Effects of glucocorticoid-receptor inhibition and arsenic on seawater acclimation

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The euryhaline killifish, *Fundulus heteroclitus*, has adapted to withstand abrupt changes in salinity. When fish move from freshwater (FW) to seawater (SW) the gills immediately secrete chloride[1]. The pituitary interrenal axis is responsible for cortisol release during this transition[2]. However, the responses cortisol regulates remain poorly characterized in killifish. Recent studies in mammalian cells revealed that arsenic inhibits cortisol-stimulated, glucocorticoid receptor (GR)-mediated transcriptional activation[3]. While little is known about the mechanisms of arsenic toxicity in fish, if arsenic blocks GR signaling then it could interfere with SW acclimation in the killifish. Accordingly, the goals of the present study were to investigate the hypotheses that 1) GR stimulation is required for killifish to move from FW to SW and 2) arsenic inhibits SW acclimation.

The GR antagonist, RU486, the mineralocorticoid receptor (MR) antagonist, spironolactone, and arsenic were used. Killifish were gradually acclimated to FW[1] and their responses (mortality, plasma chloride) to SW transfer following these treatments were measured.

RU486 interfered with SW acclimation. While mortality was not observed 24-h following transfer to SW, fish treated with RU486 were moribund and plasma chloride was significantly ($P<0.05$, $n=4$) elevated (265mM) compared to FW controls (158mM), shams (168mM) and RU486 injected fish that remained in FW (120mM). These effects are consistent with previous studies[4], which reported that fish treated with RU486 failed to stimulate transcription of CFTR following transfer to SW (24-h) and died at time points ≥48-h. MR involvement in SW acclimation was excluded as spironolactone had no effect on survival during SW transfer. We cannot eliminate the possibility that RU486 interfered with other steroid (e.g., estrogen, progesterone) receptors, although this is unlikely based on similarity of responses between male and female fish; and relative affinity for these receptors and their likely concentrations in the gills. Arsenic, at non-lethal levels (106μM), slightly increased plasma chloride levels (178mM) following SW transfer (24-h). In addition, fish were more sensitive to arsenic toxicity during SW acclimation. Arsenic (160μM) resulted in 65% and 90% mortality at 48 and 96-h following transfer to SW, whereas the same arsenic concentration was not toxic to either FW or SW acclimated fish maintained in those conditions. Collectively, these studies demonstrate that GR stimulation is required for FW to SW transfer and that arsenic disrupts chloride balance in the fish, making them more sensitive to arsenic toxicity during SW acclimation. (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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