THE ROLE OF MUSCARINIC RECEPTORS IN CADMIUM-INDUCED CONTRACTION OF VENTRAL AORTIC RINGS FROM THE SHARK, SQUALUS ACANTHIAS

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Our previous, preliminary studies (Evans & Weingarten, Bull. MDIBL 27, 84-85, 1987-88) demonstrated that isolated ventral aortic rings from the dogfish (stripped of vascular endothelium, 1 g initial tension) vasoconstrict when 10^{-6} to 10^{-4} M (0.11 to 11 ppm) cadmium is applied. These data are to be contrasted with those published for helical strips of rat aorta which constrict at Cd^{2+} concentrations below 9 x 10^{-5} M, but vasodilate at higher concentrations (Niwa, A. & Suzuki, A., J. Toxicol. Sci. 7, 51-60, 1982). There are a variety of potential sites of action of the effects of Cd^{2+} on smooth muscle contractility including: blockade of Ca^{2+} channels (producing vasodilation), or inhibition of sarcolemma Na/K or Na/Ca exchange and interaction with α-adrenergic receptors or adenylate cyclase, all of which would produce vasoconstriction (Kopp, J. In: Cadmium; E.C. Foulkes, Ed., Handbk. Exp. Pharmacol., Vol. 80, Springer Verlag, Berlin, pp. 195-280, 1986). In addition, Cd^{2+} has been shown to activate intracellular cadmodulin (Chao, S-H et al., Mol. Pharmacol. 26, 75-82, 1984), as well as myosin light chain kinase (Mazzei, G.J. et al. FEBS Letters 173, 124-128, 1984), which would also produce constriction. Lastly, Cd^{2+} has been shown to inhibit muscarinic, cholinergic receptors in the striatum and cerebral cortex of the rat brain (Hedlund, B., Brain Res. 168, 216-218, 1979) which, presumably would produce vasodilation in smooth muscle that was tonically constricted by parasympathetic innervation. Since we have recently shown that the shark ventral aortic ring is quite responsive to carbachol (Evans & Weingarten, 1987-88, Ibid.), we decided to investigate a potential interaction between the heavy metal and muscarinic receptors.

Isolated vascular rings were prepared from the ventral aorta of the dogfish shark as previously described (Evans & Weingarten, 1987-88, Ibid; 1989, this volume). Initial tension was set at 500 mg and maintained for approximately 30 minutes, until tension was stable. In control experiments, $CdCl_2$ was added cumulatively in aliquots to produce the desired concentration, accounting for slight volume changes. In another experimental series atropine (10^{-3} M) was added initially and maintained in the bath during the cumulative addition of Cd^{2+}. Rings were <u>not</u> pre-constricted or pre-dilated before addition of either substance.

As our preliminary studies (Evans and Weingarten, 1987-88, Op. Cit.) had demonstrated, Cd^{2+} produced a concentration:dependent increase in tension in the isolated aortic ring from the dogfish, with a 12% increase ($p < 0.01$) seen at 10^{-5} M and a 24% increase at 10^{-4} M ($N = 9$). Addition of the muscarinic receptor blocker atropine to the dogfish ring before the Cd^{2+} <u>inhibited</u> the contraction significantly (65% at 10^{-5} M and 50% at 10^{-4} M, $N = 9$, $p < 0.02$). Addition of the 10^{-3} M atropine alone produced variable, but small responses (2 ± 2% increase in tension) which suggests that parasympathetic nerve endings may not be present, or that atropine may act as a partial agonist at such high concentrations.

Our data are consistent with the proposition that some 50% of the vasoconstrictory action of Cd^{2+} on this isolated vascular smooth muscle is secondary to some step involving muscarinic receptors. Whether this interaction involves direct stimulation of the muscarinic receptor, or stimulation of resident parasympathetic nerve endings remains to be determined. It is clear that a detailed immunohistochemical analysis of the innervation of the shark

ventral aorta is in order. Nevertheless, our data demonstrate, for the first time, that Cd-induced vasoconstriction may involve either the direct stimulation of the muscarinic receptors of the parasympathetic nervous system, or possibly activation of the release of acetylcholine itself. (Supported by NSF DCB-8801572 to DHE and NIEHS 1 P50 ES 03828-03 to the Center for Membrane Toxicity Studies).