

**IONIC REGULATION IN SOFTWATER-ACCLIMATED RAINBOW  
TROUT: A ROLE FOR GLUCOCORTICOID AND  
MINERALOCORTICOID RECEPTORS**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

The main corticosteroid hormone in fish, cortisol, plays a dual role in contributing to the regulation of both carbohydrate metabolism and salt/water balance. Until recently, both the mineralocorticoid and glucocorticoid actions of cortisol were considered to be mediated by a single corticoid receptor of the glucocorticoid receptor (GR) subtype (Mommsen et al., 1999). The cloning from rainbow trout testis of a novel steroid receptor that exhibited high homology to mammalian and amphibian mineralocorticoid receptor (MR) cDNA sequences at both nucleotide and amino acid levels, as well as steroid binding characteristics consistent with those of other MR, strongly suggests the presence in trout of a mineralocorticoid-like receptor (rtMR) (Colombe et al., 2000). Whereas GR have been characterised in a number of fish species and tissues, the tissue distribution, physiological function and regulation of MR in fish are not yet clear.

In the present study, the acclimation of rainbow trout to ion-poor water has been used as a tool to investigate the function and regulation of GR and MR in the freshwater fish gill. When acclimated to soft water, rainbow trout experience a proliferation of the ion-transporting chloride cells of the branchial epithelium, which contributes to the maintenance of ionic homeostasis by enhancing

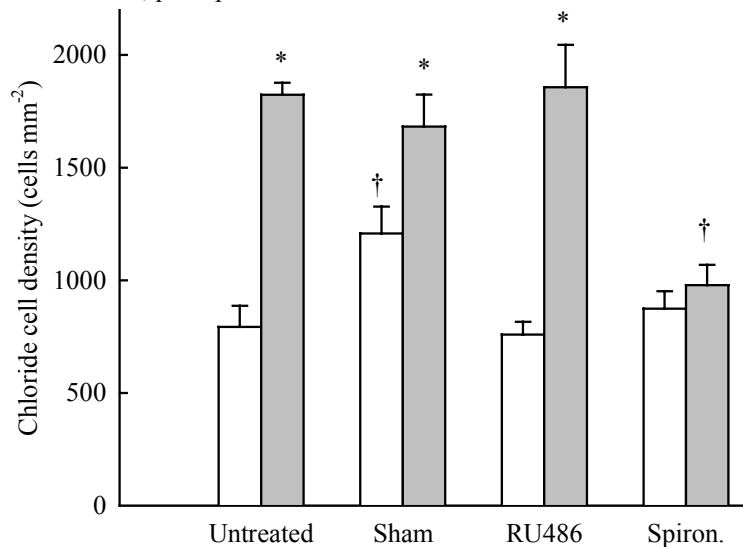
branchial ion uptake. Chloride cell proliferation in softwater-acclimated trout is thought to be stimulated by cortisol, first, because several studies have reported transient elevations of circulating cortisol concentrations during exposure to ion-deficient water (e.g. Perry and Laurent, 1989), and second, because cortisol treatment (in the absence of an ionoregulatory challenge) has been found to induce chloride cell proliferation (e.g. Bindon et al., 1994).

Rainbow trout (*Oncorhynchus mykiss*) were either maintained in dechlorinated city-of-Ottawa tap water (tap water; in mmol L<sup>-1</sup>, [Na<sup>+</sup>] = 0.163, [Ca<sup>2+</sup>] = 0.397, [K<sup>+</sup>] = 0.021 and [Cl<sup>-</sup>] = 0.173) or were provided with dechlorinated tap water diluted with reverse osmosis water (artificial soft water; in mmol L<sup>-1</sup>, [Na<sup>+</sup>] = 0.032, [Ca<sup>2+</sup>] = 0.096, [K<sup>+</sup>] = 0.007 and [Cl<sup>-</sup>] = 0.050). Fish were exposed to the softwater condition by a gradual increase in the proportion of RO water over a 24 h period, and exposure periods ranged from 24 h to 7 days. Trout in each acclimation condition were allocated to one of four treatment groups; untreated (control), a single intraperitoneal injection of warm coconut oil (sham; 0.005 mL g<sup>-1</sup> body mass), coconut oil containing the GR antagonist RU486 (RU486; 0.5 mg g<sup>-1</sup> body mass), or coconut oil containing the MR antagonist spironolactone (spironolactone, 0.1 mg g<sup>-1</sup> body mass). Plasma cortisol and ion concentrations, and branchial chloride cell proliferation, Na<sup>+</sup>-K<sup>+</sup>-ATPase activity, GR protein expression, and GR and MR mRNA abundance were examined as a function of acclimation condition and/or treatment.

Softwater acclimation elicited chloride cell proliferation, a response that was blocked by treatment with the MR antagonist spironolactone, but not by the GR antagonist RU486 (Fig. 1). This finding provides further evidence for the presence of a mineralocorticoid-like receptor in rainbow trout, as proposed by Colombe et al. (2000), and implicates MR rather than GR in eliciting the response to the ionoregulatory challenge of softwater exposure. Indeed, branchial GR protein content decreased significantly as a result of acclimation to ion-poor water. Chloride cell proliferation in softwater-acclimated fish occurred in the absence of any significant elevation of plasma cortisol levels, or significant change in branchial Na<sup>+</sup>-K<sup>+</sup>-ATPase activity. Plasma ion levels were unaffected by softwater acclimation, implying that the physiological responses to this ionoregulatory challenge were sufficient to maintain ionic homeostasis.

In conclusion, the results of the present study support the hypothesis that rainbow trout possess both GR and MR, and that these receptors may be differentially regulated. Further, the data confirm that cortisol plays an

important role in mediating the physiological responses to softwater acclimation in rainbow trout, perhaps via MR.



**Figure 1.** The effect of acclimation condition and treatment on chloride cell density. Values are means  $\pm$  SEM ( $N = 4$ ). \* indicates a significant difference between tap water and softwater-acclimated fish within the same treatment group; † indicates a significant difference within an acclimation condition from the untreated group (two-way ANOVA,  $P < 0.05$ ). Modified from Sloman et al. (2001).

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