Pioneering Neuroscience, 2014, **14**, 65-68.

Spermidine may alter EPSP amplitude at the crayfish neuromuscular junction

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ABSTRACT

Spermidine is a protein that is known to enhance NMDA receptor function but seems to depress EPSP amplitudes. Our experiment tested the effects of spermidine on EPSP amplitudes at neuromuscular junctions in crayfish. The EPSP amplitudes were measured with intracellular electrodes exposed to a control saline solution and a 100μM spermidine solution. Our results were varied as spermidine increased EPSP amplitudes in some trials and decreased EPSP amplitudes in others. While it's unclear how spermidine affects EPSP amplitude it's clear that it changes the post-synaptic cell activity in the neuromuscular junction of crayfish.

INTRODUCTION

Spermidine is an endogenous polyamine involved in cellular metabolism in all eukaryotic cells. Spermidine is known to regulate Ca^{2+} influx by glutamatergic N-methyl-d-aspartate (NMDA) receptors. Research shows that spermidine affects NMDA receptors during synaptic transmission. Spermidine has been found to enhance NMDA receptor function but, surprisingly, to depress excitatory postsynaptic potentials (EPSPs) amplitude (DiScenna *et al.*,1994). Spermidine enhances NMDA receptor function by increasing the receptor's affinity for glutamate, a co-activator of the receptor (Ransom *et al.*, 1988). These results led to our hypothesis that spermidine within the postsynaptic cell would increase the EPSP amplitude because NMDA receptors have a higher affinity for glutamate.

The amino acid glutamate plays a key role in both the normal and abnormal functioning of the central nervous system (CNS) and other neuromuscular junctions (Newcomer *et al.*, 2000). Glutamate is the main excitatory neurotransmitter in the CNS, estimated to be released at up to half of the synapses in the brain (Newcomer *et al.*, 2000). Additionally, glutamate is also an excitotoxin that can destroy neurons by excessive activation of excitatory receptors on dendritic and somal surfaces (Newcomer *et al.*, 2000). Glutamate exerts excitotoxic activity through three receptor subtypes, which belong to the ionotropic family. These three receptors are named after agonists to which they are differentially sensitive, NMDA, amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA) (Kew *et al.*, 2005). The NMDA receptor has been the most extensively studied and the most frequently implicated in CNS diseases. The NMDA receptor is important in generating synaptic plasticity and memory function (Kew *et al.* 2005). Spermidine and spermine modulate learning and memory by interacting with the polyamine binding site at the NMDA receptor (Guerra *et al.*, 2006). This is why studying NMDA receptors in the crayfish neuromuscular junction is significant. We chose crayfish because it's neuromuscular junction is simpler to study compared to synapses in the CNS. It is easier to control factors and to determine what is effecting synaptic transmission.

We measured spermidine's effects on the NMDA receptor by measuring EPSPs while spermidine was diluted in the solution and compared them to EPSPs taken during the control saline trials. EPSPs result from an action potential arriving at the presynaptic terminal which causes an influx of calcium. This allows the vesicles holding the neurotransmitters to move towards and fuse with the membrane and release the neurotransmitters into the synaptic cleft. The neurotransmitters diffuse across the synaptic gap and bind to receptors on the postsynaptic terminal. The amplitude of an EPSP is dependent upon how many neurotransmitters bind to receptors in the postsynaptic cell and properties intrinsic to the receptors themselves. The more neurotransmitters that bind, the higher the EPSP amplitude.

Our hypothesis that spermidine within the postsynaptic cell would increase the EPSP amplitude because NMDA receptors have a higher affinity for glutamate wasn't conclusively supported by our results. In a majority of the trials the spermidine increased EPSP amplitude within the range of 4.5% and 135%; however, in multiple trials EPSPs decreased after the addition of spermidine though not as noticeably.

