SYNTHETIC ATRIOPEPTIN II AND SHARK CARDIAC EXTRACTS ARE POTENT VASOCONSTRICTORS OF THE EFFERENT BRANCHIAL CIRCULATION OF THE DOGFISH SHARK PUP (SQUALUS ACANTHIAS)

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In recent years various peptides isolated from atrial myocytes have been found to produce marked natriuresis and systemic hypotension (e.g. DeBold et al., Life Sci. 28, 89-94, 1981; Currie et al., Science 221, 71-73, 1983; Pegram et al., Am. J. Physiol. 249, H265-H271, 1985; Wakitani et al., Am. J. Physiol. 249, F49-F53, 1985; Winquist, Life Sci. 37, 1081-1087, 1985). These atrial natriuretic factors (ANFs) are now considered to play a physiologic role in the renal responses to volume expansion in higher vertebrates, although it is unclear if the renal responses involve alteration of renal vascular resistance (Increase: Camargo et al., Am. J. Physiol. 246, F447-F456, 1984; Decrease: Hintze et al., Am. J. Physiol. 248, H587-H591, 1985) or not (Murray et al., Am. J. Physiol. 249, F603-F609, 1985). Recent studies in the intact shark have demonstrated that the rectal gland responds to an increase in extracellular volume with a significant increase in Cl^- secretory rate (Solomon et al., Am. J. Physiol., 248, R638-R640, 1985). Furthermore, synthetic atriopeptin II (APII) is able to stimulate both the intact and in vitro perfused rectal gland (Solomon et al., Am. J. Physiol. 249, R348-R354, 1985). A hemodynamic component to these responses is apparently present since both a volume load and APII increased in vitro blood flow to the gland (Solomon et al., Am. J. Physiol. 246, R67-R71, 1984). In addition, APII decreased systemic blood pressure. Thus it appears that APII is vasodilatory to both the rectal gland and systemic vasculature of the shark. The branchial vasculature of the shark receives blood directly from the heart, and has been shown to be sensitive to other vasoactive substances such as epinephrine (Evans & Claiborne, J. Exp. Biol. 105, 363-371, 1983), carbachol (Evans & Claiborne, Bull. MDIBL 21, 9-11, 1981) and adenosine (Evans, unpublished observations); therefore we examined the effect of both synthetic APII and shark cardiac extracts on the resistance of the branchial vasculature of the isolated, perfused head of the shark pup.

Pup heads were perfused as described previously (Evans & Claiborne, J. Exp. Biol., 1983, op. cit.; Evans & Robbins, this bulletin). In the first series of experiments the head was perfused (afferent pressures of 20-30 torr) with hormone-free shark Ringer's for an initial control period, followed by perfusion with Ringer's containing 500 ng/ml (2.1×10^{-7} M) synthetic APII (Peninsula Laboratories). The synthetic peptide produced significant increases (over a baseline of ca. 30 torr) in branchial resistance (10.5 ± 5.3 torr, N = 3; Mean $\pm$ SE). In a single experiment, APII (2.1×10^{-7} M) was added to the perfusate in the presence of 10^{-5} M epinephrine (which produces substantial net vasodilation--5.1 torr, via stimulation of both alpha- and beta adrenergic receptors; Evans & Claiborne, 1983, op. cit.). Under this protocol the cardiac peptide produced a similar increase in afferent pressure (11.1 torr), suggesting that a-adrenergic receptors were not involved in the response to APII since they were already stimulated by the presence of 10^{-5} M

epinephrine. Thus, contrary to mammalian systemic and shark systemic and rectal gland vasculature, the branchial vessels of the pup respond to synthetic APII by vasoconstriction, rather than vasodilation.

Because the perfused head presumably still has intact cholinergic nerves to the branchial vasculature and similar levels of vasoconstriction (7.5 torr) had been seen previously when 5×10^{-6} M carbachol was added to the perfused pup head (which was inhibited by 1×10^{-5} M atropine; Evans & Claiborne, 1981, op. cit.), we tested for a role of muscarinic cholinergic receptors in the response, and the vasoactivity of shark cardiac extracts in the same experiment. In this series of experiments 10^{-5} M atropine was added to the perfusate (after an initial control period), followed by perfusion with 10^{-5} M atropine plus either shark atrial or ventricular extracts (50 ng/ml; prepared as described in Solomon et al., 1985, op. cit.). Both extracts produced substantial vasoconstriction (Atrial: 3.7 and 5.0 torr; Ventricular: 5.2 and 13.1 torr) in the presence of atropine, suggesting that muscarinic cholinergic receptors were not involved in the vasoconstrictory response of the branchial vasculature to shark cardiac extracts.

Since the site of action of vasoactive substances can be inferred based upon effects on venous vs. dorsal aortic effluent flow (Claiborne & Evans, J. Comp. Physiol. 138, 79-85, 1980; Evans & Claiborne, 1983, op. cit.), we perfused a third series of heads with synthetic APII while measuring the venous and dorsal aortic effluent flow. Perfusion with 10^{-8} M APII increased the afferent pressure by 4.2 ± 0.8 torr (N = 3), decreased perfusate efflow from the dorsal aorta by $25.3 \pm 1.8\%$ and increased the venous perfusate efflow by $13.6 \pm 5.9\%$. This suggests that vasoconstriction took place at a site distal to the separation of arterial and venous return from the branchial vasculature. The actual site of action is unknown but it is generally accepted that the distal region of the efferent filamental artery is the site of action of acetylcholine (Smith, Am. J. Physiol. 233, R222-R229, 1977; Nilsson, Autonomic Nerve Function in the Vertebrates, Springer Verlag, Berlin, pp. 121, 1983).

Finally, since the lower limits of the vasodilatory sensitivity of mammalian systemic vessels to synthetic ANFs is of the order of 10^{-10} M (Winquist, 1985, op. cit.), we examined the sensitivity of the shark branchial hemodynamic response by perfusion with different concentrations of APII. We found that in five experiments utilizing concentrations in the range of 10^{-11} to 10^{-17} M the heads responded with significant increases in afferent pressure and decreases in dorsal aortic outflow. Thus, it is clear that even extremely low concentrations of APII are still effective in producing the changes in afferent pressure and distribution of perfusate flow characteristic of the response of the shark branchial vasculature to these peptides.

A vasoconstrictory response seems at odds with the shark systemic and rectal gland vessel response (see above), and the established pattern of response of mammalian vessels to the ANFs (Winquist, 1985, op. cit.); however, it has recently been found that the mammalian coronary arteries also constrict in response to the addition of synthetic APII (Wangler et al., Science 230, 558-561, 1985) and Camargo et al. (1984, op. cit.) demonstrated that rat atrial extracts (but not ventricular extracts) produced an increase in the renal vascular resistance of isolated, perfused rat kidneys. It is interesting to note that embryological evidence points to a common origin of the fish branchial and tetrapod coronary vasculature (Keys & Bateman, Biol. Bull. 63, 327-336, 1932).

Our findings suggest strongly that ANFs may be extremely important agents in the control of shark branchial hemodynamics and therefore important effectors in such crucial physiologic processes as gas exchange, acid-base regulation, and ion/water balance. Moreover, its extreme sensitivity makes the perfused pup head a potential bioassay for cardiac extracts. Supported by NSF PCM 8302621 to DHE and a grant from the American Heart Association, Rhode Island Affiliate to RS.