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The Role of Homer1c in Metabotropic Glutamate Receptor-dependent Long-Term Potentiation

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Abstract

Group I metabotropic glutamate receptors (mGluR1/5) play a role in synaptic plasticity and they demonstrate direct interactions with the neuronal Homer1c protein. We have previously shown that Homer1c can restore the plasticity deficits in Homer1 knockout mice (H1-KO). Here, we investigated the role of Homer1c in mGluR-dependent synaptic plasticity in wild-type mice, H1-KO, and H1-KO mice overexpressing Homer1c (KO+H1c). We used a form of plasticity induced by activation of mGluR1/5 that transforms short-term potentiation (STP) induced by a subthreshold theta burst stimulation into long-term potentiation (LTP). We have shown that although acute hippocampal slices from wild-type animals can induce LTP using this stimulation protocol, H1-KO only show STP. Gene delivery of Homer1c into the hippocampus of H1-KO mice rescued LTP to wild-type levels. This form of synaptic plasticity was dependent on mGluR5 but not mGluR1 activation both in wild-type mice and in KO+H1c. mGluR1/5-dependent LTP was blocked with inhibitors of the MEK-ERK and PI3K-mTOR pathways in KO+H1c mice. Moreover, blocking Homer1c–mGluR5 interactions prevented the maintenance of LTP in acute hippocampal slices from KO+H1c. These data indicate that Homer1c–mGluR5 interactions are necessary for mGluR-dependent LTP, and that mGluR1/5-dependent LTP involves PI3K and ERK activation.

Keywords

hippocampus; Homer1 knockout mice; molecular switch; ERK; mTOR

INTRODUCTION

Homer1 is a neuronal protein that regulates glutamatergic and intracellular calcium signaling in the brain (Xiao et al., 1998; Kammermeier et al., 2000; Yuan et al., 2003). Homer1 interacts with Group I metabotropic glutamate receptors (mGluR1/5) in the postsynaptic density although the function of this interaction is not completely understood (Ronesi and Huber, 2008; Ronesi et al., 2012). Homer1–mGluR1/5 direct interactions have been shown to link to protein synthesis signaling machinery (Giuffrida et al., 2005; Ronesi and Huber, 2008) and disruption of this link blocks activation of the MEK-ERK and/or PI3K-mTOR

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pathways in different brain regions (Rong et al., 2003; Mao et al., 2005; Orr et al., 2012; Ronesi et al., 2012).

Using Adeno-associated viral gene delivery of Homer1c (rAAV-H1c), we reversed learning impairment and hippocampal long-term potentiation (LTP) deficits in Homer1 knockout mice (H1-KO [Gerstein et al., 2012]), and rescued learning in aged impaired rats (Gerstein et al., 2013). However, the cellular mechanism by which Homer1c can improve memory and synaptic plasticity in these rodent models is currently unknown.

In this study, we tapped the ability of mGluR1/5 activation to change a below-threshold stimulus that alone results in short-term potentiation (STP) into one that successfully elicits enduring LTP (Bashir et al., 1993; Bortolotto et al., 1994; Cohen and Abraham, 1996). This ability of mGluR1/5 to change STP into LTP has been termed the molecular switch property of mGluR1/5 (Bortolotto et al., 1994). This molecular switch is reliant on new protein synthesis (Raymond et al., 2000; Bortolotto et al., 2005), and has been shown to be mGluR5 specific (Bortolotto et al., 2005). The cellular cascades triggered by this molecular switch remain unknown. Here, we show that this form of mGluR-dependent synaptic plasticity is impaired in Homer1 knockout mice, and it can be restored by gene transfer of Homer1c into the hippocampus of H1-KO mice. Moreover, we demonstrate that the molecular switch is dependent on mGluR5 activation, requires Homer1c-mGluR5 direct association, and it involves both PI3K and ERK activation.

