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Characterization of the Response of TRPV4 to Chemical Stimulation

Jacob Schluns, University of Arkansas

Abstract

Traumatic Brain Injury (TBI) is a source of acute and chronic health issues for many patients. One of the components of the brain's response to injury is astrogliosis, in which astrocytes that normally function to repair the brain instead form scar tissue that halts repair processes. Transient Receptor Potential Vanilloid Type 4 (TRPV4) is a trans-membrane calcium channel involved in astrogliosis. Through Fura-2AM based calcium imaging, the base activity of this channel in mouse astrocyte cells was recorded. The cells were then subjected to TRPV4 agonist and antagonist stimulation and their subsequent activity levels were recorded. The data showed that increasing agonist levels garnered higher TRPV4 activity and increasing antagonist levels suppressed TRPV4 activity, though to a lesser extent. With further characterization of the exact nature of TRPV4's role in TBI response, potential treatment plans for TBI could be developed.

Background

TBI is a common injury source for those involved in contact sports, car accidents, and other sources of significant force to the brain. One of the pathological occurrences associated with TBI is astrogliosis, also known as astrocytosis. With astrogliosis, astrocytes around the injury site that would normally promote a return to homeostasis instead cause scar formation and inhibit axon regeneration. This reduces the brain's already limited ability to self-repair and characterizing this activity is thus of interest. One of the cellular proteins associated with this pathology is Transient Receptor Potential Vanilloid Type 4 (TRPV4), a Ca^{2+} permeable channel made up of six transmembrane helices. It is found throughout a variety of bodily tissues, including the kidneys, lungs, and the brain [2]. TRPV4 is also involved in osmoregulation within the brain and in the brain's response to mechanical and chemical stimuli [2,4]. Ion balance, mechanical forces, and signaling proteins in the wound healing pathway are all important to the body's response to injury. This confluence of responses makes TRPV4 an ideal choice for study in relation to TBI.

Materials and Methods

Mouse astrocytes were chosen as the cell line for this experiment because they are known to contain TRPV4 and are involved in the mouse brain's response to injury. These cells were grown in T-125 flasks with DMES Modified Eagle Medium containing supplemental Fetal Bovine Serum, L-Glutamine, and Gentamicin. After the cells reached confluency, they were plated on cover slips that had previously been sonicated for 30 minutes and treated with Laminin for 24 hours. Confluency here refers to cells

covering 70-80% of the adhesion surface. Sonication is a technique utilized for sterilization, while Laminin enhances cell adhesion to the coverslips. The cells were then incubated at 37°C for 48 hours on the coverslips. Immunocytochemistry images of TRPV4 were utilized to gain a qualitative knowledge of the relative concentration of TRPV4 channels on the astrocytes.

In order to characterize the initial response and subsequent activity of TRPV4 when it is faced with chemical stimuli, a calcium imaging protocol developed in the lab of Dr. Kartik Balachandran was chosen. This protocol makes use of Fura-2-acetoxymethyl ester (Fura-2AM). Cell membranes are permeable to this molecule, which acts as a ratio-metric dye that indicates calcium concentration within the cell. Fura-2AM has an emission peak at 505 nm and its excitation peak shifts from 340 nm to 380 nm when it has bound to a calcium ion [1]. A graph of this fluorescence behavior can be seen in Figure 1.

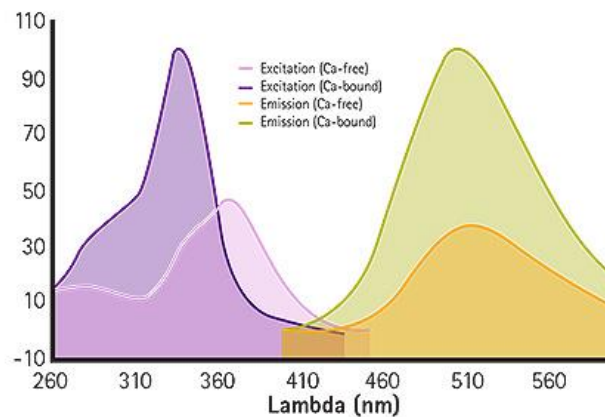


Figure 1. Excitation and emission spectra for Fura-2AM, in calcium-bound and -unbound states.

