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Nitric Oxide inhibition shortens long-term facilitation at the neuromuscular junction of the crayfish.

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ABSTRACT

This experiment examined the effects of nitric oxide on long-term facilitation in crayfish tail extensor muscle cells. I expected that a 10 second period of high frequency neuron stimulation would have the effect of increasing excitatory postsynaptic potentials in a control condition, but that such an effect would not occur in a condition in which endogenous nitric oxide production was inhibited with the chemical L-NAME. I stimulated a crayfish motor neuron and measured postsynaptic potentials in the tail extensor muscle cells in a saline solution in order to determine the effects of nitric oxide inhibition on long-term facilitation. Although the addition of a nitric oxide inhibitor did not eliminate the effect of high frequency stimulation, it did shorten the duration of long-term facilitation in my experiment. Further research into the role of nitric oxide in long-term facilitation is warranted.

INTRODUCTION

Long-term facilitation (LTF) is a phenomenon in which excitatory postsynaptic potentials (EPSP) in a cell increase following a period of high frequency stimulation of a presynaptic neuron at frequencies above 10 Hz (Wojtowicz and Atwood, 1988). Previous studies have found that a strong but quickly decaying effect is observable for 10-20 minutes after stimulation, and then decay occurs slowly with higher than baseline EPSPs occurring for anywhere from hours to a day (Lnenicka and Atwood, 1985, as cited in Wojtowicz and Atwood, 1988). Many studies have looked at the similar phenomenon of long-term potentiation (LTP), which is the name given to LTF when it occurs in the mammalian hippocampus or cerebellum. LTP has been most extensively examined in the CA1 region of the mammalian hippocampus, suggesting that these phenomena are involved in neural plasticity, most notably learning and memory (Malenka and Bear, 2004; Szabadits et al., 2007; Arancio et al., 1996). The underlying mechanism of LTF was once thought to be sodium dependent with calcium playing a role (Wojtowicz and Atwood, 1988), but studies into LTP have produced evidence for a more complicated process, and have implicated nitric oxide (NO) as having a role (Arancio et al. 1996).

Numerous studies have identified NO as a neuromodulator in the nervous system (Aonuma, Nagayama, and Takahata, 2000; Lindgren and Laird, 1994; Thomas and Robitaille, 2001). Much of the research on NO has focused on synapses at the neuromuscular junction due to the ease of observations and measurement, and the results suggest that NO plays an inhibitory role in the release of neurotransmitter at the presynaptic cell (Lindgren and Laird, 1994; Zefirov et al., 2002). One model species on which research at the neuromuscular junction is often conducted is the crayfish, including research on NO (Aonuma, Nagayama, and Takahata, 2000). However, to my knowledge, the effect of NO on LTF has never been explored at the crayfish neuromuscular junction. Research into the role of NO in LTF has important implications for the neuroplasticity of mammalian brains, and is therefore of great interest to the general scientific community.

In order to explore the effects of NO on LTF at the crayfish neuromuscular junction, I conducted a study comparing the effects of LTF on crayfish in 5.5mM Ca2+ saline solution (approximately 40% Ca concentration of standard solution) and 5.5mM Ca2+ saline solution with an endogenous NO inhibitor, L-NAME. I measured excitatory postsynaptic potentials in exposed dorsal tail extensor muscle cells with a glass 3M KCl electrode after stimulating exposed motor neurons with a suction electrode and stimulator.

Based upon previous research, I expected to see no increase in EPSP strength following high frequency stimulation in the L-NAME condition, indicating that LTF had not occurred. The results were consistent with the hypothesis that there would not be an average increase in EPSPs following high frequency stimulation over an extended period of time, although I did observe an increase immediately following the high frequency stimulation. This observation indicates that facilitation did occur, but over a shortened period of time, suggesting a role for NO in LTF.

MATERIALS AND METHODS

Materials

The experiments were conducted with the dorsal section of a crayfish tail that had been dissected along the abdomen just above the indentation with a pair of scissors and separated from the ventral part by hand. Any ventral muscles remaining after separation were gently pushed out by thumb as to expose the dorsal tail extensor muscles, and the tail was then put into a gel bottom container, pinned down, and placed in a low calcium saline solution so that the dorsal extensor muscles were exposed for a glass electrode and suction electrode. The glass electrodes were made from thin glass tubes using a PUL-1 World Precision Instruments puller, filled with 3M KCl, and rinsed in a standard saline solution to wash off any residual KCl on the electrode. Electrical stimulations were created using the suction electrode and a stimulator. Membrane potentials were measured using the 3M KCl glass electrodes (resistance 5-20 MΩ), Scope software, and a microscope to see the electrode. The treatment solution was created using a P-200 pipette, a 100 mL graduated cylinder, 5.5mM Ca^{2+} saline solution, and L-NAME chemical. We diluted 300 µL of 1M L-NAME into 100 mL of 5.5mM Ca^{2+} saline to create a final concentration of L-NAME of 0.3mM.

Experimental Procedure

After pinning down the dissected crayfish and filling the dish with saline solution, I used the microscope to find an exposed motor neuron and sucked the neuron into the suction electrode using a syringe. I then placed our glass microelectrode in the saline solution and zeroed the software to account for junction potentials, used the microscope to find the dorsal extensor muscles, and punctured a crayfish extensor muscle cell in the same segment and side as the motor neuron. Once it was determined that the electrode was measuring a resting potential inside a cell, I stimulated the motor neuron at a frequency of .5 Hz, .6 ms delay, 11 ms duration, and varying voltages in an attempt to record an excitatory postsynaptic potential (EPSP) in the aforementioned muscle cell. Once a voltage was found to produce consistent EPSPs, I allowed the stimulator to run for approximately a minute while recording EPSPs with the Scope software to get a baseline level for EPSPs. I then switched the stimulator frequency to 50 Hz for a period of 10 seconds (high frequency stimulation period), after which we switched the frequency back to .5 Hz and resumed recording EPSPs. Every EPSP was recorded for approximately 30 seconds after high frequency stimulation, and then EPSPs were recorded every 30 seconds thereafter until it was determined that the EPSPs were unchanging.

After the EPSPs were determined to be unchanging, recordings were taken every minute for approximately 10 minutes, at which time the measurements indicated that the EPSPs had returned to the baseline level as measured in the first part of the experiment. At that point, I paused stimulation, sucked approximately half of the saline out of the dish, and refilled the dish with the L-NAME solution. I waited approximately 3 minutes to allow the L-NAME to inhibit NO production and repeated the steps taken in the first part of the experiment.

Data Analysis

Data were graphed and analyzed using Microsoft Excel software and Scope software. I determined the strength of an EPSP by measuring the change in voltage between a baseline point after stimulation and the highest voltage point thereafter. I compiled EPSP strengths into averages using Excel to compare conditions to see if the inhibition of endogenous NO production by L-NAME had any significant effects on the process of long-term facilitation.

RESULTS

The objective of this experiment was to explore the effects of NO on LTF at the crayfish neuromuscular junction by inducing an LTF condition (high frequency stimulation) and adding an endogenous NO inhibitor (L-NAME) to determine if NO inhibition would result in different EPSP strengths as compared to a control condition. Baseline strengths were determined when EPSPs were stable, and post-high frequency stimulation strengths were measured for a period of 5 minutes after the high frequency stimulation.

As shown in Figure 1, the EPSP strengths increased following a period of high frequency stimulation in the control condition. When the L-NAME chemical was added, the overall strength of EPSPs decreased, and the EPSPs decreased following the high frequency stimulation. There were not enough trials to run any significance tests.

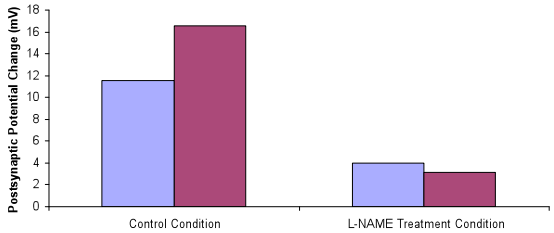
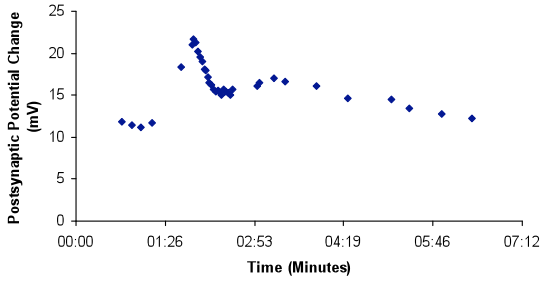


Figure 1. Excitatory postsynaptic potential strengths were measured before and after a 10 second period of high frequency stimulation. The before data represents measurements taken when postsynaptic potentials were stable at low frequency stimulation. The after data represent measurements taken in a 5 minute period after the conclusion of the high frequency stimulation.

However, as shown in Figure 2 (B), EPSP strengths in cells treated with L-NAME did increase for a short period of time following high frequency stimulation, but quickly fell to a level lower than the baseline strength. Figure 2 (A) shows a much more gradual return to baseline strength in the control solution.

(A)



(B)

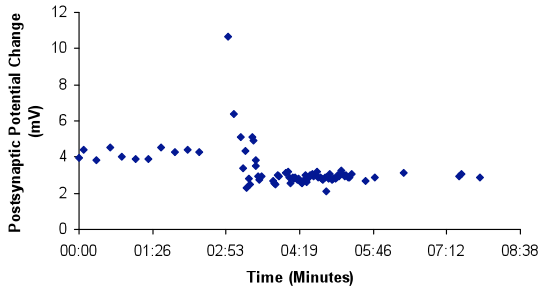


Figure 2. (A) Excitatory postsynaptic potential strengths were measured both before a period of high frequency stimulation and for a period of 5 minutes afterwards in a control condition. (B) Excitatory postsynaptic potential strengths were measured both before a period of high frequency stimulation and for a period of 5 minutes afterwards in a treatment condition with an NO inhibitor (L-NAME).

DISCUSSION

My purpose in this experiment was to explore the potential role of NO in LTF of postsynaptic potentials at the neuromuscular junction in crayfish dorsal tail extensor muscle cells. I stimulated crayfish motor neurons using an electrical stimulator and suction electrode and then measured postsynaptic potentials with glass electrodes filled with 3M KCl solution. In order to determine the effects of NO in LTF, I compared the EPSPs in a crayfish extensor muscle cell before and after inducing LTF (high frequency stimulation) in a control condition and an inhibited NO condition. The control condition seemed to be consistent with previous studies on LTF (Wojtowicz and Atwood, 1988; Arancio et al. 1996); EPSPs increased following high frequency stimulation. The results for the treatment condition partially support my hypothesis, depending on how the data is analyzed. Overall, the data supports my hypothesis that LTF would not occur when NO production was inhibited. However, a close look at the results shows us that facilitation did occur, but the effect was shorter lived and less pronounced in terms of EPSP strength than in the control condition. Additionally, there was an observable depression in EPSP strength to below baseline level within a minute after high frequency stimulation in the L-NAME condition. These observations support the hypothesis of NO having a role in LTF, suggesting that NO impacts the duration of the effect, and are consistent with previous studies on NO regarding its inhibitory role in synaptic transmission (Lindgren and Laird, 1994; Zefirov et al., 2002).

Unfortunately, very few trials were analyzed for this study. I am unable to make any claims of significance, and any trends seen in this data should be understood in this context. However, the results do provide some interesting pathways for exploration. My results suggest that the inhibition of NO had the effects of lowering the strength of all EPSPs and shortening the facilitation brought on by high frequency stimulation. Initially, the EPSPs increased in both conditions following the high frequency stimulation, but whereas the EPSP strengths fell gradually over the 5 minute period in the control condition (with NO), they fell rapidly in the NO inhibited condition, suggesting that NO may have a role not in the facilitation itself but in the lasting effect of the facilitation. It is also possible, though, that NO production was not inhibited enough by the concentration of L-NAME used in this study, and that higher concentrations of inhibitor could reveal a crucial role for NO in the occurrence of LTF and not just its duration. The results of this study provide a starting point for research on the role of NO in LTF at the crayfish neuromuscular junction. Future research using more trials, more conditions with different concentrations of L-NAME, and possibly conditions to examine the effects of the addition of exogenous NO will expand our knowledge about LTF, and could possibly lead us to valuable information about our own learning and memory processes.

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