Effects of cortisol on development of the stress axis in zebrafish (*Danio rerio*)

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Introduction

Fish, like other vertebrates, respond to the perception of a stressor by producing glucocorticoid stress hormones.[1] Activation of the hypothalamic-pituitary-interrenal (HPI) axis (see Figure 1) by a stressor leads to the production of cortisol, which mediates physiological and behavioural responses to the stressor by regulating gene expression through binding to the glucocorticoid receptor (GR).

Studies indicate that maternal stress in fish has a variety of effects on offspring, including changes in survival, growth and behaviour, as well as the occurrence of physical abnormalities.[2] Maternal stress also may impact development of the stress axis and stress responsiveness in offspring (unpublished observations; JD Jeffrey, N Sopinka and KM Gilmour). The mechanisms underlying the effects of maternal stress on offspring remain unclear, but maternal transmission of cortisol to eggs is one possibility.

Therefore, the present study aimed to determine how exposure of zebrafish (*Danio rerio*) fertilized eggs to cortisol affects development of the HPI axis. We hypothesized that maternal stress affects development of the stress axis by elevating egg cortisol levels. Based on this hypothesis and in accordance with the observation that maternal stress attenuates the stress response of offspring, we predicted that cortisol-treated embryos would exhibit reduced mRNA abundance of key genes in the stress response (GR, StAR and P450scc), as well as elevated mRNA abundance of 11β-HSD2.

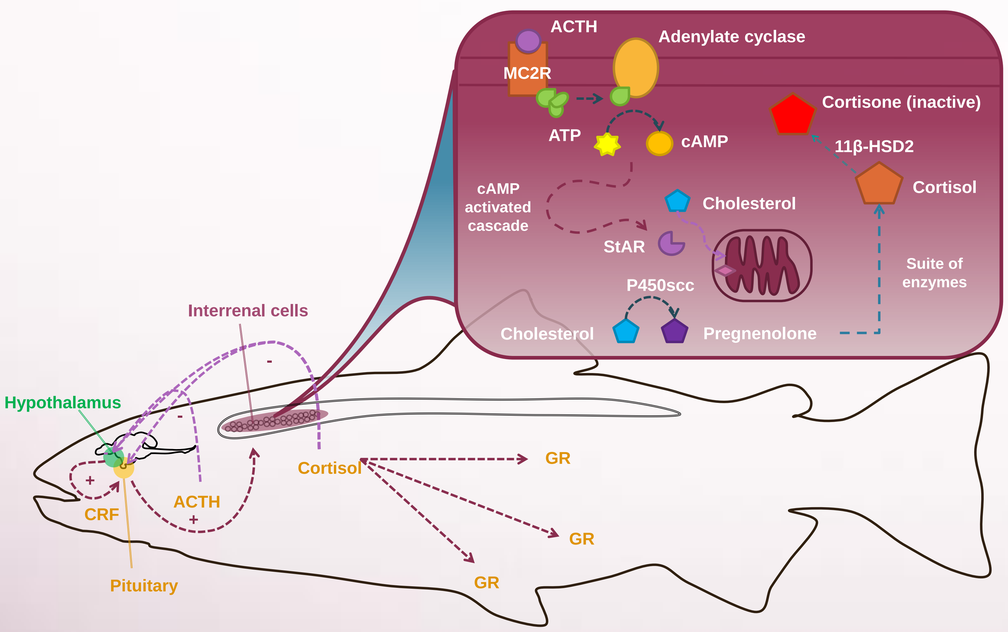

Figure 1. Hypothalamic-pituitary-interrenal axis. GR = glucocorticoid receptor. StAR = steroidogenic acute regulatory protein. P450scc = cholesterol side-chain cleavage enzyme. 11β-HSD2 = 11-β-hydroxysteroid dehydrogenase 2. CRF = corticotropin releasing factor. ACTH = adrenocorticotropic factor. MC2R = melanocortin-2 receptor.

Methods

Female zebrafish (*Danio rerio*; 8-12 fish) were stripped of their eggs which were pooled together and fertilized *in vitro*. Eggs were allocated to two groups, control and cortisol-treated, with the cortisol-treated group being exposed to 500 ng ml^{-1} cortisol (a level often achieved in response to a stressor) for 2 h immediately after fertilization. Samples were collected 25, 49 and 97 h post-fertilization (hpf), and mRNA abundances of GR, StAR, P450scc and 11β-HSD2 were evaluated using semi-quantitative real-time RT-PCR.

Results

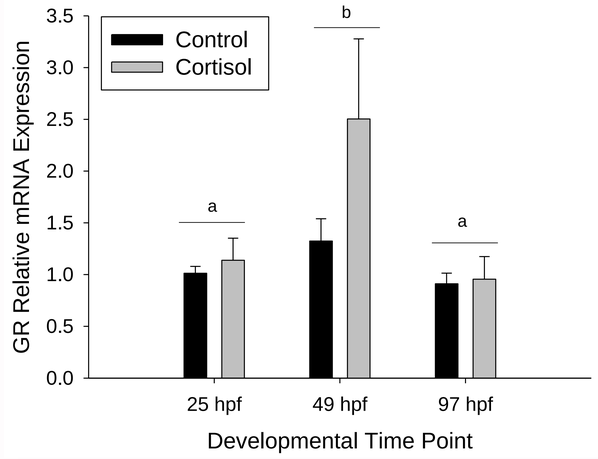

Figure 2. GR relative mRNA expression across treatment groups and time points. Data are means (±SEM) with n=5-8 for each group. Time points that do not share a letter are significantly different from one another. p=0.008 for the effect of developmental time. At 49 hpf, a Studen'ts t-test yielded p=0.059.

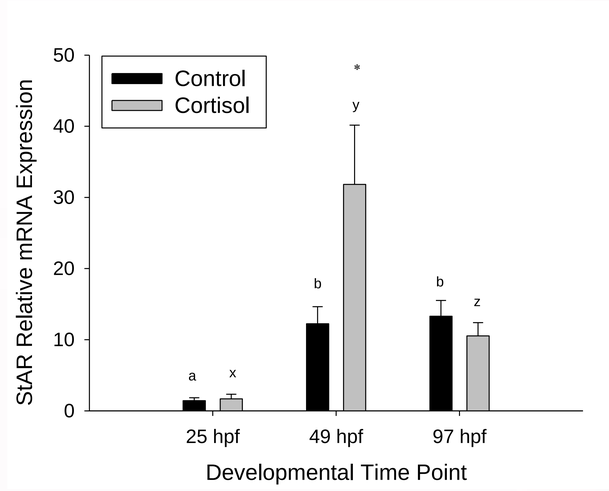

Figure 3. StAR relative mRNA expression across treatment groups and time points. Data are means (±SEM) with n=5-8 for each group. Time points within a treatment group that do not share a letter are significantly different from one another and * represents a significant difference between treatment groups. p<0.001 for the effect of developmental time and p=0.013 for the interaction of developmental time x treatment.

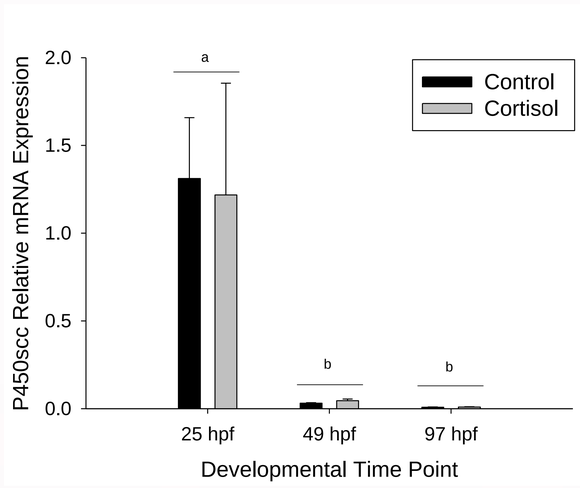

Figure 4. P450scc relative mRNA expression across treatment groups and time points. Data are means (±SEM) with n=5-8 for each group. Time points that do not share a letter are significantly different from one another. p<0.001 for the effect of developmental time.

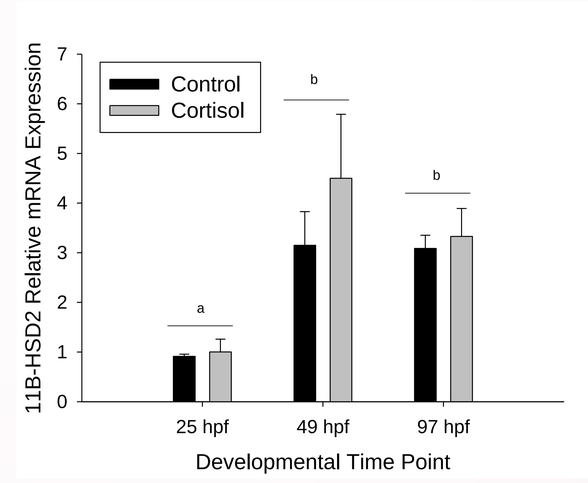

Figure 5. 11β-HSD2 relative mRNA expression across treatment groups and time points. Data are means (±SEM) with n=5-8 for each group. Time points that do not share a letter are significantly different from one another. p<0.001 for the effect of developmental time.

Discussion

Treatment effects

• A short 2 h exposure to elevated cortisol levels had long term effects on stress axis development

•Cortisol effects at 49 hpf (elevated StAR and GR mRNA abundance) were opposite to the prediction that mRNA abundance of genes in the HPI axis would decrease with cortisol exposure. The increase in mRNA abundance may reflect an auto-regulatory mechanism in which mRNA abundance increases in response to a fall in protein levels induced by cortisol exposure. A similar effect was observed in rainbow trout treated with cortisol; liver GR protein levels fell but GR mRNA abundance increased.[3]

Time point effects

• The mRNA abundance of most genes involved in the HPI axis increased over developmental time, likely a reflection of development of the HPI axis, which becomes functional around 97 hpf.[4] These results were in agreement with previous studies on development of the HPI axis in zebrafish.[4]

•P450scc mRNA levels declined after 25 hpf which reflects the potential importance of this steroidogenic enzyme during early embryogenesis.[5]

•Interpretation of data for StAR and P450scc is complicated by the fact that these proteins are involved in the biosynthesis of many steroids, not only cortisol.

References

[1] **Wendelaar Bonga.** (1997). Physiol. Rev. **77**, 591-625.
[2] **Eriksen et al.** (2007). J. Fish Biol. **70**, 462-473.
[3] **Vijayan et al.** (2003). Gen. Comp. Endocrinol. **132**, 256-263.
[4] **Alsop and Vijayan.** (2008). Am. J. Physiol. **294**, R711-R719.
[5] **Hsu et al.** (2009). Mol. Cell Endicrinol. **312**, 31-34

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