FURTHER CHARACTERIZATION OF MUSCARINIC AND ENDOTHELIN RECEPTORS IN THE AORTIC VASCULAR SMOOTH MUSCLE OF THE DOGFISH SHARK, SQUALUS ACANTHIAS

David H. Evans[1] and Mark Gunderson[2]
[1]Department of Zoology, University of Florida, Gainesville, FL 32611
[2] St. Olaf College, Northfield, MN 55057

Our preliminary experiments have suggested that the aortic vascular smooth muscle (AVSM) from the dogfish shark expresses both muscarinic and endothelin receptors (Evans and Cegelis, Bull. MDIBL 33: 114-115, 1990). These initial studies had indicated that the AVSM muscarinic receptor is of the M_3 type, based upon the greater sensitivity of acetylcholine-induced contraction to the M_3 antagonist 4-DAMP ($pK_2 \approx 9.7$) compared to the M_1 antagonist pirenzepine ($pK_2 \approx 6.7$), and the insensitivity to the M_2 antagonist, gallamine. The presence of M_3 receptors in smooth muscles in other species has been well established (e.g., Eglen et al., Trends in Physiol. Sci. 15: 114-119, 1994). Our studies also suggested that the shark AVSM endothelin receptors are of the ET_B type, based upon similar contractile efficacy to ET-1 and ET-3 and inability of the ET_A-specific antagonist, BQ-123, to inhibit the response. This was an unexpected finding because mammalian AVSM always have been found to express ET_A receptors, while ET_B receptors are common in the central nervous system, kidney, veins and some smaller arteries, as well as the endothelium itself (e.g., Rubanyi and Polokoff, Pharm. Revs. 46: 325-415, 1994). The following experiments were carried out to characterize further these two families of receptors in the shark AVSM.

The experimental protocols for the use of isolated, AVSM rings from Squalus acanthias have been described previously (e.g., Evans, J. Comp. Physiol. 162: 179-183, 1992). In order to test the hypothesis that M_3 muscarinic receptors are expressed in this tissue, we examined the efficacy of additional receptor-specific antagonists in displacing the concentration-response curve produced by cummulative addition of acetylcholine (ACh). p-F-HHSiD, which has a specificity of $M_3 > M_1$ in mammalian VSM, did inhibit the ACh-induced contractions in a concentration-dependent manner (N = 4-6 for 3 concentrations of antagonist), with a $pK_2 \approx 7.4$, in the same range as that described for M_3 receptors in mammalian VSM (Eglen, op. cit.). The M_2 antagonist methoctramine did not inhibit the ACh-induced contractions, even at 10 μM (N = 6), a concentration nearly 3 orders of magnitude greater than its pK_2 in mammalian M_2 systems (Eglen, op. cit.). These data support our hypothesis that the major muscarinic receptor sub-type expressed in this tissue is M_3.

Using the same protocols, we also tested our hypothesis that ET_B receptors are expressed in this tissue by examining other receptor-specific endothelin agonists and antagonists. Our hypothesis was supported by the following findings: 1. Sarafotoxin S6c, a specific ET_B agonist, contracted the aortic rings with an EC_{50} in the same range as we described previously for ET-1 (20 nM, N= 6), 2. Two specific ET_B agonists, BQ-3020 (Saeki et al., Biochem. Biophys. Res. Commun. 179: 285-292, 1991) and IRL-1620 (Takai et al., Biochem. Biophys. Res. Commun. 184: 953-959, 1992), produced concentration-dependent contractions. However, their efficacy was significantly lower than described for mammalian ET_B receptors ($EC_{50} \approx 100$ nM, N = 5 in both cases). Interestingly, the recently described ET_B-specific antagonist, RES 701-1 (Morishita et al., J. Antibiotics 47: 276-280, 1994), was without effect in our system, even 1 μM did not shift the ET-1 concentration-response curve to the right (N= 6). Thus, we have no reason to reject our initial hypothesis that the shark AVSM expresses primarily ET_B receptors, despite the fact that these receptors have not been described in mammalian aortae. (Supported by NSF IBN-9306997 to DHE and EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies).