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The effects of a Group III metabotropic glutamate receptor antagonist at the crayfish neuromuscular junction.

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ABSTRACT

This experiment sought to obtain information about the role of metabotropic glutamate receptors (mGluR) in higher brain function. The study particularly looked to verify the presence of group III metabotropic glutamate receptors at the crayfish neuromuscular junction, analogous to a synapse of the human brain, through the use of a group III specific antagonist. Measurements of EPSPs elicited by stimulating nerves of the crayfish extensor muscles were compared in the presence of normal crayfish saline and saline solutions with different concentrations of the antagonist added, where a significant change in amplitude of the EPSPs would indicate the antagonist was interacting with mGluRs present in the presynaptic or postsynaptic cell. When comparing the results of the study in the control solution to the antagonist modified solution data showed no significant decrease in the mean amplitude of elicited EPSPs, but did show a significant increase in the mean lowest voltage needed to elicit an EPSP. The latter suggests the antagonist was operating, and thus that mGluRs were present in the cell; however, the results of the study are not conclusive enough to demonstrate that metabotropic glutamate receptors of group III are present in the crayfish neuromuscular junction.

INTRODUCTION

Glutamate is an amino acid and is the most commonly used neurotransmitter in the human brain (Golan et al., 1996). Metabotropic glutamate receptors play a modulatory role in synaptic transmission at many glutamatergic synapses. Current research suggests that there are multiple types of metabotropic glutamate receptors which behave in distinct ways and respond differently to certain antagonists; the receptors are most often categorized into three different groups. Much research has been done regarding the pharmacology of agonists for metabotropic glutamate receptors in groups II and I but very little research has been done regarding selective ligands and the pharmacology of antagonists that are specific to group III (Kew et. al., 2005).

Metabotropic glutamate receptors in group III are G-protein-coupled receptors; thus, they activate a G protein which inhibits adenlyl cyclase, an enzyme involved in the creation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) (Kew et. al., 2005). Metabotropic glutamate receptors in group III are also known to affect excitatory and inhibitory postsynaptic potentials in the brain cortex (Kew et. al., 2005).

The long term objective of this study is to further our understanding of synaptic transmission and higher brain functions by researching the role of metabotropic glutamate receptors in synaptic transmission. The more immediate goal of this study is to better understand the role played, if any, of group III metabotropic receptors on synaptic transmission at the crayfish neuromuscular junction. We chose to use the crayfish neuromuscular junction because it is analogous to synapses in the human brain. Crayfish are evolutionarily simple; they are not particularly complicated in structure but still carry out the essential processes of life. Also, crayfish are easy to obtain and allow for a fast and easy dissection.

We tested this by using the group III antagonist (R,S)-α-methylserine-*O*-phosphate (MSOP). We observed MSOP's effects by comparing the amplitude of an EPSP at the crayfish neuromuscular junction in a standard solution verses the amplitude of an EPSP at the crayfish neuromuscular junction in modified solutions containing varying concentrations of MSOP.

Additionally, we recorded the mean lowest voltage necessary to elicit an EPSP in each of the solutions. We chose to measure EPSP amplitude for evidence of altered synaptic transmission because EPSP amplitude reflects the strength of synaptic communication in regards to the amount of neurotransmitter released and how sensitive the muscle receptors are.

MATERIALS AND METHODS

Dissection

We cut the removed tail of a frozen crayfish longitudinally along the edges of the shell, from the base of the tail to the opposite tip. We then peeled

back the ventral shell, and scraped off muscle tissue to expose the lateral extensor muscles.

Set Up

A pulled microelectrode was filled with 3M KCl and attached to a micromanipulator. A second reference electrode was placed in a dish of standard saline solution (205 mM NaCL, 5.4 mM KCL, 13.5 mM CaCl2, 2.6 mM MgCl2, 10.0 mM Tris Buffer pH 7.40). Both electrodes were grounded through a metal base plate and routed all information to a computer display of the relative voltage potential between the electrodes. A microscope helped us to guide the microelectrode into the crayfish muscle cells to record voltages. The resistance was continually tested and kept within a range of 10-20 MΩ to ensure that the electrodes were able to accurately record.

We then attached a suction electrode to a second micromanipulator that, like the micro-electrode, was accompanied by a reference electrode placed in the solution. Routed to a stimulating circuit, the suction electrode transmitted charge through the captured nerve. Any excitatory postsynaptic potentials (EPSP) elicited were recorded by the recording electrode.

Data Collection

The dissected crayfish tail was immersed in the dish of standard saline solution and pinned to the sylgard base of the dish. The suction electrode was then used to suck up a nerve, while the microelectrode was inserted into a nearby muscle cell. The nerve was then stimulated by electrical pulses to elicit an EPSP, which was displayed by a graph in the program Scope v4.03 on the computer. We observed and recorded the amplitude of the EPSP as displayed on the graph. We also recorded the lowest voltage at which we could elicit the EPSP. During the procedure the saline was replaced every fifteen minutes by way of siphon to avoid both disturbing and contaminating the sample. This data served as the control data.

After EPSPs were successfully recorded from a crayfish tail in the standard saline, the tail was immersed in one of two modified saline solutions. We made these solutions from a 20 mM stock solution of MSOP. The first solution was composed of 100mL of the regular saline solution with .47mM of MSOP (we referred to as .47mM antagonist solution). The second modified solution was composed of 100 mL of the regular saline solution with .94mM of MSOP (referred to as .94mM antagonist solution). The same procedure that was used in the standard saline solution was followed in the modified solutions and the amplitude of the EPSPs as well as the lowest voltage at which an EPSP was elicited was recorded. However, because we only had a small amount of the antagonist, the modified solutions were not replaced every fifteen minutes.

RESULTS

Our experiment sought to determine whether or not group III metabotropic glutamate receptors are present in the crayfish neuromuscular junction. We investigated this by measuring EPSP amplitude at the crayfish neuromuscular junction in a standard saline solution (205 mM NaCl, 5.4 mM KCl, 13.5 mM CaCl2, 2.6 mM MgCl2, 10.0 mM Tris Buffer pH 7.40) and in two altered solutions containing different concentrations of the group III antagonist methyl serine O phosphate (MSOP). The first solution was 100 mL standard saline and .47mM of MSOP; the second solution was 100 mL standard saline and .94mM of MSOP.

We found that although the mean EPSP amplitude in the control condition was 9.0 mV and the mean EPSP amplitude in the .47mM antagonist condition was 3.9 mV the difference between them was not statistically significant. Additionally, we found the mean EPSP amplitude in the .94mM antagonist condition was 7 mV. This also was not statistically significant when compared to the mean EPSP amplitude of the control condition. Despite the lower mean EPSP amplitudes in the antagonist conditions, which suggest that the receptors are present and were inhibited by the antagonist, in all cases the differences between the means were not statistically significant.

Our results demonstrated a 3.1 mV difference between the mean EPSP amplitude in the .47mM antagonist solution and the .94mM antagonist solution with a higher mean in the .94mM antagonist solution. This difference could be due to human error or to unique behavior of MSOP in different concentrations. It is also possible that MSOP behaves the same way in any concentration and that there appears to be a difference between the means in each concentration because too few measurements were taken (in .47mM antagonist solution n=6, in .94mM antagonist solution n=5). Even if we assumed MSOP behaves the same way in all concentrations the pooled mean amplitude for all of the antagonist data is 5.3 mV. When compared to the control mean EPSP amplitude the pooled mean EPSP amplitude in the antagonist solutions is still not statistically significant.

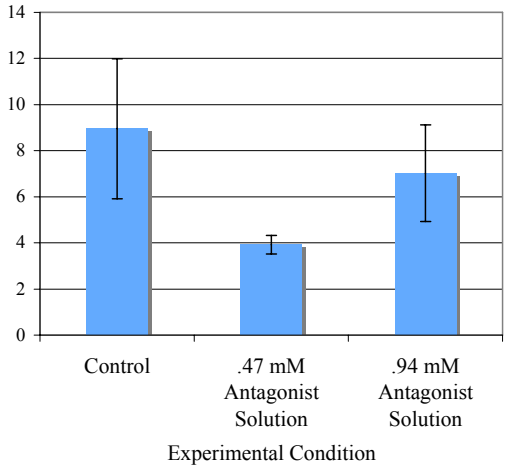

Figure 1. The mean EPSP amplitude in each experimental condition. Control n=10, .47 mM antagonist solution n=6, .94 mM antagonist solution n=5. In all conditions p>.05. Error bars represent 1 S.E.

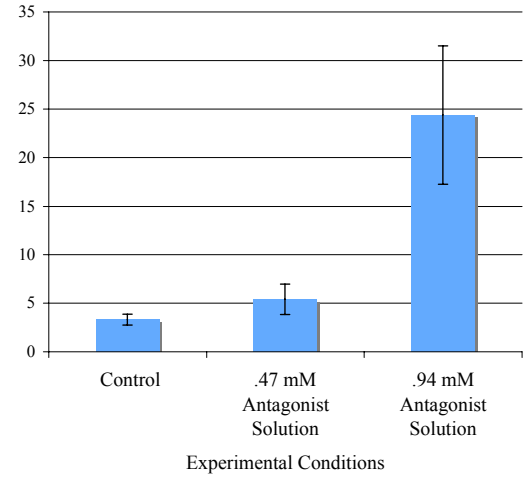

Figure 2. The mean lowest voltage for an EPSP in each experimental condition. Control n=10, .47 mM antagonist solution n=6, .94 mM antagonist solution n=5. In independent t tests p values< .05 for control vs. .94 mM antagonist solution, and .47 mM antagonist solution vs. .94 mM antagonist solution, p value >.05 for control vs. .47 mM antagonist solution. Error bars represent 1 S.E.

During data collection we also recorded the mean lowest voltage or the threshold voltage needed to elicit an EPSP in each experimental condition. Figure 2 demonstrates the trend that a higher mean voltage was needed to elicit an EPSP in the antagonist solutions. The data also shows that a higher mean voltage was needed for an EPSP when twice was much antagonist was added to the crayfish extracellular fluid. The mean lowest voltage needed for an EPSP in the control condition was 3.3 volts as compared to the mean in the .47mM antagonist condition of 5.4 volts or the mean in the .94mM antagonist condition of 24.4 volts. The difference between the control mean and the mean of the .94mM antagonist condition is statistically significant (Fig. 2, p value = .0009). Similarly, the difference between the mean lowest voltage in the .47mM antagonist solution and the mean lowest voltage in the .94mM antagonist solution is statistically significant (Fig. 2, p value = .02).

DISCUSSION

Our results indicate that mean EPSP amplitude decreased between the control and the .47mM solution, as well as between the control and the .94mM antagonist solution. However, we did not find these differences to be statistically significant. Additionally, we had expected the mean EPSP amplitude to show a change in both the modified solutions. The hypothesis stated that group III glutamate receptors are present in the crayfish neuromuscular junction. Thus the synaptic transmission at the neuromuscular junction will be inhibited by MSOP. Because we did not see statistically significant decreases in amplitude with the addition of the antagonist, we were not able to support our hypothesis.

Our study did show a statistically significant trend of increase of mean lowest voltage needed to elicit an EPSP as the antagonist concentration was increased. This suggests that the presence of the MSOP antagonist raises the threshold needed to elicit an EPSP. Furthermore, this would suggest that the group III glutamate receptors are present and are affected by the presence of the MSOP antagonist, thereby supporting our hypothesis.

In resolving the discrepancy concerning the data we collected regarding mean EPSP amplitude, which did not support our hypothesis, and the data we collected regarding mean lowest voltage, which does support our hypothesis, we considered the reliability of each data set. It should be noted that some variability may exist within the voltage data due to the use of numerous recording electrodes. This was not a confounding factor in the amplitude data. Therefore we believe the mean EPSP amplitude data is more accurate and cannot state conclusively that group III mGluRs are present in the crayfish neuromuscular junction.

Other scientific studies on glutamate and synaptic transmission suggest that mGluR antagonists inhibit synaptic transmission and show a decrease in EPSP amplitude where mGluRs are present (Vaughan, 1997). Additional studies on the subject show similar yet varied responses of group I and II mGluRs to antagonists (Moroni, et al., 1996). Our

study investigated these responses in the context of group III glutamate receptors. Despite the evidence offered by the studies cited above, and others, the fact that we found no statistically significant decrease in EPSP amplitude while MSOP was present does not allow us to assume the presence of group III glutamate receptors at the crayfish neuromuscular junction. However, because of the positive results elicited by previous studies we do not rule out the possibility that group III glutamate receptors do exist in the crayfish neuromuscular junction.

While our hypothesis was not supported by our results, the trends that we encountered surrounding the mean lowest voltage needed to elicit an EPSP do suggest that group III glutamate receptors exist in the crayfish neuromuscular junction. Initially we had intended on furthering our understanding of synaptic transmission and higher brain function as they relate to the metabotropic glutamate receptors and their antagonists. While this study illuminated further the role of mGluRs in synaptic transmission, we suggest that future studies center on the responses of group III glutamate receptors to higher concentrations of the MSOP antagonist to gain conclusive data about the role of glutamate receptor antagonists in synaptic transmission. This would hopefully lead to a better understanding of metabotropic receptors, synaptic transmission, and higher brain function.

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