Effects of cortisol and arsenic on seawater acclimation in killifish (*Fundulus heteroclitus*)

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The killifish, *Fundulus heteroclitus*, can withstand large changes in salinity, which require the gills to rapidly switch between NaCl absorption (freshwater, FW) and secretion (seawater, SW) to maintain salt balance. In SW, Cl secretion is accomplished by CFTR Cl channels. Relatively few studies have focused on the mechanisms whereby CFTR increases Cl secretion when fish move from FW to SW[1]. While Marshall et al.[1] have shown that this transition causes a dramatic increase in plasma cortisol that precedes increased CFTR mRNA, direct evidence for cortisol regulation of CFTR is lacking. Nonetheless, Singer et al.[2] have identified a consensus sequence of a putative glucocorticoid response element in the regulatory region of the killifish CFTR gene. Recent studies in cell culture have demonstrated that arsenic inhibits cortisol mediated transcriptional activation[3]. In killifish arsenic increases plasma Cl during the move from FW to SW, which was also when they were most vulnerable to arsenic toxicity[4]. These results are consistent with an inhibition of CFTR mediated Cl secretion. Accordingly, the goals of our research were to test the hypotheses that 1) cortisol stimulates CFTR gene expression in killifish and is required for FW to SW movement, and 2) arsenic inhibits cortisol stimulated CFTR gene expression.

Killifish were gradually acclimated to FW[5] and their response to SW challenge for 24-h was studied (i.e., direct transfer, FW to SW). Treatments included cortisol (40 nmol/g) and a cortisol-receptor antagonist, mifepristone (100 nmol/g) injected IP, arsenic (8000ppb) dissolved in swimwater, and controls (FW, SW, Sham/vehicle). CFTR transcript was quantified by real-time PCR[5].

CFTR mRNA levels increased when FW fish were injected with cortisol. Likewise, CFTR mRNA increased following SW challenge (which naturally increases cortisol levels) and this increase was even greater in cortisol injected animals. Furthermore, the increase in CFTR mRNA expression was blocked (with/without SW challenge) by mifepristone. If cortisol is essential for stimulating the physiological changes occurring during the transition from FW to SW, then the combination of mifepristone and SW challenge should be toxic. While this combination was not overtly toxic over 24-h, almost all (>80%) fish were moribund and this was the only treatment that produced such effects. These observations were confirmed with toxicity tests, as the same treatments produced 65% and 90% mortality after 48-h and 96-h (controls <10% at 96-h). Arsenic blocked CFTR mRNA expression following SW challenge (with/without cortisol). Decreased CFTR mRNA expression is consistent with the decreases in Cl secretion[5] and increases in plasma Cl[4] that have been observed following similar arsenic exposures. Future investigations will concentrate on defining the cellular mechanisms responsible for the arsenic inhibition CFTR expression. (Supported by an MDIBL New Investigator Award, Center for Membrane Toxicity Studies (NIEHS P30 ESO3828-18), Superfund Basic Research Program (NIEHS ESO7373), NCRR MBRIN (1-P20-RP-6463-01) and NSF REU (DBI-0139190).

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