REGULATION OF GLOMERULAR FILTRATION RATE IN A MARINE ELASMOBRANCH, THE DOGFISH (SQUALUS ACANTHIAS)

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The kidney is the major organ for extracellular volume regulation in vertebrates. In fishes, the water influx and therefore the required rate of water excretion are greatly affected by the salinity of the environment. Alteration of the rate of glomerular filtration (GFR) is a primary means for regulating the rate of water excretion in these animals. In the present study, we investigated the response of the kidney of an elasmobranch, the dogfish (Squalus acanthias) to acute changes in environmental salinity and attempted to elucidate the hormonal bases for the response.

Methods Female dogfish (BW 4.51 ± 0.32 kg, mean ± SE, n=11) were kept in live cars until use. Fish were placed in flowing aerated sea water at 13°-15°C in a tank fitted with partitions to prevent turning. Two indwelling non-occluding polyethylene cannulas (PE50) were inserted percutaneously into the dorsal aorta via the caudal artery of unanesthetized dogfish for the measurement of arterial pressure, the collection of blood samples and the administration of drugs. Tubing was inserted into the urogenital papilla and tied securely for urine collection. Inulin (5 ml of 15 g% solution in elasmobranch Ringer) was administered as a bolus injection intraarterially for GFR determination. The fish were allowed to recover at least 24 h before experimentation. Control renal clearance measurements were performed continuously for several hours to ensure steady-state conditions. In experiments with fish undergoing environmental dilution, the water salinity was altered to 90% sea water (osmolality ca. 870 mOsm) by appropriate adjustment of sea water and fresh water delivery rates to the tank. Renal function was continuously monitored immediately upon transfer and throughout dilution period to assess the time course of changes in renal function. After the steady-state values were obtained for the dilution period, the fish were returned to full-strength sea water with renal function being continuously monitored.

In animals receiving hormone agonists or antagonists both in full-strength sea water and in 90% sea water, the renal function was assessed, after a control period, while the agents were infused intraarterially at rates from 10 to 50 µl/min for 2 hours to attain the desired dosage. This was followed by a recovery period of several hours. Dorsal aortic pressure was continuously monitored with a calibrated Statham P-231D transducer, or in some cases, the pressure was read directly in cm H_2O from the arterial catheter. Blood and urine samples were frozen for later determinations of inulin and hormones (catecholamines, arginine vasotocin). Data are expressed as means ± SEM and Student's t test was used to assess statistical significance.

Results and Discussion

Within 2-5 h upon transfer of the dogfish into 90% sea water, there were changes in renal function. These results are shown in Table 1. The rates of glomerular filtration and urine flow increased approximately 3-fold while the ratio of urine-to-plasma inulin concentration decreased by half indicating a reduction in tubular water reabsorption. These changes in renal function occurred with no changes in dorsal aortic pressure. The hematocrit tended to drop slightly. The enhanced GFR and urine flow rate persisted throughout the stay in dilute sea water. It should be noted that the dilution imposed in the present study (90% SW) is much less than in previously published studies (e.g. Myers et al. Bull. MDIBL 15: 59, 1975) which typically imposed dilution to 70%SW over longer time period. The rapid renal response to environmental dilution and presumably to increased water influx indicate that the shark possesses sensitive regulatory mechanism which quickly responds to maintain volume homeostasis. Upon return to full-strength sea water, GFR and urine flow dropped quickly toward control levels.

Several hormones have been suggested as mediators for changes in the rate of glomerular filtration in response to change in salinity in euryhaline teleost fish. Among these are angiotensin II (Brown et al. Am. J. Physiol. 239: R509, 1980) and catecholamines (Elger and Hentschel, Comp. Biochem. Physiol. 75C: 253, 1983). Elasmobranchs lack the renin-angiotensin system (Nishimura et al. Am. J. Physiol. 218: 911, 1970), and angiotensin II has been found to have no effect on either glomerular filtration or urine flow rates in the dogfish (Churchill et al. Bull. MDIBL 17: 85, 1977).

Table 1

Acute Effects of Environmental Dilution on Renal Function

	Control in 100% SW [0-20 h]	Transfer to 90% SW [20-70 h]	Return to 100% SW [70-115 h]
Urine flow rate (ml/kg-h)	0.28 ± 0.08	0.84 ± 0.04**	0.46 ± 0.02
GFR (ml/kg-h)	0.93 ± 0.17	2.57 ± 0.60*	1.01 ± 0.03
[U/P] inulin	5.7 ± 2.7	2.8 ± 0.7	2.4 ± 0.2
Dorsal aortic pressure (mm Hg)	22.8 ± 1.3	21.6 ± 0.1	21.1 ± 2.0
Hematocrit (%)	18.4 ± 1.0	16.9 ± 1.1	17.1 ± 2.6

-Values are means ± SEM from the same 4 animals. Values for each animal represent the means of steady-state values of 3-11 clearance periods (at least 1 h in duration): in 100% SW - 20 h before dilution, in 90% SW - 3 h after dilution, and - 4 h after return to 100% SW.
-Significantly different from 100% SW values at $P<0.05$(*) and $P<0.01$(**) by t-test.

