NaCl transport across the opercular epithelium of Fundulus heteroclitus is inhibited by an endothelin to NO, superoxide, and prostanoid signaling axis

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epithelium was removed by dissection under a microscope. The tissue was mounted over a 3 mm diameter aperture recessed in a Lucite plate and held in place by a small amount of silicone grease and a Lucite ring to minimize edge damage. The plate was inserted into a Lucite Ussing Chamber (Jim’s Instruments) so that the 3 mm epithelial circle separated two chambers, each containing 2 ml of killifish Ringer (26) bubbled with 100% oxygen. Each chamber had ports to allow for aeration, solution addition, and removal and the insertion of Ag-AgCl2 electrodes to measure transepithelial electrical potential and resistance on Isc. The output from these electrodes was monitored by a University of Iowa model 742C dual voltage clamp and recorded and saved by a Biopac MP100 data-acquisition system, using AcqKnowledge software on a Macintosh computer. Experiments were initiated only if and when a stable Isc (Isc > 50 nA/cm2) and resistance (>35 ohms/cm2) were reached, usually within 30 min. During the course of each experiment, electrical resistance was monitored by the automatic generation of a ±1 mV pulse every minute by the voltage clamp and recording the Isc deflection. No systematic changes in tissue resistance were observed in the course of any of the experiments. In all experiments, equal volumes of solution (agonist/inhibitor vs. carrier) were added to both sides of the tissues (experimental and control) to avoid volume and osmotic effects, because our initial studies confirmed that even a 2% osmolarity differential across this epithelium can affect the Isc (33).

Experimental chemicals were solubilized according to manufacturer’s instructions, and the final concentrations used were determined from our earlier studies with these agonists/inhibitors or from published studies from other laboratories. In each case, the substance was dissolved in the appropriate solvent, subdivided, stored either frozen (−70 or −20°C) or at 4°C, and made up to the desired concentration by further dilution in the solvent and/or in the experimental medium (killifish Ringer) the day of the experiment. The final volume of agonist/inhibitor added ranged from 0.1 to 4% of the initial volume of the experimental medium. ET-1 (human) and SRX6S6c (American Peptide, Sunnyvale, CA) were dissolved in 1% acetic acid and 50% DMSO, respectively, lyophilized (ET-1 only), and stored at −20°C. Sodium nitroprusside (Sigma, St. Louis, MO), TEMPOL (4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl; Sigma), and t-NNAME (N5-nitro-L-arginine methyl ester; Cayman Chemical, Ann Arbor, MI) were dissolved in killifish Ringer and stored at 4°C; 1-benzylimidazole-1[1-phenylmethyl]-H-imadazole; Cayman was dissolved in killifish Ringer and stored at −20°C. Indomethacin [1-[4-chlorobenzoyl]-5-methoxy-2-methyl-H-indole-3-acetic acid; Sigma] was dissolved in 100 mM NaHCO3, ethanol (3:1) and stored at 4°C. PGE2, carbaprostacyclin (6,9a-methylenene-11a,15-dis-hydroxy-prosta-5E,13E-dien-1-oic, Sigma), PGD2, PGF2a, U-46619 (9,11-dideoxy-9a,11a-dihydroxy-prosta-5Z, 13E-dien-1-oic acid), I-BOP {1S-[1-methoxy-2-[2-hydroxy(4-iodophenox)-1-butenyl]-7-oxa-bicyclo[2.2.1]hept-5-enoic acid}, SC550 [5-[4-chlorophenyl]-1-(4-methoxyphenyl)-3- (trifluoromethyl)-H-pyrrole], NS-398 [N-[2-(cyclohexyloxy)-4-nitrophenyl]-methylene sulfonamide], butaprost (9-oxo-11a,16R-dihydroxy-17-cyclobutyl-prosta-13E-en-1-O-acid, methyl ester), and sulprostone [N-(methylsulfonfonyl)-9oxo-11a,15R-dihydroxy-16-phenoxy-17,18,19,20-tetranor-prosta-5Z,13E-dien-1-amide] (all from Cayman Chemical) were dissolved in DMSO and stored at −20°C. Spermine NONOate (1-[2]-N-[3-aminopropyl]-N-[4-[3-amino- pylumamino]butyl]-amino)[diaceto-1-ium,2,1-diolate] was dissolved in 0.01 N NaOH on ice, purged with N2 and stored at −70°C.

