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The Effects of Heart Medication on the Heart Rates of Drosophila Melanogaster

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ABSTRACT

Background
Current animal models of human cardiac disease may be similar in anatomy and physiology but are often expensive and tedious to work with. The current need is for a model organism that is more efficient to work with in the lab but that still provides an accurate model of human cardiac disease. *Drosophila melanogaster (D. mel)* is such a candidate. While 74% of the genes coding for protein are conserved between *D. mel* and human hearts, it is unknown if cardiac medication used in humans, such as atropine and propranolol hydrochloride, similarly affect heart rate. I hypothesized that administration of atropine and propranolol hydrochloride to third instar larvae would cause an increase and decrease respectively in the heart rates of *D. mel*.

Methods
After larvae hatched and reached the second instar larval phase, they were moved to fresh vials. The control group larvae were transferred to vials containing no medication, and the experimental group larvae were transferred to vials with 1mM atropine or 1mM propranolol hydrochloride. The larvae inhabited the new vials for twenty-four hours to reach the third instar larvae stage. Larvae were removed, placed individually on a microscope slide, and observed using the 4X objective lens of a Leica compound microscope. Heart rates of fifty larvae per group were recorded in triplicate over fifteen second intervals.

Results
We observed elevated heart rates of 406 ± 3.18 beats per minute in atropine treated larvae when compared to rates of 388 ± 2.07 in control larvae, a 4.83% increase. Moreover, heart rates were slowed to an average of 274 ± 2.70 beats per minute in propranolol hydrochloride treated hearts, a 29.18% decrease. Both changes in heart rate when compared to the control were found to be statistically significant ($p<0.001$).

Conclusion
Administration of propranolol hydrochloride and atropine increased and decreased the heart rates of *D. mel* respectively. This data supports the hypothesis that *D. mel* can serve as an experimental model for human cardiovascular disease. Future work should build on this study and focus on the use of *D. mel* in preliminary pharmaceutical testing for new medication treating cardiovascular conditions.

Keywords: *drosophila melanogaster*, atropine, propranolol hydrochloride, heart rate

INTRODUCTION

Models of Cardiovascular Disease
According to the American Heart Association, one American dies of cardiac disease every forty seconds (Mozaffarian et al., 2015), and the causation, prevention, and treatment of cardiac disease are areas of active research. Organisms of mammalian origin are predominantly chosen for studying cardiac disease and include baboons, pigs, sheep, dogs, rabbits, rats, and mice (Hasenfuss, 1998). Mammals are typically used due to their similarity in physiology, making them candidates to research new treatment methods (Patel et al., 2001). Smaller mammals, such as rats and mice, are also useful because they can be genetically manipulated and subsequently used to determine the effects of mutations in genes relating to cardiac function (Rosenthal and Brown, 2007). Similarly, zebrafish have recently emerged as another model for human cardiac disease research and have been used to model congenital heart defects and cardiomyopathies, as well as to determine mechanisms that can lead to cardiac disease (Bakkers, 2011).

These organisms have been pivotal in developing current knowledge regarding the physiology of the human cardiovascular system as well as the development and treatment of cardiovascular disease. Unfortunately, many of these organisms are inefficient to use in the laboratory. Costs to obtain and maintain these organisms in a laboratory setting are not trivial. According to the Jackson Laboratory website, purchasing mice for research would cost approximately $10.75 per mouse ("Jax mice pricing information," 2018). Genetically modified mice are even more expensive, and, according to the Cyagen Biosciences website, could range from $250 to nearly $7,000 depending on the desired method of inserting genes into the mouse genome ("Regular transgenic mice," 2018). Rabbits, according to the Charles River website, range from $160 to $330 per rabbit depending on weight ("New Zealand white rabbit," 2018). In addition, many of these animals can be difficult to handle and manage within the lab to ensure they are cared for humanely throughout research. Mice, for example, can move quickly and may attempt to bite the hands of researchers while handling (Buerge and Weiss, 2004). Dogs and primates, according to the Johns Hopkins University Animal Care and Use Committee, may require tranquilization if they are aggressive and difficult to work with. To advance research in this area, it would be advantageous to find an organism that is more efficient to utilize in the lab but that still provides an accurate model of human cardiac disease.

Drosophila melanogaster
D. mel is a candidate for modeling human cardiac disease and is an efficient organism to use in the lab for many reasons. One reason is the well documented short lifespan of the organism (Linford et al., 2013). *D. mel* is used as a model of aging due to its short life span of approximately fifty days from fertilization of the egg to the death of the adult fly. This lifespan is significantly lower than those of typical mammals. A rat, for example, has an estimated mean life expectancy of twenty-two months (Baati et al., 2012). A shorter lifespan allows researchers to study organisms in different stages of life over a shorter amount of time.

Another contributor to the efficiency of using *D. mel* is the immense research that has been done to sequence the genome of the organism (Pandey and Nichols, 2011). Scientists know many of the genes in the DNA of *D. mel* and are thus able to use this information to determine whether genes code for disease or for resistance to disease. Further, *D. mel* can incur mutations in genes homologous to genes of human disease

naturally or by manipulation (Bier and Bodmer, 2004). This ability allows *D. mel* to be a model for discovering the outcome of mutations in genes necessary for physiological function and maintenance of health.

Further benefits include the feasibility of raising the organisms in lab and the inexpensive cost (Doke and Dhawale, 2015). *D. mel*, in contrast to the prices of mammalian models, can be purchased for $8.10 per vial containing twenty-five to thirty flies for most wildtype and mutant strains. In addition, all of the materials necessary for culturing can be purchased for $65.50 ("Carolina easy fly drosophila cultures, living," 2018).

In addition to being a practical organism to work with, *D. mel* is a candidate for human cardiac disease research. A study performed by Cammarato et al. (2011) determined that the proteome, or complete protein makeup, of the *D. mel* heart contains 498 genes vital to heart function. Seventy-four of these genes (15%) were protein products that are also produced in humans. Additionally, 73% of the genes were determined to be orthologs of genes found in humans and mice. Other experiments researching the genetic makeup of the *D. mel* heart have shown that they are physiologically similar to human hearts. The hearts of *D. mel* can develop structural defects and suffer from arrhythmias (irregular heartbeats) or cardiomyopathies (hereditary cardiac disease) (Pandey and Nichols, 2011). Another study used *D. mel* to model the development of age-related heart failure (Ocorr, Akasaka, and Bodmer, 2007). Due to genetic similarity, *D. mel* hearts develop heart failure caused by errors in pacing as well as arrhythmias, key factors in researching age-related heart failure. This research suggests that *D. mel* has a promising future in determining genetic contributions to cardiac disease. Anatomically, however, the heart of *D. mel* is different from that of a human **(Figure 1).**

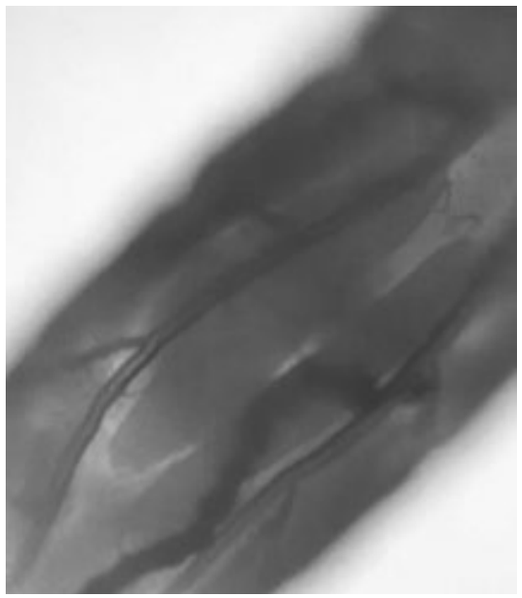

Figure 1: *D. mel* cardiac tube captured during the recording of heart rate.

The image was taken of a *D. mel* larvae under the 4X objective lens of a *Leica* compound microscope. The outline of the head of the cardiac tube can be observed. Surrounding the cardiac tube is a single layer of cardiomyocytes.

In a study identifying the genetic components of heart development and function, it was noted that the *D. mel* heart has only one chamber, referred to as the cardiac tube, and lacks coronary arteries (Seyres, Röder, and Perrin, 2012). This structure is different than that of a human heart, in which there are four highly vascularized chambers. Another study notes that the *D. mel* heart has only one layer of cardiomyocytes (heart cells) whereas the human heart has two sections of cardiomyocytes, the myocardium and endocardium (Medioni et al., 2009). Despite the anatomical differences though, the similarity in genetics and proteome present a convincing case for *D. mel* as a model of human cardiovascular disease.

Despite current advances in understanding genomic similarities between *D. mel* and other organisms typically used as models, it is still not known whether the *D. mel* cardiovascular system is capable of responding similarly to heart medication. Understanding physiological responses of the *D. mel* heart to heart medication could further qualify it as a model of human cardiovascular disease and open new doors of research.

Atropine

Atropine is frequently prescribed to increase heart rate in instances of hypotension ("Atropine," 2014). The drug is administered to those suffering from bradycardia, a condition of extremely low heart rate to increase firing of the SA node in the heart (Al, 2014).

Atropine increases heart rate in humans by preventing acetylcholine from binding to sinoatrial and atrioventricular nodes. It does this by blocking muscarinic acetylcholine receptors (mAchRs) and, as a result, contraction of these pacemaking nodes increases (Kinkade, 2012). For this drug to effect *D. mel* in a similar fashion, the organism must have conserved receptors with the same capability of being blocked by atropine. One study determined which G-protein receptors in *D. mel* were coupled to mAchRs, the active site of atropine (Ren, Folke, Hauser, Li, and Grimmelikhuijzen, 2015). They found that mammals have five mAchRs and that *D. mel* has only two, an A-type and B-type. Specifically, the A-type mAchRs in *D. mel* were determined to have a similar structure to the mammalian receptors. For these receptors to function similarly to human mAchRs, the *D. mel* heart would need to be similarly innervated. It was originally thought that the *D. mel* heart was not innervated. It was discovered, however, that the *D. mel* heart is indeed innervated (Dulcis and Levine, 2003). At the larval stage, heart rate is controlled by a pacemaker structure thought to be located in the caudal region of the heart. With all of this in mind, it is still not known whether these receptors can invoke the same response on the innervating structure of the *D. mel* heart. If the mAchRs of *D. mel* can respond to atropine similarly to humans, this would support our knowledge of *D. mel* as a model for human cardiac disease and medicinal research.

Propranolol hydrochloride

While the effect of atropine increases heart rate, propranolol hydrochloride is a β-blocker that decreases heart rate and is prescribed by physicians for patients suffering

from heart failure (Coppola, Froio, and Chiumello, 2015). Results of one study suggest that β-blockers benefit patients by reducing heart rate and thereby inducing relaxation which may have an effect on diastolic filling of the heart (Dobre et al., 2007).

β-blockers reduce heart rate by blocking *beta1-adrenergic receptors* (βARs), reducing sinoatrial node automaticity and therefore heart rate (Gibson and Raphael, 2014). A study measuring the effects of βARs agonists versus antagonists on *D. mel* showed that antagonist β-blockers, such as propranolol hydrochloride, decreased mortality by 6.4% (Spindler et al., 2013). The same study also showed that *D. mel* does not have βARs but possesses a family of G-protein receptors that are structurally and functionally related to βARs. However, it is not known whether treatment with propranolol produces an effect on heart rate. If the βAR-like receptors in *D. mel* can respond to propranolol hydrochloride in a similar way to humans, this would further support *D. mel* as a model for human cardiac disease and medicinal research.

The Effects of Heart Medication on *D. mel*

While there is proteomic support for a conserved mechanism of regulation, it is unknown if heart medications such as atropine and propranolol hydrochloride have a similar effect on heart rate. We hypothesized that atropine and propranolol hydrochloride in the growth media of third instar larvae would cause an increase and decrease respectively in the heart rates of *D. mel*. Characterization of the pharmacologic activity of these two drugs on *D. mel* cardiac activity would further clarify if there are conserved mechanisms of heart rate regulation between the invertebrate *D. mel* and mammals and would lend further support to using *D. mel* as a model for human cardiac disease.

METHODS

Materials and *D. mel* Culturing

Wild type *D. mel* were purchased from Carolina Biological and atropine and propranolol hydrochloride were purchased from Sigma Aldrich. To maintain a constant living environment, the flies were kept at 22°C and transferred to new vials to mate. The vials were made by mixing equal amounts of Instant Drosophila Medium purchased from Carolina Biological with sterile deionized water in drosophila culturing vials with sponge plugs. The flies were given several days to lay eggs, and once larvae were seen in the vials the adults were moved to new vials to repeat the process and obtain stock vials of adult flies.

Treatment with Atropine and Propranolol Hydrochloride

The experimental procedure required a time span of seven days to complete **(Figure 2)** and was completed three times in succession. On the first day, adult flies from stock vials were placed in three new vials to mate. Over the following four to five days, the adult flies laid eggs in the medium that hatched into larvae and began to mature. By the sixth day, the larvae reached the second instar larval phase. Larvae at this stage were preferred due to their burrowing nature in which they tunnel deep into their growth media, thereby increasing exposure to medication in the media. Second instar larvae were transferred to fresh vials that contained the experimental treatment. For the control group, fifty second instar larvae were moved to vials containing media made with sterile deionized water. For the first experimental group, fifty second instar larvae were transferred to vials with 1mM propranolol hydrochloride in the media. For the second experimental group, fifty second instar larvae were transferred to vials with 1mM atropine in the media. 1mM concentrations of each medication were utilized after performing a preliminary test of a tenfold range of molar concentrations, of which 1mM concentrations were found to display an effect on heart rate without mortality of the larvae. The second instar larvae inhabited the new vials for twenty-four hours, maturing into third instar larvae during this time at which point heart rates were observed.

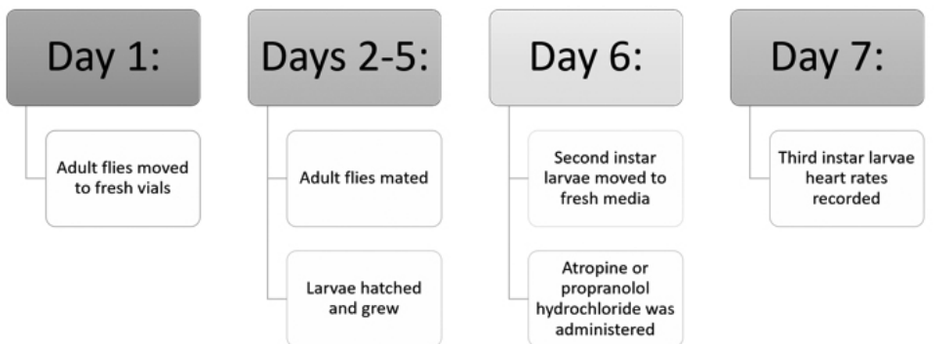

Figure 2: Timeline of procedure detailing when flies were moved to new vials and when heart rates were recorded. On Day 1, adult flies were moved to new vials to mate. Over the next few days, the flies were left to lay eggs. Once the larvae hatched from the eggs and entered the second instar larval stage of development, they were moved to fresh media. At this stage, atropine or propranolol hydrochloride were administered to the media. After twenty-four hours, the third instar larval heart rates were recorded.

Heart Rate Assessment of Third Instar Larvae

The heart rate of each third instar larvae of the control and treatment groups was observed three times in fifteen second intervals. Larvae were removed, placed individually on a microscope slide, and observed using the 4X objective lens of a Leica compound microscope. Beating hearts were visualized through the transparent skin of the dorsal side when larvae were placed on their ventral side. Once the microscope was properly focused on the larva, a fifteen second timer with a ten second interval of rest was started. A tap counter was used to count the number of times the heart beat during each fifteen second interval. After a ten second rest, the process was repeated until a total of three heart rates had been recorded for the larva.

Values were averaged for each larva, with the resulting fifty averages used to calculate the average heart rate and standard error for the group. To determine the significance of the two experimental groups from the control group, a T-Test was performed. The statistical analysis was done using Microsoft Excel.

RESULTS

Second instar *D. mel* larvae were treated with 1mM atropine or 1mM propranolol hydrochloride for twenty-four hours, after which the heart rates of fifty larvae were assessed at the third instar larval stage for each treatment. These averages were compared to that of untreated control larvae **(Figure 3).** Atropine treatment resulted in a heart rate of 406 ± 3.18 beats per minute, which represented a 4.83% increase in heart rate over control hearts (388 ± 2.07 bpm). On the contrary, propranolol hydrochloride treated larvae had hearts rates of 275 ± 2.70 beats per minute, which was a 29.18% decrease in heart rate when compared to controls. Both changes in heart rate were statistically significant ($p<0.001$). These results indicate that both heart rate medications influenced a change in the heart rates of *D. mel* larvae.

DISCUSSION

This research aimed to test whether *D. mel* larvae can respond similarly to humans when treated with heart medication. We observed a significant difference in the heart rates of the larvae that were treated with propranolol hydrochloride. Control larvae had an average heart rate of 388 ± 2.07 beats per minute while the propranolol hydrochloride treated larvae had an average heart rate of 275 ± 2.70 beats per minute ($p<0.001$). This supports the hypothesis that propranolol hydrochloride decreases the heart rate of third instar larvae and demonstrates that *D. mel* responds to heart medication similarly as humans. Further, we also observed a difference in the heart rates of the larvae treated with atropine. Larvae treated with atropine had an increased heart rate of 406 ± 3.18 beats per minute ($p<0.001$). This supports the hypothesis that atropine increases the heart rate of third instar larvae and demonstrates that *D. mel* can respond to heart medication similarly as humans.

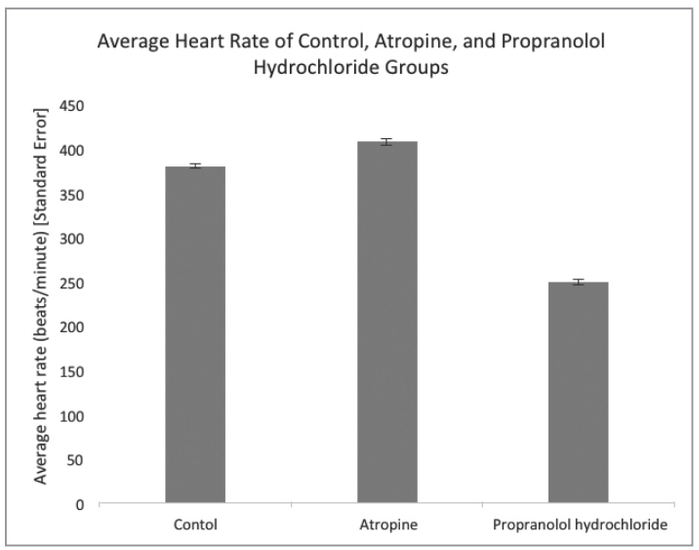

Figure 3: Atropine and Propranolol Hydrochloride act as respective positive and negative chrontropes of *D. mel* heart rates. Average heart rates of treated third instar larvae in the control, atropine (1mM), and propranolol hydrochloride (1mM) groups were assessed by observing the heart rate using the 4X objective lens of a *Leica* compound microscope. Heart rates were recorded in triplicate for fifty in each group. Data are presented as mean plus and minus the standard error. Respective increase and decreases of heart rate due to atropine and propranolol hydrochloride treatments were statistically significant ($p<0.001$).

The effects of the two medications on the heart rates of *D. mel* support previous work that demonstrated the conserved mAchRs and βARs in *D. mel*. One study determined the protein content of the two mAchRs found in the *D. mel* genome (Ren et al., 2015). They concluded that one of the two mAchRs is pharmacologically similar to that of humans; however, they did not demonstrate that *D. mel* was capable of responding to atropine in a similar way to humans. The increase in heart rate due to atropine supports this research, further supporting the notion that the mAchRs in *D. mel* are pharmacologically similar to human mAchRs. Another study used propranolol hydrochloride to study the effects of β-blockers on the lifespan of *D. mel* (Spindler et al., 2013). In this research, it is explained that *D. mel* do not have the same βARs as humans but rather utilize a family of G-protein receptors that may function very similarly to that of humans. The decrease in heart rate due to propranolol hydrochloride that was observed in this study would support this research and suggest that the heart of *D. mel* has conserved receptors capable of responding to the β-blocker propranolol hydrochloride in a similar manner to humans.

To evaluate the similarity between the effects of atropine and propranolol on the heart rates of *D. mel* larvae and humans, it is necessary to compare the results of this experiment to those found by other studies. After the administration of atropine, there was an observed 4.83% increase in larval heart rate. One study, which measured heart rate after the endobronchial administration of atropine in humans, found a 16% increase in heart rate at a dosage of 0.02 mg/kg (Paret et al., 1999). The difference between this 16% increase in heart rate from the observed 4.83% increase in heart rate is due to differences in atropine dose as well as the method of delivering the medication. Another study found that atropine increased heart rate in humans by 13.1 beats per minute (bpm) after 80 minutes at a dosage of 0.15 μg/kg/min (Bruck, Ulrich, Gerlach, Radke, and Brodde, 2003). The difference between the 13.1 bpm increase from the approximately 18 bmp increase observed in this experiment is due to differences in dose. There was also an observed 29.18% increase in larval heart rate after the administration of propranolol hydrochloride. In one study, participants who received 40mg of propranolol hydrochloride were found to have resting heart rates of 62 bpm after five hours as opposed to 72 bpm in participants who received the placebo (Joannides et al., 2006). The difference in recorded decrease in heart rate is due to the difference in dose administered and time between doses. Larvae in this experiment were exposed to propranolol hydrochloride for twenty-four hours before heart rate was recorded; however, participants in the study described above were exposed to the drug for only five hours. This difference in time could influence the observed decrease in heart rate in the two experiments. Through comparing the results of this experiment to those of other studies, it is apparent that the effects of the drugs on the heart rates of *D. mel* larvae are similar. To confirm this conclusion, future studies need to employ concentrations of the drugs that more closely parallel those used in human studies.

There were limitations in the study design that should be recognized and improved upon in future research. Most importantly, the research should have been completed as a blinded study. This would allow for experimental recording of heart rates while

unaware of the group being observed. Performing the experiment in this way would eliminate the possibility of bias. Further, there are more precise methods of recording heart rate that could have been used. At heart rates as rapid as that of *D. mel*, human error in manually counting is inevitable. With additional funding, software could be purchased which would allow for computer-generated analysis of videos of the beating larval heart that would more accurately assess the heart rate (Vogler and Ocorr, 2009).

While further research will add to our knowledge in this area, the data collected in this experiment suggest that the *D. mel* heart responds to atropine and propranolol hydrochloride as the human heart does. If this is indeed the case, future research could be performed to discover whether *D. mel* has the capability of responding to other heart medications. In the future, *D. mel* could be used as a model organism for research being performed on the effects of these heart medications.

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