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Effect of a calcium ionophore in the process of hormesis at the Crayfish Neuromuscular Junction.

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ABSTRACT

The concept of hormesis is applicable to many fields of biological inquiry. Hormesis is the process in which a cell adapts to certain stressors by first being exposed to a low level of stress, then being exposed to higher levels of the same stress. Our aim for this project was to test the principle of calcium overload and neural adaptation. We conducted experiments on three different setups to observe the effects of different phases of hormesis. We used a calcium ionophore A23187 to speed calcium penetration into the cells. We hypothesized that we would observe the process of hormesis when the crayfish tail was exposed to different levels calcium and A23187. We used the reduction in the membrane potential as an indicator of cell death. Our results indicate that while significant changes occurred in the membrane potentials within setups, our results were not statistically significant when we compared the data between setups.

INTRODUCTION

Hormesis, a biological concept that refers to the adaptation to low levels of stress in order to withstand higher levels of stress, is represented in various biological fields; scientists have exposed cells to small dosages of toxins to prevent the likeliness of the toxin later on killing the cell. We study these biological concepts on model organisms and through this knowledge we can better understand the functions of cell processes in higher organisms and humans. We performed our experiment on crayfish, because previous research tested this concept on other types of animals, but not crustaceans (Mattson, 2007). Previous studies suggest that calcium overloads can cause the cell to age and die because overloads of calcium can cause lipid peroxidation, a membrane oxidative stress that is a chief reason for cell death, and can also induce cell apoptosis, programmed cell death (Nixon, 2003; Culmsee & Mattson, 2005; Mattson, 2007).

We exposed crayfish extensor muscle cells to low levels of calcium, allowed the muscles to adapt to the calcium, and then exposed it to a high level of calcium. We predicted that if a cell were directly exposed to a high level of calcium, its rate of death would be much faster in comparison to a cell that is allowed to acclimate to the stressor.

To test this process of hormesis, we needed our preparations to remain alive in the solution. Because the cells cannot for long after dissection, we used a calcium ionophore A23187, which speeds calcium penetration by creating channels in the cell plasma membrane. We then measured the membrane potential of muscle cells in solutions of different calcium concentration and used low membrane potentials as an indicator of cell death.

After conducting our experiment, we used the statistical tool ANOVA to analyze our data. Our differences were not statistically significant when comparing the setups against each other, leading us to conclude hormesis cannot be sped up.

MATERIALS AND METHODS

Preparations

We used the muscle cells of *Orconectes rusticus*, a species of crayfish. The neuromuscular junction of the crayfish acts as a model system that we could use to test the concept of hormesis.

After cutting the tail off the crayfish, we removed the ventral surface and scooped out all the ventral muscles leaving the superficial extensor muscles attached to the dorsal part of the tail. This was then pinned to a sylgand-lined petri dish and placed it under the microscope. We used four crayfish tails.

Solutions

The concentrations of ions and A23187 in the four solutions used for this experiment are described in Table 1. Standard crayfish saline and low calcium crayfish saline were used, with and without low concentrations of A23187. Each preparation was left in the solution for an hour before measuring the resting membrane potential.

	Standard crayfish saline	Standard crayfish saline with calcium ionophore	Low calcium crayfish saline	Low calcium crayfish saline with calcium ionophore
KCl	5.4 mM	5.4 mM	5.4 mM	5.4 mM
NaCl	196 mM	196 mM	196 mM	196 mM
MgCl	2.6 mM	2.6 mM	16.1 mM	16.1 mM
Na Hepes Buffer	10 mM	10 mM	10 mM	10 mM
CaCl	13.5 mM	13.5 mM	0.1 mM	0.1 mM
A23187		0.01 mM		0.01 mM

Table 1: The concentrations (all given in mM) and makeup of the solutions used in the different setups used in this experiment.

Electrophysiology

We measured the membrane potential with glass electrodes prepared using pipette puller made by WPI, and filled with 3 M-KCl. After filling the electrodes, we dipped them in normal saline to prevent any extra drops of 3 M-KCl from changing the concentrations of solutions. The electrodes' resistance was over 20 mega ohms. For the purpose of this experiment, we measured resting membrane potentials.

