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The Role of the NMDA Receptor in Synaptic Transmission at the Crayfish Neuromuscular Junction

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ABSTRACT

The N-methyl-D-aspartate (NMDA) receptor is a predominant molecular device responsible for many crucial aspects of postsynaptic signal transmission including memory formation and synaptic plasticity. Studies suggest that NMDA application increases potentiation of signal transmission in mammalian hippocampal cells (Broutman and Baudry, 2001). In our study, we explored the prevalence of the NMDA receptor in the neuromuscular junction of crayfish. We measured excitatory postsynaptic potentials (EPSPs) in control settings and after applying a NMDA bath. Our findings suggest that the application of NMDA did not have a significant effect on potentiation of EPSPs.

INTRODUCTION

The N-methyl-D-aspartate (NMDA) receptor plays an important role in synaptic transmission in the neuromuscular junction of many living organisms. The NMDA receptor is a predominant molecular device for controlling synaptic plasticity and memory formation (Bevers et. al., 2009).

In transmission of signals, neurons release neurotransmitters across the synaptic gap. These neurotransmitters bind to the postsynaptic receptors, which activate chemical-gated ion channels. The resulting diffusion of ions into the postsynaptic cell leads to a depolarization, which is known as an EPSP (excitatory postsynaptic potential). EPSPs are a good measure synaptic activity. As both a voltage and chemically gated ion channel, the NMDA receptor is unique because it must be activated by the presence of both a ligand and electrical depolarization to open the ion channel. Its normal agonist is glutamate. Previous studies show that application of NMDA in rat hippocampal cells results in long term potentiation (LTP) of signal transmission (Broutman and Baudry, 2001). Questions still remain about the specific function of the NMDA receptor. In this study, the role of the NMDA receptor will be examined in the crayfish neuromuscular junction, a relevant and accessible model for the human central nervous system. Thus, observations made regarding synaptic plasticity and neurotransmission in our study are relevant to medical and pharmacological application in humans. Our study is connected to the field of medical research concerning neurodegenerative diseases including Alzheimer's disease. Such diseases are caused in part by an impairment of synaptic function, a process that can be caused by a myriad of factors including excitotoxic cascade involving the NMDA receptor (Nimmerich et. al., 2010). The goal of this area of research is to determine methods to prevent neuronal decline and synaptic deficits.

Furthermore, the NMDA receptor and ion channel is permeable to calcium, which plays an important role in controlling neuronal function including the activation of calpain (Wu and Lynch, 2006). Calpain is an enzyme which also plays an important role in learning and memory and mechanisms of neuronal death (Wu and Lynch, 2006). Calpain has been shown to have a role in cleaving many postsynaptically localized proteins, including voltage-gated sodium channels, density proteins, kinases and phosphatases (Silverman-Gavrila et al. 2013). This suggests that calpain may also interact with the NMDA receptor on the postsynaptic membrane. Our intended experiment was to examine the role of calpain in the anticipated LTP after NMDA application. Because the application of NMDA did not result in LTP, exploring the role of calpain was no longer relevant. Our results indicated that in crayfish neuromuscular junctions, NMDA application does not significantly affect potentiation of signal transmission.

MATERIALS AND METHODS

Dissection

Our exploration of neurotransmission takes a closer look at the crayfish neuromuscular junction. For this preparation, we used *Procambarus clarkii*. The crayfish was anesthetized in ice until the tail was removed at the base. We exposed the dorsal extensor muscles and nerves by removing the ventral surface of the crayfish tail and the remaining bulk of the muscle tissue,. The dorsal exoskeleton was pinned to a dissecting dish and covered with 100 ml crayfish saline.

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Microelectrodes and electrophysiology

To accurately measure intracellular voltage of the muscle cell and EPSPs, we used glass electrodes. To minimize variation in the electrodes, they were heated and pulled using the same electrode puller. One was filled with 3M KCl and placed in a electrode holder and micromanipulator. This electrode has a resistance range of 4-10 MΩ. The second, the suction electrode, was gently filed with emery paper, allowing a wider diameter to accommodate a nerve, placed in an electrode holder and micromanipulator. The second electrode was used to suck up a nerve using a syringe before the intracellular electrode entered a muscle cell. Both electrodes were strategically placed in the same segment of the crayfish.

Stimulation and recording

Artificial stimulation was used to elicit a postsynaptic response from the muscle cells. The simulator we used was set at a frequency of 1 second, a delay of 5ms and duration of 10ms. Measurements were recorded on the computer program Scope. The voltage change recorded from the muscle cell as a result of stimulation was amplified on a simultaneous screen as EPSPs. For each group (control and experimental), recordings from at least four different muscle cells were taken and averaged to account for differences in individual cell resting potentials.

Solutions

The control solution was a crayfish saline to mimic extracellular ion concentration in living crayfish. We used 100ml of crayfish saline, consisting of 196mM NaCl, 5.4mMKCl, 13.5mM Cacl2 and 2.6 mM MgCl2 with a pH of 7.4. 20µl of DMSO was added as a control for the experimental condition in which calpain would be inhibited with MDL-28170.

The NMDA solution was created by adding 25ml crayfish saline containing 200µM NMDA to 75 ml of control crayfish saline. The final concentration of NMDA in the solution was thus 50µM. The above 25ml NMDA solution was previously made by diluting 200µl of NMDA stock solution (50mM) into 25ml of control solution. To allow intracellular recording to continue, we added the NMDA solution by suctioning out 25ml of the original solution and replacing it with 25ml of the NMDA solution . This transfer was done gently to prevent dislodging either of the electrodes.

