EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON RENAL FUNCTION IN SQUALUS ACANTHIAS: Richard Solomon, Anjani Dubey, Patricio Silva, and Franklin Epstein.
New York Medical College and Harvard Medical School.

INTRODUCTION: Atrial natriuretic peptide (ANP) is a circulating hormone derived from cardiac myocytes which increases the renal excretion of salt and water, vasodilates resistance vessels, and inhibits the renin–angiotensin–aldosterone system in mammals. In Squalus acanthias, we have previously demonstrated the presence of an immunoreactive ANP within cardiac muscle (unpublished data) and found that exogenous rat ANP was capable of stimulating chloride secretion by the in vivo and in vitro rectal gland (Solomon et al., Am J Physiol 249:R348–354,1985). We hypothesized that endogenous ANP may therefore play a role in the maintainance of extracellular volume and electrolyte composition. To further test this hypothesis, we studied the effects of acute dilution of the external environment to 70% seawater (SW) and the responsiveness of the shark kidney to exogenous ANP during chronic adaptation to this dilute environment.

METHODS: Acute dilution to 70% seawater: Male sharks were studied without anaesthesia or pithing. For urine collections, sharks were restrained ventral side up and a urinary catheter (PE 260) was inserted into the renal papilla and secured by occlusive ligature. Urine was collected every 30 minutes via aspiration. The dorsal aorta was catheterized twice with PE 90 tubing using a Touhy needle. The open end of one catheter was positioned proximal to the origin of the renal arteries for monitoring of mean arterial pressure (MAP) and injection of ANP. The second catheter was placed distal to the renal arteries for withdrawal of blood samples. Two to three clearance periods were obtained in a holding tank with running 100% SW. Tap water was then introduced into the holding tank at a rate previously determined to result in 70% SW at equilibrium (usually within 20 minutes). Collection periods were obtained every 30 minutes and continued without disturbing the animal for an additional 2 hours. A control group in which the external environment was not diluted was also studied.

Chronic dilution to 70% seawater: Sharks were transfered directly into 70% SW for 24 hours prior to study (n=6). A control group of similar size sharks caught at the same time and left in the 100% SW livecars was also studied (n=5). After 24 hours, sharks were transferred to the holding tank of similar salinity and prepared for urine collections as described above. The intraarterial administration of rat ANP III, 2 μg/kg, (Peninsula Labs) was preceded by 2–3 baseline clearance periods and followed by 4 clearance periods of 30 minutes each.

RESULTS: Acute dilution studies: Following acute dilution of the external environment to 70%, urine volume and chloride excretion increased during the first half–hour and continued to rise for the subsequent 2 hours (Figure 1a). No change in blood pressure was observed during this time and although serum chloride and osmolality indicated dilution of the extracellular space, the changes were not significant (Table 1).

Table 1: The effect of acute dilution from 100% to 70% seawater on renal chloride excretion and serum electrolytes in Squalus acanthias.

PARAMETER	100%SW	70%SW−1h	70%SW−2h	Δ CHANGE*	p
MAP (mmHg)	18.89±1.9	19.3±1.9	20.2±1.7	1.3±1.0	NS
Serum Osmolality(mosm/kg)	994±3	992±5	987±3	−7±3	NS
Serum Chloride (mmol/l)	262±5	261±3	258±3	−5±3	NS
Hematocrit (%)	20.1±3.0	19.1±1.8	17.5±1.4	−2.6±1.0	<.05
Chloride Excretion (μEq/min/kg)	0.91±0.40	1.69±0.55	2.35±0.79	1.44±0.40	<.02

* Comparison between 100%SW and 70%SW−2h by paired t test. MAP=mean arterial pressure

Chronic dilution studies: Animals adapted to 70% SW had a higher mean arterial pressure compared to 100%SW adapted fish. Plasma chloride and plasma osmolality were appropriately reduced after 24 hours. Urinary volume was increased nearly sixfold and urinary chloride excretion threefold compared to the 100%SW adapted fish (Table 2).

Table 2. Differences between sharks adapted to 100% SW and 70% SW for 24 hours.

GROUP (%SW)	Wt. (kg)	Hct (%)	MAP (mmHg)	Pchl. (mEq/l)	Posm (mosm/kg)	Uvol•	Uosm (mosm/kg)	Uchl. (meq/l)	Uchl.V•
100%	1.99 ±.11	17.5 ±2.8	13.0 ±1.9	278 ±10	984 ±8	3.9 ±2.3	866 ±24	318 ±21	1.14 ±.67
70%	2.01 ±.15	20.4 ±0.9	23.3* ±1.9	235* ±3	866* ±5	23.8* ±3.6	585* ±61	161* ±17	3.64* ±.43

Data are mean±SE. * p<.05 compared to 100%SW group. • units in μl/min/kg and μEq/min/kg respectively.

Following the intraarterial administration of ANP III, urinary chloride excretion and volume increased further in the sharks adapted to 70%SW but not in the 100%SW adapted sharks (Figure 1b). There were no significant effects of ANP on MAP in either group (data not shown). Urinary chloride concentration was also not affected by ANP III.