RESULTS

We first tested whether mGluR1/5 activation with the mGluR1/5 selective agonist 3,5-dihydroxyphenylglycine (DHPG, 10 μ M) could induce LTP in wild-type mice (LM-WT) using a half train theta burst stimulation that alone induces STP (0.5 TBS) (Bashir et al., 1993; Bortolotto et al., 1994; Cohen and Abraham, 1996). After initial induction of LTP, slices incubated in DHPG maintained robust LTP for the time tested, whereas control slices that received only 0.5 TBS showed a decaying STP (Fig. 1A; main effect of treatment [–DHPG vs. +DHPG], $F_{(1,24)} = 18.15$, $P < 0.0003$). This successful transformation of STP into a persistent LTP in LM-WT mice recapitulates the findings by the previous groups in Sprague–Dawley rats (Bortolotto et al., 1994; Cohen and Abraham, 1996; Cohen et al., 1998; Raymond et al., 2000; Piccinin et al., 2008). In addition to the persistent LTP induced by this protocol, there was an increase in the magnitude of LTP induction during stimulation in LM-WT exposed to DHPG versus the LM-WT that received only 0.5 TBS (Fig. 1A, $P < 0.001$, 2-min poststimulation).

To test the hypothesis that Homer1c plays a role in mGluR1/5-dependent LTP, we investigated this form of plasticity in H1-KO mice in the absence or presence of Homer1c. We have previously shown robust transgene expression in the dorsal hippocampus using rAAV delivery of Homer1c and green fluorescence protein (GFP) in H1-KO mice (Gerstein et al., 2012).

We injected H1-KO mice with either rAAV–GFP (KO+GFP) or rAAV–Homer1c (KO+H1c). GFP injection does not affect synaptic plasticity or behavior in these animals and therefore is

a good control for surgery and transgene expression (Gerstein et al., 2012). We found that H1-KO mice show deficits in this form of synaptic plasticity. H1-KO+GFP cannot induce LTP when a 0.5-TBS is preceded by mGluR1/5 activation (Fig. 1B). Expression of Homer1c in H1-KO mice resulted in LTP persistence upon priming with DHPG (Fig. 1C; main effect of treatment $F_{(1,8)} = 40.68$, $P < 0.0001$). This plasticity profile is highly comparable to LM-WT (LM-WT+DHPG vs. KO+H1c+DHPG, no main effect of genotype, $F_{(1,22)} = 0.2438$, $P = 0.6269$). The maintenance of LTP seen in the KO+H1c was significantly better than that of the KO+GFP slices (Figs. 1B, C; KO+H1c+DHPG vs. KO+GFP+DHPG, main effect of treatment, $F_{(1,17)} = 13.35$, $P < 0.0021$). There was also an increase in the magnitude of LTP induction during stimulation in KO+H1c+DHPG versus KO+H1c-DHPG (Fig. 1C, $P < 0.001$, 2-min poststimulation). Thus, Homer1c expression in the hippocampus of H1-KO is sufficient for mGluR1/5 activation to convert STP into a persistent LTP.

Next, we set to determine whether mGluR1 or mGluR5 is the specific receptor subtype activating this molecular switch in our animal model. The mGluR5-selective noncompetitive antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), and mGluR1 α -selective competitive antagonist LY367385 were used to block LTP. Preincubation of wild-type hippocampal slices with the MPEP but not LY367385 blocked LTP in the presence of DHPG (Fig. 2A; main effect of treatment WT+DHPG vs. WT+DHPG+MPEP; $F_{(1,30)} = 37.94$, $P < 0.0001$; WT+DHPG vs. WT+DHPG+LY367385 $F_{(1,30)} = 0.6872$, $P < 0.4139$). LTP from H1-KO slices overexpressing Homer1c was also selectively blocked by MPEP and not LY367385 (Fig. 2B, KO+H1c+DHPG+MPEP vs. KO+H1c +DHPG, $F_{(1,12)} = 11.4$, $P < 0.0062$; KO+H1c+DHPG vs. KO+H1c+DHPG+LY367385: $F_{(1,11)} = 2.114$, $P < 0.1766$). Together, these results indicate that this form of plasticity is mGluR5 dependent as it has been shown previously (Bortolotto et al., 2005).

To demonstrate that Homer1c–mGluR5 direct interactions are required for the maintenance of LTP upon activation of mGluR1/5, we disrupted Homer1c/mGluR5 interactions in our KO+H1c mice with a peptide that mimics the carboxyterminal tail of mGluR5 (tatmGluR5) (Mao et al., 2005; Ronesi and Huber, 2008; Tronson et al., 2010; Ronesi et al., 2012). A control peptide was used that contains a dual-point mutation that makes it unable to bind Homer (tatmGluR5 mut) (Tronson et al., 2010). Acute hippocampal slices were stimulated (DHPG+0.5 TBS) in the presence of the mGluR5 peptides. We found that H1-KO+H1c slices were unable to maintain LTP in the presence of tatmGluR5 peptide but not tatmGluR5 mut (Fig. 3A, main effect of treatment H1-KO+H1c+DHPG vs. H1-KO+H1c+DHPG +tatmGluR5, $F_{(1,9)} = 6.663$, $P < 0.0326$; H1-KO+H1c+DHPG+ tatmGluR5 vs. H1-KO+H1c +DHPG+tat-mGluR5mut, $F_{(1,8)} = 6.912$, $P < 0.034$). These results indicate that Homer1c–mGluR5 interactions are necessary for the maintenance of mGluR-dependent LTP function.

Finally, as both the PI3K-mTOR and the MEK-ERK pathways have been implicated in mGluR1/5 function, we examined the putative involvement of each pathway in the rescue of LTP in H1-KO mice expressing Homer1c. A group of slices from animals in each group were treated with pharmacological blockers of PI3K signaling (wortmannin), and the MEK-ERK signaling MEK inhibitor (U0126) during LTP induction with DHPG. We found that both U0126 and wortmannin prevented the maintenance of LTP in H1-KO+H1c mice (Fig. 3B, main effect of treatment H1-KO+H1c+DHPG vs. H1-KO+H1c+DHPG+U0126, $F_{(1,9)} =$

5.882, $P < 0.0415$; H1-KO+H1c+DHPG vs. H1-KO+H1c+DHPG+wortmannin, $F_{(1,9)} = 16.28$, $P < 0.0024$). Both UO126 and wortmannin attenuated the induction of LTP during stimulation relative to control slices (Fig. 3C, $P < 0.001$, 2-min post-TBS). These results suggest that activation of both PI3K and ERK contributes to this form of mGluR1/5-dependent plasticity.

DISCUSSION

This study investigated the possible role of *Homer1* in the mGluR1/5 activation of a molecular switch that converts STP to LTP. The main purpose of using this form of synaptic plasticity is that it allowed us to dissect the cellular mechanism by which Homer1c might be involved in mGluR1/5 activation of synaptic plasticity by using H1-KO mice. We show that priming with the mGluR1/5 agonist DHPG produced a persistent LTP after induction with a below-threshold stimulus in wild-type mice. We also found that H1-KO animals showed deficits in this form of plasticity, being unable to maintain LTP. Gene targeting of Homer1c into the dorsal hippocampus of H1-KO mice reversed the deficits found in these animals to wild-type levels. Thus, Homer1c expression in the H1-KO hippocampus is sufficient for successful maintenance of mGluR-dependent LTP.

The importance of Homer1 isoform binding in the function of mGluR1/5 in heterologous cell lines and neuronal cell cultures is well documented, showing that Homer1c plays a key role in the trafficking, targeting, and positioning of mGluR1/5 (Tu et al., 1998; Roche et al., 1999; Ango et al., 2000) and in their insertion into the cell membrane (Ciruela et al., 1999). Furthermore, Homer1 binding to mGluR1/5 has been shown to be essential for hippocampal long-term depression (LTD) (Ronesi and Huber, 2008). However, the direct role of Homer binding to mGluR1/5 has not been studied in the context of mGluR-dependent LTP. We used a peptide that disrupts Homer–mGluR1/5 interactions and found that this peptide successfully disrupts the conversion of STP into LTP in the molecular switch paradigm, and thus demonstrating that direct interaction between Homer and mGluR5 is vital for mGluR-dependent LTP.

In addition, the signaling cascades activated by the molecular switch have not been fully elucidated. We found that blocking either the PI3K–mTOR or the MEK–ERK pathways prevented the maintenance of LTP. This is in agreement with the mechanism of mGluR1/5 activation in LTD, which has been shown to involve recruitment of both ERK and mTOR pathways. In the case of LTD, only the mTOR involves Homer1c (Ronesi and Huber, 2008), whereas our LTP studies suggest that both the mTOR and the ERK cascades involve Homer1c and might converge in a common end target. PI3K activation occurs at a point upstream of MEK activation in the amygdala following LTP-Inducing Tetanic Stimulation (Lin et al., 2001). Also, activation of PI3K is required for ERK/MAPK activation in contextual memory retrieval in the hippocampus (Chen et al., 2005). Akt activation has also been shown to be upstream from ERK activation of estrogen-induced enhancement of object recognition memory in the hippocampus (Fan et al., 2010), and in the protein synthesis-dependent induction of LTP (Gelinis et al., 2007). Thus, it is possible that both pathways act sequentially in the maintenance of LTP (Tsokas et al., 2007).

