Int J Physiol Pathophysiol Pharmacol 2013;5(3):169-176
www.ijppp.org /ISSN:1944-8171/IJPPP1307006

Original Article

Regulation of $GABA_A$ receptors by fragile X mental retardation protein

Baosong Liu[1*], Lijun Li[1*], Juan Chen[2], Zefen Wang[2], Zhiqiang Li[2], Qi Wan[1,2,3]

[1]Toronto Western Research Institute, University of Toronto, Toronto, Ontario, Canada, M5T 2S8; [2]Department of Physiology, School of Medicine, Wuhan University, 185 Donghu Road, Wuhan 430071, China; [3]University of Nevada School of Medicine, 1664 North Virginia Street, MS0352, Reno, NV 89557. [*]These authors contributed equally to this work.

Received July 18, 2013; Accepted August 2, 2013; Epub September 10, 2013; Published September 15, 2013

Abstract: Fragile X syndrome (FXS) is caused by the loss of fragile X mental retardation protein (FMRP). The deficiency of $GABA_A$ receptors ($GABA_A$Rs) is implicated in FXS. However, the underlying mechanisms remain unclear. To investigate the effect of FMRP on $GABA_A$Rs, we transfected FMRP cDNAs in rat cortical neurons. We measured the protein expression of $GABA_A$Rs and phosphatase PTEN, and recorded $GABA_A$R-mediated whole-cell currents in the transfected neurons. We show that the transfection of FMRP cDNAs causes increased protein expression of $GABA_A$Rs in cortical neurons, but $GABA_A$R-mediated whole-cell currents are not potentiated by FMRP transfection. These results suggest the possibility that intracellular signaling antagonizing $GABA_A$R activity may play a role in inhibiting $GABA_A$R function in FMRP-transfected neurons. We further show that FMRP transfection results in an enhanced protein expression of PTEN, which contributes to the inhibition of $GABA_A$R function in FMRP-transfected neurons. These results indicate that $GABA_A$Rs are regulated by FMRP through both an up-regulation of $GABA_A$R expression and a PTEN enhancement-induced inhibition of $GABA_A$R function, suggesting that an abnormal regulation of $GABA_A$R and PTEN by the loss of FMRP underlies the pathogenesis of FXS.

Keywords: Fragile X syndrome, fragile X mental retardation protein, $GABA_A$ receptor, PTEN

Introduction

Fragile X syndrome (FXS), caused by the loss of fragile X mental retardation protein (FMRP), is the most common inherited form of mental retardation [1-3]. The trinucleotide CGG expansion that inactivates the fragile X mental retardation 1 (*FMR1*) gene prevents the expression of the encoded FMRP protein [4]. FMRP is a selective RNA-binding protein that regulates the local translation of a subset of mRNAs at synapses [5]. The major symptoms of FXS are mental retardation, autistic behaviors, attention deficit, hyperactivity, alteration in sleep patterns and epileptic seizures [6, 7].

The γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) [8]. The GABA subtype A receptors ($GABA_A$Rs) are generally localized on the postsynaptic membranes and responsible for most fast inhibitory synaptic transmission in the CNS through opening bicuculline-sensitive Cl^- channels [9]. $GABA_A$Rs are assembled from several different classes of subunits (α1-6, β1-3, γ1-3, δ, θ, π and ε) and the α1β2γ2 combination is the most abundant $GABA_A$Rs expressed in the brain [10, 11]. Recent evidence indicates that the mRNA levels of seven subunits of $GABA_A$R, including α1, α3, α4, β1, β2, γ1 and γ2, are decreased in the cortex of *FMR1* knockout mice [12]. The protein level of $GABA_A$R β subunit is also reduced in cortex, hippocampus, diencephalons and brainstem of fragile X mice [13]. Electrophysiological studies suggest that the GABAergic efficiency and the tonic $GABA_A$R currents may be suppressed in the fragile X mice [14-16]. Moreover, anatomical defects have been observed in the neocortical GABAergic inhibitory circuits [16]. In agreement with the alterations of $GABA_A$Rs, the ratio between inhibitory (taurine and GABA) and excitatory (aspartate and glutamate) amino acids is decreased in brainstem, hippocampus and caudal cortex of fragile X mouse [17]. These findings suggest that the absence of FMRP may

be involved in mediating the suppressed activities of $GABA_A$Rs in FXS. As dysfunction of $GABA_A$R channels is implicated in symptoms that are also disturbed in fragile X patients, such as anxiety, depression, epilepsy, insomnia, and learning and memory [18], it is likely that the decreased $GABA_A$R function may underlying the behavioral and epileptic phenotype associated with FXS [19].

