An interesting calculation can be made from these data. The free pericardial cavity volume is the physical factor limiting maximum ventricular stroke volume. The product of free pericardial cavity volume and the maximum heart rate characteristic of intact fish, or the maximum heart rate observed in the isolated heart preparations (about 34 beats/min in either case) gives a theoretical maximal achievable cardiac output in a dogfish, assuming that the heart fills to the limit imposed by the free pericardial cavity volume and empties completely at each stroke. Using our data, this value would be about 440 ml/min, or about 2.9 times the calculated resting cardiac output derived by use of Murdaugh's cardiac index. This can be compared to a factor of about 6x in man or dog.

The discrepancy between actual cardiac output from isolated heart preparations and the output of hearts in intact fish raises a serious question about the viability and performance of *all* isolated heart preparations. The preparation described here should be superior to traditional isolated heart preparations of the Langendorff type because the hearts are not subjected to physical trauma, since they operate in the unopened pericardial cavity. However, it is evident that essential components of cardioregulatory function must have been removed. These could include neural, hormonal and chemical stimulators of cardiac activity, critical nutrients and an imbalance of respiratory gases, with attendant acid-base disturbances. The preparation offers an excellent opportunity to study the factors responsible for maintaining normal cardiac function. The future challenge is to make this isolated preparation perform on a par with the heart of an intact dogfish. Supported by USPHS Grant No. 18868.

EXPERIMENTS WITH ANGIOTENSIN ANTAGONISTS IN DOGFISH

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The curious circumstance that the dogfish, *Squalus acanthias*, exhibits a pressor response to a variety of natural and synthetic angiotensins I and II, even though this species is not known to make either renin or angiotensin, caused us to examine the action of some potent angiotensin antagonists. The situation is even more interesting because the pressor response to AII in dogfish is apparently mediated only by release of adrenergic catecholamines. There is no evidence for the presence of vascular angiotensin receptors in dogfish which directly cause vasoconstriction. Therefore, any antagonist activity exhibited in dogfish would involve the mechanism for catecholamine release. Three synthetic angiotensins that inhibit the pressor response to [Asn^1-Val^5] AII (teleost AII) in mammals were tested in dogfish. These were [Sar^1-Thr^8] AII, [Sar^1-Ile^8] AII and [Ile^8] AI. All were synthesized by the Research Division, Cleveland Clinic Foundation.

The dorsal aortas of dogfish were catheterized via the caudal artery. The fish were then lightly anesthetized with 10 mg/kg sodium pentobarbital and the gills bathed in running seawater through tubes placed in each spiracle. Another catheter was placed in the lateral abdominal vein under local anesthesia (procaine) and advanced to the posterior cardinal sinus. Dorsal aortic pressure and heart rate were monitored via the dorsal aortic catheter. Infusions of the antagonists were given through the lateral abdominal vein, but challenge doses of AII were injected through the dorsal aortic catheter. The test procedure was as follows: Challenge doses of AII were given at 3 dose levels, 1.5, 3.0 and 6.0 µg/kg at 10 min intervals and in random order before beginning the infusion of an antagonist. These dose levels cover the range of doses producing submaximal to maximal pressor responses. The pressor response is brief (1-2 min). The dogfish does not exhibit tachphylaxis to AII. Infusion of one of the antagonists was commenced at a rate of 2 µg/kg/min ten min after the last challenge dose of AII. Five min after beginning the antagonist infusion the sequence of challenge doses of AII was repeated at 10 min intervals. The responses were recorded continuously during each trial.

Bolus injections of the antagonists were given at higher dose levels (40-80 µg, total dose) in another series of experiments on unanesthetized and unrestrained dogfish in order to better characterize the agonist properties of these polypeptides.

None of the three polypeptides tested exhibited agonist pressor activity during or after the first 5 min of infusion (compare controls, Table 1). Neither [Sar^1-Thr^8] AII or [Ile^8] AI showed any antagonistic activity to the pressor response elicited by challenge doses of AII. There was no significant inhibition of the pressor response by these antagonists at any level of challenge.

