Exploring 5-HT2 Receptors as Targets for Treating Epilepsy in Fragile X Syndrome: A Preclinical Study of Fmr1 Knock-out Mice
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INTRODUCTION
Epilepsy prevalence is ~12% in children with autism spectrum disorder (ASD) and ~25% in children with fragile X syndrome (FXS), a monogenic neurodevelopmental disorder characterized by intellectual disabilities, severe anxiety, attention deficit hyperactivity disorder, and sensory hypersensitivity—FXS is also the most common, known cause of ASD. Seizure risk increases by three times in individuals with comorbid FXS and autism.

Recently, (P2R-P2S) Fmr1 knock-out mice—a genetic model of FXS/ASD—have a high prevalence of sound-induced (audigenic) seizures, recapitulating the increased incidence of seizures and sensory hypersensitivity in FXS and ASD. Several early studies linked alterations in serotonin (5-hydroxytryptamine, 5-HT) to ASD, and selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed medicines to treat psychiatric symptoms in ASD and FXS. Critically, there are at least 14 genetically-encoded 5-HT receptors (5-HTRs)—all indirectly activated by SSRIs—yet detailed information about the impact of specific 5-HTRs on seizures in FXS and ASD is lacking.

A recent, small clinical trial reported that lorcaserin (Belviq®), a moderately selective 5-HT2c receptor antagonist for obesity, reduced seizure frequency in children with Dravet syndrome (Griffin et al., 2017; PMID: 28073700) and Lennox-Gastaut syndrome (Toite et al., 2018; PMID: 30258026). This project explores the anti-epileptic potential of 5-HT, receptor antagonists in FXS and ASD, beginning with tests of lorcaserin to prevent audiogenic seizures in Fmr1 knock-out mice.

METHODS
Fmr1 knock-out (FVB.129P2-Poteb-Tyr-c Knockout JrlPrkTB10)) and wild-type control (FVB.129P2-Poteb-Tyr-c/JrlPt) mice were purchased from the Jackson Laboratory to develop a colony. Genotyping of litters by PCR was performed based on established protocols.

Audiogenic seizures were assessed by exposing male and female juvenile Fmr1 knock-out mice to a 120 dB radiant (Radio Shack) for 5 min, 30 min after intraperitoneal administration of vehicle (MilliQ water), lorcaserin (AdoDo Bioscience) 1 (L1), 3 (L3), 5.6 (L5.6), or 10 mg/kg (L10), M100907 0.03 mg/kg (M0.03), M0.03 L5.6, or MPEP. Juvenile wild-type mice were treated with vehicle and radiant before exposing to the alarm. Responses, including climbing and jumping (WRJs), tonic-clonic seizures (TCS) with and without respiratory arrest, were scored and recorded.

Radioligand saturation binding was performed using cell membranes of brain tissue collected from juvenile male and female wild-type and Fmr1 knock-out mice, as previously described (Canal et al., 2011; PMID: 20166943). [3H]Mesulergine and [3H]ketanserin (PerkinElmer) with cold ligands to block off-targets were used to label 5-HT1A and 5-HT5 receptors, respectively.

Adult, male C57BL/6J mice were used to test in vivo interactions between lorcaserin and racemic (±)-2,3-dimethoxy-4-methamphetamine (DOI); specifically effects on the head-twitch response (HTR) and locomotion. 5-HT2c receptor phosphosinositide hydrolysis assays were performed as previously described (Chen et al., 2019; PMID: 30845376) using human 5-HT2c and 5-HT5 receptors transfected in HEK-293 cells.

LORCASERIN PHARMACOLOGY

lorcaserin exhibits full agonist activity at human 5-HT3Rs and 5-HT6Rs, with ~17-fold selectivity for 5-HT3Rs (Fmr1−/− mice). Lorcaserin (Belviq®)

EFFECTS OF LORCASERIN ON AUDIGENIC SEIZURES IN JUVENILE FMR2 KNOCK-OUT MICE

Significantly less wild type mice that received vehicle and Fmr1 knock-out (KO) mice that received MPEP (positive control) displayed WRJs and TCS seizures (Fisher’s exact test, *P=0.001 and *P=0.05, respectively) and died due to seizure induced lethality (Fisher’s exact test, **P=0.002 and **P=0.05, respectively). In contrast, a different subset of mice did not respond to the pre-procedural significance level (Fisher’s exact test, P=0.371 and P=0.0585, respectively).

No significant sex differences were observed, however, qualitatively, males were more susceptible to AS in all treatment groups as they displayed more severe phenotypic manifestations such as a higher percentage of lethality and decreased onset latency to responses ultimately leading to early death. Females.

ASSESSMENT OF 5-HT2A AND 5-HT2C RECEPTOR EXPRESSION IN BRAINS OF FMR2 KNOCK-OUT MICE

Preliminary data suggest decreased expression of cortical 5-HT2cRs in juvenile male mice (A) and decreased expression of 5-HT2cRs in cortical sections containing striatum and frontal lobe in juvenile female Fmr1 knock-out (KO) mice (B) relative to wild type (WT) FVB mice.

DISCUSSION
Lorcaserin (3 mg/kg) reduced seizure prevalence and latency, but its efficacy over WT may not be statistically significant. Overall, lorcaserin did not significantly prevent or prolong latency to or shorten duration of audiogenic seizures; instead, at doses other than 3 mg/kg, lorcaserin potentiated seizures, displaying mild but not statistically significant) proconvulsant effects. At 5.6 mg/kg, onset latency to all responses, number, and duration of WRJ before TCS increased, thus, prolonging the time spent in seizure avoidance behavior. Coadministration of M0.03 and L5.6, increased seizure prevalence but decreased severity of seizures with a similar pattern of anticonvulsant activity (increasing onset latency) observed at 5.6 mg/kg. Thus, lorcaserin’s 5-HT2c-R-specific efficacy at higher doses, may involve potential anticonvulsant action. These complex mixed effects of lorcaserin in FXS may involve molecular mechanisms that are distinct from seizure attenuation effects in Dravet and Lennox-Gastaut syndromes. Preliminary results suggest decreases in 5-HT2c- and 5-HT5Rs in brains of juvenile Fmr1 knock-out mice. These data suggest 5-HTRs are altered in Fmr1 knock-out mice, and might be therapeutic targets for FXS or ASD.

Lorcaserin is marketed as a selective 5-HT2c agonist but its efficacy over WT may not be statistically significant. This reveals novel targets for preclinical studies, and in drug discrimination tests, the full efficacy. 5-HT2c agonist, 8-OH-DPAT occasioned greater than 90% lorcaserin (0.56 mg/kg) lever responding in 57.9% of rats tested (Kraske et al., 2016; PMID: 27640338). According to the FDA briefing document, brain levels of lorcaserin could reach 1.7 μM from the clinical dose. At this concentration, lorcaserin could activate 5-HT2cRs and 5-HT5Rs in addition to 5-HT2cRs. Pharmacodynamic effects of lorcaserin for individuals with ASD or FXS, therefore, remain uncertain.

Future experiments will assess potential contributions of 5-HT5Rs, and will evaluate other 5-HT, ligands, including DOI.

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