[3]

By measuring the emitted fluorescence at both wavelengths and computing the ratio of the two, relative calcium levels can be observed. For the experiment, the cells were exposed to Fura-2AM for 30 minutes. After a Phosphate-buffered saline (PBS) wash to remove the Fura-2AM and any residue, the cells were subsequently treated with Tyrode's Buffer, which seals the Fura-2AM inside of the astrocytes so that there is no loss in signal during the experiment. The buffer was left on the cells for 15 minutes. A final wash with PBS was performed, after which a fresh 2 mL of Tyrode's buffer was placed back on the cells. They were then transported to the OPTIX optical microscope (Ion Optix, (Westwood, MA)), shown in Figure 2, for calcium imaging.



Figure 2. Optix Microscopy System

There were two forms of chemical stimuli chosen for this experiment: the first was a known TRPV4 agonist, GSK1016790A (GSK 101). The second was known TRPV4 antagonist GSK205. Once the cells were placed in the imaging station shown in Figure 2, calcium imaging began with a minute of imaging without outside influence so that

calcium levels could equilibrate. The cells were then treated with varying levels of the agonist, antagonist, or a buffer containing a high level of calcium, which acted as a positive control. With varying levels of agonist and antagonist exposure, it was hypothesized that a change in TRPV4 activity would be observed.

