Metabotropic Receptor-Dependent Long-Term Depression Persists in the Absence of Protein Synthesis in the Mouse Model of Fragile X Syndrome

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Nosyreva, Elena D. and Kimberly M. Huber. Metabotropic receptor-dependent long-term depression persists in the absence of protein synthesis in the mouse model of fragile X syndrome. J Neurophysiol 95: 3291–3295, 2006. First published February 1, 2006; doi:10.1152/jn.01316.2005. Fragile X syndrome (FXS), a form of human mental retardation, is caused by loss of function mutations in the fragile X mental retardation gene (FMR1). The protein product of FMR1, fragile X mental retardation protein (FMRP), is an RNA-binding protein and may function as a translational suppressor. Metabotropic glutamate receptor–dependent long-term depression (mGluR-LTD) in hippocampal area CA1 is a form of synaptic plasticity that relies on dendritic protein synthesis. mGluR-LTD is enhanced in the mouse model of FXS, Fmr1 knockout (KO) mice, suggesting that FMRP negatively regulates translation of proteins required for LTD. Here we examine the synaptic and cellular mechanisms of mGluR-LTD in KO mice and find that mGluR-LTD no longer requires new protein synthesis, in contrast to wild-type (WT) mice. We further show that mGluR-LTD in KO and WT mice is associated with decreases in AMPA receptor (AMPAR) surface expression, indicating a similar postsynaptic expression mechanism. However, like LTD, mGluR-induced decreases in AMPAR surface expression in KO mice persist in protein synthesis inhibitors. These results are consistent with recent findings of elevated protein synthesis rates and synaptic protein levels in Fmr1 KO mice and suggest that these elevated levels of synaptic proteins are available to increase the persistence of LTD without de novo protein synthesis.

INTRODUCTION

Patients with fragile X syndrome (FXS) have altered dendritic spine structure, suggesting that abnormal postsynaptic function, development, or plasticity contributes to the cognitive deficits of this disease (Hinton et al. 1991; Irwin et al. 2001). Much is known about the molecular function of fragile X mental retardation protein (FMRP), the protein product of the fragile X mental retardation gene (FMR1), and has prompted specific hypotheses regarding the role of FMRP in synaptic function and plasticity (for reviews see Bagni and Greenough 2005; O’Donnell and Warren 2002). FMRP is an RNA-binding protein and associates with large, presumably translating, polyribosomes (Corbin et al. 1997; Feng et al. 1997; Stefani et al. 2004). However, both in vitro and in vivo studies suggest that FMRP functions as a suppressor of protein synthesis (Laggerbauer et al. 2001; Li et al. 2001). Evidence that FMRP suppresses translation in vivo comes from a recent study that measured increased protein synthesis rates in selected brain regions of knockout (KO) mice, including area CA1 of the hippocampus (Qin et al. 2005). These increased protein synthesis rates result in elevated steady-state levels of neuronal proteins including CaMKII (Ca2+/calmodulin–dependent protein kinase II), Arc (activity-regulated cytoskeletal-associated protein), and MAP1B (Lu et al. 2004; Sung et al. 2003; Zalfa et al. 2003).

Clues to the synaptic role of FMRP emerged when FMRP was found to be synthesized in synaptoneurosomes in response to group 1 metabotropic glutamate receptor (mGluR) activation (Weiler et al. 1997). In turn, FMRP regulates mGluR-dependent long-term depression (LTD). mGluR-LTD is enhanced in the mouse model of FXS, Fmr1 knockout (KO) mice, whereas N-methyl-D-aspartate (NMDA) receptor–dependent LTD is unchanged (Huber et al. 2002). The fact that mGluR-LTD requires dendritically synthesized proteins suggests that FMRP may normally suppress translation of the protein required for LTD, such that in the absence of FMRP, LTD is enhanced (Huber et al. 2000).

Contrary to this hypothesis, a deficit in mGluR-stimulated protein synthesis exists in KO mice, suggesting that FMRP facilitates translation of synaptic proteins (Todd et al. 2003; Weiler et al. 2004). Alternatively, the findings of enhanced protein synthesis rates and protein levels in KO mice suggest that synaptic protein synthesis is at a maximum such that ex vivo stimulation of mGluRs does not further increase protein levels (Qin et al. 2005; Zalfa et al. 2003). In the context of mGluR-LTD, we hypothesized that there are increased levels of proteins required for the persistence of LTD at the synapses of KO mice, which predicts that mGluR-LTD would no longer require new protein synthesis. Here we present data in support of this hypothesis. Our findings provide functional evidence that elevated levels of synaptic proteins in KO mice can be used for long-term synaptic change.

METHODS

Drugs

D,L-2-Amino-5-phosphonovalerate (D,L-AP5, Tocris Cookson, Elsivlle, MO), anisomycin, and cycloheximide (Sigma, St. Louis, MO) were prepared fresh in artificial cerebrospinal fluid (ACSF), which consists of (in mM) NaCl, 124; KCl, 5; NaH2PO4, 1.25; NaHCO3, 26; MgCl2, 1; CaCl2, 2; and dextrose, 10.

Electrophysiology

Hippocampal slices (400 μm) were prepared from 30- to 60-day-old wild-type (WT) or Fmr1 KO littermates bred from the congenic C57Bl/6 strain (provided by Dr. Steve Warren; Emory University) (Bakker 1994) as previously described (Huber et al. 2002; Nosyreva et al. 2005).
and Huber 2005). All experiments were performed blind to the genotype of the mouse. Extracellular field potentials (FPs) were measured in the str. radiatum of CA1 elicited by Schaffer collateral stimulation. mGluR-LTD was induced by pairs of stimuli (50-ms interstimulus interval) delivered at 1 Hz for 20 min [2,400 pulses; paired-pulse low-frequency stimulation (PP-LFS)], or by application of 100 μM 3,4-dihydroxyphenylglycine (DHPG) for 5 min. The duration of PP-LFS was increased from 15 to 20 min for this study compared to our previous study (Huber et al. 2002) to increase the magnitude of mGluR-LTD and our ability to measure effects of protein synthesis inhibitors. Synaptic strength was measured as the initial slope (10–40% of the rising phase) of the FP. LTD magnitude was compared at 60–70 min after the onset of DHPG or PP-LFS between inhibitor-treated and control interleaved slices. Independent t-tests were used to determine statistical significance.

Biochemical measurements of surface expressed AMPA receptors

Biotinylation experiments were performed on hippocampal slices from WT or KO mice as described (Nosyreva and Huber 2005). Slices were treated with 100 μM DHPG (5 min) or ACSF (control) in the presence of 100 μM N-[L-2-amino-5-phosphonovalerate (D,L-AP5). By 60 min after treatment, surface proteins were biotinylated on ice for 10 min. GluR1 and GluR2/3 immunoreactive bands were visualized using enhanced chemiluminescence (ECL). Duplicate conditions within one animal were averaged to obtain an animal average for that condition. Significant differences between raw ratio values from treated and within-animal control slices were determined using a paired t-test.

Results

mGluR long-term depression in Fmr1 KO mice occurs in the presence of protein synthesis inhibitors

We first investigated the protein synthesis dependency of chemically induced (100 μM DHPG; 5 min) mGluR-LTD in acute hippocampal slices prepared from WT or KO mice. As observed in rats, preincubation in the protein synthesis inhibitor anisomycin (20 μM) inhibited DHPG-induced LTD in WT mice (ACSF: 76 ± 2% of baseline 1 h post-DHPG; n = 16 slices from 11 mice; anisomycin: 90 ± 3%; n = 9 slices from eight mice; P < 0.001; Fig. 1A) (Huber et al. 2000). In contrast, anisomycin had no effect on DHPG-induced LTD in KO mice (ACSF: 68 ± 2%; n = 11 slices from 9 mice; anisomycin: 70 ± 4%; n = 10 slices from seven mice; P = 0.77; Fig. 2B). Under control conditions, DHPG-induced LTD was enhanced in KO mice compared to WT mice (P = 0.01) as previously described (Huber et al. 2002). However, the differences in LTD between WT and KO mice are much more pronounced in the presence of anisomycin (Fig. 1C; P = 0.003).

