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The Effects of Mercury on the Classes of Fast Axonal Transport

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I. Mercury treated mitochondria have a lower membrane charge compared to control mitochondria.

<>II. Introduction

Mercury is a naturally occurring metal that can take many forms. The organic form of mercury is called methyl mercury and is a common contaminant in fish and shellfish. Methyl mercury is formed when the elemental form of mercury gets into lakes, rivers and oceans. From there the mercury is converted into methyl mercury by microbes found in the water. The result of this biochemical reaction is methyl mercury. Methyl mercury (MeHg) is a neurotoxicant that can affect many organ systems in the body however; the most targeted is the central nervous system (Leong et al., 2000). Methyl mercury can cause the most damage to the body is during fetal development where the body is most vulnerable. Being a neurotoxicant, methyl mercury can disrupt brain growth especially during the developmental stage. Pregnant mothers who take methyl mercury into their system can easily pass it to the placenta. The methyl mercury will then get transported to the brain where it disrupts and damages nerve cell division and differentiation. The effects of methyl mercury also compromise brain structure. High doses of methyl mercury also result in many complications including low birth weight sever mental retardation, cerebral palsy, deafness, blindness, and seizures. To further examine the effects of mercury on the CNS, we isolated sympathetic neurons from embryonic chick. More specifically we quantitatively examined the effects of methyl mercury on axonal transport and organelle activity. I hypothesized that mercury would decrease the potential of organelle activity. This topic is very interesting because mercury has broad toxic effects on nervous system and function. To properly examine the effects we wanted to examine at the molecular level the specific organelles and their function that are being affected. From this research, we then can properly examine from which molecule or function a certain process is disrupted. This helps researchers properly examine diseases as well as fully understand the effects of neurotoxins and how to properly treat them. My collaborator was Jasmine Bhatia. J. Bhatia examined axonal transport by using time-lapse video imaging to measure the rate at which organelles traveled along microtubules through axons. Both experiments reflected the condition of the neuron and how successful it was at performing a specific function, which, in this was growing. By conducting these experiments we were able to further examine the effects of mercury on the growth capabilities of the cell by specifically looking at the organelles involved. The development of time-lapse and still imaging techniques for intact isolated neurons, using cell culture systems, has allowed direct observations of axonal transport as well as organelle activity. Therefore, the primary objective of this study was to determine whether mercury had an effect on membrane charge of mitochondria found in axons.

<>III. Materials and Methods

Materials:

Animals: Experiments were performed on sympathetic neurons dissected from 8-10 chicken embryos, dissociated, and plated on sterile coverslips coated with poly-lysine and laminin. Coverslips were put into incubator at 37 degrees C to allow growth for 20-24 hours. All tissue culture media and supplements were obtained from Sigma Chemical Co. (St. Louis, MO). Rhodamine 123 was obtained from Molecular Probes, Inc. (Eugene, OR). Methyl mercury was obtained

through Jani Benoit, Assistant Professor of Chemistry, Wheaton College, Ma.

Cell culture: Sympathetic chain ganglia were dissected from 8-10 day-old cicken embryos and cultured as whole ganglia or dissociated and grown as single cells on sterile coverslips. Cells were grown for 20-24 hours in a 37 degree C incubator in Liebovitz L-15 medium (HBSS) supplemented with 10% fetal bovine serum, 0.6% glucose, 2 mM L-glutamine, 100 mg/ml penicillin, 100 mg/ml streptomycin, 50 ng/ml nerve growth factor, and 0.5% methyl cellulose (F+ medium). The coverslips were treated with 1mg/ml poly-lysine followed by a laminin medium for 20-60 minutes prior to plating cells. Cultures were allowed to grow for 20-24 hours after cells were plated. (Morris et al., 1993)

***Application of heavy metal solution*:** All neurons that had adhered to the poly-lysine substratum could be used for the experiment. Neurons were allowed to extend for 20-24 hours after plating before exposure to heavy metal solution. Metals were obtained from Jani Benoit (Wheaton College, MA) Working solution concentration was 10 nM. Two culture dishes with 1x concentration of DRG were obtained and labeled. F+ medium was removed from both culture dishes and Hg working solution + HBSS was added to experimental culture.. Control culture received 0.5% HCL + HBSS. Solutions were delivered using individual Pasteur pipettes. Both cultures were incubated for 10 minutes at 37 degrees C.

Application of Rhodamine:

Once cells were removed from incubator. All solution was removed from cultures. Rhodamine 123 (R123) was added to both cultures in order to view mitochondria in living drug treated cells. Both cultures received 0.1 mg/ml R123 and were incubated for 10 minutes at 37 degrees C.

Preparation of Chip Chambers:

Coverslips were removed from culture dishes and placed on chip chambers with the cell-side down. A drop of Hanks was used on the chip chamber (glass chips placed in circle on slide) prior to transfer to keep cells from flattening. Excess HBSS was removed from the edges of the slide to prevent leakage. All edges were then sealed with VALAP. Following a 2-minute wait period for the VALAP to dry. The coverslips were rinsed with distilled water in order to remove all excess salt. Slides were dried again with kimwipes and then were used for imaging.

Imaging Technique:

Immunofluorescence microscopy was used in order to view the mitochondria in living, mercury treated cells. The upright microscopes, E600 and E400 were used for viewing the cells, and Spot Advanced captured all fluorescent images. Cultures were placed on E400 stage with a 37 degree C stage warmer and R123 stained mitochondria were viewed using fluorescent microscopy. Fluorescent images were taken under Phase 3 on E400.

Data Collection:

The experiment was conducted three times, and two culture dishes were used in every experiment. Membrane charge was quantified as a level of brightness using immunofluorescent microscopy. Fluorescent still images were captured using Spot Advantage. Each image was then transferring into Photoshop where brightness could be measured. Brightness was measured by selecting areas of each cell that were expected to contain mitochondria. This included all axons that were visible in each fluorescent image. The brightness of mitochondria was used to quantify the health of the cell. Brightness was measured and quantified in mercury treated and controlled images. The three experimental and control values were each averaged and compared.

IV. Results

To determine if membrane charge in sympathetic neurons were affected by the presence of mercury we adapted previously published techniques for methyl mercury exposure time and concentrations (Leong et al., 2000). Although the effects of methyl mercury on neurons have been extensively studied, we introduced a new aspect into the experiment and used primary culture chick sympathetic neurons. In general when viewing the mercury treated neurons compared to the control neurons there appeared to be much less movement in the cell. When immonofluorescent time lapse was used, one could see that there appeared to be fewer organelles in the axons of the neuron.

The average brightness for mercury treated axons was quantified as 36.007 %, where as the control axons had a brightness of 48.07%. Similarly, when observing axonal transport there seemed to be a decrease in the occurrence of organelles passes a certain point on similar specific regions on neurons.

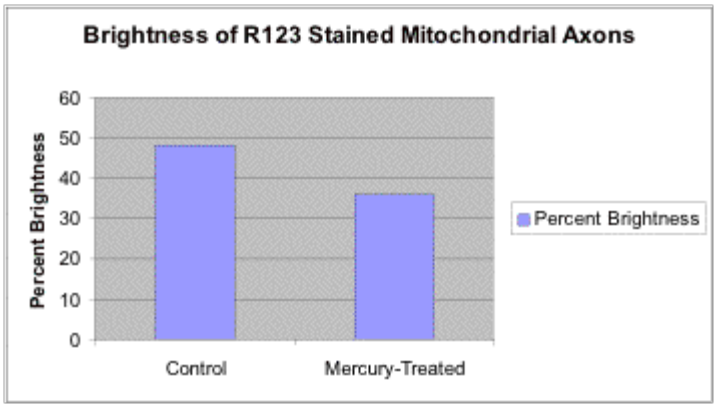

Fig 1 – Brightness of R123 stained mitochondria varied between the mercury exposed axons and the controlled axons. Membrane charges of mitochondria in mercury treated axons were lower compared to the membrane charges of mitochondria in controlled axons. n =2

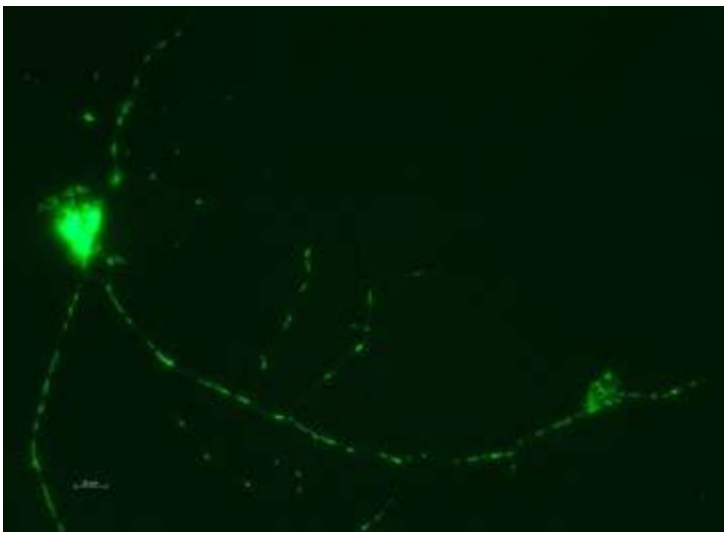

Fig 2- Sympathetic neurons were digitally imaged using the Nikon Eclipse E400 microscope. Membrane charged mitochondria were imaged through the use of Rhodamine 123. Membrane charge was measured as a level of brightness.

V. Discussion and Conclusion

The results of the experiment described herein demonstrates that exposure to methyl mercury does have an effect on organelle activity. In order to test this we used well-characterized techniques to grow neurons and then exposing them to mercury (Loeng et al., 1993). R123 was used to image individual mitochondria in the presence of mercury. The results reflect that membrane charges of mitochondria in methyl mercury treated axons are lower than membrane charges of mitochondria in controlled axons.

The question arises as to whether any source of error could have occurred in the data. A fixed source of error occurred when comparing cells during quantification. This error was unavoidable due to the procedure of the experiment. Two cells are never the same and therefore cannot be compared as the same. However, trends were seen throughout all experiments and therefore averages were taken to minimize this error.

These findings are consistent with J. BhatiaÕs results indicating that the presence of mercury decreases the rate of axonal transport in neurons treated with mercury. The number of occurrences of organelles in mercury treated neurons is significantly lower then in cells that were not treated with mercury although they were under the same conditions.

Future experiments would involve more cultures and more trials. I feel if this experiment was given more time the results would be more valid and refined. Another aspect of the experiment I would change would be the quantification process. If this was more defined to specific regions of the neuron and there were more guidelines regarding the selection process I feel it would help validity of quantification. Examining the effects on other organelles that are involved in axonal transport could extend the results from this experiment. In addition, the level of mercury could be examined further. In this experiment we used one tenth the amount of mercury used in C.C.W. Loeng used in his experiment. In future experiments it may be interesting to increase this dosage and see the effects of membrane charge.

VI. Bibliography

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