was performed with propylene phenoxetol, (Bagenal, 1963; Adam, Schirner & Walvig, 1962) from Nipa-Laboratories Ltd, Treforest, Pontypridd, Glamorgan, G.B., at a concentration of 0·9 ml/500 μl seawater. The gills were perfused via an oral tube with 23 ml/min seawater thermostatted at 6–8 °C, and the kidneys laid open by a midline incision beginning 2 cm in front of the cloaca.

To achieve a potassium load, the animals were kept in K-enriched seawater (20 mM-K) for 40 h.

2. *Single nephron glomerular filtration rate* (*SNGFR*). The glomerular ultrafiltrate was collected quantiatively by catheterization of the archinephric duct adjacent to the glomerulus whose function was under study, using calibrated tubings (PP10, Portex, Hythe, Kent, G.B.). Care was taken to avoid backpressure which probably affects glomerular filtration. Pressure in the aorta or cardinal vein was measured with a water column device attached to indwelling glass capillaries. No more than four to five glomeruli were perfused via segmental branches of the aorta. For calculation of the SNGFR the total urinary volume was divided by the number of glomeruli.

The perfusion pressure was monitored through an L-shaped glass tube in parallel with the perfusion device (Stolte & Eisenbach, 1973). The perfusion fluid contained: 530 mM-Na^+, 9·0mM-K^+, 6·0 mM-Ca^{2+}, 18 mM-$MgSO_4$, and 1·67 mM-glucose. 1 mg/ml ^{14}C-inulin (spec. activity 0·23 mCi/91·8 mg, NEN-Chemicals, Boston, Mass., U.S.A.) was added as marker for fluid reabsorption.

3. *Electrolyte and glucose determinations.* Na^+ and K^+ concentrations were measured by micro-flame photometry in 3 nl samples (Müller, 1958; Stolte *et al.* 1969). Ca^{2+} and Mg^{2+} determinations were performed by atomic absorption spectrophotometry (Perkin-Elmer model 107). Osmolalities were measured by freezing point depression with a Ramsay–Brown osmometer (Ramsay & Brown, 1955). Glucose was measured by an enzymatic micromethod (Zwiebel *et al.* 1969) and chloride was determined by coulometric titration (Buchler-Cotlove chloridometer). Sulphur and phosphorus were determined by C. Lechene by electron probe microanalysis (Lechene & Warner, 1977).

4. *Urine and plasma.* Urine was collected under free flow conditions by cannulation of the distal archinephric duct; Blood samples were obtained by puncturing the aorta close to the glomeruli and archinephric duct segment under study with glass capillaries or, at the end of the experiment, by heart puncture.

5. *Stopped flow perfusion.* The archinephric duct was tied off so that a section of 7 to 8 cm, draining about 15 glomeruli, was separated from the rest of the archinephric duct. This segment was flushed and then filled with perfusion fluid. 60 min later the perfused archinephric duct segment was emptied and the fluid analysed. Glomeru

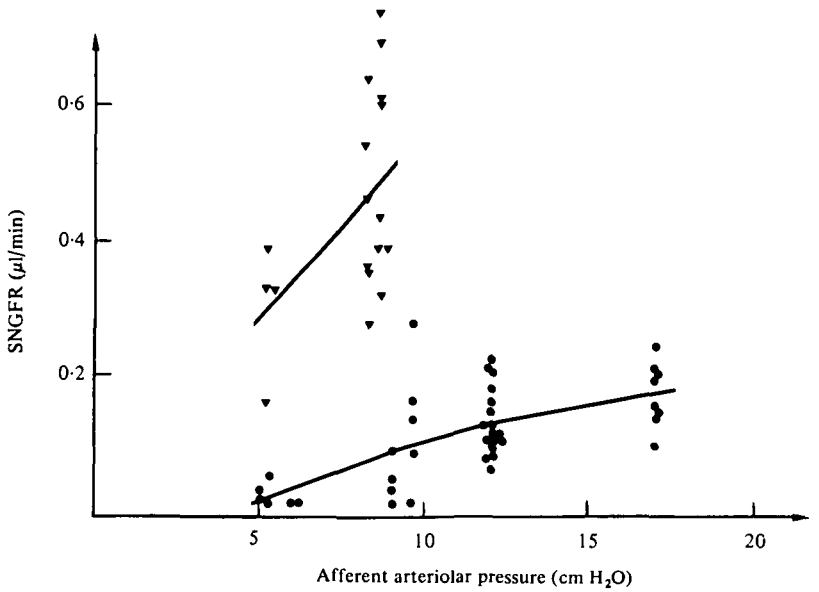

Fig. 1. The increase of single nephron GFR with rising afferent arteriolar pressure. ●, Cardinal venous pressure 0 cm H_2O. ▼, Cardinal venous pressure 4·8–5·3 cm H_2O.

ultrafiltration was stopped by counter-balancing the filtration pressure. Lissamine green was added to the perfusion fluid in order to control possible further filtration.

