EFFECT OF ATRIAL NATRIURETIC PEPTIDE IN LOPHIUS AMERICANUS: A PRELIMINARY REPORT. R. Solomon, G. Solomon, A. Landsberg, and F. H. Epstein, New York Medical College and Harvard Medical School.

Atrial natriuretic peptide (ANP) is a hormone synthesized in cardiac myocytes. Target tissues for this hormone in mammals include the kidney, vascular smooth muscle, and the adrenal gland. In both in vivo and in vitro studies, ANP produces a marked natriuresis/chloruresis and diuresis. A direct effect of ANP to inhibit tubular sodium/chloride reabsorption has not been identified although indirect studies suggest such inhibition in the more distal segments of the nephron (Sonnenberg, H., Fed. Proc. 45:2106-2110, 1986). On the other hand, the acute administration of ANP is usually associated with transient hemodynamic effects which include an increase in whole organ and single nephron GFR, and an increase in total and medullary blood flow. These hemodynamic effects usually precede the natriuresis and diuresis and prevention of these hemodynamic effects significantly attenuates the subsequent natriuresis and diuresis (Gellai, M. et al, Fed. Proc. 45:2387-2391, 1986). Thus the precise mechanism for the renal excretory effects of ANP remains unclear.

We chose to study the effects of ANP in the goosefish, Lophius americanus, which lacks glomeruli and forms a urine solely via tubular secretion and reabsorption (Beyenbach, K., Renal Physiol 8:222-236, 1985). Such a model appears to represent a unique opportunity for evaluating the role of renal hemodynamic effects in mediating the response to ANP.

METHODS: Fish were obtained by nets from Frenchman Bay, ME and were studied usually within 1 to 3 days of capture. Fish were restrained without anesthesia on a board with their ventral side facing the surface of the water. PE 90 tubing was inserted into the dorsal aorta via a Tuohy needle and patency was maintained by systemic heparinization. This catheter was used for monitoring of dorsal aortic pressure and the injection of ANP. PE 260 tubing was placed in the bladder and a purse-string suture applied at the papillary orifice. Urine was collected via gravity drainage into a calibrated cylinder at 30 minute intervals. Three to five baseline collections were obtained and ANP was administered only after serial baseline collections did not show more than 20% variation in flow rate. Dorsal aortic pressure was recorded immediately prior to ANP administration and at 15 minute intervals thereafter for the first one hour.

Synthetic rat atriopeptin II (sANP) was given as a 10 ug bolus in 1 ml of dogfish Ringers. Urinary chloride was measured using a Buchler chloridometer. Cardiac extracts were prepared from other goosefish as previously described for Squalus acanthias (Solomon, R., et al, Am. J. Physiol. 249:R348-354, 1985) and stored in the refrigerator until use the following day.

RESULTS: Six goosefish weighing an average of 5.92±49 kg were studied. The effect of synthetic sANP on the renal excretion of chloride is shown in Figure 1. Chloride excretion varied between 50-70 uEq/min during baseline and was not increased after administration of ANP. In fact an acute fall

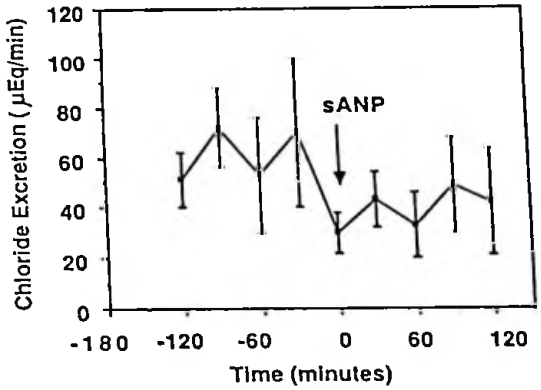

Figure 1. The effect of sANP on chloride excretion in the urine of the goosefish.

in chloride excretion followed by a gradual return toward baseline levels was observed. Urine flow rate paralleled the changes in chloride excretion. The effect of sANP on dorsal aortic pressure is shown in Figure 2. Mean pressure fell immediately following sANP administration and returned to baseline levels by 30 minutes.

Finally, the injection in one fish of an extract of Lophius americanus atria failed to elicit a diuretic or a chloruretic,response. Unfortunately the blood pressure response was not monitored in that animal.

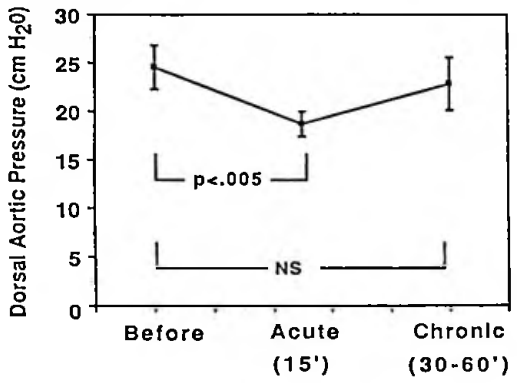

Figure 2. The effect of sANP on the dorsal aortic pressure of the goosefish.

DISCUSSION: Synthetic rat atriopeptin II failed to elicit a diuretic and/or chloruretic response in the aglomerular kidney of Lophius americanus. While it would be facile to attribute this negative effect to the absence of glomeruli and thus hemodynamic influences, a number of other factors must first be considered.

We tested only one dose of sANP, 10 ug, which, if distributed into total extracellular fluid, would result in a concentration of approximately 8 ng/ml. Plasma concentration in mammals is in the order of 10-400 pg/ml and thus in our experimental model, 20-1000 fold greater plasma levels would be predicted. Although species variation in receptor affinity and cross reactivity to the rat peptide are confounding variables, we nevertheless did observe a biologic effect on systemic blood pressure in this model. This hypotensive action was similar in magnitude to that observed in mammals and the elasmobranch (Solomon,R. Ibid). It might be argued that the fall in blood pressure might have reduced renal excretory function although there appears to be little evidence that perfusion pressure alters excretory function in aglomerular rish. The aglomerular kidney lacks a renal arterial blood supply and is perfused from a low pressure portal system (Beyenbach, K, Ibid). Finally a crude atrial extract from the goosefish also failed to stimulate renal excretory function, although similarly prepared extracts from Squalus acanthias do stimulate rectal gland chloride secretion in that species. (Solomon, R., Ibid).

It might be argued that the nephron of Lophius americanus lacks receptors for this peptide hormone. The nephron of this species lacks a loop of Henle or distal tubule segment (Beyenbach, K., Ibid). However, the glomerular kidney of Squalus acanthias also fails to respond to sANP despite both a hypotensive effect and a stimulatory effect on rectal gland chloride secretion (Solomon, R., Ibid). Until more species are examined, it is not possible to determine when in phylogenetic development the renal responsiveness to ANP appeared.

In addition to these theoretical concerns, there are methodological factors that must be considered as well. The experimental design did not incorporate a positive control. That is to say, no maneuvers were employed to confirm that the kidney could have responded with a diuresis and chloruresis. This is particularly relevant as Forster reported that Lophius americanus undergoes a "laboratory diuresis" when maintained in captivity

for long periods of time (Forster,R.P., J. Cell. Comp. Physiol. 42:487-509, 1953). Although we attempted to study animals soon after capture the average urine flow rate (55 ml/kg/day) and chloride concentrations (220 mEq/l) were similar to those found by Forster in "laboratory diuresis". Although maximum flow rates of 75 ml/kg/d were seen by Forster, the possibility exists that our animals were already undergoing a maximum diuresis and could not respond further to a diuretic stimulus.