MATERIALS AND METHODS

Crayfish Dissection

We immersed crayfish in a crushed ice bath for approximately 15 minutes to anesthetize them. Crayfish are cold blooded so their body temperature lowered until their nerves no longer sent signals to their brain and they felt no pain. We made two vertical cuts with scissors along the ventral side and removed the ventral tissue, isolating the dorsal extensor muscle. The muscle and attached exoskeleton were pinned down in a dish and submerged in 100 ml of a ringer solution composed of 5.4 mM KCl, 200.7 mM NaCl, 12.3 mM g Cl_26H_2O, 5 mM Sodium Hepes Buffer, and 6.5 $CaCl_22H_2O$ mM at a pH of 7.4 to preserve normal neural activity.

Intracellular data recording

Intracellular recording was used to report EPSPs at the neuromuscular junctions. We used intracellular recording electrodes filled with 3M KCl attached to an electrode holder. The electrodes were fabricated using a PUL-1 electrode puller machine. We removed air bubbles to reduce resistance. The resistance of our intracellular electrode consistently leveled between 8-10 MΩ. Data was measured with Power Lab.

Extracellular nerve stimulation

To apply voltage to the nerve endings, we used extracellular electrodes pulled by a PUL-1 electrode maker and then blunted with sandpaper. These were then attached to another electrode holder, and used to backfill solution into the electrode and the holder, with a suction syringe, until it surrounded the wire inside the electrode, and then the nerve ending.

Control tests

To establish a baseline to compare our experimental findings, a control test was performed before and after each experimental test. About 40 pages of EPSPs were recorded for every control and experimental trial. We stimulated the crayfish by applying voltage (usually 11V) to a nerve ending of an extracellular electrode, and measuring it with an intracellular electrode in the muscle.

Chemical dilution/application

To determine the effects of spermidine on NMDA receptor function we elected to use 100µM solution of spermidine diluted in the typical ringer solution. . To achieve this, we calculated that we needed 125 µL per 250 mL of prepared Ringer solution when the stock solution of spermidine was 800 mM. Once this was prepared, we removed the 25ml of normal Ringer solution, which was used to record baseline readings, with a suction syringe, and then added 25 ml of 400 µM spermidine solution in order to create a 100 µM Spermidine bath. When switching to the second control period we performed a saline wash. We removed 50 ml of the spermidine solution and added 50 ml of saline and repeated the process 3 times to cleanse the crayfish of residual spermidine.

RESULTS

Our goal was to determine the effects of spermidine on NMDA receptor function at the neuromuscular junction. We measured the difference in height of EPSPs between the control and the 100µM spermidine solution. We then diluted the saline solution and measured the EPSPs as a second control. We use this method of control-spermidine-control to say with greater confidence that any change in EPSPs we record is due to the addition of spermidine and not decomposition of the cell. This experiment can also display the reversibility of spermidine's effect on EPSPs.

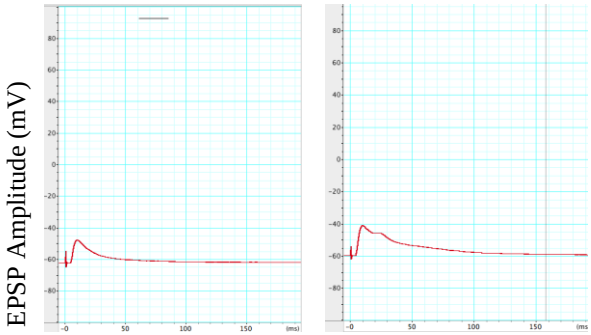

Figure 1. These are EPSPs measured during control and spermidine trials. Figure 1a displays an EPSP obtained during the control period while Figure 1b displays the following EPSP after the spermidine solution was inserted.

Figure 1 displays the EPSPs that were used to produce the amplitudes from the intracellular electrodes. The minimum values were subtracted from the maximum values on Figure 1a and Figure 1b.

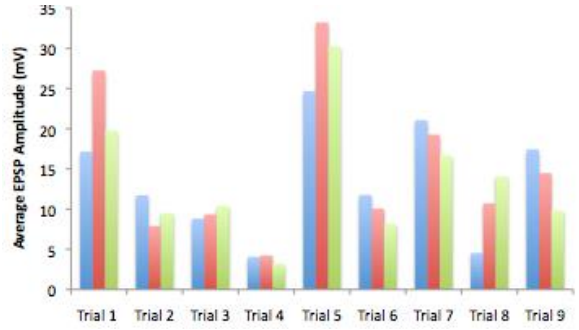

Figure 2. This graph shows the average EPSP amplitude for the control, spermidine, and control 2. It compares the three categories for every one of the nine trials.

We calculated averages for every group of EPSPs taken, including the control, spermidine, and control 2, the measurement taken after the rinsing. Figure 2 shows that Trials 1, 3, 4, 5, and 8 displayed an increase in EPSP amplitude compared to the amplitude of the control. Our results were varied and in 5 out of 9 trials spermidine increased EPSP amplitude and in the remaining trials it decreased EPSP amplitude, although the change was greater during the trials where the amplitude increased. Our results also show that in 5 out of 9 of the trials spermidine's effect on EPSPs was not reversible. This is shown clearly in Figure 3. After performing a Students t-test we obtained a p value of 0.35 when comparing the control values with the spermidine values to determine whether spermidine affected EPSP amplitudes. Since it was greater than .05 our results are not statistically significant.

As shown in figure 2, in all of the trials the control 2 amplitudes correlated with the spermidine measurements. If spermidine increased, the control 2 was higher than control 1. If spermidine decreased, control 2 was lower than control 1.

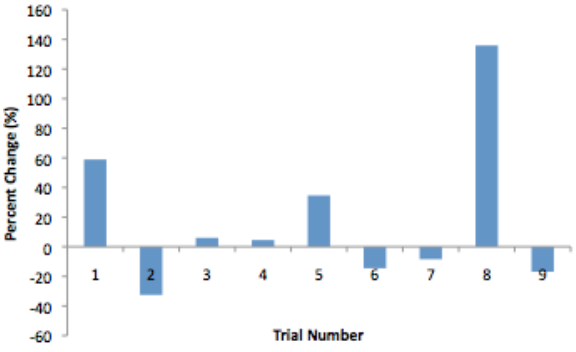

Figure 3.Percent changes from Control 1 to Spermidine. This graph displays the percent change in EPSP amplitude calculated from the measurements taken during the experiments.

Figure 3 shows that the increased percent change between Control 1 and spermidine is, on average, larger than the decreased percent change between a Control 1 and spermidine. It shows that Trials 1, 3, 4, 5, and 8 increased amplitude by 58.9%, 6%, 4.5%, 34.6% and 135.9%. Trials 2, 6, 7, and 9 show spermidine changed the amplitude by -32.5%, -14.5%, -8.4% and -16.9%.

DISCUSSION

Our hypothesis stated that spermidine would increase the EPSP amplitude because it is known to enhance NMDA receptor function at the neuromuscular junction. Five out of nine of our trials supported our hypothesis with increased EPSP amplitude while four of the trials had decreased EPSP amplitudes which is in accordance with previous research (DiScenna *et al.*,1994).

Additionally, we observed that control 2 followed spermidine's trend. This may have indicated that spermidine permanently altered the NMDA receptor function and caused irreversible effects or that we did not wash out the spermidine completely. We couldn't completely replace the spermidine because some solution had to surround the cell we were measuring. In future experiments, it would be good to test the permanence of spermidine's effects on NMDA receptor function. We would also like to control for more variables that might have caused the conflicting data such as: temperature, differences between crayfish, time, spermidine application, and wash method. Washing out the solution more thoroughly or using a different method may also lead to better results.

While five of our nine trials showed increased amplitude we would need to do several more trials to determine whether this trend holds true. Our experiment further supports the contradicting evidence that has been found in tests with spermidine at the neuromuscular junction. Past research has yielded conflicting results where it is unclear of the effect of spermidine on EPSPs.

ACKNOWLEDGEMENTS

We would like to thank Professor Lindgren for his advice on our topic and guidance towards helpful associated research. We would also like to thank Sue Kolbe for teaching us correct lab practices and preparing our stock solution. Thank you to Allie Byrne and Jason Park for helping with crayfish dissection and answering general questions. Thank you to the Grinnell College Biology department for funding our project and providing the necessary equipment.

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