The dogfish pressor response to intravascular infusion of AII is cruious in light in the inability of AII to induce in vivo or in vitro constriction of dogfish vascular smooth muscle. Pharmacological evidence indicates that alpha-adrenergic receptors mediate the response to AII, possibly due to the release of stored catecholamines. To test the ability of AII to cause the direct release of vasoconstrictor agents from dogfish chromaffin tissue, the muscle bath procedure was modified. Islets of chromaffin tissue were isolated from dogfish kidney, blotted dry, and added to the muscle bath after the artery had reached its stress-relaxation point. After a steady state had been reached, 1 ml of saline was added, followed 3 minutes later by the addition of 20 μg [Val^5] AII in 1 ml. The responses to saline and AII were compared 60 seconds after exposure to each by a paired t-test, alpha= .01. Addition of [Val^5] AII to the chromaffin-artery muscle bath significantly raised the force of contraction from 2.9$\pm$3.0 mg to 40.6 $\pm$ 11.3 mg. This demonstrates that AII is capable of acting directly on the chromaffin tissue of the dogfish to liberate a vasoconstrictor substance. (see Fig. 2)

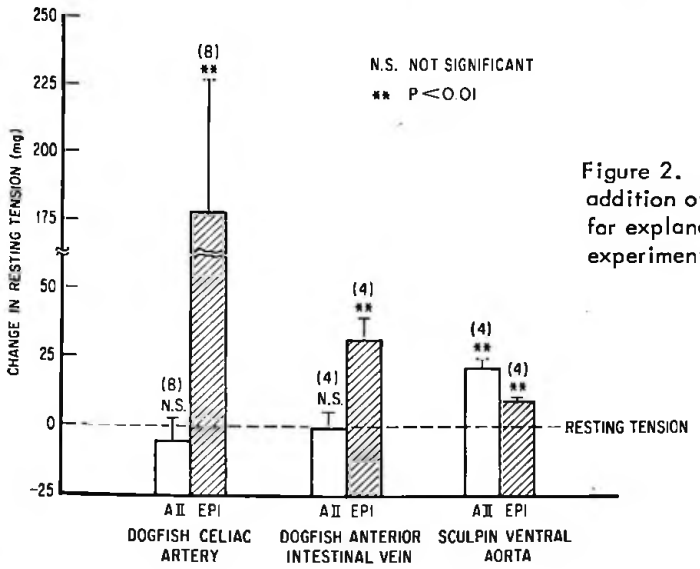

Figure 2. Reactivity of the dogfish celiac artery following addition of chromaffin tissue to the muscle bath (see text for explanation). Values shown are mean $\pm$ SEM for 4 experiments.

This study shows that AII has both a direct and an indirect mechanism for exerting a pressor effect. In the elasmobranch dogfish shark, AII demonstrated neither in vivo nor in vitro direct vascular activity. Additional evidence for an indirect vascular effect, one in which the octapeptide acts directly on chromaffin tissue to cause the release of vasoactive catecholmines, has been shown. AII has a direct vasoconstrictor effect both in vitro and in vivo in the longhorn sculpin. The possible contribution of catecholamine release to a pressor response in the sculpin has not been examined, but some is to be suspected in light of a similar contribution of catecholamines to the pressor response to AII in the teleost freshwater eel (Nishimura et al, Am. J. Physiol. 235:H95-H103, 1978).

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ARTERIO-VENOUS ANASTOMOSES IN THE DOGFISH GILL

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The vascular organization of the dogfish gill was described in the Bulletin last year (Olson, et al, Bull MDIBL (18) 112-116, 1979). Corrosion casts of the gill circulation were made and were studied with the scanning electron microscope. Two circulations were described. The first was the usual respiratory pathway involving blood flow through the lamellae. The second was an extensive collateral circulation which connected to the interlamellar vessels, interdigitated with the lamellar circulation, supplied vessels to areas beneath the water channels between filaments

and drained into the venous side of the circulation. Recent work (Boland and Olson, Cell Tissue Res. 198, 487-500, 1979) has shown that a similar intralamellar circulation in teleost gills is supplied by small vessels which branch from the prelamellar afferent circulation. The present study was carried out to determine the origin of the collateral circulation in the dogfish gill.

A methyl methacrylate resin and catalyst (Mercox) were used to make vascular casts of the gills as described earlier (Bull MDIBL (18), 112-116, 1978). Four anesthetized (20 mg/Kg pentobarbital) heparinized (1000 u) fish were used. Mercox was pumpsed into either the ventral aorta or the dorsal aorta at pressures close to 25 mm Hg. After digestion of the tissue with 20% NaOH the specimens were examined with a Cambridge Stereoscan 600 scanning electron microscope at the Notre Dame University Biology Department.

Small vessels from two different origins can be found joining the sinus-like interlamellar vessels. The first (Fig. 1) are vessels arising from the medial afferent sinus. When the collateral circulation is removed to expose the afferent medial sinus beyond the point of departure of the afferent lamellar arterioles, small vessels are exposed which run from the medial afferent sinus along the width of the filament to anastomose with the interlamellar vessels. These vessels are relatively straight, occur at the same frequency as the pre-lamellar arterioles and generally anastomose with an interlamellar vessel underlying the lamella supplied by the nearest prelamellar arteriole. The anastomoses are often at sharp angles with a stricture of less than 10 μ in diameter proximal to the actual junction.

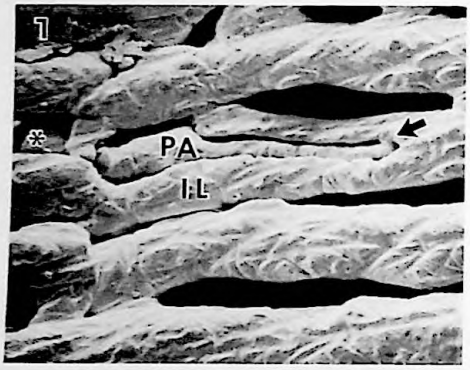

Figure 1. Prelamellar arteriovenous anastomosis (PA) between medial afferent sinus (*) and interlamellar vessels (IL). Note the right angle formed at the PA-IL junction (arrow). (270 X)

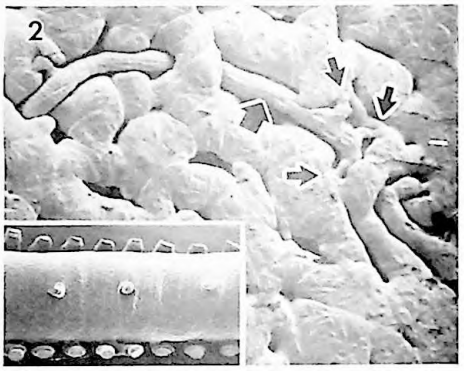

Figure 2. Postlamellar anastomoses (small arrows) between a nutrient artery (large arrow) and the interlamellar vessels (150X). (Insert, the origin of three nutrient arteries from the medial border of the efferent filamental arteries. Other vessels are the ends of the efferent lamellar arterioles, (70 X).

The efferent filamental artery gives rise to a second series of small vessels which join the interlamellar vessels (Fig. 2). Midway between every 3rd or 4th pair of efferent lamellar arterioles (Fig. 2 insert) a small vessel is seen leaving the efferent filamental artery and entering the adjacent interlamellar area where it joins an interlamellar vessel.

The vessels arising from the afferent medial sinus serve as connective channels between the arterial afferent circulation and the venous interlamellar circulation. These are arterio-venous anastomoses and their location makes them analogous to the AVA's found in teleost gills. The presence of these AVA's has important implications in the balancing of the osmoregulatory and respiratory functions of the dogfish gill. For instance, it is well known that resistance to blood flow in the gill increases when the dogfish is exposed to a hypoxic environment. It may be that under hypoxic conditions blood is shunted away from respiratory surfaces where unfavorable ion movement might take place with little compensation in oxygen uptake. An increase in resistance in the respiratory pathway would lead to an increased perfusion of the interlamellar system via the prelamellar AVA's. The diameter of the AVA's is small enough to impede the passage of red cells into the

