

Axon Growth in the Presence of Mercury: The Effects on Cell Adhesion

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Introduction

Cell adhesion molecules (CAMs) are proteins located on the cell surface involved in binding with other cells or with the extracellular matrix (Kamiguchi, 2007). CAMs can be broken down into three major classes: integrins, cadherins and immunoglobulin superfamily (Kamiguchi, 2007). CAMs are involved in the development of neurons (Kamiguchi, 2007). During development CAMs play a crucial role in static cell adhesion as well as dynamic cellular events such as cell migration and process outgrowth (Kamiguchi, 2007). Neural cell adhesion molecule (NCAM) mediates side-to-side adhesion of axons (fasciculation) and controls axon guidance via a mechanism called selective fasciculation (Kamiguchi, 2007). Cadherins mediate cell-to-cell adhesion and regulate axon elongation via a homophilic binding mechanism (Kamiguchi, 2007). Cell adhesion is beneficial to axon growth (Kamiguchi, 2007). In order to study cell adhesion quantitatively one must find a way to measure where along two adhered axons cell adhesion is no longer present, this can be measured by the point where branching begins.

Branching of neuronal cells can occur in three different modes: splitting, delayed and interstitial (Acebes & Ferrús, 2000). The splitting mode involves the divergence of a growth cone, which yields two similar structures that continue their paths in opposite directions (Acebes & Ferrús, 2000). This type of branching is most often exhibited by dendrites (Acebes & Ferrús, 2000). In the delayed form of branching, a region of the axon with unstable cytoskeleton and frequent filopodial activity left behind the growth cone develops a branch that leaves the axon in the appropriate direction toward its target (Acebes & Ferrús, 2000). This form of branching is most often seen in axons (Acebes & Ferrús, 2000). In interstitial branching axon collaterals emerge as a single outgrowth from a stable axon (Acebes & Ferrús, 2000). Delayed branching was the mode of branching observed in this study. The point where axon branching occurred was used to mark the end of cell adhesion. Axon branching provides a neuron with the ability to establish synaptic contacts with multiple targets and is therefore critical for assembly of highly interconnected networks (Kalil et al., 2000)

Mercury is a neurotoxin that has been shown to lead to neuron degeneration (Xu et al., 2012). Mercury is known to disrupt synaptic connectivity by altering cytoskeleton structure and inhibiting growth of axon processes (Xu et al., 2012). Effects such as these produced by exposure to high levels of mercury are also observed in a variety of

neurodegenerative diseases and developmental disorders (Xu et al., 2012). As previous research has shown that mercury has detrimental effects on neurons it is reasonable to hypothesize that mercury will have degenerative effects on components of axon growth such as cell adhesion.

The focus of this study was axon growth in the presence of mercury. One specific property of axon growth was studied and measured. This property was CAM activity. The hypothesis tested was that cell adhesion measured by branching of axons is depleted in the presence of mercury. This hypothesis is interesting to study because through researching how mercury negatively impacts components of axon growth insight can be gained into how proper axon growth affects function of neurons. This can lead to insight into how mercury is related to developmental disorders and what levels are detrimental to the developing brain. Research on the toxic effects of mercury on neurons could also be beneficial to research on treatments for neurodegenerative diseases as the effects produced by mercury are observed in many neurodegenerative diseases.

In order to test this hypothesis *Gallus gallus* embryos were dissected before the tenth day of development. Sympathetic ganglia and dorsal root ganglia were dissected and then grown into sympathetic neurons, which were treated with mercury solutions. The neurons were treated with varying concentrations of mercury and cell adhesion measured by branching axons was observed.

Materials and Methods

In this study *Gallus gallus* embryos were dissected before the tenth day of development and sympathetic ganglia and dorsal root ganglia were removed and grown using growth medium and an incubator following the same procedure used to culture chick embryonic peripheral neurons (Morris, 2013a). Four flow chambers were created and ganglia were placed in flow chambers following the same procedure used to observe live unlabeled cells (Morris, 2013b). Three solutions with varying mercury concentrations were created using a dilution technique. The first solution consisted of 100 nanomolar (nM) mercury. Then using Tyrode's salt solution a 10nM and a 1nM mercury solution were created. Each solution was applied to a separate flow chamber following the same procedure that was used to apply vital dye to cells (Morris, 2013c). One flow chamber of cells was given pure Tyrode's Salt Solution to be used as a control sample.

Flow chambers were observed in the ICUC of the Mars Science Center at Wheaton College, MA. A group of neurons was found on the slide using a Nikon Eclipse E200 (C92) microscope under 10x objective magnification and phase optics. The microscope had a 1.0X C-mount adapter connected to a Sony DFW-X700 camera. The images were viewed on an Apple i-mac computer that used Mac OS X version 10.5.8. The images were collected using the software program BTV. The images were analyzed using the software program ImageJ. An image was captured and saved on the

computer every five minutes over the course of thirty minutes for each flow chamber of mercury treated cells as well as the control sample.

In order to measure cell adhesion the images taken at zero minutes and thirty minutes were compared side by side for each flow chamber of cells. Using ImageJ adhesion length of axons was measured. Axons adhered to another axon that eventually branched were located on the before and after image. Then the length of cell adhesion was measured in the before image and the after image. Cell adhesion length was measured by right clicking at the point where the axon is distinct from a cell body and then dragging the mouse until the point where branching of the axons began. Ideally a minimum of three measurements would be taken for each flow chamber created. In this experiment for the 1nM and 10nM mercury solution treated cells three different measurements were taken at three different locations. For the control only one measurement was available. For the 100nM mercury solution treated cells no measurements were available. ImageJ recorded the lengths in pixels. In order to convert the pixels to millimeters (mm) an image of a ruler was captured under the microscope at the same magnification (10x) and the number of pixels was measured in mm, then this was used to convert the pixels to mm in the cell adhesion measurements. A percent change in cell adhesion length was calculated for each set of measurements. Using the initial length and the final length of cell adhesion the change in length was calculated. This change in length was then converted into a percentage. The percentages for each treatment group and the control sample were averaged to come up with an overall average percent change for each treatment group. The average percent change for each solution was used to create a graph.

Results

Figure 1



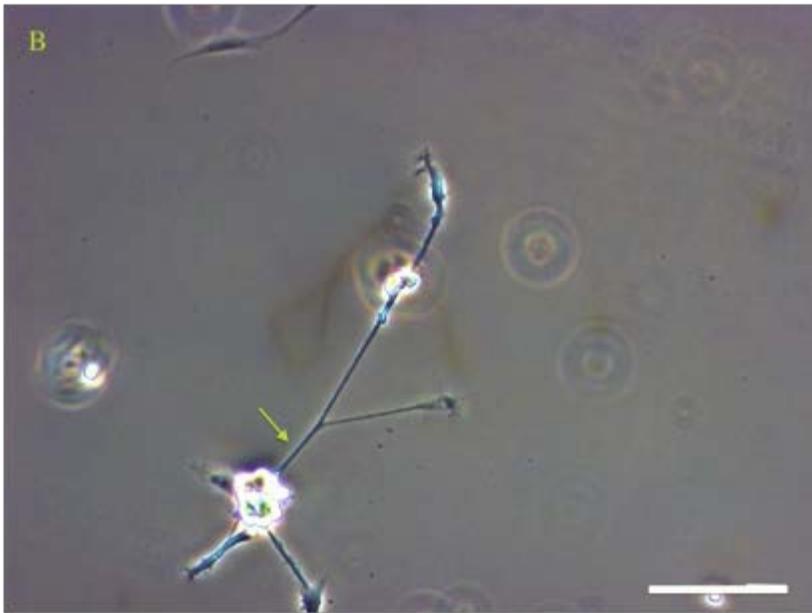
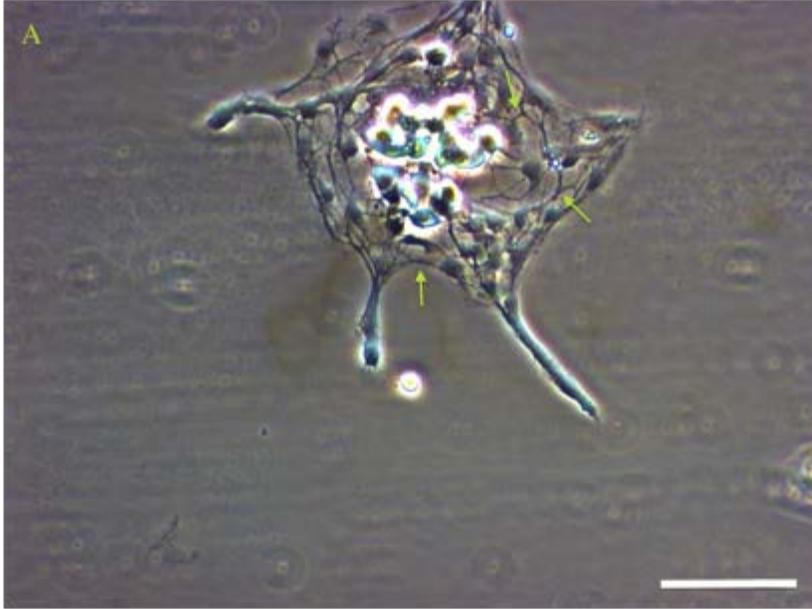


Figure 1. Sympathetic neurons left untreated taken with 10x objective with Nikon phase optics at 0 minutes (A) and 30 minutes (B). Arrow indicates where cell adhesion was measured. Bar, 0.1mm.

Figure 2



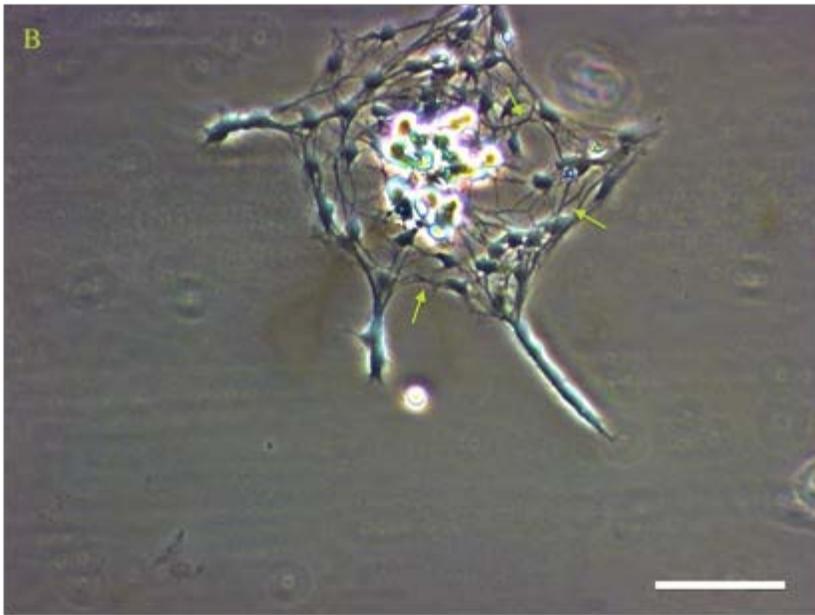


Figure 2. Sympathetic neurons exposed to 1nM mercury solution taken with 10x objective with Nikon phase optics at 0 minutes (A) and 30 minutes (B). Arrows indicate where cell adhesion was measured. Bar, 0.1mm.

Figure 3



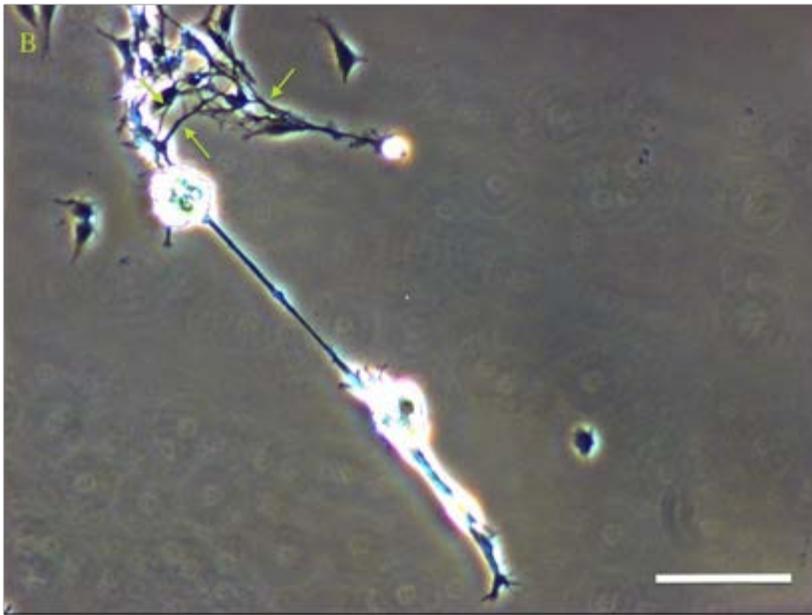


Figure 3. Sympathetic neurons exposed to 10nM mercury solution taken with 10x objective with Nikon phase optics at 0 minutes (A) and 30 minutes (B). Arrows indicate where cell adhesion was measured. Bar, 0.1mm.

Figure 4

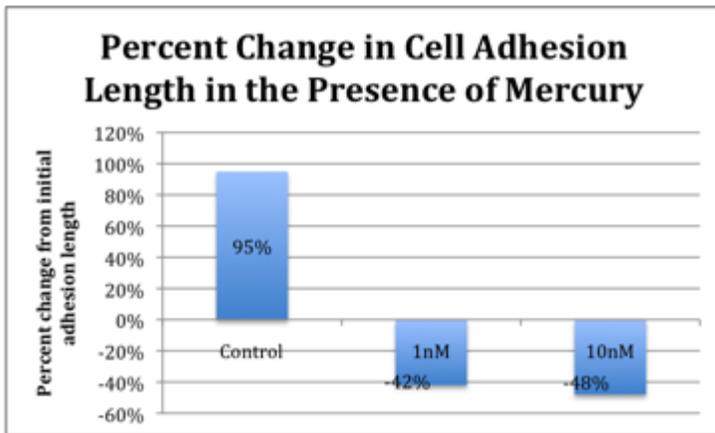


Figure 4. Average percent change in cell adhesion length in cells treated with mercury solutions and untreated cells. One cell adhesion length was measured in the control sample and three different cell adhesion lengths were measured in each the 1nM and 10nM treatment groups.