Calculations. All values are given as mean ± S.D. Statistical significance was calculated by means of the Student's *t* test or by the paired *t* test. For calculation of differences from 1, the 99% confidence intervals of the U/P ratios were used.

RESULTS

Single nephron GFR. Fig. 1 demonstrates the rise in single nephron GFR with rising arterial pressure from the non-perfused value of 5·4 ± 0·38 cm H_2O (n = 12). With venous pressure at 0 cm H_2O, the rate rose less steeply than when venous pressure was held between 4·8–5·3 cm H_2O. The U/P ratio of inulin in these experiments was 1·0 ± 0·08 (n = 33). At a perfusion pressure of 5·0–6·0 cm H_2O, which is close to the normal arterial pressure, the single nephron GFR was 24·2 ± 15 nl/min (n = 6).

Electrolytes in seawater, plasma and urine. Electrolyte concentrations and the osmolalities are compiled in Table 1. The K^+-concentration in hagfish plasma is 66% of the concentration in seawater; plasma Ca^{2+} is 74% and plasma Mg^{2+} is 44% of the seawater concentration. These differences are highly significant. Na^+ and osmolality in plasma are almost the same as in seawater; however, the scatter is too large to caulculate significant differences.

In urine, the K^+-level is higher (8·6 ± 4·6, n = 8) than in plasma (5·9 ± 0·9, n = 10, $P < 0.05$), whereas Ca^{2+} is 41% lower ($P < 0.0005$). The Mg^{2+}-concentration in urine is slightly lower than in plasma ($P < 0.05$, paired *t* test). Chloride and sulphate have virtually the same concentrations in plasma and urine. Phosphate seems to be

Table 1. *Electrolyte concentrations in* Myxine glutinosa

	Osmolality (m-osmol/kg)	Na^+ (mM)	K^+ (mM)	Ca^{2+} (mM)	Mg^{2+} (mM)	Cl^- (mM)	P (mM)	S (mM)
Seawater	898 ± 65	424 ± 31	$8{\cdot}9 \pm 0{\cdot}9$	$9{\cdot}7 \pm 0{\cdot}3$	$44{\cdot}6 \pm 3{\cdot}6$	511 ± 27	1·08	19·8
	$n = 7$	$n = 7$	$n = 6$	$n = 5$	$n = 6$	$n = 6$		
Plasma	980 ± 104	439 ± 29	$5{\cdot}9 \pm 0{\cdot}9$	$7{\cdot}2 \pm 1{\cdot}4$	$19{\cdot}5 \pm 6{\cdot}6$	455 ± 45	$1{\cdot}0 \pm 0{\cdot}1$	$17{\cdot}6 \pm 8{\cdot}8$
	$n = 11$	$n = 9$	$n = 10$	$n = 9$	$n = 9$	$n = 9$	$n = 5$	$n = 5$
Urine	1053 ± 99	462 ± 22	$8{\cdot}6 \pm 4{\cdot}6$	$4{\cdot}2 \pm 1{\cdot}3$	$14{\cdot}7 \pm 1{\cdot}8$	430 ± 82	$2{\cdot}0 \pm 1{\cdot}9$	$17{\cdot}7 \pm 2{\cdot}2$
	$n = 13$	$n = 11$	$n = 8$	$n = 6$	$n = 6$	$n = 7$	$n = 5$	$n = 5$
U/P ratio	$1{\cdot}07 \pm 0{\cdot}1$	$1{\cdot}06 \pm 0{\cdot}05$	$1{\cdot}5 \pm 0{\cdot}7$	$0{\cdot}5 \pm 0{\cdot}1$	$0{\cdot}77 \pm 0{\cdot}21$	$1{\cdot}0 \pm 0{\cdot}2$	$3{\cdot}1 \pm 2{\cdot}0$	$1{\cdot}15 \pm 0{\cdot}14$
	$n = 13$	$n = 11$	$n = 8$	$n = 6$	$n = 6$	$n = 7$	$n = 5$	$n = 5$

(The animals were caught and processed in Maine. The values are $\bar{x} \pm$ S.D.)

Table 2. *Regional differences in electrolyte concentrations in* Myxine glutinosa

	Osmolality (m-osmol/kg)		Na+ (mM)		K+ (mM)	
	Maine	Drøbak	Maine	Drøbak	Maine	Drøbak
Seawater	898 ± 65* $n = 7$	1037 ± 31* $n = 13$	424 ± 31 $n = 7$	465 ± 64 $n = 13$	8·9 ± 0·9 $n = 6$	10·9 ± 2·0 $n = 13$
Plasma	980 ± 104* $n = 11$	1162 ± 111* $n = 14$	439 ± 29 $n = 9$	495 ± 135 $n = 14$	5·9 ± 0·9 $n = 10$	8·2 ± 2·8 $n = 14$
Urine	1053 ± 99 $n = 13$	1153 ± 150 $n = 12$	462 ± 22 $n = 11$	555 ± 109 $n = 12$	8·6 ± 4·6* $n = 8$	21·0 ± 7·6* $n = 12$

(* Indicates significant differences ($P < 0{\cdot}0005$). The values are $\bar{x} \pm$ S.D.)

Table 3. *Stopped flow perfusion of the kidney of* Myxine glutinosa

	Osmolality (m-osmol/kg)	Na^+ (mM)	K^+ (mM)	Glucose (mM)	Inulin
Test-solution	1132 ± 162 $n = 11$	522 ± 99 $n = 8$	9·2 ± 2·4 $n = 11$	2·6 ± 0·4 $n = 4$	
After perfusion	1042 ± 253 $n = 11$	519 ± 144 $n = 6$	15·3 ± 5·4 $n = 11$	1·6 ± 0·3 $n = 8$	
Ratio	0·97 ± 0·16 $n = 11$	1·03 ± 0·2 $n = 6$	1·68 ± 0·5 $n = 11$	0·62 ± 0·22 $n = 8$	0·99 ± 0·04 $n = 4$

(The animals used for this study were caught in Maine. The values are $\bar{x} \pm$ S.D.)

Table 4. *The effect of potassium loading on renal function in* Myxine glutinosa

	Osmolality (m-osmol/kg)	Na^+ (mM)	K^+ (mM)
Seawater	926	438	20·1
Plasma	812 ± 8*	399 ± 9*	15·9 ± 5·4*
Urine	818 ± 22*	461 ± 21	21·2 ± 8·0*

(These experiments were performed on five animals in Maine. * Indicates significant differences from controls (see Table 2). The values are $\bar{x} \pm$ S.D.

excreted by the renal system as shown by the U/P ratio of 3·07. Even though the scatter is large, the mean U/P ratio is different from 1 ($P < 0{\cdot}01$).

Regional differences. The values of Table 1 were measured in animals caught and processed in Maine. Since 1975, comparative experiments have been made with hagfish caught in Oslo Fjord, where seawater osmolality is significantly higher. Plasma osmolality of these hagfishes was also higher, but such that the ratio of plasma to seawater osmolality was the same (1·09 in Maine, 1·12 Oslo Fjord). Na^+ and K^+ were also slightly higher in Oslo Fjord seawater and in the plasma of hagfish living there. However, more striking was the difference in the U/P ratio of K^+ which was much higher in the Norwegian hagfish (2·56) than in animals caught in Maine (1·47, $P < 0{\cdot}0005$; see Table 2).

Stopped flow perfusion. As is clearly shown in Table 3, neither the osmolality nor the Na or inulin concentration within the archinephric duct changed. Only the K^+

concentration rose significantly ($P < 0.05$) and the glucose concentration fell. The K^+-secretion was even more marked in experiments performed in Drøbak. In these animals the concentration ratio of K^+ in the perfusion fluid after perfusion to before was 2.06 ± 0.24 ($P < 0.0025$).

Potassium load. Absolute K^+-concentrations in plasma and urine increased, but the concentration ratios plasma/seawater and urine/plasma did not change (Table 4). The osmolality in plasma and urine was lower in potassium loaded animals than in controls. So was the ratio plasma osmolality/seawater osmolality and the Na^+-concentration in the plasma.

