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Inhibition of glutamate transporters decreases the amplitude of excitatory postsynaptic potential at the crayfish neuromuscular junction.

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ABSTRACT

The aim of our experiment was to determine the effect of the chemical trans-2, 4 PDC, a glutamate transport inhibitor, at the crayfish neuromuscular junction. Trans-2,4 PDC prevents glutamate transporters from binding onto glutamate and removing it from the synaptic cleft. We hypothesized that the addition of the chemical to the neuromuscular junction would result in an increase in the amplitude of the Excitatory Post-Synaptic Potentials (EPSP). We recorded the amplitude of several EPSPs of each crayfish while it was in normal saline, then added trans 2,4 PDC and recorded the EPSPs again. We observed a decrease in the amplitude of the EPSP after the chemical was added. We conclude from this that, contrary to our hypothesis, the decrease in the amplitude of the EPSPs could be attributed to the fact that down regulation occurred or that there was a lack of glutamate recycling in the presynaptic cell.

INTRODUCTION

In recent years, neuroscientists have looked increasingly into the importance of glutamate transporters in synaptic transmission. Glutamate transporters regulate the concentration of extracellular glutamate by binding to glutamate in the synaptic cleft and transporting it into glial cells, such as astrocytes (Cechova and Zou 2006). Since glutamate is toxic in high concentrations, these glutamate transporters play a crucial role in maintaining cellular health and thus the ability of nerve cells to participate in synaptic transmission. They also reduce stimulation of post-synaptic receptors by preventing all glutamate released in pre-synaptic cells from binding to receptors. Recent research suggests that a failure of glutamate transporters to operate may contribute to certain medical conditions, such as epilepsy. These findings have spawned an increase in studies on the mechanism of these transporters (Huang and Bergles 2004). Glutamate inhibition in newborn rats has also been found to adjust electrical activity in the brain, inducing seizures along with stints of hypoactivity (Milh 2006).

Until now, glutamate transporters have largely been studied in mammals (Malandro and Kilberg 1996). Thus, here we studied the role of glutamate transporters in synaptic transmission at the crayfish neuromuscular junction. We chose to use crayfish because they are cheap and easy to work with as a model organism; this research can be used to establish the ability of the crayfish to be useful in future research. Furthermore, an earlier study has indicated that experimentation in this area on the crayfish could be fruitful (Dudel 2006). Previous research has used the chemical L-trans-pyrrolidine-2,4-dicarboxylate (trans 2,4 PDC) to inhibit glutamate transporters in certain vertebrates (Robinson, Djali and Buchhalter 1993). We introduced this chemical to the crayfish neuromuscular junction through its addition to the saline and studied its effects on the amplitude and duration of excitatory post-synaptic potentials (EPSP).

We hypothesized that trans 2,4 PDC would cause the amplitude of the EPSPs to increase. By inhibiting glutamate transporters, trans 2,4 PDC would cause more glutamate to reach the glutamate receptors and thus increase stimulation of the EPSP. Our results showed that the amplitude of the EPSPs actually decreased due to the drug, which suggests either down-regulation or a shortage of glutamate was occurring.

MATERIALS AND METHODS

This experiment seeks to investigate the varied effects of trans-2, 4 PDC on the crayfish neuromuscular junction; considering that trans-2, 4 PDC is a glutamate transmitter transporter inhibitor. This chemical reverses glutamate transporters and elevates extra cellular glutamate levels in the crayfish neuromuscular junction (Rothstein and Dykes-Hoberg 1996).

Preparation

Using a pair of scissors we cut the tail off a *Pacifastascus lenisculus* crayfish that had been cooled in ice for at least 15 minutes. We slit the lateral ends of the cut tail and pulled off the shell and the ventral extensor muscles, leaving the dorsal extensor muscles exposed.

Solution

We created 100μM solution of trans 2, 4 PDC and saline solution. There were two different saline solutions that we used at one time or another, each with different composition. The normal saline solution consisted of 5.4mM KCl, 19.6mM NaCl, 2.6mM MgCl, 13.5mM CaCl, and 10mM Hepes buffer. The low calcium solution, with 2mM Ca2+, consisted of 5.4mM KCl, 196mM NaCl, 14.1mM MgCl, 2mM CaCl, and 10mM Hepes Buffer. The glutamate transporter inhibitor was from Tocris Bioscience.

Electrophysiology

We made recording electrodes from glass pipettes inserted into the World Precision Instruments PUL-1 electrode puller. These electrodes were filled with 3M KCL solution and then rinsed in saline solution prior to use. The microelectrodes we used ranged between 5-20MΩ.

Method

In order to undertake this experiment, the crayfish tail was dissected as described earlier in order to use the neuromuscular junction and then was completely submerged in saline solution. Once we sucked up a crayfish nerve cell using the suction electrode, we impaled a muscle cell with a microelectrode filled with KCL. We recorded the membrane potential and then proceeded to record the EPSP. A nerve cell which was sucked up using a suction electrode was stimulated via a Grass Stimulator at a frequency of 1 stimulus per second 3 times, adjusting the voltage in order to achieve an EPSP. If the EPSPs showed signs of an action potential, we removed the regular saline solution, and added the low calcium saline solution before taking our readings in order to get more accurate data. If we used the low calcium saline solution to find our data, we also mixed the trans 2,4 PDC in the low calcium saline.

After getting three readings with the regular saline solution, we removed the saline and replaced it with 100μM trans 2, 4-PDC and got new readings with stimulation of the same nerve cell.

We used the Scope 4 software to record and observe the amplitude of the EPSPs during the experiment. We used a t-test to compare the amplitude, membrane potential and half-life of the control measurements and the measurements after the drug was added. The t-test was performed for each individual crayfish and on all of the data combined. A p-value less than 0.05 allowed us to reject the null hypothesis. The half-max duration was measured by finding the time it took for the membrane potential to reach half what it was at its maximum point during the EPSP.

RESULTS

In order to find the effect of inhibition of glutamate transporters on the amplitute of EPSPs, we took control readings of EPSPs while the crayfish was submerged in normal saline. Then we measured new EPSPs after adding the glutamate transporter inhibitor trans 2,4 PDC to the solution. Our results show that the glutamate inhibitor trans 2,4 PDC has a statistically significant effect on the amplitude of excitatory post-synaptic potentials (EPSPs) at the crayfish neuromuscular junction (p-value<0.05). While there is some evidence that it also increased the half-life of the EPSPs, the data was not statistically significant, and there is no evidence that trans 2,4 PDC effects the membrane potential at the crayfish neuromuscular junction (Table 1).

	Average (Control)	Average (2,4-PDC)	P-Value
Amplitude (mV)	23.58	11.04	0.013
Membrane Potential (mV)	-48.67	-47.01	0.608
Half-max Duration (mS)	15.28	20.9	0.066

TABLE 1 Average amplitude of EPSPs, membrane potential and the half-max duration of the control readings and the readings taken after trans 2,4 PDC was applied to the crayfish. Also lists the p-values for each of these three data sets.

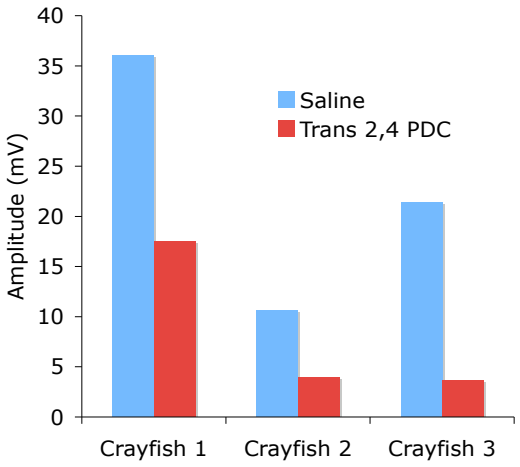

FIGURE 1A The graph shows the average amplitude of the EPSPs for each crayfish studied before and after the drug was added.

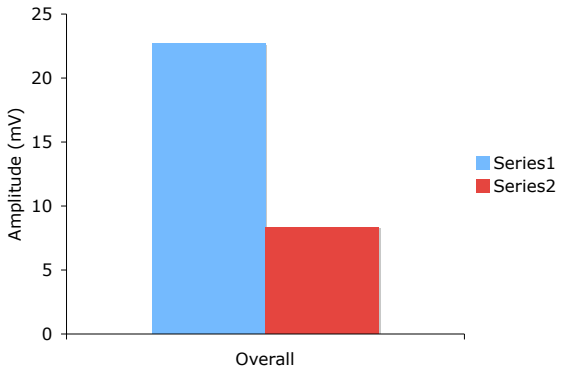

FIGURE 1A & 1B: The graph on the left (1A) shows the average amplitude of the EPSPs for each crayfish studied before and after the drug was added. The graph on the right (1B) shows the overall average amplitude.

The amplitude of the EPSPs was on average lower in all three crayfish experimented on (Figure 1A), as well as on the average of all three crayfish (Figure 1B). For each individual crayfish, there was a statistically shown difference between the amplitudes (p-value<0.05). This data includes two readings on the third crayfish in which the amplitude measured was close to zero as we believed the drug may have eliminated the possibility of achieving an EPSP in that particular crayfish. When those two readings were not included, our overall p-value was 0.03, so our findings were still statistically significant. However, the readings for the third crayfish are no longer statistically significant (p-value=.3).

DISCUSSION

In our attempt to understand the impact of glutamate transporters on the crayfish neuromuscular junction, we applied the glutamate inhibitor trans 2,4-PDC and measured the amplitude and half-max duration of the excitatory post-synaptic potentials (EPSPs) at the crayfish neuromuscular junction, as well as the membrane potential of the crayfish muscular cells. Our results showed that the amplitude of the EPSPs was decreased by application of trans 2,4 PDC. This disproves our hypothesis, which was that the amplitudes would increase due to increased stimulation of the post-synaptic cell. Since application of trans 2,4 PDC did not affect the membrane potential of the crayfish muscle cells, cellular death is not the reason for this decrease. The decrease is most likely due to either down-regulation of the glutamate receptors, which causes receptors to become desensitized to glutamate due to over-exposure and thus not respond, or a failure of the system to recycle glutamate, thus resulting in a shortage of glutamate in the pre-synaptic cell. Another study found that dihydrokainate, another glutamate transporter inhibitor, did prevent glutamate recycling (Yasuyuki 2002).

In order to differentiate between these two possibilities, more experimentation is needed. One possible experiment could simply replicate the experiment described in this paper, then add glutamate to the saline after trans 2,4 PDC is added. If the decrease was due to down-regulation, the amplitude of the EPSPs would either stay the same or become even smaller since the receptors would continue to be desensitized to glutamate. If the decrease was caused by a failure to recycle glutamate, the amplitude of the EPSPs would most likely increase once again now that glutamate was available to be released from the pre-synaptic cell.

However, our experiment does give preliminary results which show that trans 2,4 PDC does have an effect on the EPSPs at the crayfish neuromuscular junction, thus showing a similarity between the crayfish and mammals used in other experiments (Robinson, Djali and Buchhalter, 1993). Experiments involving glutamate transporters could use the crayfish neuromuscular junction in the future, as well as trans 2,4 PDC as a way to inhibit those transporters.

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