THE ROLE OF PROSTAGLANDINS IN THE INHIBITION OF NaCl TRANSPORT ACROSS THE OPERCULAR SKIN OF THE KILLIFISH, *FUNDULUS HETEROCLITUS*

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Our initial studies demonstrated that both endothelin (ET) and nitric oxide (NO) could produce measurable inhibition of the short-circuit current (Isc) across the killifish opercular epithelium (Evans, D.H. et al. Bull. MDIBL 39: 17, 2000), the standard model for the teleost chloride cell (e.g., Evans, D.H. et al. J. Exp. Zool. 283: 641-652, 1999). More recently, we found that approximately 20% of the ET-induced inhibition of the Isc is actually mediated by the release of NO (Evans, D.H. et al., Bull. MDIBL 41: 8, 2002). Because we and others have found that prostaglandin E (PGE_2) can also inhibit the Isc across the opercular epithelium (Eriksson, O. et al., Acta Physiol Scand 125: 55-66, 1985; Van Praag, D. et al., Gen Comp Endocrinol 67: 50-7, 1987; Evans, D.H. et al., Bull MDIBL 41: 9, 2002), the present study was undertaken to investigate further the pathways involved in the inhibition produced by ET.

The experimental protocol was as described previously (Evans, D.H. et al., *Op. Cit.*, 2000), with agonists or inhibitors added to the basolateral side of the opercular epithelium mounted in an Ussing chamber. None of the experimental treatments altered transepithelial resistance.

Addition of the cyclooxygenase (COX) inhibitor indomethacin (10^{-5}M) to the basolateral side of the opercular epithelium (N = 5) had no effect on the Isc, contrary to the addition of the NOS inhibitor L-NAME (Evans, D.H. et al., Bull MDIBL 41: 8, 2002), suggesting that there is no tonic release of PGs under non-stimulated conditions. Subsequent addition of the ET_B receptor agonist SRX S6c inhibited the Isc by 46 ± 9 % (SE; N = 8) in control skins, but only by 5.2 ± 1.3% in the skins that had been pretreated with Indomethacin. This nearly 90% reduction in the effect of SRX suggests that the vast majority of the SRX inhibition of the Isc is mediated by the release of a prostaglandin. To determine if PGs other than PGE might also mediate this inhibition, we tested the ability of PGD, $PGF_{2\alpha}$, and thromboxane to alter the Isc. Neither PGD nor $PGF_{2\alpha}$ produced any change in the Isc (N = 5), and two thromboxane agonists (U-46619 and I-BOP) also were without effect (N = 4); all agents were tested at 10^{-10} to 3 x 10^{-6} M. In most experiments, 10^{-7} M SRX was added at the end of the cumulative addition of the putative agonist, and significant inhibition was always observed, suggesting that experimental tissues were responsive to the usual ET-receptor mediated signaling system. Finally, we delineated the subtype of COX involved by comparing the ability of specific inhibitors of COX-1 and COX-2, SC-560 and NS-398, respectively, to attenuate the SRX effect. In the presence of SC-560, SRX inhibited the Isc by 24 ± 3.7 % (N = 5), but only by 4.8 ± 1.1 % in the presence of NS-398. Moreover, addition of NS-398 to the unstimulated skin produced a 16 ± 1.2 % (5) increase in the Isc. These two experiments suggest that COX-2 mediates the release of PG upon stimulation of ETB receptor in killifish opercular epithelium. (Supported by NSF IBN-0089943 to DHE)