The Effects of Protein Synthesis on Long-Term Synaptic Depression at the Crayfish Neuromuscular Junction

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ABSTRACT

Long-term synaptic depression (LTD) has been implicated as a mechanism of information storage in the human brain. We studied the role of protein synthesis on LTD at the crayfish neuromuscular junction, whose synaptic connectivity resembles the human central nervous system. Previous studies have investigated presynaptic calcium current at the phasic F3 motoneuron and suggested that Ca\(^{2+}\) influx induces a protein synthesis pathway that leads to LTD. In this study, we successfully induced LTD at the F3 neuromuscular junction by applying a 46V stimulus at a frequency of 0.6 pps for two hours. Moreover, we examined LTD by measuring the change in EPP amplitude. In addition, we attenuated LTD after exposing the crayfish preparation to cycloheximide (CHX), which inhibits protein synthesis. Our results provide evidence that protein synthesis is necessary for LTD induction and may support the theory that LTD in crayfish is presynaptic in origin.

INTRODUCTION

Long-term synaptic depression (LTD) has been implicated as a possible neural process involved in learning and memory (Linden and Connor, 1995). Depression is characterized by a decrease in neurotransmitter release, which can be measured by a reduction in amplitude of synaptic potentials. Past research has been inconclusive about the precise mechanism of LTD. For example, it has not been determined whether LTD is presynaptic or postsynaptic in origin; there are many arguments in favor of either hypothesis (Linden and Connor, 1995). Our study explored a theory established by Hong and Lnenicka (1993) that implicates protein synthesis as the intermediary between presynaptic calcium influx and LTD.

The crayfish neuromuscular junction has been extensively used in studies of synaptic transmission because its synaptic connectivity resembles the human central nervous system (Crawdad manual). In particular, the crayfish neuromuscular junction and human central nervous system exhibit similar synaptic plasticity. Therefore, research on LTD in crayfish may shed light on mechanisms of LTD in humans.

Using the crayfish model, Hong and Lnenicka (1993) have observed LTD and found that this LTD is dependent on an influx of calcium in the F3 motoneuron, an abdominal neuron that innervates the fast flexor muscle in the tail. They further studied the Ca\(^{2+}\) channels in F3 and identified an activity-dependent reduction in voltage-dependent calcium influx (Hong and Lnenicka, 1995). Although the underlying mechanism is unknown, they postulated that Ca\(^{2+}\) influx induces a protein synthesis pathway that leads to the inactivation and/or degradation of Ca\(^{2+}\) channels (Hong and Lnenicka, 1997). Hong and Lnenicka’s study implies that LTD in crayfish is presynaptic in origin. Additionally, presynaptic Ca\(^{2+}\) has been identified as a modulator of neurotransmitter release and, therefore, the effects of LTD should also be observable postsynaptically.

We studied the role of protein synthesis on LTD at the same F3 neuromuscular junction as Hong and Lnenicka. While Hong and Lnenicka (1993,1995,1997) described LTD by measuring change in Ca\(^{2+}\) currents in the presynaptic terminal, we examined LTD by measuring the change in amplitude of end plate potentials (EPPs). To investigate the importance of protein synthesis in inducing LTD, we treated the preparation with a protein synthesis inhibitor, cycloheximide (CHX). Since CHX inhibits protein synthesis and Hong and Lnenicka (1997) have shown that protein synthesis is integral to LTD, we hypothesized that the addition of CHX would effectively attenuate LTD as measured by postsynaptic EPPs.

When the F3 motoneuron was continuously stimulated for two hours under normal conditions we observed an extinction of the post-synaptic response. In contrast, the same stimulation in the presence of CHX elicited diminished but present EPPs. These results confirmed our hypothesis that CHX inhibits LTD, which supports Hong and Lnenicka’s theory and may suggest that LTD in crayfish is presynaptic in origin.
MATERIALS AND METHODS

All stimulation took place at the phasic motoneuron F3 and recording occurred at the fast flexor muscles that F3 innervates in the third abdominal segment of the crayfish.

Preparation of Crayfish Tail

Crayfish were placed in an ice bath for 15 minutes or until they stopped moving. Then the tail was isolated and dissected along the midline of the third abdominal segment. The superficial flexor musculature and cuticle were removed, exposing the underlying phasic muscle. Using fine scissors, the deep phasic muscles were also removed in order to uncover the F3 motoneuron and the fast flexor muscle that it innervates. Preparations were kept in standard crayfish Ringer solution with 10% Ca\(^{2+}\) Ringer solution for dissection and recording. Ca\(^{2+}\) levels were maintained at 10% in Ringer solution to reduce large muscle contractions which interfere with recording.

EPP Measurement

EPPs were measured intracellularly in the fast flexor muscle innervated by the F3 motoneuron using microelectrodes filled with 3 M KCl (resistance 5-10 M\(\Omega\)). It was performed using standard neurophysiological protocol as described in the Crawdad manual and analyzed using Scope v3.6.3 software. During measurement, five EPPs were averaged under both conditions in order to obtain statistically reliable results. For these measurements the F3 motoneuron was stimulated with an extracellular suction electrode. We used a minimum voltage in order to elicit a measurable EPP. The voltage varied between 10V and 100V.