Both LM-WT and KO+H1c hippocampal slices primed with DHPG showed a significant increase in the induction of LTP during stimulation versus nonprimed slices (Figs. 1A, C). Further, blocking MEK or PI3K resulted in a decrease in LTP during 0.5 TBS+DHPG in KO+H1c (Fig. 3C), suggesting that activation of these kinases might be involved in the induction of LTP at the time of stimulation. Indeed, inhibition of MEK activation attenuates induction of LTP during stimulation by reducing the likelihood of action potential firing during TBS (Selcher et al., 2003). In this study, Selcher et al. also showed that *N*-methyl-D-aspartate (NMDA) block only partially attenuates the increased action potential firing probability during the period of stimulation, whereas blocking both NMDA and ERK activation further attenuated this action potential firing probability during TBS. These studies point to both NMDA- and ERK-dependent process as a mechanism for TBS induction of LTP. Other factors might contribute to the induction mechanism such as facilitation of the release of other neurotransmitters such as norepinephrine by NMDA activation (Cohen et al., 1999).

Our results shed light into the role of Homer1c–mGluR5 interactions in triggering molecular cascades that result in LTP, and might explain the role of Homer1c in restoring the learning and memory deficits in *Homer1* KO mice, and in aged, learning-impaired rats. Future experiments will define the downstream effector proteins activated by MEK and PI3K in mGluR-dependent LTP, and will also help define the role of Homer1c in cognitive impairment associated with neurological disorders toward finding therapeutic strategies based on the gene replacement or pharmaceutical intervention for memory loss.

DETAILED METHODS

Animal Subjects and Vector Injections

The mice used in these experiments have been described previously (Yuan et al., 2003). All animals were single housed under a 12/12-h light/dark cycle and given access to food and water ad libitum. Protocols were approved by the University of Wisconsin-Madison Animal Care and Use Advisory Committee and were in accordance with the guidelines established by the U.S. Public Health Policy on Humane Care and Use of Laboratory Animals.

rAAV was purified by iodixanol gradient purification followed by FPLC (Zolotukhin et al., 2002). Each animal was injected with a total of 2.4×10^{10} vector genomes of rAAV5–Homer1c or 1.8×10^{11} vector genomes of rAAV5–GFP. The rAAV–Homer1c vector and detailed procedure for vector injection has been described earlier (Gerstein et al., 2012).

Electrophysiology

For extracellular recordings of field excitatory postsynaptic potentials (fEPSP), acute hippocampal slices (400 μ m) were prepared from the experimental mice as described earlier (Gerstein et al., 2012). Enameled bipolar platinum–tungsten stimulating electrodes were placed in Schaffer collaterals. fEPSPs were recorded from CA1 stratum radiatum. Baseline responses were set to an intensity that evoked an fEPSP with a slope of 66% that of the maximum evoked response (Raymond et al., 2000). Half-Train theta burst (0.5 TBS)

consisted of a total of five bursts (each burst consisting of four stimulations at a frequency of 100 Hz) with an interburst interval of 200 ms.

LTP was induced by priming with DHPG followed by 0.5 TBS as follows. After obtaining a stable baseline, 10 μ M of DHPG was applied for 10 min to the chamber, followed by a 20-min washout to ensure return to baseline prior to the 0.5-theta burst stimulation. After electrophysiological recordings, slices were frozen and saved for Western blot analysis or fixed in paraformaldehyde and resectioned for immunohistochemistry to confirm transgene expression in the recorded slices. All numerical data were expressed as mean $\pm$ SEM. Data were analyzed by two-way ANOVA (treatment and time) with repeated measures (mixed model) and Bonferroni post hoc tests.

Drug Treatment

DHPG, MPEP, and LY367385 were purchased from Tocris Bioscience (Missouri, USA). 20 mM DHPG and 4 mM MPEP stock solutions were prepared in H₂O. 4 mM LY367385 stock solutions were prepared in dimethyl sulfoxide (DMSO). All solutions were stored in aliquots at -20°C . MPEP and LY367385 solutions were used within 4 weeks of time after preparation. DHPG solutions were used within 2 weeks of preparation.

Wortmannin (10 mM) and U0126 were purchased from Sigma (Missouri, USA). They were both diluted to the appropriate concentration in DMSO and used within 2 weeks of preparation. The tat-mGluR5 peptides were synthesized at the University of Wisconsin Biotechnology Center. Stock solutions were prepared in H₂O and used within 2 weeks of preparation. Peptides were used at a final concentration of 5 μ M in ACSF supplemented with 10 μ M of HEPES buffer, pH 7.4, and 0.05% of bovine serum albumin. The peptide sequences have been described earlier (Ronesi et al., 2012). All solutions were stored in aliquots at -20°C .

MPEP (40 μ M), LY367385 (100 μ M), U0126 (30 μ M), and wortmannin (500 nM) were applied 55 min before stimulation for 15 min (-55 to -40 min) removed from the bath solution and allowed to wash out for 10 min (-40 to -30 min). After washout of the drugs, the slices were exposed to DHPG for 10 min (-30 to -20 min). DHPG was then removed and allowed to wash out for 20 min before 0.5 TBS (-20 to stim). All drugs were added at least 30 minutes after a stable baseline was obtained.

The tat-mGluR5 peptides were added 35 min after the slices were added to the rigs and left on for at least 2 h before 0.5 TBS. The peptide was allowed to remain for the entire recording session.

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FIGURE 1.

Homer1c restores mGluR-dependent LTP in *Homer1* KO mice. (A) Priming with DHPG prior to 0.5 TBS results in robust LTP in slices from wild-type mice ($n = 17$ slices (from 9 mice)) relative to nonprimed slices ($n = 8(3)$). (B) H1-KO mice injected with GFP show an inability to transform an STP into LTP via activation of mGluR1/5 (KO+GFP+DHPG, $n = 13(6)$), KO+GFP ($n = 9(4)$). (C) H1-KO expressing Homer1c (KO+H1c+DHPG; $n = 5(3)$) show an enhanced ability to maintain a robust mGluR-dependent LTP relative to KO+H1c nonprimed slices ($n = 4(3)$). Black horizontal line indicates time period of 10 μ M DHPG application. Half-train of TBS stimulation is applied at a of time 0 min. Top: representative traces at time of 0.5 TBS and at the end of the recording (120 min; vertical scale bars 1 mV, horizontal bars, 1 msec).

FIGURE 2.

Facilitation of LTP by DHPG is dependent on mGluR5 but not on mGluR1 activation (A) in wild type (WT+MPEP, $n = 14(6)$; WT+LY367385 $n = 14(6)$, (B) and Homer1 KO mice overexpressing Homer1c (KO+H1c+MPEP, $n = 8(3)$; KO+H1c+LY367385 $n = 7(3)$. Hatched lines indicate the time of exposure to the mGluR antagonists. Black line indicates DHPG application time. Top: representative traces at time of 0.5 TBS and at the end of the recording (120 min; vertical scale bars 1 mV, horizontal bars, 1 msec.).

FIGURE 3.

(A) Homer1c–mGluR5 interactions are necessary to maintain LTP in H1-KO mice overexpressing Homer1c. KO+H1c +DHPG, $n = 5(3)$, KO+H1c +DHPG+tatmGluR5 mut, $n = 4(2)$, KO+H1c+DHPG+tatmgluR5, $n = 5(2)$. (B) Both the MEK-ERK and the PI3K-mTOR signaling pathways contribute to mGluR-dependent LTP. KO+H1c+DHPG, $n = 5(3)$, KO+H1c+DHPG+U0126, $n = 5(3)$, KO+H1c+DHPG+ wortmannin, $n = 5(3)$. Top: representative traces at time of 0.5 TBS and at the end of the recording (120 min; vertical scale bars 1 mV, horizontal bars, 1 msec.). Hatched lines indicate the time of exposure to the peptides (A), or wortmannin and UO126 (B). Black line indicates DHPG application time.