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Lifting the Mood on Treating Fragile X

Emily K. Osterweil, Peter C. Kind, and Mark F. Bear

Only a decade ago, it was believed that a genetic diagnosis of intellectual disability and autism offered little in the way of hope for a medical treatment to lessen the burden on the affected individuals and their families. However, recent research aimed at understanding the cellular and molecular mechanisms that underlie the pathogenesis of ASD has ushered in a new era of targeted treatment strategies. Studies in fragile X syndrome (FXS) have been at the forefront of this revolution, and they are forging a path that could define future approaches to the treatment of ASD.

FXS is the leading identified genetic cause of autism. Because it is a defined genetic disorder that can be effectively modeled in animals, the study of FXS has great potential to yield information about the pathophysiology of ASD. FXS is caused by a silencing of the *FMR1* gene and the loss of fragile X mental retardation protein (FMRP), a repressor of mRNA translation. Early studies suggested that synaptic protein synthesis is stimulated by activation of group 1 (Gp1) metabotropic glutamate receptors (comprising mGluR1 and mGluR5) and that one functional consequence of this protein synthesis is the long-term depression (LTD) of synaptic strength (reviewed in in Bhakar *et al.* [1]). The subsequent discovery that LTD is elevated in the mouse model of FXS ($Fmr1^{-/y}$) led to the proposal that multiple symptoms of the disease might be accounted for by heightened responsiveness to Gp1 mGluR activation. This mGluR theory of fragile X predicted that the antagonism of Gp1 mGluRs should correct pathologic changes in FXS (2). In the decade that elapsed since it was first proposed, numerous studies in a range of animal models have validated this theory. The animal data show that many aspects of FXS can be accounted for by altered Gp1 mGluR signaling at synapses. This insight is the basis for multiple clinical trials that are now underway in FXS (for extensive reviews see Bhakar *et al.* [1] and Krueger and Bear [3]).

The first clinical test of the mGluR theory was a small, open-label trial using fenobam, a Gp1 mGluR antagonist that is highly selective for mGluR5. The results of this small trial showed beneficial effects on a subset of the adult FXS patients tested; however, it is important to note that no placebo control group was included in this trial, nor was it performed in an experimenter blind fashion (4). Since this initial study, multiple large-scale placebo-controlled trials have been initiated with selective mGluR5 negative allosteric modulators— namely, STX107 (Seaside Therapeutics, Cambridge, Massachusetts), RG7090 (Hoffman-LaRoche, Basel, Switzerland), and AFQ056 (Novartis, Basel, Switzerland). Although the results of most of these trials have yet to be revealed, in post hoc analysis of Phase II data AFQ056 was reported to show an improvement on multiple behavioral tests in adult patients with a fully methylated (silenced) *FMR1* gene.

In tandem with drugs that directly interfere with mGluR5, other drugs that may indirectly target Gp1 mGluR signaling are also being tested in clinical trials. One strategy for reducing mGluR activation is to suppress glutamate release at excitatory synapses, and this can be achieved by the activation of $GABA_B$ receptors. Early studies based on this idea showed that the GABA receptor agonist baclofen could drastically reduce the incidence of audiogenic seizures in $Fmr1^{-/y}$ mice (reviewed in Krueger and Bear [3]).

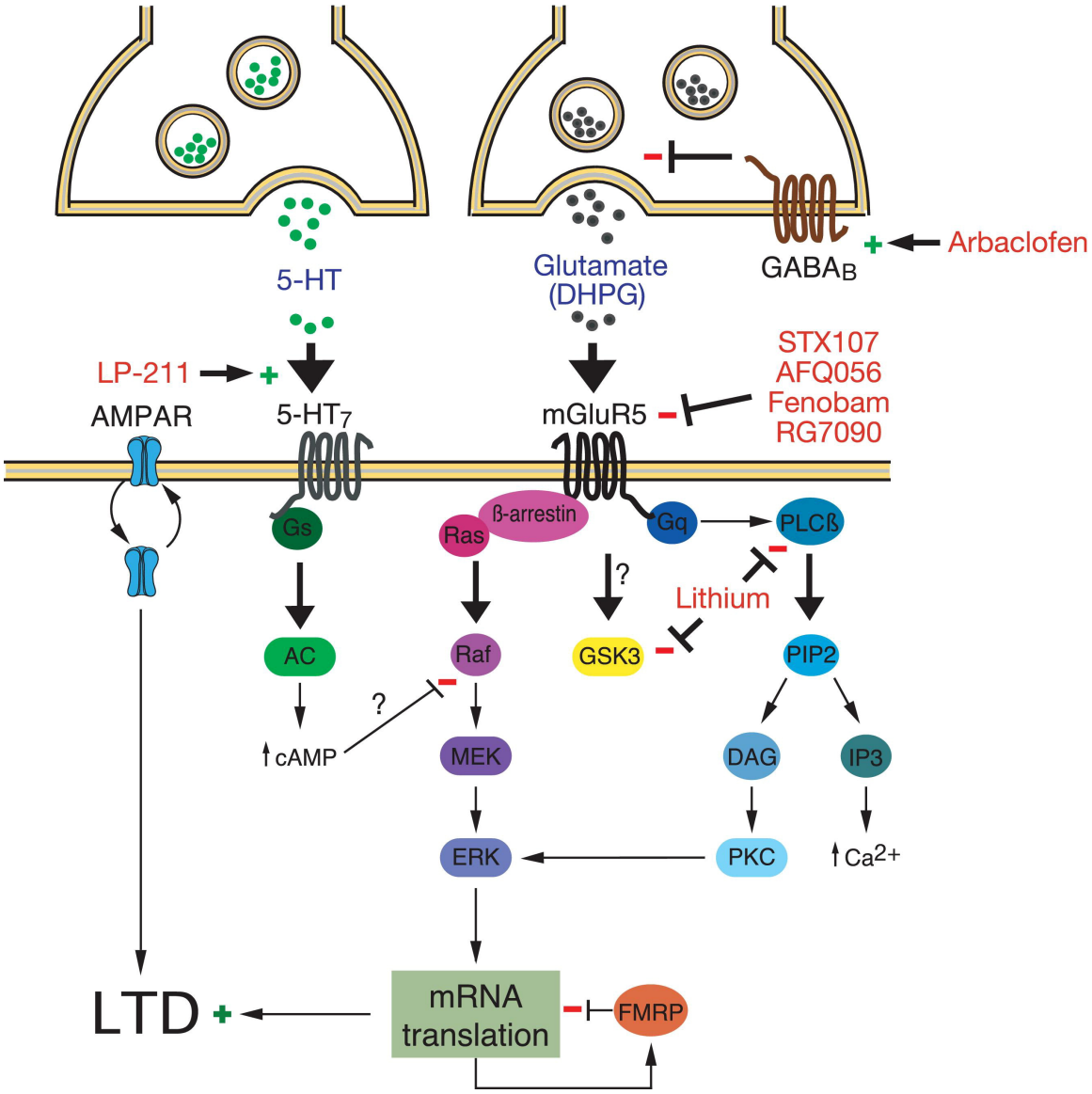

Figure 1. Inhibition of metabotropic glutamate receptors (mGluR) signaling in fragile X syndrome (FXS). Schematic shows how mGluR signaling is targeted to treat FXS. Fragile X mental retardation protein inhibits messenger (m)RNA translation downstream of constitutive activation of mGluR5, resulting in excessive protein synthesis and exaggerated mGluR long-term depression (LTD). Fenobam, STX107, RG7090, and AFQ056 negatively regulate mGluR5 at allosteric sites. Arbaclofen activates gamma-aminobutyric acid B (GABAB) receptors, thereby inhibiting vesicular glutamate release. Lithium targets both phospholipase C (PLC) and glycogen synthase kinase 3 (GSK3), two pathways that are activated downstream of Gp1 mGluRs. LP-211 enhances 5-HT7 receptor activation, stimulating the production of cAMP. This may negatively regulate extracellular signal-regulated kinase (ERK)1/2 signaling by targeting Raf1. AC, ; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AMPAR, AMPA receptor; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; DHPG, 3,5-Dihydroxyphenylglycine; FMRP, fragile X mental retardation protein; GSK3, glycogen synthase kinase 3; IP3, inositol triphosphate; MEK, MAP kinase kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; Raf, Raf serine/threonine-protein kinase; Ras, RAt sarcoma protein.

More recently, a study using the R-isomer of baclofen (arbaclofen or STX209, Seaside Therapeutics) finds that multiple phenotypes in *Fmr1*$^{-/y}$ mice are corrected, including

abnormal dendritic spine density, excessive protein synthesis, exaggerated alpha-amino-3hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor trafficking, and audiogenic seizures (Henderson *et al*. [5]). Importantly, recent results from a double-blind placebo-controlled study of 63 patients with FXS revealed arbaclofen treatment results in significant improvement in clinical presentation (Berry-Kravis *et al*. [6]). Arbaclofen is currently in Phase III clinical trials for the treatment of FXS.

Other therapeutic strategies have targeted the signaling pathways downstream of Gp1 mGluRs. The phospholipase C (PLC) and glycogen synthase kinase 3 (GSK3) signaling pathways are both stimulated by Gp1 mGluR activation, and the latter is hyperactive in the*Fmr1*$^{-/y}$.Preclinical studies show that lithium ,a drug that inhibits PLC and GSK3, can correct multiple phenotypes in the *Fmr1*$^{-/y}$, and an open-label clinical trial showed behavioral improvements in FXS patients (for review, see Krueger and Bear [3]). Another important pathway activated by Gp1 mGluRs is the extracellular signalregulated kinase 1/2 (ERK1/2) signaling cascade, which is activated by the G-protein Ras, which leads to the sequential phosphorylation of Raf1 and MEK1/2. The *Fmr1*$^{-/y}$ mouse is hypersensitive to signaling through this pathway, and inhibition of ERK1/2 corrects multiple pathologic phenotypes in the *Fmr1*$^{-/y}$, including excessive protein synthesis, hippocampal epileptiform activity, and susceptibility to AGS (for review, see Bhakar *et al*. [1]). Investigation into clinically viable strategies for targeting this pathway is ongoing.

In the current issue of *Biological Psychiatry*, Costa *et al*. (7) propose the 5-HT$_7$ serotonin receptor as a novel target for the treatment of FXS. They show that activation of 5-HT$_7$ inhibits mGluR-LTD induced by 3,5-Dihydroxyphenylglycine (DHPG), a Gp1 mGluR agonist. This effect is observed in both wild-type and *Fmr1*$^{-/y}$ hippocampus. Application of either serotonin (5-HT) or 8-Hydroxy-2dipropylaminotetralin hydrobromide (8-OH DPAT), a 5-HT$_7$ and 5-HT$_{1A}$ receptor agonist, suppresses mGluR-LTD when applied within 15 min of induction. A specific 5-HT$_7$ agonist, LP-211, also blocks the induction of mGluR-LTD. The suppressive effect of 8-OH DPAT on LTD is blocked by application of a specific antagonist to 5-HT$_7$, but not 5-HT$_{1A}$, receptors. Together, these results show that activation of 5-HT$_7$ inhibits the induction of mGluR-LTD, suggesting two hypotheses of particular interest: 1) that the Gp1 mGluR and 5-HT pathways functionally interact; and 2) that 5-HT$_7$ receptor agonists can correct pathologic changes in FXS.

5-HT modulates the release of several neurotransmitters, including glutamate and gamma-aminobutyric acid, and the expression of 5-HT receptors is widespread. Accumulating evidence suggests that 5-HT$_7$, the most recently characterized 5-HT receptor, plays a role in enhancing memory formation. Multiple behavioral studies have shown that antagonism of 5-HT$_7$ receptors impairs long-term memory, and studies using 5-HT$_7$ agonists show a beneficial effect on instrumental and associative memory formation (reviewed in Roberts and Hedlund [8]). Consistent with these findings, studies performed on 5-HT$_7$ knockout mice show a deficit in contextual fear conditioning, a hippocampal learning task, as well as deficient LTP in hippocampal CA1 (9). However,

the mechanism through which 5-HT_7 activation enhances memory formation is largely unknown. It is interesting to speculate that the suppressive action of 5-HT_7 activation on mGluR-LTD observed by Costa *et al*. is relevant to its positive impact on hippocampal learning.

Given the antagonistic effect of 5-HT_7 activation on mGluR-LTD, a pertinent question is whether other functional consequences of mGluR activation could be similarly dampened by 5-HT_7 agonists, particularly in the context of FXS. It will be important to assess in future studies if 5-HT_7 compounds can ameliorate the same broad array of *Fmr1*$^{-/y}$ phenotypes as respond to inhibition of mGluR5. In the meantime, it is interesting to consider the mechanism through which 5-HT_7 activation might suppress mGluR-LTD. Costa *et al*. find that 8-OH DPAT did not affect a presynaptically expressed form of mGluR-LTD, thus it is likely that 5-HT_7 activation blocks LTD via a postsynaptic mechanism. The inhibition of DHPG-induced AMPA receptor (AMPAR) endocytosis by 8-OH DPAT, in both acute slices and dissociated hippocampal cultures, also suggests that 5-HT_7 acts postsynaptically. The authors speculate that the inhibition of LTD by 5-HT_7 activation could be due to a change in the phosphorylation and trafficking of specific AMPAR subunits. Another enticing possibility is that 5-HT_7 activation negatively modulates a shared signaling pathway downstream of Gp1 mGluRs (Figure 1). 5-HT_7 is a G protein coupled receptor that activates Gs, which stimulates adenylate cyclase and increases the production of cyclic adenosine monophosphate (cAMP) (8). Studies from several cell types have found that cAMP can inhibit the activation of Raf1 and subsequently downregulate ERK1/2 signaling (10). Thus, it is possible that activation of 5-HT_7 inhibits mGluR-LTD by dampening the ERK1/2 pathway. In this context, it is interesting to note that a robust finding in both the *Fmr1*$^{-/y}$ mouse and in cells isolated from FXS patients is an impairment in cAMP production (11).

The study by Costa *et al*. (7) joins a growing body of research linking the negative regulation of Gp1 mGluRs to a correction of pathologic changes in FXS. Several compounds that target mGluRs and associated signaling pathways have shown promise in the clinic, and additional treatment strategies based on the mGluR theory continue to be developed (Figure 1). This has relevance not only for FXS but for the treatment of the greater ASD population. The notion that a neurodevelopmental disorder can be treated with pharmacologic intervention has heretofore been seen as naive. However, evidence to the contrary is accumulating, and it is now becoming accepted that treatment can be beneficial even when administered later in development (12). The recent studies in FXS thus represent a paradigm shift in our thinking about the treatment of neurodevelopmental disorders. The door has been opened for a new wave of targeted treatment strategies for FXS and related ASD.

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