The Effects of Cortisol on the Cardiac Development/Functionality in Zebrafish

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Abstract

Cortisol affects development, not only in zebrafish, but mammals as well. Experiments have concluded that cortisol can affect zebrafish cardiac development, but these experiments injected the embryo itself with the cortisol. The aim of my experiment was to examine the effect of cortisol on cardiac development by adding cortisol into the water, much like the way zebrafish embryos would encounter maternal cortisol in their natural environment.[1] Based off the results of previous experiments, I predicted that cortisol levels in the water would affect the cardiac development of zebrafish. In my experiments, I utilized the fast breeding and growing characteristics of zebrafish to collect clutches of eggs. I separated the clutches into two groups: cortisol-treated and a group without treatment (water). The group without the hormone served as a control group to determine the number of embryos in a selection that develop improperly, without the treatments. The cortisol-treated tank served as the experimental group. Zebrafish were acquired, maintained, and their breeding behaviors as well as developmental stages determined before experimentation. Clutches of eggs were treated and documented over the course of three days. After analysis through Chi square testing, cortisol appears to affect the cardiac functionality and development in some of the fish exposed.

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Background

Zebrafish (*Danio rerio*) are freshwater teleost, often found in home aquariums. Kept at 28.5°C [2] and between 6.8 and 7.2 pH, these fish can thrive and mate, producing thousands of eggs in under ten minutes.[3] Not only can they produce many embryos in a short amount of time, the amount of time between fertilization and hatching is between three and six days, and their translucent bodies during development allow for observations that would be difficult to analyze otherwise. Zebrafish contain many conserved genes that are similar to humans, making them an ideal model organism for studying diseases and disorders.[4] Among the observations that can be observed are cardiac development and malfunction in zebrafish. Zebrafish embryos are able to survive cardiac malformation and malfunction until the larval stages because their small size allows diffusion of nutrients and oxygen from the surrounding water to keep them viable, allowing the condition to be studied on a live specimen, with a fully formed heart produced around 30 hours post fertilization (hpf).[5]

Cortisol is a lipophilic hormone that is released when an organism is stressed. Maternal cortisol levels in zebrafish embryos play an important role in zebrafish development[6] as zygotic cortisol does not change in correspondence to stress until three to four days post fertilization.[7] As the zygote mitotically divides, the maternally-encoded receptor that receives cortisol congregates into high concentrated areas and low concentrated areas (notably the head and tail regions respectively), eventually becoming replaced with the zygotic receptor after about 24 hpf.[8]

Significance

It has been observed that cortisol affects development, not only in zebrafish, but mammals as well.[9] Experiments have concluded that cortisol can indeed affect cardiac development, but they inject the embryo itself with the cortisol. In the experiment executed, the cortisol was displaced in the water, much like the way zebrafish embryos would encounter maternal cortisol in their natural environment. The experiments performed allowed insight into the effects of maternal cortisol on their young, in this instance, zebrafish, dispersed the way the mother would provide the hormone. Understanding the effects of cortisol on zebrafish cardiac development may provide insight into the effects of cortisol/ steroid treatment on the embryos/ fetuses of pregnant women. Based off the results of previous experiments, I had predicted that cortisol levels in the water would affect the cardiac development of zebrafish. Since cortisol can affect the homeostasis of an organism, it stands to reason that an imbalance of this hormone would result in an imbalance during development and, therefore, lead to developmental issues.

Method

The purpose of this experiment was to examine the cardiac physiology of cortisol-treated zebrafish. Both resting and stressed cardiac states were observed and recorded. The following procedure was followed. Two petri dishes were set up: one containing fresh water, and the other containing cortisol-treated water. Both contained 1 mL of embryo rearing solution. All were maintained at 28.5β and had a 14 hr light/10 hr dark cycle.

Cortisol treated water was prepared as follows. Add 0.1 g of the hormone to 1.0 ml of ethanol to dissolve the lipophilic hormone. Add this ethanol/hormone solution to 100 mL of fresh water. Evapourate the ethanol and add 150 ml more of water for 200 mL hormone-treated solution. This produced a 1.38 *10-3 M solution.

Adult zebrafish tanks were 10L, and maintained at 28.0 β with a 14 hr light, 10 hr dark cycle. Fish were fed two-three times daily with dried pellets. Naturally spawned eggs were gathered from the adult tanks. Eggs were collected ten minutes after light exposure using a syphoning tube. Eggs were separated into the petri dishes, being sure to label the different petri dishes with the appropriate solution. To determine the physiological effects of cortisol on cardiac development and functionality, samples from each dish were taken at 12, 24, 36, 40, and 48 hpf, examined microscopically, photographed, and heart rates determined in the resting and agitated states.

To examine the resting state, samples of the clutches were moved from the petri dish of fish medium (200 mL of the appropriate solution) to a depression slide. After waiting five minutes, embryos were examined under the microscope. Allowing the embryos to acclimate for five minutes helps minimize the stress of moving, and allows examination of the resting state. Heart movements/beats in one minute were counted to determine resting heart rate and are reported as beats per minute (bpm). This was performed at 0, 10, 30 and 90 minutes after the initial five minute wait to maximize accuracy in resting heart rate.

To examine the agitated state, following resting state observations, the medium was gently stirred for two minutes. Again, heart movements/beats were counted at 0, 30, and 90 minutes after agitation or until resting state was achieved

Statistical analysis was performed using chi-square analysis with the non-cortisol treated (water) tank as the expected results. The chi-square result was calculated by hand using the following equation (rate of treated-rate of non-treated)2/(rate of non-treated). The Chi Square analysis was set up to with one degree of freedom since there are two variables (treated and non-treated), and the cut off was at 3.814. Any points larger than 3.814 were considered rejected results and the likelihood of the same results occurring in a natural environment were less than 5 percent.

Preliminary Results:

Preliminary microscopic observation of the embryos suggests that some cortisol treated embryos developed pericardial changes. Results from physiologic studies of heart rate of cortisol treated embryos and control (water treated) embryos in the resting state are seen in Table 1 and in the agitated state are seen in Table 2. Since three of the four non-treated eggs had died, the comparisons were taken using the one egg from the non-treated, and one singular egg from the treated. This singular egg was used for each of the trials in which data was collected.

Time (hr)	Cortisol (bpm)	Water (bpm)	Chi Square Cortisol	Accept/Reject
0	112	102	0.980	Accept
12	112	101	1.198	Accept
24	129	134	0.186	Accept
36	120	135	1.667	Accept
48	150	127	4.165	Reject
72	147	133	1.473	Accept

Table 1. Resting Chi Square results

Time (hr)	Cortisol (bpm)	Water (bpm)	Chi Square Value	Accept/Reject
0	108	116	0.552	Accept
0.5	106	110	0.145	Accept
1.25	102	127	4.921	Reject
72	144.8	123.25	3.751	Accept

Table 2. Agitated Chi Square Results

Discussion

Cortisol did appear to affect the functionality and development of some of the zebrafish exposed. The longer the fish had been exposed, the more drastic the effect as seen in some fish expressing enlarged heart cavities in the cortisol solution, yet none of the fish in the non-treated water expressed this phenotype. After exposing zebrafish to the cortisol solution for over 48 hours, the heart rate dramatically increased, and according to Chi Square analysis this jump did not occur by chance. These results are preliminary, however, due to the small number of eggs collected resulting in a small pool of data to be compared with each variable and a cortisol solution that kept precipitating out of the solution.

Future Direction:

Since three of the four non-treated eggs had died, the comparisons were taken using the one egg from the non-treated, and one singular egg from the treated. As the experiments were performed on a small number of fish, these experiments will be repeated to verify results of the original study. To further analyze cardiac development and the effects of cortisol, immunohistochemistry assays will be performed utilizing an antibody (AB-MF20) to detect the myosin light chain that controls contractility of the zebrafish heart. In a separate set of studies, aldosterone (another steroid hormone) will be used to determine whether cardiac functionality and/or development are in part due to a hormone added to the water even if the hormone is not synthesized by the organism itself.

Additional future studies will evaluate cortisol effect on cardiac physiology further into development (i.e. after hatching) and when zebrafish fry (rather than embryos) are exposed to the same treatments as the embryos in this study.

1 (Pikulkaew et al. 2011)
2 (Nesan et al. 2012)
3 ("Birds Do It, Bees Do It, Even Zebrafish Do It—Just Too Little - WSJ" n.d.)
4 (Bakkers 2011)
5 (Glickman and Yelon 2002)
6 (Pikulkaew et al. 2011)
7 (Nesan and Vijayan 2012)
8 (Pikulkaew et al. 2011)
9 (Pikulkaew et al. 2011)

References

Bakkers, Jeroen. 2011. "Zebrafish as a Model to Study Cardiac Development and Human Cardiac Disease." *Cardiovascular research* 91(2): 279–88. http://cardiovascres.oxfordjournals.org/content/91/2/279 (November 14, 2014).

"Birds Do It, Bees Do It, Even Zebrafish Do It—Just Too Little - WSJ." http://online.wsj.com/news/articles/SB10001424052970203436904577154962232874998?mg=reno64-wsj&url=http://online.wsj.com/article/SB10001424052970203436904577154962232874998.html (November 14, 2014).

Glickman, Nathalia S, and Deborah Yelon. 2002. "Cardiac Development in Zebrafish: Coordination of Form and Function." *Seminars in Cell & Developmental Biology* 13(6): 507–13. http://www.sciencedirect.com/science/article/pii/S1084952102001040 (November 6, 2014).

Kim, Ki-Hyun et al. 2007. "Lrrc10 Is Required for Early Heart Development and Function in Zebrafish." *Developmental biology* 308(2): 494–506. http://www.sciencedirect.com/science/article/pii/S0012160607011219 (December 4, 2014).

Nesan, Dinushan et al. 2012. "Glucocorticoid Receptor Signaling Is Essential for Mesoderm Formation and Muscle Development in Zebrafish." http://press.endocrine.org/doi/full/10.1210/en.2011-1559 (November 6, 2014).

Nesan, Dinushan, and Mathilakath M Vijayan. 2012. "Embryo Exposure to Elevated Cortisol Level Leads to Cardiac Performance Dysfunction in Zebrafish." *Molecular and cellular endocrinology* 363(1-2): 85–91. http://www.sciencedirect.com/science/article/pii/S0303720712003735 (November 6, 2014).

Pikulkaew, S et al. 2011. "The Knockdown of Maternal Glucocorticoid Receptor mRNA Alters Embryo Development in Zebrafish." *Developmental dynamics: an official publication of the American Association of Anatomists* 240(4): 874–89. http://www.ncbi.nlm.nih.gov/pubmed/21360790 (November 6, 2014).