interlamellar system. Indeed, when India ink is injected into the circulation of the dogfish, the collateral circulation at the tip of the filament and under the water channels is seen to turn gray while the lamellae remain red. This indicates a separation of red cells from plasma as the collateral circulation is filled. The collateral circulation supplies areas rich in chloride cells before returning to the venous system. During hypoxic vasoconstriction then, perhaps the respiratory function of the gill is attenuated without impairing the osmoregulatory function. An alternate mechanism may be suggested for control of flow in the collateral and respiratory circulations. Since the control of the gill flow resistance rise in hypoxia has been shown to be neural and not local (Kent & Peirce, Comp. Biochem. Physiol. 60C, 37-44, 1978), the nerves running in the interlamelar space may control the amount of constriction of the AVA. Dilated AVA's would tend to fill the interlamellar vessels and might even restrict flow through the prelamellar arteriole by a sluice effect at the point where the two circulations interdigitate. Constriction of the AVA's would reverse those circumstances and enhance lamellar flow.

The small vessels from the efferent filamental artery deliver oxygenated blood to the interlamellar space and vessels and are nutritive in nature. Because the direction of blood flow from the small efferent nutritive vessels is from efferent to afferent across the width of the filament, there may be opportunity for counter current exchange with blood running the opposite direction in the inner marginal channel of the adjacent lamella.

The presence of AVA's in dogfish gill vasculature opens many new possibilities of an important role of blood flow control in the regulation of respiration and osmotic equilibrium. This work was supported by Research Project #4901-01 and 02, Veterans Administration Medical Center, Bronx, New York and by NSF grant PCM 76-16840.

RENAL HANDLING OF PEPTIDE ANTIFREEZE IN NORTHERN FISHES

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Seasonal studies of freezing avoidance of marine coastal fishes from New England have revealed the presence of blood peptides with antifreeze properties (Duman and DeVries, Comp. Biochem. Physiol. 52A: 193-199,1975, Fletcher, Can. J. Zool. 55: 789-795, 1977, Petzel and DeVries, J. Exp. Zool (in press)). These antifreeze compounds lower the freezing point of the body fluids without significantly affecting the osmolarity. This non-colligative lowering of the freezing point results from adsorption of the antifreeze to the ice which then inhibits crystal growth (Raymond and DeVries, Proc. Natl. Acad. Sci. 74: 2589-2993, 1977). The peptide antifreezes have been isolated from winter flounder, Pseudopleuronectes americanus (Duman and DeVries, Comp. Biochem. Physiol. 54B: 375-379, 1976) and the shorthorn sculpin, Myoxocephalus scorpius. Their average molecular weight is 5,000 daltons and they make up two to three percent of the blood and other body fluids. However they are not found in the urine although these fishes have been shown to freely filter the glomerular markers inulin and polyethylene glycol which are similar in size. We have attempted to compare the clearance of the glomerular marker polyethylene glycol and tritium labeled peptide antifreeze in an attempt to explain why the antifreeze molecules are not found in the urine.

Materials and Methods

The fishes were otter trawled from waters south of Mt. Desert Island, Maine at a depth of 60 meters. One week after being kept in a continuously circulating open sea water system, the fish were bled for serum chemistry analysis. Serum ions, melting and freezing points were determined as described by Duman and DeVries (Comp. Biochem. Physiol. 52A: 193-199, 1975).

Renal clearance studies were conducted in unanesthetized, free-swimming fish maintained in 80 liter aquarium at ambient sea water temperatures (13 to 15°C). A PE 10 caudal vessel cannule and urinary bladder catheter were sutured in place under MS 222 anethesia. Urine was collected continuously by securing the free end of the catheter in a collection vial placed 5 cm below the level of the fish. Blood was periodically sampled from the caudal vessel cannulae or in the case of the flounder directly from the caudal vessel via a 30 guage needle. Glomerular filtration markers including ^{14}C-inulin (5,000 daltons) and ^{14}C-polyethylene glycol (PEG, 4,000 daltons) were purchased from New England Nuclear.