Loss of the Fragile X Mental Retardation Protein Decouples Metabotropic Glutamate Receptor Dependent Priming of Long-Term Potentiation From Protein Synthesis

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Auerbach BD, Bear MF. Loss of the fragile X mental retardation protein decouples metabotropic glutamate receptor dependent priming of long-term potentiation from protein synthesis. J Neurophysiol 104: 1047–1051, 2010. First published June 16, 2010; doi:10.1152/jn.00449.2010. Fragile X Syndrome (FXS), the most common inherited form of intellectual disability, is caused by loss of the fragile X mental retardation protein (FMRP). FMRP is a negative regulator of local mRNA translation downstream of group 1 metabotropic glutamate receptor (Gp1 mGluR) activation. In the absence of FMRP there is excessive mGluR-dependent protein synthesis, resulting in exaggerated mGluR-dependent long-term synaptic depression (LTD) in area CA1 of the hippocampus. Understanding disease pathophysiology is critical for development of therapies for FXS and the question arises of whether it is more appropriate to target excessive LTD or excessive mGluR-dependent protein synthesis. Priming of long-term potentiation (LTP) is a qualitatively different functional consequence of Gp1 mGluR-stimulated protein synthesis at the same population of CA1 synapses where LTD can be induced. Therefore we determined if LTP priming, like LTD, is also disrupted in the Fmr1 knockout (KO) mouse. We found that mGluR-dependent priming of LTP is of comparable magnitude in wild-type (WT) and Fmr1 KO mice. However, whereas LTD priming requires acute stimulation of protein synthesis in WT mice, it is no longer protein synthesis dependent in the Fmr1 KO. These experiments show that the dysregulation of mGluR-mediated protein synthesis seen in Fmr1 KO mice has multiple consequences on synaptic plasticity, even within the same population of synapses. Furthermore, it suggests that there is a bifurcation in the Gp1 mGluR signaling pathway, with one arm triggering synaptic modifications such as LTP priming and LTD and the other stimulating protein synthesis that is permissive for these modifications.

INTRODUCTION

Fragile X Syndrome (FXS) is the most common inherited form of intellectual disability. The disease is usually caused by expansion of a CGG triplet repeat sequence upstream of the FMR1 gene that results in transcriptional silencing and consequent loss of FMRP, the fragile X mental retardation protein (Garber et al. 2008; Pieretti et al. 1991). FMRP is an RNA-binding protein (Ashley Jr et al. 1993; Siomi et al. 1993) that has been shown to regulate, among other things, synaptic protein synthesis (Brown et al. 2001; Feng et al. 1997; Khandojian et al. 2004; Laggerbauer et al. 2001; Lu et al. 2004; Todd et al. 2003; Weiler et al. 2004; Zalfa et al. 2003). In the Fmr1 knockout (KO) mouse, basal protein synthesis in the hippocampus is significantly elevated over wild-type (WT) levels (Dolen et al. 2007; Qin et al. 2005).

Group 1 metabotropic glutamate receptors (Gp1 mGluRs) are expressed in neurons throughout the brain (Masu et al. 1991). Activation of Gp1 mGluRs has been shown to trigger dendritic mRNA translation, including the synthesis of FMRP (Weiler and Greenough 1993, 1999). In the hippocampus, one functional consequence of activating Gp1 mGluRs is induction of long-term synaptic depression (LTD) at the Shaffer collateral–CA1 synapse (Huber et al. 2001; Oliet et al. 1997; Palmer et al. 1997), expressed in part by a loss of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–type glutamate receptors (Snyder et al. 2001; Waung et al. 2008). In WT rats and mice, mGluR-stimulated protein synthesis is obligatory for stable expression of LTD (Huber et al. 2000, 2001). LTD magnitude is enhanced in the Fmr1 KO mouse, possibly due to exaggerated protein synthesis (Huber et al. 2002). Consistent with this interpretation, mGluR-LTD in the KO also no longer requires acute stimulation of protein synthesis, presumably due to constitutive overexpression of “LTD proteins” (Hou et al. 2006; Nosyreva and Huber 2006). Because LTD mechanisms are believed to be important for sculpting synaptic connections during postnatal development, a reasonable conjecture is that exaggerated LTD could be pathogenic in FXS (Huber et al. 2002). Moreover, increased LTD in hippocampal area CA1 could contribute specifically to the cognitive impairment that is characteristic of this disease.

Understanding disease pathophysiology is critical for development of therapies for FXS and the question arises of whether it is more appropriate to target excessive LTD and impaired AMPA receptor function (Lynch et al. 2008) or excessive mGluR-dependent protein synthesis (Bear 2005; Bear et al. 2004). The phenomenon of LTP priming, first described by Abraham and colleagues in rats (Cohen and Abraham 1996; Cohen et al. 1998; Raymond et al. 2000), offers an interesting opportunity to distinguish among these alternatives. Normally, weak high-frequency stimulation (HFS) elicits modest long-term synaptic potentiation (LTP) at the Shaffer collateral–CA1 synapse. However, if Gp1 mGluRs are first stimulated briefly with a low concentration of the selective agonist DHPG (R,S-dihydroxyphenylglycine; 10 μM), then the LTP caused by subsequent HFS is substantially augmented. Like mGluR-LTD induced by higher DHPG concentrations, LTP priming in WT rats is abolished by inhibitors of mRNA translation, but not by...
inhibitors of transcription. Thus LTD and LTP priming are qualitatively different functional consequences of Gp1 mGluR-stimulated protein synthesis at the Shaffer collateral–CA1 synapse. In the current study we ask whether LTP priming, like LTD, is also disrupted in the Fmr1 KO mouse.

We find, first, that priming of LTP results from weak activation of Gp1 mGluRs with DHPG in mouse CA1, as previously reported in rats. Second, although priming is quantitatively similar in Fmr1 KO and WT mice, it is blocked by a protein synthesis inhibitor only in the WT. These findings suggest, first, that proteins overexpressed in FXS are not restricted to “LTD proteins” because they apparently include those required for LTP priming as well. Second, the findings indicate that there is a posttranslational component of mGluR-dependent LTP priming. Instead of serving as a trigger for LTP induction (or LTD), dendritic protein synthesis may rather serve as a gate for synaptic plasticity that normally opens only in response to an mGluR signaling event. In fragile X, this gate is perpetually open due to excessive basal protein synthesis and overexpression of proteins that are normally rate-limiting for these forms of synaptic modification.

METHODOLOGY

Animals

Fmr1 mutant mice (Jackson Labs) were bred on the C57Bl/6J clonal background. In an effort to reduce variability due to rearing conditions, all experimental animals were bred from Fmr1 heterozygote mothers, group housed (animals weaned to solitary housing were excluded), and maintained on a 12:12-h light:dark cycle. The Institutional Animal Care and Use Committee at MIT approved all experimental techniques.

Slice preparation

Transverse hippocampal slices (350 μm thick) were prepared from 6- to 10-wk-old mice in ice-cold dissection buffer containing (in mM): NaCl 87, sucrose 75, KCl 2.5, NaH2PO4 1.25, NaHCO3 25, CaCl2 0.5, MgCl2 7, ascorbic acid 1.3, and d-glucose 10 (saturated with 95% O2-5% CO2). Immediately following slicing, the CA3 region was removed. Slices were recovered in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 3.5, NaH2PO4 1.23, NaHCO3 26, CaCl2 2, MgCl2 1, and d-glucose 10 (saturated with 95% O2-5% CO2) at room temperature for ≥3 h prior to recording.

Electrophysiology

Field recordings were performed in a submersion chamber, perfused with ACSF (2–3 ml/min) at 30°C. Field EPSPs (fEPSPs) were recorded in CA1 stratum radiatum with extracellular recording electrodes filled with ACSF. Baseline responses were evoked by stimulation of the Schaffer collaterals at 0.033 Hz with a two-contact cluster electrode (FHC) using a 0.2-ms stimulus yielding 40–60% of the maximal response. Priming was induced by applying 10 μM DHPG for 10 min (Mellentin et al. 2007). Pairs of primed and unprimed slices were recorded simultaneously. LTP was induced with a 1-s 100-Hz tetanus. Protein synthesis was inhibited by applying 60 μM cycloheximide (CHX) for 30 min as follows: 15 min of pretreatment during baseline recording, 10 min during DHPG application, and 5 min post-DHPG application; or during the equivalent time of baseline recording in unprimed slices.

fEPSP recordings were filtered at 0.1 Hz to 1 kHz, digitized at 10 kHz, and analyzed using pClamp9 (Axon Instruments). The initial slope of the response was used to assess changes in synaptic strength. Data were normalized to the baseline response and are presented as group means ± SE. LTP was measured by comparing the average response 55–60 min posttetanus to the average of the last 5 min of baseline. ANOVA and unpaired t-test were used to determine statistically significant differences. Experiments used aged-matched and interleaved WT and Fmr1 KO mice. For all experiments the experimenter was blind to genotype.

Reagents

R,S-DHPG was purchased from Tocris Biosciences (Ellisville, MO). All other reagents were purchased from Sigma (St. Louis, MO). Fresh bottles of DHPG were prepared as a 100× stock in H2O, aliquoted, and stored at −80°C. Fresh stocks were made once a week. CHX was prepared at 100× stock in H2O daily. These stocks were diluted in ACSF to achieve final concentration.
RESULTS

To confirm that there is facilitation of LTP by prior Gp1 mGluR activation in mice, we first established a tetanization protocol that produced a subsaturable level of LTP and has been shown to be amenable to priming (Cohen et al. 1998; Mellentin et al. 2007). Brief HFS (1-s 100-Hz) produced a modest but reliable level of LTP 1 h posttetanus in slices from both WT and Fmr1 KO mice (WT: 111.2 ± 2.1%, n = 9; KO: 113.8 ± 3.1%, n = 9; Fig. 1). As has been reported previously, there was no significant difference in the basal level of hippocampal LTP in Fmr1 KO mice compared with WT mice (P = 0.51) (Godfraind et al. 1996; Paradee et al. 1999).

