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NAAG Increases Synaptic Transmission at the Crayfish Neuromuscular Junction

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ABSTRACT

We examined the effects of NAAG and LY341495 on metabatropic glutamate group II receptors (mGluR Group II) at crayfish neuromuscular junctions. We added exogenous NAAG and LY341495 saline solutions to crayfish neuromuscular junctions and recording excitatory postsynaptic potentials (EPSP) through paired pulse stimulation. Our results showed that NAAG increases synaptic plasticity and LY341495 has no effect on crayfish neuromuscular junctions under baseline conditions, meaning either mGluR Group II are not present in crayfish or they are not activated under baseline conditions (i.e. low frequency stimulation).

INTRODUCTION

The relationship between the presynaptic and postsynaptic cell is critical for an effective synapse. Group II metabotropic glutamatergic receptors (mGluR) and their corresponding neurotransmitter, N-acetylaspartylglutamic acid (NAAG) moderate cell communication (Gafuroy et al., 2001). Group II mGlu receptors are important in cell communication, because they are neuroprotective and such receptors are cognitive enhancers that can protect against diseases such as schizophrenia, epilepsy and Alzheimer's (Jeffrey et al., 1997). Most importantly, mGluR group II receptors are autoreceptors that modulate synapses via receptors on the presynaptic cell. Research has also shown that mGlu Group II receptors are found in both presynaptic and postsynaptic cells (Flor et al., 1997; Moldrich et al., 2003; Schoepp et al. 2001; Kew et al., 2001; Poschel et al., 2005).

Our research seeks to reinforce the scientific evidence of the existence of mGlu Group II receptors in crayfish at the neuromuscular junction both presynaptically and postsynaptically. Crayfish are ideal for exploring mGluR group II because crayfish have good model synapses, especially glutamatergic, as they have large muscle cells and neurons, accessible neuromuscular junctions, and NAAG (Gafuroy et al., 2001). We hypothesized that the addition of NAAG will alter the EPSP response, demonstrating that mGlu Group II receptors are both present and effective in the presynaptic cell of crayfish. We also hypothesized that the addition of LY341495 would affect the EPSP response, showing that mGlu Group II receptors are also present and effective in the postsynaptic cell. Our results showed that NAAG increases synaptic transmission and LY341495 has no effect in crayfish under baseline conditions. Thus crayfish synapses are responsive to exogenous NAAG, but we have no evidence that group II metabotropic glutamate receptors are active under baseline conditions.

MATERIALS AND METHODS

Saline Preparation

We used a saline solution containing 5.4 mM KCl, 196 mM NaCl, 2.6 mM $MgCl_2$, 6.75 mM $CaCl_2$, 10 mM HEPES (pH 7.4).

Chemical Preparation

We diluted 100 µL of 10 mM NAAG into 100 mL of crayfish saline to create the NAAG saline and diluted 20 µL of 5 mM LY341495 in 100 mL of crayfish saline to create the LY341495 saline. We then added 25 mL of the NAAG saline and LY341495 saline to 75 mL crayfish saline so the final concentrations would be 2.5 µM NAAG and 0.25 µM LY341495.

Crayfish Tail Preparation

We used crayfish that were previously on ice and cut and removed the crayfish's abdomen. We then made incisions along the lateral ridges on both sides of the tail and removed the swimmerets and ventral exoskeleton, exposing the tail muscles. We gently pushed the exposed muscle and viscera towards the tail until we were able to pull and remove it from the tail. We secured the remaining extensor muscle and dorsal exoskeleton to a silicon elastomer with two pins, submerged the preparation in 100 ml crayfish saline and placed the tray under the microscope.

Microelectrode Preparation

We placed and secured 1.2 mm diameter glass tubes in a WPI PUL-1 Micropipette Puller and turned the Micropipette Puller on until the glass was heated enough to pull the tube apart into two micropipettes. We then filled the electrodes with 3.0 M KCl, making sure there were no air bubbles, and rinsed the bottom inch of the electrodes in the crayfish saline solution. We put the microelectrode in the micromanipulator. We were unable to measure the resistance of the electrodes but suspect they were between 5 and 20 MΩ.

Paired Pulse Stimulation

We used a suction electrode to suck up a nerve at the neuromuscular junction. We used a micromanipulator to move the electrode into a muscle cell at the same neuromuscular junction. We applied low frequency (0.2 Hz) stimulation to the nerve to trigger an action potential and then recorded synaptic potentials in the muscles.

RESULTS

We hypothesized that NAAG and LY341495 would affect the EPSP at a crayfish neuromuscular junction which would show that mGluR Group II are effective in crayfish either pre- or postsynaptically. We found that NAAG affected synaptic activity and that LY341495 had no effect at crayfish neuromuscular junctions. We dissected a crayfish tail, exposing the dorsal extensor muscles. We submerged the tail in 100 mL control saline and used low frequency (0.2 Hz) paired pulse stimulation to record EPSPs. We then removed 25 mL of the control saline and added 25 mL NAAG saline and recorded EPSPs for five minutes. We washed the crayfish with control saline and replaced 25 mL of the control saline with LY341495 saline and recorded EPSPs for five minutes. We compared NAAG and LY341495 EPSPs with EPSPs taken after the crayfish had been washed with control saline due to fluctuating resting membrane potentials.

