Does Caffeine Affect the Number of Filopodia in the Coelomocytes of Sea Urchins?

Researched by Jasmine Bhatia

Posted on December 9th, 2005

Introduction

I collaborated with Abbe Bosk on this study of the effects of caffeine; however we explored different aspects so and it must be noted that general explanations were co-authored.

Filopodia, or microspikes, are long and slender extensions of the cell that are supported by actin bundles. Filopodia are essential in cell motility. They allow a cell to explore its environment through movement. Cell motility through retraction and formation of filopodia is a complex and intricate process in which the assembly and disassembly of actin filaments is regulated (Cooper et. Al. , 2004).

Caffeine is a stimulant which increases metabolic activity which would mean an increase in the activity of the mitochondria cells. Mitochondria are the primary suppliers of energy to the cell and play an important role in the generation of metabolic energy in multi-cellular organisms. In this study, we tested the hypothesis that caffeine increases metabolism, which thus can be measured through an increased number of filopodia. With an increased number of filopodia, we can extrapolate that a cell has increased cellular activity because an increase in metabolism would cause the coelomocytes to have more filopodia, which would allow the cell to perhaps move faster.

Metabolism through testing effects of caffeine can be measured in the amount of energy expended since it is known to have the physiological effects of increasing a person's energy levels. Caffeine is known to cause a person to have the physiological effect of rapid, repeated movements colloquially referred to as "the jitters." We are exploring this physiological effect on a cellular basis and are taking this concept of the jitters to mean that an increased number of filopodia would be an effect of the increased metabolism because it might allow the cell to move faster, since a coelomocyte can not undergo the jitters that human beings experience when caffeine is present in the body.

Actin is a small scale version of muscle fibers, and we can use this fact to translate cellular results into relevant physiological evidence. Actin filaments in filopodia are rapidly assembled and disassembled to allow the cell to move. During this morphological transformation, actin filaments extensively reorganize from a random meshwork into tight bundles, which become the skeletons or cores of the filopodia (Otto, J. J., Bryan,J). In order for a coelomocyte to move, first its filopodia must be extended from the leading edge of the cell, then the filpodia must attach to the substratum across which the cell is migrating. Lastly the trailing edge of the cell must dissociate from the substratum and retract into the cell body.

Experiments have indicated that the extension of the leading edge involves the branching and polymerization of actin filaments. The regulation of this process involves small GTP- binding proteins. These GTP-binding proteins come from the Rho family and are thus referred to as Rho proteins. These proteins initiate local actin filament growth and branching by activating the Arp2/3 complex to create branches and ADF/coilin to sever pre-existing filaments to allow new growth from the growing end. As the new microfilaments provide pathways for the delivery of membrane vesicles and proteins needed for continued extension. Actin-bundling proteins which are needed for the formation of actin bundles and stress fibers immediately behind the leading edge. Calponin family proteins, which are found in focal adhesions are membrane associated proteins that links the Arp2/3 complex to the membrane at the leading edge. Intermediate filament proteins are also transported toward the leading edge where they are used for the reorganization of the intermediate filament meshwork. Both kinesin and myosin motors aid in these processes(Cooper et. Al. , 2004).

Cell attachment to the surface requires rebuilding cell-substratum or cell-cell adhesions. For these rapidly moving cells, focal adhesions form more diffuse contacts with the substratum. The reconstruction of focal adhesions happens in two steps, the appearance of small focal complexes containing a few microfilaments attached to integrin proteins and the growth of these focal complexes into mature focal contacts which require the development of tension between the cell and the substratum that is generated by myosin motors acting on actin bundles(Cooper et. Al. , 2004).

Finally, for the trailing edge to retract, the action of small GTP-binding proteins ,from the ARF family, break down existing focal adhesions. The contraction of actin bundles connected to the new focal adhesions formed at the leading edge generates the force necessary to pull the trailing edge forward (Cooper et. Al. , 2004).

Coelomocytes are the amoeboid immune cells of the body cavity of a sea urchin. Sea urchins are great model organisms because they share certain fundamental aspects found in humans. Human and sea urchins have been known

to be more closely related than previously thought. We used coelomocytes because they can be acquired easily and are present in abundant amounts. Further, these specific studies are significant in that filopodia are key in a cell's guidance towards a decision in human cells. Particularly, these studies can be applied to neurons because they rely heavily on the ability of their filopodia to sense and allow appropriate cell-cell connections to be made. Studies done on filopodia are very relevant and useful for people who have sustained injuries that have severed their neurons (Sem, K., et. Al). Filopodia, also called growth cones, can allow neurons to reconnect to their targets if a central nervous system injury occurs (Argiro et Al. 1985).

Materials

This section was co-authored with Abbe Bosk because we collaborated on developing a procedure and collected data together. We co-wrote a double length section as per Professor Morris's requirement for co-authorship.

Nikon Eclipse E400 Microscope with fluorescence capabilities
Diagnostic Instruments Spot Insight QE Camera, with Spot Advanced 3.5 software
Macintosh G4 with OS X 10.2
Adobe Photoshop version 7.0
Disposable transfer pipettes
Six well plate
Glass slides
Cover slip fragments
Kim wipes
Poly-lysine treated cover slips
PBS buffer
Vaseline/ paraffin wax mixture
1ug/ml Rhodamine 123
20 milli-molar solution of caffeine
Sea Urchins
3cc syringe
16 gauge needle

Methods

This section was co-authored with Abbe Bosk because we collaborated on developing a procedure and collected data together. We co-wrote a double length section as per Professor Morris's requirement for co-authorship.

Extraction of coelomocyte cells from sea urchins

A sea urchin was obtained. It was flipped onto its top side, and a 16 gauge needle was inserted into the region surrounding its mouth. The 3 cc syringe was used to draw up the coelomocyte cells from the sea urchin, and was released into a test tube.

Staining of the mitochondria and cover slip preparation:

Two cover slips and poly-lysine (0.1-.01ug/ml solution) were obtained. Two drops were applied to the tops of the cover slips on a Kim wipe. The poly-lysine was left on the cover slips for 5 minutes so that it could coat the surface. The excess poly-lysine was removed by washing the cover slips with distilled water. A Kim wipe was used to wick excess liquid off the cover slips. The coelomocyte cells were applied to the poly-lysine coated side of the cover slips. The cover slips were suspended in PBS for 5 minutes in a well plate. The PBS was removed and Rhodamine-123 was added to each cover slip. The well plate was covered with foil to prevent photo bleaching. 2 mL of 20 millimolar concentration of caffeine was added to the experimental cover slip and was again covered and incubated at 37^0C for 30 minutes. Chip chambers were prepared on two slides and a sufficient amount of PBS buffer was added. After 30 minutes, the PBS was removed and the cover slips were placed face down on the slides. The cover slips were sealed with vapolap and the slides were cleaned with distilled water and Kim wipes.

Imaging the cells:
Using The E400 Fluorescence Microscope

Observing specimens on a microscope
E400 Fluorescence- Brightfield
A bright field condenser setting was selected by turning the condenser so that "A" is visible at the front of the condenser. The microscope was turned on with the power switch located on the lower right hand side of the microscope. The stage was lowered by turning the large knob on the lower left hand side of the microscope clockwise. Then the nosepiece was turned to select the 4x objective, then 20x objective. The slide was placed on the stage and secured using stage clamps. The specimen was focused by using the focus knob used to lower the stage. Before focusing on the specimen, the specimen was viewed through the eye piece and the course focus adjustment was turned counter clockwise to rise the stage. When the specimen came into view, the fine focus adjustment knob was turned until the edges of the specimen were crisp. The condenser was adjusted by closing down the iris diaphragm. The condenser was focused until the edges of the hexagon were crisp. The iris diaphragm ring was turned clockwise to open it. The nose piece was turned to the desired viewing power and the fine focus knob was used to bring the specimen back into focus (English, C. et al. 2003)

Taking a picture with Spot
The spot camera was turned on and the spot program was opened. Once the sample was in focus, the silver knob on the head of the microscope was pulled. The **Live Image** button was pressed to show a preview of the image. The **Camera** button was pressed to capture the image (English, C. et al. 2003).

Quantifying data using Photoshop
The file was opened in spot and the entire image was selected and copied. A new image file was created in Adobe Photoshop and the image was pasted. It was zoomed and cropped so as to have a clear image of all filopodia present on the cell, Arrows were used to denote the filopodia present in the image.

Results

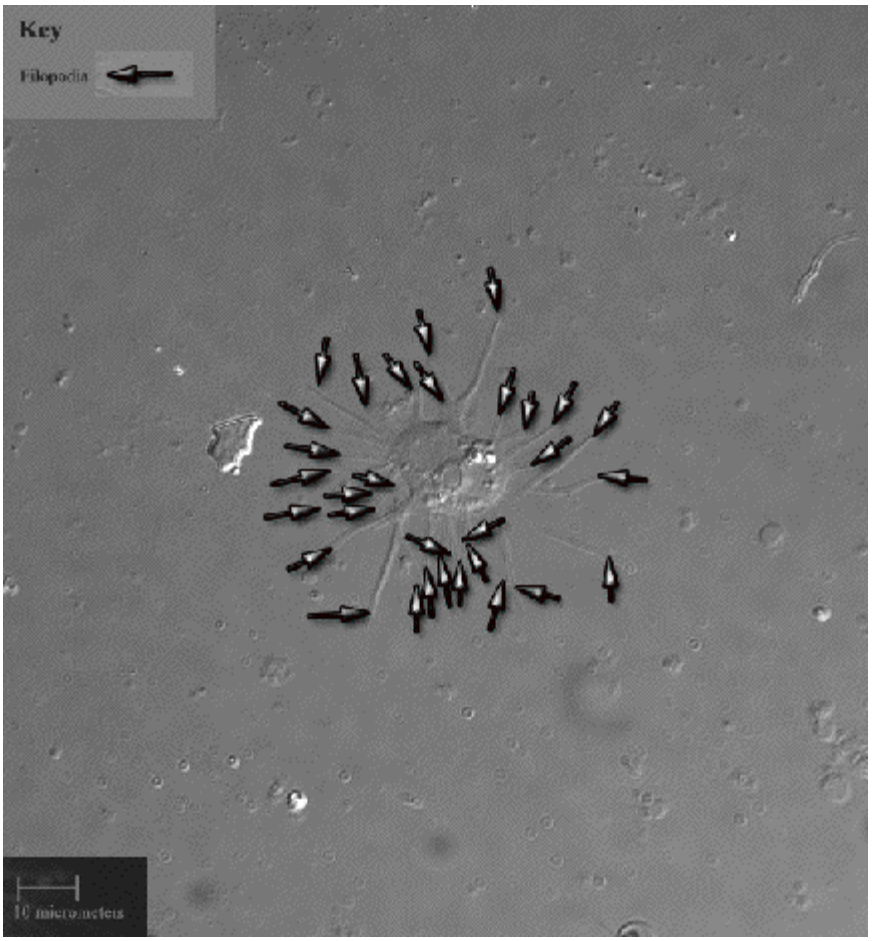

Figure 1. Experimental (caffeinated) Coelomocyte viewed at 60x
This is a coelomocyte cell treated with caffeine and viewed at 60x. The arrows indicate the presence of a filopodial extension. Arrows were added so that they could be counted in a systematic way. A scale bar was created by taking a

ruler and placing it on the Nikon Eclipse E400 Microscope and viewing it at 60x. Since all images were viewed through the same magnification, one scale bar can be applied to all images taken on the 60x setting.

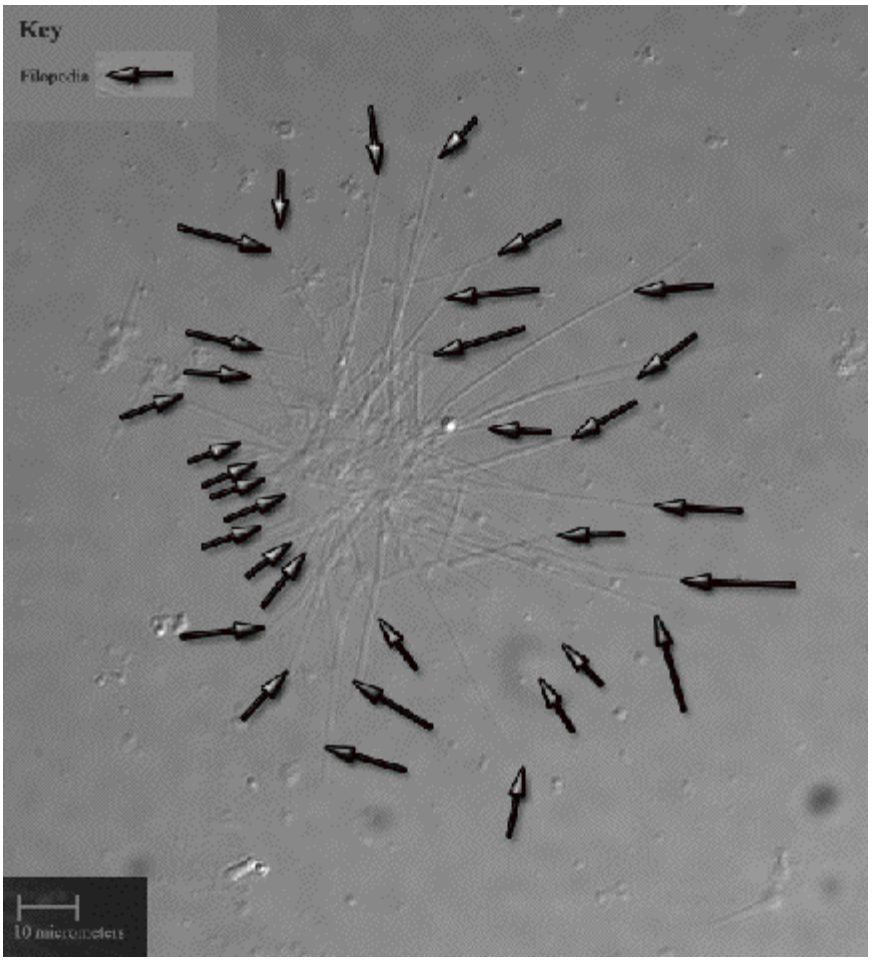

Figure 2. Control (non-caffeinated) Coelomocyte viewed at 60x
This is a coelomocyte cell that had no caffeine added to it and was viewed at 60x. Again, the arrows indicate the presence of a filopodial extension and were added so that the number of filopodia could be counted in a systematic manner. The scale bar was added in the previously mentioned manner.

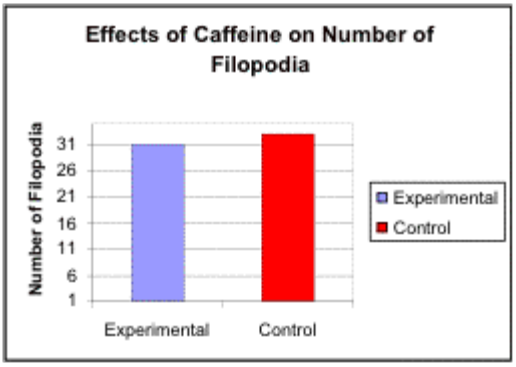

Figure 3. Graph of data

Discussion

Our preliminary studies indicate that caffeine decreases the amount of filopodia on the coelomocyte cells of sea urchins. Although it can be concluded that there are a lesser number of filopodia on the cell in the experimental group, one must note that these results were based on the fact that the difference in the amount of filopodia present in the experimental and control images is minimal. This decrease in number of filopodia possibly suggests that caffeine may slow down cell motility. This can be attributed to the pathway of filopodial extension and retraction. Caffeine may act to increase the amount of time it takes for the cytoskeletal attributes to assemble and disassemble so that filopodial formation and retraction happens at a slower rate. The actin bundles are regulated by kinesin and myosin motors that aid in these processes and may actually be hindered so that the filopodia do not get formed as quickly or as numerously as they would in a caffeinated cell.

The results from this initial study indicate that caffeine may perhaps be more effective than previously thought. It is known that caffeine mimics adenosine, a molecule that binds to its receptors to produce cyclic AMP (cAMP). When adenosine molecules bind to their receptors, adenylyl cyclase, an enzyme, is stimulated and large amounts of cAMP are produced. The cAMP activates alpha kinase , another enzyme, to phosphorylate specific proteins. The activation of alpha kinase phosphorylation is the stimulation of the breakdown of glycogen into glucose and inhibition of glycogen synthesis from glucose. Thus cAMP controls metabolic rates which controls mitochondrial activity (Cooper et. Al. , 2004).