As seen in Figure 1, in the control sample cell adhesion length increased over time. As seen in Figure 2, in the sample exposed to 1nM mercury solution cell adhesion decreased over time. As seen in Figure 3, in the sample exposed to 10nM mercury solution cell adhesion decreased over time to a greater degree than in the sample exposed to 1nM mercury solution. In the sample exposed to 100nM mercury solution, no cell adhesion activity was observed. No branching activity was observed, axons retracted and blebbing occurred. In the untreated cells cell adhesion length increased over time. In the mercury treated cells cell adhesion length decreased over time. The higher the concentration of mercury the more cell adhesion length decreased over time. As seen in Figure 4 the average percent change in cell adhesion in the control sample was high. The average percent change in cell adhesion in the sample exposed to 1nM

mercury solution was quite low in comparison, and the average percent change for the sample exposed to 10nM mercury solution was even lower. The positive value of the control sample indicates that axon elongation of adhered axons occurred while the negative values indicate that retraction of adhered axons occurred.

Discussion and Conclusions

The data supports the hypothesis that in the presence of mercury cell adhesion as measured by branching, depletes. In the control sample cell adhesion increased over time and in the presence of mercury cell adhesion decreased over time. Cell adhesion decreased to a greater degree the higher the concentration of mercury applied. The data supports the claim that mercury diminishes cell adhesion, which suggests that mercury diminishes cell adhesion molecules. The data also supports the claim that by depleting CAMs axon growth is negatively impacted. In the mercury treated samples where CAMs were depleted retraction of adhered cells was observed, where as in the control sample elongation of axons was observed. If more research was done and the same conclusions were made then it could be argued that cell adhesion is an important factor in axon growth and that depleting CAMs with the presence of mercury causes considerable negative effects on axon growth.

This data does not directly explain how mercury depletes CAMs. However, mercury is known to cause cell body damage and degeneration of neurons (Xu et al., 2012). One of the mechanisms by which mercury causes degeneration of neurons is by disrupting the proteins of the cytoskeleton such as F-actin and β -tubulin (Xu et al., 2012). It is possible that by disrupting the cytoskeleton structure proteins located on the cell surface such as CAMs are also disrupted and eventually depleted. The data supports the previous findings that CAMs contribute to axon growth (Goldberg, 2003). Axon retraction was observed when CAMs were depleted and elongation of axons was observed when CAMs were present. There is more than one possible explanation as to why axon growth would be negatively impacted by depleting CAMs. One possibility is due to the fact that CAMs are a critical substrate along which axons extend (Goldberg, 2003). CAMs are identified as axon guidance molecules not only because they aid in elongating axons but also because they activate growth cones, which are responsible for generating the forward tension on the elongating axon (Goldberg, 2003). By depleting CAMs axon's outgrowth can no longer be guided and growth cones are not activated, therefore axon growth is negatively impacted and retraction of axons occurs. Another possible explanation of how depleting CAMs would impact axon growth is by eliminating communication between interactions at the plasma membrane to the cytoskeleton (Goldberg, 2003). Integrins and cadherins are some of the receptor families that recruit specific adaptor and scaffolding proteins to the site of cell adhesion to transduce signals from the membrane directly to the cytoskeleton (Goldberg, 2003). It is possible that by depleting CAMs and therefore communication between the plasma membrane and the cytoskeleton axon growth is negatively impacted.

If this experiment were to be repeated it would be beneficial to test more samples. By exposing more samples to mercury solution more data could be collected conclusions could be more strongly supported. Also in this experiment cells were observed at 10x magnification, which is a low magnification to observe such effects. This experiment can be improved by using a higher magnification so that more data could be collected and the data collected would be more accurate.

This experiment displayed how mercury depletes cell adhesion molecules, which in turn disrupts axon growth and how in high enough concentrations mercury can cause death of neurons. Future experiments should study other ways in which depleting CAMs can affect neurons. Future research should also focus on how disrupting the structure of neurons in the brain can contribute to impaired function of neurons and how this is related to degenerative diseases or developmental disorders. Data from this experiment suggests that CAMs contribute to axon growth. Previous research suggests that undisrupted axon growth is important to the proper development of the structure of neurons (Shapiro & Colman, 1999). Previous research has also suggested that axon structure is related to function in the formation of synaptic connections and that CAMs are important to forming synaptic connections because they act as synaptic specifiers (Shapiro & Colman, 1999). Future research could use these findings to advance knowledge on how impaired synaptic connectivity is related to degenerative or developmental disorders.

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