EVIDENCE FOR A ROLE OF SUPEROXIDE AND PEROXYNITRITE IONS IN VASOREACTIVITY OF THE AORTIC VASCULAR SMOOTH MUSCLE IN THE DOGFISH SHARK, *SQUALUS ACANTHIAS*.

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Surprisingly, nitric oxide (NO) induces constriction, not dilation, of smooth muscle rings from the ventral aorta of the dogfish shark (Evans, D.H. and Gunderson, M.P., Am. J. Physiol. 274: R1050-R1057, 1998). Recent evidence suggests that many of the cytotoxic effects of NO are secondary to the interaction of NO with superoxide ions (SO) and the generation of the very reactive peroxynitrite ion (PN; e.g., Beckman, J.S. and Koppenol, W.H., Am. J. Physiol. 271: C1424-1437, 1996). In fact, generation of these molecules is now thought to be the basis for a variety of diseases, including arthritis, atherosclerosis, diabetes, ischemia-reperfusion injury, and Alzheimer's disease. We hypothesized that SO and/or PN may be involved in the NO-induced constriction of vascular rings from the shark ventral aorta. Last year, we demonstrated that the addition of a membrane-permeable superoxide dismutase mimetic (Tempol; 4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl; e.g., Nilsson, U.A. J. Biol. Chem. 264: 11131-11135, 1989) dilated aortic rings from the dogfish and reduced the NO-induced constriction (Evans, D.H. and Hagen, J.E.C., Bull. MDIBL 39: 16, 2000). These data suggest that at least SO plays a role in the NO response. The following experiments were designed to examine a putative role for SO and PN more directly.

Isolated rings of the ventral aorta from *S. acanthias* were prepared as described previously (Evans and Gunderson, *Op. Cit.*). In all experiments, the rings were not pre-contracted (ca. 500 mg tension) and had an intact endothelium. To examine a putative role for SO, paired rings were exposed to either 5 mM Tempol or the same volume (100 μl) of the carrier, distilled water. Both rings also had 100 μM L-NAME added to inhibit the production of NO, which could have reduced the concentration of SO by generation of PN. After the tension in the rings stabilized, 10 μM pyrogallol was added to each ring. Pyrogallol has been shown to produce SO (e.g., Xie, Y. W. and Wolin, M.S., Circulation 94: 2580-6, 1996). Pyrogallol alone constricted the rings significantly (124 $\pm$ 64.5 mg (SE); $p = 0.004$; Wilcoxon test, two tailed; $N = 8$), and 5 mM Tempol inhibited the response to pyrogallol significantly (constriction was 29.5 $\pm$ 10.2 mg; $p = 0.016$, two tailed, $N = 8$). Interestingly, the constriction produced by pyrogallol in the presence of Tempol, which should have bound all of the pyrogallol-generated SO, was still significant ($p = 0.031$, two tailed). This suggests that either excess SO was produced (unlikely since the Tempol concentration was 500X the pyrogallol concentration) or some unknown constrictory factor was present. The fact that Tempol only reduced the NO-induced constriction by 50% in our earlier experiments (Evans, D.H. and Hagen, J.E.C. Bull. MDIBL 39: 16, 2000) supports this conclusion. Nevertheless, our data support our previous hypothesis that superoxide ions may play a role in vasoactivity in the ventral aorta of the dogfish shark.

To determine if peroxynitrite ions are also involved in the reactivity of the shark ventral aorta, paired rings were exposed to either the PN scavenger ergothioneine (e.g., Aruoma, O. I., et al., Biochem Biophys Res Commun 231: 389-91, 1997) or carrier (90 ul of distilled water). In four of the experiments, both rings were secondarily exposed to 0.1 mM sodium nitroprusside (SNP, a known NO donor). Addition of 1 mM ergothioneine dilated the rings significantly, compared to the control rings (-158 ± 37 mg; $p = 0.002$, Wilcoxon test, two tailed; $N = 10$). Moreover, ergothioneine inhibited the constrictory effect of SNP (2.25 ± 4.47 mg vs. 28.29 ± 3.61; Student's t-test, paired, two tailed). These data suggest that PN is tonically released from the rings, partially maintaining constriction, and that the constrictory effect of NO can be completely inhibited by scavenging PN. Interestingly, SNP did not dilate the rings in the presence of ergothioneine, which suggests that all of the generated NO reacted with tissue SO to produce PN, which was scavenged by the ergothioneine.

Our data support the following hypothesis: NO is tonically released by the aortic tissue (either endothelium or smooth muscle), but it immediately becomes a constrictory rather than dilatory agent because of the presence of SO and production of PN. The role this axis plays in normotension in fish blood vessels remains to be seen. (Supported by NSF IBN-9604824 to DHE)