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Proteasome Inhibition Augments New Protein Accumulation Early in Long-Term Synaptic Plasticity and Rescues Adverse Aβ Effects on Protein Synthesis

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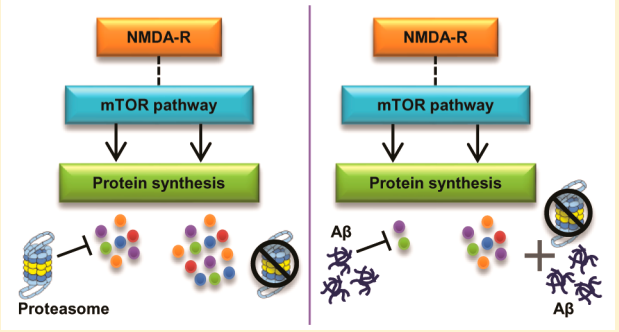
ABSTRACT: Protein degradation plays a critical role in synaptic plasticity, but the molecular mechanisms are not well understood. Previously we showed that proteasome inhibition enhances the early induction part of long-term synaptic plasticity for which protein synthesis is essential. In this study, we tested the effect of proteasome inhibition on protein synthesis using a chemically induced long-lasting synaptic plasticity (cLTP) in the murine hippocampus as a model system. Our metabolic labeling experiments showed that cLTP induction increases protein synthesis and proteasome inhibition enhances the amount of newly synthesized proteins. We then found that amyloid beta (Aβ), a peptide contributing to Alzheimer's pathology and impairment of synaptic plasticity, blocks protein synthesis increased by cLTP. This blockade can be reversed by prior proteasome inhibition. Thus, our work reveals interactions between protein synthesis and protein degradation and suggests a possible way to exploit protein degradation to rescue adverse Aβ effects on long-term synaptic plasticity.



KEYWORDS: *Translation, proteolysis, amyloid beta, mTOR, protein turnover, metabolic labeling, pulse-chase*

The central nervous system stores information through synaptic plasticity, the ability to change the strength of synaptic connections between neurons. Synaptic plasticity can be short-term and last for just a few minutes or long-term and can last up to several hours and days. Mechanistically, the two forms of plasticity are different. Short-term synaptic plasticity depends on posttranslational modifications of preexisting proteins.[1] Long-term synaptic plasticity requires translation of pre-existing mRNA for induction and new gene expression and proteins synthesized from the newly transcribed mRNA for maintenance.[2] Research during the last several years has found that protein degradation by the ubiquitin-proteasome pathway is crucial for both short- and long-term synaptic plasticity.[3−6]

Previously we showed that proteasome inhibition enhances the early induction part of long-term synaptic plasticity, whereas it blocks the late maintenance part.[7] In addition, we established that proteasome inhibition increases induction of synaptic plasticity by stabilizing translational activators such as eukaryotic initiation factors 4E (eIF4E) and eukaryotic elongation factor 1A (eEF1A).[8] We found that the blockade of maintenance is through stabilization at a later time-point of translational repressors such as polyadenylate-binding protein interacting protein 2 (Paip2) and eukaryotic initiation factor 4E-binding protein 2 (4E-BP2). All of these translational factors are part of the signaling pathway regulated by a key protein kinase called the mammalian target of rapamycin (mTOR).[9,10] These past results suggested that the interplay between protein synthesis and protein degradation played a key role during long-term synaptic plasticity. Therefore, in this investigation, we directly measured new protein synthesis and tested how protein degradation affects protein synthesis. Since synaptic plasticity is impaired in many neurodegenerative diseases such as Alzheimer's disease,[11] we also tested whether amyloid beta (Aβ), a peptide that is a key contributor to Alzheimer's disease pathology, affects protein synthesis in long-term synaptic plasticity.

To investigate the interaction between protein synthesis and protein degradation, we metabolically labeled proteins in hippocampal slices. We used a long-lasting chemically induced long-term potentiation (cLTP) as a model for long-term synaptic plasticity.[7] Electrically induced LTP modifies only a subset of synapses, and hence, detection of molecular changes in the background of many unchanged synapses is difficult. Since cLTP modifies a large proportion (if not all) of the synapses, we can readily and routinely detect molecular changes with this method. cLTP induced by cAMP analogues or reagents that stimulate cAMP production is similar to electrically induced late-phase LTP (L-LTP).[12−14] We previously rigorously established by electrophysiological recording after cLTP and by measuring induction of the CREB-inducible gene *Bdnf*, that cLTP lasts long and is equivalent to electrically induced L-LTP.[7]

We found that overall protein synthesis increased with cLTP (Figure 1B and D). Protein synthesis increases early in cLTP with a peak at around 60 min followed by a decline in protein synthesis (cLTP, 60 min: 260.7% ± 21.6% relative control taken

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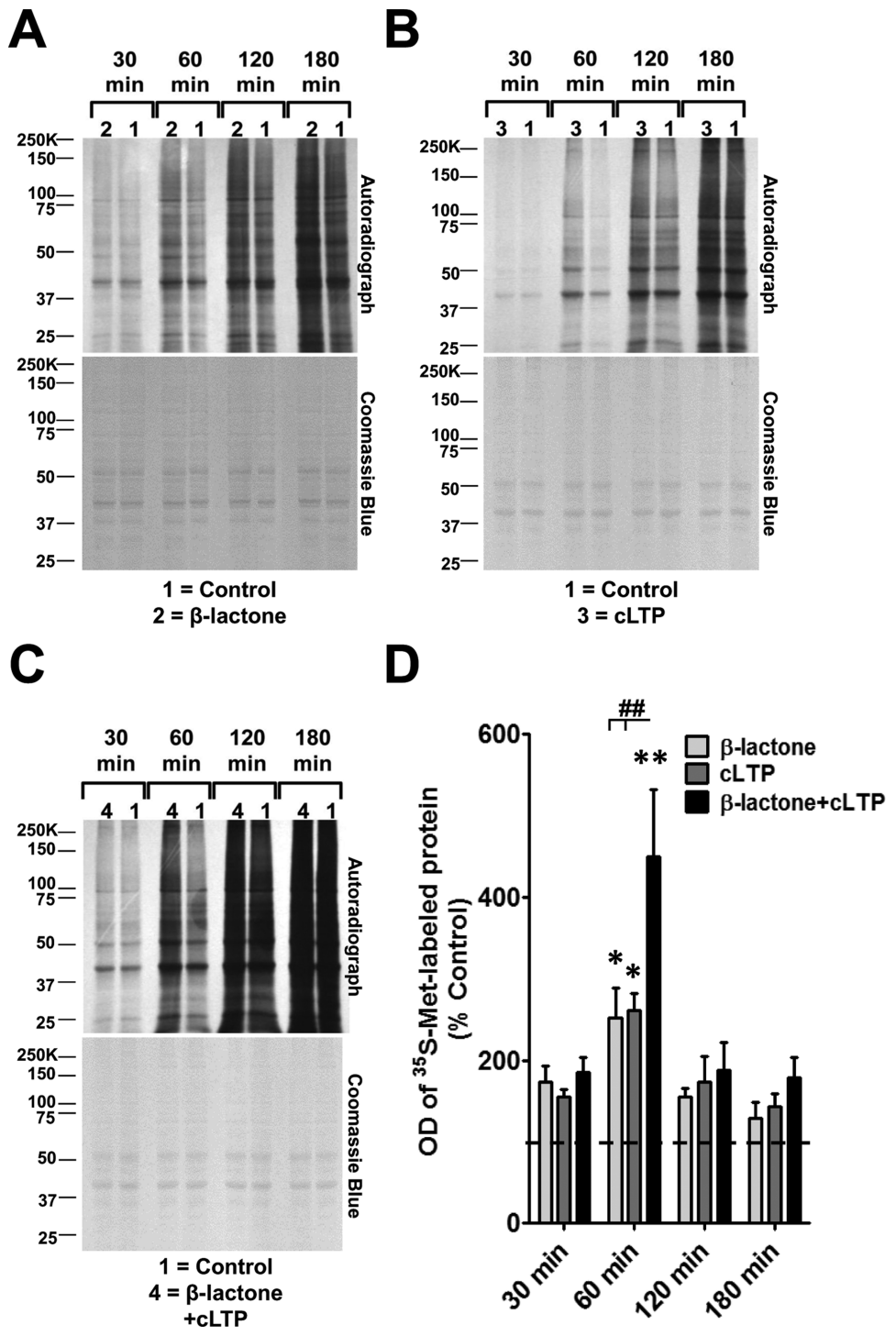

Figure 1. Overall protein synthesis increases during cLTP and newly synthesized proteins are stabilized by proteasome inhibition. Incorporation of ^{35}S-methionine into proteins with no treatment (Control), with pretreatment with β-lactone (A, top), with cLTP induction (B, top), and with cLTP induction after pretreatment with β-lactone (C, top). The gels stained with Coomassie Blue (bottom panels in A−C) were used as loading controls. Quantification of ^{35}S-methionine incorporation into proteins shows that when β-lactone is used prior to cLTP induction, proteins are stabilized and overall protein synthesis significantly increases at the peak time-point of 60 min (C, D). (*$p < 0.05$ compared to time-matched control, **$p < 0.01$ compared to time-matched control, $^{\#\#}p < 0.01$ between experimental groups, $n = 6$, one-way ANOVA, Tukey posthoc test.)

as 100%; $p < 0.05$) (Figure 1D). The decline in new protein synthesis at later time points might be due to accumulation of translational repressors.[8] The increase in protein synthesis during cLTP is greatly enhanced with incubation of the hippocampal slices with the proteasome inhibitor β-lactone for 30 min prior to cLTP induction (β-lactone + cLTP: 449.1% ± 83.4% vs cLTP, 60 min: 260.7% ± 21.6%; $p < 0.01$). Although incubation with β-lactone alone increased the quantity of new proteins (Figure 1A and D), the extent of protein synthesis with cLTP induction after preincubation with β-lactone was much greater than the protein synthesis with cLTP alone and β-lactone alone (β-lactone alone: 252.4% ± 36.4% vs β-lactone + cLTP:

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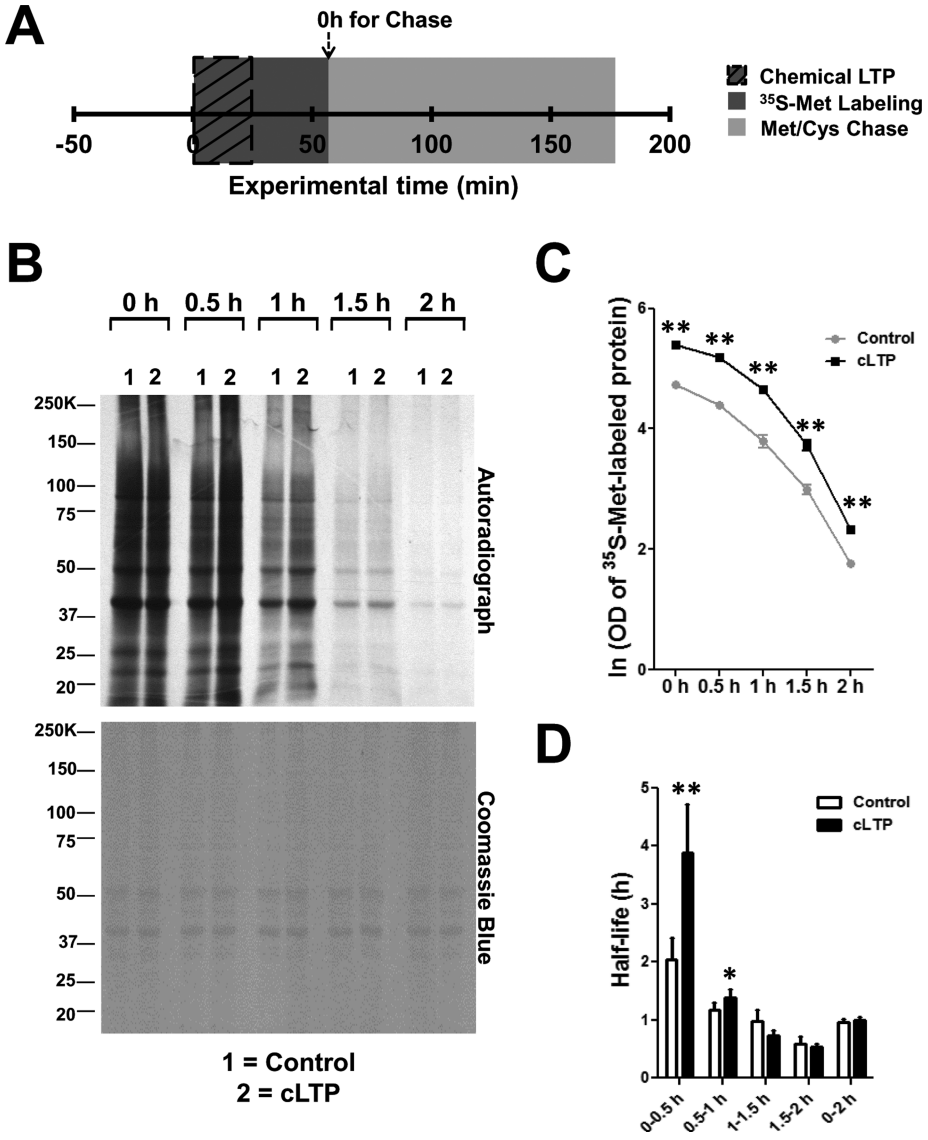

Figure 2. Pulse-chase experiment with or without cLTP. Schematic outline of the experiment (A). Decay of ^{35}S-methionine-labeled proteins after a 1 h pulse under no treatment (Control) or with cLTP induction (B top, C). The gel stained with Coomassie Blue (bottom panel in B) was used as loading control. Total labeled protein during chase after cLTP is increased at all time points up to 2 h after the end of pulse compared to control (C). Calculation of half-lives at each 30 min segment shows significantly longer half-lives during the first and second 30 min segments (D). (*$p < 0.05$ compared to time-matched control, **$p < 0.01$ compared to time-matched control, $n = 5–7$, one-way ANOVA, Tukey posthoc test for C and paired t tests for D.)

449.1% ± 83.4%; $p < 0.01$) (Figure 1C and D). It is likely that preincubation with β-lactone increases a different but overlapping subset of proteins compared to those synthesized during cLTP, and induction of cLTP leads to synthesis of specific plasticity-related proteins such as synaptic tags.[4]

Next, we sought to test the effect of cLTP induction on protein turnover. To do this, we radiolabeled the proteins with a pulse of ^{35}S-methioinine and chased them with unlabeled methionine and cysteine (Figure 2A). We started the chase procedure after the amount of newly synthesized proteins reached its peak value at 60 min and compared the fate of pulse-labeled proteins (after the chase) with or without cLTP induction. We found that the amount of radiolabeled proteins increased following cLTP relative to controls at all time-points measured (Figure 2B and C). In addition, using the decay rate represented in Figure 2C, we determined that the half-life of proteins was only slightly increased in cLTP (0.99 ± 0.07h) relative to controls (0.96 ± 0.05 h) when the decay during the entire 2 h chase was taken into consideration. In protein half-life experiments, when the decay of proteins is analyzed using a semilogarithmic plot, generally a straight line is observed. In our data analysis, however, "inflection points" were seen during the first 30 min and the second 30 min. These inflection points are thought to indicate distinct processes driving the rate of decay.[15] Therefore, we analyzed the half-life by dividing the 2 h time course into 30 min segments and found that, during the first 30 min, the half-life with cLTP was 3.88 ± 0.83 h as compared to 2.04 ± 0.38 h under control conditions ($p < 0.01$). In the second 30 min segment, the half-life with cLTP was 1.38 ± 0.15 h as compared to 1.18 ± 0.13 h under control conditions ($p < 0.05$). After the first hour, the rate of decay of proteins under cLTP conditions accelerated and was not significantly different from that in the control conditions (1−.5 h: Control 0.97 ± 0.20, cLTP 0.74 ± 0.09; 1.5−2 h: Control 0.59 ± 0.11, cLTP 0.53 ± 0.06) (Figure 2D). These results suggest that cLTP-inducing stimuli reduce the rate of degradation initially but the degradation rate increases at later time points,

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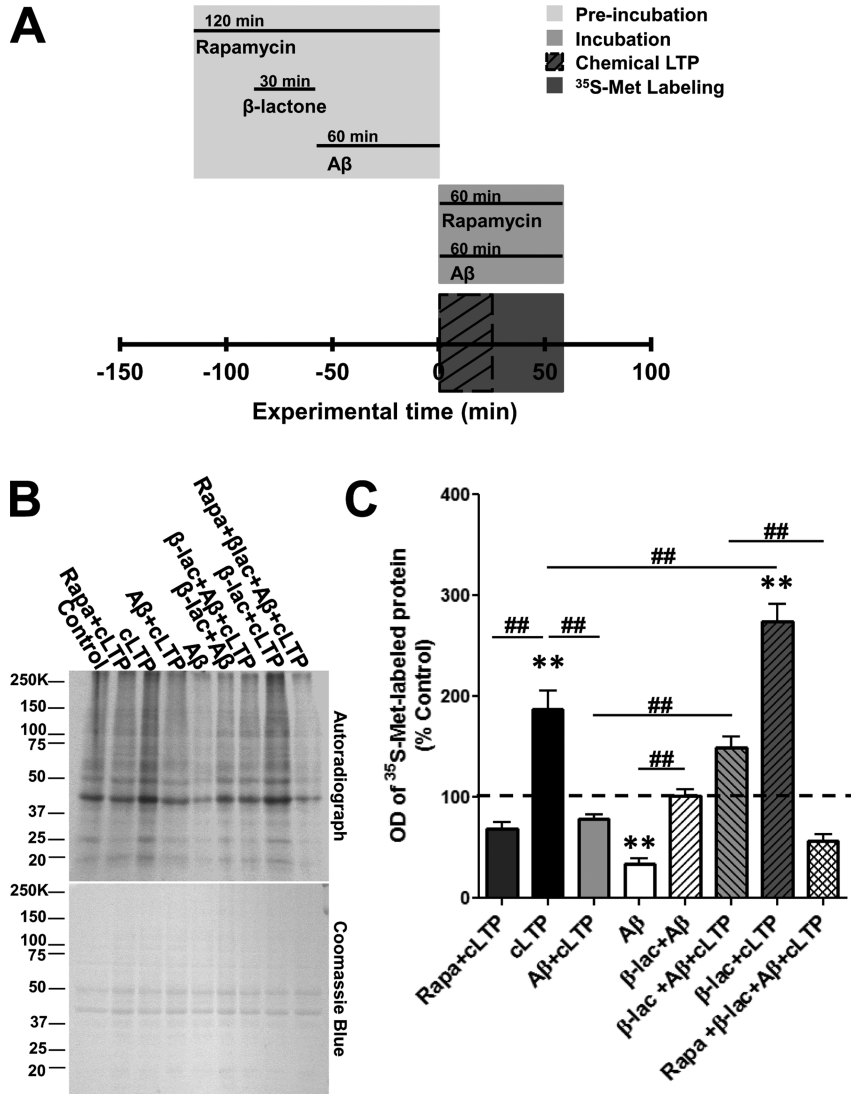

Figure 3. Exposure to Aβ oligomers decreases overall protein synthesis, and proteasome inhibition prevents this decrease. Schematic representation of the incubation protocol for the experiments (A). Aβ oligomers decrease protein synthesis in both basal and cLTP conditions (B top, C). The gel stained with Coomassie Blue (bottom panel in B) was used as loading control. Preincubation with β-lactone overcomes the adverse effect of Aβ under basal as well as cLTP conditions. The restoring effect of β-lactone on protein synthesis is blocked by rapamycin (C). (*$p < 0.05$ compared to time-matched control, **$p < 0.01$ compared to time-matched control, $^{\#\#}p < 0.01$ between experimental groups, $n = 6-10$, one-way ANOVA, Tukey posthoc test.)

although definitive proof of this concept will have to await future confirmative studies.

The molecular pathways underlying long-term synaptic plasticity are often perturbed in neurodegenerative diseases. In Alzheimer's disease, synaptic dysfunction precedes overt brain pathology.[11] A key contributor to Alzheimer's disease pathology is the amyloid β (Aβ) peptide. Specifically, oligomers of Aβ are thought to have a role in the cognitive dysfunction seen in Alzheimer's disease patients.[16] This observation is replicated in animal models of Alzheimer's disease as well.[17,18] Previous studies showed that Aβ affects several signaling pathways critical for synaptic plasticity including dysregulation of the mTOR pathway.[19,20] Even though such studies hinted at impairment of protein synthesis in Alzheimer's disease, there has been no direct evidence to link any of the Alzheimer's disease pathology to possible alterations in protein synthesis. Therefore, we tested the effect of Aβ oligomers on protein synthesis in hippocampal slices.

Our experiments showed that Aβ oligomers significantly reduced basal protein synthesis (Aβ: 33.8% ± 5.5% relative to control taken as 100%, $p < 0.01$) as well as the enhancement in protein synthesis observed with cLTP (Aβ + cLTP: 78.9% ± 3.6%; cLTP: 186.6% ± 19.2%; relative to control taken as 100%, $p < 0.001$) (Figure 3). Thus, the adverse effect of Aβ on long-term synaptic plasticity might be due to prevention of protein accumulation needed during the induction phase. If this were so, could this adverse effect be prevented by blocking protein degradation by the proteasome? To test this idea, we incubated the hippocampal slices with β-lactone before treatment with Aβ oligomers. We found that pretreatment with β-lactone restored the level of protein synthesis observed with cLTP (cLTP: 186.6% ± 19.2% vs β-lactone + Aβ + cLTP: 149.3% ± 10.2%; $p > 0.05$). Pretreatment with β-lactone also overcomes the adverse effect of Aβ on basal protein synthesis and brings it back to control levels (β-lactone + Aβ: 101.2% ± 6.1% relative to control taken as 100%; $p > 0.05$). Since our previous work suggested that proteasome inhibition stabilizes proteins synthesized through the mTOR pathway,[8] we tested whether the new protein synthesis in cLTP is sensitive to inhibition by rapamycin. We

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found that rapamycin significantly blocked cLTP-induced protein synthesis (rapamycin + cLTP: 68.6% ± 6.6% vs cLTP: 186.6% ± 19.2%, $p < 0.001$). Next, we tested whether rapamycin had an effect on the ability of β-lactone to prevent the decrease in protein synthesis caused by Aβ oligomers. We found that rapamycin greatly diminished the corrective effect of β-lactone on Aβ-mediated blockade of protein synthesis (rapamycin + β-lactone + Aβ + cLTP: 57.2% ± 6.4% vs β-lactone + Aβ + cLTP: 149.3% ± 10.3%; $p < 0.001$) (Figure 3). These data indicate that proteasome inhibition overcomes the deleterious effect of Aβ oligomers on protein synthesis by stabilizing the proteins synthesized through the mTOR pathway.

Our results suggest that protein degradation limits the extent of new protein synthesis during induction of long-term synaptic plasticity. This is exemplified by the fact that cLTP induction causes an increase in new protein synthesis which is significantly increased with proteasome inhibition by β-lactone. Based on these observations, it appears that protein degradation acts as a gatekeeper for induction of long-term synaptic plasticity. In the absence of sufficient synaptic stimulation, protein degradation limits the accumulation of proteins needed for induction of long-term synaptic plasticity. With strong stimulation that exceeds a certain threshold, the balance between protein degradation and synthesis is shifted toward a net increase in protein synthesis. How might this be achieved? A net gain in protein synthesis could come about by increased protein synthesis or reduced protein degradation or both. Our data indicate that new protein synthesis does increase significantly during cLTP relative to controls. Our results also suggest that protein degradation is slowed down during induction of long-term synaptic plasticity because the half-life of newly synthesized proteins is increased with cLTP. What could be the mechanism by which cLTP prolongs the half-life of newly synthesized proteins? It is possible that cLTP-inducing stimuli stabilize the proteins by making them resistant to ubiquitin-proteasome-mediated degradation through posttranslational modification such as phosphorylation. Previous investigation on neurons as well as other cell types showed that phosphorylation can confer resistance to degradation.[21−24]

Our data also showed that Aβ significantly reduces protein synthesis. This negative effect of Aβ on protein synthesis is likely to be at least partly due to blockade of mTOR signaling that regulates translation required for L-LTP. This notion is also reinforced by the fact that proteasome inhibition rescues the adverse effect of Aβ on accumulation of newly synthesized proteins and preincubation with rapamycin blocks the beneficial effects of proteasome inhibition. Previously, we showed that proteasome inhibition increases the amounts of proteins synthesized through the mTOR pathway during L-LTP induction.[8] Proteasome inhibition prior to incubation of the slices with Aβ restores the amounts of new protein synthesis to levels seen with cLTP but not to the levels observed with cLTP after β-lactone pretreatment. This is because with Aβ treatment, protein synthesis remains low. Proteasome inhibition can only act against the degradation process that prevents the net increase in protein accumulation during cLTP. Thus, devising a way to increase protein synthesis and block degradation in the presence of Aβ might be beneficial in reversing the deleterious effect of Aβ on long-term synaptic plasticity.

METHODS

Animals. For all experiments, 6−8 week old C57BL/6 male mice (Harlan Laboratories, Frederick, MD) were used for experimental protocols approved by the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences. Coronal hippocampal slices (400 μm) were made using a tissue chopper and allowed to recover in oxygenated artificial cerebrospinal fluid (ACSF) at 32 °C for 1 h. Chemical LTP (cLTP) was induced using 200 nM NMDA in 0 Mg^{2+} ACSF followed by 0.1 μM rolipram + 50 μM forskolin also in 0 Mg^{2+} ACSF.[7]

Metabolic Labeling. After 1 h of recovery, slices were incubated in oxygenated ACSF with or without 25 μM β-lactone for 30 min. Slices were then incubated in oxygenated ACSF with 13 nM ^{35}S-methioinine (1175 Ci/mmol, PerkinElmer, Waltham, MA) with or without cLTP-inducing chemicals followed by ^{35}S-methioinine alone until collection at 30, 60, 120, and 180 min. At collection, slices were quickly spun, all liquid was removed, and slices were immediately frozen on dry ice.

Analysis of Radiolabeled Proteins. After the experiments with ^{35}S-methioinine, slices were homogenized with radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 2 mM EDTA, 50 mM NaF) including protease inhibitor cocktail (EMD Millipore, Billerica, MA). The homogenate was incubated on ice for 1 h, centrifuged at 15 000g for 30 min, and the supernatant was collected and processed for bicinchoninic acid (BCA) protein estimation assay (Thermo Scientific, Waltham, MA). After SDS-PAGE of 50 μg of protein from each sample, the gels were stained with Coomassie Blue stain as a loading control measure. After destaining, gels were incubated for 1 h in En^{3}Hance liquid autoradiography enhancer (PerkinElmer) and then rinsed in ice-cold water for 30 min, dried, and exposed to Kodak BioMax Light film. The autoradiographic images were taken using Gel Doc (Bio-Rad, Hercules, California), and optical density was quantified using ImageJ (NIH, Bethesda, MD). Background was subtracted from each measurement, and protein loading differences were factored in using the optical density from Coomassie blue gel stain. Experimental groups for each time point were normalized to their time-matched control.

Pulse-Chase. After 1 h of recovery, slices were incubated in oxygenated ACSF with 13 nM 35S-methioinine (PerkinElmer) with or without cLTP-inducing chemicals followed by ^{35}S-methioinine alone for a total of 1 h of ^{35}S-methioinine labeling. This 1 h pulse was followed by 50 mM cold methionine and cysteine chase in oxygenated ACSF until the end of the experiment. We empirically determined that a cold methionine and cysteine chase was more effective than cold methionine alone. This observation can be explained by the fact that methionine is a precursor for synthesis of cysteine in vivo through what is called the transsulfuration reaction[25] which is fully functional in the brain.[26] For the pulse-chase experiments, the slices were collected at 60, 90, 120, 150, and 180 min from the start of cLTP and quickly spun down and frozen on dry ice. After autoradiography, the proteins were quantified by densitometry, and a semilogarithmic plot was generated. Half-life of proteins was calculated by using the formula $t_{1/2} = \ln(2)/k$, where k is the slope of the decay curve.[27,28]

Testing the Effect of Aβ on Protein Synthesis. Oligomeric Aβ_{1-42} was prepared as described previously.[29,30] Briefly, the lyophilized peptide (American peptide, Sunnyvale, CA) were resuspended in 10% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (concentration: 1 mM). The solution was divided into aliquots, the HFIP was allowed to evaporate, and the peptide was dried under vacuum and stored at −20 °C. The aliquots were resuspended in dimethyl sulfoxide (DMSO, 1 mM) and sonicated for 10 min. Oligomeric Aβ_{1-42} was obtained by diluting Aβ_{1-42} in DMSO into a smaller volume of ACSF (100 μM), vortexing for 30 s, and incubating at 4 °C for 24 h. Before use, the peptide was further diluted in ACSF to yield 200 nM final concentration. This preparation is referred to as Aβ oliogmers throughout the text.

Slices were recovered for 1 h before beginning preincubation in the chemicals necessary for each group: 1 μM rapamycin (EMD Millipore), β-lactone, and/or 200 nM Aβ oligomers. Slices were then subjected to cLTP induction with or without Aβ oligomers and/or rapamycin in a 13 nM ^{35}S-methioinine oxygenated 0 Mg^{2+} ACSF. Following cLTP protocol, slices were incubated with regular ACSF with ^{35}S-methioinine for 35 min, for a total of 1 h of metabolic labeling. All slices were collected at 1 h, quickly spun down, and frozen on dry ice.

Statistical Analysis. Data are expressed as mean ± standard error of the mean. The sample size (n) corresponds to the number of animals

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used for each experiment, not the number of slices. Data on half-life were analyzed by paired *t* tests. All other data were analyzed by one-way ANOVA followed by Tukey posthoc test.

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Author Contributions

K.A.H. and A.N.H. designed the study. K.A.H., T.K.S., and C.J.P. carried out the experiments. K.A.H. and C.J.P. analyzed the data under A.N.H.'s supervision. K.A.H. and A.N.H. wrote and edited the manuscript.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Kandel, E. R., and Schwartz, J. H. (1982) Molecular biology of learning: Modulation of transmitter release. *Science 218*, 433−443.

(2) Yin, J. C., and Tully, T. (1996) CREB and the formation of long-term memory. *Curr. Opin. Neurobiol. 6*, 264−268.

(3) Hegde, A. N. (2010) The ubiquitin-proteasome pathway and synaptic plasticity. *Learn. Mem. 17*, 314−327.

(4) Cai, F., Frey, J. U., Sanna, P. P., and Behnisch, T. (2010) Protein degradation by the proteasome is required for synaptic tagging and the heterosynaptic stabilization of hippocampal late-phase long-term potentiation. *Neuroscience 169*, 1520−1526.

(5) Fioravante, D., and Byrne, J. H. (2011) Protein degradation and memory formation. *Brain Res. Bull. 85*, 14−20.

(6) Jarome, T. J., and Helmstetter, F. J. (2013) The ubiquitin-proteasome system as a critical regulator of synaptic plasticity and long-term memory formation. *Neurobiol. Learn. Mem. 105*, 107−116.

(7) Dong, C., Upadhya, S. C., Ding, L., Smith, T. K., and Hegde, A. N. (2008) Proteasome inhibition enhances the induction and impairs the maintenance of late-phase long-term potentiation. *Learn. Mem. 15*, 335−347.

(8) Dong, C., Bach, S. V., Haynes, K. A., and Hegde, A. N. (2014) Proteasome modulates positive and negative translational regulators in long-term synaptic plasticity. *J. Neurosci. 34*, 3171−3182.

(9) Hay, N., and Sonenberg, N. (2004) Upstream and downstream of mTOR. *Genes Dev. 18*, 1926−1945.

(10) Graber, T. E., McCamphill, P. K., and Sossin, W. S. (2013) A recollection of mTOR signaling in learning and memory. *Learn. Mem. 20*, 518−530.

(11) Selkoe, D. J. (2002) Alzheimer's disease is a synaptic failure. *Science 298*, 789−791.

(12) Frey, U., Huang, Y. Y., and Kandel, E. R. (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science 260*, 1661−1664.

(13) Makhinson, M., Chotiner, J. K., Watson, J. B., and O'Dell, T. J. (1999) Adenylyl cyclase activation modulates activity-dependent changes in synaptic strength and Ca^{2+}/calmodulin-dependent kinase II autophosphorylation. *J. Neurosci. 19*, 2500−2510.

(14) Otmakhov, N., Khibnik, L., Otmakhova, N., Carpenter, S., Riahi, S., Asrican, B., and Lisman, J. (2004) Forskolin-induced LTP in the CA1 hippocampal region is NMDA receptor dependent. *J. Neurophysiol. 91*, 1955−1962.

(15) Stewart, D. J., Behrens, C., Roth, J., and Wistuba, I. I. (2011) Exponential decay nonlinear regression analysis of patient survival curves: Preliminary assessment in non-small cell lung cancer. *Lung Cancer 71*, 217−223.

(16) Naslund, J., Haroutunian, V., Mohs, R., Davis, K. L., Davies, P., Greengard, P., and Buxbaum, J. D. (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA, J. Am. Med. Assoc. 283*, 1571−1577.

(17) Mucke, L., Masliah, E., Yu, G. Q., Mallory, M., Rockenstein, E. M., Tatsuno, G., Hu, K., Kholodenko, D., Johnson-Wood, K., and McConlogue, L. (2000) High-level neuronal expression of Aβ 1−42 in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. *J. Neurosci. 20*, 4050−4058.

(18) Puzzo, D., and Arancio, O. (2006) Fibrillar beta-amyloid impairs the late phase of long term potentiation. *Curr. Alzheimer Res. 3*, 179−183.

(19) Lafay-Chebassier, C., Paccalin, M., Page, G., Barc-Pain, S., Perault-Pochat, M. C., Gil, R., Pradier, L., and Hugon, J. (2005) mTOR/p70S6k signalling alteration by Abeta exposure as well as in APP-PS1 transgenic models and in patients with Alzheimer's disease. *J. Neurochem. 94*, 215−225.

(20) Ma, T., Hoeffer, C. A., Capetillo-Zarate, E., Yu, F., Wong, H., Lin, M. T., Tampellini, D., Klann, E., Blitzer, R. D., and Gouras, G. K. (2010) Dysregulation of the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of Alzheimer's disease. *PLoS One 5*, e12845.

(21) Musti, A. M., Treier, M., and Bohmann, D. (1997) Reduced ubiquitin-dependent degradation of c-Jun after phosphorylation by MAP kinases. *Science 275*, 400−402.

(22) Yamamoto, N., Hegde, A. N., Chain, D. G., and Schwartz, J. H. (1999) Activation and degradation of the transcription factor C/EBP during long-term facilitation in Aplysia. *J. Neurochem. 73*, 2415−2423.

(23) Vosper, J. M., Fiore-Heriche, C. S., Horan, I., Wilson, K., Wise, H., and Philpott, A. (2007) Regulation of neurogenin stability by ubiquitin-mediated proteolysis. *Biochem. J. 407*, 277−284.

(24) Mukherjee, S., Poddar, R., Deb, I., and Paul, S. (2011) Dephosphorylation of specific sites in the kinase-specificity sequence domain leads to ubiquitin-mediated degradation of the tyrosine phosphatase STEP. *Biochem. J. 440*, 115−125.

(25) Brosnan, J. T., and Brosnan, M. E. (2006) The sulfur-containing amino acids: An overview. *J. Nutr. 136*, 1636S−1640S.

(26) Vitvitsky, V., Thomas, M., Ghorpade, A., Gendelman, H. E., and Banerjee, R. (2006) A functional transsulfuration pathway in the brain links to glutathione homeostasis. *J. Biol. Chem. 281*, 35785−35793.

(27) Belle, A., Tanay, A., Bitincka, L., Shamir, R., and O'Shea, E. K. (2006) Quantification of protein half-lives in the budding yeast proteome. *Proc. Natl. Acad. Sci. U. S. A 103*, 13004−13009.

(28) Eden, E., Geva-Zatorsky, N., Issaeva, I., Cohen, A., Dekel, E., Danon, T., Cohen, L., Mayo, A., and Alon, U. (2011) Proteome half-life dynamics in living human cells. *Science 331*, 764−768.

(29) Stine, W. B., Jr., Dahlgren, K. N., Krafft, G. A., and LaDu, M. J. (2003) In vitro characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. *J. Biol. Chem. 278*, 11612−11622.

(30) Puzzo, D., Vitolo, O., Trinchese, F., Jacob, J. P., Palmeri, A., and Arancio, O. (2005) Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity. *J. Neurosci. 25*, 6887−6897.

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