APPARENT LACK OF THE NITRIC OXIDE/SOLUBLE GUANYLYL CYCLASE AXIS IN THE AORTIC VASCULAR SMOOTH MUSCLE OF THE SHARK, SQUALUS ACANTHIAS

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It is now generally accepted that the major endothelium-derived relaxing factor (EDRF), first described in 1980 (Furchgott and Zawadzki, Nature 299: 373-376, 1980), is actually nitric oxide (Bredt and Snyder, Ann. Rev. Biochem. 63: 175-195, 1994). Current understanding is that NO is synthesized by oxidation of L-arginine in the vascular endothelium after stimulation by secretagogues such as acetylcholine (ACh), bradykinin, endothelin, histamine, and thrombin, and physical factors such as hypoxia and shear stress (Brenner et al., J. Clin. Invest. 84: 1373-1378, 1980). The reactive gas permeates the underlying vascular smooth muscle (VSM) and activates soluble guanylyl cyclase (sGC), which generates the cascade of cyclic GMP, protein kinases, phosphorylated proteins, reduced intracellular Ca^{2+}, and subsequent muscular relaxation (Schmidt et al., Biochim. Biophys. Acta 1178: 153-175, 1993). The presence of this NO/sGC axis in mammalian vessels is used as a diagnostic for the presence of an intact endothelium--if ACh produces dilation of a given vessel, the endothelium is assumed to be intact; ACh-induced contraction is characteristic of vessels after the endothelium has been removed. Our investigation of the expression of ACh and endothelin (ET) receptors in shark aortic VSM has suggested that the NO/sGC axis may be lacking in this system since both substances produced contraction whether the endothelium was left intact or removed (Evans and Cegelis, Bull. MDIBL 33: 113, 1994). Therefore, we set out to examine this hypothesis more directly by testing the effect of L-arginine and a sGC stimulant, sodium nitroprusside (SNP), on endothelium-intact, aortic VSM from the dogfish shark.

The experimental protocols for the use of isolated, aortic VSM rings from Squalus acanthias have been described previously (e.g., Evans, J. Comp. Physiol. 162: 179-183, 1992); however, in this case, the endothelial lining of the aortic rings was not removed. To test for the presence of endothelial NO synthesis via oxidation of L-arginine, we applied 0.1 mM of either L-arginine or D-arginine (inactive analog) to paired rings (N = 5) after first testing the viability of the rings with two doses of 0.1 mM ACh. In all experiments, the rings responded with maximal tensions after ACh was applied, which could be rinsed off with elasmobranch Ringer's (ERS), but neither L- nor D-arginine produced dilation, as it does in mammalian VSM. These data suggest strongly that the endothelium of the shark aortic VSM does not produce an EDRF via oxidation of L-arginine, contrary to the situation in mammals, but corroborating earlier studies which suggested that EDRF in fishes may be a prostanoid (Olson et al., Am. J. Physiol. 260: H1214-1223, 1991; Miller and Vanhoutte, in "Endothelial Regulation of Vascular Tone", ed. by Ryan and Rubanyi, Marcel Dekker, Inc., New York, pgs. 3-20, 1992). To test for the presence of the other limb of the axis, sGC in the VSM, we applied 0.1 mM SNP to intact or endothelium-free aortic rings after an initial stimulation with 0.1 mM ACh, followed by a rinse with ERS. In all cases (N = 6 for both experiments), both ACh and SNP produced contractions, suggesting that sGC is not present in this tissue. Importantly, application of 0.1 μM porcine C-type natriuretic peptide always produced dilation, as we have shown previously (Evans et al. J. Exp. Zool. 265: 84-87, 1993), demonstrating that particulate GC is present in this tissue. Our finding that SNP does not produce dilation in elasmobranch aortic VSM is at odds with the finding by Olson and Villa (Am. J. Physiol. 260: R925-R933, 1991) that trout VSM dilates when SNP (10 μM) is applied. In conclusion, it appears that the NO/sGC axis is not involved in production of endothelium-dependent dilation of shark aortic VSM. Just when this system evolved in vertebrate vasculature remains to be determined, but frog aortic rings do dilate when NO is applied (Miller and Vanhoutte, op. cit.). (Supported by NSF IBN-9306997 to DHE and EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies).