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Making the Daphnia Heart Rate Lab Work: Optimizing the Use of Club Soda and Isopropyl Alcohol

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MAKING THE *DAPHNIA* HEART RATE LAB WORK: OPTIMIZING THE USE OF CLUB SODA AND ISOPROPYL ALCOHOL

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ABSTRACT

Students commonly test the effects of chemical agents on the heart rate of *Daphnia magna*, a small crustacean. We investigated whether club soda and isopropanol are suitable test agents. Treatment groups contained 6-12 animals. Club soda caused a dose-dependent decrease in heart rate, presumably because of the anesthetic effects of CO_2. Ten percent, 30%, and 50% club soda reduced mean heart rates to 78%, 57%, and 47% of initial values. The effect was transient; heart rates recovered quickly to control values even though the club soda remained present. Isopropanol's effect was dose-dependent and sustained. Three percent, 5%, and 10% isopropanol reduced mean heart rate to 45%, 35%, and 12% of initial values. Removal of the isopropanol failed to fully reverse its effects. Ten percent isopropanol proved fatal to one animal out of the eight tested at that concentration. Both club soda and isopropanol are suitable agents for students to test. If reversibility is to be investigated, club soda should not be used. Its effects wear off even when the club soda is still present. Isopropanol is best used at 3-10% as it causes marked heart rate suppression and partial reversibility.

Key Words: *Daphnia*, heart rate, isopropanol, club soda

INTRODUCTION

The water flea *Daphnia magna*, a small crustacean, is a common study subject in teaching laboratories. Its visible heart allows students to easily determine the effects of various substances on heart rate. Several biological supply companies advocate the use of their *Daphnia* for this purpose. Numerous procedures can be found on the internet. When the first author incorporated this exercise into a course in research design, he found that none of the commonly tested agents caused statistically significant effects. No one had ever determined what agents should be tested, and at what doses, to achieve particular results. Since then, the best parameters for the use of ethanol have been determined (1) but the other two agents investigated, nicotine and caffeine, proved unsuitable for use in the laboratory exercise. Nicotine's effects are complex and vary with dose and exposure time while caffeine, surprisingly, has little or no effect on *Daphnia* heart rate (1).

Our goal was to broaden the range of compounds that could be tested in the *Daphnia* heart rate lab. Ethanol is an excellent test agent (1), but teachers may be reluctant to use it. Students might be tempted to drink it, even if it is denatured. It should be easy to convince them not to drink rubbing alcohol, so here we describe the optimal parameters for the use of isopropanol. Any effect of isopropanol would likely stem from its ability to dissolve in cell membranes (2), presumably altering viscosity. Because carbon dioxide is a general anesthetic, historically used on fish (3), we also chose to investigate the effects of club soda. Carbon dioxide's anesthetic effect is attributed to its ability to perturb cell membranes and its ability to reduce blood pH (4). If *Daphnia* could be used to illustrate carbon dioxide's toxicity, then students would be reminded why animals need circulatory and respiratory systems to remove it.

MATERIALS & METHODS

Daphnia magna were obtained from Ward's Natural Science. They were kept in the solutions in which they were shipped and fed Ward's *Daphnia* food (Ward's catalog number 88W5950). Commercially available 91% isopropyl alcohol (Cumberland Swan, Smyrna, TN) and club soda (Schweppes) were diluted in aged tap water to their final concentrations. Control solutions consisted of aged tap water alone. Aliquots were coded to prevent observer bias and frozen. Six to 12 *Daphnia* were tested at each concentration.

For testing, animals were placed in a 1.5 ml glass well with aged tap water and enough cotton to limit their movement. Heart activity was recorded in iMovie with a video camera attached to a dissecting microscope. Ten-second clips were played back in slow motion to allow accurate counting of heart beats. Three recordings were made at 10 min intervals before replacing the tap water with a test solution. Heart activity was recorded immediately after applying the substance and then three more times at 10 min intervals. The well was then flushed three times with aged tap water. Three more recordings were made at 10 min intervals to check for recovery.

RESULTS

Both club soda and isopropanol caused dose-dependent decreases in mean heart rate. Within 10 min of its application, 3%, 5%, and 10% isopropanol reduced mean heart rate to 45%, 35%, and 14% of initial values (Figure 1A). Ten percent isopropanol also brought about marked irregularities in rhythm, brief periods of asystole, and proved fatal to one animal out of the eight tested. Heart rates of the surviving animals increased after removal of the isopropanol, but they failed to return fully to pre-exposure levels. Club soda diluted to 10%, 30%, and 50% reduced mean heart rates to 78%, 57%, and 47% of initial values (Figure 1B). The effect of club soda was transient. Mean heart rate dropped immediately after the club soda was applied but returned to previous levels within 10 min even though the club soda remained (Figure 1B). None of the animals that were exposed to club soda died.

