PHARMACOLOGIC CHARACTERIZATION OF AN ET_B RECEPTOR IN THE GILL OF THE SHARK, *SQUALUS ACANTHIAS*

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We have proposed that the endothelin receptor that mediates the constriction of the shark ventral aorta produced by endothelin is of the ET_B type (Evans, D.H. et al., *J.Comp. Physiol.* B 165: 659-664, 1996). The ET_A endothelin receptor is usually expressed in the arterial tissue of mammals, although ET_B sometimes mediates contractions (e.g.,Stjernquist, M. *Cell Tiss. Res.* 292: 1-9, 1998). To determine if the gill of the shark also expresses an endothelin receptor (and characterize its type) we froze shark gills in liquid nitrogen and shipped them back to Gainesville. After thawing, gill filaments were scraped with a scapel to remove the gill epithelium and associated blood vessels from the underlying cartilage, and membrane fragments were prepared for radioligand binding studies using the techniques we have previously described for our characterization of the receptors for natriuretic peptides in the same tissue (Donald, J.A. et al., *Gen.Comp.Endocrinol.* 106: 338-347, 1997). All calculations were performed using Prism (GraphPad Software, Inc.)

Specific ET-1 binding was maximal after 90 min. incubation and was saturable at a single site, with an apparent K_d of 68.8 ± 10.9 pM (mean ± s.e.) and a B_{max} of 780 ± 59.7 fmol.mg $protein^{-1}$ (N = 3), calculated by non-linear regression. Unlabeled ET-1 competed with ^{125}I-ET-1 at a single site with an IC_{50} of 5.11 ± 0.99 nM (N = 10; K_i = 3.57 ± 0.689 nM). ET-3 also displaced ^{125}I-ET-1 with an IC_{50} of 8.93 ± 4.90 nM (N = 8), not significantly different from the IC_{50} of ET-1 (p = 0.4), suggesting the presence of an ET_B receptor. The ET_B-specific agonists sarafotoxin S6c, IRL 1620, and BQ-3020 also displaced the radiolabeled ET-1 with high efficacy: IC_{50} = 1.77 ± 1.31 nM (N = 8), 32.8 ± 18.7 nM (N = 7), and 37.2 ± 13.5 nM (N = 7), respectively. All competitions were at a single site. Only the IC_{50} of BQ-3020 was significantly above (p = 0.01) that of ET-1. Our data support the conclusion that the dogfish gill tissue expresses an endothelin receptor of the ET_B-type, as does the ventral aorta. The functional site of the receptor is unknown but could be vascular or on the pillar cells (either site of which could control gill perfusion). In fact, Sundin and Nilsson (*J. Comp. Physiol.*, in press) have recently shown that endothelin can redistribute blood flow in the trout gill lamellae by contracting pillar cells. On the other hand, the ET_B receptor may be on the gill epithelial cells (pavement and mitochondrion-rich) which control such important functions as gas exchange, osmoregulation, nitrogen excretion, and acid-base balance. Recent evidence suggests that endothelin can inhibit Na^+-K^+-ATPase in the inner medullary collecting duct of the mammal (Zeidel, M., *Am. J. Physiol.* 265: F159-F173, 1993), but stimulates the same transport protein in the adrenal zona glomerulosa (Pecci, A. et al. *J. Steroid Biochem. Molec. Biol.* 50: 49-53, 1994) and stimulates both Na^+-K^+-ATPase and the Na-K-2Cl cotransporter in the cerebral capillary endothelium (Kawai, N. et al., *J. Neurochemistry* 65: 1588-1596, 1995). Since these transporters play fundamental roles in fish osmoregulation (e.g., Karnaky, K., in *"The Physiology of Fishes"*, 2nd Edition, ed. D. Evans, Boca Raton, CRC Press, pgs. 157-176, 1998), further experiments to determine the cellular site of the ET_B receptor, and its effect on gill function are warranted. (Supported by NSF IBN-9306997 and IBN-9604824, and AHA 9507715S)