INITIAL CHARACTERIZATION OF ENDOTHELIN RECEPTORS IN VASCULAR SMOOTH MUSCLE OF THE SHARK, SQUALUS ACANTHIAS

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Endothelin (ET) is now considered to be the most potent vasoconstrictor known (see Masaki, Endocr. Revs. 14: 256-268, 1993 for a recent review). ET plasma concentrations increase in a variety of pathological states including essential hypertension and stable chronic renal failure, but it is more likely that ET is a paracrine, rather than a hormone, and its actual role in normal and pathological states remains to be determined. To date, three ETs have been sequenced, termed ET-1, ET-2, and ET-3. It is now clear that ETs are synthesized in a variety of tissues. Secretion of ET from endothelial cells is stimulated by a variety of secretagogues, including angiotensin, vasopressin, epinephrine, hypoxia, and shear stress. Secretion is inhibited by both natriuretic peptides and endothelium-derived relaxation factor (EDRF=nitric oxide). Three receptors for the endothelins have been cloned and designated ET_A, ET_B, and ET_C. ET_A is apparently found exclusively on vascular smooth muscle (and is considered to be the only ET receptor in aortic VSM), is much more sensitive to ET-1 (and ET-2) than ET-3 and is inhibited specifically by compounds such as BQ 123. ET_B has a more general distribution (including the vascular endothelium itself, as well as venous VSM) and displays equal sensitivities to the three ET peptides. ET_C, selectively stimulated by ET-3, has just recently been cloned (Karne et al., J. Biol. Chem. 268: 19126-19133, 1993) from Xenopus dermal melanophores, but its distribution in mammalian tissues is unstudied. The endothelins have been shown to produce a variety of actions in addition to vasoconstriction including inotropic and chronotropic stimulation of the heart and decrease in glomerular filtration rate and Na reabsorption in the medullary collecting duct. Of particular interest is the early finding that ET-1, which produces vasoconstriction when released abluminally from endothelial cells, can actually act as a paracrine to produce vasodilation, via interaction with luminal endothelial ET_B receptors which stimulate the production of NO and, hence, relaxation of VSM. This dual receptor system apparently mediates the often described transient hypotension followed by hypertension produced by injecting endothelins into intact mammals.

The role of ET in fishes is relatively unstudied. ET-1 immunoreactivity has recently been described in the medaka brain, gill, and kidney, as well as the brain of the lamprey (Kasuya et al., J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S463-S466, 1991). ET-1 also has been shown to contract vascular rings from both trout (Olson et al., Am. J. Physiol. 260: H1214-H1223, 1991) and catfish (Poder et al., Can. J. Physiol. Pharmacol. 69: 215-217, 1991) mesenteric arteries and cardinal veins, but the trout ventral aorta was refractory. On the other hand, Miller and Vanhoutte (in Ryan and Rubanyi, "Endothelial Regulation of Vascular Tone, Marcel Dekker, Inc., New York, pgs. 3-20, 1992) did show ET-1 mediated contraction of trout ventral aorta.

The present study was undertaken to examine the sensitivity of shark VSM to ETs and to try to distinguish the type of receptor involved. The experimental set-up and protocols have been described previously (e.g., Evans, J. Comp. Physiol. 162: 179-183, 1992). Two types of endothelium-free VSM were used: ventral aorta and anterior intestinal vein. ET-1 produced a concentration-dependent contraction in both preparations ($N = 6$), with EC_{50s} of approximately 15 nM in the aorta and 1 nM in the vein. ET-3 was nearly as efficacious in both preparations, suggesting that ET_B rather than ET_A receptors were involved. The fact that even 3 uM BQ 123 did not inhibit the ET-1 induced contraction of the aorta ($N = 4$) supports this hypothesis. Thus, our preliminary data suggest strongly that ET receptors are indeed present in shark vascular smooth muscle, and display high sensitivity to even the mammalian peptides. Most importantly, these data also suggest that ET_B receptors are present, even in the aortic VSM. This is the first description of such receptors in aortic VSM in the vertebrates. (Supported by NSF IBN-9219122 and IBN-9306997 to DHE as well as EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies)