Bryostatin-1 Restores Hippocampal Synapses and Spatial Learning and Memory in Adult Fragile X Mice

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ABSTRACT

Fragile X syndrome (FXS) is caused by transcriptional silencing in neurons of the FMR1 gene product, fragile X mental retardation protein (FMRP), a repressor of dendritic mRNA translation. The lack of FMRP leads to dysregulation of synaptically driven protein synthesis and impairments of intellect, cognition, and behavior, a disorder that currently has no effective treatments. Fragile X mice were treated with chronic bryostatin-1, a selective protein kinase C (PKC) activator with pharmacological profiles of rapid mGluR desensitization, synaptogenesis, and synaptic maturation/repairing. Differences in the major FXS phenotypes, synapses, and cognitive functions were evaluated and compared among the age-matched groups. Long-term treatment with bryostatin-1 rescues adult fragile X mice from the disorder phenotypes, including normalization of most FXS abnormalities in hippocampal brain–derived neurotrophic factor expression and secretion, postsynaptic density–95 levels, glycogen synthase kinase–3β phosphorylation, transformation of immature dendritic spines to mature synapses, densities of the presynaptic and postsynaptic membranes, and spatial learning and memory. Our results show that synaptic and cognitive function of adult FXS mice can be normalized through pharmacologic treatment and that bryostatin-1-like agents may represent a novel class of drugs to treat fragile X mental retardation even after postpartum brain development has largely completed.

Introduction

Fragile X syndrome (FXS) is the most common monogenically inherited form of intellectual disability (Kaufmann et al., 1999; Santoro et al., 2012). The FXS includes general deficits in cognition (Van der Molen et al., 2010; Yang et al., 2013), abnormal in memory (Koekkoek et al., 2005), and anxiety and autistic-like behavior (Sabaratnam et al., 2003; Garber et al., 2008).

In nearly all of the cases, FXS is caused by an expansion of an untranslated CGG repeat in the 5′-untranslated region of the X-linked gene fragile mental retardation 1 (FMR1; Verkerk et al., 1991; Ludwig et al., 2014). The expansion leads to DNA methylation of FMR1 and transcriptional silencing, thus loss of the fragile X mental retardation protein (FMRP). FMRP is a selective RNA-binding protein (Schaeffer et al., 2003), regulating the translation of dendritic mRNAs (Ashley et al., 1993; Darnell and Klann, 2013; Goncalves et al., 2013).

The lack of FMRP alters signal processing at synapses, such as that involving metabotropic glutamate receptor (mGluR) signaling (Weiler et al., 1997; Lüscher and Huber, 2010; Bhatacharya and Klann, 2012). The leading “mGluR theory of FXS” proposes that overactive mGluR signaling, normally balanced by FMRP, underlies much of the brain changes of FXS (Bhogal and Jongens, 2010; McLennan et al., 2011; Santoro et al., 2012; Hajós, 2014). Indeed, a mouse model that combines FMR1 inactivation with a 50% reduction in mGluR5 expression rescues several anatomic and behavioral consequences of FXS. mGluR5 antagonism has also been shown effective in several FXS phenotypes (Connor et al., 2011; Vinuexa Veloz et al., 2012; Hajós, 2014).

One of the major goals of FXS research is to develop effective therapies. Despite extensive efforts, therapeutic options for FXS remain limited. Although preclinical studies with mGluR5 antagonism appear promising, therapeutic values of mGluR inhibitors for FXS are still not clear. One problem with inhibition of the mGluR signaling, for instance, is that the inhibition exaggerates spine immaturity in the fragile X mice (Cruz-Martin et al., 2010), an effect opposite to the intended therapeutic outcomes. Here, we show that bryostatin-1 (Nelson et al., 2009; DeChristopher et al., 2012), a highly potent and relatively specific protein kinase C (PKC) ε activator (also of PKCζ), with pharmacologic profiles of rapid mGluR desensitization, synaptogenesis, and synaptic maturation/repairing (Hongpaisan and Alkon, 2007; Sun et al., 2008; Hongpaisan et al., 2011), rescues synaptic and memory functions and other phenotypic features in adult fragile X mice. Bryostatin-1 has a much lower ED₅₀ for inducing PKCζ translocation than its ED₅₀s for PKCζ or PKCβ (Szallasi et al., 1994). The structural features of the bryostatins have been well defined by leading experts in

The authors claim no conflict of interest.

