# THE ANGIOTENSIN II RECEPTOR ON THE KILLIFISH OPERCULAR EPITHELIUM

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The evidence of a role for the renin-angiotensin system in regulating fluid balance in fish is primarily derived from the effects of angiotensin II (AII) and its analogs on drinking rates (Takei, Y. and Tsuchida, Am. J. Physiol.279:R1105-R1111, 2000) and on blood pressure (Qin, Z-L., et al., Gen. Comp. Endo.115:122-131, 1999). In terms of AII regulation of ion transport at the tissue level, recent evidence has indicated that the chloride cells of the seawater eel gill possesses AII type 1 (AT1) receptors which reveal a biphasic response in the catalytic activity of the Na/K-ATPase (Marsigliante, S. et al., J. Mol. Endo.18:67-76, 1997). Electrophysiological evidence is lacking as to the effects of AII on chloride cells. Furthermore, the nature of the AII receptor sub-type depends upon whether the tissue is vascular or epithelial.Thus, we determined the electrophysiological response of the killifish opercular membrane to doses of fish AII and determined the nature of the receptor sub-type with AII analogs and receptors antagonists.

The opercular epithelium of the killifish is a sheet epithelium that contains chloride cells which have similar ion transport properties as the chloride cells found in the gill (Evans, D., et al., J. Exp. Zool. 283:641-652, 1999). The advantage of the opercular epithelium is that solution substitutions may be made on the basolateral (blood) and apical (seawater) sides of the tissue. Killifish were collected at Northeast Creek and placed in aquaria at MDIBL for at least two weeks prior to experimentation. The serum osmolality of the killifish prior to removal of the opercular epithelium was 354±4 mOsm/kg (mean±sem, n=13 fish). Opercular epithelia were bathed on both sides with physiological saline (354±2 mOsm/kg, pH 7.8, n=6) at room temperature. Addition of teleost angiotensin II (Asn-Arg-Val-Tyr-Val-His-Pro-Phe, Sigma) revealed a dose-dependent inhibition of short-circuit current and transepithelial voltage with an $EC_{50}$ of 1.93μM (n=6 opercular epithelia, OE) The effects of fish AII on opercular electrophysiology were observed only upon addition to the basolateral side and were dependent on the presence of chloride ions in the basolateral saline. At 1μM fish AII inhibited short-circuit current by 69±12% (n=6 OE). Pre-treatment of the opercular epithelium with100μM [$Sar^1$, $Thr^8$]-angiotensin II (Sar-Arg-Val-Tyr-Ile-His-Pro-Thr) followed by addition of 1μM fish AII resulted in a significant inhibition of short-circuit current of 36±9% (n=6 OE, $p<0.05$ compared with AII alone). Other angiotensin receptor antagonists including 100μM [$Sar^1$, $Val^5$, $Ala^8$]-AII, 100μM Losartan (AT1 receptor antagonist, a gift from Merck) and 100μM PD123319 (AT2 receptor antagonist, a gift from Parke-Davis) were without effect on the fish AII response at 1μM. Our results indicate that the chloride cells in the opercular epithelium contain AII receptors which are located on the basolateral membrane and are dependent on extracellular chloride. These receptors are blocked by [$Sar^1$, $Thr^8$]-AII but are not affected by AT1 nor AT2 angiotensin II receptor antagonists. These observations are similar to the angiotensin II receptor characterization studies of the vasopressor responses in the toadfish (Qin, Z-L., et al., 1999) in which [$Sar^1$, $Ile^8$]-AII completely blocked and Losartan and PD123319 partially blocked the angiotensin II vasopressor response while evidence presented by Marsigliante, S. et al., 1997 suggested the presence of an AT1 in the eel gill. The findings of AII receptors in the OE indicate that chloride cells may regulate plasma volume and may provide a model to study the effects of AII on chloride cell ion transport. Supported by NSF-OPP9613738 (DP), NIEHS-P30 ES03828 (MDIBL Center for Toxicity Studies, to KK) and MDIBL Dahlgren Fund (DP) and Senior (KK) Fellowships.