vasodilation in the in vitro perfused rectal gland. For example, both VIP and adenosine reverse norepinephrine induced vasoconstriction in this model and the vasodilatory effect is not prevented by furosemide inhibition of secretion (Shuttleworth, et al, Bull. MDIBL 21:59-62, 1981). Thus, a direct vasodilatory effect of VIP and adenosine can be inferred from these observations.

However, on the basis of the experiments with somatostatin and theophylline infusion during volume expansion, only VIP appears to be a candidate as hormonal mediator of the secretory response. If VIP independently stimulates vasodilation and chloride secretion, the membrane receptors on smooth muscle and epithelial cells must respond differently to somatostatin as only the latter are inhibited by this agent. An alternative hypothesis is that another hormonal factor is responsible for the vasodilatory response.

DRINKING IN MARINE STENOHALINE FISH

David N. Shier, Debbie S. Beasley and Richard L. Malvin, Department of Physiology, University of Michigan, Ann Arbor, Michigan

Although a considerable amount of research has linked the renin-angiotensin system with drinking behavior in mammals, much less is known of this system in non-mammalian species. Previous work from this laboratory has provided evidence that endogenously produced angiotensin II is a stimulus for drinking in the euryhaline killifish, Fundulus heteroclitus (Malvin, R.L., et al, Am. J. Physiol. 239, 1980), but the role of angiotensin in drinking behavior has not been evaluated in marine stenohaline fish.

METHODS--The current study was performed using two marine stenohaline fish, the flounder, Pseudopleuronectes americanus, and the long-horned sculpin, Myoxocephalus octodecimspinosus. For each model, drinking in response to four interventions was compared to control drinking rates. These four interventions were: 1) injection of antiotensin II (13 ug/100 g body weight in .5 ml); 2) injection of the angiotensin I converting enzyme inhibitor Captopril (250 ug/100 g body weight in .25 ml); 3) hemorrhage (1% body weight by caudal vein puncture); 4) hemorrhage following injection of Captopril. In the flounder, one additional series of experiments examined the effect of hemorrhage in the presence of Saralasin, an angiotensin II competitive antagonist (20 ug/100 g body weight in .2 ml). In all cases the drugs were administered by a single intramuscular injection. Control fish received a similar volume of vehicle (.19 M NaCl) in the same manner.

On the morning of an experiment the fish were placed in tanks containing sea water at 12°-16°C. Temperature was adjusted to this range with ice bags but was to some degree dependant on ambient temperature. After injections were made, all fish were placed in tanks containing 3% polyethylene glycol (PEG) in sea water. After one hour the fish were removed, placed in PEG-free sea water and kept there for another fifteen minutes. Drinking rate during the hour in PEG was determined by killing each fish and flushing the gut contents into a test tube with 12 ml of tap water, combining a portion of this "wash" with an equal volume of 30% TCA, and comparing the absorbance of this mixture at 650 mu with that of PEG standards. Non-specific absorbance was determined by analyzing the gut contents of fish not exposed to PEG, and these values were used to calculate "blank"drinking rates which were averaged and subtracted from all other drinking rates. The fifteen minutes in PEG-free sea water prior to killing allowed ingested PEG to move sufficiently down the gut for adequate recovery in the wash. Separate experiments assured that the transit time through the GI tract for PEG was not exceeded in these studies. Data from experimental groups and control groups were compared using Student's t-test. The control drinking rates in flounder were higher during the last month of this study. For this reason, drinking rates were compared to controls from the same period in the flounder experiments.

RESULTS--Results are summarized in Figures 1 and 2. Control rates of drinking in both groups were less than

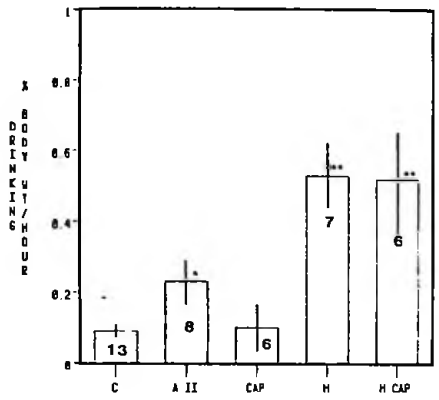

Figure 1.--Drinking rates for sculpin.
* $p < .02$; ** $p < .002$.