TABLE I

EFFECT OF ANGIOTENSIN ANTAGONISTS ON DORSAL AORTIC PRESSURE RESPONSE TO [Asn^1-Val^5] AII IN SQUALUS ACANTHIAS

	RESPONSE TO 3 DOSE LEVELS OF Asn^1 Val^5 AII BEFORE AND DURING INFUSION OF ANTAGONISTS							
			BEFORE INFUSION			DURING INFUSION		
	AII DOSE µg/Kg	DORSAL AORTIC PRESS.	CONTROL TORR (±1SE)	RESPONSE TORR	AII DOSE µg/Kg	DORSAL AORTIC PRESS	CONTROL TORR*	RESPONSE TORR
[Sar^1 Thr^8] AII 2.0 µg/Kg/min. n = 5	1.5	SYST.	29.9 ± 5.6	39.0 ± 7.4	1.5	SYST.	29.7 ± 3.6	40.9 ± 7.4
		DIAST.	27.0 ± 2.4	31.8 ± 4.3		DIAST.	24.3 ± 2.4	31.1 ± 3.6
	3.0	SYST.	30.4 ± 8.3	40.3 ± 12.2	3.0	SYST.	29.7 ± 5.1	42.2 ± 7.4
		DIAST.	25.5 ± 4.1	32.3 ± 5.4		DIAST.	24.5 ± 2.9	32.5 ± 2.6
	6.0	SYST.	27.9 ± 3.8	44.6 ± 3.4	6.0	SYST.	30.0 ± 5.9	45.6 ± 6.7
		DIAST.	24.5 ± 2.6	35.3 ± 2.2		DIAST.	24.4 ± 3.2	34.5 ± 1.2
[Sar^1 Ile^8] AII 2.0 µg/Kg/min. n = 5	1.5	SYST.	25.5 ± 5.5	33.4 ± 6.9	1.5	SYST.	25.8 ± 5.2	29.9 ± 7.0
		DIAST.	22.7 ± 5.7	28.8 ± 6.6		DIAST.	21.4 ± 5.6	26.5 ± 6.4
	3.0	SYST.	26.2 ± 5.0	38.0 ± 4.3	3.0	SYST.	25.9 ± 6.7	34.4 ± 7.7
		DIAST.	21.2 ± 2.6	30.1 ± 6.6		DIAST.	21.9 ± 6.2	27.6 ± 7.1
	6.0	SYST.	25.1 ± 5.6	37.1 ± 6.5	6.0	SYST.	27.8 ± 7.1	35.3 ± 11.3
		DIAST.	22.5 ± 5.5	31.1 ± 6.8		DIAST.	23.2 ± 6.4	27.3 ± 8.7
[Ile^8] AI 2.0 µg/Kg/min. n = 5	1.5	SYST.	28.8 ± 6.6	40.1 ± 10.6	1.5	SYST.	26.9 ± 4.0	37.7 ± 7.1
		DIAST.	23.2 ± 4.4	32.4 ± 7.3		DIAST.	22.0 ± 3.2	29.7 ± 4.2
	3.0	SYST.	27.6 ± 6.5	38.8 ± 6.3	3.0	SYST.	26.4 ± 6.7	38.9 ± 7.0
		DIAST.	23.3 ± 5.4	30.6 ± 3.8		DIAST.	21.2 ± 4.8	30.6 ± 3.4
	6.0	SYST.	28.0 ± 6.4	45.6 ± 8.6	6.0	SYST.	23.9 ± 4.7	41.3 ± 7.3
		DIAST.	23.4 ± 4.7	35.2 ± 4.8		DIAST.	19.7 ± 3.5	31.6 ± 2.5

* after 5 min. infusion

On the other hand, [Sar -Ile] AII gave a highly significant inhibition providing the individual responses at all 3 dose levels are pooled (n = 15). The pooled data show an average reduction of systolic dorsal aortic pressure from 142.9 ± 19.0% to 124.9 ± 11.7% ($p < 0.005$) and a reduction in diastolic pressure from 137.1 ± 19.1% to 119.5 ± 9.2% ($p < 0.005$). Antagonist activity was significant at the 1.5 and 6.0 µg/kg challenge level with this antagonist, but fell just short of significance ($\alpha = 0.05$) at the 3.0 µg/kg dose.

No significant changes in heart rate were observed either in response to the infusion of antagonists or to the challenge doses of teleost AII.

Bolus injections of the 3 antagonists were made in a separate series of experiments in order to test for agonist pressor activity more rigorously. Only [Sar^1-Ile^8] AII caused a significant pressor response (see Table 2). No changes in heart rate were observed in any of the trials.