The results shown in Figure 1 demonstrate that the addition of NAAG affects EPSPs marginally significantly. On average, NAAG increased EPSPs by 34.4%. Figure 1 also represents the change in EPSPs due to the addition of LY341495. Although insignificant, this addition resulted in a 20% decrease. Figure 2 represents the paired pulse ratio between EPSP1 and EPSP2. We observed a 5.5% increase in the EPSP ratio for NAAG and a 7.9% decrease in the EPSP ratio for LY341495; however, these changes were not statistically significant.

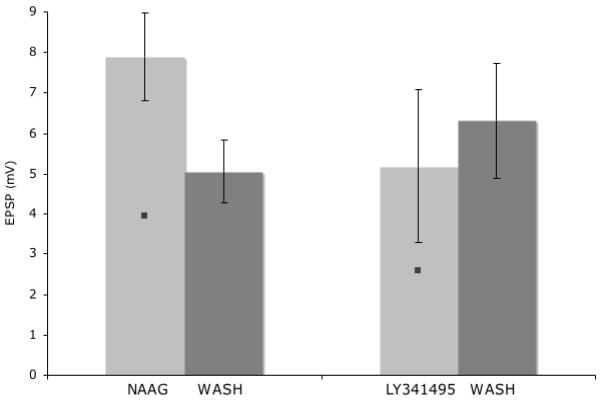

Figure 1- The effects of adding NAAG and LY341495 on EPSPs at a crayfish neuromuscular junction. The figure represents ten individual readings for NAAG and seven readings for LY341495. Error bars represent ± 1 S.E. with NAAG p> 0.05 and LY341495 p> 0.05.

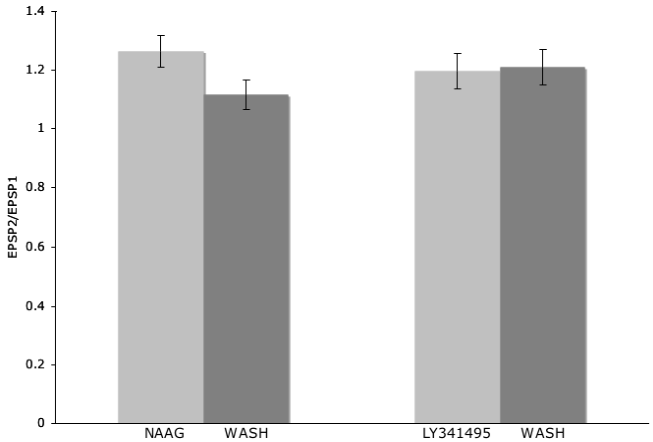

Figure 2- The effects of NAAG and LY341495on the paired pulse ratio at a crayfish neuromuscular junction. The figure represents ten individual readings for NAAG and seven readings for LY341495. Error bar represents ± 1 S.E. P-values for NAAG and LY341495 were > 0.05.

DISCUSSION

Our results indicate exogenous NAAG affects the crayfish neuromuscular junction and LY341495 has no effect on EPSPs of crayfish neuromuscular junctions under baseline conditions. Our results do not significantly support previous research that showed NAAG acts as an agonist to mGluR Group II (Pascale et al., 1994) or our hypothesis that NAAG is an agonist to mGluR Group II at neuromuscular junctions in crayfish.

Upon analysis of the average EPSP, we observed that the addition of NAAG increased synaptic transmission by 34.4%. While only marginally significant, this increase indicates that synaptic transmission increases with the addition of NAAG. The increase in the average EPSP indicates that NAAG is binding to a receptor and acting as an agonist. Previous research, in conjunction with our

results, suggests that NAAG binds to group II metabatropic glutamate receptors (Gafuroy et al., 2001). The presence of these receptors in crayfish at the neuromuscular junction remains unclear. While research in mammals indicates that mGluR group II are located on the presynaptic cell (Flor et al.,1997; Moldrich et al., 2003; Schoepp et al. 2001; Kew et al., 2001), our results suggest otherwise. Our addition of NAAG did not result in a significant alteration of the paired pulse ratio, indicating no presynaptic effects. Thus, our results indicate that NAAG affects synaptic transmission postsynaptically. While NAAG affected synaptic transmission, what it binds to and where these receptors are located is still inconclusive.

The addition of LY341495 resulted in insignificant changes in both the average EPSP and the paired pulse ratio in crayfish at the neuromuscular junction. Although LY341495 is an antagonist to mGluR group II in other animals (Howson et al., 2003), our results do not support LY343195's role as an antagonist in crayfish at neuromuscular junctions. The results of this experiment suggest that either mGluR group II receptors are not present in crayfish neuromuscular junctions or they require conditions other than baseline in order to be activated.

Considerations for further experimentation could be determining whether mGluR group II are present at crayfish neuromuscular junctions. Under baseline conditions we observed no change following the application of LY341495, but different conditions such as a higher frequency might be required for activation. If antagonistic effects are observed with LY341495, the presence of mGluR group II can be tested. One could determine whether mGluR Group II are present by adding NAAG and LY341495 saline simultaneously. If LY341495 blocks NAAG's agonistic effects, it can be concluded that mGluR group II are present in crayfish neuromuscular junctions.

Important research may also be done regarding NAAG's differing effects presynaptically and postsynaptically. Our addition of NAAG increased synaptic transmission, and the paired pulse ratio was increased by 5.5% when NAAG was added, indicating increased presynaptic plasticity. Although insignificant, further research may be done to determine the full extent of these presynaptic effects. Where NAAG binds, as well as where it is effective, remains inconclusive.

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