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Alzheimer's Disease and Down Syndrome Rodent Models Exhibit Audiogenic Seizures

Cara J. Westmark, **Pamela R. Westmark**, and **James S. Malter**
Department of Pathology & Laboratory Medicine and Waisman Center for Developmental Disabilities, University of Wisconsin, Madison, WI 53705

Abstract

Amyloid β-protein precursor (AβPP) is overexpressed in Alzheimer's disease (AD), Down syndrome (DS), autism and fragile × syndrome (F×S). Seizures are a common phenotype in all of these neurological disorders; yet, the underlying molecular mechanism(s) of seizure induction and propagation remain largely unknown. We demonstrate that Alzheimer's disease (AD) and Down syndrome (DS) mice exhibit audiogenic seizures (AGS), which can be attenuated with antagonists to metabotropic glutamate receptor 5 ($mGluR_5$) or by passive immunization with anti-Aβ antibody. Our data strongly implicates AβPP or a catabolite in seizure susceptibility and suggests that $mGluR_5$ mediates this response.

Keywords

Alzheimer's disease (AD); amyloid β-protein precursor (AβPP); audiogenic seizure (AGS); amyloid beta (Aβ); Down syndrome (DS); metabotropic glutamate receptor 5 ($mGluR_5$)

Fmr-1$^{-/-}$ mice lack expression of fragile × mental retardation protein (FMRP), overproduce AβPP and amyloid beta (Aβ) and are highly susceptible to audiogenic seizures (AGS) [1-3]. FMRP binds to and represses the dendritic translation of AβPP mRNA [2], thus, we hypothesized that increased levels of AβPP or a catabolite of AβPP near synaptic connections in *fmr-1*$^{-/-}$ mice contributed to AGS sensitivity and that other mouse models that overexpress AβPP would exhibit AGS. Seizures and myoclonus are prevalent phenotypes in AD and DS [4-5]. In this study, we employed established AD (Tg2576) [6] and DS (Ts65Dn) [7] mouse models as well as FRA×AD mice, which overexpress human AβPP with the Swedish familial mutation ($hA\beta PP_{SWE}$) in an *fmr-1*$^{-/-}$ background [8], to study the role of AβPP on AGS susceptibility.

We assessed AGS in WT, *fmr-1*$^{-/-}$, Tg2576 and FRA×AD mice all in a C57BL/6 background. Mice were generated, bred and housed as previously described [8]. All strains were tested at postnatal day 21 (P21), the peak of AGS sensitivity [9]. Mice were transferred to a Plexiglas box (13″L × 8″W × 7″H) and exposed to a high-pitched siren (118 dB) from a personal body alarm (LOUD KEY^{TM}). We scored the number of mice exhibiting wild running (WR), tonic seizures (AGS) and death, and statistical significance was assessed by Chi Square analyses. All husbandry, seizure and euthanasia procedures were performed in accordance with NIH guidelines and an approved University of Wisconsin animal care protocol.

Address correspondence to: Dr. Cara J. Westmark, Waisman Center for Developmental Disabilities, Room T507, 1500 Highland Avenue, Madison, WI 53705, Phone: (608) 262-9730, westmark@wisc.edu.

Pure C57BL/6 mice are resistant to AGS [10] and we observed only a 5% seizure rate in WT mice (Figure 1). In *fmr-1*$^{-/-}$ mice, 56% exhibited WR, 44% AGS and 38% death resulting from seizures. Thus, as seen previously, *fmr-1*$^{-/-}$ mice exhibit a strong AGS phenotype and WT controls do not [9,11-13]. Tg2576 exhibited very similar susceptibility to AGS as *fmr-1*$^{-/-}$ mice. This is the first report that an AD mouse model is susceptible to AGS, although elevated susceptibility to PTZ-induced seizures has been reported [14]. FRA×AD mice showed nearly double the AGS susceptibility as the parental *fmr-1*$^{-/-}$ and Tg2576 lines. The increased susceptibility to audiogenic stimulation in the FRA×AD compared to the Tg2576 is also apparent by the decreased latency time to onset of WR (data not shown). ELISA analyses of brain lysates revealed the highest levels of Aβ in FRA×AD mice followed by Tg2576, *fmr-1*$^{-/-}$ and WT [2,8]. Thus, there was a significant increase in seizure sensitivity in all of the AD and F×S mouse strains tested compared to WT controls, which correlated with aggregate Aβ levels.

To further strengthen our hypothesis, we tested AGS susceptibility in Ts65Dn mice, which like *fmr-1*$^{-/-}$ over-express mouse AβPP (mAβPP) and mAβ. Trisomic mice displayed 75% WR, 56% AGS and 50% death rates (Figure 1). The Ts65Dn and littermate control (Cn) mice are in a mixed background (mothers: B6EiC3Sn a/A-Ts(17^{16})65Dn; fathers: B6EiC3Sn (C57BL/6JEi × C3H/HeSnJ) F1. The WT controls in the mixed background exhibited an increased propensity for WR and AGS compared to the C57BL/6 WT mice, but significantly less than their trisomic littermates. In aggregate these results suggest that AβPP over-expression contributes to AGS.

Antagonists to $mGluR_5$ have been shown to revert many *fmr-1*$^{-/-}$ phenotypes [9,15-17]. MPEP is a specific and potent noncompetitive antagonist of $mGluR_5$ that is capable of crossing the blood brain barrier [18-19], attenuating AGS in *fmr-1*$^{-/-}$ mice [9], and blocking $mGluR_5$-mediated up-regulation of AβPP synthesis [2]. We treated WT, Tg2576 and FRA×AD mice with 30 mg/kg body weight MPEP 30 minutes prior to AGS induction. $mGluR_5$ blockade completely attenuated WR, AGS and death in Tg2576 and reduced these phenotypes in FRA×AD mice (Table 1). FRA×AD mice produce significantly more $Aβ_{1-40}$ by 2 weeks of age than Tg2576 as assessed by ELISA of whole brain lysates [8], which may account for the inability of a single treatment with MPEP to completely attenuate AGS. To corroborate these results, we tested a second $mGluR_5$ antagonist, fenobam, which can be orally administered in chow to rodents. Pups were weaned at P18 and transferred to the fenobam-supplemented feed for 3 days prior to AGS testing at P21. Fenobam significantly reduced the number of deaths in Tg2576 and Ts65Dn mice (Table 1). For the mice that did exhibit seizures, the latency times to WR and AGS were longer (at least 1.8-fold) after fenobam treatment (data not shown). This data demonstrates that $mGluR_5$ blockade significantly reduces AGS in mice that overexpress AβPP.

Finally, we assessed AGS rates in Tg2576 mice after passive immunization with an anti-AβPP/Aβ antibody, sc-28365LS (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). This monoclonal antibody was generated against amino acids 672-714 of hAβPP, but epitope information is not available. Thus, sc-28365LS recognizes AβPP and Aβ and possibly sAβPPα if the antigen recognition site is amino-terminal to the α-secretase cleavage site within Aβ. The antibody (12.5 μg) was administered by I.P. injection to 18-day-old Tg2576 and AGS sensitivity was tested at P21. Passive immunization with the anti-Aβ antibody significantly reduced the death rate in Tg2576 mice (Table 1).

Due to the heterogeneity of seizures, the underlying cellular and molecular mechanisms that induce and propagate these abnormal electrical discharges in the brain remain poorly understood. An increased incidence of seizure activity or lower thresholds to chemically-induced seizures are apparent in multiple AD mouse models [8,14,20-25]. MPEP reduces

the severity of chemically-induced seizures in Tg2576 and FRA×AD mice [26]. Herein, we demonstrate that AD, FRA×AD and DS mice are highly susceptible to audiogenic-induced seizures at rates that match or exceed *fmr-1*$^{-/-}$ mice and that $mGluR_5$ blockade or passive immunization with anti-Aβ reduces AGS and deaths. These data support roles for AβPP, or an AβPP catabolite, in seizure induction as well as FMRP-dependent and independent $mGluR_5$ signaling pathways [9] in signal propagation. AβPP plays critical physiological roles in synapse formation and maturation and altered expression or processing likely contributes to lower seizure threshold. Our data strongly suggests that therapies that reduce AβPP expression, block $mGluR_5$ signaling or increase clearance of Aβ could be beneficial in controlling seizures.

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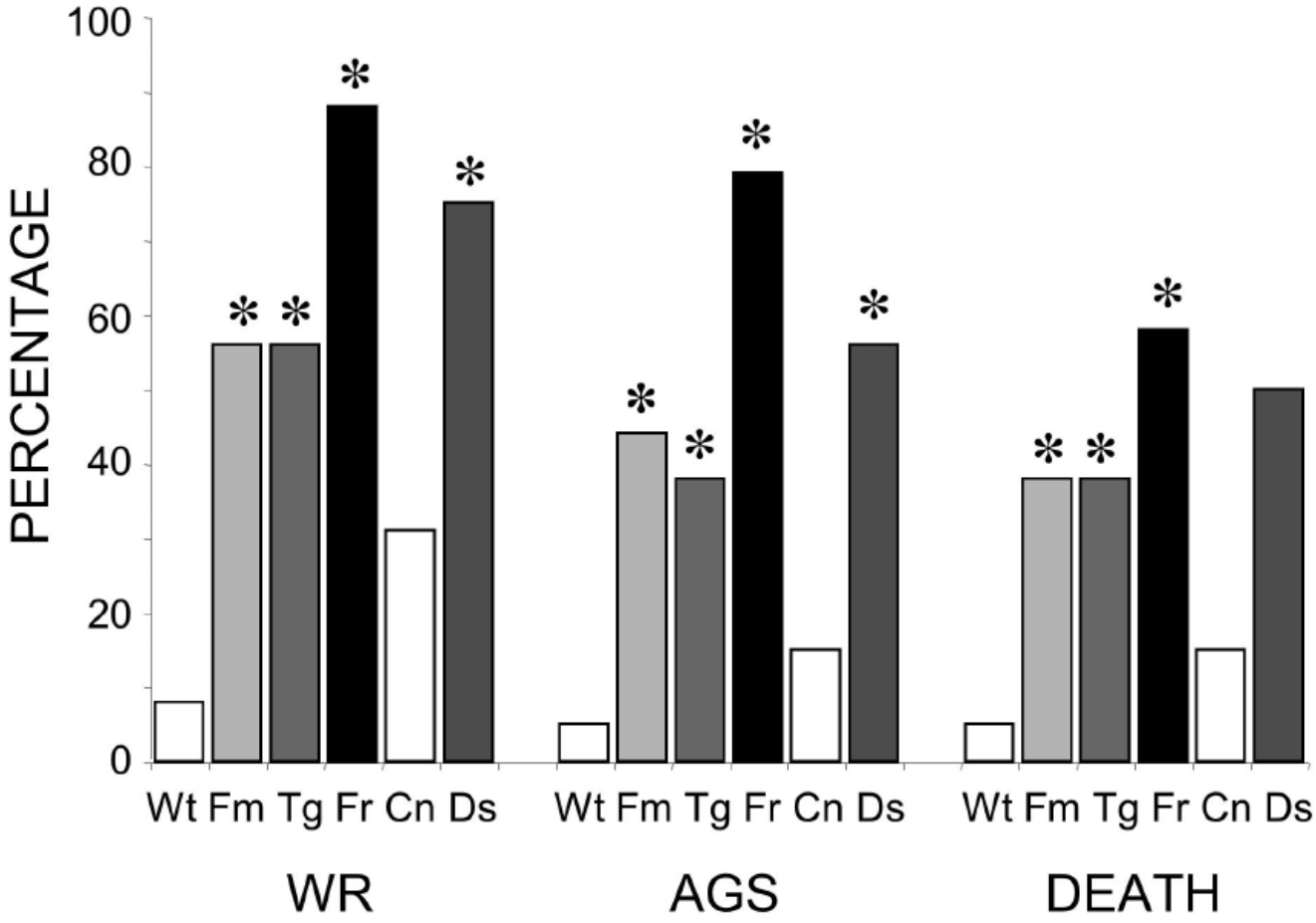

Figure 1.

WR, AGS and Death Rates in WT, *fmr-1*$^{-/-}$, Tg2576, FRA×AD and Ts65Dn Mice. Mice (age P21) were exposed to 118 dB siren and the percentage exhibiting WR, AGS and death was plotted versus genotype for WT (Wt, n=39), *fmr-1*$^{-/-}$ (Fm, n=16), Tg2576 (Tg, n=16), FRA×AD (Fr, n=24), littermate controls for Ts65Dn (Cn, n=13), and Ts65Dn (Ds, n=16). All mice were in a C57BL/6 background except for Ts65Dn and littermates, which were in a mixed background. Statistically significant differences between Tg2576 or FRA×AD compared with WT and between Ts65Dn and littermate controls were assessed by Chi Square analyses (*) ($p<0.03$).

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Table 1

Attenuation of AGS in APP/Aβ-Overexpressing Mice

Genotype[XO]	n	Treatment	% WR	% AGS	% death
WT	39	untreated	8	5	5
Tg2576	16	untreated	56*	38*	38*
FRA×AD	24	untreated	88*	79*	58*
Cn	13	untreated	31	15	15
Ts65Dn	16	untreated	75^	56^	50
WT	19	MPEP	0	0	0
Tg2576	15	MPEP	0#	0#	0#
FRA×AD	15	MPEP	60#	27#	7#
WT	26	Fenobam	4	4	4
Tg2576	27	Fenobam	30	15	0#
Cn	11	Fenobam	0#	0	0
Ts65Dn	12	Fenobam	58	0#	0#
WT	18	αAβ	6	0	0
Tg2576	13	αAβ	8#	8	0#

[X] WT, Tg2576 and FRA×AD are in a C57BL/6 background

[O] Ts65Dn and control littermates (Cn) are in a mixed background

* statistically different from untreated WT, $p<0.005$

^ statistically different from Cn $p<0.03$

statistically different from untreated of same genotype, $p<0.05$