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Nitrendipine and Cadmium Decrease Synaptic Transmission in the Crayfish Neuromuscular Junction While ω-conotoxin Does Not

GLENN CLARK, HANNA LIEBL, and SOFIA SKRABA
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

Calcium channels have proven to be essential in the optimal functioning of chemical neurotransmission. Previous researchers have found that different types of channels exist and are inhibited by various chemicals. We tested three different calcium channel blockers - cadmium, Nitrendipine, and ω-conotoxin – and using intracellular recording techniques witnessed their effects on the excitatory post-synaptic potentials (EPSPs) in the crayfish neuromuscular junction. We hypothesized that all of the blockers would decrease the EPSP amplitudes, and that cadmium, the general blocker, and ω-conotoxin, the N-type channel blocker, would have similar effects in decreasing the amplitude more than would Nitrendipine, the L-type blocker. This hypothesis proved incorrect, as ω-conotoxin increased the average EPSP amplitude, suggesting perhaps compensation for the blocked channels or that the chemical we used was too old to be effective.

INTRODUCTION

Calcium is an extremely important ion in the process of synaptic transmission and communication between nerves. The influx of calcium into the pre-synaptic cell, caused by the depolarizing effect of an action potential, allows synaptic vesicles to fuse to the cell membrane and begin exocytosis, the release of neurotransmitters into the synaptic cleft. The neurotransmitters bind to post-synaptic neuroreceptors, which in turn open ion channels, causing a depolarization in the post-synaptic cell. This depolarization is called an excitatory post-synaptic potential (EPSP), and a decrease in the amplitude of this depolarization reflects a decrease in synaptic transmission.

Given this process, blocking the entrance of calcium into the pre-synaptic cell should decrease EPSP amplitudes. Calcium enters the cell through special voltage-gated ion channels known as calcium channels. However, there are different types of these calcium channels in the pre-synaptic cell, defined in part by their responses to different pharmacological elements. Our research reveals whether more than one type of calcium channel supports neurotransmitter release. That is, it explores the importance of the existence of different types of channels and their abilities to function under different physiological conditions.

Calcium channels under different physiological conditions have been studied in the past. Rivier et al. (1986) studied ω-conotoxin and its effects on blocking N-type channels, and Abbas et al. (2006) found that the chemicals nifedipine and nicardipine block L-type calcium channels. Additionally, Cooper and Manalis (1983) used cadmium as a general blocker and noted its effects on decreasing EPSP amplitude. We sought to determine whether more than one type of calcium channel supports neurotransmitter release at the crayfish neuromuscular junction. We chose the crayfish neuromuscular junction because of its similarity to the human neuromuscular junction; effects observed in the crayfish are likely to be mirrored in humans.

On the basis of previous research, we hypothesized that the EPSP amplitudes at the crayfish neuromuscular junction would decrease when we applied various calcium channel blockers. We used ω-conotoxin to block N-type channels, Nitrendipine to block L-type channels, and cadmium as a general calcium blocker. Since Krieger et al. (1999) found that N-type channels played the most prominent role in neurotransmitter release, we also hypothesized that blocking the N-type channels would have an effect most similar to that of cadmium, which, as a general blocker, blocks all types of calcium channels.

In our research we found that cadmium and Nitrendipine reduced the average EPSP amplitude from the control, but applying ω-conotoxin increased the average EPSP amplitude. Our hypothesis was therefore incorrect, and we concluded that we might have been witnessing compensation for the blocked N-type channels resulting from the application of ω-conotoxin, or perhaps that the ω-conotoxin we used was too old to be effective. We also concluded that further research needs to be done to examine the unexpected results we found with ω-conotoxin.

MATERIALS AND METHODS

Dissection

After exposing the crayfish to very low temperatures for about twenty minutes to anesthetize

them for the dissection, we exposed the superficial extensor muscle of the crayfish. We put the tail and the muscle of the crayfish in a dish with 100mL of normal Ringer's solution (See Table 1). We changed the Ringer's solution every 15min to keep the tissue alive for the length of time necessary to complete the study. We then repeated this process for the three other crayfish that we studied.

Chemical	Concentration (mM)
NaCl	205
KCL	5.4
$CaCl_2$	13.5
$MgCl_2$	2.6
Tris Buffer	10

Table 1. Composition of Ringer's solution.

Experiment

Using a fire polished microelectrode as our stimulating electrode, we stimulated various nerve cells by capturing a nerve bundle and passing electrical current through it between a reference electrode and the stimulating electrode, to elicit excitatory post-synaptic potentials (EPSPs). We recorded these EPSPs using standard intracellular recording techniques, a microelectrode filled with 3M KCL connected to a voltage-measuring device. These electrodes were made by pulling 1.2 mm capillary tubing, and each microelectrode had between 5MΩ and 20MΩ of resistance (Green et al. 2005). These readings were displayed and collected on a MacLab station. In our lab notebooks we recorded the time, voltage inside the cell, and voltage applied to elicit an action potential. To reduce variability between individuals, we took a control recording from each crayfish studied before applying the chemical blockers.

Manipulation

In each manipulation we added a specific calcium channel blocker (CCB) into the solution of Ringer's. We used three CCBs: cadmium, Nitrendipine, and ω-conotoxin. We used ~10uM concentrations of cadmium and Nitrendipine as per earlier research in the subject, and a concentration of ~0.6uM for ω-conotoxin. Nitendipine is insoluble in water and first had to be dissolved in 10 uM DMSO. The dilution factor was 1:1000, so 0.1 % of the final solution was composed of DMSO. To see the change, recordings were then taken at five minute intervals from the addition of the blocker.

Wash

After these readings were taken, we replaced the solutions with normal crayfish Ringer's in an attempt to reverse the effects of the chemical. Once again, readings were taken at five minute intervals from the addition of the new solution. Each time a solution was changed, we kept the same nerve and muscle cell, so the readings for each crayfish would come from the same muscle and nerve cell; this was done to cut down on variability between muscle and nerve cells.

Data Analysis

In each manipulation we took a control reading using the same nerve and muscle cell as the manipulation to cut down on variability between individual crayfish and individual cells. Thus, when analyzing our data, we first compared our data to its control by subtracting the average EPSP amplitude at each level from the average of the control. We also performed t-tests to determine the significance of these differences.

RESULTS

We tested the effect of various calcium channel blockers on EPSP amplitude in the crayfish neuromuscular junction. Our findings indicate that after five minutes cadmium proved to be effective in blocking calcium channels. In fact, cadmium showed the greatest decrease in EPSP amplitude relative to the control while the chemical was in the solution as shown in Figure 1. From this decrease after five minutes, the crayfish in the cadmium solution showed a significant increase in EPSP amplitude after the wash ($p<0.05$).

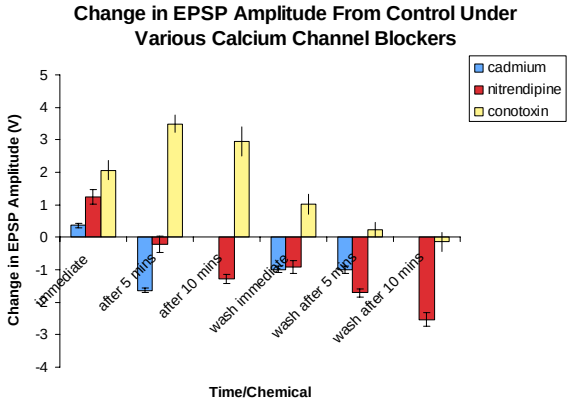

Figure 1. Average change in EPSP amplitude from the control under various calcium channel blockers. Average changes in EPSP amplitude resulting from cadmium are shown in blue, from nitrendipine shown in red, and ω-conotoxin shown in yellow. Error bars represent the standard error of the mean. With ω-conotoxin, significant increases in EPSP amplitude compared to the control occurred when it was initially added ($n=5$, $p<0.01$), after five minutes ($n=10$, $p<0.01$), and after ten minutes ($n=8$, $p<0.01$). With nitrendipine, the immediate reading is significantly greater than the control ($n=9$, $p<0.05$), after 5 minutes, the change in EPSP amplitude is not significant, after 10 minutes, a significant decrease in EPSP amplitude is observed ($n=12$, $p<0.01$). Although the immediate cadmium reading gives no significant change, the reading after 5 minutes is significant ($n=6$, $p<0.01$). With both post-cadmium washes, we see a significant rise in EPSP amplitude as compared to the reading with cadmium ($n=5$, $p<0.01$).

DISCUSSION

The results for our experiment on the effect of cadmium, ω-conotoxin, and Nitrendipine on the EPSPs at the crayfish neuromuscular junction showed that the EPSPs decreased from the application of both Nitrendipine and cadmium, but increased for ω-conotoxin. Therefore, our hypothesis that ω-conotoxin would have an effect most similar to cadmium was incorrect; in fact, ω-conotoxin's effect on the EPSPs was opposite than what we hypothesized it to be—instead of decreasing the EPSP amplitude, ω-conotoxin increased it.

These results are in stark contrast to those of Rivier et al. (1986), who found 50% calcium channel inhibition in chick brains at a ω-conotoxin concentration of 10^{-8} M. Differences in the test animal used and the time allowed for the ω-conotoxin to act on the EPSPs could explain some differences in our results from previous research, however. We were constrained by time and material limitations, and could not let the solution sit for 15 minutes in our preparation, as Rivier et al. (1986) did.

We might also have been seeing examples of compensation, however. Ghijsen et al. (2003) discovered that when nearly all P/Q-type channels were blocked in mice by a gene mutation, the N-type channels fully compensated for this loss. When we blocked the N-type channels, perhaps other channels took over the regulation of calcium influx and allowed EPSPs to continue forming with even greater amplitudes. If what we found were an example of compensation, however, the time frame in which we witnessed it would be significantly shorter than the time frame in which Ghijsen et al. (2003) witnessed compensation.

Another possible explanation is simply that the ω-conotoxin we used was too old to be effective. The sample we used was not fresh and had been sitting in the freezer for a few months before we used it.

In addition to what we found with ω-conotoxin, there were some interesting trends with the Nitrendipine. After the wash, the average EPSP amplitudes from the subjects applied with Nitrendipine continued to drop. This unexpected result is most likely due to either the cells in the crayfish beginning to die because the solution was getting too old, or because the wash did not manage to get all of the Nitrendipine out of the dissection.

On the whole, our research demonstrates that there are different types of calcium channels in the crayfish neuromuscular junction supporting neurotransmitter release, and that different pharmacological conditions have effects on the EPSP amplitudes. However, why blocking N-type channels raised the average amplitude of the EPSPs and presumably helped in neurotransmitter release remains an important unanswered question in our research. Further research could look into the results we obtained with ω-conotoxin to discover if the results do truly point to compensation, or simply to a lack of time spent bathing the crayfish in the solution. Instead of measuring only EPSPs, finding a way to actually measure the influx of calcium into the cell would also be useful.

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