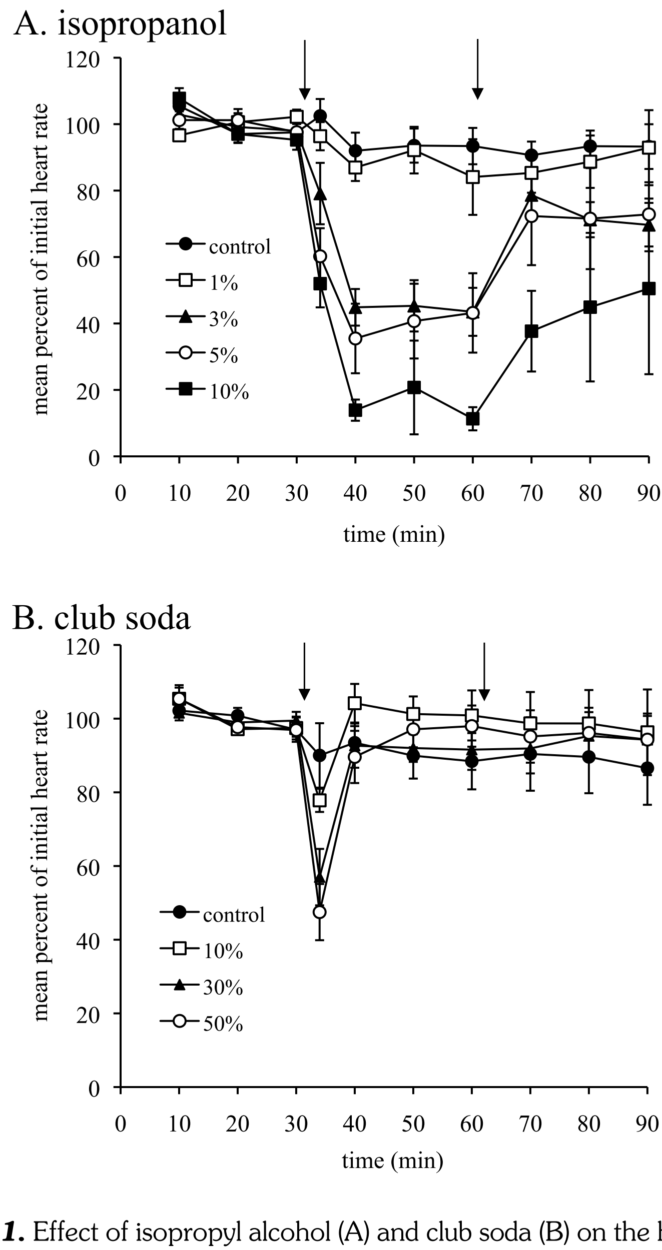

Figure 1. Effect of isopropyl alcohol (A) and club soda (B) on the heart rate of *Daphnia magna*. Values shown are mean percent of initial heart rate. Initial heart rate was determined as the average of the first three heart rate determinations for each animal. Arrows mark the replacement of the bath with the test substance at 30 min and its removal 30 min later. Bars show standard error of the mean. Six to 12 *Daphnia* were tested at each concentration. Results from the animal that died in 10% isopropanol are not included.

DISCUSSION

Isopropyl alcohol and club soda are both highly suitable agents to test in a *Daphnia* heart rate lab. Since the effects of both substances vary with concentration, either one could be used to illustrate the principle of dose-dependency. One percent isopropanol has little effect, while 3% and 5% have about the same effect, so to show dose-dependency we recommend testing 3% and 10% isopropanol as well as a control solution. Isopropanol's full effect is evident within 10 min of its application, so students should wait at least that long before collecting data. One hundred milliliters of 10% isopropanol can be made from 11 ml of 91% or 14 ml of 70% rubbing alcohol, the remainder being tap water. Dilute 10% isopropanol one-to-two with tap water to yield 3%. For club soda, pilot experiments showed that is fatal to *Daphnia* if it is not diluted, so it is best to use 50% club soda as the highest dose. The effect of 30% club soda was similar to that of 50% while 10% had little effect, so we recommend testing 20% in addition to 50% and a control solution. Unlike the case with isopropanol, where students should wait 10 min for it to take effect, data on the effect of club soda must be collected within a few minutes of its application.

In addition to dose-dependency, isopropanol could also be used to illustrate reversibility. Although animals do not recover fully following removal of the isopropanol, the recovery that occurs should be clearly evident to students. Club soda, on the other hand, cannot be used to demonstrate reversibility. Its effects are transient, ending within 10 min or so, even when the club soda remains present. If students remove the club soda, and note that heart rate recovers, they would erroneously conclude that it was club soda's removal that brought about the recovery. In fact, heart rates recover even if the club soda is never removed.

Many internet protocols advocate testing complex mixtures like tobacco extract or caffeinated soda. While this practice may foster curiosity on the part of the student, what valid scientific conclusions can be drawn from the results? By testing known substances, students can at least attribute their results to those substances. Ethanol, isopropanol, and carbon dioxide all penetrate cell membranes easily due to their lipophilic nature (2,5). Their effects on *Daphnia* heart are likely achieved by altering membrane viscosity. Discussing this point with students reinforces their knowledge of cell membrane structure, its fluidity and hydrophobic core in particular. In addition, some of carbon dioxide's effects likely result from its conversion to carbonic acid within the *Daphnia* and subsequent reduction of hemolymph pH. This likely explains why carbon dioxide is a more potent anesthetic than one would expect given its ability to penetrate cell membranes alone (4). Thus, testing club soda on *Daphnia* gives teachers the opportunity to cover carbon dioxide, carbonic acid, bicarbonate ions, and pH - an important topic in both physiology and environmental science.

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