The effect of Cytochalasins on Psudopodia Formation in Amoebae

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Introduction:

Experimental evidence involving macrophages suggests that pseudopods are extended to grab foreign proteins.

They also appear to flatten when viewed under a scanning electron microscope and are thought to be “composed of interdigitating filaments.” The concentration of filaments in pseudopodia appear high, all of which suggests that contractile proteins are involved in generating the forces necessary to extend a pseudopod used to retrieve a particle, in this case a cytochalasin drug. (Goldman et. Al., 1976)

In amoebae, pseudopodia are formed in multiple ways. They can form in de novo formation in which “a hyaline cap appears on the cell surface.” The cytoplasm is drawn inward and a new pseudopodium advances as it forms. Pseudopodium can also be formed by a “reversal of polarity in an already existing pseudopod. A pseudopod can also subdivide to form two new ones. Multiple endoplasmic streams are usually the cause for such formation. (Jeon, 1973)

Amoeba motility generally depends on pseudopodia formation; the cell moves by extending one or more pseudopods. Based on multiple experiments, it is likely that particles that attach to the pseudopodium tip advance with the pseudopodium. Different amoeba stream in their particles differently. Some of them, such as *Chaos* and *A. Proteus* channel an inrush of particles along multiple streams. It has also been observed that some particles within an amoebae pseudopod run countercurrent to the particles coming in.
(Jeon, 1973)

Previous research indicates that Cytochalasins inhibit actin depolymerization. (Alberts et. Al, 2002). Cytochalasins are experimentally known to result in shape changes of a cell and a deficiency in cell behaviors. Previous experiments on pseudopodia formation, such as those done by Kappner in 1961, suggest that the median number of undisturbed pseudopodia in *Chaos* amoebae is ten and the maximum is fifteen, and that lowering pH deminsishes the number of pseudopodia formed. (Jeon, 1973) Since pseudopod formation depends on the depolymerization of actin filaments and the rapid polymerization of microtubules, pseudopodia formation in an amobae cell is likely to decrease after a cytochalasin drug is applied to the cell.

Materials and Methods:

A flows chamber was prepared using two coverslips and a glass slide. Valap was used to paste the coverslip over the glass slide. The cover slip was elevated on two sides by glass chips forming a chip chamber. An amoeba was adhered to the slide using a pipette. The slide was placed on a Nikon E200 microscope which was aligned for Kohler illumination, under a 10X lens. Once an amoeba cell was located, a SPOT camera was used to take a picture of the cell. An image sequence program was run and was set to take 4 images over 20 minutes. Each image observed was drawn as best to scale as possible in the lab notebook and the number of pseudopodia observed in each image were counted and

later averaged, (see Glass et. Al). After 20 minutes the chamber was placed on a paper towel and a kim wipe was placed underneath the slide. Cytochalasin dissolved in Dimethyl-Oxylsulfide dissolved in water was collected in a test tube. A pipette was used to administer a drop of the Cytochalsin solution to the slide and then was repeated 3 times. A photo was taken and the again to minutes later and the number of pseudopodia observed were counted and averaged. A pseudopod was counted if there was convex region of the plasma membrane in which its distal tip was at least 200μm. (Glass et. Al, 2008)

Results:

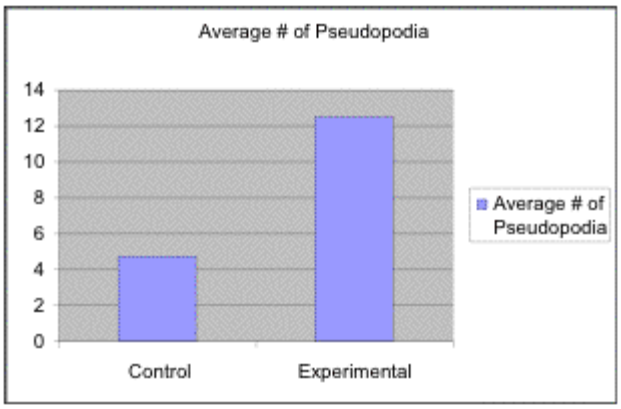

Figure 1: Quantity of pseudopodia formed in an amoeba before and after application of a cytochalasin drug.

The control group consists of the number of pseudopodia counted before application of the cytochalasin drug. The experimental group accounts for the number of pseudopodia formed after application of the drug.

The averages for each quantitative measurement of pseudopodia were collected from various slides over a time lapse sequence taken by a SPOT camera. The interval for the control group was 20 minutes with four photos taken, while the experimental group was only recorded over two minutes, with two photos taken. However the variation of pseudopodia amongst the photos snapped was very little; only one shot has differed in pseudopodia by an integer of one. The difference however between the control group and the experimental group is noticeable; the quantity of pseudopodia changed on the amoebae within the first two minutes after application of the drug.

Discussion:

The extended time of the control and the increased number of shots was only meant to emphasize the minimum degree of change between pseudopodia formation at moments when the amoebae was not exposed to cytochalasin. The rapid change in the average number of pseudopodia observed within the first two minutes following drug application indicates a significant response to the drug from the cell.

Our hypothesis did not match our results; the amount of pseudopodia actually increased after the cytochalasin

drug was applied. This suggests that the cell responded in some way to the presence of the drug. Perhaps the cell tried to increase its pseudopodia formation rapidly upon detection of the drug. This could make sense since it is the pseudopodia themselves that extend from the cell to receive the drug in the first place. (Goldman, et. Al., 1976). Also the intake of the drug may have been distributed among multiple pseudopodia and a sudden division of streaming caused the longer ones viewed before the drug to subdivide into smaller ones. Since the length of each pseudopod was not measured in this experiment, it is quite likely that based on the previous research mentioned in the introduction, the cytochalasin drug did inhibit the growth of each pseudopod, but in the length of formation and not in number.

Works Cited:

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