Do the interrenal cells of rainbow trout (*Oncorhynchus mykiss*) exhibit a dose-dependent response to ACTH?

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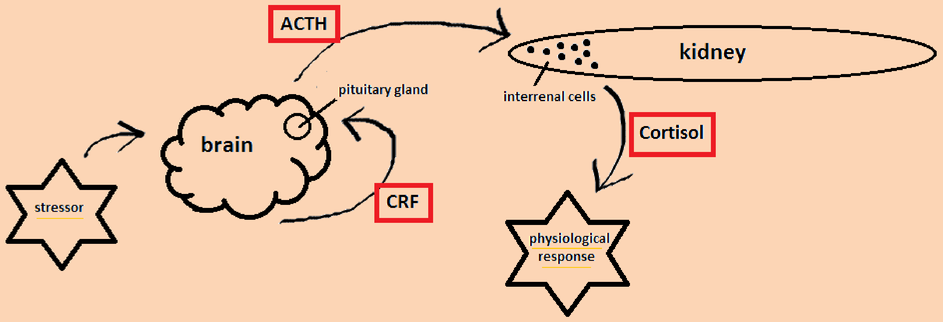
Introduction

Rainbow trout (*Oncorhynchus mykiss*) are a salmonoid fish found in North America and Asia. When these fish are put under stress, their bodies will mount a physiological response. This response involves what is known as the hypothalamic-pituitary-interrenal axis (HPI axis), and the pathway is as follows:



Upon encountering a stressor, the hypothalamus produces corticotropin releasing factor (CRF), which is sent to the pituitary gland, located in a different section of the brain. The anterior pituitary then releases adrenocorticotropic hormone (ACTH) to the interrenal cells in the kidney via the blood stream. (Gilmour *et al.*, 2005) Upon arrival, the cells are stimulated to produce and release cortisol, the primary stress hormone.

The purpose of the experiment was to see if different concentrations of ACTH elicited different levels of cortisol production. We believed that at higher [ACTH], more cortisol would be produced.

This research project uses some data collected this past summer. One set of data concerns concentrations of ACTH and cortisol production; the same as described above. The other set of data looks at concentrations of cAMP, another biochemical involved in the HPI-axis, and at what time post-stimulation the concentration of it peaks.

Methods

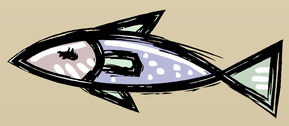
Mature fish were taken directly from the stock tank, blood samples were immediately taken, and the fish were quickly euthanized.

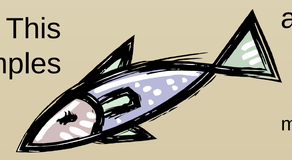
The trout were dissected, and 20 mL of saline solution was flushed through the circulatory system to remove any remaining blood cells from the kidney, as we only wanted interrenal cells to remain.

We removed the head kidney, filtered it and reconstituted it to create a solution of 50 000 000 interrenal cells per millilitre.

The cells were incubated in wells at 13°C for two hours with gentle agitation, and following that were either treated with a ACTH solution of 1, 2, or 10 IU/mL, or with a simple media solution as a control. They were incubated again under the same conditions, but this time only for one hour. Cortisol production was stopped, and the solutions were collected and stored.

At a later date, a cortisol assay was run on the solutions. This involved using “hot” cortisol to compare amounts of cortisol in our samples to known standards.





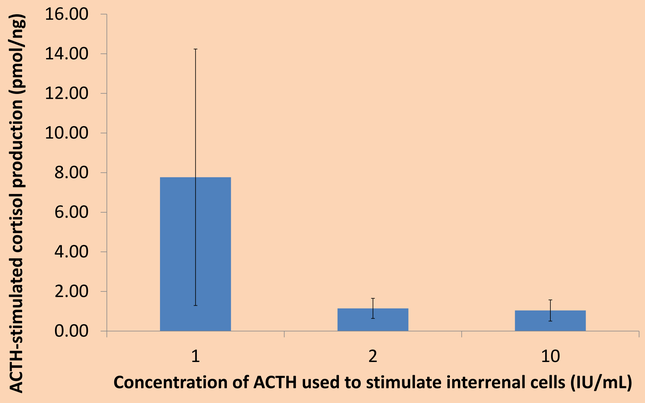


Figure 1: Average amount of cortisol produced by rainbow trout interrenal cells depending on ACTH concentration. (paired t-test, P = 0.188) (Data collected in July 2011)

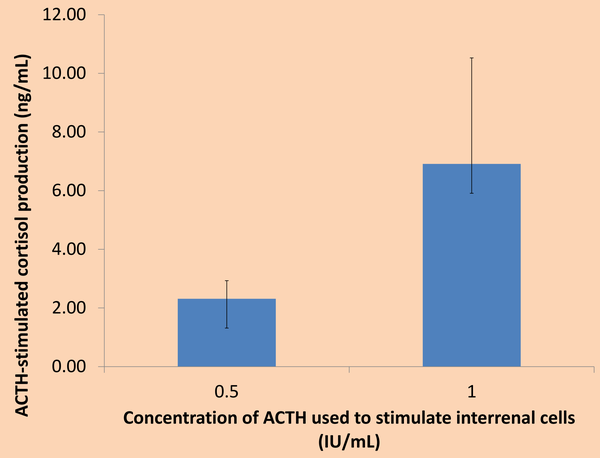


Figure 2: Average amount of cortisol produced by rainbow trout interrenal cells depending on ACTH concentration. (one-way RM ANOVA, P = 0.653) (Data collected during Fall 2011)

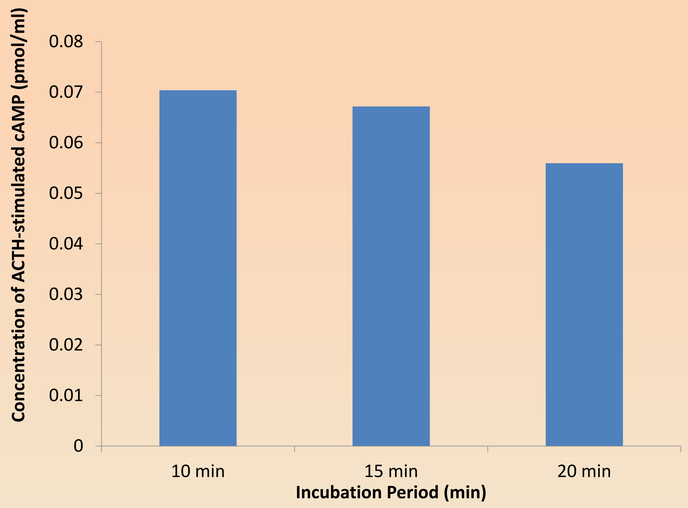


Figure 3: Average amount of cAMP produced by rainbow trout interrenal cells depending on incubation period. Please note that this assay was completed by Jen Jeffrey. (n=2) (Data collected during July 2011)

Results

The results showed that using different concentrations of ACTH to stimulate the interrenal cells produced no significant differences in cortisol production. Between 0.5 and 1 IU/mL, there was a trend showing increased production at the higher concentration. (see Figure 1). At the higher concentrations (1, 2, 10 IU/mL), there was a trend showing decreased production at higher concentrations. (see Figure 2) None of the differences were statistically significant at p=0.05.

The results obtained from the cAMP work showed a trend of decreasing concentrations with time (see Figure 3). However, we can not say with any confidence that this is conclusive, as the n number is so low for this data (n=2).

Discussion

Do the interrenal cells show a dose-dependent response to ACTH? It appears that they do slightly, but not drastically. In the cortisol experiments, it could be that 1 IU/mL is simply the most ACTH a cell can process. We could hypothesize that higher doses are toxic to these cells, as cortisol production decreased above 1 IU/mL. Cell death would explain the apparent drop in cortisol production.

The data regarding cAMP implicated that concentrations diminish quite quickly after stimulation. This is consistent with known fact, as cAMP is degraded quickly by enzymes, so its presence could only be detected for a short period after stimulation. (Mommsen et al, 1998)

The data presented in this experiment will be used create and alter protocols that will be used in further experiments. Knowing how much ACTH to use, and when we should take concentration readings will allow us to alter and confidently quantify other variables. We conclude that 1 IU/mL is an adequate concentration of ACTH to use to stimulate interrenal cells. If testing for cAMP levels, the optimal time to measure at would be ten minutes post-stimulation, but those results are preliminary; a larger n number is needed.

References

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