The effect of mercuric chloride on the concentration of mitochondria along the axon

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Introduction

The purpose of this study was to investigate how mercury affects the concentration of mitochondria along the axon. This is a relevant question because while the effects of mercury on the growth cone have been studied previously (Leong et. al., 2000), extensive research has not been done on how the organelles of the neuron are affected by mercury. Other studies have discussed how mercury can change the metabolism of mitochondria, but not in regards to its effect on mitochondria position and movement (Allen et. al., 2001). Since mercury has been shown to cause retraction of the growth cone (Leong et. al., 2000) I expected that this retraction would also correspond with mitochondrial movement away from the end of the growth cone. It has also been demonstrated that blocking axonal outgrowth results in uniform distributions of mitochondria along the axon (Morris & Hollenbeck, 1993). Therefore when a toxic agent such as mercury is introduced, I hypothesized that the mitochondria would redistribute to show lower concentrations near the growth cone. In this study, I tested the hypothesis that the application of mercuric chloride ($HgCl_2$) to growing sympathetic neuron cell cultures would cause a shift in the concentration of mitochondria along the axon of the neuron, with higher concentrations of the organelle closer to the cell body and more sparse concentrations further away.

The system used in this study was the domestic chicken, *Gallus gallus*. This system has been used frequently to study neurological function because it has a nervous system similar to that of humans. They are also advantageous because they can be observed at all stages of development and they are easy to manipulate (Hollenbeck & Bamburg, 2003). In this study, I treated *Gallus gallus* sympathetic neurons isolated from 10-day embryos with mercury solutions and labeled mitochondria to examine the effects of mercury on mitochondrial distribution, evaluated using fluorescence microscopy.

Materials and Methods

Neurons were dissected from ten-day-old chick embryos. The full materials and methods for the dissection procedure were performed as has been previously described (Morris, (2011a)). Ten-day embryos were ideal because

the ganglia are not too large or too small at this stage of development. Dorsal root ganglia (DRG) and sympathetic chains were collected from the spinal cord of these embryos. Dissociated DRGs and whole sympathetic chains were plated in growth medium on coverslips treated with laminin and poly-lysine. Cells were then incubated at 37° C, for at least overnight to see abundant growth. Cultures were examined for the presence of growth cones two days later using the microscope Nikon Eclipse E200, an Insight FireWire2 camera and the computer program Spot.

After cell cultures were obtained treatment and data collection began. Staining and microscopy observation procedures were followed as has been previously described (Morris, (2011b)). Both control and experimental cells were treated with 50 mg/mL Rhodamine-123 for ten minutes to label mitochondria. Cultures were kept in the dark as much as possible from this point on because Rhodamine-123 is light sensitive. Experimental cultures were then exposed to mercuric chloride at a concentration of 100 nM for twenty minutes. Mercury treatment was applied to the coverslips while they were still in the petri dish, and the mercury was removed after twenty minutes using three washes with growth medium. The coverslip was then sealed to a clean chip chamber slide. Microscopy was employed to locate growth cones to use for measurement. For the experimental group all images were taken only after mercury exposure. Fluorescence and phase pictures were then taken with a 40x objective for each growth cone on a Nikon Eclipse E200 microscope with a mercury 100W lamp, using an Insight FireWire2 camera and the computer program Spot. The optimal exposure time was determined to be two seconds for the fluorescence images. Control trials were also carried out, using the same procedure for treating with Rhodamine-123 but with no treatment with mercury; cultures remained in growth medium for twenty minutes to control for timing effects. One control trial and one experimental trial were performed one week apart, with five sets of control images and four sets of experimental images collected. The different images for each group were all taken within a fifteen-minute span. Images were taken from different areas on the coverslip so as to reduce the effects of bleaching from exposure to fluorescent light. Three images from each group were used for data analysis.