Like DHPG-induced LTD, synthetically induced mGluR-LTD (using PP-LFS) was also insensitive to protein synthesis inhibitors in KO mice (ACSF: 80 ± 4%; n = 8 slices from four mice; anisomycin: 82 ± 3%; n = 8 slices from four mice; P = 0.77; Fig. 2B) in contrast to WT mice (ACSF: 80 ± 2%; n = 6 slices from four mice; anisomycin: 105 ± 5%; n = 8 slices from four mice; P < 0.01; Fig. 2A). Therefore in the presence of anisomycin, the magnitude of synthetically induced mGluR-LTD between WT and KO is strikingly different (Fig. 2C; P = 0.005). Similar results were observed with another protein synthesis inhibitor, cycloheximide (60 μM). Cycloheximide inhibited synthetically induced mGluR-LTD in WT mice (ACSF: 74 ± 3%; n = 8 slices from five mice; cycloheximide: 91 ± 3%; n = 11 slices from five mice; P = 0.001), but not in KO mice (ACSF: 76 ± 3%; n = 6 slices from five mice; cycloheximide: 77 ± 2%; n = 8 slices from five mice; P = 0.8). Therefore mGluR-LTD was different between WT and KO mice in the presence of cycloheximide (P = 0.001).

mGluR decreases in AMPAR surface expression are independent of protein synthesis in Fmr1 KO mice

We recently discovered a developmental switch in the protein synthesis dependency and synaptic mechanisms of mGluR-LTD (Nosyreva and Huber 2005). mGluR-LTD in neonatal rats (P8–P15) does not require protein synthesis and...
is not associated with decreases in postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) surface expression, but is mediated primarily by a decrease in presynaptic function (Bolschakov and Siegelbaum 1994; Feinmark et al. 2003; Fitzjohn et al. 2001; Nosyreva and Huber 2005; Zakharenko et al. 2002). As synapses mature (P21–P35), mGluR-LTD becomes protein synthesis dependent and is associated with long-term decreases in the postsynaptic AMPAR surface expression (Nosyreva and Huber 2005). To determine whether mGluR-LTD in KO mice is the mature postsynaptic surface expression, we tested whether LTD was associated with long-term decreases in the postsynaptic AMPAR surface expression as in WT mice and is not the immature presynaptic form of mGluR-LTD.

Long-term decreases in postsynaptic AMPAR surface expression induced by mGluR activation also rely on new protein synthesis (Nosyreva and Huber 2005; Snyder et al. 2001). Like mGluR-LTD, we would expect these decreases in AMPAR surface expression to occur without the need for new protein synthesis in KO mice. To test this, we measured mGluR-induced decreases in AMPAR surface expression in slices preincubated in anisomycin (20 μM). Anisomycin blocked DHPG-induced decreases in AMPAR surface expression in WT mice [GluR1: 99 ± 5%; n = 6 mice; P = 0.56 (control vs. DHPG), GluR2/3: 109 ± 5%; n = 5; P = 0.39; Fig. 3, C and D]. In contrast, DHPG-induced decreases in AMPAR surface expression persisted in anisomycin in KO mice [GluR1: 73 ± 6%; n = 7 mice; P = 0.008; GluR2/3: 64 ± 8%; n = 6; P = 0.02; Fig. 3, C and D]. Consequently, in the presence of
anisomycin, mGluR-induced decreases in AMPAR surface expression are greater in KO mice than in WT mice (WT vs. KO; GluR1: \( P = 0.009 \); GluR2/3: \( P = 0.004 \)). Thus at the ages we have examined (30–60 days) mGluR-LTD in KO mice is most likely mediated by a postsynaptic expression mechanism as in WT mice, but differs in its requirement for new proteins.