The research protocols in this study were approved by IACUC committees at the University of Florida and the Mt. Desert Island Biological Laboratory.

All data are expressed as means ± SE. P values for statistical differences were calculated by the appropriate, two-tailed, paired or unpaired Student’s t-tests, and concentration-dependence data were analyzed using repeated-measures ANOVA and the appropriate post-tests. In the data analysis of all putative inhibitor experiments, each tissue served as its own control when testing the effect of the
inhibitor(s), but paired tissues (experimental vs. control) were compared when determining the effect of most of the putative inhibitors on the SRXS6c-mediated inhibition of the \( I_{sc} \). The exception was the COX-1 vs. COX-2 inhibitor study, where SC560 and NS-398 were applied to paired epithelia before SRXS6c was added, and the effect of either on the SRXS6c-mediated inhibition of the \( I_{sc} \) was compared with the sum of the control effects of SRXS6c in the previous experiments (l-NAME, indomethacin, etc.). In this case, unpaired statistical analyses were used. In all cases, \( P < 0.05 \) was taken as significant. Specific statistical analyses were performed using Prism (GraphPad Software, San Diego, CA) and are indicated in the text and figure legends.

RESULTS

Preliminary experiments determined that the cumulative addition of ET, sodium nitroprusside (SNP; NO donor), and PGE2 to both sides of the isolated tissue inhibited the \( I_{sc} \) across the operculum, so the ET receptor distribution was examined by comparing the effect of basolateral vs. apical addition of \( 10^{-7} \) M ET-1. Addition of ET-1 to the basolateral side of the opercular epithelium inhibited the \( I_{sc} \) to the same extent (31.5 ± 3.3%; \( n = 6 \)) as addition to both sides of the tissue (37.3 ± 8.6%; \( n = 5 \); \( P = 0.52 \); unpaired \( t \)-test, 2 tailed), but addition to the apical side did not produce a significant inhibition of the \( I_{sc} \) (8.0 ± 3.9%; \( n = 7 \); \( P = 0.09 \); \( t \)-test vs. zero), suggesting that the endothelin receptors are located on the basolateral surface. Because the effect was maximal after basolateral addition, all subsequent experiments tested the effects of putative agonists or inhibitors after addition to the basolateral solution.

To confirm that the response of the \( I_{sc} \) to ET-1 was concentration dependent, and to attempt to delineate the role of ET\(_{A}\) vs. ET\(_{B}\) receptors, the effects of the cumulative addition of ET-1 (agonist for both ET\(_{A}\) and ET\(_{B}\) receptors) or SRXS6c (ET\(_{B}\) specific) were monitored. Both agonists produced a concentration-dependent inhibition of the \( I_{sc} \), becoming significant at \( 10^{-8} \) M and reaching 30–40% at \( 10^{-7} \) M in each case (Figs. 1B and 2). ET-1 and SRXS6c were equipotent at all concentrations tested. Because SRXS6c produced significant responses (suggesting the presence of ET\(_{B}\) receptors), all subsequent experiments used SRXS6c as an ET agonist, to constrain the study to ET\(_{B}\)-mediated effects.

Similar experiments determined the concentration dependence of the response of the \( I_{sc} \) to either of two NO donors, SNP (Fig. 1C) and spermine NONOate (SPNO) or the prostanooids PGE2 (Fig. 1D) and carboxabaptacystein (CPR; stable analog of PGL2). Both NO donors (SNP and SPNO) produced a small, concentration-dependent inhibition of the \( I_{sc} \), reaching significance at \( 10^{-7} \) and \( 10^{-6} \) M, respectively (Fig. 3). The efficacy of both donors was the same at all concentrations. Both PGE2 and CPR also produced what appeared to be a concentration-dependent inhibition of the \( I_{sc} \), but only the effect of PGE2 reached statistical significant (Fig. 4). PGE2 was somewhat less effective than either ET or SRXS6c at equivalent concentrations.