The octapeptide [Ile^8] AII was made available and also tested in the bolus injection series. In contrast to [Ile^8] AI, this octapeptide caused a highly significant pressor response at a relatively low dose as shown in Table 2. The result is of considerable interest because it indicates that the dogfish does not possess an enzyme which will convert tht [Ile^8] AI decapeptide to the corresponding octapeptide. It is known that dogfish are capable of converting naturally occurring decapeptide angiotensins (snake, fowl, teleost and equine) to the active AII (octapeptide) form. [Ile^8] AI has been shown to be a potent inhibitor of catecholamine release in mammals (Ackerly et al., European J. Pharm. 42:391-401, 1977). However, it does not appear to have such activity in dogfish, in fact, [Sar^1-Ile^8] AII appears to be a better antagonist than [Ile^8] AI. These results suggest that either the mechanism

TABLE 2

Response of Dogfish Dorsal Aortic Pressure to Bolus Injections of Three Angiotensin II Antagonists and [Ile^8] AII

TREATMENT	DORSAL AORTIC PRESSURE*			
	Control		Response	
	Systolic	Diastolic	Systolic	Diastolic
[Sar^1-Ile^8] AII				
40 µg**	27.7 ± 4.4	21.7 ± 3.8	35.5 ± 3.7	27.5 ± 2.1
n = 4			(p < 0.025)	(p < 0.01)
[Sar^1-Thr^6] AII				
40 µg**	27.2 ± 4.4	21.4 ± 5.1	28.2 ± 5.4	22.4 ± 5.0
n = 5			(N.S.)	(N.S.)
[Ile^8] AI				
40 µg**	26.2 ± 4.7	20.4 ± 4.3	27.6 ± 4.7	21.3 ± 4.8
n = 7			(N.S.)	(N.S.)
[Ile^8] AII				
20 µg***	26.5 ± 5.0	18.7 ± 4.2	35.0 ± 7.0	22.2 ± 5.0
n = 4			(p < 0.005)	(p < 0.05)

* Pressure in Torr ± 1 SE; ** Larger fish (6-8 kg) received 80 µg;
*** Larger fish received 40 µg

for catecholamine release in dogfish is different from that of mammals, or, that dogfish have a unique complement of adrenergic catecholamines and/or adrenergic catecholamine receptors. Supported by Grant No. 18868, National Institutes of Health.

GLUCOSE OXIDATION: THE RESPONSE TO DIAMIDE IN THE SCULPIN AND DOGFISH CORNEA AND LENS

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Corneal transparency in vertebrates is preserved by the expenditure of metabolic energy. This energy is utilized in keeping the hydrophilic stroma at a minimum of physiological hydration. Swelling of this transparent tissue results in opacification. It has previously been shown that the limiting layers of the cornea (epithelium and endothelium) are sites of active ion transport, effecting the osmotic movement of water out of the hydrophilic stroma. In aquatic animals the environmental osmotic stress further complicates the maintenance of corneal transparency, and various anatomical adaptations have evolved to prevent hydration. In cyclostomes a primary spectacle containing sutural fibers protects the cornea (Van Horn et al., J. Ultrastruct. Res. 26:454, 1969). In sharks a thick epithelium is present and the stroma is nonswelling as a result of sutural fibers (Smelser, Invest. Ophth. 1:1, 1962; and Goldman and Benedick, Invest. Ophth. 6:574, 1968). These sutural fibers are lost in the teleosts. The corneas of marine species have been shown to be divided into an inner and outer layer capable of becoming hydrated (Fischer and Zadunaisky, Exp. Eye Res. 25:149, 1977). By comparison in fresh water teleosts the cornea is completely fused and possesses a thick epithelium which essentially makes it impermeable (Edelhauser et al., Invest. Ophth. 4:290, 1965). Regardless of structural adaptations that have evolved, the corneas of all species contain an epithelium, endothelium and stroma which depend upon metabolic energy for the maintenance of their pump function, cell division, collagen secretion and ultimately corneal transparency. We have previously reported the QO_2 and Q_{10} values of marine fish corneas (Bull. MDIBL 17:6, 10, 1977) and have recently measured the hexose monophosphate shunt (HMS) in the component layers of the rabbit cornea in the normal and after diamide treatment