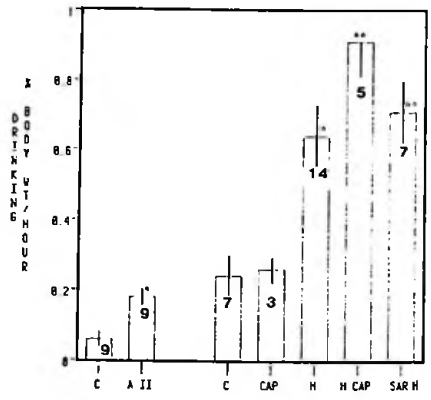

Figure 2.--Drinking rates for flounder.
* $p < .02$; ** $p < .002$.

that measured in Fundulus heteroclitus in this laboratory using the same method (Malvin, R.L., et al, Am. J. Physiol. 239, 1980). However, angiotensin II also stimulated drinking in both flounder and sculpin 2.5 - fold. Hemorrhage stimulated drinking in both groups approximately 6-fold. In spite of the fact that angiotensin II stimulated drinking, neither Captopril nor Saralasin attenuated the drinking response to hemorrhage, nor did Captopril alter resting drinking rates in either group.

DISCUSSION--Because angiotensin II appears to play a physiological role in the drinking behavior of the salt-water adapted killifish, we investigated whether angiotensin II is similarly involved in the drinking behavior of the flounder and long-horned sculpin. Drinking rates in salt-water adapted euryhaline killifish previously reported by this laboratory and by others (Potts, W.T.W. and D.H. Evans, Biol. Bull. 132, 1967) are on the order of 1% body weight/hour. In the present study, control drinking rates in the marine stenohaline flounder and long-horned sculpin were on the order of 0.1% body weight/hour. Thus, against similar osmotic stress, these stenohaline fish maintain volume homeostasis with an order of magnitude less drinking.

Exogenous angiotensin II significantly stimulated drinking in both groups of fish in the present study. To test the hypothesis that endogenous angiotensin II is a normal stimulus for drinking in these animals, Captopril, an inhibitor of angiotensin I converting enzyme, was administered. Since this would be expected to reduce the circulating levels of angiotensin II, any angiotensin II-dependant "thirst" drive should be attenuated, and the drinking rate should decrease. However, Captopril had no significant effect on drinking rate in either flounder or sculpin. However, drinking rates in these fish are relatively low, as described above, and angiotensin II may be an important dipsogenic factor only in times of volume depletion. We tested this hypothesis by creating a volume deplete state secondary to a hemorrhage of 1% body weight. This degree of hemorrhage was associated with a marked increase in drinking rate, and Captopril failed to attenuate this response in both groups of fish. In addition, Saralasin, a competitive antagonist of angiotensin II was equally ineffective at blunting the drinking response to hemorrhage in flounder.

Thus, our attempts to evaluate whether angiotensin II plays a physiological role in determining drinking behavior in marine stenohaline fish have generated negative results. Angiotensin II is dipsogenic in these animals, but blockers of the renin-angiotensin system fail to alter control drinking rates as well as the enhanced drinking rates induced by hemorrhage. One possible explanation is that the blocking agents used in this study are for some reason ineffective in flounder and sculpin. Another is that the route of administration resulted in a dose and distribution of the

blocking agents inadequate for inhibition of the renin-angiotensin system. However, angiotensin II administered the same way did increase drinking rates in both groups. A third possibility is that drinking behavior in these animals is under redundant control, and that other mechanisms drive "thirst" if the renin-angiotensin system is blocked. Furthermore, if peripheral levels of Captopril or Saralasin compromised the maintenance of blood pressure after hemorrhage, these other stimuli of drinking might well be enhanced. Indeed, drinking in flounder after hemorrhage plus Captopril was greater than after hemorrhage alone, although the difference was not significant ($p=.10$). Blood pressure was not measured in these experiments.

In conclusion, the marine stenohaline flounder and sculpin have control drinking rates one-tenth that of the salt-water adapted killifish. Exogenous angiotensin II stimulates drinking in the flounder and sculpin, but no evidence was obtained implicating endogenous angiotensin II in the physiological control of drinking behavior either in the basal state or following hemorrhage. This work was supported by NSF grant PCM 77-16465.

THE INFLUENCE OF Ca^{2+} AND CONTRACTION FREQUENCY ON TENSION DEVELOPMENT ON TELEOST VENTRICULAR STRIPS

H. Gesser and W.R. Driedzic, Department of Zoophysiology, University of Aarhus, Aarhus C., Denmark, Biology Department, Mount Allison University, Sackville, N.B.,Canada

In common with other vertebrate hearts, teleost ventricular strips develop increased tension as $[Ca^{2+}]_o$ is increased (Gesser and Jørgensen, J. Exp. Biol. 96:405-412, 1982). This may have direct relevance to the teleost heart since blood $[Ca^{2+}]_o$ is known to increase in association with intense activity (Ruben and Bennet, Nature 291: 411-413, 1981). In situations with enhanced circulatory requirements calcium stimulated ionotropy is likely to be favorable; however, an enlargement of the calcium pool also increases demand on the relaxation system. As such, a particularly prolonged relaxation time could compromise the maximal rate of contraction. Due to these considerations isolated heart strips were examined as to their ability to maintain force at different stimulation frequencies at low and high $[Ca^{2+}]_o$.

Experiments were conducted with 3 individuals each of mackerel (Scomber scombrus), ocean pout (Macrozoarces americanus), sea raven (Hemitripterus americanus), lumpfish (Cyclopterus lumpus), and longhorn sculpin (Myoxocephalus octodecimspinosus). The hearts were rapidly excised and placed in a solution gassed with 99% O_2: 1% CO_2 and containing NaCl 150 mM, KCl 5.0 mM, $MgSO_4$ 2.0 mM, NaH_2PO_4 0.5 mM, $NaHCO_3$ 11mM, and $CaCl_2$ 1 mM. Strips of approximately 1 mm diameter were cut from the ventricle, mounted for isometric recording of force at 15°C (Gesser, J. Exp. Biol. 69:199-206, 1977), electrically stimulated at 12 min^{-1} and stretched until peak force did not increase any further. After stabilization of force development the stimulation rate was increased by steps of 12 min^{-1} to the rate of 108^{-1} or to the rate at which arrythmic responses were observed. Each rate was maintained for about 30 sec. After this, the rate of 12 min^{-1} was reintroduced and the heart strips after stabilization were subjected to increased levels of Ca^{2+} from 1 to 7 or 9 mM. At the upper level of $[Ca^{2+}]_o$ the protocol with increasing stimulation rates was again carried out.

For all of the hearts examined increases in $[Ca^{2+}]_o$ resulted in an increased twitch force. The largest response was observed with mackerel in which a change in $[Ca^{2+}]_o$ from 1 to 9 mM resulted in a 270% increase in peak tension development. Sculpin, ocean pout and lumpfish were far less responsive in that similar alterations in $[Ca^{2+}]_o$ increased peak force development by only about 50%.

The maximum rate at which strips could contract regularly in response to imposed electrical stimulation varied amongst species. Mackerel, lumpfish, and sea raven could sustain the highest rate tested which was 108 contractions per min, whereas ocean pout and sculpin achieved only 96 and 60 contractions per min, respectively. Increasing the level of $[Ca^{2+}]_o$ from 1 to 7 or 9 mM did not influence this parameter.