LACK OF AN INTRACELLULAR EFFECT OF CADMIUM AND NICKEL ON VASCULAR SMOOTH MUSCLE FROM THE SHARK (SQUALUS ACANTHIAS) VENTRAL AORTA

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We have recently shown that muscarinic receptor blockade reduced the constrictory effect of Cd^{2+} on vascular smooth muscle (VSM) rings from the ventral aorta of the dogfish shark by 50%, but did not affect the constrictory effect of Ni^{2+} (Evans et al., Toxicology 62: 89-94, 1990) on this tissue. This suggested that intracellular proteins may be alternative sites of action in these responses. Moreover, recent studies (e.g., Cheung, Ann. N.Y. Acad. Sci. 522: 74-87, 1988) have demonstrated that Cd^{2+} stimulates calmodulin (CaM, measured as phosphodiesterase activity in vitro) at concentrations between 1-20 μM, with inhibition at higher concentrations, and Ni^{2+} has also been shown to bind to CaM (Raos and Kasprzak, Fund. Appl. Tox. 13: 816-822, 1989). We have found that the permeabilized (via chemically "skinning" with a detergent) VSM from the ventral aorta of dogfish is quite sensitive to applied Ca^{2+} (Evans and Chipouras, Bull. MDIBL 30: 110-111, 1991), and apparently does not leak sufficient CaM to reduce the Ca^{2+} stimulation of contraction, as is typically found in permeabilized mammalian VSM preparations (e.g., Gardner et al., Pflugers Arch. 414: 484-491, 1989; Ruegg and Paul, Circ. Res. 50: 394-399, 1982). We have, therefore, used this system to investigate potential intracellular sites of action of these two heavy metals. Our initial studies (Evans et al., Bull. MDIBL 30: 115-116) suggested that both heavy metals could constrict chemically-skinned VSM rings, but we were unable to produce a concentration-response curve, and the lowest effective concentration of the metals was in the 1-100 pM range, far below what appeared to be physiologically relevant.

Isolated rings were prepared as described previously (Evans and Weingarten, op. cit., 1990). Permeabilization of the sarcolemma (as well as the sarcoplasmic reticulum) by incubating the mounted rings in Triton X-100, as well as determination of the effect of external addition of either Cd^{2+} or Ni^{2+}, followed the protocols described in Evans and Chipouras (op. cit., 1991). Control experiments tested the sensitivity of the permeabilized rings to Ca^{2+}. Actual free Cd^{2+}, Ni^{2+}, or Ca^{2+} concentrations in the EGTA buffer solutions were calculated using the computer program of Chang et al. (Comp. Biol. Med. 18: 351-366, 1988).

As we found previously (Evans and Chipouras, op. cit., 1991), the permeabilized rings were very sensitive to external Ca^{2+} concentrations, contracting in a concentration-dependent manner with an EC_{50} of approximately 10 nM. However, we were unable to produce any contraction of these rings when either Cd^{2+} (N=5) or Ni^{2+} (N=5) were applied over the concentration range of 0.2 pM to 1 mM (Cd^{2+}) or 200 pM to 40 μM (Ni^{2+}). Addition of 1 μM CaM in three experiments with each metal did not stimulate contraction. This was not especially surprising since our earlier studies suggested that intracellular CaM was not limiting the response to Ca^{2+} (Evans and Chipouras, op. cit., 1991). Thus, we must conclude that our original results (Evans et al., op. cit., 1991) were flawed, and despite published data suggesting intracellular proteins such as CaM as potential sites for heavy metal toxicity (see above), the sites of action of these heavy metals on VSM from the shark aorta must be entirely sarcolemmal. These studies were supported in part by NSF DCB 8916413 (DHE) and NIH EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies.