JUBLI

Journal of Undergraduate Biology Laboratory Investigations

Decrease of heart rate of *Daphnia Magna* in 0.5%, 0.75%, and 1.5% KCl environments

Sarah Alexander, Jessica Ferman, Ashley Jantz, Benjamin Abram, Shannon Reeves*
University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Room 314 Norman, OK 73019

Abstract

Research on humans and various insects has led to different conclusions regarding the effect elevated blood potassium levels has on heart rate. While reduced growth and increases in mitochondria and ribosomes in new cells are long-term effects of potassium in insects, short term effects have yet to be thoroughly investigated. *Daphnia magna*, a planktonic crustacean more commonly known as a water flea, was used in this experiment to test the effects of potassium chloride on heart rate in order to gain an understanding of the effects of high potassium environments. As the concentration of KCl increased, the *Daphnia* heart rate dropped, which resulted in death. As feldspar and mica rock formations contain large amounts of potassium which may be released in weathering and potassium fertilizers have become increasingly common, the results of this study might be useful in aiding scientists and wildlife preservers in maintaining microbial populations in susceptible areas.

Introduction

Daphnia magna have a self-regulated heart rate similar to mammals, and have been used for human drug trials of acetylcholine, tetraethyl pyrophosphate, pilocarpine, adrenaline, and rotenone to analyze the effects each has on heart palpitations (Navarro, 2003). Although humans and *Daphnia* share similar heart regulations, their nervous systems differ. Humans possess a central nervous system which includes the brain and spinal cord, while *Daphnia* possess a ventral nerve cord that implements the messages transmitted by the brain. Even though the human nervous system is more complex than the *Daphnia's* nervous system, its transparent exoskeleton allows the heart to be visible, which makes it more efficient for scientific studies (Poirier, 1988). One thing that can affect heart rate in *Daphnia* include the different ions present in heart chambers, such as potassium.

This laboratory will investigate the effect of potassium chloride on *Daphnia magna* in order to gain a better understanding of how high potassium environments interfere with action potentials. In rats, high diet potassium levels lead to metabolic acidosis, reduced weight, and larger mitochondria in cells (Keep et al., 1987). High potassium diets in Manduca sexta moth showed reduced overall growth, but increased ribosome structures in cells (Perkins et al., 2004). Both of these studies were long term, and the increased ribosome and mitochondria structures might result from an increase of energy needed to operate sodium-potassium pumps, as a larger quantity of potassium needs to be transported out for proper cell function. The lab expects that because *Daphnia magna* share similar internal structures, that high potassium environments will initially cause an increase in heart rate and therefore action potentials as *Daphnia* attempts to compensate for elevated potassium levels.

As the internal structures of humans and *Daphnia* have some similar organelles, the effects of high potassium levels in humans should also be addressed. Hyperkalemia in humans refers to elevated potassium levels in the blood. It is caused from kidney failure, metabolic acidosis, and large potassium intake. Reduced potassium levels have been associated with increased heart rate, increased risk of stroke, and muscle weakness (Aburto et al., 2013). As suggested by the rat and moth study, cells might eventually adapt to high potassium levels by changing the number of structures present in new cells. However, in short term studies, blood pressure would likely initially rise as the circulatory system attempts to compensate for high potassium levels, as there is not enough time to adapt to a new environment on the cellular level.

Hyperchloremia, which is less common and therefore has less research, results in many of hyperkalemia's opposite symptoms, including respiratory alkalosis and hypertension. For this reason, increased research on KCl and the effect it has on nervous systems is needed in order to ensure its medicinal safety, as nearly 30% of modern drugs contain chlorine atoms (Chlorine, 2009).

If *Daphnia magna* are exposed to KCl, then their heart palpitations and action potentials will initially increase, because initial exposure of potassium will cause the sodium-potassium pump to component for elevated levels in cells by overworking, therefore causing an increase in beats per minute. If the hypothesis is supported, then the *Daphnia magna* heart rate should increase with KCl concentration. If the hypothesis is not supported, then the *Daphnia* heart rate will decrease or remain the same in trials of increasing concentration.

Methods

Daphnia were extracted from the aquarium using a beaker and modified dropper, and placed onto the depression slide. To keep the *Daphnia* in place during observations, thin cotton was placed on slides. Using a microscope, the initial heart rate was recorded for each trial in ten second slow motion videos using a smartphone adapter for microscope ocular. The water was removed and different concentrations of potassium chloride were added to each trial, and their subsequent videos were recorded.

In order to investigate whether increasing KCl concentrations increase *Daphnia* heart rate, *Daphnia* heart beats were counted with a microscope under normal conditions, with water, and increasing KCl concentrations (The three trials consisted of 0.5%, 0.75%, and 1.5% KCl). Each *Daphnia* was only used in a single trial in order to avoid residual effects from different concentrations.

The *Daphnia* was placed in each solution for five minutes prior to measuring. Using the raw beats per minute data for both data sets (the percent change between the control environment without KCl and the environment with KCl) the percent change was analyzed, in order to investigate the effect KCl concentrations had on heart rate.

Percent change was calculated because the *Daphnia* heart rate differs greatly due to age, sex, and health which could cause variance. Our lab chose to display data with a bar graph because there was only a percent change collected for the 0.5% concentration of KCl, and the higher concentrations had a zero percent change.

Results

The first *Daphnia* in the controlled condition, water, had a measured heart rate of 440 bpm, but when the 0.5% KCl was added, heart rate plummeted to 172 bpm, resulting in a percent change of 60.9%. The second concentration, 0.75% KCl, was added to a new *Daphnia* that had a starting heart rate of 320, but the *Daphnia*'s heart rate ceased after five minutes into the pre-trial absorption time. Several trials of the 0.75% KCl and 1.5% KCl were conducted; however, none of the *Daphnia* survived those trials. The *Daphnia* stopped moving after around three minutes of absorbing the higher concentrations of KCl, and showed no heartbeat under the microscope. Data from other groups were incorporated into the 0.5% KCl trial shown in Figure 1, however it should be noted that these groups likely used larger *Daphnia* who were less susceptible to KCl change, as their average percent decrease was roughly 20% lower than our lab's conducted trials. Overall, *Daphnia* in 0.5% KCl trials had a ~42% decrease in heart rate.

A paired T-test was conducted to compare the effect of KCl concentration on heart rate in 0.5%, 0.75%, and 1.5% concentration. There was a significant difference between the three concentrations; $t(1) = 2.353$, $p = 0.006578$.

Discussion

It became apparent to the lab immediately that the hypothesis was not taking into account the speed at which *Daphnia* heart rates would change from an increase upon initial KCl exposure to a decrease. Our hypothesis was that the KCl would cause increased heart rate, because the heart would

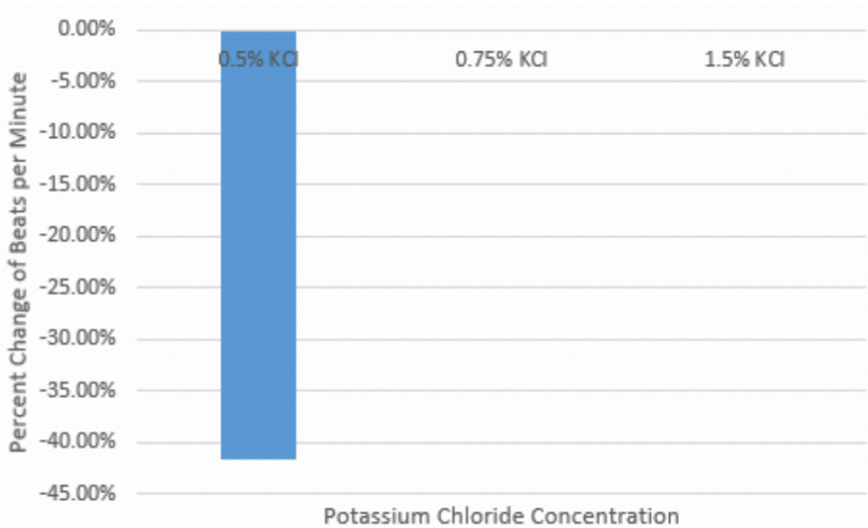

Figure 1 - The percent change of beats per minute in *Daphnia magna* in increasingly concentrated potassium chloride environments is displayed. *Daphnia magna* in 0.5% KCl exhibited a roughly 42% decrease in heart rate. All *Daphnia* in trials above 0.5% KCl died.

work faster to remove potassium and chlorine ion concentrations. While the lab believed this increase would last for several minutes and would keep *Daphnia* safe, the results showed this initial increase to last only a minute. Then, a sharp decrease of heart rate followed, resulting in *Daphnia* death in KCl concentrations above 0.5%. At first, our lab was uncertain as to why *Daphnia* would be dying, with initial reasoning being due to age, parasites, or the possibility of yeast infestation (a white film was observed on some *Daphnia*). All of these would make it difficult for the *Daphnia* to survive with the addition of KCl. However, *Daphnia* continued to die even when healthy, large *Daphnia* were chosen, suggesting compounded problems along with the strength of KCl. A previous study has shown identical results, as high KCl concentration caused *Daphnia* to die during testing, suggesting KCl to be more toxic than previously expected (Utz and Bohrer, 2001).

Another hypothesis that could have been tested is the effect of KCl on the *Daphnia* of different surface area to volume ratios. Many of the *Daphnia* used in this lab were small and likely young, their surface-to-body ratio is likely quite small, suggesting a stronger absorption of KCl. Therefore, it would have had a more drastic effect on their nervous systems.

The resulting data could be used to ensure the safety of wildlife populations living near areas with frequent potassium fertilizer use, or consistent weathering, as potassium is often stored and released in feldspar and mica rock formations. Microbial populations in highly maintained parks on top of feldspar and mica might be in danger of dying from potassium overdose, for instance. Our laboratory recommends that care is taken in maintaining the microbial populations of these susceptible areas and that further testing is conducted in order to investigate the threshold for potassium absorption before normal functioning is impaired in *Daphnia magna* and similar organisms.

Literature Cited

Marc C, Perkins, H. Arthur Woods, Jon F. Harrison, James J. Elser.(2004). Dietary Phosphorus Affects the Growth of Larval Manduca Sexta. Archives of Insect Biochemistry and Physiology. 55:153-168

Nancy J. Aburto, Sara Hanson, Hialy Gutierrez, Lee Hooper, Paul Elliot, Francesco P. Cappucio.
(2013). Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ: 1-19

Navarro, A. Rosas, E. Reyes, Y. (2003). The heart of Daphnia magna: effects of four cardioactive drugs. 136:127-134

R.F. Keep, R.D.Cawkwell, H.C. Jones. (1987). Choroid Plexus Structure and Function in Young
Rats on a High-Potassium diet. Brain Research. 413(1):45-52

Poirier, D. Westlake, G., Abernethy, S. Daphnia Magna Acute Lethality and Toxicity Test
Protocol; Queen's Printer: Ontario, 1988.

Chlorine. Chlorine in Medicine, 2009. https://ngchlorine.wordpress.com/uses-of-chlorine/chlorine-in-medicine/ (accessed March 3, 2019).

L.R.P. Utz, M.B.C. Bohrer. (2001). Acute
chronic toxicity of potassium chloride and
potassium
acetate to *daphnia similis* and *ceriodaphnia
dubia,* Bulletin of environmental
contamination and toxicology. 66(3): 379-385