Results

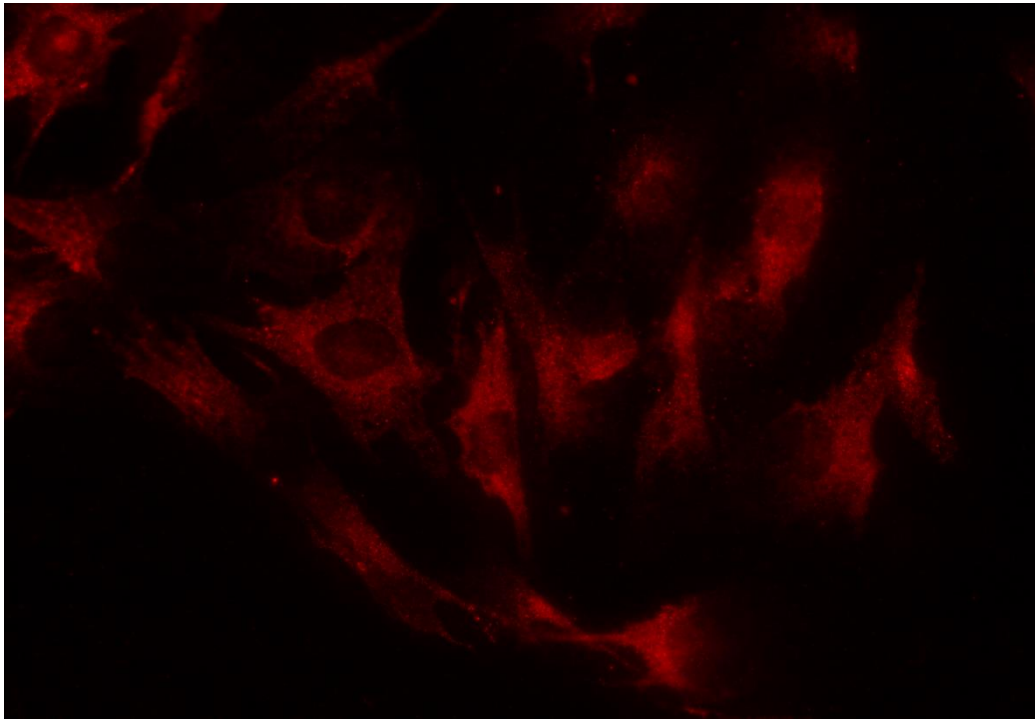


Figure 3. Immunostaining Fluorescent image showing TRPV4 on Mouse Astrocytes

Immunochemistry images of the mouse astrocyte cells showed that there is a large concentration of TRPV4 channels on the surface of these cells. As can be seen in Figure 3, TRPV4 is spread over nearly the entire surface of the cell and creates a distinctive lining around the nucleus. Images were also taken of the cells as they were visualized through the Optix microscope system, as seen in Figure 4. These show differing concentrations of cells, with one being more confluent than the other. It was

found that less confluent coverslips produced data more reliably than those with confluent cells.

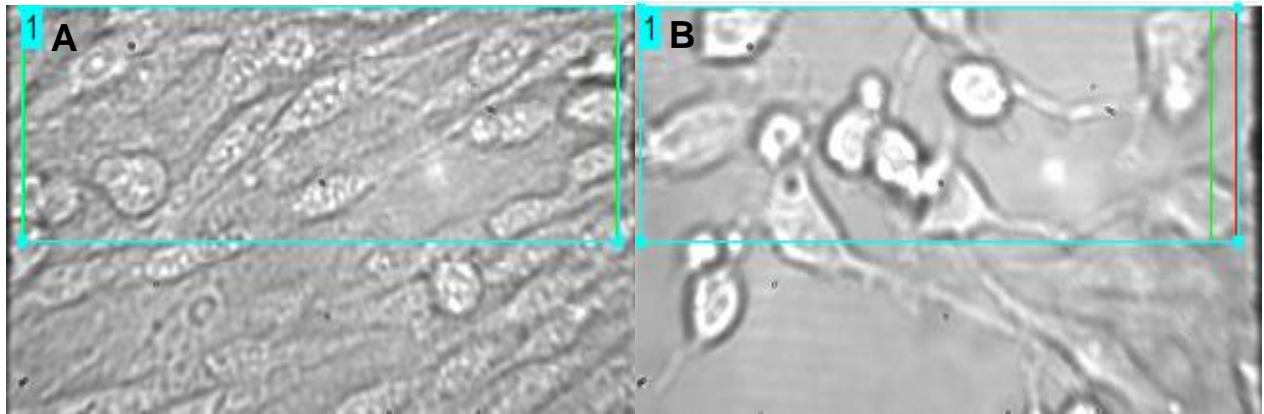


Figure 4. A and B. Optix Microscopy System Image of confluent (A) and nonconfluent (B)

Mouse Astrocyte cells.

Calcium imaging of the mouse astrocyte cells yielded a ratio R describing the relative intensities of 340 and 380 nm fluorescence excitations. Examples of these ratios are shown in Figures 5 and 6, for both the agonist and antagonist runs of the calcium imaging.

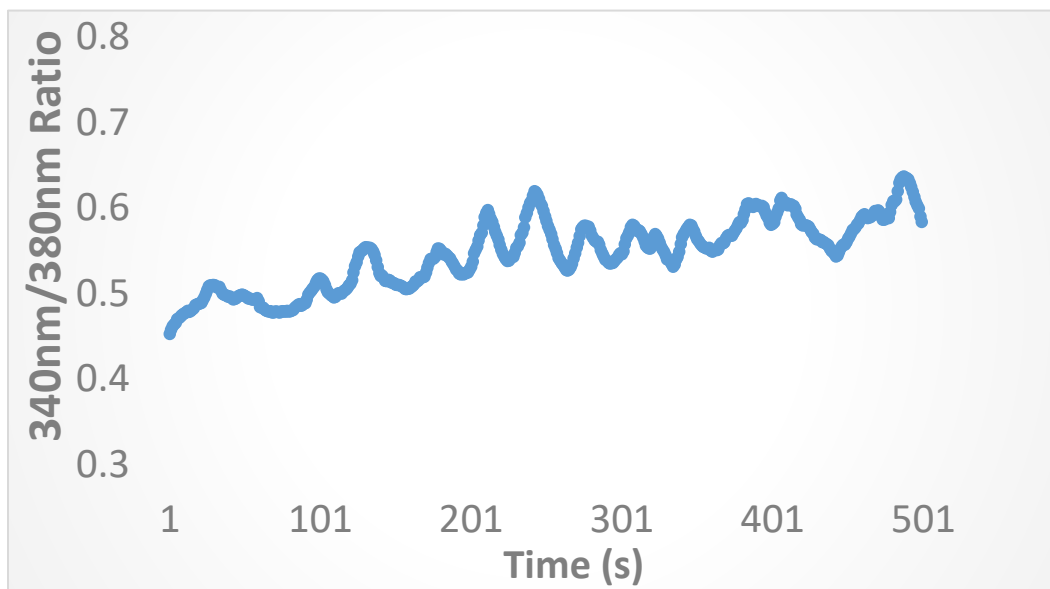


Figure 5. Graph of 340/380 nm Ratio for TRPV agonist

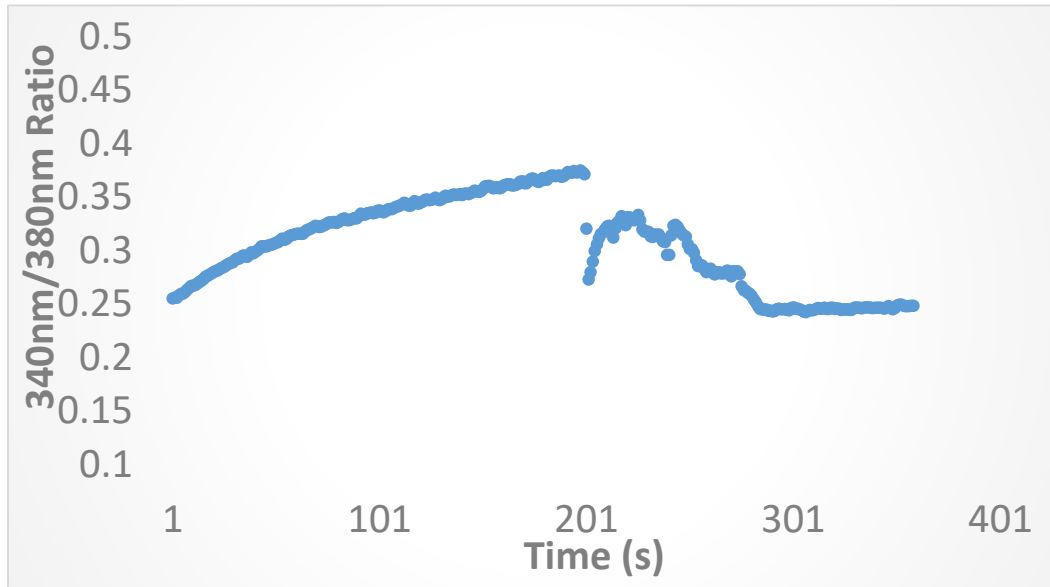


Figure 6. Graph of 340/380 nm Ratio for TRPV antagonist

Figure 5 begins at the point at which the samples were initially exposed to GSK 101, while Figure 6 shows the period of equilibration and GSK 205 introduction around 200 seconds. In order to interpret changes that occurred in the R ratio of the cells, a $\frac{\Delta R}{R}$ value was calculated. For this value, ΔR is the difference between the maximum and minimum R values for the time after the administration of the agonist or antagonist and R is an average of the initial minute of equilibration. In effect, the R forms a baseline to which ΔR are compared. These $\frac{\Delta R}{R}$ values were averaged for the samples within each of the different dosages of agonist and antagonist and are displayed in Figure 7.