PTEN (Phosphatase and tensin homolog deleted on chromosome ten) is a dual-specificity phosphatase [20]. Recently, we have provided evidence that PTEN can positively regulate both the expression and function of excitatory NMDA receptors in rat hippocampal neurons [21, 22]. Suppressing PTEN protects ischemia-induced neuronal death through both inhibiting NMDA receptor-mediated excitotoxicity and enhancing activity of cell survival-promoting kinase Akt [21, 22]. We also showed that PTEN negatively regulates $GABA_A$R function in hippocampal neurons [23].

To reveal the pathogenesis of FXS, a necessary step is to understand the biological role of FMRP in the CNS. We therefore set up to test the interactions among FMRP, $GABA_A$R and PTEN in an experimental model with FMRP overexpression in cultured rat cortical neurons.

Materials and methods

Cortex neuronal culture

Cortex neuronal cultures were prepared from Wistar rats on gestation day 18 [24]. Dissociated neurons were suspended in plating medium (Neurobasal medium, 2% B-27 supplement, 0.5% FBS, 0.5 μM L-glutamine, and 25 μM glutamic acid) and transferred to poly-D-lysine-coated coverslips in 35mm Petri dishes. After 3 d *in vitro* (DIV), half of the plating medium was removed and replaced with maintenance medium (Neurobasal medium, B-27 supplement, and 0.5 μM L-glutamine). Medium replacement was performed every 3-4 d, and cells were used at 12-15 DIV.

Immunofluorescent labeling, image acquisition and analysis

To examine the surface expression of $GABA_A$R γ2 subunits, nonpermeabilized cells were labeled with rabbit anti-$GABA_A$R γ2 antibody (Millipore Corporation, Billerica, MA), and Alexa Fluor 594 (red fluorescence) secondary antibody (Invitrogen, Burlington, Ontario, Canada). The detailed methods of surface receptor labeling are described in our previous studies [25]. To examine FMRP or PTEN expression, cells were permeabilized with 4% paraformaldehyde/PBS and 0.3% Triton X-100, and then labeled with rabbit anti-PTEN antibody (Cell Signaling Technology, Inc. Danvers, MA) or rabbit anti-FMRP polyclonal antibody (Abcam, Cambridge, MA).

Fluorescence-labeled neurons were imaged using a Zeiss LSM 510 META confocal microscope (Carl Zeiss, Germany) and analyzed as described previously [25-28]. Images were acquired using a Zeiss AxioCam digital camera in the linear range with constant settings. Each image was a *z*-series of 6-13 images, taken at 0.75-μm-depth intervals. The resultant stack was "flattened" into a single image using a maximum projection. For all experiments, we analyzed fluorescent signal in regions of interest by measuring the average fluorescence intensity per unit area. All images in all experiments were analyzed using identical acquisition parameters. During data acquisition and analysis, the investigator was blind to the treatment group. In each experiment, neurons were selected randomly under bright-field optics, and fluorescent images of each neuron acquired from a single plane were transferred for analysis. The cells in control and OGD groups from the same culture preparation were processed and imaged in parallel. Three fields were randomly selected in each culture. The fluorescence density was analyzed by Image J software (NIH) [25, 29, 30]. All the immunolabeling experiments were repeated using neuronal cultures prepared from 5-8 animals. The expression of surface receptors and whole-cell proteins represented by labeled fluorescence densities in treated groups was normalized versus that in control groups. The *n* value refers to the number of cells analyzed.

Transfection

The transfection of GFP (green fluorescence protein) cDNA, wild-type FMRP-GFP (FMRP-GFP) cDNA, scrambled PTEN siRNA (SsiRNApten) or PTEN siRNA (siRNApten) in cultured cortical neurons was done using Lipofectamine 2000 (Invitrogen) as described previously [31],

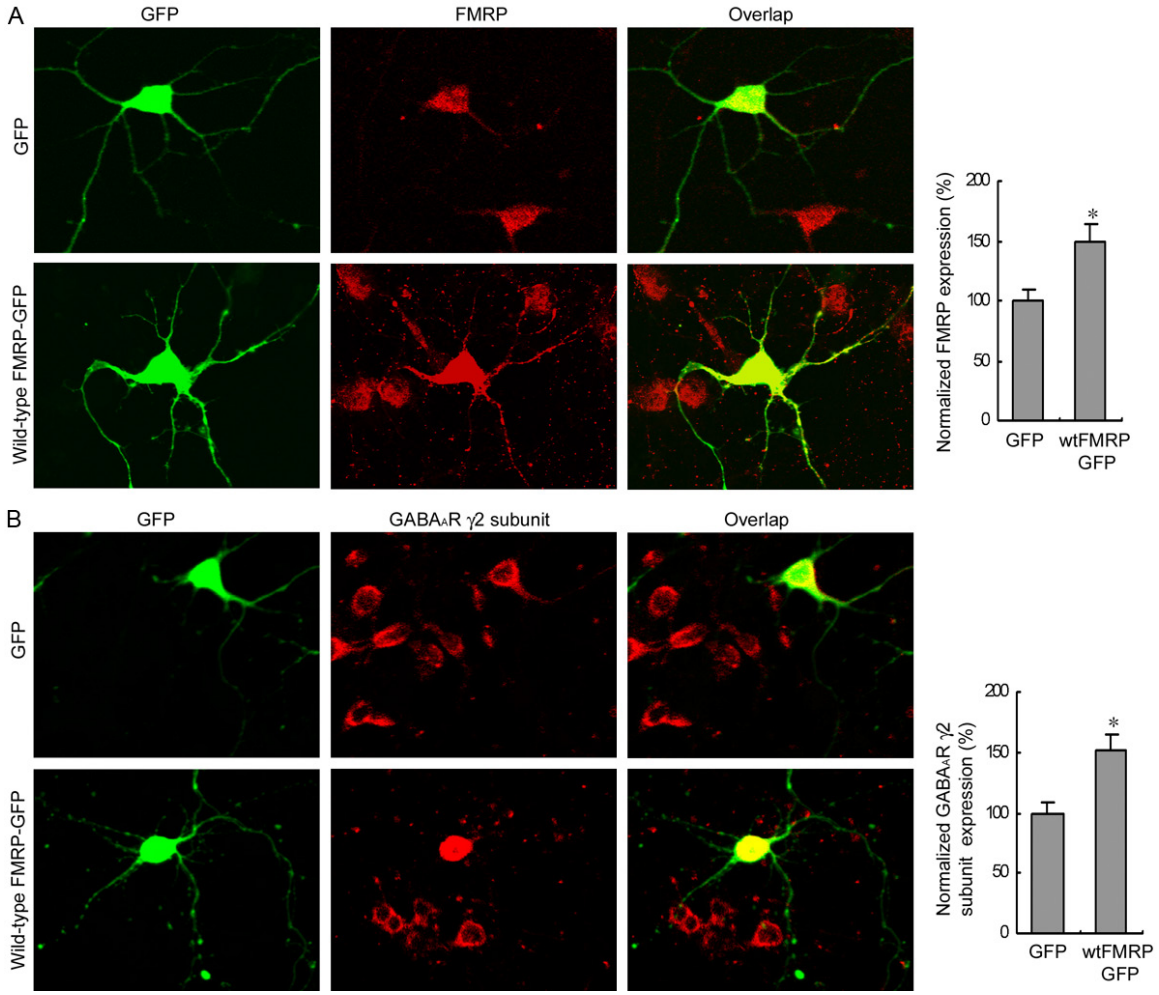

Figure 1. The surface expression of $GABA_A$R γ2 subunits is increased by FMRP upregulation. A: Immunofluorescent staining of FMRP (red) in neurons transfected with cDNAs of GFP and wild-type FMRP-GFP, respectively. Summarized data show that the expression of FMRP is increased in cultured cortical neurons transfected with FMRP-GFP (n=7 for both groups, *p<0.05, Student's t test). B: Non-permeable immunofluorescent staining of membrane surface $GABA_A$R γ2 subunits (red) in neurons transfected with cDNAs of GFP and FMRP-GFP, respectively. Summarized data indicate that FMRP upregulation increases γ2 surface expression in cortical neurons transfected with cDNAs of FMRP-GFP (n=6 for both groups, *p<0.05, Student's t test).

GFP positive cells were selected for immunostaining analysis.

Recording of $GABA_A$R-mediated whole cell currents

Whole-cell patch-clamp recording was performed as described previously [21, 25]. The recording electrode was filled with solution containing 140 mM CsCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM EGTA, 10 mM HEPES, 4 mM K2ATP, with pH=7.3, osmolality=280-290 mOsm and resistance=3-5 MΩ. The extracellular solution contains (in mM): 140 NaCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 5.0 KCl, 25 HEPES, 33 glucose (pH 7.35, osmolarity 320 mOsm/L). BpV(pic) (CalBiochem, EMD Chemicals, Inc. San Diego, CA) was added into the pipette filling solution. TTX (0.5 μM) was added into the bath solution to block voltage-gated sodium channels. Neurons were held at -60 mV under voltage clamp. $GABA_A$R-mediated whole-cell currents were recorded by pressure application of 100 μM GABA (20 kPa, 20 ms) from a micropipette with its tip located ~20 μm from the recorded cell. GABA were delivered at intervals of 30 s. Data were acquired with an Axopatch 200B amplifier and pClamp 10 software interfaced to a Digidata 1322A acquisition board (Molecular Devices, CA), and