Junction potential

We used the Power lab and Scope (AD instruments) to measure the membrane potential; to account for the voltage created by the junction potential, we "zeroed" the voltage displayed when the electrode was in the saline, not the cell. We zeroed it periodically.

Precision instruments

We used a Leica zoom 2000 microscope. We also inserted the electrodes in an electrode holder that was filled with 3 M-KCl, and was inserted in the manipulator.

Experimental Setups

We used reduction of membrane potential as an indicator of cell death. We first placed the dissected crayfish tail in a solution of standard saline containing A23187 and measured the membrane potential of four cells every five minutes to determine how long it takes for the chemical to permit the calcium in the cell (see Figure 1a). We estimated the time to be 5 to 10 minutes.

The second crayfish tail was placed in a solution of low calcium crayfish saline and A23187 for an hour and measured the membrane potential. Then, we replaced the solution with the standard crayfish saline and A23187 mixture (see Figure 1b.) and measured the membrane potential after 5 and 10 minutes. The third crayfish tail was placed in standard saline solution for an hour. Same as the previous, we measured membrane potential of four cells, added A23187, and measured the membrane potential after 5 minutes and 10 minutes (see Figure 1c.).

The fourth crayfish tail was placed in low calcium saline and allowed to sit for an hour. We measured the membrane potential of four cells, then added A23187 and measured the membrane potential after 5 and 10 minutes (see Figure 1d.).

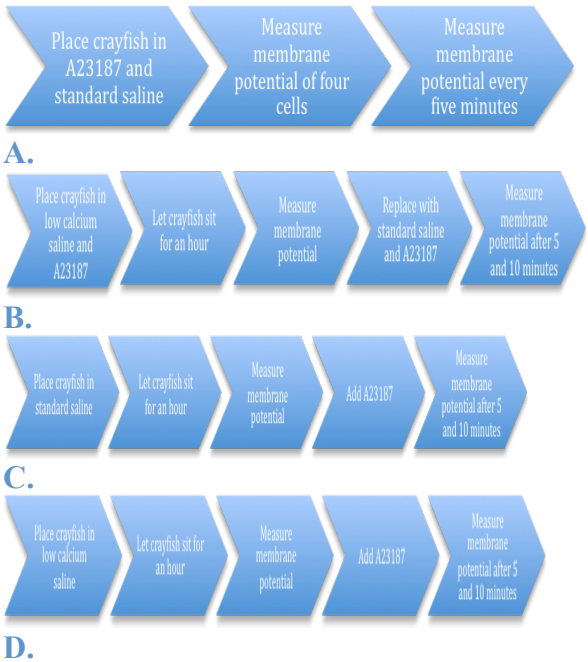

Figure 1: Experiment setups to test the effect of hormesis on the adaptation to calcium overload in crustaceans. A) The setup to determine the time it takes A23187 to take effect. B) The setup to observe a crayfish that undergoes hormesis for the entire hour and 15 minutes of observation. C) The setup to observe a crayfish that does not undergo hormesis but is still subjected to calcium overload. D) The setup to observe a crayfish that undergoes the first half, the adaptation step, of hormesis, only exposed to low amounts of calcium.

RESULTS

We structured our setups based on our ability to manipulate the way cells adapt to different calcium concentrations. We used the reduction of membrane potentials as an indicator of cell death. We measured the membrane potential in each of three different setups four times: after allowing the preparation to sit in its solution for an hour; after further adding A23187; after 5 minutes from adding the A23187; and after10 minutes from adding the A23187. We had two controls and an experimental setup; the controls were placed in high calcium solution and low calcium solution and the experimental was placed in a low calcium solution containing A23187. We then used ANOVA to compare the three setups. Table 2 shows that the experimental changes we performed on each preparation were significant ($P<0.05$). We also calculated the percent decrease of the membrane potential for each setup (see Figure 2). Our results show that cells exposed directly to high levels of calcium experienced a higher rate of death in comparison to the other two setups. We also recorded several changes in membrane potentials (see Figure 3). While we could see several outliers, the general trend indicates that our hypothesis was correct.

