The effects of Dihydroergotamine on the Developing Cardiovascular System of a Chick Embryo

*Alishia Pecorilli, Supervisors: Dr. Janowicz and Dr. Churchward
Faculty of Biology and Environmental Sciences
apecoril@student.concordia.ab.ca

Abstract

Ergot alkaloids can be life-threatening, if ingested. Four embryo treatment groups were injected and used to determine the effects of dihydroergotamine (DHE) on the developing chick cardiovascular system. Dissections and observations were made on 96-hour and 14-day old embryos to establish the relationship between heart development and different concentrations of DHE. On day 14, the dissected hearts were also used to run a glycosaminoglycan (GAG) assay. It was found that there is a significant positive correlation between DHE and impaired cardiogenesis (heart development).

Introduction

Ergot, is a mycotoxic fungus that predominantly inhabits forage grasses and feed grains (Coufal-Majewski et al. 2016). If ingested, ergot alkaloids can greatly impact normal functioning of various body-systems, and even cause death (Derso and Melaku 2015). Ergotamine, a prominent ergot alkaloid, is an agonist of the Serotonin 2B receptor subtypes (Padhariya et al. 2017). If bound to these receptors they are capable of producing biological responses; such as vulvulopathy and prothrombotic effects (disease and thickening of heart valves) (Liu et al. 2018). The receptors are found in the central nervous system, cardiovascular system, and gastrointestinal tract (Padhariya et al. 2017). Prior research has not investigated how ergotamine impacts the developmental fitness of a developing chick embryo. The purpose of this research is to identify how ergotamine effects the developing cardiovascular system of a chick embryo.

Hypothesis

I hypothesized that dihydroergotamine (DHE) would negatively impact heart and valve development in the chick embryo's cardiovascular system. I predicted that higher concentrations of DHE would disrupt angiogenesis (formation of blood vessels) more significantly. My prediction was supported by the known positive correlation of ergotamine and vasoconstriction of smooth muscles and heart valves.

Methods / Results

Methodology was adapted by (Cartwright and Smith 1995). Fertile eggs (n=104), were incubated at 37°C and 57% humidity for the duration of the experiment. Three concentrations of dihydroergotamine (DHE) were injected into embryos of 3 treatment groups (1µg/ml, 0.1 µg/ml, and 0.01µg/ml), at 1ml each. The control group did not receive DHE. 72-hour, 96-hour, and 14-day embryos were weighed. Four of the day-14 embryos, from each treatment group, were used for heart dissections and analyses. The remaining four embryos were used for a Glycosaminoglycan-assay.

As illustrated in Figure 1, as the concentrations of DHE increased there was greater damage to chorioallantoic membrane (CAM) vessels of the 96-hour embryos.

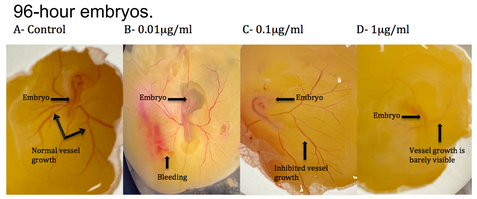

Figure 1- Chorioallantoic membrane (CAM) development of 96-hour embryos across all treatment groups. Increasing concentration from right to left.

Figure 2, shows that 14-day old chicks exposed to higher doses of DHE experienced more internal bleeding. The chicks of the highest concentration (C) also experienced cyanosis.

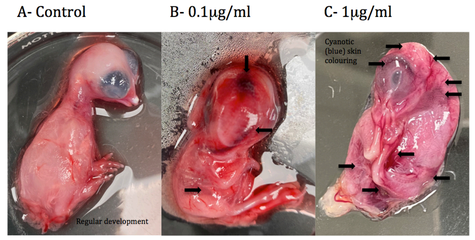

Figure 2- 14- day old chick development in different treatment groups. Internal bleeding in all images of the chicks is indicated with a (→) symbol.

Figure 3 displays 14-day old dissected chick hearts. Increased DHE concentrations lead to greater changes in regular heart development; especially in the atriums.

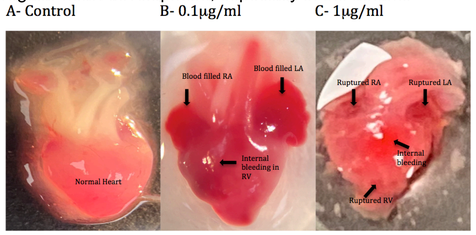

Figure 3- 14-day old dissected chick hearts. Three major structures are indicated in the images- RA, right atrium; LA, left atrium; RV, right ventricle.

Figure 4 is the graphed results of the average glycosaminoglycan GAG-assay values from the dissected hearts of 14-day old chicks. As illustrated, there is a sharp decline in the amount of GAGs found in the treatment groups versus the control.

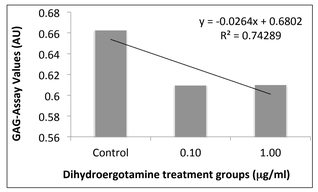

Figure 4- Average GAG-Assay values from 14-day old dissected embryo hearts.

Figure 5 displays the average 14-day chick heart weight. The data reveals that DHE did not effect to heart weight.

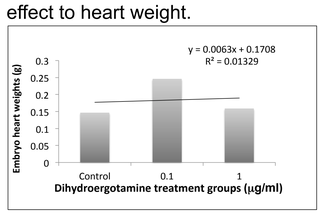

Figure 5- 14- day old dissected chick heart weights in different treatment groups

This figure outlines that the embryo weights remain constant, compared to the control, until day 14, where there is a large leap in weights of the treated embryos.

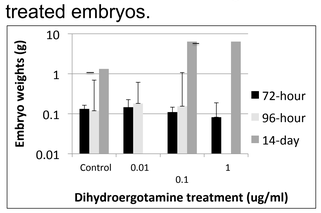

Figure 6- Graphed average weights of embryos at 72-hours, 96-hours, and 14-days at all treatment levels.

Discussion

CAM vessel development was severely impacted as the concentration of DHE increased (figure 1). Inhibition of blood flow, to the embryo, resulted in impairment of appropriate heart development and maturation of cardiac valves (Burggren and Antich 2020). Observations of 14-day chicks (figure 2) indicated that there was substantial internal bleeding in the treated embryos, as compared to the control. Vasoconstriction, due to ergotamine, leads to increased blood pressure causing spontaneous bleeding (Reddy et al. 2020). In the 14-day old dissected hearts (figure 3) damage to regular function was evident. Pooling of blood in the atriums suggested normal valve functioning impairment. 5-HT2BR is abundant in both the mitral and tricuspid valve, agonism of these receptors results in heart valve stenosis; impairment in ability of the valve to open (Zajdel et al. 2015)

Discussion (Cont'd)

GAG-assay results, from the dissected hearts, (figure 4) suggest that DHE greatly impacts GAG presence. Many studies have revealed that dysfunction in GAG levels is commonly revealed in heart valve dysfunction (Kodigepalli et al. 2020). Surprisingly, there was no significance in heart weight in relation to DHE treatment (figure 5).The P-value = 0.73, which confirms the insignificance. This will have to be explored further to identify a connection. Many of the findings are in-line with heart failure (HF). One of the leading symptoms of HF is water retention. This could account for the weight gain, seen in the 14-day embryos', weights as compared to the control (figure 6) (Pellicori et al. 2015).

Conclusion

Since the data is consistent to what we have proposed this means, there will be a significant impact on animals that ingest ergot and, therefore, needs to be explored further.

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