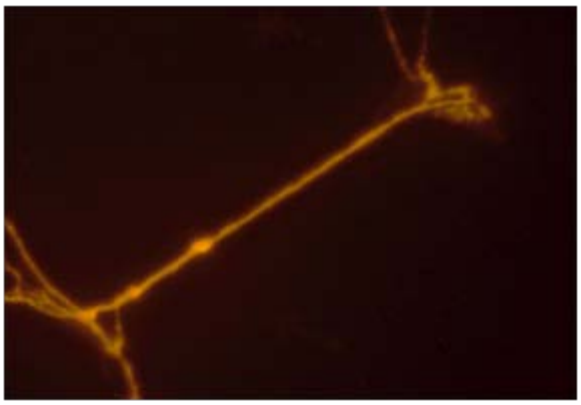
Data analysis was conducted using the software Image J and Microsoft Excel. The quantitative measure used was brightness, measured by average pixel brightness per region. Measurements were taken for lengths of 100 pixels each along the axon. Ten segments were measured for each axon. Measurements always started at the growth cone end and progressed in the direction of the cell body. The end of the growth cone was considered the point at which the filopodia ended, and was easily visible in most images as having a wider more bulbous area than the normal axon width. Measurements proceeded from this point down the axon. Average pixel brightness of the segment area was used as the quantitative measure; the average segment area brightness was subtracted from the average background brightness in order to account for the relatively high background fluorescence seen in some of the images. The average

brightness per segment was averaged for the data obtained from the three pictures measured for the control group and for the experimental group. This averaged data was plotted as a scatter plot for each group to demonstrate the differences in brightness along the axon. A bar graph was also created to demonstrate the differences in average concentrations along the axons between the experimental and control groups.

Results

The fluorescence pictures taken show that brightness levels were lower along the experimental axons than the controls (Figure 1). A higher brightness reading indicates a greater number, or concentration, of mitochondria. Background fluorescence also varied among images, with the control images having a greater level of background fluorescence. Background was controlled for in the data analysis. The brightness observed in this study indicates the overall cellular and mitochondrial charge, as I was unable to distinguish mitochondria from the cytoplasm for this data. Phase pictures were taken for each growth cone image and these images confirmed that Rhodamine-123 labeling only occurred where axons or cells were located. Fluorescence imaging settings were adjusted in order to obtain the best exposure; this ultimately resulted in an exposure time of two seconds. Visual observation of brightness before data analysis did not show any obvious trends in brightness along an axon. Brightness was variable for each image, with some showing the greatest fluorescence intensity at the very end of the growth cone and others showing sections of intense brightness in portions further down the axon.

Figure 1



A: Fluorescence image of a control growth cone stained with Rhodamine-123. The growth cone is the large structure in the upper right of the image.

B: Fluorescence image of an experimental growth cone stained with Rhodamine-123. The growth cone is located in the upper right of the image, and measurements start from where the filopodia end. Note the lower fluorescence brightness compared to the control.

Overall, the average mitochondrial brightness in the control group was greater than the experimental group for any given region (Figure 3). The average brightness of the region nearest the growth cone was 58.368 for the control group, and 33.303 for the mercury treated cells. No definitive pattern of increasing or decreasing brightness was observed with either group (Figure 2). The control group showed an overall lower brightness at the furthest region of measurement compared to the region closest to the growth cone (Figure 1A).

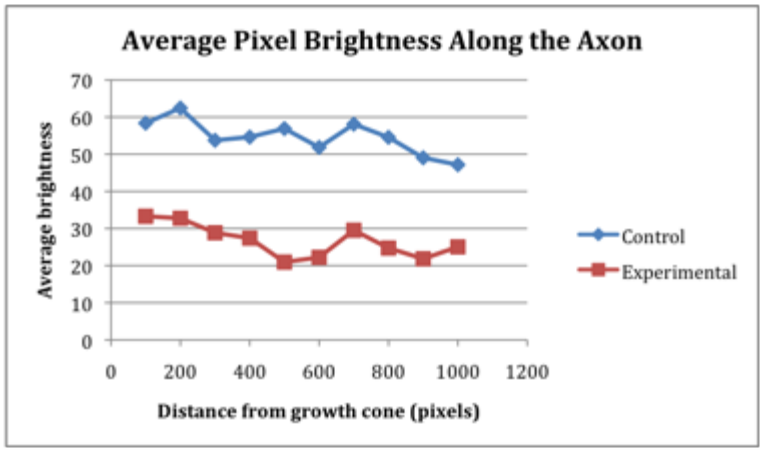


Figure 2: Graph showing the average brightness along the growth cone for control and experimental groups. All measurements start from the tip of the growth cone (n=3 for each group). No large trends in brightness change along the axon can be observed for either group.

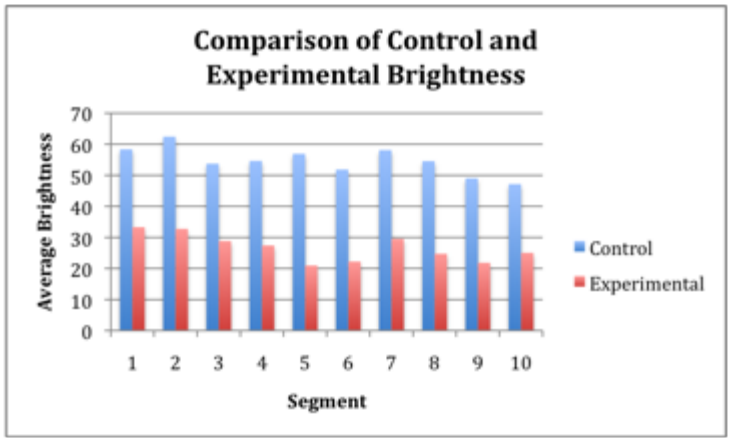


Figure 3: Bar graph comparing the average brightness of each segment measured for control and experimental groups. Control group average brightness is consistently much higher (n=3 for each group).

Discussion

The results of this study do not support my initial hypothesis. I expected to observe a much lower mitochondrial concentration near the growth cone when mercury was applied. I did not observe this trend in experimental data, and actually saw an overall higher brightness in the region nearest the growth cone compared to the region furthest away. The results from this study were unexpected because previous studies on the effects of mercury on the growth cone have shown growth cone retraction (Leong et. al., 2000), which I expected to correspond with mitochondrial redistribution as well. However, I did see that overall brightness was decreased in cells treated with mercuric chloride; thus the brightness did decrease between the control and experimental groups as expected. These results indicate that exposure to mercuric chloride does not change the ratios of mitochondrial concentration along the axon; however, it may have an effect on total mitochondrial concentration. If a larger population was studied with results found to be significant I could conclude that mercuric chloride does not change trends in mitochondrial concentration near the growth cone. The large difference in brightness between the groups could indicate the mercuric chloride causes a mass redistribution of mitochondria back towards the growth cone and out of the site measured in this study. It may also affect the ability of mitochondria to function properly, thus decreasing activity of mitochondria and resulting in a lower brightness.

Other studies have demonstrated that mercury affects the release of transmitters and membrane potential in mitochondria (Provan & Miyamoto, 1994). It has also been shown that mercuric chloride decreases the activity of mitochondria in both neurons and astrocytes (Kaur et. al., 2006). It is clear from these previous studies that mercury negatively impacts many cellular processes in mitochondria. Even though mercury was not shown to affect distribution of this organelle along neuron axons, the toxicity of mercury still has negative impacts on mitochondria in other ways.

Future experiments could include using different methods of measuring mitochondrial health and function, such

as measuring activity of different mitochondrial enzymes or effectiveness of transport. A possible source of error from this study was experimental data and control data were collected from different cultures, which may have had different mitochondrial concentration due to growth or age conditions. I would refine this experiment by observing the measured experimental growth cones before and after mercury application to observe if any retraction or other cellular effects occurred. It is possible that I did not measure far enough down the axon to see the effect I was looking for; therefore future experiments could also take measurements further down the axon.

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