# ANGIOTENSIN II RELEASES CATECHOLAMINES IN DOGFISH

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Dogfish (Squalus acanthias) exhibit a pressor response to intravascular injection of angiotensin II (AII). The pressor response is completely abolished by prior treatment with phentolamine, an alpha adrenergic blocking drug (Opdyke et al, Am. J. Physiol. 231: 1750-1753, 1976). This is in contrast to the situation in mammals and teleost fish where alpha receptor blockade only partially inhibits the pressor response to AII (Nishimura et al, Am. J. Physiol. 235:H95-103, 1978). Based on these observations, a hypothesis was advanced that the pressor response in dogfish is caused solely by catecholamine release (Opdyke and Holcombe, Am. J. Physiol. 234:R196-200, 1978). However, the hypothesis lacked direct supporting evidence that catecholamines are released in response to the administration of AII. The results reported here provide that direct evidence.

After inserting an indwelling cathether (Intramedic, PE 60) into the caudal artery of unanesthetized dogfish (2-7 kg) and advancing it into the dorsal aorta the fish were placed in a restraining tank provided with a continuous flow of cold sea-water. The catheter was used for recording phasic dorsal aortic pressure (DAP), for injecting teleost angiotesin II, [$Asn^1 Val^5$] AII, and for collecting blood samples. Control blood samples ( 6 ml) were collected from each of 9 dogfish 20-30 minutes prior to injecting 5 μg/kg AII. This is a dose that gives a maximum pressor response in most dogfish. In 7 fish a second 6 ml blood sample was withdrawn when it was observed that the injection of AII had definitely initiated a pressor response - usually within 60-90 seconds after injection. The injection of 5 μg/kg AII and the subsequent blood sampling was repeated 20-30 minutes after the first injection in 6 fish. In 2 dogfish control blood samples were drawn as described above, but in these fish 2-3 ml blood samples were taken at 1 minute intervals for several consecutive minutes after the AII injection. All blood samples were collected in heparinized syringes, quickly transferred to tubes containing EGTA and glutathione and spun down promptly in a refrigerated centrifuge. The plasma was drawn off into cooled storage vials and kept on dry ice until thawed for catecholamine analysis by radioimmunoassay.

In each fish injection of AII resulted in an increase in the plasma concentration of norepinephrine, epinephrine and dopamine. There was considerable variation in control concentrations of catecholamines and the levels measured after AII injection. Control concentration of norepinephrine ranged from 1.6 ng/ml to 13 ng/ml; concentrations ranging from 5.8 to 108 ng/ml were observed after the first injection of AII. Comparable values for epinephrine were: control range- 1-7 ng/ml; post-AII concentration- 3.5-86.5 ng/ml. For dopamine, the concentrations were: control range- 0-1.0 ng/ml; after AII injection- 0.2-4.5 ng/ml. Mean concentrations ± 1 SEM of these catecholamines before and after AII injection are shown in Figure 1. The increase in concentration over control following both the first and second AII

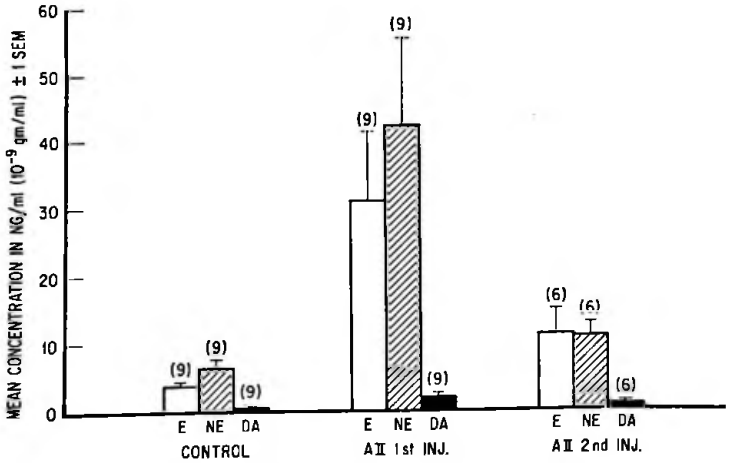


FIGURE 1

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.

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## 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.