Because these experiments determined that ET-1, PGE2, and NO donors produced concentration-dependent inhibition of the \( I_{sc} \), interactions between these putative signaling agents were examined by determining the effect of inhibition of either NOS by l-NAME or COX by indomethacin on the baseline (unstimulated) \( I_{sc} \) as well as the SRXS6c-mediated inhibition of the \( I_{sc} \). These experiments used paired epithelia from the same animal, and either \( 10^{-4} \) M l-NAME or \( 10^{-5} \) M indomethacin was added to the experimental tissue, and the same volume of carrier was added to the control tissue. Initial studies determined that any response was relatively rapid, so after 15 min, \( 10^{-7} \) M SRXS6c was added to experimental tissue, and an equal volume of the carrier was added to the control tissue, and the \( I_{sc} \) was recorded for an additional 30–60 min to equilibrium. Addition of l-NAME to the opercular epithelium eliminated the \( I_{sc} \) slightly but significantly (experimental vs. control: 130 ± 10.2 vs. 113 ± 7.99 \( \mu \)A/cm\(^2\); \( n = 25 \); \( P < 0.01 \), paired \( t \)-test, 2 tailed). Subsequent addition of SRXS6c inhibited the \( I_{sc} \), but this inhibition was reduced by 17% in those tissues that had been pretreated with the NOS inhibitor (Table 1). Inhibition of COX by indomethacin did not affect the initial \( I_{sc} \) (186 ± 37.5 vs. 191 ± 43.6 \( \mu \)A/cm\(^2\); \( n = 8 \); \( P = 0.79 \), paired \( t \)-test, 2 tailed), but it reduced the SRXS6c-mediated inhibition by nearly 90% (Table 1).

To determine if the effect of inhibition of NOS and COX (on baseline and SRXS6c inhibition) was additive, in another series of experiments both inhibitors were added to the tissue before the addition of SRXS6c. When l-NAME and indomethacin were added simultaneously, the initial \( I_{sc} \) did not change (124 ± 21 vs. 124 ± 26 \( \mu \)A/cm\(^2\); \( n = 6 \); \( P = 0.99 \), paired \( t \)-test, 2 tailed), demonstrating that the inhibition of COX abolishes the stimulation of the \( I_{sc} \) seen when l-NAME is added alone. The presence of both l-NAME and indomethacin inhibited the effect of subsequent addition of SRXS6c by 83% (Table 1), but the net effect was no greater than the effect of indomethacin alone.

Because NOS-mediated effects may be secondary to chemical interactions of NO with \( O_2^- \), another series of experiments examined the effects of the spin trap TEMPOL (\( 5 \times 10^{-3} \) M; a superoxide dismutase mimetic) on the baseline and SRXS6c-mediated inhibition of the \( I_{sc} \), as well as the effect of the simultaneous addition of l-NAME, indomethacin, and TEMPOL on these parameters. Addition of TEMPOL had no effect on the initial \( I_{sc} \) across the epithelium (256 ± 47.7 vs. 260 ± 47.9 \( \mu \)A/cm\(^2\); \( n = 7 \); \( P = 0.39 \), paired \( t \)-test, 2 tailed), but it did reduce the SRXS6c-mediated inhibition by 34% (Table 1), twice the effect of inhibition of NOS by l-NAME (Table 1). Simultaneous addition of l-NAME, indomethacin, and TEMPOL did not affect the \( I_{sc} \) (186 ± 53.4 vs. 185 ± 49.2 \( \mu \)A/cm\(^2\); \( n = 6 \); \( P = 0.90 \), paired \( t \)-test, 2 tailed), but it completely inhibited the effect of subsequent addition of SRXS6c (Table 1; percent reduction by SRXS6c not different from zero; \( P = 0.14 \)).

To attempt to differentiate between COX-1- and COX-2-mediated responses, the effects of SC560 (\( 10^{-6} \) M; COX-1-specific inhibitor) and NS-398 (\( 10^{-6} \) M; COX-2-specific inhibitor) on baseline \( I_{sc} \) and SRXS6c-mediated inhibition were studied in another series of experiments. Addition of SC560 had no effect on the initial \( I_{sc} \) (139 ± 25.5 vs. 138 ± 24.8 \( \mu \)A/cm\(^2\); \( n = 5 \); \( P = 0.89 \), paired \( t \)-test, 2 tailed), but the addition of NS-398 stimulated the \( I_{sc} \) by 16% (150 ± 23.6 vs. 129 ± 20.8 \( \mu \)A/cm\(^2\); \( n = 5 \); \( P < 0.01 \), paired \( t \)-test, 2 tailed). Subsequent addition of SRXS6c inhibited the \( I_{sc} \), but previous inhibition of COX-1 reduced this inhibition by 46%, and inhibition of COX-2 reduced the effect by 90% (Table 1).

Because PGE2 can bind to any of four receptors (termed EP\(_{1-4}\)), another series of experiments tested the effects of cumulative addition of either butaprost (EP\(_{2}\) specific) or sul-
prostane (EP1/3 specific; e.g., 3) on the $I_{sc}$ across paired opercular epithelia. Butaprost stimulated the $I_{sc}$ across the opercular epithelium in a concentration-dependent manner (reaching significance at $10^{-6}$ M); sulprostone produced the opposite effect on the $I_{sc}$, reaching significance at $10^{-7}$ M (Fig. 5).

To test the efficacy of other prostanoids, putative agonists (carbaprostacyclin for PGI2, U-46619 and I-BOP for thromboxane A2, PGF2α, or PGD) were applied to opercular tissues using the same protocol as described for the PGE2-mediated concentration-dependence experiments. In addition, the ability of the thromboxane synthase inhibitor 1-benzylimidazole ($10^{-5}$ M) to affect either baseline $I_{sc}$ or SRXS6c-mediated inhibition of the $I_{sc}$ was tested, using the protocol described for L-NAME, indomethacin, and TEMPOL. Neither of the putative TXA2 agonists ($10^{-10}$-$10^{-6}$ M U-46619 or I-BOP; $n = 4$) nor PGF2α or PGD2 ($10^{-10}$-$10^{-6}$ M; $n = 5$) produced any change in the $I_{sc}$ across the opercular epithelium (data not shown). Moreover, pretreatment with 1-benzylimidazole had no effect on the unstimulated $I_{sc}$ and did not blunt the SRXS6c-mediated...
DISCUSSION

Our data show that ET-1 and SRXS6c, two NO donors (SNP and SPNO), and PGE₂ each can inhibit the Išc across the opercular epithelium of the killifish Fundulus heteroclitus in a concentration-dependent manner (Figs. 1–4). This is the first demonstration of a putative role for ET and NO in modulating salt transport across this epithelium, which models the gill epithelium of marine teleost fishes (e.g., 26). Our finding that PGE₂ is inhibitory corroborates earlier studies using the same epithelial preparation (11, 58). The response to ET is mediated by basolateral receptors, as one might expect; the slight (and statistically insignificant) apical response may be due to leakage of the applied ET from the basolateral to apical surface, or a smaller population of apical receptors may be present, as has been described for other epithelia (e.g., 25). Since the ETₐ-specific agonist SRXS6c was as effective as ET-1 in inhibiting the Išc (Figs. 1 and 2), we conclude that stimulation of ETₐ-like receptors mediate this response to ET. Our data do not preclude the presence of ETA receptors in the opercular epithelium, however, but the response of the Išc to ET-1 appears to be wholly via ETₐ receptors.

The fact that incubation with 10⁻⁴ M L-NAME produced a small, but significant, stimulation of the Išc suggests that tonic release of NO inhibits the Išc in the unstimulated tissue. However, if COX is also inhibited by the simultaneous addition of 10⁻⁵ M indomethacin and 10⁻⁴ M L-NAME, this stimulation is lost (Table 1). Thus another explanation of the L-NAME-mediated stimulation is that the unstimulated Išc is actually set by the sum of tonic, COX-mediated stimulation and NOS-mediated inhibition. However, inhibition of COX alone by preincubation with 10⁻⁵ indomethacin did not change the unstimulated Išc, contrary to the inhibition of the Išc one might expect if this model were correct. Moreover, inhibition of COX-2 alone, using the specific inhibitor NS-398, actually
The opercular epithelium of Fundulus heteroclitus (P) prostanoid may be tonically controlling the stimulation of the ET-NO and ET-PG axes and/or the presence of other components in the inhibition produced by activation of the ET₉ receptor by SRXS6c.

It has become clear that the role of NO in a variety of signaling pathways is at least partially controlled by its effectivestantly instantaneous reaction (X = 7 × 10⁹ mol·l⁻¹·s⁻¹) with superoxide ions (O₂⁻) to form peroxynitrite (OONO⁻), an especially toxic molecule (e.g., 30). These three molecules have been termed “the good, the bad, and the ugly” (e.g., 2) because the highly oxidative O₂⁻ and OONO⁻ could produce physiological or pathophysiological responses directly, or merely because O₂⁻ removes NO from the system. The fact that the addition of NO scavenger TEMPOL (5 × 10⁻³ M) blunted the effect of subsequent addition of SRXS6c by 34%, twice the effect of the addition of l-NAME, suggests that O₂⁻ itself plays a role in this signaling axis in the opercular epithelium (Table 1). Indeed, if TEMPOL, l-NAME, and indomethacin were added simultaneously to unstimulated tissue, the effect of subsequent addition of SRXS6c was completely inhibited (Table 1). Because TEMPOL reduced the effect of SRXS6c significantly more than l-NAME (the NO-dependent component), we hypothesize that O₂⁻ itself is inhibitory and can be produced by a pathway that is stimulated by ET/SRXS6c. In fact, a recent study demonstrated that ET generated O₂⁻ in a COX-dependent pathway after brain injury in newborn pigs (1), so it could be that the TEMPO-dependenc effect on the opercular epithelium is actually the sum of reduction of NO-O₂⁻ interactions and reduction of COX-generated O₂⁻. It is notable that indomethacin alone reduced the SRXS6c effect to the same degree as indomethacin plus l-NAME or indomethacin plus l-NAME and TEMPOL, despite the fact that only the three inhibitors together produce inhibition that is statistically 100% (Table 1). This suggests that the COX-mediated prostanoid and O₂⁻ production is by far the dominant pathway in the operculum. It is clear that O₂⁻ production is not tonic, because TEMPOL did not affect the unstimulated Iₛₑ across the unstimulated Iₛₑ. Garvin’s group (42) recently showed that addition of TEMPOL increases the inhibition of Cl⁻ transport across the THAL of the rat loop of Henle produced by NO. The effect, however, appears to be via inhibition of a stimulatory response to O₂⁻ (rather than by removal of O₂⁻ and the subsequent stimulation of an NO-mediated inhibition), because exogenous production of O₂⁻ by the addition of xanthine oxidase/hypoxanthine stimulated Cl⁻ transport across the THAL. They concluded, therefore, that O₂⁻ itself is stimulatory and not just a modulator of NO concentration (44).

