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Extracellular ATP Effects on Intracellular Actin Fibrils' Location and Characteristics

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Abstract

Epithelial cells lining secretory units and ducts of bovine mammary glands perform an important role in regulating movement of various macromolecules and whole cells during normal lactation and mastitis. During mastitis, host and bacterial produced substances can affect the "barrier" function of epithelial monolayers. One potential component is adenosine triphosphate (ATP). ATP likely interacts with P2X7, a purinergic receptor, in mediating some effects associated with mastitis. Bovine mammary gland epithelial cell line, Mac-T cells, were examined for cytoskeletal changes as result of P2X7 interactions. Actin cytoskeletons were stained with phalloidin and effects were examined by fluorescent microscopy. Observable increase in actin fibril size was noted in ATP treated cells, and not seen in cells treated with P2X7 inhibitors prior to ATP exposure. Results indicate the possibility of ATP modulating epithelial cell function in bovine mammary glands, affecting the barrier function epithelial cells normally provide, through interaction with the P2X7 receptor.

Introduction

Bovine mastitis is an inflammatory condition in the mammary gland caused by bacterial infection. It has great significance for the cattle industry due to its effect on milk and milk products. We can use bovine mastitis as a disease model of inflammatory conditions of epithelial cells. This is due to elevated levels of extracellular ATP present in this disease. Similar elevated levels of extracellular ATP are found in the inflammatory condition asthma, as well as in other airway inflammations.

This project is a study of the effect of ATP (adenosine triphosphate) on cellular actin. Extracellular ATP is considered to be an inflammatory mediator and it has been previously demonstrated to have multiple effects on cells including modulating their permeability. To further focus on the permeability aspects, I examined the effect of ATP on the location and characteristics of actin within the cell.

Actin is a known structural component of the cell. Cells were treated with different exposure times to ATP. In addition, to further understand how ATP affects cells, specific inhibitors to the ATP receptor were used. This was followed by measuring actin fiber size and location using fluorescent staining methods. Changes in these characteristics were then quantified and statistical significance was determined.

Methods

- Mac-T cells, a bovine mammary glad epithelial cell line were grown until confluent
- Cells were treated with ATP for 0, 5, 15, or 30 minutes before fixation and permeabilization
- Cells were stained with phalloidin and coverslipped with an aqueous based mounting solution that contained DAPI
- Actin was examined under a fluorescent microscope and images were taken
- Images were analyzed using DiameterJ and Matlab programs

Figure 1: ATP exposure produces changes in actin fiber density

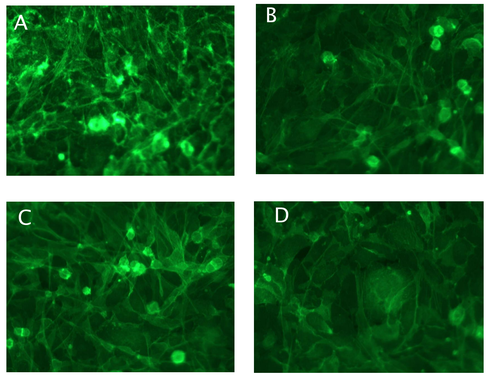

Figure 1: Mac-T cells were grown to confluence on chambered microscope slides. Cells were then exposed to 10 mM ATP or vehicle control for 5, 15, and 30 minutes and then fixed and permeabilized. The cells were then stained with phalloidin and coverslipped with a mounting solution containing DAPI. Cells were then examined and photographed with a fluorescent microscope. The photomicrographs shown above are representative images. A) Control cells not treated with ATP. Cells treated with ATP for B) 5 minutes, C) 15 minutes, and D) 30 minutes.

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Figure 2: P2X7 Receptor

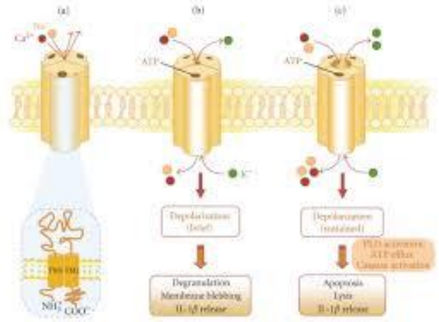

Figure 3: Histograms

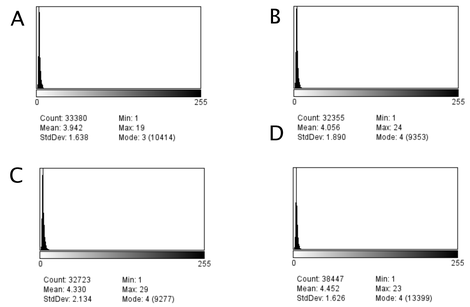

Image analysis was performed using DiameterJ and OrientationJ to produce histograms of Fiber Diameter. Representative images are shown for A) no treatment, B) 5 minute exposure, C) 15 minute exposure, and D) 30 minute exposure to ATP.

Figure 4: Fiber Diameter

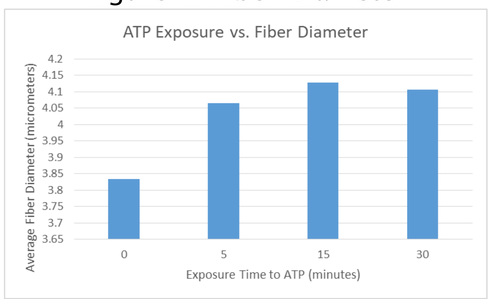

Figure 4: Mean fiber diameters were determined from histograms and averaged by time point. Data points were taken from six trials.

Discussion and Conclusions

- Individual trials appear to show an increase in actin fiber diameter between no treatment and cells exposed to ATP
- In some trials an increase in actin fiber diameter correlates with increase in time of exposure
- When averaged, there is a marked increase between fiber diameter in cells with no ATP exposure and cells treated with ATP

Future Steps

Additional experiments will be analyzed similarly, as well as with Matlab to determine if here is an effect on the amount of actin fibrils present. In the future, similar experiments will be performed and analyzed with P2X7 receptor inhibitors.

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