BLOOD PRESSURE AND CATECHOLAMINE RESPONSE TO DOGFISH AngII IN SQUALUS ACANTHIAS.

Sara M. Galli[1,2] and Melvin J. Fregly[1]
Departments of [1]Physiology and [2]Pharmacology,
College of Medicine, University of Florida, Box 100274
Gainesville, FL 32610

The effects of exogenous administration of angiotensins in the dogfish have been studied extensively by Opdyke, Caroll and co-workers (Comp. Bioch. Physiol. 70:131-134, 1981; Am. J. Physiol. 243:R65-R69, 1982). They demonstrated that Asn^1-Val^5-AngII in high doses (5-40 $\mu g \times kg^{-1}$) increased blood pressure and plasma concentration of epinephrine (E) and norepinephrine (NE). The blood pressure response to exogenous AngII was completely blocked by the α-blocking agent, phentolamine. Isolated dogfish gut vasculature did not exhibit vasoconstriction as a direct response to either AngI or AngII, as it did to an equivalent dose of epinephrine. These results led to the hypothesis that in elasmobranchs the blood pressure response to AngII is indirect and entirely mediated by the stimulatory action of AngII on catecholamine release, suggesting that specific AngII receptors mediating vasoconstriction may not be present in the vasculature of the dogfish. In support of these observations, renin and angiotensins were not found in several species of sharks studied, all of which suggested the absence of a Renin-Angiotensin System (RAS) in these elasmobranchs (Nishimura H., et al., Am. J. Physiol. 218:911-915, 1970). This hypothesis has been prevalent during the past two decades, during which specific methods for qualitative and quantitative analysis of the RAS of lower vertebrates were scarce. The present availability of specific AngII antibodies and AngII receptor blocking agents has provided new tools to search again into the presence and functional role of the RAS in elasmobranchs.

In contrast to the hypothesis mentioned above, we have identified angiotensins in plasma, kidney, brain and rectal gland of the nurse shark Gynglymostoma cirratum, and found in this species an active RAS that responds as in mammals to hypotension and hemorrhage (Galli, S.M., Fifth Int. Symp. on Fish Physiol., Odense Univ., Proc. Abstr. No. 25, 1991). In vitro studies in the nurse shark have shown production of AngI from kidney homogenates incubated with shark plasma. Further, the presence of angiotensinogen mRNA was found in liver and kidney of the nurse shark (Galli, S.M. et al., Int. Meeting Am. Biol. Soc., Cambridge Univ., Proc. Abstr. No. 29, 1992). We have also found specific AngII binding in nurse shark rectal gland tissue, that could not be displaced by either AT_1 or AT_2 receptor antagonists. This is a new type of AngII receptor that requires further characterization (Galli, S.M. and V. Cook, FASEB Abstr. 2534, 1993). The results of our in vivo and in vitro studies in the nurse shark support the hypothesis that the RAS is present in sharks and they suggest a role for angiotensin in blood-pressure and blood-volume control in elasmobranchs.

The amino acid sequence of dogfish (Triakis scyllia) AngI has recently been reported as [Asn^1, Pro^3, Ile^5, Glu^9] (Takei, H. and Hazon, N., J. of Endocrinol., 139:281-

285, 1994). Recently we synthesized dogfish AngII (DF-AngII) and used it in the in vivo studies we report here on Squalus acanthias.

One of the main objectives of this study was to investigate the effect of low physiological doses of homologous AngII, DF-AngII, on blood pressure and plasma catecholamine response in unanesthetized Squalus acanthias. A second objective was to study the involvement of the AngII AT_1 receptor in blood-pressure, E and NE responses to DF-AngII by using the AngII AT_1-receptor-subtype antagonist, Losartan (DuP 753, DuPont Merck Co.).

To choose the doses of DF-AngII to be administered in dogfish, it was necessary initially to determine the normal concentration of the peptide in a large group of control unanesthetized fish. For this purpose 18 male dogfish (BW 1.8-3.5 kg) were kept in large tanks with running sea water (SW), for 3 days after which 1 ml of blood was withdrawn by puncture of the caudal aorta and placed on iced tubes containing EDTA and orthophenanthroline. The plasma was kept in a -70°C freezer until extraction and AngII RIA procedures were performed. For the AngII RIA, labelled ^{125}I-DF-AngII, cold DF-AngII and a polyclonal antibody raised against Ile^5-AngII were used.

Another group of ten male dogfish was used for blood pressure studies. Each fish was subjected to anesthesia (MS-222, 0.01% in SW) and a PE50 plastic tubing was

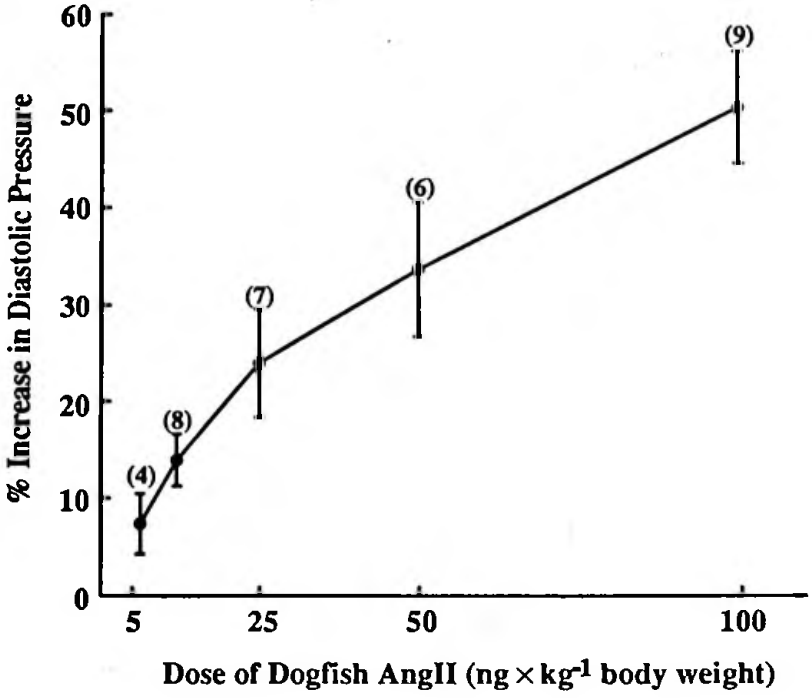

Figure 1. Blood pressure response to a bolus i.v. infusion of dogfish AngII in non-anesthetized *Squalus acanthias*. Values are mean ± SE (n).

inserted into the dorsal aorta and in the mesenteric vein via a ventral incision. The fish were transferred on a sling into a running salt water tank, where they remained undisturbed for 24-48 hrs until the experiment started. Blood pressure was measured by a Statham blood pressure transducer and recorded on a Grass polygraph (kindly provided by Dr. Ian Callard, MDIBL). Increases in blood pressure are expressed as % change in diastolic pressure after 2 min of i.v. DF-AngII infusion. The dose of DF-AngII (100 ng x kg^{-1}) that increased diastolic pressure by an average of 50 percent was used for the quantification of the plasma catecholamine response. In these experiments 500-800 μl blood was withdrawn after administration of DF-AngII for 3 min. The blood was collected in iced tubes containing EDTA and the plasma was concentrated on acid washed alumina and eluted with 0.1 M $HClO_4$. The quantification and analysis of E and NE were performed in a Bio-Rad HPLC model 1330 assay. The values presented are means ± SE (n).

The results of the RIA for plasma AngII in a control group of dogfish showed that an AngII-like peptide is present in dogfish plasma at a concentration of 119.8 ± 19.3 SE pg/ml. The doses of DF-AngII chosen to test were 1, 6, 12, 50 and 100 ng x kg^{-1}, which were within the normal dogfish plasma range. The blood pressure responses are shown in Fig. 1. DF-AngII produced a dose-dependent increase in diastolic pressure, when expressed as the mean percentage change from preinjection control values. Com-

