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Proteasome regulates the mediators of cytoplasmic polyadenylation signaling during late-phase long-term potentiation

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HIGHLIGHTS

- We tested the link between the proteasome and translation during L-LTP.
- We examined components of cytoplasmic polyadenylation signaling.
- A polyadenylation inhibitor blocks the increase in L-LTP by proteasome inhibition.
- Blocking Aurora-A kinase reduces the L-LTP increase by proteasome inhibition.
- CaMKII inhibition diminishes the L-LTP increase by proteasome inhibition.

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ABSTRACT

The ubiquitin-proteasome pathway is essential for long-term synaptic plasticity, but its exact roles remain unclear. Previously we established that proteasome inhibition increased the early, induction part of late-phase long-term potentiation (L-LTP) but blocks the late, maintenance part. Our prior work also showed that the proteasome modulates components of the mammalian target of rapamycin pathway for translation. In this study, we tested the possible role of the proteasome in regulating the cytoplasmic polyadenylation signaling required for translation during L-LTP. We found that a polyadenylation inhibitor cordycepin diminishes the enhancement of early L-LTP mediated by proteasome inhibition. Furthermore, blocking Aurora-A kinase and calcium-calmodulin-dependent kinase II reduces the increase in early L-LTP brought about by proteasome inhibition. Our results suggest a link between polyadenylation-mediated translational control and protein degradation during induction of long-term synaptic plasticity.

1. Introduction

Long-term synaptic plasticity, which underlies long-term memory, depends on new protein synthesis through gene transcription and translation of newly transcribed genes [1,2]. The induction of long-term synaptic plasticity in the mammalian central nervous system also requires local translation of pre-existing mRNAs in dendrites which is thought to confer synapse-specificity of plasticity [3]. Many studies in the past several years have indicated that protein degradation by the ubiquitin-proteasome pathway (UPP) has a role in long-term synaptic plasticity and long-term memory [4–6]. Previously, using hippocampal late-phase long-term potentiation (L-LTP) in the mouse as a model system, we showed that protein degradation has opposite roles with respect to L-LTP induction and maintenance [7]. We showed that proteasome inhibition enhances the induction part of L-LTP but blocks maintenance. Our results suggested that under normal physiological conditions, the proteasome limits L-LTP induction by degrading proteins that are locally translated from pre-existing mRNAs in dendrites but promotes L-LTP maintenance by facilitating transcription in the nucleus. There are two major signaling cascades that regulate local protein synthesis in dendrites. One is the signaling pathway mediated by mammalian target of rapamycin (mTOR) [8]. The second is the cytoplasmic polyadenylation signaling pathway [9]. We recently established that proteasome inhibition stabilizes the proteins whose synthesis is regulated by the mTOR pathway [10]. In this study, we tested the possible role of the cytoplasmic polyadenylation signaling pathway.

The translation of a subset of mRNAs in dendrites depends on extension of the poly-A tail in their 3′ untranslated regions, which occurs in the cytoplasm and is thus termed cytoplasmic polyadenylation [11–13]. The lengthening of the poly-A tail depends on a nucleotide sequence (typically UUUUUAU) called the cytoplasmic polyadenylation element (CPE). A protein called CPE-binding

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protein (CPEB) plays a key role in regulated polyadenylation of a subset of mRNAs and thus affects their translation [14].

CPEB plays a key role in activity-dependent local translation of mRNAs in dendrites [15]. NMDA receptor (NMDA-R)-mediated signaling regulates polyadenylation of mRNAs in dendrites [16]. NMDA-R activates Aurora-A kinase which phosphorylates CPEB. In the non-phosphorylated state, CPEB is bound by neuroguidin (or maskin in non-neuronal cells) which in turn is bound to the translation initiation factor eIF4E. The binding of neuroguidin prevents eIF4E from binding to eIF4G and since the interaction of eIF4E and eIF4G is critical for driving the translation forward, translation remains repressed. For relief of translational repression by neuroguidin, polyadenylation of the mRNAs needs to occur which is a highly regulated process. Regulation of polyadenylation requires two sequence elements in the 3′-UTR of the mRNAs, a hexanucleotide sequence AAUAAA and the CPE. When CPEB bound to the CPE is phosphorylated, it recruits cleavage and polyadnylation specificity factor (CPSF) to the nearby polyadenylation hexanucleotide. CPSF enables poly-A addition to the mRNA by poly-A polymerase. Poly-A binding protein (PABP) binds the elongated poly-A tail and recruits eIF4G. The PAPB-eIF4G dimer helps dislodge neuroguidin from eIF4E and allows the interaction of eIF4E with eIF4G which initiates translation [17]. CPEB is also a substrate for Calcium-calmodulin-dependent protein kinase II (CaMKII) [18].

2. Materials and methods

2.1. Hippocampal slice electrophysiology

We used C57/Bl6 mice obtained from Harlan Laboratories (Frederick, MD, USA) for experimental protocols approved by the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences. For L-LTP experiments we prepared transverse hippocampal sections (400 μm) from 6 to 12-week-old male mice using a tissue chopper in an oxygenated and chilled artificial cerebrospinal fluid (ACSF) as described previously [7]. All recordings were obtained from slices maintained at 32 °C. We used theta-burst stimulation (TBS) protocol to induce L-LTP. The stimulation protocol consisted of 4 pulses at 100 Hz, with bursts repeated at 5 Hz and each tetanus including 3 ten-burst trains separated by 30 s [7]. The same stimulus was repeated after 5 min. For L-LTP experiments on isolated dendrites, we separated the dendrites by means of a surgical cut to the slice which has been shown to be effective [7,19–21]. We placed the hippocampal slices in ice-cold ACSF containing sucrose instead of NaCl and $MgCl_2$ instead of $CaCl_2$ (replacement chemicals were of same molarity), and made a cut below the cell body layer of CA1 region. The slices were then placed in ACSF and allowed to recover for 2 h before the experiment. L-LTP was induced as described previously [7,22].

2.2. Pharmacological reagents

Cordycepin was purchased from R & D Systems (Minneapolis, MN, USA). MLN8237 was from Selleck Chemicals (Houston, TX, USA). KN-93 and KN-92 were obtained from EMD Millipore (Billerica, MA, USA). Stock solutions of all these reagents were prepared in DMSO and were diluted to the final concentrations in ACSF. For these reagents, the same concentration of DMSO alone was used for controls. A membrane-permeant proteasome inhibitor β-lactone (25 μM final; Cayman Chemical, Ann Arbor, MI, USA) was

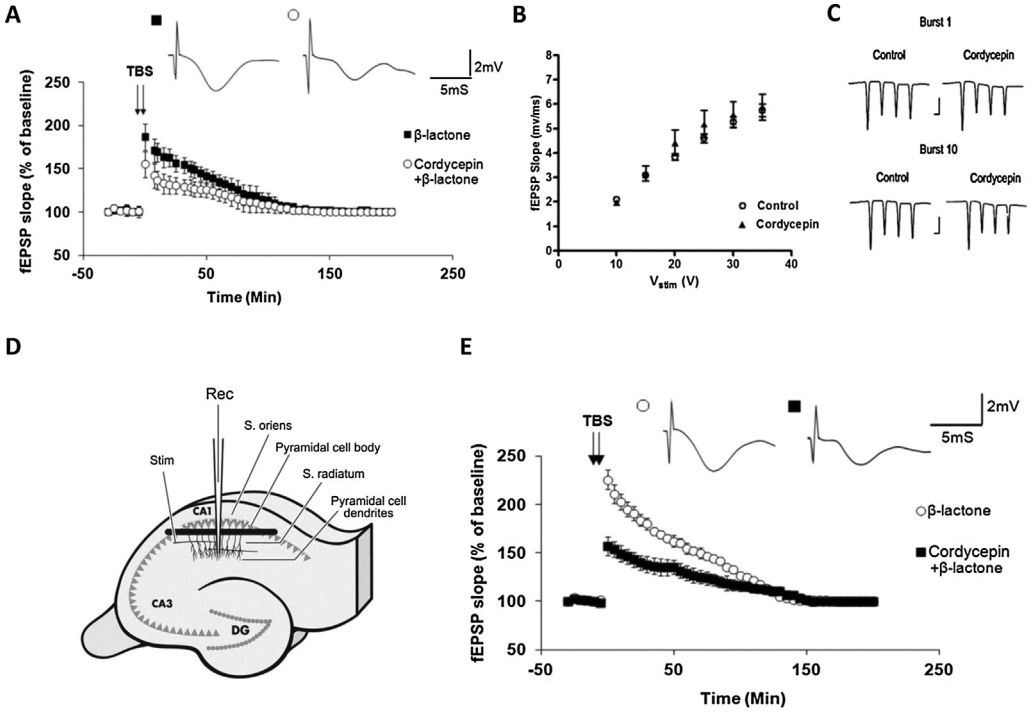

Fig. 1. Cordycepin treatment significantly decreases β-lactone-mediated enhancement of Ep-L-LTP in intact slices and isolated dendrites. (A) Incubation of intact hippocampal slices with cordycepin prior to β-lactone significantly ($P < 0.01$) reduces the extent of Ep-L-LTP. Inset: Representative traces taken at 30 min for treatment with β-lactone alone and Cordycepin + β-lactone. TBS: theta-burst stimulation. (B) Normal basal synaptic transmission in cordycepin-treated hippocampal slices ($n = 6$) compared to vehicle (DMSO)-treated control ($n = 6$) slices. The graph shows input-output curves of fEPSP slope (mV/ms) versus stimulus at the Schaffer collateral pathway upon treatment with the vehicle (Control) or cordycepin. (C) Cordycepin does not affect the pattern of theta-burst stimulation (TBS). Representative traces collected after the first burst and the tenth burst under vehicle (DMSO)-treated control hippocampal slices and cordycepin-treated slices. Calibration: 2 mV, 20 ms. (D) Schematic illustration of isolation of dendrites. A cut (black bar) is made just below the pyramidal cell body layer in the CA1 region. Recording electrode (Rec) is placed in the dendritic layer of pyramidal cells. Position of the stimulation electrode (Stim) and the layers stratum radiatum and stratum oriens are also indicated. DG: dentate gyrus (Figure modified from Dong et al 2008 and reprinted with permission from Cold Spring Harbor Laboratory Press). (E) Treatment of cut slices with cordycepin prior to β-lactone significantly ($P < 0.001$) decreases the magnitude of Ep-L-LTP in isolated dendrites. Inset: Representative traces taken at 30 min for β-lactone alone and Cordycepin + β-lactone.