VASOACTIVE EFFECTS OF ADENOSINE, VASOACTIVE INTESTINAL PEPTIDE, AND ATRIOPEPTIN ON VENTRAL AORTIC RINGS FROM THE SHARK, SQUALUS ACANTHIAS

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We have previously demonstrated that isolated ventral aortic rings from the dogfish (stripped of vascular endothelium) are responsive to epinephrine (vasodilatory; EC_{50} ca. 5×10^{-7} M) and carbachol (vasoconstrictive; EC_{50} ca. 5×10^{-7} M) (Evans & Weingarten, Bull. MDIBL 27, 84-85, 1987-88). The current study extends these studies to include the putative vasoactive agents adenosine, VIP, and atriopeptin.

Isolated vascular rings were prepared from the ventral aorta of the dogfish shark taken between the third and fourth branchial arches. The rings were stripped of vascular endothelium and mounted in 10 ml of elasmobranch Ringer's solution as previous described (Evans & Weingarten, Ibid). Initial tension was set at 500 mg and maintained for approximately 30 minutes, until tension was stable. 2-chloroadenosine (Sigma) was dissolved directly in distilled water and VIP (Sigma) was dissolved directly in phosphate buffered saline (FLOW labs). Atriopeptin ($AP_{101-126}$, rat; Bachem) were dissolved in 0.1N acetic acid, aliquoted, lyophilyzed and stored at -70°C until re-dissolved in distilled water before use. All hormones were added cumulatively in aliquots to produce the desired concentration, accounting for slight volume changes. Rings were <u>not</u> pre-constricted or pre-dilated before addition of the vasoactive agents.

All three putative vasoactive agents produced significant reductions in the tension of the aortic ring of the dogfish shark. Adenosine produced a biphasic effect with slight (max. ca. 6%, $p < 0.01$) vasoconstriction at concentrations between 10^{-8} M and 10^{-5} M and substantial (max. ca. 20%, $p << 0.01$) vasodilation at 10^{-4} M and 10^{-3} M. In mammals, adenosine generally uces vasodilation, especially in the coronary arteries (Bern, R.M., Cir. Res. 47, 807-813, 1980), except in renal vasculature, where vasoconstriction appears to predominate (Osswald, H., in Bern, R.M. et al. Regulatory Function of Adenosine, pp. 399-415, 1983). Very little information has been published on vasoactive actions of adenosine on fish vasculature. Shuttleworth (J. Exp. Biol. 103, 193-204, 1983) demonstrated that 10^{-5} M adenosine produced significant vasodilation of the <u>Scyliorhinus</u> (European dogfish) rectal gland vasculature, and the gill vasculature of both the in vivo trout and the perfused trout head appear to vasoconstrict when adenosine is applied at a concentration of approximately 10^{-6} M (Colin, D.A. et al., J. Comp. Physiol. 130, 325-330, 1979). Interestingly, Forrest and co-workers have demonstrated stimulation of Cl^- secretion by the shark rectal gland at relatively high adenosine concentrations (10^{-4} M; Forrest et al., Bull. MDIBL 20, 152-155, 1980), but inhibition at lower concentrations (10^{-6} M) (Kelley, G. et al., Bull. MDIBL 23, 86-88, 1983). It is unclear what role vascular changes could have played in these secretory responses, but the biphasic response seen in the present experiments suggests that vasoconstriction of the rectal gland vasculature in low adenosine concentrations, and vasodilation at higher concentrations may have played some role. Most importantly the current data demonstrate for the first time that release of adenosine from the shark heart could produce substantial changes in the rates and pressure of perfusion of the branchial vasculature, with concomitant alterations in gas exchange and osmoregulation.

Very low concentrations (10^{-8} and 10^{-7} M) of VIP produced significant vasodilation (max. ca. 10% at 10^{-7} M, $p < 0.02$) of the aortic rings, in the

same concentration range as that described for its effects on mammalian gut epithelia (e.g., Rosselin, G. et al., Mol. Cell. Endocrinol. 27, 243-262, 1982). VIP is a known vasodilator of the peripheral vasculature in mammals (Said, S.I., J. Endocrinol. Invest. 9, 191-200, 1986), and has also been shown to vasodilate the perfused intestinal loop of the catfish, Ictalurus (Holder, F-C et al., C.R. Acad. Sci. Paris, 296, 783-788, 1983). VIP has also been found to be a potent (at 10^{-8} to 10^{-6} M) stimulant of Cl^- secretion by the perfused Squalus rectal gland (e.g. Stoff, J.S. et al., Am. J. Physiol. 237, F138-F144, 1979), and a vasodilator (at 2 x 10^{-8} M) of the rectal gland vasculature in that species, but not in Scyliorhinus (Shuttleworth, T.J., Op. Cit., 1983). The role of vasodilation in the stimulation of the rectal gland in Squalus remains unstudied. VIP-containing nerves have not been described in the shark ventral aorta, but they are certainly present in the walls of the coeliac and mesenteric arteries, as well as the gut and rectal gland, of Squalus (Holmgren, S. & Nilsson, S., Cell Tiss. Res. 234, 595-618, 1983). The role of VIP in controlling shark, or teleost, gill hemodynamics is unstudied.

Finally, atriopeptin also produces vasodilation of the shark aortic ring, with a significant effect ($p < 0.02$) at 10^{-9} M, and an apparent EC_{50} of approximately 5 x 10^{-9} M. The efficacy of the synthetic, rat ($AP_{101-126}$) peptide was more than 2 orders of magnitude greater than of the more truncated AP II ($AP_{102-125}$) on dogfish ventral aortic rings preconstricted with carbachol (Solomon, R.J. et al., Bull. MDIBL 25, 146-149, 1985), and maintained at an initial tension of 1g. Importantly, the EC_{50} in the present experiments, using the heterologous, mammalian peptide is similar to that described in a variety of mammalian vascular studies (e.g. Winquist, R.J., Life Sci. 37, 1081-1087, 1985), suggesting that the fish atriopeptin is structurally very similar to the mammalian peptide. This is supported by our recent radioimmunoassay data, but, as has been found in mammals, the apparent physiological EC_{50} is some two orders of magnitude above the apparent plasma AP concentrations (see Evans and Weingarten, this volume). Whether this discrepancy is produced by silent, "clearance" receptors as described for the rat kidney (Maack, T. et al., Science 238, 675-678, 1987) remains to be investigated.

In summary, our studies have demonstrated that adenosine, VIP, and atriopeptin produce measurable vasodilation of the isolated shark ventral aortic ring, with adenosine producing constriction at concentrations at or below 10^{-5} M. Since all three are probably present in shark blood, at least under certain conditions, it is apparent that they may play major roles in branchial hemodynamics, and secondarily, gas exchange and osmoregulation. (Supported by NSF DCB-8801572 to DHE and NIEHS 1 P50 ES 03828-03 to the Center for Membrane Toxicity Studies).