**Discussion**

Here we demonstrate that mGluR-induced long-term changes in synaptic plasticity in Fmr1 KO mice persist without the need for new protein synthesis. Our previous work demonstrated that mGluR-LTD was enhanced in KO mice, but NMDA-receptor–dependent LTD, which does not rely on protein synthesis, was unchanged (Huber et al. 2002). Here we show that the synaptic mechanisms of mGluR-LTD are similar in both WT and KO mice, in that LTD is associated with decreases in AMPAR surface expression, but the regulation of these synaptic changes differs. Because mGluR-dependent decreases in AMPAR surface expression are associated with LTD in KO mice (Fig. 3) this suggests that LTD is the mature postsynaptic form and not a developmentally arrested presynaptic form of mGluR-LTD (Nossyeva and Huber 2005). The loss of the protein synthesis dependency of LTD and AMPAR surface decreases may instead arise from the loss of translational suppression of proteins required for LTD in the absence of FMRP.

The present results add to the recent findings of enhanced protein synthesis rates and protein levels in KO mice and demonstrate that these changes alter synaptic plasticity properties (Lu et al. 2004; Qin et al. 2005; Zalfa et al. 2003). Our data also help to reconcile the findings of enhanced mGluR-dependent plasticity with that of reduced mGluR stimulated protein synthesis (Huber et al. 2002; Todd et al. 2003; Weiler et al. 2004). Our current findings are consistent with a model in which FMRP suppresses protein synthesis rates in vivo, such that in its absence, there is an increase in the steady-state level of synaptic proteins, which can be used for maintenance of LTD and decreases in AMPAR surface expression, what we term “LTD” proteins. Our data suggest that stimulation of mGluRs is required to initiate AMPAR endocytosis to capture or use the “LTD” proteins. Alternatively, changes in mGluR5 signaling resulting from a reduced association with Homer or altered trafficking of AMPARs in KO mice could contribute to the differences in mGluR-LTD properties (Giuffrida et al. 2005; Mao et al. 2005).

The increase in mGluR-LTD in KO mice has prompted a hypothesis that other mGluR- and protein synthesis–dependent processes are enhanced, which contributes to the multitude of symptoms in FXS, termed the mGluR theory of Fragile X Syndrome (Bear et al. 2004). Recent findings that mGluR-LTD in the cerebellum and mGluR-dependent epileptiform bursts in hippocampal area CA3 are enhanced in KO mice support this theory (Chuang et al. 2005; Koekkoek et al. 2005). Interestingly, the enhanced epileptiform bursts in KO mice retain their protein synthesis dependency, suggesting that proteins required for mGluR-LTD and epileptiform bursts are regulated differently in the absence of FMRP (Chuang et al. 2005).

Unlike mGluR-LTD, long-term potentiation (LTP) is unaffected in the area CA1 (Godfraind et al. 1996; Paradee et al. 1999). However, an LTP deficit in the amygdala and neocortex was recently identified in KO mice (Larson et al. 2005; Li et al. 2002; Zhao et al. 2005). The specific cortical LTP deficit could be related to the decreased levels of GluR1 observed in the neocortex, but not hippocampus (Li et al. 2002), as well as the dendritic spine abnormalities observed on neocortical KO neurons (Irwin et al. 2002; Nimchinsky et al. 2001). Although it is unknown whether mGluR-LTD is altered in the neocortex of KO mice, these data indicate that FMRP differentially affects synaptic plasticity across brain regions.

How the alterations in hippocampal mGluR-LTD in KO mice contribute to the cognitive deficits in FXS is unknown. A recent study reported enhanced mGluR-LTD in the cerebellum and incorporated this quantitative change into a computational model of associative learning in a cerebellar circuit (Koekkoek et al. 2005). Their model makes testable predictions of how enhanced mGluR-LTD leads to reduced eye-blink conditioning observed in KO mice and FXS patients and therefore provides a tractable paradigm to link plasticity alterations with learning in humans (Koekkoek et al. 2005).

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