RESULTS

Intracellular electrodes were used to quantitatively measure synaptic transmission in the crayfish neuromuscular junction. We used a voltage above threshold to stimulate the nerve and elicit a postsynaptic response from the muscle cell. Due to the nature of the NMDA receptor and previous research, we expected to see a potentiation of EPSPs after the application of NMDA with stimulation. However, our data was not consistent with prior studies. In each organism, we measured the amplitudes of EPSPs in four cells. Our recorded data reflect the average of three recordings per cell, to minimize variability. For the first organism, the average EPSP amplitude in the control solution was 23.8mV. Of the entire experiment, this set of data represented the largest range of values, as amplitudes ranged from 12.34mV to 51.44mV.

We then applied the NMDA solution, waited approximately 10 minutes, and again took three recordings each from four different cells. After applying the NMDA bath, the average amplitude of EPSPs were 13.55mV. In the second organism, the average EPSP amplitude before and after NMDA application was 46.43mV and 52.79mV, respectively. This data is represented in Figure 1. We performed a t-test for the first and second trial, we calculated p values of .18 and .22, respectively. Because both p values are greater than .05, we conclude that these figures are not statistically significant. According to our data, the application of NMDA does not significantly affect the potentiation of signal transmission in crayfish neuromuscular junctions.

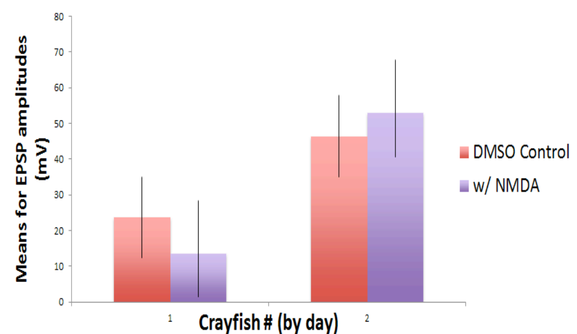

Figure 1. This graph depicts our results by day. Each cluster represents a different animal, with the left column as the DMSO control and the right as the NMDA. Crayfish 1 in the control solution had average EPSP amplitudes of 23.8mV (values ranged between 12.34mV-51.44mV); with NMDA the EPSP amplitudes averaged 13.55mV (values ranged between 7.22mV-17.43mV). Crayfish 2 had an average EPSP amplitude of 46.43mV (range between 36.85mV-53.46mV) in the control solution and 52.79 (range between 51.82mV-53.35mV) after NMDA application. (All averages represent n=4 cells, each of which were recorded 3 times). Error bars represent standard errors of the mean).

DISCUSSION

As is shown in Figure 1, our tests on two different crayfish resulted in opposite effects. For Crayfish 1, average EPSPs decreased when we added NMDA while the opposite was true for Crayfish 2. Thus, our results do not significantly support previous research showing the application of NDMA to rat hippocampal cells as a cause of LTP (Broutman and Baudry, 2001). When we added NMDA to our crayfish bath, we observed inconsistent changes in EPSP amplitude. T-tests for both days render our data insignificant.

It is possible that future trials conducted on crayfish neuromuscular junctions utilizing different concentrations of NDMA will reveal results more similar to established literature. We based the NMDA concentrations of our crayfish baths on a study done by Broutman and Baudry (2001) and therefore consistently used a 50 μM concentration. However, Bevers et al. (2009) used concentrations of ≥200 μM to obtain significant results in their study of the relative role of m-calpain and μ-calpain in a primary hippocampal neuron model of NMDA-mediated excitotoxicity. Both aforementioned studies tested variables on rat hippocampal slice cultures. Yet, the effects of NMDA application on crayfish neuromuscular junctions is variable in different species and therefore different concentrations should be used for different animals. Fujii et al. (1995) demonstrated that variable levels of acetylcholine (ACh) are present in the blood of different species and that the distribution of ACh in blood constituents varies by species. Because Ach is another prevalent neurotransmitter, conclusions could be extrapolated to the concentration of NMDA receptors. It is therefore likely that crayfish have a different concentration of NMDA receptors than rats. Thus, future experiments could use a range of concentrations to determine a more appropriate NMDA concentration.

Additionally, future trials should be done to test the effect of DMSO on NMDA receptors. Because we anticipated using a calpain inhibitor in a DMSO solution, DMSO was added to the crayfish saline throughout our experiment to serve as a control. However, because we did not continue to examine the specific effects of calpain inhibition, the prevalence of DMSO became an extra variable to account for in our results. Thus, DMSO may affect signal transmission and plasticity as relating to the NMDA receptor and ion channel in the neuromuscular junction of crayfish.

As mentioned above, we opted not to move on to the second part of our experiment for lack of significant results in the first part. We planned to apply a calpain inhibitor to observe calpain's role in conjunction with NMDA on LTP because studies show that inhibiting calpain prevents NMDA-induced cell death in rat hippocampal slice cultures (Nimmrich et al., 2010). If more significant results are achieved by altering variables, including NMDA concentration and the application of DMSO, further studies should test the effect of inhibiting calpain on NMDA-bathed crayfish neuromuscular junctions.

Our results suggest that concentrations of NMDA that are ≤ 50μM may not be strong enough to affect EPSP amplitude in crayfish neuromuscular junctions within 20 minutes of application. It is also possible that DMSO in the solution can affect the results. Should further studies establish the effect of NMDA on crayfish neuromuscular junctions, researchers should continue by inhibiting calpain in order to test its reaction to NMDA.

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