MUSCARINIC RECEPTORS ARE NOT INVOLVED IN THE NICKEL-INDUCED CONSTRICTION OF VASCULAR SMOOTH MUSCLE OF THE DOGFISH SHARK (SQUALUS ACANTHIAS) VENTRAL AORTA

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We have previously demonstrated that endothelium-free, vascular smooth muscle (VSM) rings from the ventral aorta of the dogfish shark, Squalus acanthias, contracted significantly when either cadmium or nickel were applied, but were substantially unaffected by exposure to other chemically-related (Nieboer & Richardson, Environ. Pollution (Series B), 1, 3-26, 1980) metals such as Hg^{2+}, Sn^{2+}, Pb^{2+}, Co^{2+}, Cu^{2+}, or Zn^{2+} (Evans & Weingarten, Bull. MDIBL 28, 6, 1989; Evans & Weingarten, Toxicology, in press, 1990). Since this vessel is homologous to mammalian aortic and pulmonary vessels (Goodrich, Studies on the Structure and Development of Vertebrates. New York: Dover Publications, Inc. 837 pp., 1958) and possesses adrenergic, cholinergic (Evans et al., Bull. MDIBL 27, 84-85, 1988) purinergic, and atrial natriuretic peptide receptors (Evans & Weingarten, Bull. MDIBL 28, 4-5, 1989), it may be used as a model system to investigate the VSM membrane and intracellular effects of toxicants such as heavy metals. To date, the role of metals in the etiology of hypertension is unclear (e.g., Goyer, In: Casarett and Doull's Toxicology, eds. Klaassen, Amdur and Doull, 582-635. New York, Macmillan Publishing Co., 1986; Kopp, In: Cadmium, ed. E.C. Foulkes, Handbk. Exp. Pharmacol., Vol. 80, Springer Verlag, Berlin, pp. 195-280, 1986), despite the fact that a rather limited database demonstrates that both Cd^{2+} and Ni^{2+} can produce constriction in mammalian vascular smooth muscle (e.g., Niwa & Suziki, J. Toxicol. Sci. 7, 51-60, 1982; Rubanyi et al., J. Mol. Cell. Cardiol. 13, 1023-1026, 1981), somewhat surprising since they are generally considered to be excellent Ca^{2+} channel blockers (i.e., Kopp, op. cit., 1986; Tsien et al., TINS 11, 431-438, 1988), and should therefore produce vasodilation.

Given the complex cascade of excitation-contraction coupling events in vascular smooth muscle, a myriad of potential stimulatory sites exist, some of which have been implicated in metal toxicity. These include: (1) Stimulation of presynaptic release of acetylcholine (Asai et al., Br. J. Pharmacol. 75, 561-567, 1982) or norepinephrine (Williams & Laubach, Life Sci. 23, 1929-1934, 1978); (2) agonistic interaction with a post-synaptic, cholinergic, α-adrenergic (Rubanyi et al., Acta Physiol. Acad. Sci. Hung. 52, 61-167, 1982) or A_1-purinergic receptor; (3) inhibition of membrane-bound Na, K-activated ATPase (Kramer et al., Nephron 44, 329-336, 1986); (4) inhibition of membrane-bound Na-Ca exchange; (5) increase in the conductance of membrane Ca-channels (Koller et al., Acta Physiol. Acad. Sci. Hung. 59, 287-290, 1982); (6) inhibition of membrane-bound Ca-activated ATPase (Verbost et al., J. Biol. Chem. 264, 5613-5615, 1989); (7) inhibition of adenylcyclase (Nathanson & Bloom, Mol. Pharmacol. 12, 390-398, 1976); (8) stimulation of intracellular inositol triphosphate (Smith et al., J. Biol. Chem. 264, 7115-7118, 1989); (9) stimulation of intracellular calmodulin (Cheung, N.Y. Acad. Sci. 522, 74-87, 1988); or (10) stimulation of myosin light-chain kinase (Mazzei et al., FEBS Letters 173, 124-128, 1984).

Last year we (Evans & Weingarten, Bull. MDIBL, 28, 10-11, 1989) demonstrated that approximately 50% of the Cd^{2+}-induced vasoconstriction of the shark ventral aorta could be inhibited by the addition of atropine to the experimental bath, suggesting that muscarinic receptors are involved in that metal's vasotoxicity. The present study examined the role of muscarinic receptors in Ni^{2+}-induced contractions of this tissue.

The preparation and mounting of the VSM rings has been described previously (Evans & Weingarten, Bull. MDIBL 27, 84-85, 1987-88). Ni^{2+} (as the chloride salt) was added cumulatively to achieve a concentration range of 10^{-9} M to 10^{-4} M. At the end of all experiments, 10^{-3} M carbachol was added to monitor viability of the tissue and to

determine the degree of inhibition produced by atropine. To examine the effect of blockade of muscarinic receptors, atropine (sulfate; Sigma) was added from a stock solution to make a 10^{-4} M solution, before the metal was added.

The results are presented in Table 1; it is obvious that, contrary to Cd^{2+}-induced vasoconstriction, the effect of Ni^{2+} on shark aortic VSM is not attenuated at all by the blockade (88%) of muscarinic receptors by the addition of atropine.

TABLE 1 The effect of muscarinic blockade on the Ni-induced contraction of aortic vascular smooth muscle from Squalus acanthias

	10^{-5} M Ni^{2+}	10^{-4} M Ni^{2+}	10^{-4} M Carbachol
Control (9)	15 ± 13 mg	137 ± 15 mg	281 ± 67 mg
Atropine (12) (10^{-4} M)	31 ± 20 mg	112 ± 27 mg	35 ± 8 mg

All data are expressed as mean ± S.E. (N) of net change in tension. Initial tension was approximately 500 mg.

These data are consistent with the conclusion that muscarinic receptors are not involved in the constrictive response of the shark aortic VSM to Ni^{2+}. Thus, our previous determination that some 50% of the vasoactive effects of Cd^{2+} are mediated via muscarinic receptors must be specific for that metal, and not a generic response. Experiments are planned to investigate other potential sites of action for Ni^{2+}. Supported by NIH EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies and NSF DCB-8801572 to DHE.