Pioneering Neuroscience, 2007, **8**, 1-4

Reduction of endoplasmic reticulum internal calcium stores via BHQ decreases EJP amplitude

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ABSTRACT

Synaptic transmission requires Ca^{2+} to initiate the release of neurotransmitters. While the mechanism that allows this is not known, we do know that Ca^{2+} allows the vesicles containing these neurotransmitters to fuse with the neuron's membrane. Ca^{2+} is released from internal Ca^{2+} stores like the endoplasmic reticulum when a signal is sent to the nerve terminal. This Ca^{2+} re-enters the endoplasmic reticulum internal stores through SERCA pumps. These pumps can be inhibited by the chemical butylhydroquinone (BHQ). This causes a depletion of Ca^{2+} within the internal stores and reduces the removal of free Ca^{2+} from the cytosol. The inhibition of these internal stores has both an immediate and long-term effect on the amplitudes and duration of the EJPs. In the present work we found that amplitude initially fell after the inhibition, but eventually rose until it reached the values from before the BHQ was added. Duration grew steadily longer after the BHQ was added. These results show that the role of endoplasmic reticulum as internal Ca^{2+} stores is important in maintaining both functional amplitude and duration.

INTRODUCTION

The influx of Ca^{2+} into the cytosol of a terminal causes membrane-bound vesicles containing neurotransmitters to bind to the terminal's cell membrane, releasing neurotransmitters into the synaptic gap (see diagram 1). Because of the fact that Ca^{2+} ions are so critical to synaptic communication, their role in neuronal pathology and neurodegenerative diseases is being studied extensively. By understanding the means by which neurons communicate with each other, we can gain a further awareness of how disorders and diseases of this system occur on a cellular or even molecular level, and how they might be treated or prevented (Verkhratsky 1998). In neurons, Ca^{2+} used in synaptic transmission is supplied by two sources; Ca^{2+} that exists in the extracellular fluid and flows into the cell via ion channels and Ca^{2+} stored in and released from organelles that function as internal Ca^{2+} stores within the cell (Verkhratsky 1998). One such store is the endoplasmic reticulum (ER). Calcium ions are cycled in and out of the ER by the SERCA pump and through $InsP_3R$ and RyR, respectively.

The inhibition of the reuptake of Ca^{2+} by internal stores has been studied extensively. Many chemicals, such as BHQ and thapsigargin, inhibit reuptake by blocking the action of SERCA pumps in the endoplasmic and sarcoplasmic reticulum (Fiumelli, Cancedda, and Poo 2005). It has been shown that the inhibition of SERCA pumps and simultaneous stimulation of $InsP_3R$ and RyR channels by nifedipine and Bay K 8644 leads to the depletion of Ca^{2+} from the endoplasmic reticulum (Low, Lang, and Daniel 1993). We intended to examine the effects of inhibited internal Ca^{2+} store function on the amplitude and duration of EJPs generated at the neuromuscular junction.

By inhibiting Ca^{2+} reuptake in the endoplasmic reticulum and recording EJPs across the juncture, we were able to see general trends in the affected cells. We had hypothesized that the amplitudes of EJPs generated under the effect of BHQ would increase immediately after the application of the chemical, and then eventually decrease gradually until no EJPs are generated. In our experiment, we found that after the BHQ addition, the amplitudes were smaller than those before the addition. We also observed that the EJPs recorded after the application of the chemical had a longer duration than those before.

MATERIALS AND METHODS

Methods

To experiment on internal calcium stores, we used the superficial extensor muscle and nerve cells of the crayfish. The crayfish were chilled in a box of ice for 10-15 minutes. After that, the tail was cut from the crayfish. We cut along the ventral

surface of the tail, and the ventral shell surface and all muscles ventral to the gut were removed, revealing the superficial extensor muscle. The tail was then pinned onto a dish containing 100 ml crayfish saline. After measuring a baseline for EJP amplitude in the normal saline, we siphoned out the saline and replaced it with a solution containing BHQ in addition to standard saline. After recording EJPs in this solution, we washed out the modified saline and replaced it with normal saline to determine how permanent the effects of BHQ were.

Equipment

1.2 mm glass capillary tubes were pulled to make electrodes with a resistance of 5-20 MΩ. These electrodes were filled with 3M KCL, rinsed in crayfish saline, and attached to a micromanipulator and amplifier, which is connected to the Scope computer program. We used a suction electrode that was connected to a Grass SD9 Stimulator. We would stimulate the nerve through the suction electrode, and measure the voltage across the membranes of superficial extensor cells with the pulled electrode.

Solutions

The crayfish saline we used is a mixture of 5.4 mM KCL, 196 mM NaCl, 2.6 mM MgCl2.6H20, 10.0 mM Sodium Hepes Buffer, and 13.5 mM CaCl2.2H20, adjusted to a pH of 7.4. Our readings before the addition of the butylhydroquinone (BHQ) were in this solution. After our first readings, we removed this solution and replaced it with a solution of 10 μM BHQ and 100 mL crayfish saline. We recorded the EJPs while the cells were in the BHQ solution to observe its affects on the amplitude and duration, and later removed the BHQ solution and washed it out, and recorded the EJPS to see if the affects of the BHQ were permanent.

RESULTS

We hypothesized that the inhibition of the endoplasmic reticulum's role as internal calcium stores would result in initially increased EJP amplitudes, and eventually end in the nerve terminal's failure to fire EJPs or action potentials. In our first trial, there were no recordable EJPs after the application of the BHQ. We attribute this to an error in our methodology. We think that the time lost between removing and adding each liquid contributed to the lack of recordable EJPs. We were able to measure EJPs in the second trial. However, as time passed in our experiment, the amplitudes started to get larger, until they finally returned to their previous values (see Fig. 1).