Table 1. Effect of various inhibitors on the SRXS6c-mediated reduction of the Iₛₑ across the opercular epithelium of Fundulus heteroclitus

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>%Reduction in Iₛₑ: SRXS6c Only (n)</th>
<th>%Reduction in Iₛₑ: SRXS6c After Drug Pretreatment (n)</th>
<th>F Value; Control vs. Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁶ M l-NAME</td>
<td>63.9±5.64(14)</td>
<td>53.0±6.19(14)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>10⁻⁷ M Indomethacin</td>
<td>46.4±9.07(8)</td>
<td>5.15±1.33(8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>10⁻⁸ M l-NAME + 10⁻⁵ M indomethacin</td>
<td>34.4±4.93(6)</td>
<td>5.78±1.78(6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5 × 10⁻³ M TEMPOL</td>
<td>28.4±6.25(7)</td>
<td>16.6±4.77(7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5 × 10⁻³ M TEMPOL + 10⁻⁴ M l-NAME + 10⁻⁵ M indomethacin</td>
<td>34.7±3.60(6)</td>
<td>4.58±2.59(6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10⁻⁶ M SC560</td>
<td>45.8±3.61(41)</td>
<td>24.4±3.65(5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>10⁻⁶ M NS-398</td>
<td>45.8±3.61(41)</td>
<td>4.76±1.15(5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. n values in parentheses. Iₛₑ, short-circuit current; TEMPOL, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl; l-NAME, N⁴-nitro-L-arginine methyl ester.
The fact that pretreatment with NS-398, but not SC560, inhibited the $I_{sc}$ suggests that there may be a COX-2-mediated, tonic inhibition of the $I_{sc}$, as is the case with NO (see above), in contrast to the indomethacin experiments that suggested that there is COX-mediated, tonic stimulation of the $I_{sc}$. At this point, we cannot differentiate between these two alternatives, but these experiments do demonstrate that COX-mediated, tonic production of prostanoids may have effects on salt transport across the epithelium, and they also suggest that there may be different roles played by COX-1 vs. COX-2. Because inhibition of COX-2 was twice as effective in attenuating the SRXS6c-mediated inhibition of the $I_{sc}$ as inhibition of COX-1 (Table 1), it appears that both COX-1 and -2 are involved in the production of prostanoids after ETB stimulation in this tissue but that COX-2 is the major effector. The physiological importance of COX-1 and COX-2 has become appreciated in the past few years, and both are expressed in the rat nephron. For instance, a recent study has shown that COX-1 mRNA predominates in the glomerulus, distal tubule, and collecting duct, whereas COX-2 message is localized in the glomerulus and medullary THAL (59). The genes for the homologues of COX-1 and/or COX-2 have been cloned for the zebrafish (21), two trout species (46, 51), and dogfish shark (62), where the clone was amplified from the rectal gland, a functional analog of both the marine teleost gill and THAL of the mammalian nephron (e.g., 40, 52). The latter study found that the specific COX-2 inhibitor, NS-398, reduced the Cl$^-$ secretion rate of the shark rectal gland. This study suggests that prostanoids are stimulatory, rather than inhibitory, contrary to the present findings and what has been published in the renal literature (e.g., 3). On the other hand, stimulation of salt secretion by the shark rectal gland has the same effect as inhibition of salt uptake by the mammalian nephron: increased salt excretion.

Because the PGE$_2$ receptors EP$_1$ and EP$_3$ predominate in mammalian renal tubules (e.g., 3), we attempted to differentiate between putative receptors in the killifish operculum by comparing the efficacy of the relatively specific agonists, butaprost (EP$_2$), and sulprostone (EP$_{13}$) in this system. The fact that butaprost produced a concentration-dependent stimulation and sulprostone produced a concentration-dependent inhibition (Fig. 5) suggests that EP$_2$ and EP$_1$ (and/or EP$_3$) receptors are present and that release of PGE$_2$ in the opercular epithelium can produce stimulation or inhibition, depending on the distribution of the respective receptors. Our data suggest that inhibitory receptors (EP$_1$ and/or EP$_3$) predominate, but the finding that the 1-NAME-induced stimulation of the $I_{sc}$ is inhibited by the simultaneous addition of indomethacin suggests the presence of at least some stimulatory receptors. The actual receptors present could be identified by immunological or molecular techniques, as well as measurement of intracellular second messengers in the future. It is important to note, however, that early work on the killifish operculum demonstrated that the $I_{sc}$ could be stimulated by isoproterenol and inhibited by epinephrine and arterenol (10) and subsequent studies showed that the stimulation vs. inhibition is mediated by $\beta$-adrenergic and $\alpha$-adrenergic receptors, respectively (37). In the killifish operculum, $\beta$-adrenergic receptors stimulate intracellular cAMP (38) and $\alpha$-adrenergic receptors stimulate intracellular inositol triphosphate (34), parallel to at least EP$_2$ and EP$_1$, respectively. Thus the intracellular second messen-

