The Effect of Mercury on Neuron-Glia Interaction

Introduction

Mercury is a form of heavy metal known to be toxic to humans. It has been shown to cause cell death due to the collapse of cytoskeletal structure, because it has a high affinity for binding to tubulin and interfering with the building of microtubules in the cell (Castoldi, 2000). Methylmercury is the most common type of mercury found in the environment (US Geological Survey, 2006). It consists of a methyl group bound to a single mercury atom (US Geological Survey, 2006), and the majority of human exposure to it comes from eating fish and shellfish (Environmental Protection Agency, 2006). Because unborn babies and young children have nervous systems that are still developing, they are most at risk for contracting severe mercury poisoning if exposed (Environmental Protection Agency, 2006). The Environmental Protection Agency and Food and Drug Administration have agreed on a dosage of .1 µg/kg of body weight/day as one which would have no recognized adverse effects (Environmental Protection Agency, 2006).

The primary purpose of this study was to determine the effect that methylmercury has on communication between a neuron and its glial cells. In order to do this, peripheral neurons from embryonic chicks were incubated in a solution containing mercury, and then the number of connections they formed with their glia was compared to the number of connections formed between neurons and glia in samples which had not been exposed to mercury. The hypothesis for this study was that there would be a significant decrease in the amount of interaction between neurons and glial cells in the samples which had been exposed to methylmercury.

The experimental methods used here were adapted from a 2000 study by Leong et al. which analyzed the effects of mercury on the growth cones of snail neurons. They were testing to determine if mercury had an effect on tubulin, and their results showed that there was indeed a collapse of structural integrity in the cells as a direct result of mercury being applied to them (Leong, 2000). There were nine other experiments done in conjunction with this one, which studied the effects of mercury on cell structure, growth, and organelle transport.

This study was closely related to that of Sara Tower, who analyzed the effect that mercury had on interactions between neurons. By studying the same slides and some of the same interacting areas, collaborative information was collected in which it was determined that mercury has a similar effect on both types of communication.

Studying the effects of mercury on organisms like chicks is a critical step in understanding its potential impact on humans. It is known that mercury is toxic, in particular to developing fetuses, but further research may produce new information about the reasons for these effects and the particular problems that mercury may cause on a cellular level.

Materials and Methods

Primary Culture of Chick Embryonic Peripheral Neurons: Ten day chicken eggs were used for this culture. First, the egg was set with blunt end up and sprayed with ethanol to sterilize. The dissection was performed in a 110mm Petri dish filled with 5ml Hanks Balanced Salt Solution (HBSS). Once the egg was dry, sterile forceps were used to tap through the shell and lift the top away, and then to lift the embryo out of the egg and place it in HBSS. The head was removed and returned to the egg for disposal. Once in the dish, the legs, wings, and viscera were removed, and the body was oriented with its ventral side up. All tissue was removed to expose the spinal cord. Forceps were then used to tease away the sympathetic chains and pull out dorsal root ganglia by the tails. The dissected ganglia were then placed in a 25mm Petri dish with HBSS.

Dissociation of Ganglia: The debris was cleaned from the ganglia and they were washed with HBSS. The HBSS was then removed and replaced with trypsin solution (Ca/Mg-free HBSS with .25% trypsin). This was incubated for 15-20 minutes at 37°C. Then the trypsin was removed and the cells were resuspended in a small amount of HBSS, then triturated with a drawn Pasteur pipette until dissociated.

Preparation of Substrata: In order to coat the surface of the coverslip with 1mg/ml poly-L lysine, drops of polylysine were placed on the inside lid of a Petri dish and one sterile coverslip was set on each for 20 minutes. The cover slips were then rinsed with sterile water and allowed to dry. Once dry, they were coated with laminin in HBSS for 20-30 minutes or longer. The cover slips were kept wet until plating by leaving them on laminin or in a dish of HBSS. Before plating, they were rinsed with HBSS.

Plating Cells: Dissociated cells were mixed into F+ medium (Leibovitz L1 5 medium plus 10% fetal calf serum, .6% glucose, 2 mM L-glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, 10-50 ng/ml NGF) and placed into a small Petri dish, laminin side up. The dish was then filled with medium and cells. The cells were allowed to settle onto the cover slip for 24 hours.

Application of Mercury: The mercury solution consisted of 10 µM $HgCl_2$ in .5% HCl, diluted 1:1000 in HBSS, which produced 10 nM $HgCl_2$ HBSS. Control dosages were the same concentration of HCl without the mercury. The growth

medium was removed from the Petri dish and experimental or control solutions were added. This was then allowed to incubate for 20 minutes at 37°C. After this period of time, the dishes were removed from the incubator and the solution was extracted. The growth medium was replaced, and the dishes were allowed to sit for 10 minutes.

Creation of Chip Chamber: Cover slip chips were placed on a slide, and a drop of medium was placed in the middle of the chips. The medium was removed from the Petri dish and the cover slip was picked up with forceps. The back of the slip was dried with a kimwipe and deposited laminin side down on the slide. The edges were sealed with VALAP and any salt remaining on the slide was rinsed off with DDi water. The slip and slide were then dried with a kimwipe.

Observation and Data Collection: The slide was placed on a microscope at magnification 40x. The program BTV Pro was used to capture time lapse videos of cell movement, one photo each 30 seconds for 20 minutes. Overall, there were ten videos collected, five of which showed neurons in control solution, and five of which contained neurons which had been exposed to mercury. The movement of neurons and glia were observed, with the number of connections formed over the course of each video being counted and written into a chart. In this case, a connection was defined as two extensions, one from a neuron and one from a glia, coming together and forming a single channel between the two cells (see Figure 1). In some cases, there were preexisting links between the cells. These were not included in the data collection. Only connections that actively formed during the time they were being observed were counted. Once each video had been carefully observed, the total connections formed were written down, and the sums of the connections made in control and experimental conditions were found. These two numbers were then compared to see if there was a difference between connections formed in samples with mercury and samples without.

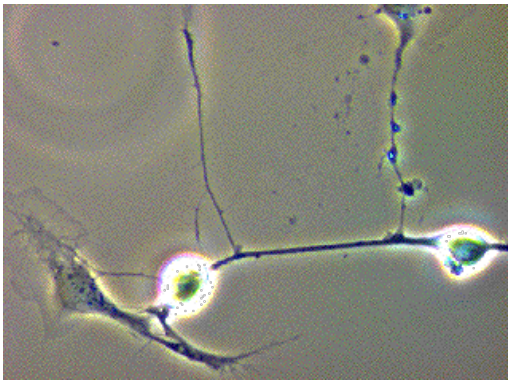

Figure 1: Two neurons with preexisting connections to glial cells in a sample containing mercury.

Results

Ten videos were taken, showing the movements that the cells made over the course of twenty minutes. There

was a similar amount of movement between the cells with mercury and the cells without, but those without mercury formed more connections with their glia than those which had been put in the presence of mercury.

In the five videos which contained control cells, there were a total of fifteen of these connections formed. In the other five videos, containing cells which had been incubated in the presence of methylmercury, there were only three of these channels formed in the same amount of time. These data can be seen in Figure 2.

Figure 2: Graph of the connections formed between neurons and their glia in control samples and samples containing mercury.

Discussion

The results of this experiment show that exposure to mercury does indeed have some bearing on the way that a neuron interacts with its surrounding glial cells. Those cells which had been incubated in the presence of mercury displayed a decreased tendency to form connections with the glia around them, while those which had been grown in control solutions formed many channels over the course of the time they were observed. These findings appear to support the hypothesis of this study, indicating that mercury does have a detrimental effect on the communication between neurons and glia.

If this study were to be repeated, the observations would be more precise if they contained more videos. Due to time constraints, only ten were able to be collected, and the data would perhaps show more accurate results if there had been a larger sample size observed.

The results of this experiment were closely related to those of Sara Tower's study, in which she looked at the effect that mercury had on communication between neurons themselves. Her data also showed that there was a marked decrease in the number of triple points that formed between adjacent axons in samples which had been incubated with mercury compared to those which had been grown in control conditions (Bhatia et al., 2006). She calculated the rate of triple point formation and discovered that the rate was much higher in the control cultures (Bhatia et al., 2006).

Future experiments in this field should focus on the reasons for this apparent decrease in the number of

connections that form between neurons and glia when they are in the presence of mercury. Mercury has been shown to have a profound effect on the structure of growth cones in neurons by inhibiting GTP binding to beta tubulin and preventing the formation of microtubules (Leong, 2001). Further study could determine whether that is a contributing factor in the development of extensions that reach the glia around them, and a reason why mercury inhibits such communication.

References

Bhatia, J., Chick, B.J., Cummings, S.C., Fess, J.M., Jeffrey, B.A., Kovacs, E.A., Shorey, L.E., Silverio, A.L., Tower, S.A., Yen, R., & Morris, R.L. 2006. The effects of mercury on primary culture chick sympathetic neurons. Presented at NEURON conference, Hunter College, New York, 8 April 2006.

Castoldi, A.F., Barni, S., Turin, I., Gandini, C., & Manzo, L. 2000. Early acute necrosis, delayed apoptosis and cytoskeletal breakdown in cultured cerebellar granule neurons exposed to methylmercury. *Journal of Neuroscience Research*. 59: 775-787.

Environmental Protection Agency. Mercury: basic information. 14 April 2006. <http://www.epa.gov/mercury/about.htm>. [accessed 2006 April 30]

Leong, C.C.W., Syed, N.I., & Lorscheider, F.L. 2001. Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following *in vitro* exposure to mercury. *Membrane Biophysics and Biochemistry*. 12: 4 26, 733 737.

U.S. Geological Survey. Toxic substances hydrology program: methylmercury. 12 April 2006. <http://toxics.usgs.gov/definitions/methylmercury.html>. [accessed 2006 April 30]