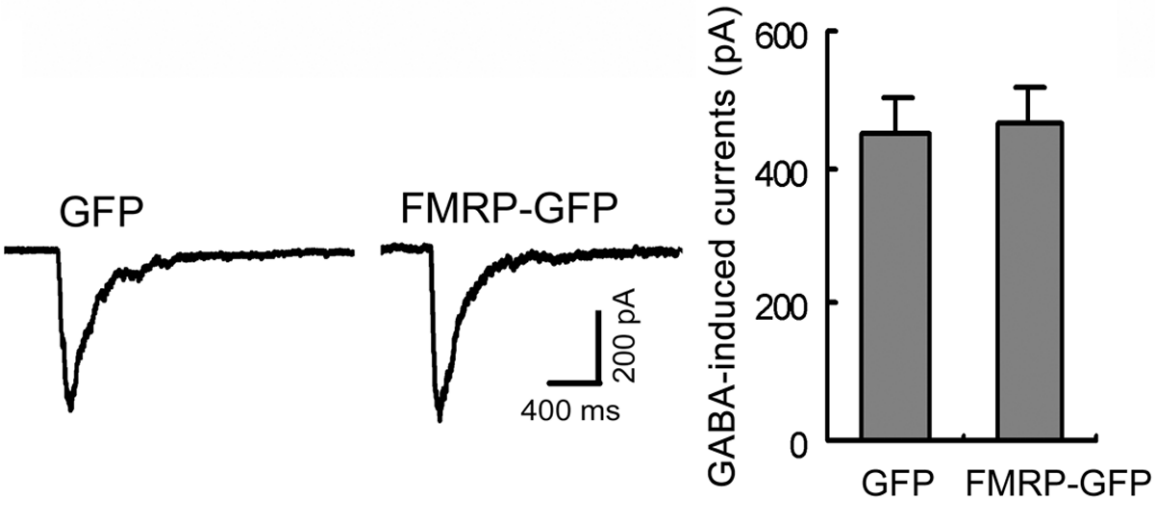

Figure 2. The $GABA_A$R-mediated whole-cell currents are not altered by FMRP upregulation. Left, sample traces of $GABA_A$R-mediated whole-cell currents recorded in neurons transfected with GFP and FMRP-GFP, respectively. Right, the summarized data show that FMRP does not alter $GABA_A$R-mediated whole-cell currents (n=6 for GFP group, n=8 for FMRP-GFP group; *p<0.05, Student's t test).

signals were filtered at 2 kHz and digitized at 10 kHz.

Statistics

Student's t test or ANOVA test was used where appropriate to examine the statistical significance of the differences between groups of data. Significance was placed at p<0.05.

Results

FMRP enhances the surface expression of $GABA_A$Rs

To determine whether FMRP regulates $GABA_A$Rs, we examined the membrane expression of $GABA_A$Rs in FMRP-overexpressed cortical neurons. A non-permeable staining method was used to examine the surface expression of $GABA_A$R γ2 subunits in the cultured cortical neurons transfected with wild-type FMRP cDNAs that was conjugated with GFP [25]. As illustrated in **Figure 1A**, neurons transfected with FMRP-GFP exhibit increased expression of FMRP protein. Using a polyclonal antibody against the extracellular N-terminus of $GABA_A$R γ2 subunit, we showed that the surface expression of γ2 subunits was significantly increased in neurons transfected with FMRP-GFP, compared with that in neurons transfected with GFP alone (**Figure 1B**). These data indicate that FMRP can positively regulate the protein expression of $GABA_A$R γ2 subunits in the membrane surface of cortical neurons.

FMRP does not alter the function of $GABA_A$Rs

As the increased surface $GABA_A$R expression might contribute to an enhanced function of these channels, we recorded $GABA_A$R-mediated whole-cell currents in cultured cortical neurons transfected with FMRP-GFP or GFP alone. Surprisingly, our results showed that $GABA_A$R-mediated currents were not significantly increased in neurons transfected with FMRP-GFP compared with neurons transfected with GFP alone (**Figure 2**). Among many possibilities, a simple explanation for this result is that the FMRP up-regulation of $GABA_A$R function may be antagonized by intracellular signaling that are also regulated by FMRP.

FMRP increases PTEN expression

Our recent study shows that the phosphatase PTEN negatively regulates $GABA_A$Rs in rat hippocampal neurons [23]. We therefore hypothesized that the enhancement of $GABA_A$R function by FMRP may be suppressed by the increased PTEN expression in FMRP-overexpressed neurons. Indeed, our results showed that FMRP overexpression significantly increased protein expression of PTEN in cultured neurons (**Figure 3**). These data suggest that the increased PTEN expression in FMRP-over-

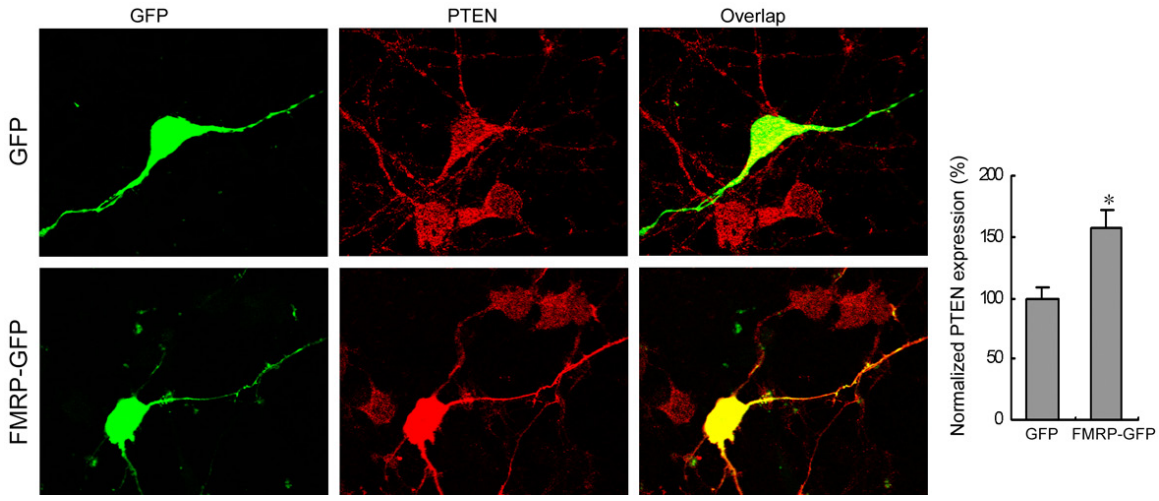

Figure 3. FMRP overexpression enhances PTEN expression in cortical neurons. Left, representative images showing immunofluorescent staining of PTEN (red) in neurons transfected with cDNAs of GFP and FMRP-GFP, respectively. Right, summarized data show that PTEN expression is increased in FMRP-overexpressed neurons (n=7 for GFP group, n=6 for FMRP-GFP group; $p<0.05$, Student's *t* test).

expressed neurons may inhibit $GABA_AR$ function.

FMRP suppresses $GABA_AR$ function through upregulation of PTEN

To determine whether an increased PTEN expression in FMRP-overexpressed cortical neurons could inhibit $GABA_AR$ function, we tested the effects of PTEN inhibitor BpV(pic) on $GABA_AR$-mediated whole-cell currents in cultured neurons transfected with cDNAs of GFP or FMRP-GFP [32, 33]. Our data showed that PTEN inhibition by BpV(pic) significantly increased the peak currents of $GABA_ARs$ in neurons transfected with FMRP-GFP (**Figure 4A**), suggesting that the upregulation of endogenous PTEN by FMRP inhibits $GABA_AR$ function in cortical neurons. Thus, the elevated PTEN expression counteracts the effect of FMRP-induced increase of $GABA_AR$ expression. To provide evidence that BpV(pic) is a specific PTEN inhibitor in rat cortical neurons, neurons transfected with scrambled PTEN siRNA (SsiRNApten) or PTEN siRNA (siRNApten) were treated with BpV(pic). We showed that while SsiRNApten had no effect on BpV(pic)-induced potentiation of $GABA_AR$ currents, siRNApten introduction occluded BpV(pic)-induced potentiation of $GABA_AR$ currents (**Figure 4B-D**), indicating the specificity of BpV(pic) in inhibiting PTEN activity in our experimental conditions. Taken together, this study reveals that $GABA_ARs$ are regulated by FMRP through both an up-regulation of $GABA_AR$ expression and a PTEN enhancement-induced inhibition of $GABA_AR$ function.

Discussion

Epileptic seizure is a disorder of recurrent, spontaneous episodes of aberrant synchronization in neural networks [34]. It has been reported that about 10-20% of FXS patients suffered from seizures [35]. Increasing evidence suggests that $GABA_AR$ deficiency may contribute to the occurrence of epileptic seizures in FXS [35]. Based on our experimental evidence obtained from the FMRP overexpression model, we reason that the absence of FMRP in FXS may lead to reduced protein expression of both $GABA_ARs$ and PTEN. As PTEN suppression can potentiate $GABA_AR$ function, the effect of suppressed $GABA_AR$ expression in FMRP-deficient neurons that is supposed to cause increased seizure occurrence, would be antagonized by FMRP loss-induced PTEN suppression. If this is true, the PTEN inhibition-mediated $GABA_AR$ upregulation may explain in part why only 10-20% of fragile X patients have seizure occurrence [35].

Our previous study demonstrates that PTEN increases NMDA receptor activity by physically associating with NR2B-containing NMDA receptors [21, 22]. It is possible that FMRP loss-induced PTEN suppression may also act

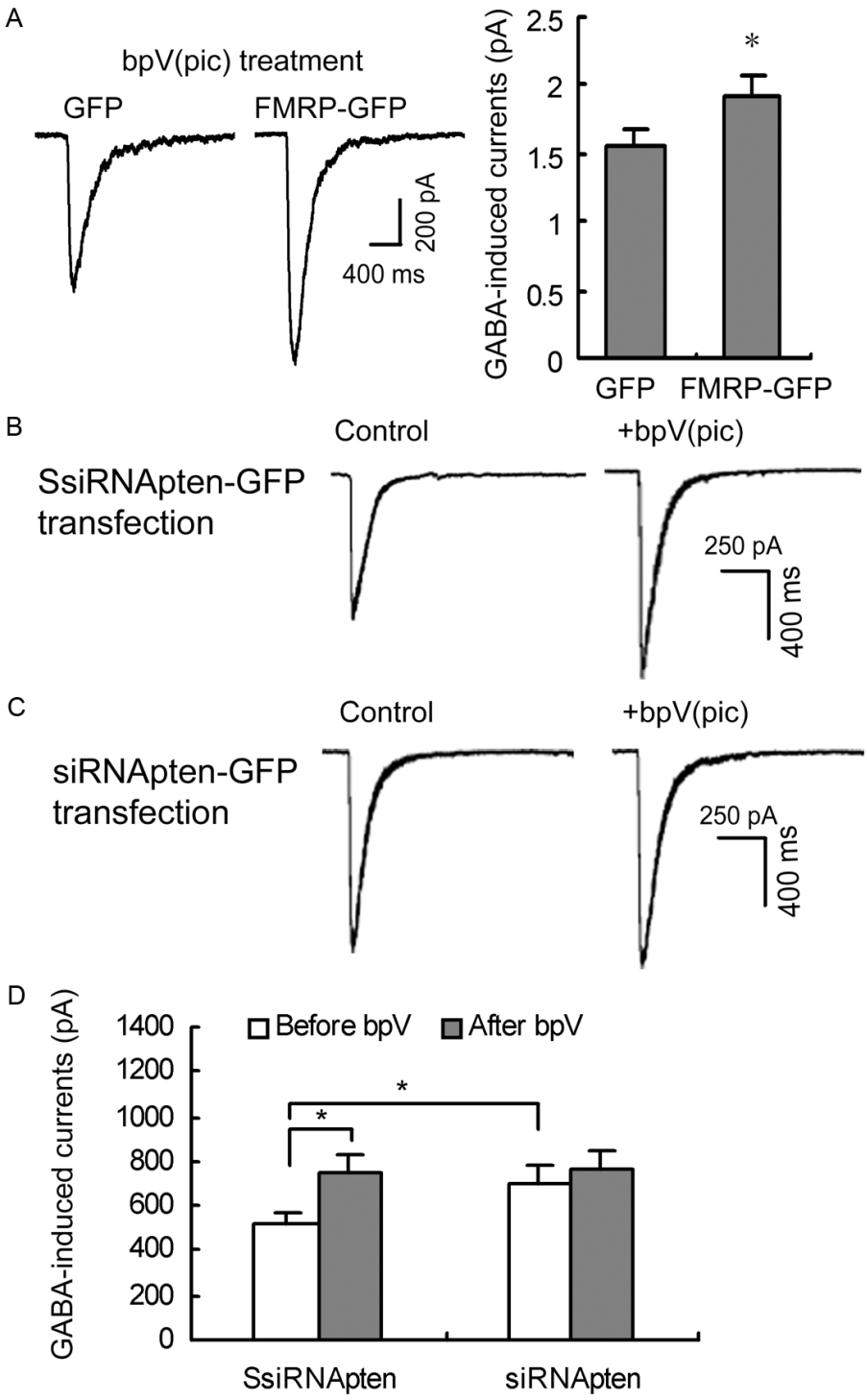

Figure 4. FMRP inhibits $GABA_AR$ function through upregulation of PTEN. A: Sample traces of $GABA_AR$-mediated whole-cell currents recorded in neurons transfected with GFP and FMRP-GFP, respectively. Summarized data show that PTEN inhibition by bpV(pic) increases the peak amplitude of $GABA_AR$ currents in neurons transfected with FMRP-GFP (n=7 for GFP group, n=8 for FMRP-GFP group; *p<0.05, Student's t test). B & D: SsiRNApten transfection has no effect on bpV(pic)-induced potentiation of $GABA_AR$ currents (n=7 for GFP group, n=7 for FMRP-GFP group; *p<0.05, ANOVA test). C & D: siRNApten introduction occludes bpV(pic)-induced potentiation of $GABA_AR$ currents (n=8 for GFP group, n=8 for FMRP-GFP group; *p<0.05, ANOVA test).

through inhibiting NMDA receptor-mediated excitatory activity to counteract seizure occurrence in FXS.

Yet, it is unclear how PTEN exerts its effect on $GABA_ARs$. Our future studies will investigate whether PTEN regulates $GABA_AR$ function through a direct protein-protein interaction as PTEN regulation of NMDA receptors [21]. If not, intracellular signaling mediates PTEN regulation of $GABA_ARs$ will be investigated. We will also investigate in detail whether the channel properties of $GABA_ARs$ and the $GABA_AR$-mediated synaptic responses are regulated by FMRP.

