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Role of group 1 metabotropic glutamate antagonists in facilitation of post-tetanic plasticity is unclear in crayfish neuromuscular junction

JONATHAN DOWELL, YUE YU, and ALEXANDRA KARAGIARIDI
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

Previous research suggests that glutamate receptors play a role in drug-seeking behaviors. To observe the effect of glutamate receptors, we were looking for the relationship between group I glutamate receptors and postsynaptic plasticity. We had predicted that an increase in the concentration of group 1 metabotropic glutamate receptor antagonist SIB 1757 would decrease depression in the crayfish neuromuscular junction. We applied the antagonist in concentrations of .4 μM and 4 μM, and stimulated nerves in the crayfish tail at 20 Hz for 10 seconds each. Depression or potentiation occurred depending on the nerve that was stimulated. No connection between antagonist and post-tetanic plasticity was observed.

INTRODUCTION

Glutamate receptors are distinguished into two types, ionotropic and metabotropic. An ionotropic glutamate receptor directly forms an ion channel pore when glutamate binds to a receptor, through which ions would be transferred. Metabotropic glutamate receptors activate G proteins that release second messengers, which either activate ion channels or other intermediate molecules.

We focused on metabotropic glutamate receptors because they are associated with prolonged stimuli and have been shown to be a key determinant in the understanding of drug-seeking behaviors, as part of the reward system. Metabotropic glutamate receptors are split into Group I and Group II & III. Group I mGluRs receptors are subdivided into mGluR1 and mGluR5 and have both excitatory and inhibitory effects, allowing scientists to isolate and identify them more easily. Group II & III include subtypes 2,3,4,6,7 and 8 involved in presynaptic inhibition and reduction of the activity of postsynaptic potentials.

When trying to establish a connection between group I metabotropic glutamate receptors (mGluR) and drug addiction, it was shown that the inhibition of the receptors can actually reduce drug reward, and if properly modulated they can even reverse the cognitive deficits related to long-term drug abuse (Olive, 2010). Therefore inhibiting these receptors are expected to be helpful in drug-addiction treatments. Apart from the group I metabotropic glutamate receptors, there are also the group II and III ones that could possibly affect synaptic plasticity. Nevertheless, experiments carried out by previous Grinnell Bio-150 students showed that inhibition of these types of metabotropic glutamate receptors does not affect the plasticity of the crayfish neuromuscular junction, following tetanic stimulation applied at different frequencies and durations (Elias et al, 2011).

We focused on the effect of group I metabotropic glutamate receptors on synaptic plasticity. The question that we addressed is ***"What is the effect of a type I metabotropic glutamate receptor antagonist on post-tetanic depression in the crayfish neuromuscular junction?"***. Based on a previous study by Neale (2001) showing that group I mGluRs can create depression in the postsynaptic cell, we hypothesized that the presence of a group I metabotropic glutamate receptor (mGluR5) antagonist, SIB 1757, would reduce post-tetanic depression amplitude in the crayfish neuromuscular junction. mGluR5 receptors are particularly connected to understanding drug addiction, thus our focus was primarily on this Group I subtype. To be more accurate, we predicted that an increase in the antagonist's concentration (0.4μM to 4μM), would cause a decrease in the percent change of the EPSPs' amplitude after application of tetanic stimulation. If a link between group I mGluRs and processes involving drug addiction does exist, an antagonist that reduces intensity of cell depression would open the door to more research on effectively treating drug addiction.

Finally, our results did not suggest any relationship between group 1 mGluR antagonist SIB 1757 and post-tetanic plasticity in the crayfish neuromuscular junction. Both depression and

potentiation were observed, so we analyzed them separately.

MATERIALS AND METHODS

Preparation of 0.4μM and 4μM of SIB 1757

We prepared 4 μM of SIB 1757 by diluting 250 μl of the drug, initially prepared at 4mM, in 250 mL of crayfish saline and inverting the flask several times to mix. To prepare 0.4 μM of SIB 1757 we diluted the 4 μM antagonist in 1:10 with crayfish saline by taking 25 ml of the 4 μM antagonist solution into a flask and filling the flask to 250 ml with saline. The composition of the crayfish saline solution was 5.4mM KCl, 196 mM NaCl, 2.6 mM $MgCl_26H_2O$, 10 mM Sodium Hepes Buffer, 13.5 mM $CaCl_22H_2O$ at pH of 7.4.

Dissection of Crayfish

Before dissection, we placed the crayfish in an ice bucket until it had stopped moving,for ethical purposes. We cut the crayfish's tail off the main body and separated the ventral part of the abdomen from the rest of the abdomen, cutting just at the angle between the ventral surface and the side and going along that line. We removed the deep muscle tissue in the dorsal part of the abdomen and left the superficial abdominal extensor muscles, and placed it in a disc container filled with crayfish saline.

Glass Electrode Preparation

With a Pul-1 heat puller we pulled the glass electrodes and filled them with 3M KCl solution. To remove any concentrated KCl amounts left on the electrodes we rinsed the filled electrodes in saline solution. Also we checked the resistance of the tips to be >13 MΩ so that they would make accurate measurements. We also made suction electrodes by breaking the tips with a sand paper.

Electrophysiology

For cellular measurements we inserted the filled glass electrode into the crayfish's muscle cell after zeroing it in the saline solution. We also detected the nerve connected to the muscles and sucked it with the suction-stimulating electrode. Using the PowerLab software, we observed the EPSP generated in the crayfish's muscle cell. After applying tetanic stimulation at 20Hz for 10 seconds, we measured the difference in the EPSP magnitudes achieved. When exposing the crayfish's tail in 0.4μM and 4μM SIB 1757 solutions, we left it in the antagonist solution for 5 minutes to soak, prior to making measurements.

RESULTS

We collected 10 initial EPSP readings in the crayfish cell at 0.5Hz. 5 minutes after applying a tetanic stimulus of 20Hz for 10s, we took 11 readings of the EPSP of the cell from 4'50'' to 5'10'', at 0.5Hz. Then we averaged both sets of data, and calculated the percent difference in EPSP amplitude with the formula

((EPSPafter - EPSPinitial) / EPSPinitial) x 100%

We conducted five trials for each category (control, 4 m and 0.4 m) and the results are shown in Figure 1.

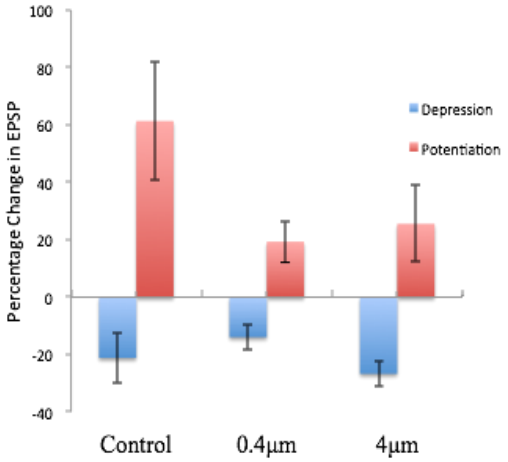

Fig.1 The average percentage difference of EPSP after tetanic stimulation. Percentage differences observed for depression were 21% (r=±8.91, n=3) for the control, 14% (r=±8.91, n=3) with 0.4μm antagonist, and 27% (r=±4.40, n=2) with 4 μM antagonist. Percentage difference observed for potentiation were 61% (r=±20.69, n=2) for the control, 19% (r=±7.19, n=2) with 0.4μm antagonist, and 26% (r=±13.37, n=3) with 4 μM antagonist The error bars show the standard error of percentage change in EPSP. n=11.

Both potentiation and depression occurred to the crayfish muscle cell after the tetanic stimulation. Therefore we decided to analyze them separately. Percentage differences observed for depression were 21% (r=±8.91, n=3) for the control, 14% (r=±8.91, n=3) with 0.4 m antagonist, and 27% (r=±4.40, n=2) with 4 M antagonist. The percentage difference in EPSP was not lower when we applied the antagonist in a higher concentration.

When potentiation was observed, percentage differences were 61% (r=±20.69, n=2) for the

control, 19% (r=±7.19, n=2) with 0.4μm antagonist, and 26% (r=±13.37, n=3) with 4 M antagonist. Similar to the depression, percent change in EPSP did not decrease as we applied higher-concentration of the antagonist to the crayfish.

We observed a general trend that under 0.4 m SIB 1757, which is the IC50 concentration value of the drug, the percentages of both depression and potentiation were mostly decreased. However, we did not have enough trials, and were unable to conduct a student's T-test. Thus it is possible that the differences in average percentage change of EPSP were due to chance variability.

DISCUSSION

We observed a surprising range of post-tetanic plasticity during our experiments. Prior to testing, we only anticipated post-tetanic depression to occur in the crayfish neuromuscular junction. Instead, both depression and potentiation were observed after applying 20 Hz stimulation for 10 seconds. The cell's ability to either depress or potentiate after stimulus was consistent among single nerves, but not among different crayfish tail specimens. Research by Jimbo with cortical cell cultures found similar results (Jimbo et. al., 1999). Jimbo and his colleagues (1999) observed that the same tetanic stimulation resulted in depression or potentiation in different individual nerve pathways. We noted that cells with larger EPSP amplitude tended to depress, and consequently those with lower amplitude tended to potentiate. Postsynaptic potential amplitude could be a differing property among nerve pathways that experience different post-tetanic plasticity. Activation of type 5 mGluRs in the hippocampus has been shown to modulate both LTP and LTD (Cleva, 2011). If type 5 mGluR function similarly in the crayfish neuromuscular junction, then antagonizing these receptors would still allow both potentiation and depression to occur.

We had hypothesized that as we increased the concentration of group 1 mGluR antagonist SIB 1757, post-tetanic depression in the crayfish neuromuscular junction would decrease. The occurrence of post-tetanic potentiation was a surprise, but nerves that potentiated tended to have a more significant reduction in amplitude change with presence of the drug. With nerves that were depressed, no significant change in EPSP amplitude was observed with the antagonist present. Antagonist concentration did not seem to have a significant effect on amplitude change during either potentiation or depression. Thus the hypothesis was not supported by our data.

Because both potentiation and depression occurred, a low number of trials for each group prevented in-depth statistical analysis of our data. It is possible that the drug is effective at low concentrations, or that the drug simply does not affect changes in post-tetanic plasticity. If group 1 mGluR antagonists indeed have no effect on neural plasticity, then group 1 mGluRs may not have as large of an effect on learning as we had hypothesized.

Based on our results, we are unable to infer on the connection between glutamate receptors and learning involving drug addiction. We recommend further investigation on nerve pathways in crayfish that depress versus those that potentiate.

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