FUNCTIONAL LOCALIZATION OF M_3 MUSCARINIC RECEPTORS IN AORTIC VASCULAR SMOOTH MUSCLE OF THE SHARK, SQUALUS ACANTHIAS

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Our recent studies demonstrated that 50% of the cadmium-induced contraction of shark aortic vascular smooth muscle (VSM) rings was inhibited by atropine, suggesting that a major site of action of this heavy metal is probably muscarinic receptors (Evans et al., Toxicology 62: 89-94, 1990). Muscarinic receptors now can be defined (see Caulfield, Pharmac. Ther. 58: 319-379, 1993 for a recent review) either pharmacologically (designated M_1-M_4) or via expression of one or more of five cloned genes (designated m1-m5). The product of the m5 receptor gene has not been pharmacologically defined to date. Pharmacological characterization of M_1-M_4 receptor subtypes depends on displacement or competition curves generated in the presence of relatively specific antagonists, including pirenzepine (M_1 and possibly M_3), gallamine (M_2) and 4-DAMP (M_3). Using such criteria, it has been suggested previously that cholinergic-mediated contraction in various endothelium-free, mammalian VSM preparations is mediated by M_1, M_2, or M_3 receptors (e.g., Entzeroth et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 341: 432-438, 1990; Caulfield, Op. Cit., 1993). The intent of this study was to provide the first characterization of muscarinic receptor type(s) in fish VSM, and to provide a basis for further studies of the interaction between cadmium and muscarinic receptors.

The experimental set-up of shark aortic VSM rings and basic concentration-response protocols have been described previously (e.g., Evans, J. Comp. Physiol. 162: 179-183, 1992). Efficacy of putative receptor antagonists was determined by running a concentration-response curve for acetylcholine (ACH) in the presence of increasing concentrations of pirenzepine, gallamine, or 4-DAMP, after a 30 minute incubation period of the ring in the putative antagonist. Data were analyzed by Schild Plot and the pK_2 ($-\log_{10}$ of competitive antagonist that reduces the effect of the agonist by 50%) calculated. Gallamine had no effect on ACH-stimulated contraction at concentrations of 0.1, 1, and 100 μM ($N = 4 - 8$), suggesting that M_2 type receptors are not involved. On the other hand, both pirenzepine and 4-DAMP inhibited ACH-induced contraction in a concentration dependent manner ($pK_2 \approx 6.7$ and 9.7, respectively; $n = 4 - 8$). The fact that 4-DAMP is approximately 1000-fold more competitive than pirenzepine suggests strongly that the shark aortic VSM expresses primarily M_3 type muscarinic receptors. Further studies, utilizing other antagonists and receptor binding protocols using radiolabeled agonist, are planned to test this hypothesis. (Supported by NSF IBN-9219122 and IBN-9306997 to DHE as well as EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies)