VASOCONSTRICTIVE ACTION OF SHARK (SQUALUS ACANTHIAS) CARDIAC AND BRAIN EXTRACTS ON SHARK VENTRAL AORTIC SMOOTH MUSCLE

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Using the isolated, perfused dogfish pup head, we have previously demonstrated that relatively high concentrations (2.1×10^{-7} M) of rat atriopeptin II (AP103-125) produced an increase in branchial resistance, presumably secondary to vasoconstriction (Evans et al., Bull. MDIBL 25, 168-169, 1985). This was particularly surprising since there is overwhelming evidence that AP produces vasodilation in a variety of mammalian vascular tissues (e.g., Genest & Cantin, Rev. Physiol. Biochem. Pharmacol. 110, 2-147, 1988). However, it has been shown that AP can vasoconstrict coronary arteries (Wangler et al., Science 230, 558-561, 1985) and the increase in glomerular filtration rate secondary to AP addition in mammals is associated with constriction of efferent glomerular arterioles (Genest & Cantin, op. cit., 1988). Our subsequent studies on isolated, vascular smooth muscle rings from the shark have demonstrated a vasodilatory response to rat AP (101-126) over the concentration range of 10^{-9} to 10^{-7} M, with an EC_{50} of 5×10^{-9} M, in the same range as that described for mammalian VSM (Evans & Weingarten, Bull. MDIBL 28, 4-5, 1989). Since our work has also determined that measurable quantities of immunoreactive AP are present in the heart and brain of the dogfish (Evans, Bull. MDIBL 28, 39-41, 1989), we decided to investigate the vasoactivity of heart and brain extracts when applied to aortic rings of that species.

The preparation and mounting of dogfish aortic VSM rings (initial tension ca. 500 mg) has already been described (Evans & Weingarten, Bull. MDIBL 27, 84-85, 1987-88), as has our initial protocol for preparation of tissue extracts (termed extract #1; Evans, 1989, op. cit.). In conjunction with another study (Karnaky et al., this volume), a second extract was prepared using the same procedure except that the sep-pak extraction was with 40% acetonitrile in 0.01% trifluoroacetic acid (termed extract #2). In this case the total protein concentration (1.4 mg protein/ml) and immunological competitive binding activity (1 µg ANP/ml; against rat synthetic ANP) was determined. In our initial study, using extract #1, addition of extract equivalent to 10% of a single dogfish atrium to the 10 ml experimental bath produced a 83 mg (17%) increase in tension within 25 sec. Addition of 10% of a ventricle to another ring from the same shark produced no significant effect. Addition of more extract (in increments to make a total of 100% of a single atrium or ventricle) did not further increase the tension in the ring exposed to atrial extracts, but did stimulate constriction in the ring exposed to ventricular extracts (initial response at 30% of one ventricle, reaching a total of 45 mg (9%) after the addition of the equivalent of a single ventricle). In both cases the rings were finally exposed to isoproterenol (10^{-5} M) and carbachol (10^{-4} M), producing the usual dilation and constriction, demonstrating viability of the tissue. Three additional experiments using extract #1 demonstrated that extract from a single atrium produced a 110 ± 66 mg (22%; SE) increase in tension; extract from an entire ventricle produced a 107 ± 38 mg (21%) increase in tension. Extract from a single brain also produced a 93 ± 34 mg (n = 4) increase in tension when subsequently applied to the rings that had received heart extracts. Isoproterenol and carbachol were again effective at the end of these experiments. Addition of 5 µl of extract #2 (5 ng/10 mls = ca. 0.2×10^{-9} M ANP) produced a rapid increase in tension of 80 mg (16%), followed by a secondary decline to 50 mg higher than the control tension. Subsequent addition of 50 µl of extract #2 (2×10^{-9} M) produced an additional 25 mg increase in tension, with no secondary decline.

Thus, these pilot studies support our earlier study on the intact branchial vasculature (Evans et al., Bull. MDIBL 25, 168-169, 1985) and suggest that rather crude extracts from dogfish atrial, ventricular, and brain tissues are constrictory to homologous aortic VSM. Our finding is especially interesting because similar extracts have been shown to contain immunoreactive AP (Evans, Bull. MDIBL 28, 39-41, 1989; Karnaky et al., this volume), and shark aortic VSM rings are dilated by mammalian AP (Evans & Weingarten, Bull. MDIBL 28, 4-5, 1989). The bases for this discrepancy between a heterologous peptide producing the "expected" response, and a homologous extract producing the opposite response are unknown. Supported by NIH EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies, NSF DCB-8801572 to DHE, and a grant from the Lucille B. Markey Charitable Trust to KJK.