APPARENT LACK OF MUSCARINIC AND ENDOTHELIN RECEPTORS IN THE ENDOTHELIUM OF THE AORTA OF THE SHARK, SQUALUS ACANTHIAS

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In recent years it has become clear that the vascular endothelium is a vital endocrine (actually paracrine) tissue in mammals; a recent review refers to it as the "body's largest endocrine organ" (Anggard, J. Endocr. 127: 371-375, 1990). Its importance was first demonstrated when Furchgott and Zawadzki (Nature 288: 373-376, 1980) found that acetylcholine-mediated relaxation of vascular smooth muscle (VSM) was dependent upon an intact endothelium. Removal of the endothelium actually resulted in contraction of the VSM. In fact, loss of acetylcholine-mediated VSM relaxation is routinely used as an empirical test of endothelium removal in mammalian vessels. The endothelium-derived relaxation factor (EDRF) secreted in response to cholinergic stimulation is now thought to be nitric oxide (Moncada et al., Biochem. Parmacol. 37: 2495-2501, 1988). It has also been shown that the endothelium of mammalian vessels secretes EDRF in response to the stimulation by the peptide endothelin released from adjacent endothelial cells. Like acetylcholine (ACH), endothelin (ET) produces vasoconstriction if the endothelium is removed. Thus, one can test for the presence of these endothelial receptors by noting the effects of ACH or ET on intact and endothelium-free VSM preparations. In addition, one can empirically test for the presence of a functional endothelium by demonstrating non-receptor mediated release of endothelium EDRF (either NO or prostanoids) by treatment of endothelium-intact VSM with the calcium ionophore A23187 (e.g., Miller and Vanhoutte, Blood Vessels 23: 225-235, 1986). Based upon this criterion, there is some evidence that teleost fish blood vessels contain a functional endothelium that does not express ACH or ET receptors. For instance it has been shown that both ACH and ET contract rings of aortic VSM from the trout, whether the endothelium is present or not, but A23187 produces endothelium-dependent relaxation (Miller and Vanhoutte, Ibid.; Olson and Villa, Am. J. Physiol. 260: R925-R933, 1991; Miller and Vanhoutte, in "Endothelial Regulation of Vascular Tone, ed. by Ryan and Rubanyi, Marcel Kekker, Inc., New York, pgs. 3-20, 1992).

The present study was undertaken to examine the role of the endothelium in the sensitivity of the ventral aortic VSM from the shark to ACH or ET, and to test for non-receptor mediated endothelium release of putative EDRFs after stimulation with A23187. The experimental set-up and protocols have been described previously (e.g., Evans, J. Comp. Physiol. 162, 179-183: 1992). Concentration-response curves for both ACH and ET (ET-1) were generated in intact rings or endothelium-free rings produced by gently rubbing the aortic vessel with roughened PE-90 tubing before cutting into rings. ACH produced contraction in the shark aortic VSM rings, whether the endothelium was present or not ($N = 4$; EC_{50} ca. 0.1 μM in both cases). ET-1 also produced concentration-dependent contractions in both intact and endothelium-free aortic VSM ($N = 6$, $EC_{50} = 10$ nM in both cases). In addition, A23187 produced dose-dependent, endothelium-dependent relaxation in acetylcholine-contracted rings at concentrations above 1 μM.

These data support the hypothesis that shark aortic VSM, like that in the trout, has a functional endothelium that does not express either ACH or ET receptors, even though both are obviously present in the underlying VSM itself. It is apparent that these endothelium receptors evolved later in vertebrate evolution, possibly during the transition to terrestriality since ACH-mediated contraction of frog aortic rings is somewhat attenuated by the presence of an intact endothelium (Miller and Vanoutte, Op. Cit., 1986). (Supported by NSF IBN-9219122 and IBN-9306997 to DHE as well as EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies)