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2-Butoxyethanol and Dimethyl Sulfoxide influence potentiation by changing the efflux of Calcium from the presynaptic terminal in the crayfish neuromuscular junction

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ABSTRACT

Past research by Mayahara *et al.* (1982) has shown that 2-butoxyethanol increases and dimethyl sulfoxide (DMSO) decreases the efflux of calcium from the sarcoplasmic reticulum, which releases calcium when the voltage changes across the membrane. Based on this result, we hypothesized that, when applied extracellularly, those two chemicals would have the same effect on the outer cell membrane and therefore would influence potentiation. We maintained high-potassium depolarization in the crayfish neuromuscular junction and measured excitatory post-synaptic potentials (EPSPs) in each experiment with 2-butoxyethanol and DMSO. Our results show that 2-butoxyethanol decreases and DMSO increases potentiation during high-potassium depolarization, which suggests that our hypothesis is true.

INTRODUCTION

Calcium plays an important role in synaptic transmission. It rapidly enters the presynaptic terminal when the action potential arrives and causes the release of neurotransmitter. When there is a higher influx of calcium, more neurotransmitter is released and larger excitatory post-synaptic potentials (EPSPs) result. It has already been shown by Mayahara *et al.* (1982)that 2-butoxyethanol increases and DMSO decreases the efflux of calcium out of the sarcoplasmic reticulum (SR). We wanted to examine whether 2-butoxyethanol and dimethyl sulfoxide have the same effect on the cell membrane as on the SR and consequently influence potentiation in the crayfish neuromuscular junction. Wojtowicz *et al*. (1983) reported that potentiation can be caused by maintaining the low-level depolarization. Based on the results from the experiments by Mayahara *et al*. (1982) and Wojtowicz *et al*.. (1983), we predicted that, if 2-butoxyethanol and DMSO were applied extracellularly during low-level depolarization, they would have a stronger effect on the cell membrane than on the organelles. In our control experiment, high-potassium depolarization caused potentiation, presumably by building up residual calcium. We hypothesized that in a 2-butoxyethanol solution we would not observe as much potentiation as in the control and that we would observe the opposite results in the solution with DMSO. As predicted, our results show that 2-butoxyethanol reduces potentiation while dimethyl sulfoxide increases it.

MATERIALS AND METHODS

We made micropipettes from 1.2mm glass capillary tubes using the PUL-1 microelectrode puller. We filled each micropipette with a 3M KCl solution to serve as a conductor and placed it in a microelectrode holder filled with the same solution. We then fixed the holder with the

microelectrode in it on the microelectrode manipulator. We made sure that the resistance of the microelectrodes was between 5-10 MΩ before we started the experiment.

We cooled the crayfish in the ice for 15 minutes, cut the tail and removed the dorsal section of it. We took out the gut and placed the dorsal shell in a Sylgard-lined preparation dish. Then we plugged the microelectrode and the stimulating electrode in one of the segments of the tail. We took all of the measurements in the lateral muscles because the microelectrode was pushed out of the cell by muscle contractions when we tried to take measurements in the medial muscle. The pH of all solutions was always kept constant at 7.4 throughout the whole experiment. The duration of the stimulus was always 5 ms and the frequency was 1pps. We created the low-level depolarization by increasing the concentration of potassium in the bathing solution.

In our control experiment, we used a control solution of normal crayfish saline solution (5mM K), and an experimental solution, made up of normal crayfish saline solution with a high concentration of potassium (15 mM K). We prepared the 15mM potassium solution by taking 90ml of the normal crayfish solution and 10ml of 100mM of KCl. We measured EPSPs in the control solution, then in the experimental solution and one more time, in the control solution. After changing from the control to the experimental solution, we waited for half an hour to let the high potassium affect the muscle before we took the measurements. We used a stimulus that was between 15-58V to get EPSPs for this experiment.

In the second experiment we used the solution of 0.1% 2-butoxyethanol. To make the solution, we mixed 99.9ml of the normal crayfish saline solution with 0.1ml of 2-butoxyethanol, using the stir plate. We added the solution to the preparation dish, waited for 5 minutes and then measured EPSPs. After that, we measured EPSPs in the solution of 0.1% 2-butoxyethanol with a high concentration of potassium and in the 0.1% 2-butoxyethanol solution again. We prepared the solution of 0.1% 2-butoxyethanol with high concentration of potassium by adding 0.1ml of 2-butoxyethanol to 99.9ml of 15mM KCl solution. The range of the stimulus for this experiment was 20-100V.

In the third experiment, we measured EPSPs in 0.1% DMSO solution, which was prepared by mixing 99.9ml of normal crayfish saline solution and 0.1ml of DMSO on the stir plate. Then we measured EPSPs in the solution of 0.1% DMSO with high potassium and in the solution containing 0.1% DMSO again. We prepared the DMSO high potassium solution in the same way as the 2-butoxyethanol high potassium solution. The range of the stimulus we used for this experiment was 60-100V.

RESULTS

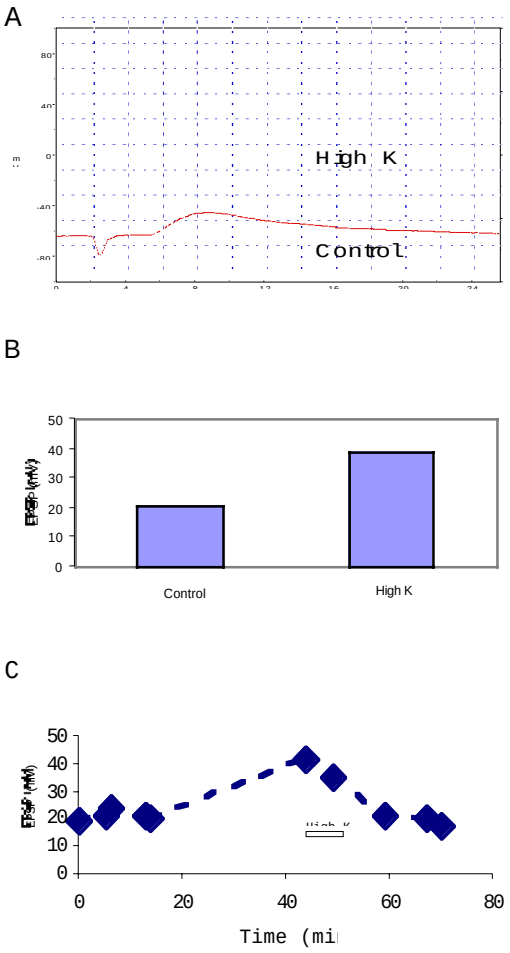

Figure 1. Potentiation of EPSPs by high potassium in the experiment with the normal crayfish saline solution. (A) Effect of high potassium depolarization on the EPSP compared to the EPSP in the experiment with the normal crayfish saline solution.. Note that the peak of the EPSP is markedly increased in the experiment with high-potassium solution. It indicates low-level depolarization. (B) Mean of the amplitude of the EPSP in the experiment with the normal crayfish saline solution compared to one in the experiment with the high-potassium solution. (C) Time course of the peaks of the EPSP in the experiment with the normal crayfish saline solution and with the high-potassium solution. The peaks during high-potassium depolarization are increased significantly and returned quickly to the original values.

In our control experiment, the resting membrane potentials were between –75 and –46 mV. The range of the EPSPs was 20.3 ± 1.7 mV for the control solution, but after using high-potassium depolarization, the range was 38.5 ± 4.9 mV.

Fig.1(A) shows the peaks of the EPSPs from the control and the experiment with high concentration of potassium. The EPSP increased by 88% in the high-potassium experiment compared to the control [Fig.1(B)]. The graph in Figure 1(C) depicts the time course over which the change in EPSPs occurred in the control solution and the experimental solution. It shows that after changing to the high potassium solution, the peaks of the EPSP nearly double and quickly return to the original amplitude when the control saline is returned to the bath.

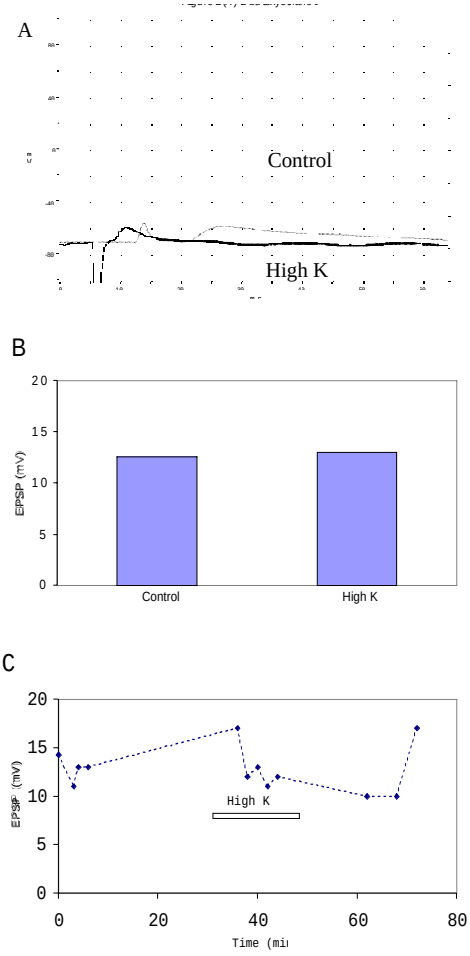

Figure 2. Potentiation of EPSPs by high potassium in the solution with 2-butoxyethanol. (A) Effect of high potassium depolarization on the EPSP compared to the EPSP in the experiment with the normal crayfish saline solution. (B) Mean of the amplitude on the EPSP in the experiment with the normal crayfish saline solution compared to one in the experiment with the high-potassium solution. (C) Time course of the peaks of the EPSP in the experiment with the normal crayfish saline solution and with the high-potassium solution.

The range of the EPSPs in our experiment with 0.1% 2-butoxyethanol was 12.6 ± 2.7 mV for the control and 13.0 ± 2.3 mV for the potentiation experiment. These results suggest that 2-butoxyethanol decreases the amplitudes of EPSPs compared to the EPSPs in our first experiment. Fig 2(A) shows the peaks of EPSPs from our 2-butoxyethanol control solution and high-potassium 2-butoxyethanol solution. The average peak of the EPSPs increases by 3.17% after low-level depolarization [Fig. 2(B)]. Fig. 2(C) shows that although the EPSP amplitude fluctuated over time, there was only a small change that could be correlated with the high K treatment.

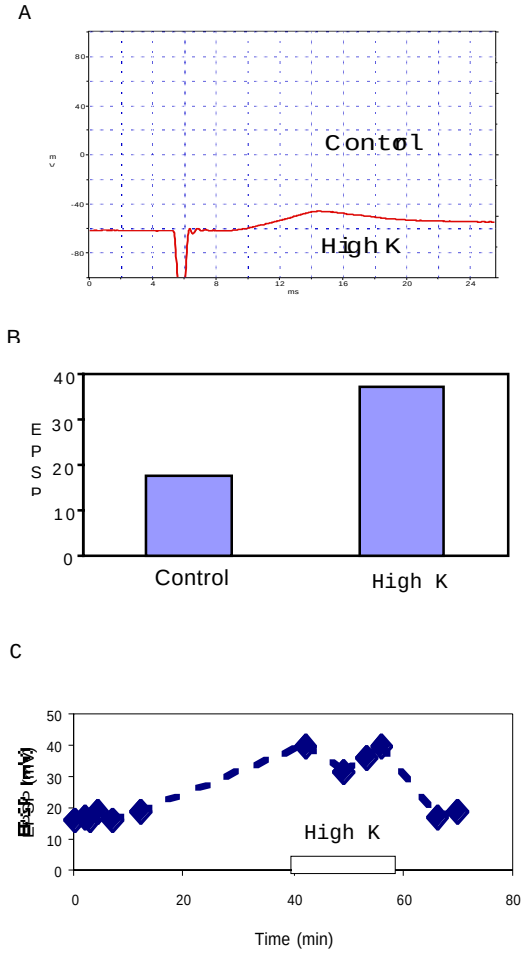

Figure 3. Potentiation of EPSPs by high potassium in the solution with dimethyl sulfoxide. (A) Effect of high potassium depolarization on the EPSP compared to the EPSP in the experiment with the normal crayfish saline solution. (B) Mean of the amplitude on the EPSP in the experiment with the normal crayfish saline solution compared to one in the experiment with the high-potassium solution. (C) Time course of the peaks of the EPSP in the experiment with the normal crayfish saline solution and with the high-potassium solution.

Fig. 3(A) gives an example of a normal EPSP in the DMSO control solution and in the high-potassium DMSO solution. In this experiment, the range of the EPSPs for our control was 17.6 ± 1.4 mV and that for the solution with high potassium was 37.0 ± 3.8 mV. We also got some EPSPs during high K depolarization that seemed to be high enough to create a spike of an action potential. We ignored the higher peak of the spike and measured EPSPs from the lower peak. The average difference between potentiated EPSPs and normal EPSPs was 19.6mV [Fig. 3(B)], which is an increase of 113% of potantiated EPSPs compared to normal EPSP in this experiment with DMSO. Figure 3(C) supports that the EPSP increases during high-potassium depolarization.

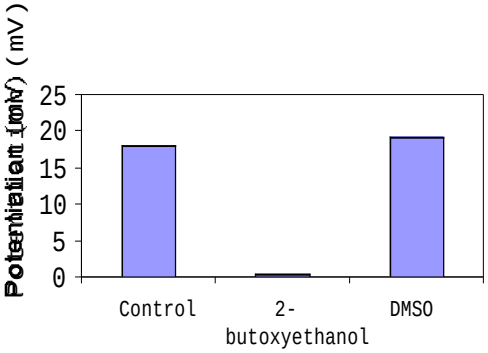

Figure 4. The difference between the means of the EPSPs in control experiments and high-potassium depolarization. The differences were calculated for the three experiments: with 2-butoxyethanol and with DMSO.

Fig. 4 is the comparison of the differences of the means of EPSPs from the control and the high-potassium depolarization in each trial with the normal crayfish saline solution, with 0.1% 2-butoxyethanol and with 0.1% DMSO. This graph suggests that,

compared to the data from the control with normal saline solution, the amount of high K potentiation is markedly smaller in the experiment with 2-butoxyethanol and is higher in the experiment with DMSO.

DISCUSSION

Our results support our prediction that 2-butoxyethanol and DMSO do not only affect the efflux of calcium from the sarcoplasmic reticulum as reported by Mayahara *et al.* (1982) but that they also have the same effect on the cell membrane. Potentiation is induced by an increase of residual calcium in the presynaptic terminal, as has been shown in earlier research (Kandel, E.R. 1981; Katz, B. and Miledi, R. 1986). Since the application of 2-butoxyethanol prevented potentiation, which is normally caused by low-level depolarization (Wojtowicz, J.M. and Atwood, H.L. 1983), 2-butoxyethanol presumably increased the efflux of calcium from the presynaptic terminal. DMSO had the opposite effect and therefore presumably decreases the efflux of calcium from the presynaptic terminal.

It is essential to use low concentrations of DMSO. When we used high concentrations of DMSO—20% as reported by Mayahara *et al.* (1982)—we were not able to measure EPSPs because in high concentrations DMSO causes muscle relaxation (Mayahara *et al.*, 1982). When DMSO was used in concentrations of more than 0.1%, its effect of decreasing the efflux of calcium from the sarcoplasmic reticulum was increased and therefore we could not observe any EPSPs.

We suggest that the two chemicals may be useful for studying potentiation and other kinds of plasticity, which depend on calcium. It may also be useful to study the level of toxicity of the chemicals in order to know if it is possible to inject them into a living animal. If they are not harmful, future research can show whether potentiation, which, as our research has shown, can be surpressed with 2-butoxyethanol, is the mechanism responsible for learning. Future research is also needed to show how 2-butoxyethanol and DMSO effect the cell membrane. Since our research shows the effects of 2-butoxyethanol and dimethyl sulfoxide on the crayfish neuromuscular junction, the crayfish may be an ideal model for future research of the interaction of these two chemicals on potentiation and Ca-dependent plasticity.

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