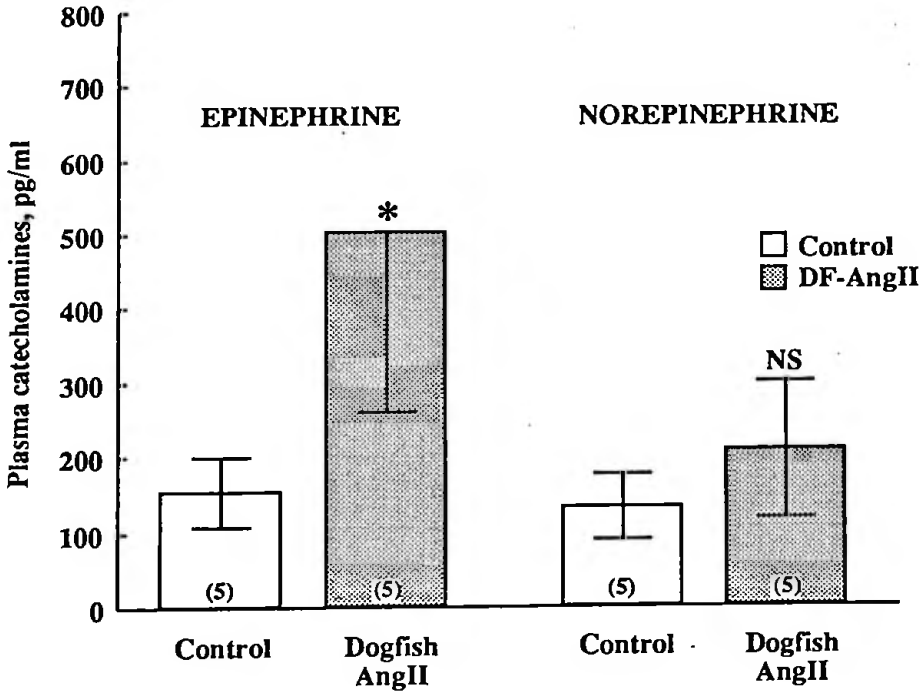

Figure 2. ***Squalus acanthias*** **plasma E and NE concentration in response to the i.v. administration of a single dose of Dogfish AngII, 100 ng × kg^{-1}. Number of observations shown in parentheses. *Values significantly different from control, as determined by a paired t-test, $P < 0.05$. NS: not significantly different from controls.**

parison of the logarithm of the dose with the percentage change in diastolic pressure by a linear regression analysis yields a significant correlation ($r = 0.97$; $p < 0.017$) for the doses tested. A small dose of 1 ng x kg^{-1} did not induce a change in B.P., but doses above 12 ng x kg^{-1} induced a marked increase in diastolic pressure due to either a direct or an indirect vasoconstrictor effect of DF-AngII.

The effect of DF-AngII on plasma concentrations of E and NE is shown in Fig. 2. A dose of 100 ng x kg^{-1} produced a significant rise in plasma E concentration ($p < 0.05$) and only a moderate increase in plasma NE concentration. This response to DF-AngII is similar to the one found in teleost fish in which Asn^1-Val^5-AngII preferentially releases E (Caroll, R.G., Am. J. Physiol. 240:R139-R143, 1981).

In a group of 5 dogfish, Losartan at a dose of 0.8, 1.5, or 2.0 mg x kg^{-1} did not abolish the blood-pressure response to doses of 12, 25 or 100 ng x kg^{-1} DF-AngII. These results suggest that either the AngII type 1 receptor is absent in the dogfish vasculature or that the vascular response to AngII is mediated by a completely different type of AngII receptor. In the nurse shark we have previously reported the presence of a new AngII receptor subtype in the rectal gland, that does not resemble the AT_1 or AT_2 AngII receptor (Galli, S.M. and V. Cook, FASEB Abstr. 2534, 1993).

In summary, we have shown (1) that DF-AngII increases blood pressure in Squalus acanthias in a dose-dependent fashion, and (2) that E is released by DF-AngII. (3) Preliminary data indicate that Losartan does not inhibit the dogfish blood-pressure response to DF-AngII. Therefore we conclude that in dogfish the AngII receptors involved in blood pressure are not of the AT_1 receptor subtype.

Supported by grants from EPS CoR and NIH HL-27334.

We acknowledge the kind gift of AngII antibody from Dr. M. Ian Phillips, and of Losartan (DuP 753) from the DuPont Merck Co.