AABBREVIATIONS: BDNF, brain-derived neurotrophic factor; Dil, 1,1′-dioctadecyl-3,3′,3′-tetramethylindocarbocyanine perchlorate; ELISA, enzyme-linked immunosorbent assay; FMR1, fragile mental retardation gene 1; FMRP, fragile X mental retardation protein; FXS, fragile X syndrome; GAP, growth-associated protein; GSK, glycogen synthase kinase; mGluR, metabotropic glutamate receptor; PBS, phosphate-buffered saline; PKC, protein kinase C; PSD, postsynaptic density; TBS-T, Tris-buffered saline plus 0.1% Tween 20.
function-oriented synthesis (Wender et al., 1988, 2011). Bryostatin-1–like agents may represent a novel class of drugs for treating FXS.

Materials and Methods

Animals and Drug Treatment. Two types of mice (male; The Jackson Laboratory, Bar Harbor, ME; 9 or 10/group) were used as follows: FVB:129P2-Pdcd6b 9 TgMyr-Ch/Fnr1cm1UC/J (formerly identified as FVB:129P2-Fnr1cm1UC/J or fragile X mice) and FVB:129P2-Pdcd6b 9 TgMyr-Ch/Anj (as the controls). These mice do not suffer from blindness. They were housed in a temperature-controlled (20–24°C) room for at least a week, allowed free access to food and water, and kept on a 12-hour light/dark cycle.

All mice were randomly assigned to different groups. We began to treat the fragile X mice when they reached an age of 2 months with bryostatin-1 (20 µg/mg, tail i.v., two doses/week for 13 weeks). The dose was chosen, based on our preliminary dose-response studies showing that smaller doses were not effective against induced synaptic and cognitive impairments. Nontreated groups received the same volume of vehicle at the same frequency. Synaptic and memory functions and other phenotypic features were evaluated 10 days after the last dose.

Total RNA Isolation and Reverse Transcription–Polymerase Chain Reaction. Bilateral hippocampi were dissected (n = 5) for total RNA isolation, using an RNasesy Mini kit (Qiagen, Valencia, CA). RNA purity was confirmed by spectrophotometry (A260/A280 > 1.8), and RNA integrity was visualized by agarose gel electrophoresis. For reverse transcription–polymerase chain reaction (RT-PCR), 500 ng of total RNA was reverse-transcribed, using oligo(dT) primer and Superscript III (Invitrogen, Carlsbad, CA) at 50°C for 1 hour. Real-time PCR was performed for 40 cycles with SYBR Green I PCR Master mixture and processed on a LightCycler 480 II (Roche, Indianapolis, IN) machine. The following primers (Origene, Rockville, MD) were used: mouse BDNF, 5'-GGCTGACACTTGGAGCACGTC-3' and 5'-CTCTAAAGGCACCTTGACGCTG-3' and mouse GAPDH, 5'-TGAACGGGAAGCTCAGTGCCAT-3' and 5'-TCAAGATCTGCTGCTCACCT-3' as a control. Threshold cycle (Ct) for BDNF was normalized on the housekeeping GAPDH (dCt), and every experimental sample was referred to its ddCt. Relative change values were expressed as 2­ddCt.

Western Blot Analysis. Bilateral hippocampi (n = 5) were homogenized in ice-cold lysis buffer—10 mM Tris-Cl, pH 7.4, 5 mM EDTA, 1% SDS, and 1× complete protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA). After protein concentration measurement with the BCA method, the same number of proteins (25 µg/lane) was then separated using 18% (for BDNF) or 8% (for FMRP, PSD-95, and 5µm bFGF) polyacrylamide gels. As a loading control, mouse β-tubulin (Cell Signaling Technology) was used to control for loading differences among lanes, membranes were stripped and reprobed using mouse anti-α-tubulin (1:15000; Sigma-Aldrich) and mouse anti-β-actin (1:10000; Jackson Immunoresearch Laboratories, West Grove, PA) in TBS-T for 1 hour at room temperature, and immunoreactive bands were visualized by SuperSignal West Pico chemiluminescent substrate. For densitometric analysis of films, using the ImageJ 1.44a system, and normalized to α-tubulin levels.

Enzyme-Linked Immunosorbent Assay. Bilateral hippocampi were homogenized in ice-cold homogenization buffer containing 20 mM Tris-Cl (pH 7.4) buffer containing 137 mM NaCl, 1% Triton X-100, 10% glycerol, and 1× protease inhibitor cocktail (Thermo Fisher Scientific) and used for measuring BDNF. The homogenates were centrifuged at 14,000g for 30 minutes. From the resulting supernatants, total BDNF amounts were measured with the mouse BDNF ELISA technique (Insight Genomics).

Confocal Microscopy for Dendritic Spine Morphology. Mice were deeply anesthetized with chloral hydrate (400 mg/kg i.p.) and perfused through the heart by gravity with phosphate buffered saline (PBS) at room temperature. They were then perfused with 4% paraformaldehyde in PBS (20 ml for light fixation) at room temperature to avoid the negative impact of hypothermia on the number of dendritic spines (Kirov et al., 2004). Right dorsal hippocampi were dissected and sectioned with a vibratome (Leica VT1000S). A series of sections were selected from the right dorsal hippocampus by starting at approximately 1200 µm and selecting one section every 400 µm.

The number of distinct shapes of dendritic spines on individual dendritic shafts was studied with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Invitrogen) staining, as previously described (Hongpaisan and Alkon, 2007).

Dendritic spines in the stratum radiatum were imaged (≥510 nm/568 nm; excitation/emission) with a Zeiss Axio Observer Z1 microscope equipped with a 710 confocal scanning system, using the 100× Plan-APO Chromat om immersion objective (1.4 numerical aperture). A series of confocal images were collected and confocal images (1024 × 1024 pixels) were confocally scanned over every 0.45 µm. Individual spines identified on a single image were verified on adjacent images of series of confocal images to approximate the three-dimensional structure of the spines with the ImageJ program (http://rsb.info.nih.gov/ij/).

The criteria to differentiate the morphology of dendritic spines were based on the relative ratios between spine, head, and neck lengths, as described by Sorra and Harris (2000).

Densities of Pre- and Postsynaptic Structures and BDNF Immunohistochemistry. For immunohistochemical studies, hippocampal sections were incubated free-floating overnight at room temperature with primary antibodies (Millipore/Chemicon, Billerica, MA; mouse monoclonal anti–growth-associated protein (GAP)-43/43B-50 (1:2000), polyclonal rabbit anti-neurogranin (1:400), and mouse monoclonal anti-synaptophysin (1:2000). Tissue sections were then incubated with either Alexa Fluor 568 goat anti-rabbit IgG (1:200; Invitrogen) for 3 hours at room temperature or biotinylated anti-mouse secondary antibody (1:20; Vector Laboratories, Burlingame, CA) for 3 hours at room temperature and then streptavidin-conjugated Alexa Fluor 488 (1:100; Invitrogen) for 3 hours at room temperature. Sections were mounted with VECTASHIELD mounting medium (Vector Laboratories) with 4',6-diamidino-2-phenylindole to counterstain nuclei.

Confocal images (Zeiss confocal microscope, 63× objectives) were acquired of hippocampal sections between 0.6-µm thick, in-line scan mode, and with a pinhole of approximately 1.00 Airy unit. Confocal images with similar levels of 4',6-diamidino-2-phenylindole fluorescence intensity were quantified with the ImageJ program.

The densities per 63 × 63 × 0.6 µm3 volume of the CA1 stratum radiatum (where apical dendrites of CA1 pyramidal neurons are located) of postsynaptic membranes (neurogranin grains), presynaptic membranes (GAP-43 grains), presynaptic axonal terminals (the presynaptic vesicle membrane protein synaptophysin grains), and presynaptic vesicle concentration (synaptophysin fluorescence intensity) were measured. The confocal images lateral resolution was 20 nm in XY and 100 nm in Z, and its point-spread function was automatically collected by the microscope software. By collecting confocal images at 0.6-µm thickness, the overlap of synaptic membranes or axonal boutons was minimized.

Electron Microscopy. Electron microscopy was used to evaluate synaptic details. Mice at an age of 2 months were treated with bryostatin-1 (25 µg/kg i.p., twice/week) for 5 weeks. Under anesthesia...
During double-blind quantification, electron micrographs (64 μm² CA1 area at 8000× magnification and a pixel resolution of 266,859 pixels/μm) were imaged with a charge-coupled device camera (UltraScan, Gatan, Pleasanton, CA).

Results

Bryostatin-1 Increases BDNF Levels, PSD-95, and GSK-3β Phosphorylation. FMRP was only highly expressed in the wild type, but not in the fragile X mice (Fig. 1A). Bryostatin-1 had no significant impact on its expression (Fig. 1A).

The quantitative RT-PCR study revealed that the hippocampal BDNF mRNA levels did not differ significantly between the fragile X mice and the wild type. Bryostatin-1 increased the BDNF mRNA level by approximately 2.1-fold in the wild type (F<sub>1,58</sub> = 19.64, P < 0.001) and by approximately 2-fold in the fragile X mice (F<sub>1,58</sub> = 17.23, P < 0.001; Fig. 1B).

The BDNF protein levels in hippocampal extracts were measured with Western blot analysis. Bryostatin-1 increased production of the 14-kDa BDNF form by ~45% in the wild type (F<sub>1,28</sub> = 4.727, P < 0.05) and 44% (F<sub>1,28</sub> = 4.59, P < 0.05) in the fragile X mice, respectively (Fig. 1C). By use of the sandwich ELISA method, no significant differences were found between the untreated groups, but bryostatin-1 enhanced BDNF production by ~2.43-fold (F<sub>1,54</sub> = 8.682, P < 0.001; Fig. 1D). By use of immunohistochemistry and confocal microscopy, we also observed significant group differences in BDNF expression (Fig. 1E; F<sub>3,114</sub> = 3.633, P < 0.01). Bryostatin-1 restored BDNF levels in the hippocampal CA1 stratum radiatum in the fragile X mice (Fig. 1F).

We determined the expression of PSD-95, a postsynaptic marker protein, to explore whether bryostatin-1 can rescue functional synapses. PSD-95 expression increased in the hippocampus of the fragile X mice by 34% (F<sub>1,28</sub> = 4.289, P < 0.05, versus the wild type; Fig. 1G). Bryostatin-1 enhanced PSD-95 expression by ~51% (F<sub>1,28</sub> = 4.935, P < 0.05) and rescued the PSD-95 levels in the fragile X mice (F<sub>1,28</sub> = 7.793, P < 0.01; Fig. 1G).

GSK-3β is highly active in FXS and is one of the core phenotypes of FXS. There were no group differences in the total GSK-3β levels. However, levels of phosphorylation of GSK-3β on Ser9 were lower by approximately 33% in the hippocampus of the fragile X mice by 34% (F<sub>1,28</sub> = 9.884, P < 0.01; Fig. 1H), indicating that GSK-3β was more active in the hippocampus of the fragile X mice. Bryostatin-1 increased phosphorylation of GSK-3β (Ser9) by approximately 35% (F<sub>1,28</sub> = 10.43, P < 0.01) in the wild type and totally rescued phosphorylation of GSK-3β (Ser9) in the hippocampus of the fragile X mice (F<sub>1,28</sub> = 11.96, P < 0.01).

Bryostatin-1 Prevents the Loss of Mature Dendritic Spines and Pre- and Postsynaptic Membranes and Presynaptic Vesicles in the Fragile X Mice. After water maze learning, dendritic spines on individual apical dendritic shafts of the CA1 pyramidal neurons (stratum radiatum) were stained with Dil and imaged with confocal microscopy by serial scanning, three-dimensional reconstruction, and a double-blind analysis (unknown subject and treatment; Fig. 2A). There was a significant overall group difference for mushroom-shaped spine density (F<sub>3,94</sub> = 2.959, P < 0.05; Fig. 2B) and all-shape dendritic spine (mushroom, thin, and stubby spines together; F<sub>3,94</sub> = 4.245, P < 0.01; Fig. 2C). Both mushroom-shaped spine (P < 0.05) and all-shape dendritic spine (P < 0.01) densities were significantly lower in the fragile X mice (Fig. 2, B and C). A significant overall group difference was found for the density of immature dendritic spines (F<sub>3,94</sub> = 8.347, P < 0.001; Fig. 2, A and D). We found a significant (P < 0.001) increase in immature dendritic spine density in the fragile X mice, versus the wild type (Fig. 2D).

Bryostatin-1 significantly reduced the density of immature dendritic spines (P < 0.01; Fig. 2D), but increased the density of mushroom and all dendritic spines (P < 0.01; Fig. 2, B and C), suggesting that bryostatin-1 enhanced the transformation of immature dendritic spines to the mature dendritic spines.

Immunohistochemistry and confocal microscopy were used to study the densities of presynaptic membranes (GAP-43...
grains) and postsynaptic membranes (neurogranin grains) in the CA1 stratum radiatum (Fig. 2E). An analysis of variance revealed a significant overall group difference for presynaptic membranes ($F_{3,139} = 4.753$, $P < 0.01$; Fig. 2F) and postsynaptic membranes ($F_{3,167} = 7.713$, $P < 0.001$; Fig. 2G).

Compared with the wild type, the fragile X mice had lower density of presynaptic membranes (Fig. 2F; $P < 0.01$) and postsynaptic membranes (Fig. 2G; $P < 0.001$) that were reversed by bryostatin-1 ($P < 0.05$).

The presynaptic vesicle membrane protein synaptophysin was used as a marker for presynaptic axon boutons (Graham and Redman, 1994). At the resolution of the confocal microscope, one synaptophysin granule equals one presynaptic axonal bouton/terminal (Fig. 2H). No significant group differences were found for the number of synaptophysin-labeled endings (Fig. 2I), suggesting that the number of the axon boutons was not affected by the loss of FMRP or bryostatin-1.

The concentration of presynaptic vesicles was studied by measuring fluorescence intensity of the presynaptic vesicle membrane synaptophysin. There was a significant overall group difference for synaptophysin intensity ($F_{3,122} = 3.431$, $P < 0.01$; Fig. 2J). The concentration of presynaptic vesicles decreased in the fragile X mice, versus the wild type ($P < 0.01$). In the fragile X mice treated with bryostatin-1, the presynaptic vesicle density was restored ($P < 0.01$) and reached a level higher ($P < 0.01$) than that in the wild type.

Density of synapses in the hippocampal CA1 stratum radiatum was also studied with electron microscopy (a double-blind analysis). There were significant overall group differences for synaptic density ($F_{2,114} = 2.968$, $P < 0.05$; Fig. 3, A and B) and presynaptic vesicle concentration within axonal boutons ($F_{2,114} = 8.121$, $P < 0.001$; Fig. 3, C and D). The fragile X mice had lower synaptic density (Fig. 3, A and B; $P < 0.05$) and presynaptic vesicle concentration (Fig. 3, C and D; $P < 0.05$), versus the wild type. Bryostatin-1 reversed the decrease in synaptic density in the fragile X mice (Fig. 3, A and B; $P < 0.05$). With bryostatin-1, the reduction of presynaptic vesicle number was not only rescued ($P < 0.001$; the fragile X mice + bryostatin-1 versus the fragile X mice) but also enhanced ($P < 0.05$; the fragile X mice + bryostatin-1 versus the wild type; Fig. 3, C and D), confirming the results in Fig. 2J.

As one dendritic spine may form more than one synapse, we further assessed the density of all dendritic spines, using an antibody against the dendritic spine–specific protein...
spinophilin (Fig. 3E). A significant group difference was found ($F_{3,149} = 15.273, P < 0.001$). Bryostatin-1 increased dendritic spine density in the fragile X mice ($P < 0.001$; Fig. 3F), suggesting that the bryostatin-1–induced increase in synaptic density is correlated with an increase in the number of dendritic spines rather than an increase in multiple synapses of preexisting dendritic spines.

**Bryostatin-1 Restored Spatial Learning and Memory of the Fragile X Mice but Did Not Alter Sensorimotor Ability.** There were significant group differences in learning ($F_{3,623} = 5.214, P = 0.001$; Fig. 4A). Bryostatin-1 significantly improved the learning of the fragile X mice (the fragile X mice versus the fragile X mice with bryostatin-1: $F_{1,319} = 15.556, P < 0.001$) to the level of the controls (the wild-type versus the fragile X mice with bryostatin-1: $F_{1,319} = 0.827, P > 0.05$), indicating that bryostatin-1 repaired the learning of the fragile X mice.

The results in the probe test (Fig. 4, B–E) were analyzed using the target quadrant ratio (dividing the target quadrant distance by the average of the nontarget quadrant values during the probe test; Fig. 4F). There were significant group differences in the ratio ($F_{3,38} = 3.016, P < 0.05$), indicating differences in the spatial memory. Detailed analysis reveals that bryostatin-1 significantly improved the memory recall in the fragile X mice versus the mice without bryostatin-1 ($F_{1,19} = 6.640, P < 0.05$), to the level of the control (the wild-type versus the fragile X mice + bryostatin-1: $F_{1,15} = 0.028, P > 0.05$).
A visible platform test, determined after the probe test, revealed no significant group differences ($F_{3,38} = 1.042; P > 0.05$) (Fig. 4G), indicating that there were no group differences in sensorimotor ability or escape motivation.

**Discussion**

The results of the present study reveal a novel pharmacologic profile of bryostatin-1 (Nelson et al., 2008; DeChristopher et al., 2012) for treating FXS after the FXS phenotypes have already been established in the fragile X mice (Bhattacharya and Klann, 2012). Bryostatin-1 rescues the hippocampus from the FXS phenotypes, including a decrease in PSD-95, a decrease in GSK-3β phosphorylation, a decrease in density of presynaptic and postsynaptic membranes, an increase in immature synapses, a decrease in learning-induced mature synapses, and an impairment of hippocampus-dependent spatial learning and memory. Learned-induced formation of dendritic spines...
is severely impaired in the fragile X mice (Padmashri et al., 2013), probably involving a loss of some dendritic channels (Routh et al., 2013), but can be rescued with bryostatin-1 treatment.

FMRP is an mRNA-binding translation regulator that mediates activity-dependent control of synaptic structure and function (Niere et al., 2012). Its lack results in an overactivity of the mGluRs, a decreased GABAergic system or delayed developmental switch in GABA polarity (D’Hulst et al., 2006; Olmos-Serrano et al., 2010; He et al., 2014), and an elevated activity of GSK-3β (Guo et al., 2012). Potential therapeutics, therefore, include mGluR inhibitors (Vinueza Veloz et al., 2012), GABAergic enhancers (D’Hulst and Kooy, 2007; Olmos-Serrano et al., 2010; Paluszkiwicz et al., 2011; Heulens et al., 2012), and inhibitors of GSK-3β (Yuskaitis et al., 2010; Guo et al., 2012). Because intellectual ability, as well as retardation (Wang et al., 2012) involves multiple players in signal processing, bryostatin-1–like agents, for their multitargeting actions, may represent a more effective class of therapeutics than agents that target a single factor in this complex pathologic process (Vislay et al., 2013).

First, some PKC isozymes play an essential role in various phases and types of learning and memory (Alkon et al., 2005). PKC activators not only increase activity of PKC isozymes and thereby restore PKC signal activity, including neurotrophic activity (Alkon et al., 2007; Sun et al., 2008), synaptic/structural remodeling, and synaptogenesis, but also reduce the accumulation of neurotoxic amyloid (Alkon et al., 2007; Hongpaisan et al., 2011), which may play pathologic roles in the fragile X mice (Westmark et al., 2011). The effects of bryostatin-1 on disorder-induced learning and memory impairments are evidenced in the present study in that it fully rescued the spatial learning and memory in the fragile X mice. The results are consistent with an earlier observation that infusion of BDNF restored synaptic function in slices from the fragile X mice (Lauterborn et al., 2007). PKCα,ε enhances BDNF expression and via the mRNA-stabilizing protein HuD increases expression of other synaptogenic factors, such as GAP-43, insulin-like growth factor, neurotrophic factor, and nerve growth factor (Nelson et al., 2008).

Second, PKCε and other isoforms are known to phosphor-ylate mGluR5 at multiple sites, inducing a relatively rapid form of desensitization (Gereau and Heinemann, 1998), an action that directly targets the mGluR-mediated overactivity. Third, bryostatin-1 effectively increases the hippocampal PSD-95 levels in the fragile X mice. The mGluR5 dysfunction includes an altered Homer scaffold interaction (Ronesi et al., 2012). Consistent with others’ findings, the synaptic scaffold...
protein PSD-95 was downregulated in the hippocampus, probably because of an alteration in mRNA stability (Zalfa et al., 2007).

Fourth, bryostatin-1 rescues the GSK-3β level to that of the wild type. GSK-3β is a core component of FXS pathology (Yuskaitis et al., 2010) and is mainly regulated by phosphorylation on an N-terminal serine, Ser-9-GSK-3β, for its inhibition. This inhibitory serine-phosphorylation of GSK-3β is impaired in the fragile X mice (Min et al., 2009) but can be rescued by bryostatin-1.

Fifth, bryostatin-1 induces transformation of immature dendritic spines to mature synapses in the hippocampus of the fragile X mice and thus avoids the undesired impact of a pure mGluR inhibition on spine immaturity (Cruz-Martin et al., 2010). Bryostatin-1 was administered at a dose at which it promotes learning-induced synaptogenesis and synaptic maturation (Hongpaisan and Alkon 2007, Hongpaisan et al., 2011). Mice lacking expression of FMR1 show an abundance of immature-looking lengthened dendritic spines (Cruz-Martin et al., 2011). In FXS patients (Grossman et al., 2006). The hyper-abundance of immature-lengthened dendritic spines could be the results of a failed/delayed maturation (Cruz-Martin et al., 2010; Harlow et al., 2010) and activity-dependent synaptic elimination (Pfeiffer et al., 2010). In short, bryostatin-1-like agents (Sun and Alkon, 2005; Nelson et al., 2009; DeChristopher et al., 2012) may have important therapeutic value for the treatment of adult FXS.

Authorship Contributions

Participated in research design: Sun, Hongpaisan, Lim, Alkon.

Conducted experiments: Sun, Hongpaisan, Lim.

Performed data analysis: Sun, Hongpaisan, Lim.

Wrote or contributed to the writing of the manuscript: Sun, Hongpaisan, Lim.

References


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