Conditioning

To induce LTD, the F3 motoneuron was stimulated with an extracellular suction electrode by applying a 46 V electrical stimulus 0.11 ms in duration at a frequency of 0.6 pps for two hours. EPP measurements were taken both before and after stimulation.

Induction of LTD in F3 motoneuron in 10% Ca\(^{2+}\) Ringer solution

We successfully induced LTD at the F3 neuromuscular junction after two hours of stimulation. We observed EPPs of an average 34 mV prior to stimulation and no apparent EPPs afterwards. This reduction translated to a 100% decrease in synaptic transmission (Figure 2).

Attenuated LTD in F3 motoneuron in 10% Ca\(^{2+}\) Ringer solution with CHX

The crayfish preparation was exposed to 0.6mM CHX in Ringer solution for 4 hours prior to stimulation. We observed EPPs of an average 8.5 mV and 1.2 mV after stimulation. This reduction translated to an 86% decrease in synaptic transmission (Figure 2).

Difference in pre-conditioning EPP amplitudes

Average EPP amplitudes were taken prior to conditioning for both 10% Ca\(^{2+}\) Ringer solution and 10% Ca\(^{2+}\) Ringer solution with 0.6 mM CHX. We observed the 10% Ca\(^{2+}\) Ringer solution preparation had an average EPP amplitude 25 mV higher than that for the CHX preparation.
DISCUSSION

Our successful attenuation of LTD by application of CHX confirms our hypothesis and supports Hong and Lnenicka’s theory that the mechanism of LTD induction involves protein synthesis and may support the theory that LTD in crayfish is presynaptic in origin.

The research of Hong and Lnenicka (1997) has implicated presynaptic Ca$^{2+}$ influx into the F3 motoneuron in LTD induction. This Ca$^{2+}$ could cause synaptic change by either modifying pre-existing proteins or by altering protein synthesis. Moreover, past research has demonstrated that when CHX is applied two hours before stimulation, there is no reduction in EPP amplitude, but when CHX is applied immediately before stimulation, synaptic depression is observed (Nguyen and Atwood 1990).

Our research synthesizes the research from both Hong and Lnenicka (1997) and Nguyen and Atwood (1990). Hong and Lnenicka recorded presynaptic Ca$^{2+}$ currents and neurotransmitter release, but did not measure the post-synaptic response (1997). On the other hand, Nguyen and Atwood did measure EPPs, but used the crayfish claw preparation and not at the F3 motoneuron. We measured post-synaptic EPP responses like Nguyen and Atwood, but chose the F3 motoneuron because it, like all phasic neurons, is known for its tendency to become rapidly depressed (Nguyen et al., 1997).

The results from our study show that protein synthesis is integral to the expression of LTD at the F3-fast flexor neuromuscular junction. With a 10% Ca$^{2+}$ Ringer solution, we observed a complete extinction of EPPs after two hours of stimulation. In contrast, when bathing the preparation in 10% Ca$^{2+}$ Ringer solution with 0.6 mM CHX LTD-inducing stimulation still diminished the EPPs, but did not eliminate them entirely. These results support the hypothesis that protein synthesis contributes to LTD, but it appears as though it may not be the sole mechanism by which the synapse becomes depressed since there still was a considerable decrease in amplitude even in the presence of CHX. Another possibility is that CHX failed to stop all protein synthesis. Repeating this study with a longer CHX exposure would reveal whether or not this was the case.

The average EPP amplitude measured before the LTD-inducing stimulation was markedly diminished when the preparation was treated with CHX. These findings suggest that CHX not only attenuates LTD, but also reduces the average EPP before stimulation. This phenomenon has also been observed by Nguyen and Atwood (1990).

The limited sample size of our study is due in part to difficulties in recording. The F3 motoneuron innervates a large muscle bundle and therefore above-threshold stimulation generated large contractions of the muscle. Such contractions have been previously observed (Nguyen et al., 1997). One of our main challenges, therefore, was keeping the intracellular electrode in the muscle fiber during recording. These difficulties prevented us from recording during the LTD-inducing stimulation, and we were forced to record from multiple muscle fibers and average the EPPs observed. In order to reduce the magnitude of these muscle contractions, we lowered the Ca$^{2+}$ level to 1 mM in Ringer solution. Even with decreased Ca$^{2+}$, the muscle contractions were still strong and hindered our ability to record.

Lastly, the size of F3 required that we create
especially large diameter suction electrodes in order to stimulate the entire nerve bundle. These electrodes may not have been as uniformly shaped as the electrodes produced by an electrode puller, so the seal may have not been perfect. This imperfect seal may have been responsible for the extracellular electrode detaching from the F3 nerve bundle multiple times, compounding the difficulties in recording.

Our results illustrate the importance of protein synthesis in LTD induction. Also, these results may support Hong and Lnenicka’s theory that LTD in crayfish is presynaptic in origin. With a better understanding of the mechanisms that underlie LTD these results may help to elucidate the pathways of learning and memory. Moreover, our results were derived from a combination of techniques and preparations from a number of previous studies. In this way, our results link those of many laboratories and may help complete the understanding of the crayfish model.

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REFERENCES