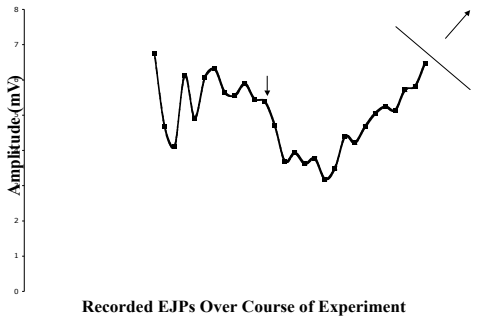

Fig. 1 shows the amplitudes in mV of the recorded EJPs over the course of the experiment. The readings were not taken in equivalent increments. The point where the BHQ was added is indicated by the arrow on the graph. After the BHQ wa added the amplitudes decreased immediately, and eventually they returned to the amplitude values before the BHQ was added. As indicated by the arrow to the right, the amplitude continued to rise steadily until action potentials were generated.

In this trial, after the addition of BHQ, the EJPs we generated in the extensors had longer durations following the application of BHQ. The durations grew longer as time passed in the experiment, as can be seen from Fig. 2.

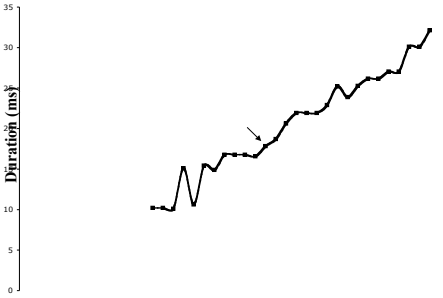

Fig. 2 shows the duration in ms of the recorded EJPs over the course of our experiment. The arrow on the graph indicates the point where the BHQ was added. The durations increased significantly after the addition and continued to increase as time passed.

We chose to do a wash after the BHQ to determine whether or not the affects were permanent. After the wash, we could not get any EJPs, only action potentials. The presence of action potentials meant that the synapse had modulated and adapted to the new conditions, and required less voltage to trigger an action potential, an example of neuronal plasticity.

The following table gives the means we calculated for EJP amplitude and duration before and after the addition of BHQ:

	Mean
EJP amplitude before BHQ addition	5.6 mV
EJP amplitude after BHQ addition	4.6 mV
EJP duration before BHQ addition	13.9 ms
EJP duration after BHQ addition	24.6 ms

DISCUSSION

The main finding of this study is that the amplitudes of EJPs, contrary to our hypothesis, do not become greater after the inhibition of the SERCA pumps in the endoplasmic reticulum. Rather, the immediate affects of the inhibition were a decrease in amplitude followed by a gradual increase until the amplitudes returned to the pre-BHQ levels. While duration was not initially a part of our research question, we found the data on duration to be particularily intriguing. After the addition of BHQ, the duration of the EJPs grew steadily longer, which is quite different than the duration of the pre-BHQ EJPs.

This can be explained by the fact that the inhibition of the SERCA pumps reduces the amount of stored Ca2+, which is exacerbated by the mechanism of calcium-induced Ca2+ release (CICR). Less Ca2+ is being released from these depleted stores, and so less is being used to facilitate neurotransmitter release. This explains the initial decrease in EJP amplitude after SERCA inhibition. After the concentrations of Ca2+ in the cytosol become large enough, though, amplitudes begin to increase as the higher concentrations cause increased neurotransmitter release. Also, the removal of cytosolic Ca2+ is slowed by the inability of the pumps to return Ca2+ to the stores, causing longer stretches of neurotransmitter release.

Our experiment, rather than providing insight into the roles played by internal Ca^{2+} stores on neuronal transmission, more directly aided our understanding of how the mechanism of neurotransmitter release is connected indirectly to intracellular Ca^{2+} concentrations. This adds to the information found by Low et al. (1993) and Verkhratsky et al. (1998). We found out more about how actual process of conveying a nerve impulse physically across the synaptic gap is dependant on and limited by the amount of Ca^{2+} within a cell than about how that Ca^{2+} amount is created. We also confirmed the assertion that Ca2+ stores in the endoplasmic reticulum are crucial for the maintenance of cytosolic Ca2+ homeostasis, and that any alteration to this mechanism results in abnormal Ca2+ concentration and ultimately in affected transmission (Scott and Rusakov 2006). In a more general sense, any research into the field of neuronal function and synaptic transmission is of value to the scientific community by virtue of its connection to the function of the mind. Any progress in this field might have significant importance in the overall understanding of how our minds and the minds of other creatures work. Though this study probably will not independently herald any immediate development, research of this nature could be built on later for more substantial work in the future.

Our study opened up several additional avenues of research that could be pursued. One might test the ability of the cells' ability to perform in a low Ca^{2+} environment. This could be affected by performing this experiment using crayfish saline without Ca^{2+}. In addition, the response of other internal Ca^{2+} stores to the inhibition of one of the primary stores could be examined. In our experiment we meant to do this, but we did not have the means of isolating how the other stores' functionality affected transmission without taking into account other factors, such as the limiting of neurotransmitter release. If one could isolate the functional changes of these stores, they could take a closer look as how this affects transmissions. Another study could examine the affect of increased Ca^{2+} release from the internal stores via caffeine, to gauge the effect of increased intracellular Ca^{2+} without reuptake inhibition. This study, like ours, would also look at how increased intracellular Ca^{2+} affects synaptic transmission across the neuromuscular junction.

ACKNOWLEDGEMENTS

We would like to thank our professor, Clark Lindgren, and laboratory assistant, Susan Kolbe, for their invaluable time and effort spent helping us design and perform this study. Our gratitude also goes to our classmate Colin Higgins for enlightening us as to the mathematical and statistical powers of Microsoft Excel.

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