A ROLE FOR SUPEROXIDE IN THE INHIBITION OF NaCl TRANSPORT ACROSS THE OPERCULAR SKIN OF THE KILLIFISH, *FUNDULUS HETEROCLITUS*

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We have demonstrated that the transport of NaCl across the killifish opercular epithelium (the model for salt extrusion by the marine teleost gill) is inhibited by an axis of signaling agents that involve the ET_B (endothelin) receptor stimulating the production of both nitric oxide (NO) and prostaglandin (PGE), each of which inhibit the short circuit current (Isc) across this epithelium (Evans, D.H. et al. Bull. MDIBL 39: 17, 2000; Evans et al., Bull. MDIBL 41: 8, 2002; Evans and Rose, this volume). Since some apparent effects of NO can be mediated by interactions with superoxide ions (SO) (Beckman, J.S. and Koppenol, W.H. Am. J. Physiol. 271: C1424-37, 1996), we tested the effect of the superoxide dismutase mimetic Tempol on the Isc across the skin, as well as on the inhibitory effect of the ET_B-specific agonist SRX S6c, which can initiate the cascade involving NO and PGE.

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 skin, mounted in an Ussing chamber. No changes in resistance were seen for any of the treatments.

When 5×10^{-3} M Tempol was added to the unstimulated opercular skin, there was no net change in Isc (N = 7), indicating that tonic production of SO does not have an effect on the Isc. However, when 10^{-7} M SRX S6c was added subsequently, the Isc was inhibited by 28.4 ± 6.25 % (SE; N = 7) in the control skins, but by only 18.6 ± 4.77 % ($p < 0.01$ compared to control) when Tempol was present. Since Tempol, like SOD, metabolizes SO, inhibiting its direct effects and/or any interaction with NO to produce peroxynitrite (PN), a very reactive species (e.g., Beckman and Koppenol Op. Cit., 1996), our data suggest that SO does play a role in the inhibition of the Isc of the killifish opercular epithelium produced by stimulation of the ET_B receptor. To clarify whether SO is acting directly or via interaction with NO, we compared the ability of the NOS inhibitor L-NAME (10^{-5} M) plus the COX inhibitor indomethacin (10^{-5} M) to inhibit the SRX effect with the effect of these two inhibitors plus Tempol (5×10^{-3} M). The fact that inhibition of NOS plus COX inhibited the SRX effect by 84% (N = 6), compared to 100% (N = 6) when Tempol was also added, suggests that at least some of the effect of SO on the Isc is direct, not through interaction with NO.

Thus, our working hypothesis is that stimulation of ET_B receptors increases the production of prostaglandins (see Evans and Rose, this volume), NO (Evans et al., *Bull. MDIBL* 41: 8, 2001), and SO, each of which can inhibit the Isc across the killifish opercular epithelium. (Supported by NSF IBN-0089943 to DHE)