Does cortisol regulate carbonic anhydrase during acid-base challenges in zebrafish (*Danio rerio*)?

K. McDonald and K.M. Gilmour
Department of Biology, University of Ottawa

Introduction

Overall Goal – To determine whether cortisol regulates the expression of carbonic anhydrase (CA) in zebrafish exposed to acid-base challenges.

UROP Goal – To measure cortisol levels in zebrafish exposed to the acid-base challenges of low pH water or hypercapnia. A separate group of zebrafish was fed cortisol-treated food to assess this approach as a means of manipulating cortisol levels *in vivo*.

Hypothesis: We hypothesize that acid-base challenges will elevate circulating cortisol concentrations, and that the elevated cortisol levels will cause an increase in carbonic anhydrase expression that contributes to acid-base compensation.

Carbonic anhydrase is an enzyme that catalyzes the reaction:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

Therefore, this enzyme is involved in many physiological processes including acid-base and ionic regulation, gas exchange, metabolism and the ossification of bones.

Fish use metabolic compensation to restore pH during acid-base challenges. Cytosolic CA (gill and kidney) provides H^+ and HCO_3^- for export across the gill, and renal CA acidifies urine.

CA expression is adjusted during acid-base challenges, but the factors regulating CA expression remain uncertain. Cortisol is one possibility.

If cortisol regulates CA expression during an acid-base challenge, then a rise in cortisol would be expected to occur during acid-base challenges such as exposure to pH 4.5 water or to 1% CO_2 (hypercapnia).

Cortisol responses to acid-base challenges were the focus of the UROP project. A radioimmunoassay was used to measure whole-body cortisol levels in fish exposed to an acid-base challenge or fed cortisol-treated food.

Methods

Zebrafish were exposed to pH 4.5 water or water equilibrated with 1% CO_2 for 1, 6, 24 or 48 h and then euthanized. Matched control groups were held in 28°C water aerated with an airstone.

Following a 7-day training period, fish were fed cortisol-treated food for 7 days and were then euthanized 2, 5 or 24 h after the last cortisol-treated meal. A matched control group was fed ethanol-treated food. The methods used were similar to those described by DiBattista et al. (2005).

Cortisol was extracted from whole body samples according to the procedure of Ramsay et al. (2006). A commercial radioimmunoassay (ICN) was then used to analyze cortisol levels in the extracted samples.

Results

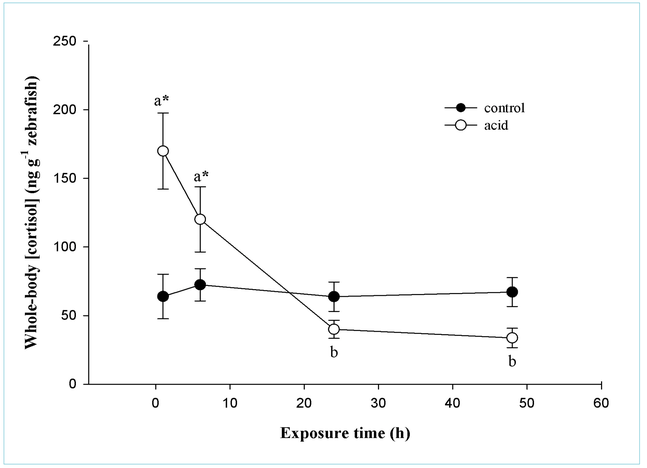

Figure 1. Effect of exposure to pH 4.5 water on whole-body [cortisol] in zebrafish. Within the acid-exposed treatment group, cortisol levels were elevated at 1 and 6 h relative to 24 and 48 h (indicated by the use of different letters). At 1 and 6 h, cortisol was significantly higher in fish exposed to pH 4.5 water than in the control group (indicated with asterisks; data were analyzed by two-way ANOVA with treatment group and time as factors).

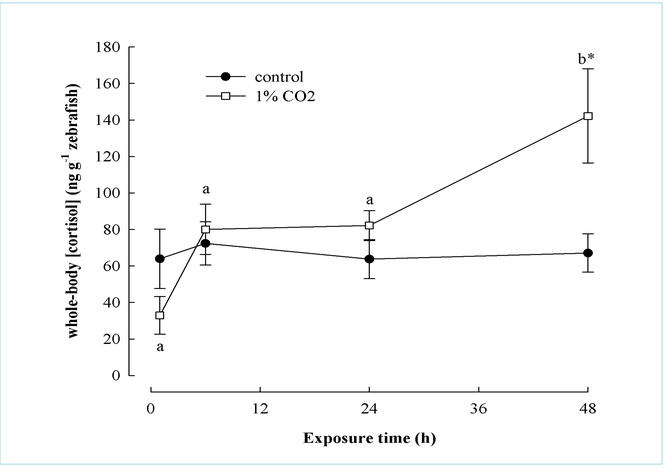

Figure 2. Effect of hypercapnia on whole-body [cortisol] in zebrafish. Within the hypercapnic treatment group, cortisol levels were elevated at 48h relatibe to 1h, 6h and 24h (indicated by the use of different letters). At 48h, cortisol was significantly higher in fish exposed to 1% CO_2 than in the control group (indicated with asterisks; data were analyzed by two-way ANOVA with treatment group and time as factors).

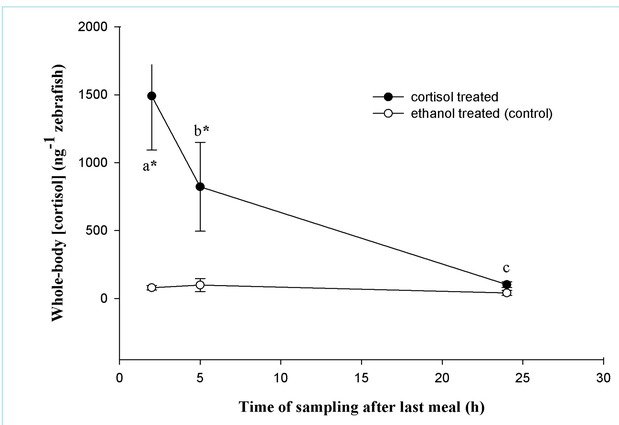

Figure 3. Effect of cortisol food treatment on whole-body [cortisol] in zebrafish. Within the cortisol treated group, cortisol levels were significant between the 2h, 5h and 24h groups (indicated by the use of different letters). At 2h and 5h, cortisol was significantly higher in fish fed cortisol treated food than the control group (indicated with asterisks; data were analyzed by two-way ANOVA with treatment group and time as factors).

Discussion

Acid-base and hypercapnic challenges

Cortisol responses to metabolic acidosis (pH 4.5 water) and respiratory acidosis (hypercapnia) were quite different. Exposure to pH 4.5 water initially elevated [cortisol], which then steadily declined over time. The rapid recovery of cortisol suggests that pH 4.5 water does not pose a substantial challenge to zebrafish, unlike many other teleost fish (Audet and Wood 1988).

By contrast, hypercapnia caused a gradual increase in [cortisol]. It will be necessary to extend the time course of this treatment to determine whether elevated [cortisol] is maintained.

In both cases, cortisol responded to acid-base challenges, suggesting that it may play a role in regulating CA expression. Additional experiments are now needed to correlate changes in CA expression with acid-base challenges to these observed changes in cortisol. In addition, cortisol should be manipulated to assess the impact of elevated cortisol on CA expression directly.

Treated food

The results of the treated food experiment suggested that cortisol-treated food provides a means of elevating cortisol in zebrafish, at least for short periods of time – cortisol remained elevated only for 5 h following the last meal of treated food. This approach can now be used to assess the effects of cortisol on CA expression directly.

References

Audet et al. (1988). *Can. J. Fish Aquat. Sci.* 45, 1399-1405.
Crocker et al. (2000). *American Journal of Physiology, 279*(2 48-2),617-628.
Gilmour, K. M., & Perry, S. F. (2009). *Journal of Experimental Biology, 212*(11), 1647-1661.
Ramsay et al. (2006). *Aquaculture,* 258(1-4), 565-574.

Acknowledgements

Special thanks to Jen Jeffery and Jenny Dupuis for all of their guidance with lab procedures. Thank you to my supervisor, Dr. Gilmour, and thank you to UROP for funding the project.