Table 2

Effects of α-Adrenegic Antagonist on Renal Function during Environmental Dilution

	ΔDAP (% Control)	$\dot{V}$ (ml/kg-h)	GFR (ml/kg-h)	[U/P]inulin
100% SW		0.29 ± 0.08*	1.45 ± 0.34	5.8 ± 2.5
90% SW	0	0.74 ± 0.12	2.82 ± 0.84	3.8 ± 0.8
90% SW + Phenoxybenzamine (10mg)	-30.2	0.43 ± 0.07*	1.34 ± 0.36	3.2 ± 0.8

Values are means ± SEM of 1 h clearance period from 3 animals.
ΔDAP are changes in dorsal aortic pressure as percent of control 100% SW value. $\dot{V}$ is the urine flow rate.
Phenoxybenzamine was administered as a 2.5mg bolus injection followed by an infusion of 5mg/ml over 1 h.
*$P<0.05$ (paired-test) from values in 90% SW.

Table 3

Renal Response of S. acanthias in 100% SW to Hormonal Agonists

Agonist (Dosage)		ΔDAP (% of Control)	GFR (ml/kg-h)	$\dot{V}$ (ml/kg-h)	[U/P]inulin
Norepinephrine (10.1 ± 0.7 μg/kg)					
n=5	Control		1.39 ± 0.28	0.42 ± 0.05	3.2 ± 0.4
	1st h infusion	+12.8 ± 1.3	3.02 ± 0.94*	0.84 ± 0.17*	3.6 ± 0.6
	2nd h infusion	+11.2 ± 1.5	4.81 ± 3.50	1.01 ± 0.48	3.2 ± 1.0
	Recovery		1.64 ± 0.26	0.41 ± 0.08	5.0 ± 1.4
Phenylephrine (2.3 ± 0.2 μg/kg)					
n=5	Control		1.45 ± 0.27	0.23 ± 0.05	6.8 ± 1.3
	1st h infusion	0	3.19 ± 0.64*	0.55 ± 0.16*	7.2 ± 1.5
	2nd h infusion	0	4.41 ± 1.63	0.57 ± 0.15	7.4 ± 1.6
	Recovery		1.75 ± 0.26	0.28 ± 0.06	7.1 ± 1.5
Epinephrine (11.2 ± 1.2 μg/kg)					
n=5	Control		1.33 ± 0.34	0.30 ± 0.08	6.3 ± 2.3
	1st h infusion	+44.5	2.75 ± 0.77	0.68 ± 0.23	5.8 ± 2.3
	2nd h infusion	+55.5	3.65 ± 1.20*	1.00 ± 0.18*	4.4 ± 2.1
	Recovery		1.39 ± 0.24	0.38 ± 0.12	6.2 ± 2.0
Isoproterenol (2.5 ± 0.3 μg/kg)					
n=5	Control		2.09 ± 0.19	0.39 ± 0.10	6.4 ± 2.2
	1st h infusion	−35.7 ± 2.9	0.16 ± 0.16*	0.03 ± 0.03	5.9 (1)
	2nd h infusion	−41.5 ± 0.5	0.07 (1)	0.01 (1)	7.3 (1)
	Recovery		1.69 (2)	0.18 (2)	10.6 (2)
KCl (1.4 ± 0.1 nmol/kg)					
n=4	Control		1.83 ± 0.39	0.39 ± 0.12	5.2 ± 2.7
	1st h infusion		2.19 ± 0.49	0.59 ± 0.19	4.5 ± 1.0
	2nd h infusion		5.20 ± 1.17*	1.40 ± 0.47	5.1 ± 1.4
	Recovery		2.40 ± 0.76	0.44 ± 0.14	5.4 ± 1.0
Arginine vasotocin (1.1 ± 0.1 ng/kg)					
n=4	Control		1.64 ± 0.22	0.28 ± 0.06	6.2 ± 0.8
	1st h infusion	+11.9	3.12 ± 0.38	0.69 ± 0.22	5.2 ± 2.5
	2nd h infusion	− 0.8	0.79 (2)	0.19 (2)	4.3 (2)
	Recovery		1.25 ± 0.19	0.26 ± 0.05	5.2 ± 1.0
Arginine Vasotocin (10.9 ± 7.0 ng/kg)					
n=3	Control		1.41 ± 0.24	0.29 ± 0.09	5.9 ± 0.9
	1st h infusion	+ 2.7	0.78 ± 0.20	0.13 ± 0.02	6.0 ± 1.1
	2nd h infusion	− 3.2	2.22 (2)	0.33 (2)	6.4 (2)
	Recovery		1.69 ± 0.37	0.30 ± 0.04	5.6 ± 0.5