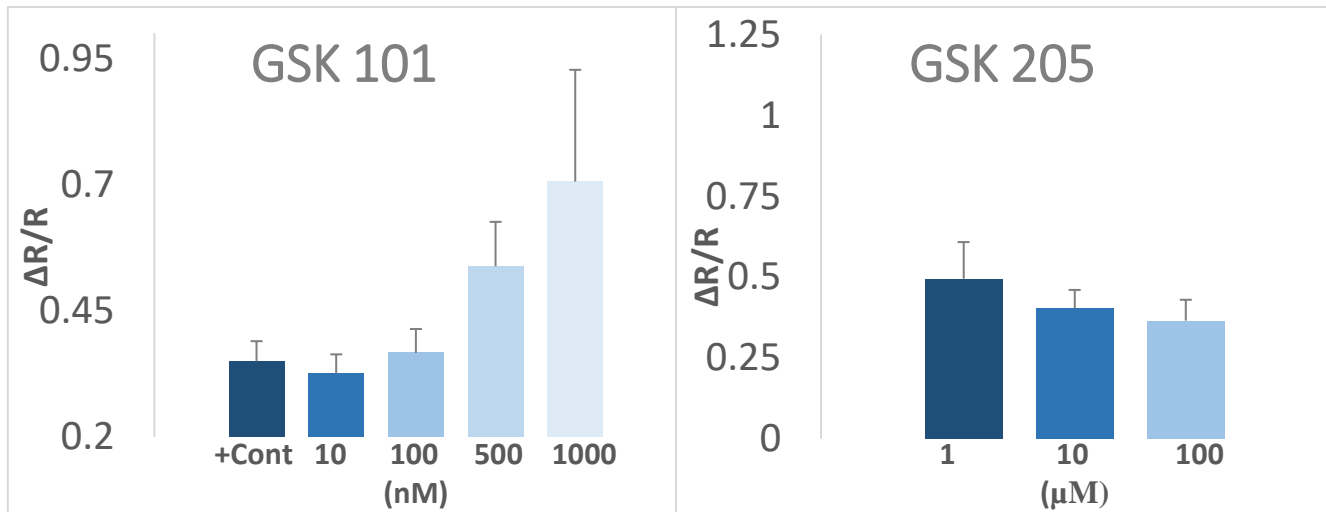


Figure 7. $\frac{\Delta R}{R}$ values for GSK 101 (agonist) and GSK 205 (antagonist)

Discussion

Through immunocytochemistry images such as Figure 3, it was established that TRPV4 can be readily found on the surface of Mouse Astrocyte cells. This supports previous studies' findings that TRPV4 is a key part of astrocytes' role in osmoregulation within the brain. In addition to this, the experimental results showed an increase in activity when GSK 101 was introduced to the samples. The measured change in $\frac{\Delta R}{R}$ increased with increasing doses of the agonist, with a maximum being achieved at 1000 nM. Similarly, the introduction of GSK 205, the TRPV4 antagonist, decreased the TRPV4 activity. Although less significant than the decrease associated with the agonist, it still shows that increasing amounts of the antagonist produce decreasing levels of TRPV4 activity.

These results provide a characterization of the response in TRPV4 activity levels to stimulation with various chemicals. This is significant because it builds a framework for

future experimentation on TRPV4 and validates the Fura-2AM protocol for measuring the calcium levels within Mouse Astrocyte cells.

Future Work

With this base knowledge of TRPV4 activity, a further characterization of TRPV4's involvement in the response of Astrocytes to TBI could be achieved. This would involve simulating physical injury to the cells and monitoring the TRPV4 activity level as this occurs. As well, techniques such as CRISPR-Cas9 could be utilized to knock-out the expression of TRPV4. Cells with this knockout could also further characterize the role that TRPV4 plays in TBI.

References

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