Effects of Nocodozole on Pseudopodial Formation in Amoeba proteus

Daniel C. Stein
x4830

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I. Introduction

Microtubules are the largest components of the cytoskeleton in eukaryotic cells. Made of polymers of ß-tubulin and alpha-tubulin dimers, the microtubule is a ring of thirteen polymers of tubulin (Cooper, Hausman 2004). They perform essential functions in transporting organelles throughout the cell, as well as aid movement of the cell within its environment (Howard, Hyman 2003). By binding to vesicles carrying proteins along the secretory pathway microtubules regulate the transport of proteins, some of which deliver signals to the actin cortex directing it to polymerize out from the cytoplasm to form a pseudopod. The Amoeba proteus is a large member of the genus Amoeba ranging from 100-500 microns at it's longest measureable length. Extensions of the cell membrane called pseudopodia (Latin for false foot) enable the cell to move within its environment and help it to ingest food through phagocytosis. For the purposes of this experiment, a pseudopod is defined as an extension of the plasma membrane having a length to width ratio of no less than 1.5:1. In my study of the Amoeba proteus I tested the effect of Nocodozole, a drug known to depolymerize microtubules, on pseudopodial formation in Amoeba proteus. Nocodozole is known to bind tubulin and prevent the addition of further ß-tubulin and alpha-tubulin dimers to the plus end of the microtubule (Childs 1996). This action prohibits the transport of vesicles that secrete signal proteins that direct pseudopodial formation.

By adding Nocodozole I expected to find that cell movement would be slowed. Due to the decreased ability of microtubules to polymerize at rates sufficient to extend the plasma membrane (through the growth and catastrophe of the microtubules), the rate of formation of pseudopodia should decrease. As a larger species of the genus amoeba, the Amoeba proteus is particularly useful for this study because their size is more conducive to imaging on light microscopes. This experiment is useful in helping to understand the movement of cells under stress introduced from outside their environment. Understanding pseudopodial formation in protista such as amoeba is important to our understanding of cell movement because through cell movement a motile organism is able to acquire food to maintain homeostasis.

II. Materials and Methods

Materials:  Nikon Eclipse E200 light microscope
Nikon SMZ660 dissection microscope
Spot Insight QE camera

Control. I examined the cells of *Amoeba proteus* under the Nikon Eclipse E200 light microscope at a magnification of 400x. Cells were obtained from a culture dish and extracted using a pasture pipette while looking for the Amoeba under a Nikon SMZ660 dissection microscope and on the computer using BTV Pro with video imaging from a Dage MTI-DC200, which was connected to the dissection scope. The cells were placed on a slide within the chamber created by placing cover slip chips in a space large enough to place a whole cover slip over it. The cover slip was placed on the chips by touching the slip to the water droplet on the slide and then laying it over the drop to avoid creating air bubbles. Any water that escaped the boundaries of the cover slip was cleaned with a Kim wipe. Using this chamber allows for amoebae move on the slide while being examined. Images were taken with a Spot Insight QE camera and imaged with the program Spot 3.5.5 on a Apple G4 computer with OS X. The first and last images were taken using the <Extract images> function in Spot 3.5.5. After extracting the images, a file was made of each image and opened in the program ImageJ. Using the <Measure> function in ImageJ, I was able to measure the length and width of a pseudopod by drawing a straight line using the line on the toolbar. The length is computed in pixels which I converted to microns using the standard conversion factor of 0.183microns/pxl computed empirically for the Spot Insight QE camera.

Experimental. Cells treated with Nocodozole were examined at a magnification of 100x. Cells were prepared using the same method for the control with the additional step of placing one drop of Nocodozole on the slide. A 0.08 µg/ml solution of Nocodozole was dropped on the slide where, through dilution, its effective concentration was 0.04 µg/ml (Borokowski, 2003). Imaging and measuring was done with the same procedure as for the control.

I compared the change in size of the pseudopod of the control and that of the experimental cell. One trial of each the control and experimental were performed. Through this I was able to examine the effect of Nocodozole on pseudopodial formation.

III. Results
Figure 1. This is the first picture taken of *Amoeba proteus* under control conditions. The above circled pseudopod corresponds to the measurements below. The original image was 1600 pixels (pxl) by 1400pxl. This was reduced to 640pxl by 480pxl to produce the above figure without losing the conversion factor of 0.183µm/pxl. This converts to a length across the image of 117µm, which is consistent across all images in this paper because they are all 640pxl across.
Figure 2. This picture was the tenth in a series of ten images taken every 30 seconds. This picture represents the movement of the control amoeba after 5 minutes. Again the circled region is the region that was a pseudopod in the beginning of the image series and changed its shape over the course of five minutes. The calculations below for the tenth picture correspond to this figure.

The first picture of the control showed a pseudopod with a width of 74.7 pixels and a length equal to 112.6 pixels (pxl), and the tenth picture showed the same pseudopod at a width of 130.6pxl and a length of 142.6pxl. In microns, this size change went from a LxW (length by width) size of 20.6x13.7 to one of 26.1x23.9. This last image had altered its size so that it was no longer a pseudopod according to my definition (length/width ratio of at least 1.5:1). The control pseudopod was seen to change only a minor amount in viewing the first and final pictures, but this change was enough to make it no longer defineable as a pseudopod because it did not have a length to width ratio of at least 1.5:1.
Figure 3. This is the first image in a series of 10 taken over 5 minutes a rate of one every 30 seconds after treating *Amoeba proteus* with nocodozole. The circled pseudopod corresponds to the measurements below.
Figure 4. Seen here, the *Amoeba proteus* has formed a smaller extension of the plasma membrane (in comparison to the control) five minutes after adding nocodozole. The circled pseudopod corresponds to the measurements below.

In Figure 3, the pseudopod is 51.0 pixels in width and 84.9 pixels in length. This computes to dimensions in microns of 9.3x15.5, width by length, respectively. In Figure 4 the pseudopod examined was measured by ImageJ to be a width of 46.2pxl and a length of 65.8pxl, which in microns is a width of 8.5 and a length of 12.0. The change in length of the experimental pseudopod was 3.5µm and the change in width was 0.9µm. The change seen in the control was on the order of -10.2µm in width (meaning the control grew in width) and -5.5µm in length (demonstrating a growth in length as well). In comparing the change in shape of the control after five minutes to that of the experimental after five minutes, it can be seen that the original pseudopod in the control changed more drastically than the original in the experimental. Though both were no longer pseudopodia after the five minutes span, the difference in growth and deconstruction rates was not great. However, this difference is very small.

**IV. Discussion and Conclusions**

From this experiment it can be said that the data does indicate that nocodozole has an effect on the formation and deconstruction of pseudopodia in *Amoeba proteus*. Through changing the number of pseudopodia that the cell has or causing more or less to form from the plasma membrane, cell movement is increased or decreased in a way that is undesirable to the amoeba's need for food. When the organism needs to obtain food, if the pseudopodia are or too few, the cell may not reach its target food, thus potentially placing the cell in danger of starvation. Evidence for this comes from the fact that amoebae acquire their food through phagocytosis which requires the extension of the actin cortex and plasma membrane outward to engulf a food particle and digest it once inside the cell (Cooper, Hausman 2004). Also, the decreased ability of amoebae to move through their environment which requires employing their pseudopodia would diminish access to food.

Based on the data the pseudopodia seem to form and deconstruct at a slower rate when treated with nocodozole. This indicates that nocodozole does in fact slow polymerization and depolymerization of cytoskeletal filaments that help the
cell to extend its plasma membrane. By binding to tubulin dimers, nocodozole prevented the polymerization of microtubules. Without microtubules to carry signal proteins to the actin cortex, transport vesicles in the cytosol did not signal the actin cortex to extend outward and form a pseudopod. My hypothesis was supported by the data, although somewhat weakly. The difference between the first and final sizes of both the control and experimental cell were of a magnitude that suggests that if I had used a longer span of time than five minutes to determine the effects of nocodozole, I might have seen more dramatic result that would have given greater support to my hypothesis. Another possible source of error may be considered in my measuring, which was not done from a fixed point on each pseudopod. My collaborator, Nguni Phakela, worked with the same protocol to see the effects of cytochalasin on actin filaments. His data reflected similar weak connections to his hypothesis.

Such weakness may point to a need to change my methods. In future experiments I may want to use a different method to measure the pixels of the image I obtain. In addition, the method that both my collaborator and myself employed to define a pseudopod may confine the interpretation of a pseudopod to an unnecessary degree. My collaborator and I would also have similar sources of error due to our choice not to measure multiple pseudopodia in the control and average the changed sizes. Again, this also points to a need to broaden our definition of a pseudopod.

Finally, I believe that this experiment can be done itself with the implementation of some of the considerations I have mentioned above. If it were to be carried further I would suggest studying the effect of nocodozole (or cytochalasin) on nerve cells in chordates. Such a study might yield useful information regarding nerve signalling and anesthesiology. It may be that nocodozole could be an effective anesthetic at low dosages. I would suspect that large doses would damage neurons too greatly to enable them repolymerize enough of their microtubules to regain their full function.

V. Bibliography


5. Collaborator, Nguni Phakela.