![Diagram of ET-inhibited NaCl transport](http://ajpregu.physiology.org/10.1152/ajpregu.00330.2004)

**Fig. 6.** Working hypothesis for the putative pathways of ET-inhibited NaCl transport across the fish gill. Two cells are diagramed, but the system may be expressed within a single cell. Width of the arrows is proportional to the presumed importance of the specific pathway in the axis. See text for details. ET-1, endothelin; ET$_B$, endothelin B receptor; NOS, nitric oxide synthase; COX-2, cyclooxygenase-2; NO, nitric oxide; EP$_1$, PGE$_2$ receptor; O$_2^-$, superoxide ion; ONOO$^-$, peroxynitrite ion.
(EP1/3) PGE receptors appear to be involved, although inhibition appears to be the most significant response. Our data do not allow a definitive model for the roles of NO and prostanoids in maintaining the unstimulated salt transport across this epithelium, but some aspects of this work provide evidence for some role for both effectors without any stimulation of the ET receptor. Figure 6 summarizes our current working hypothesis for the interactions between ET, NO, O2−, and PGE2 in inhibiting salt extrusion by the marine teleost gill epithelium, as modeled by the killifish opercular epithelium.

Perspectives

Because the opercular epithelium is the generally accepted model for the marine teleost fish branchial epithelium (see introduction), our data provide the first evidence that salt extrusion by the gill can be modulated by release of paracrine agents, in this case: ET, NO, O2−, and PGE2. Although we provide some evidence for a stimulatory prostanoid pathway, the bulk of the data suggest ET-stimulated and NO-O2−-PGE2-mediated inhibition of salt transport. The result would be salt retention, because the gill is the dominant site of salt secretion in marine teleosts (e.g., 24). This signaling pathway, therefore, has the opposite result from that found in the mammalian kidney, where ET, NO, and prostanoids are predominantly natriuretic because of inhibition of uptake in the renal tubules (see introduction). Because salt retention is important in fish in hypooosmotic environments (e.g., 13), we hypothesize that this paracrine modulating system is most important in freshwater species or euryhaline species as they enter freshwater. Indeed, gill tissue from the eel produces significantly more prostanoids (actually, PGD2 and 6-keto-F1α after acclimation to freshwater (5). Interestingly, PGE2 concentrations were very low in this tissue, as well as trout gill, in this study, and no significant changes in PGE2 concentrations were seen in either species after acclimation to freshwater vs. seawater (5).

The cellular site of ET, NO, and prostanoid production (as well as cellular receptors) in the teleost gill is currently under investigation. Zaccone’s group (64) demonstrated immunoreactivity for big-ET (ET prohormone) and NOS in what they term “neuroendocrine” cells in the branchial epithelium from a variety of teleosts and elasmobranchs. Our preliminary studies (12; and K. A. Hyndman, P. M. Piermarini, and D. H. Evans, unpublished observations) have localized immunoreactive NOS in cells distinct from the mitochondrion-rich cells (MRC; Cl− transporting) in both the killifish opercular epithelium and gill epithelium, but it is not clear if these are “neuroendocrine,” mucous, or pavement cells. In the stingray gill, we find immunoreactive big-ET is in the MRC and COX is expressed in the filamental central venous sinus, but in the killifish gill big-ET can be localized to epithelial cells distinct from the MRC, and COX is seen in the MRC. Thus it is clear that species differences may exist; nevertheless, localization of the effectors and receptors for this new signaling axis is of great interest for comparative vertebrate physiology and may provide new insights into paracrine control axes in the mammalian kidney.

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