We then replicated the previously reported mGluR-dependent priming of LTP in WT slices (Cohen et al. 1998; Mellentin et al. 2007). The Gp1 mGluR agonist DHPG (10 μM) was bath applied to slices for 10 min after a stable 20-min baseline recording period. DHPG application produced a transient depression of synaptic responses that recovered to baseline levels after a 30-min washout. The same tetanus protocol as above (1-s, 100-Hz) now produced a significantly larger magnitude of LTP compared with unprimed slices (unprimed: 111.2 ± 2.1%, n = 9; primed: 123.9 ± 3.8%, n = 10; P = 0.012; Fig. 1A).

These findings in mice are consistent with those previously reported in rats (Cohen et al. 1998; Mellentin et al. 2007).

We next characterized the effect of DHPG application on subsequent LTP in Fmr1 KO mice. As was the case in WT animals, the DHPG priming protocol also enhanced LTP in slices from Fmr1 KO mice (unprimed: 113.8 ± 3.1%, n = 9; primed: 133.7 ± 6.7%, n = 11; P = 0.016; Fig. 1B). However, there was no significant difference in the magnitude of facilitation seen in primed Fmr1 KO slices as compared with primed WT slices (P = 0.22).

Finally, we examined the role of protein synthesis in DHPG-induced priming in both WT and Fmr1 KO mice. As expected (Raymond et al. 2000), a brief application of the protein synthesis inhibitor CHX (60 μM, 30 min) completely abolished DHPG-induced priming in WT slices (unprimed: 117.5 ± 7.0%, n = 7; primed: 118.6 ± 6.0%, n = 9; P = 0.94; Fig. 2A). However, this same treatment had no effect on DHPG-induced priming in slices from Fmr1 KO mice (unprimed: 118.5 ± 7.0%, n = 7; primed: 149.6 ± 11.0%, n = 8). These findings are consistent with those previously reported in rats (Cohen et al. 1998; Mellentin et al. 2007).

FIG. 2. DHPG-induced priming of LTP does not require protein synthesis in Fmr1 KO mice. Delivery of the protein synthesis inhibitor cycloheximide (60 μM CHX, 30 min; gray bar) before and during DHPG priming prevented facilitation of LTP in slices from WT mice (A; unprimed: 117.5 ± 7.0%, n = 7 slices from 6 animals, open black circles; primed: 118.6 ± 6.0%, n = 8 slices from 6 animals, closed black circles; P = 0.94); however, this treatment had no effect on DHPG-induced priming in slices from Fmr1 KO mice (B; unprimed: 118.5 ± 6.1%, n = 7 slices from 6 animals, open gray circles; primed: 149.6 ± 11.0%, n = 8 slices from 7 animals, closed gray circles; P < 0.05). Representative field potential traces (average of 10 sweeps) were taken at times indicated by numerals.

FIG. 3. Summary of DHPG-induced priming of LTP and its protein synthesis dependence in wildtype and Fmr1 KO mice. Bar graphs represent the average percentage LTP observed 55–60 min posttetanus. Wildtype unprimed: open black; wildtype primed: closed black; Fmr1 KO unprimed: open gray; Fmr1 KO primed: closed gray. Asterisks denote significant differences (unpaired Student’s t-test, P < 0.05).

FIG. 4. Model. The finding that LTP priming by mGluR activation occurs in the Fmr1 KO without a need for acute protein synthesis suggests a bifurcation in the signaling pathway. The priming step (1) occurs in response to mGluR activation via a mechanism involving posttranslational modification of synaptic proteins (possibly the AMPA receptor itself). In WT animals, priming is not possible without (2) concurrent mGluR activation of mRNA translation and synthesis of protein(s) that gate plasticity. In the absence of the translational repressor FMRP, the gating proteins are constitutively overexpressed, rendering priming no longer sensitive to protein synthesis inhibitors. The identity of the hypothetical gating proteins remains to be determined. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.
These results show that although the magnitude of LTP enhancement induced by DHPG priming is not quantitatively different in Fmr1 KO mice, induction of priming is qualitatively different in that it no longer requires the synthesis of new proteins (Fig. 3; Supplemental Fig. S1).1

**DISCUSSION**

In this study we characterized mGluR-dependent priming of LTP in the fragile X background. In WT mice we confirmed previous reports that application of the Gp1 mGluR agonist DHPG at a low dose enhances the magnitude of subsequent LTP and that this priming of LTP is protein synthesis dependent. In the Fmr1 KO we determined that although mGluR-dependent priming of LTP is not significantly enhanced, it no longer requires acute protein synthesis at the time of induction. These experiments show that the dysregulation of mGluR-mediated protein synthesis seen in Fmr1 KO mice has multiple consequences on synaptic plasticity, even within the same population of CA1 synapses.

Although our goal was to determine whether the impact of excessive protein synthesis in area CA1 of population of CA1 synapses.

**REFERENCES**


1 The online version of this article contains supplemental data.
ALtered mGluR-Dependent LTP Priming in Fmr1 KO Mice


