injections are significant ($p < 0.05$) in all cases. However, the second injection of AII resulted in significantly smaller increases in the concentrations of norepinephrine, epinephrine and dopamine than were observed after the first AII injection, ($\alpha = .05$) indicating an incomplete restoration of catecholamine stores.

The control plasma norepinephrine/epinephrine ratio (NE/E) is 1.72:1, which is similar to the ratios observed in cats, dogs, rabbits and man (von Euler, Hormones in Blood, Vol. II, Little, Brown and Co., Boston, 1957). The NE/E ratio after stimulation with AII was 1.35:1, which, due to the large standard deviation, may not be significantly different from the control NE/E ratio. The NE/E ratio in dogfish chromaffin tissue has been reported to be 2.7:1 (Shepherd et al, Nature, 172: 509, 1953), which is in contrast to the NE/E ratio of 1:1, or less, found in most mammalian adrenal glands (von Euler, Comparative Endocrinol. Vol. I., U.S. von Euler and H. Heller, Editors. Little, Brown and Co. Boston, 1963).

Serial blood sampling after AII injection in 2 fish revealed that plasma catecholamine concentrations continued to rise after the onset of the pressor response. In both fish the highest concentration of catecholamines were measured 3 minutes after injection of AII. In 7 fish blood samples were taken only at 1-1 1/2 minutes after injection. The concentrations of catecholamines found in these samples are probably not the highest achieved during the response. Even so, the average increase in plasma concentration of either epinephrine or norepinephrine measured after AII injection is sufficient to cause a significant pressor response in dogfish as determined by reference to norepinephrine or epinephrine dogfish dose-response curves (Carroll, unpublished).

These results, together with our failure to demonstrate a direct vasoconstrictor effect of AII on vascular resistance vessesl (Opdyke et al, Bulletin, MDIBL, 17:31-33, 1977), greatly strengthens our hypothesis that the pressor response to angiotensin in the dogfish is catecholamine mediated.

This research was supported in part by a grant from the Monmouth County Chapter of the New Jersey Affiliate, American Heart Association.

THE VASCULAR RESPONSE OF THE DOGFISH AND SCULPIN TO ANGIOTENSIN II

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Although extensively studied in mammals, the physiological functions of the Renin-Angiotensin System (RAS) in lower vertebrates remains obscure. Anatomical studies indicate that the RAS appeared early in evolution, renin-containing granules having been identified in several species of bony fishes (Nishimura et al, Am. J. Physiol. 224:950-956, 1973). Vascular activity of the octapeptide Angiotensin II (AII), however, appears to antedate the native capacity for its synthesis as a component of the RAS, as intravascular infusion of AII elicits a pressor response from the dogfish shark (Opdyke and Holcombe, Am. J. Physiol. 231:1750-1753, 1976). This observation led to an *in vivo* and *in vitro* examination of AII's vascular role in the elasmobranch spiny dogfish shark *Squalus acanthias* and the teleost longhorn sculpin *Myoxocephalus octodecimspinosus*.

Direct observation of the microcirculation provided *in vivo* information on the vascular activity of AII. Animals were anesthetized with a 25 mg/kg urethane- 25 mg/kg sodium pentabarbital solution administered through a dorsal aortic catheter (3 dogfish) or by placing them in 0.25 g/liter Tricaine Methanesulphonate (MS-222) in seawater (4 sculpins). The cervical spinal cord of one unanesthetized fish of each species was severed to serve as a control for the effects of anesthesia. After placing the exposed intestine and its mesentery under a Zeiss surgical microscope (25 x magnification), the field was bathed in a continuous drip (20 drops/min.) of the appropriate saline solution. Drugs were applied topically through a Pasteur pipette for 1 minute. Following topical application of a drug, the saline drip was resumed to wash the field. Statistical significance was determined by Fisher's exact probability test, alpha = .01.

Although 5 applications of Epinephrine (Epi, 25 μg/ml) consistently slowed or stopped blood flow, 5 applications of teleost AII ([Asn^{1}Val^5] AII, 25 μg/ml) failed to elicit any change in the dogfish microcirculatory blood flow. The sculpin, however, showed consistent, reversible decreases in blood flow following 5 applications of teleost AII (10 μg/ml). No difference in response was noted between the anesthetized and cervically transected fish. Topical treatment with the alpha-adrenergic blocking agent phentalamine (0.5 mg) blocked the vasomotor response to Epi, but did not attenuate the response to teleost AII. [Sar^{1}Thr^8] AII (100 μg/ml) and [Ile^8] AII (50 μg/ml), both of which are competitive inhibitors of AII in mammals, failed to attenuate the response to teleost AII. The results from the microcirculation studies are summarized in Table 1.

TABLE 1

Microcirculatory Response

	Dogfish	Sculpin	Sculpin + alpha-blockade
Epinephrine	+	+	0
Teleost AII	0	+	+

Measurement of the force of contraction of an isolated blood vessel provides a more quantitative in vitro determination of AII vascular action. Following transection of the cervical spinal cord, the dogfish celiac artery and anterior intestinal vein, and the sculpin ventral aorta were removed, and placed in the appropriate saline solutions oxygenated with 95% O_2/ 5% CO_2. Each vessel was cut helically and suspended from a Statham strain gauge transducer used in conjunction with an IR-4 Electronics-for-Medicine amplifier and recorder. The muscle was then placed in a 30 ml saline bath, maintained at 16°C by the flow of cold seawater through a jacket. Dogfish blood vessels were allowed to equilibrate for a minimum of 10 minutes at the stress-relaxation point, resting tension > 2 g. The sculpin aorta was used after it had reached a steady-state, rather than at its stress-relaxation point. All drugs were added to the muscle bath in a volume of 1 ml, so the final concentrations was 1/31 of the concentration of the drug added. Changes in resting tension 60 seconds following the addition of the drug were compared to the control period 60 seconds preceding drug addition by a paired t-test, alpha = .01.

Epi (10 μg) caused significant increase in resting tension of both the dogfish celiac artery (178 ± 48.7 mg; mean ± SEM) and the anterior intestinal vein (31.6 ± 9.0 mg). Teleost AII (25 μg) did not significantly alter the resting tension of the dogfish artery (-5.6 ± 8.2 mg) or veing (-0.5 ± 6.0 mg), indicating that Teleost AII is unable to induce vascular smooth muscle contraction directly in this elasmobranch. The sculpin aorta, however, contracted significantly in response to both 25 μg teleost AII (21.1 ± 3.7 mg) and 25 μg Epi (9.2 ± 1.3 mg), indicating that teleost AII is capable of causing vascular smooth muscle contraction directly in this saltwater teleost. The results are summarized in the figure below.

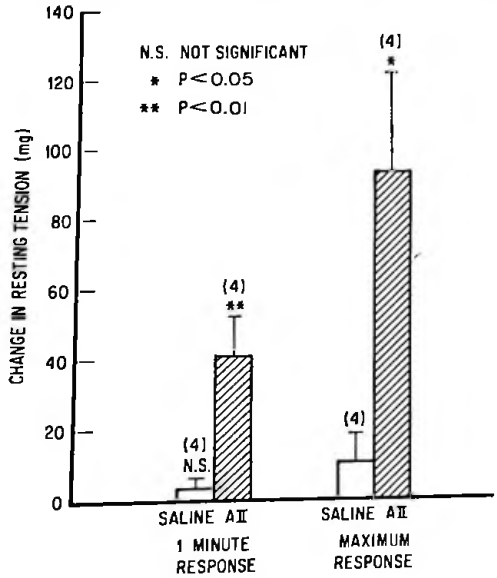

Figure 1. Reactivity of helically cut blood vessels to epinephrine and teleost AII. Values shown are mean ± SEM, and the number of trials shown above each bar. Resting tension at a pre-load of > 2 gm taken as the zero point.

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)

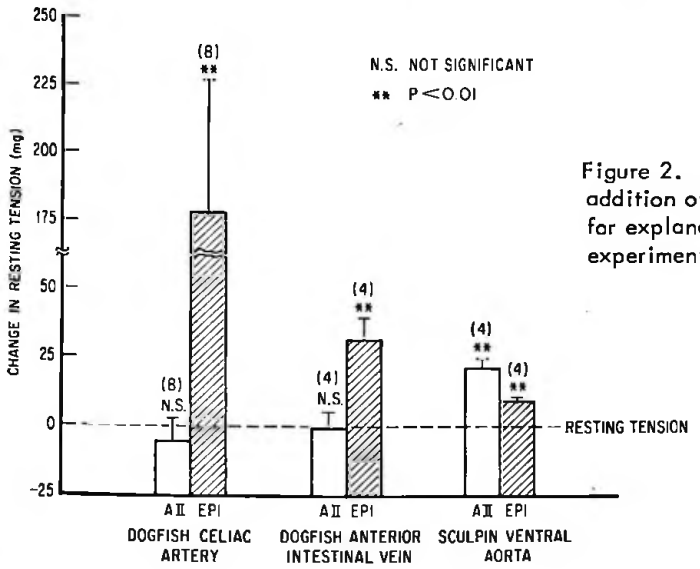

Figure 2. Reactivity of the dogfish celiac artery following addition of chromaffin tissue to the muscle bath (see text for explanation). Values shown are mean $\pm$ SEM for 4 experiments.

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).

This research was supported by grants from the Monmouth County Chapter, New Jersey Affiliate, American Heart Association and the CMDNJ-Graduate School Biomedical Research Fund.

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