In summary, the present study provides evidence that FMRP and PTEN play opposite roles in regulating $GABA_ARs$ in cortical neurons. While FMRP enhances $GABA_AR$ expression, it also increases the protein expression of PTEN, which in turn antagonizes FMRP-induced potentiation of $GABA_ARs$. These results suggest that PTEN downregulation may

play a protective role in reducing $GABA_AR$ deficiency-induced incidence of epileptic seizures in FXS.

Acknowledgements

This work was supported by grant from the Canadian Institutes of Health Research (CIHR) to Q.W. and postdoctoral fellowship awards from Fragile X Research Foundation of Canada and CIHR to B.L. and L.L. We thank Michael Yixiang Wan for the review of the manuscript.

Address correspondence to: Dr. Qi Wan, Department of Physiology, School of Medicine, Wuhan University, 185 Donghu Road, Wuhan 430071, China. Tel: 027-68759392; Fax: 027-68758766; E-mail: qwan@whu.edu.cn

References

[1] O'Donnell WT and Warren ST. A decade of molecular studies of fragile X syndrome. Annu Rev Neurosci 2002; 25: 315-38.

[2] Bardoni B, Davidovic L, Bensaid M, Khandjian EW. The fragile X syndrome: exploring its molecular basis and seeking a treatment. Expert Rev Mol Med 2006; 8: 1-16.

[3] Crawford DC, Acuna JM and Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. Genet Med 2001; 3: 359-71.

[4] Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 1991; 65: 905-14.

[5] Bassell GJ and Warren ST. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron 2008; 60: 201-14.

[6] Oostra BA and Chiurazzi P. The fragile X gene and its function. Clin Genet 2001; 60: 399-408.

[7] Musumeci SA, Hagerman RJ, Ferri R, Bosco P, Dalla Bernardina B, Tassinari CA, De Sarro GB, Elia M. Epilepsy and EEG findings in males with fragile X syndrome. Epilepsia 1999; 40: 1092-9.

[8] Sivilotti L and Nistri A. GABA receptor mechanisms in the central nervous system. Prog Neurobiol 1991; 36: 35-92.

[9] Mody I, De Koninck Y, Otis TS, Soltesz I. Bridging the cleft at GABA synapses in the brain. Trends Neurosci 1994; 17: 517-25.

[10] Brandon N, Jovanovic J and Moss S. Multiple roles of protein kinases in the modulation of gamma-aminobutyric acid(A) receptor function and cell surface expression. Pharmacol Ther 2002; 94: 113-22.

[11] McKernan RM and Whiting PJ. Which GABAA-receptor subtypes really occur in the brain? [see comments]. Trends Neurosci 1996; 19: 139-43.

[12] D'Hulst C, De Geest N, Reeve SP, Van Dam D, De Deyn PP, Hassan BA, Kooy RF. Decreased expression of the GABAA receptor in fragile X syndrome. Brain Res 2006; 1121: 238-45.