-For each animal, the control and recovery period values represent the means of at least 3 clearance periods each of 1 h duration, immediately preceding or following the agonist administration.
-All agonists were infused intraarterially over a 2 hour period at the rate of 10 to 50 ml/min.
-ΔDAP represents the mean of average changes in dorsal aortic pressure during agonist administration expressed as in Table 2.
-*P<0.05 (paired-test) between the experimental and control periods.

We, therefore, investigated the potential role of catecholamines as mediators of the change in GFR during environmental dilution in the dogfish. The role of catecholamines was assessed by the use of antagonists to block the renal response to salinity change. The results are shown in Table 2. Administration of phenoxybenzamine, an α-adrenergic blocking drug, to fish in 90% sea water resulted in decreases in both GFR and urine flow rate to the levels previously observed when fish were in full-strength sea water. In a single animal, propanolol, a β-adrenergic blocker, on the other hand, produced conflicting effects. A low dose (100 µg) of propanolol decreased GFR slightly whereas a high dose (1 mg) resulted in a marked (2-fold) increase in GFR and urine flow. These observations support the role of catecholamines, especially α-adrenergic effector, in the regulation of GFR and urine flow rate in the dogfish during environmental dilution.

We further tested the involvement of catecholamines in the regulation of GFR in elasmobranchs by infusing various adrenergic agonists into the dogfish in full-strength sea water to determine if these agents could mimic the changes observed during dilution. Table 3 summarizes the results of the administration of various agonists. Both norepinephrine (α-adrenoceptor agonist) and epinephrine (both α and β-adrenoceptor agonist) at the doses of 10 µg/kg significantly increased GFR and urine flow rate by their actions on α-adrenergic receptors, since only the prototype α-agonist, phenylephrine, could produce the diuretic response. The administration of the β-agonist, isoproterenol, on the other hand, resulted in a marked drop in GFR and urine flow rate. It should be noted that the α-agonist phenylephrine, produced its renal effects without changes in systemic blood pressure, suggesting that its site of action was within the renal vasculature.

The administration of small amounts of potassium (~1.4 nmol/kg) resulted in significant increases in GFR and urine flow. This response may be mediated by catecholamines since increased plasma potassium normally causes the release of catecholamines, especially norepinephrine, into the plasma in the dogfish (Opdyke et al. Am. J. Physiol. 241: R228, 1981).

The effects of other hormones on elasmobranch renal function were also investigated. Arginine vasotocin (AVT), the antidiuretic hormone in non-mammalian terrestrial vertebrates, produced biphasic responses (diuretic and antidiuretic) with both low and high doses. However, radioimmunoassay measurements of AVT levels in dogfish plasma indicated no significant difference between sharks in full-strength sea water (17.1 ± 2.2 pg/ml, n=3) and those in 90% sea water (15.4 pg/ml, n=2). These preliminary results suggest that AVT does not play a role in the renal response to environmental dilution.

The possible role of cardiac natriuretic factor in the renal response of dogfish to salinity change was also investigated. Dogfish cardiocytes possess a substance analogous to mammalian atrial natriuretic factor (ANF) which is released during volume expansion (Solomon et al. Am. J. Physiol. 249: R348, 1985). The administration of synthetic atriopeptin II (Peninsula) at the dose of 2 µg/kg produced a drop in dorsal aortic pressure and decreases in GFR and the urine flow rate. These observations suggest that ANF does not cause a renal natriuresis although it does stimulate rectal gland secretion (Solomon et al. ibid.).

Interestingly, prolactin, given as a bolus injection in a single animal, was found to have a transient but marked diuretic effect in the dogfish. Prolactin has been implicated in long-term changes in teleost renal function in response to salinity changes (Foster, Gen. Comp. Endocrinol. 27: 153, 1975). This preliminary observation warrants further investigation into the role of prolactin in the regulation of GFR.

In conclusion, we have preliminarily identified a role for catecholamines in the increase in GFR in elasmobranchs during environmental dilution.

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