RESPONSE OF VENTRAL AORTIC RINGS FROM THE SHARK, SQUALUS ACANTHIAS, TO CADMIUM AND OTHER VASOACTIVE SUBSTANCES

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Earlier studies have demonstrated that the branchial vasculature of the dogfish shark, Squalus acanthias, is responsive to both epinephrine and carbachol (Evans and Claiborne, Bull. MDIBL 21: 9-11, 1981; J. Exp. Biol. 105: 363-371, 1983), and that isolated vascular smooth muscle (VSM) rings from the ventral aorta of this species are responsive to isoproterenol and carbachol (Solomon et al., Bull. MDIBL 25: 146-149, 1985). Preliminary investigations of the effects of cadmium on the isolated, perfused head of the dogfish demonstrated that the branchial vasculature is also extremely sensitive to this heavy metal, with vasoconstriction at 10^{-7} M (11 ppb), and more significant vasoconstriction produced at concentrations between 10^{-6} to 10^{-4} M (Evans et al., Bull. MDIBL 26: 139-141, 1986). This finding of a consistent vasoconstrictory action of Cd^{2+} on the VSM of the shark gill was of some interest because the role of Cd^{2+} in hypertension is still quite controversial, and various studies on isolated VSM rings and strips have usually demonstrated vasodilation at micromolar concentrations, but vasoconstriction at concentrations above approximately 10^{-4} M in rats (see Kopp, In: Cadmium, E. C. Foulkes, Ed., Springer-Verlag, Berlin, pgs. 195-280, 1986). In addition, it has recently been suggested that the vascular endothelium may be the target for acute cadmium toxicity (Nolan and Shaikh, Life Sci. 39: 1403-1409, 1986). Since the perfused head maintains intact cranial and spinal nerves, which could allow feedback systems affecting branchial hemodynamics in response to applied vasoactive substances, as well as a vascular endothelium which could not be experimentally removed, we examined the effects of Cd^{2+} and other vasoactive substances on isolated, VSM rings from the ventral aorta of Squalus acanthias.

Rings of VSM were removed from the ventral aorta of the dogfish shark between the third and fourth branchial arches, stripped of vascular endothelium by abrasion with a roughened, wooden rod, and mounted in 10 ml of elasmobranch Ringer's solution bubbled with 1% CO_2 in air (pH 7.8) in an organ chamber. The VSM ring was suspended with wire loops between a stationary pole and a Gould-Statham strain transducer inputted to a Gilson Duograph. Initial tensions were set at 1 g for at least 30 minutes before addition of vasoactive substances. At this tension the rings had an internal diameter of approximately 2 mm.

Epinephrine produced vasodilation (N = 6; maximal response: 400-800 mg decrease in tension) at concentrations above 10^{-8} M (EC_{50} approximately 5 x 10^{-7} M), indicating that the ventral aorta is somewhat less sensitive than the intact branchial vasculature (Evans and More, submitted to J. Exp. Biol.). However, this sensitivity to epinephrine was still at least twice that determined previously for isoproterenol (Solomon et al., Op. Cit., 1985). Carbachol produced vasoconstriction (N = 7; maximal response: 2-4 g increase in tension) at concentrations of 10^{-7} M and above (EC_{50} approximately 5 x 10^{-7} M), indicating a somewhat greater sensitivity of these rings than that described previously for carbachol (Solomon et al., Op. Cit., 1985). Interestingly, if the carbachol concentration:response curve was generated after the rings had been exposed to increasing epinephrine concentrations (final concentration = 10^{-4} M; in an effort to maximize potential

vasoconstriction by pre-dilation) the sensitivity was significantly reduced. The EC_{50} was now approximately 10^{-6} M (N = 6), suggesting that there is some antagonism between some component(s) of the two stimulus-response coupling mechanisms. Torphy et al. (J. Pharmacol. Exp. Ther. 227: 694-699, 1983) found similar functional antagonism between the effects of methacholine and isoproterenol on canine tracheal smooth muscle, and determined that methacholine inhibited the isoproterenol-stimulated cyclic AMP-dependent protein kinase activity in a dose-dependent manner.

$CdCl_2$ produced vasoconstriction (maximal response: 100-800 mg increase in tension) of the rings at concentrations of 10^{-5} M and 10^{-4} M (17 ± 6% maximal tension, S.E., N = 7, at 10^{-5} M) when they had been pre-dilated by the addition of 10^{-5} M epinephrine. However, if the rings were not pretreated with epinephrine (N = 3), Cd^{2+} produced vasoconstriction at 10^{-6} M, and vasoconstriction at 10^{-5} M (51 ± 29%) greater than that found with pre-dilated rings. This finding suggests that the stimulus-response coupling pathways of epinephrine and Cd^{2+} may interact. However, these data indicate that the VSM rings are somewhat less sensitive to Cd^{2+} than the branchial vasculature itself, since our earlier study indicated sensitivity to concentrations as low as 10^{-7} M (Evans et al., op. cit.). This apparent reduced sensitivity of isolated rings vs. intact branchial bed is consistent with other data (e.g. Jones and Gwirtz, Drug Devel. Res. 7: 3-22, 1986), but the responses usually have been shown to be qualitatively the same when comparing isolated rings or strips and intact vascular beds. Finally, it is important to note that, since the isolated rings in the current experiments did not have functional endothelial cells, it is apparent that this tissue is not critical to the vasoactive effects of Cd^{2+} on shark VSM.

In summary, these studies demonstrate that the isolated, aortic ring from the shark, Squalus acanthias, is sensitive to Cd^{2+}, and that the vascular endothelium is not necessary for the vasoconstrictive response. Moreover, we have confirmed that this tissue is responsive to both epinephrine and carbachol, and have preliminary data suggesting that the stimulus:response coupling pathways of these two substances interact, as do the pathways for epinephrine and Cd^{2+}. We intend to use this model system to investigate further the mode(s) of action of Cd^{2+} on vascular smooth muscle. It is obvious that the consistent vasoconstrictory action of Cd^{2+} on this tissue cannot be explained by simple blockade of Ca^{2+} channels, which is usually suggested as the model of action of this metal (e.g. Kopp, Op. Cit., 1986). Our initial focus will be on the interaction of Cd^{2+} with the effects of extrinsic, vasoactive agents such as epinephrine, carbachol and Ca^{2+} on the isolated VSM ring. (Supported by NIEHS grant 1 P50 ES 03828-01 to the Center for Membrane Toxicity Studies and NSF PCM 8302621 to DHE.)