An increase in cell signaling in neurons creates more filopodia at neuronal growth cone decision points, which occurs when a neuron must transition and does so by determining its environment in order to grow in a certain direction (Morris, 2005). Our results suggest that caffeine may actually slow down this pathway more than adenosine does. By slowing down this pathway, caffeine is lessening the signaling that triggers metabolism making it so that metabolism is actually suppressed. With suppressed metabolic activity, the number of filopodia are actually decreased because of this decreased signaling as opposed to rapid and vigorous filopodia formation when signaling is active (Argiro et. Al 1985).

As seen in Figure 3, the difference between the two groups is slight and does not provide strong enough evidence that the number of filopodia in a caffeinated cell is significantly different from a non-caffeinated cell. In order to make significant and more meaningful results, one must repeat the procedure many times until the data collected plateaus, meaning that the data being collected still shows the same results.

We based our results on the images taken from the microscope and relied on the technology to give us clear, focused images of the cells so that we could count the filopodia. A source of error in collecting the data may have occurred if the camera and microscope failed to show us other filopodia that may have been present but that we could not count because we simply could not view them. Also, we are viewing a 2-dimensional image taken from the microscope when in reality the cell is 3-dimensional and has extensions coming from all angles of the cell that we simply cannot view. More filopodia may be present in one cell than another at a different angle, but we did not take that into consideration because we viewed several two dimensional images at the same angles to collect data. In order to have a full count of filopodia present on the cell, we would have to take many images at different angles so that we could take all filopodia into account. Both the experimental and control face the same bias which makes counting these other filopodia is fairly irrelevant however it does not give a curious and interested person the true number of filopodia that could be present on a coelomocyte cell.

This conclusion would force us to consider another way of measuring the effects of caffeine on a cell. One cannot definitively say that caffeine has no effect on cells based on this single study so we might test another quantifiable effect of caffeine on cells such as cell shape, cell surface area, and length of filopodia. One can observe that the cell bodies are actually shaped quite differently. This could be a phenomena or trend that an interested scientist could study. Also, one can note that the filopodial extensions are shaped quite differently, one could stud the effects of caffeine on the shape of filopodia. Along with each of these possible studies, one could use different concentrations of caffeine to measure the full effects of it. Perhaps the 20 millimolar solution, a standard used in several other studies, was not a high enough concentration to affect the filopodia of a cell. One could also study the effects of caffeine on mitochondria in caffeinated and non-caffeinated cells using different techniques, namely fluorescence.

My associate and collaborator Abbe Bosk has studied the metabolic activity of mitochondria in caffeinated and non-caffeinated cells. Her studies indicated that caffeine does actually increase the metabolic activities of mitochondria in cells. She quantified the brightness of cells used in the study and found that the caffeinated cells emitted on average a

brighter fluorescence than those that did not have caffeine added to them. This is evidence of the effects of caffeine because of the increased activity of the mitochondria.

References Cited

Argiro, V., Bunge, M.B., Johnson, M.I., (1985) A Quantitative Study of Growth Cone Filopodial Extension. *Journal of Neuroscience Research* 13. 149-162.

Bosk, Abbe, personal communication 2005

Cooper, Geoffrey M., Hausman, Robert E., (2004) *The Cell- A Molecular Approach*; 435-589

English, C., Kyes, D., Collins, G., Morris, R., August 2003
<http://icuc.wheatonma.edu/icuc_protocols/fluorescence_protocol.html> . [Accessed 1 December 2005]

Otto, J. J., Bryan,J. The Incorporation of Actin and Fascin into the Cytoskeleton of Filopodial Sea Urchin Coelomocytes
< http://www3.interscience.wiley.com/cgi-bin/abstract/109909824/ABSTRACT> [Accessed 6 December 2005]

Sem, K., Yu, F., Bachman, D., Lim, L., Goh, H., Tan, Y., Leo, A., Bu, W
Molecular Mechanism of Filopodia Formation
<http://www.med.nus.edu.sg/phys/Projects_Molecular_Sohail.htm>. [Accessed 1 December 2005]

Morris, Robert L., personal communication 2005