[13] El Idrissi A, Ding XH, Scalia J, Trenkner E, Brown WT, Dobkin C. Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. Neurosci Lett 2005; 377: 141-6.

[14] Curia G, Papouin T, Séguéla P, Avoli M. Downregulation of Tonic GABAergic Inhibition in a Mouse Model of Fragile X Syndrome. Cereb Cortex 2009; 19: 1515-20.

[15] D'Antuono M, Merlo D and Avoli M. Involvement of cholinergic and gabaergic systems in the fragile X knockout mice. Neuroscience 2003; 119: 9-13.

[16] Selby L, Zhang C and Sun QQ. Major defects in neocortical GABAergic inhibitory circuits in mice lacking the fragile X mental retardation protein. Neurosci Lett 2007; 412: 227-32.

[17] Gruss M and Braun K. Age- and region-specific imbalances of basal amino acids and monoamine metabolism in limbic regions of female Fmr1 knock-out mice. Neurochem Int 2004; 45: 81-8.

[18] Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. Proc Natl Acad Sci U S A 1999; 96: 12905-10.

[19] D'Hulst C, Heulens I, Brouwer JR, Willemsen R, De Geest N, Reeve SP, De Deyn PP, Hassan BA, Kooy RF. Expression of the GABAergic system in animal models for fragile X syndrome and fragile X associated tremor/ataxia syndrome (FXTAS). Brain Res 2009; 1253: 176-83.

[20] Maehama T and Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 1998; 273: 13375-8.

[21] Ning K, Pei L, Liao M, Liu B, Zhang Y, Jiang W, Mielke JG, Li L, Chen Y, El-Hayek YH, Fehlings MG, Zhang X, Liu F, Eubanks J, Wan Q. Dual neuroprotective signaling mediated by downregulating two distinct phosphatase activities of PTEN. J Neurosci 2004 Apr 21; 24: 4052-60.

[22] Chang N, El-Hayek YH, Gomez E, Wan Q. Phosphatase PTEN in neuronal injury and brain disorders. Trends Neurosci 2007; 30: 581-6.
[23] Liu B, Li L, Zhang Q, Chang N, Wang D, Shan Y, Li L, Wang H, Feng H, Zhang L, Brann DW, Wan Q. Preservation of GABAA receptor function by PTEN inhibition protects against neuronal death in ischemic stroke. Stroke 2010; 41: 1018-26.
[24] Brewer GJ, Torricelli JR, Evege EK, Price PJ. Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. J Neurosci Res 1993; 35: 567-76.
[25] Liu B, Liao M, Mielke JG, Ning K, Chen Y, Li L, El-Hayek YH, Gomez E, Zukin RS, Fehlings MG, Wan Q. Ischemic insults direct glutamate receptor subunit 2-lacking AMPA receptors to synaptic sites. J Neurosci 2006; 26: 5309-19.
[26] Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997; 275: 1943-7.
[27] Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, Malenka RC. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat Neurosci 2000; 3: 1291-300.
[28] Passafaro M, Piech V and Sheng M. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat Neurosci 2001; 4: 917-26.
[29] Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF. Internalization of ionotropic glutamate receptors in response to mGluR activation. Nat Neurosci 2001; 4: 1079-85.
[30] Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, Adams SR, Garner CC, Tsien RY, Ellisman MH, Malenka RC. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. Nat Neurosci 2004; 7: 244-53.
[31] Ning K, Pei L, Liao M, Liu B, Zhang Y, Jiang W, Mielke JG, Li L, Chen Y, El-Hayek YH, Fehlings MG, Zhang X, Liu F, Eubanks J, Wan Q. Dual neuroprotective signaling mediated by down-regulating two distinct phosphatase activities of PTEN. J Neurosci 2004; 24: 4052-60.
[32] Schmid AC, Byrne RD, Vilar R, Woscholski R. Bisperoxovanadium compounds are potent PTEN inhibitors. FEBS Lett 2004; 566: 35-8.
[33] Rickle A, Behbahani H, Ankarcrona M, Winblad B, Cowburn RF. PTEN, Akt, and GSK3beta signalling in rat primary cortical neuronal cultures following tumor necrosis factor-alpha and trans-4-hydroxy-2-nonenal treatments. J Neurosci Res 2006; 84: 596-605.
[34] Noebels JL. Targeting epilepsy genes. Neuron 1996; 16: 241-4.
[35] Berry-Kravis E. Epilepsy in fragile X syndrome. Dev Med Child Neurol 2002; 44: 724-8.