DISCUSSION

(A) *Glomerular filtration.* The filtration barrier of the hagfish glomerulus is similar in ultrastructure to that of mammalian kidney (Kühn, Stolte & Reale, 1975). Functionally, however, interesting differences have been found. As Riegel (1978) demonstrated, the glomerular filtration in the hagfish seemingly contradicts Starling's hypothesis, according to which fluid leaves the glomerular capillaries due to an excess of hydrostatic pressure over colloid oncotic pressure. The hydrostatic pressure in the aorta under control conditions is 5·4 cm H_2O and the mean colloid osmotic pressure of hagfish plasma is about 10·5 cm H_2O (Riegel, 1978). Even though perfusion experiments presented in this paper have been performed with colloid-free solutions, the SNGFR was only slightly higher (24.2 ± 6.8 nl/min, $\bar{x} \pm$ S.E.M., $n = 6$) than under normal conditions as measured by Riegel (20.3 ± 2.1 nl/min, $\bar{x} \pm$ S.E.M.), the hydrostatic pressure being about 5 cm H_2O in both experiments. This discrepancy may be explained by the method of measuring plasma colloid osmotic pressure which depends upon the choice of membranes used for the determination. Since no ciliated cells have been demonstrated within the tubules of the archinephric duct (Heath-Eves & McMillan, 1974) there is no reason to assume that glomerular filtration is aided by reduced pressure from this source.

The increase in SNGFR with increasing venous pressure may be caused by a pressure rise in the efferent arterioles via the cardinal vein which diminishes the pressure drop within the glomerular capillaries, thus utilizing a greater capillary surface for filtration (Stolte & Eisenbach, 1973; Stolte, 1977). It is possible that the hagfish kidney works mainly as a secretory kidney at low intravascular pressure, whereas at increased pressures the kidney becomes primarily a filtering kidney. It is likely that the extracellular volume is regulated by changes in glomerular filtration rate. As has been shown by Chapman, Jensen & Wildenthal (1963), even small increases in intravascular volume increase the arterial pressure with a consequent rise in filtration rate, thus reducing the intravascular volume back to normal. A valve action of the neck segment has been postulated. Therefore fluid excretion is not regulated by tubular absorption but by glomerular filtration.

(B) *Tubular function.* The archinephric ducts have taken over some of the functions attributed to kidney tubules of other vertebrates. Archinephric ducts also serve as urinary bladders (Fänge, 1963). Since no net fluid reabsorption occurs in the hagfish kidney it is safe to say that the urine flow equals the filtration rate (Stolte & Eisenbach, 1973; Munz & McFarland, 1964). This also implies that if the concentration of a

substance in the urine is higher than in the plasma this substance has been secreted. The results show that K^+ is excreted into the urine in contrast to glucose which is being reabsorbed. These results were confirmed by stopped-flow perfusion experiments. The concentrations of Ca^{2+} and Mg^{2+} are found to be lower in urine than in plasma.

Ca^{2+} is excreted by the gall bladder (unpublished observations; Rall & Burger, 1967). Our results showing Mg^{2+}-reabsorption are contradictory to the values found by Munz & McFarland (1964) who used different methods and species (*Eptatretus stouti*), but similar to the values measured by Rall & Burger (1967). Morris (1965) found a reduced Mg^{2+}-secretion as a consequence of anaesthesia whereas the Ca^{2+}-secretion was not reduced. An influence of propylene phenoxetol anaesthesia cannot be excluded. Our values differ in some respects from those cited by Robertson (1963), especially the values for K^+ in the plasma which he found to be higher. The determinations of K^+ by Munz & McFarland (1964), however, are in good agreement. The regional differences described above may also complicate the comparison of measurements. Moreover, the metabolic state of the animals influences renal function (Raguse-Degener *et al.* 1980) and little is known about seasonal changes and sex differences.

The response of the hagfish kidney to changes in the animals environment has constantly been a matter of interest. As has been reported by several authors, hagfish cannot live in diluted seawater for more than some hours (Robertson, 1963), though McInerney (1974) kept Pacific hagfishes successfully in 80% seawater, the osmolality of which was kept normal by the addition of sucrose. The experiments with K^+-loaded animals described above suggest that hagfish can regulate the K^+-concentration gradient, but not the K^+-concentration itself.

Uncomplicated as the hagfish kidney may be in comparison to the kidney of higher vertebrates, it still seems to be capable of active tubular transport which surely is responsible for renal regulation of some ion concentrations. As *Myxine* lacks osmoregulation and as no fluid uptake by the archinephric duct can be detected, volume regulation probably takes place by changes in glomerular filtration rate. The gills may also play an important role in electrolyte regulation as do the gall bladder and the gut.

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