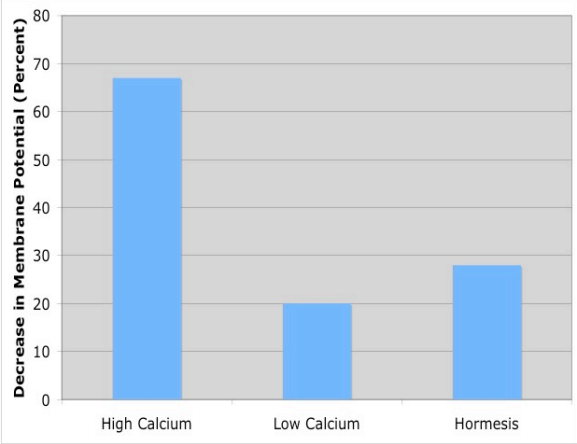

Figure 2: The percent decrease in membrane potential based on change in membrane potential from beginning to end for each experimental setup.

	df	MS	F	P
High calcium (control)	0	384	9	0.002
Low calcium (control)	3	75.5	7.8	0.003
Low calcium and A23187 (experimental)	3	184.9	11	0.0009

Table 2. Displays the statistical results for the ANOVA test.

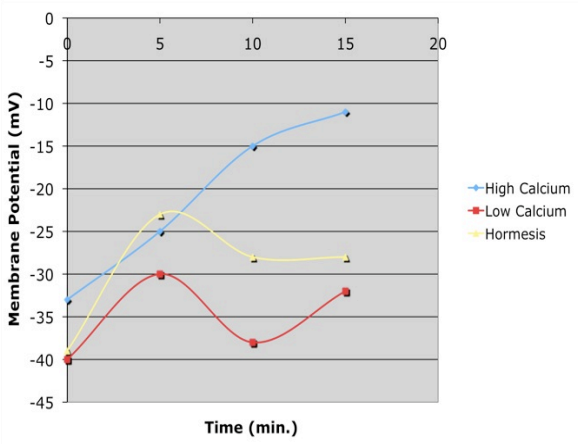

Figure 3: The average membrane potentials of cells measured every five minutes after the addition of A23187 to high calcium saline (blue line), low calcium saline (red line), and the addition of calcium and A23187 (yellow line).

DISCUSSION

Our experiment suggests that manipulating hormesis is difficult due to the fact that the process takes several hours, even days, to work (Mattson, 2007). Our different setups indicated that the cell was, in general, adapting to the low levels of calcium because of the difference in membrane potentials between the setups where we introduced high levels of calcium without adaptation and where we let the cell adapt to a lower level of calcium.

We used the statistical tool ANOVA to analyze the ratio of the variation among groups to the variation within groups. We recorded high F-values fore each setup individually, meaning that the variation between the groups was based on experimental manipulation. However, our F-value for the three setups combined was a low 1.6, suggesting that there exists no relationship between the F-values of the groups.

The problem with using ANOVA is that it does not show the paired differences between groups, only overall differences. This means that certain data we collected could have different meanings if we could use the ANOVA test to analyze paired differences.

Our research shows that the process of hormesis does occur in crayfish assuming that cells are given enough time to acclimate to different levels of calcium. Our statistical tests showed that under . This project is new in essence because it tries to apply to concept to crustaceans, specifically the crayfish (a model organism).

From this we can learn how toxins behave in humans and the concept of gradual adaptation can be applicable to several fields of science, including medicine. Past research has suggested that neuron response to stress can activate "cell-resistance" proteins, which can be beneficial when treating neurological

disorders (Mattson, 2008). In addition, biomedical research suggests that drug addicts show a hormetic dose-response to the drug they use. This research questions common knowledge concerning drug addiction, and further understanding of hormetic processes in humans can help us understand addiction.

Further research will have to explore the minimum time a cell requires to fully adapt to a low stressor. Further research will also have to compare the effects of this process on humans and see the extent to which this is applicable.

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