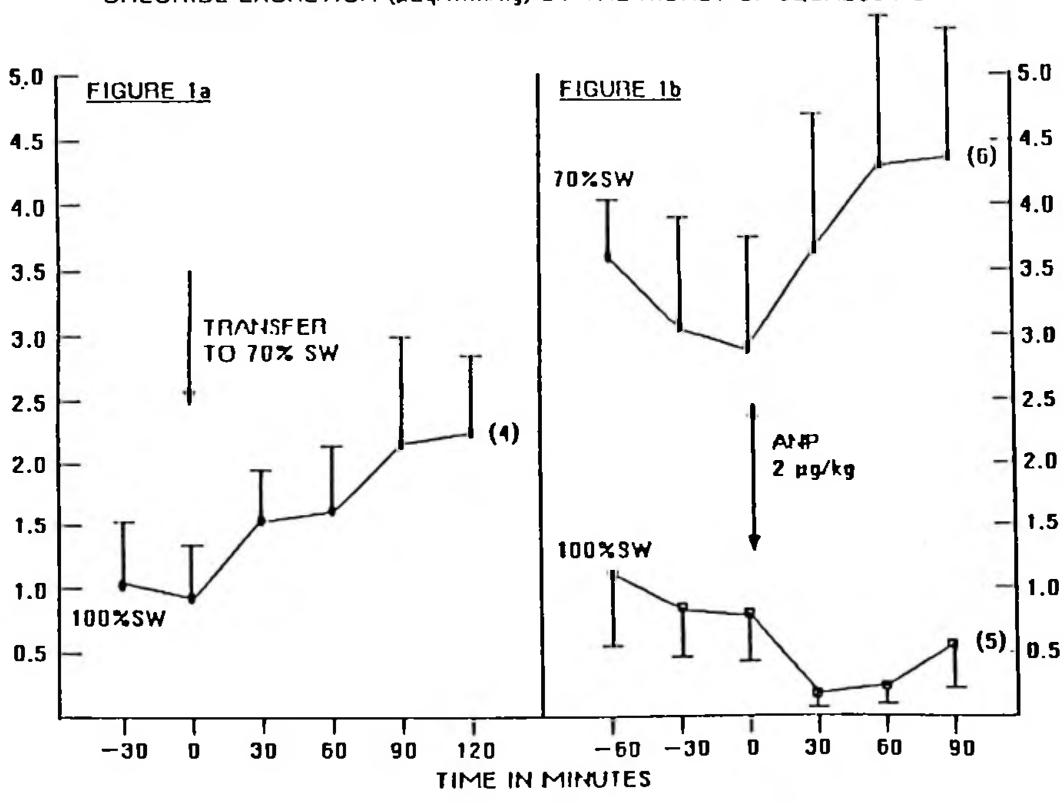
DISCUSSION: The 100%SW adapted elasmobranch has a higher urine flow rate compared to seawater adapted teleosts because of a continuous flux of water from the external environment to the extracellular space. This water movement results from the slight hypertonicity of the elasmobranch extracellular fluid compared to seawater. Sodium and chloride also enter the extracellular space moving down their respective concentration gradients. This inward flux of water and salt is excreted by the kidney and rectal gland in order to maintain extracellular volume and electrolyte balance. Transfer of the shark to a dilute external environment enhances the osmotic gradient for water movement while maintaining the electrolyte gradients and is reflected in an increase in both urine and rectal gland fluid and electrolyte outputs (Wong and Chan, J Exp Zool

200:85–96,1977). How the increase in water and electrolyte flux signals the kidney and rectal gland to increase their respective excretions is unknown. We have previously hypothesized that ANP may be one such mediator of this homeostatic response.

This hypothesis is supported by the finding of an immunoreactive endogenous ANP in the atria and ventricles of Squalus acanthias (unpublished data). A physiologic role for this ANP has been suggested by the findings that both the in vivo and in vitro perfused rectal gland increases chloride secretion in response to exogenous, rat ANP (Solomon et al, ibid). Thus ANP may be a mediator of the rectal gland response to volume expansion. However, we and others (Yokota and Benyajati, Bull MDIBL 26:87–90,1986) have not been able to demonstrate an effect of exogenous ANP on renal function in Squalus acanthias suggesting that the renal response to volume expansion was not mediated by ANP.

In the present experiments, acute dilution of the external environment led to an increase in urinary volume and chloride secretion commencing within 30 minutes. This increase was not accompanied by changes in systemic blood pressure or significant effects on serum osmolality or electrolyte composition. Although the change in renal function was significant, adaptation for 24 hours to a dilute environment was associated with even greater increases in urine volume and chloride excretion. In the 24 hour adapted animals, significant effects on systemic blood pressure, serum osmolality and chloride concentration were also noted. Most relevant to our hypothesis, the kidneys of these 24 hour adapted animals were now responsive to exogenous ANP.

CHLORIDE EXCRETION (µEq/min/kg) BY THE KIDNEY OF SQUALUS ACANTHIAS



While these observations must be considered preliminary, they are consistent with the formulation that ANP mediates volume and electrolyte balance in the elasmobranch. The lack of a renal effect of atriopeptin under conditions of 100%SW adaptation, reported by us previously, may have been a reflection of the high doses (10 μg/kg) used and the observed fall in systemic blood pressure. In the present study, a 2 μg/kg dose was used which was not associated with a fall in MAP in either the 100%SW or 70%SW adapted fish. It is also possible that, as is the case in mammals, a complex interplay of hormonal, neuronal, and hemodynamic factors regulates renal function. These factors may attenuate the renal response to ANP in the 100% SW environment.

Suppoorted by NSF#DCB8502826.