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Research report

Complex effects of mGluR5 antagonism on sociability and stereotypic behaviors in mice: Possible implications for the pharmacotherapy of autism spectrum disorders

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ABSTRACT

Balb/c mice display deficits of sociability; for example, they show reduced locomotor activity in the presence of an enclosed or freely-moving social stimulus mouse. Transgenic mice with defective or diminished expression of NMDA receptors manifest impaired sociability, while a partial and full agonist of the obligatory glycine co-agonist binding site on the NMDA receptor improved sociability in the Balb/c mouse strain. Because 2-methyl-6-(phenylethynyl)-pyridine (MPEP), an antagonist of the mGluR5 metabotropic glutamate receptor (mGluR), reduced self-grooming behavior in BTBR T+tfJ (BTBR) mice, another inbred genetic mouse model of autism spectrum disorders (ASDs), and mGluR5 antagonism is emerging as an experimental treatment for the 'fragile X syndrome," which has a high prevalence of co-morbid ASDs, we examined the effects of MPEP on sociability and stereotypic behaviors in Balb/c and Swiss Webster mice in a standard paradigm. MPEP had complex effects on sociability, impairing some measures of sociability in both strains, while it reduced the intensity of some spontaneous measures of stereotypic behaviors emerging during free social interaction in Swiss Webster mice. Conceivably, mGluR5 antagonism exacerbates diminished endogenous tone of NMDA receptor-mediated neurotransmission in neural circuits relevant to at least some measures of sociability in Balb/c mice; the mGluR5 receptor contributes to regulation of the phosphorylation status of the NMDA receptor. In any event, although stereotypies are an important therapeutic target in ASDs, medication strategies to attenuate their severity via antagonism of mGluR5 receptors must be pursued cautiously because of their potential to worsen at least some measures of sociability.

1. Introduction

Currently, there are no approved medications that target the symptom domain of "qualitative impairment in social interaction," which contributes significantly to the functional disability of persons with autism spectrum disorders (ASDs) [1]. Importantly, defective or diminished expression of the NMDA receptor is associated with quantitative impairments of sociability in transgenic mice [22,25]; also, D-cycloserine and D-serine, a partial and full agonist of the obligatory glycine co-agonist binding site on the NMDA receptor, respectively, improved measures of sociability in mice without known defects of NMDA receptor expression [15,16,24,28]. For example, D-cycloserine improved measures of sociability in both resident and intruder mice in the "resident mouse-intruder mouse model of social interaction [28]." Further, D-cycloserine and D-serine improved quantitative measures of impaired sociability in the genetically-inbred Balb/c mouse that is behaviorally hypersensitive to MK-801 (dizocilpine) [15,16,24], a noncompetitive NMDA receptor antagonist, although its immunoreactive protein content for six of the eight identified splice variant isoforms of the NR1 subunit, and NR2A and NR2B subunits in cerebral cortex and hippocampus did not differ from an outbred comparator strain [31]. Examples of the behavioral hypersensitivity of the Balb/c strain to MK-801 include greater sensitivity to the elicitation of irregular episodes of intense jumping behavior, referred to as "popping;" antagonism of electrically precipitated seizures; and induction of circling behavior, compared to other inbred and outbred comparator strains; these data suggest that the endogenous tone of NMDA receptor-mediated neurotransmission is altered in Balb/c mice, which may be causally related to its impaired sociability [4,6,18,17]. However, the reason for the altered tone may not be at the level of expression of the NMDA receptor itself, but rather due to abnormalities within circuits that utilize NMDA receptors for

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neurotransmission. Also, D-cycloserine promoted pro-socialization "partner preference" in female prairie voles that socially bond with males in this socially monogamous species [29].

Fragile X syndrome (FXS) is associated with a high prevalence of co-morbid ASDs; excessive protein synthesis in basilar dendrites due to absent expression of the "fragile X mental retardation protein (FMRP)" is implicated in the pathophysiology of this neurodevelopmental disorder [3,19,37]. FMRP inhibits translation by complexing with mRNA associated with polyribosomes in basilar dendrites, leading to unopposed stimulation of protein synthesis, mediated by the mGluR5 subtype of group 1 metabotropic receptors, a G_q-protein linked receptor positively coupled to phospholipase C [9,14,35,36]. The ability of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a relatively selective, potent (IC_{50} value in the 30 nM range), blood–brain barrier permeable non-competitive mGluR5 antagonist, to dampen protein synthesis in basilar dendrites is given as the explanation of its beneficial effects on the behavioral phenotype and histopathology of the *Fmr1* knockout mouse, a transgenic mouse model of FXS deficient in expression of FMRP [20,37,38]. The G protein signaling pathway initiated by stimulation of group 1 mGluRs may also be involved in "cross talk" with NMDA receptors, regulating the sensitivity of NMDA receptors by influencing the phosphorylation status of the S890 serine residue in the intracellular C-terminal domain of the NR1 subunit [2,10,11,26,27,32,35]. Conceivably, MPEP's ability to affect NMDA receptor activation may also contribute to its beneficial effects in the *Fmr1* knockout mouse. MPEP binds to a site in the seven transmembranous hydrophobic domain of the mGluR5 receptor, which is a site that also binds positive allosteric modulators (PAMs) of this metabotropic glutamate receptor [11]; PAMs may potentiate NMDA receptor-mediated neurotransmission.

Because MPEP improves the phenotype of transgenic mouse models of FXS [38], and FXS and ASDs are frequent comorbid disorders, MPEP was studied in BTBR T+tf/J (BTBR) mice, a genetically-inbred mouse strain with phenotypic similarities to all of the prominent symptom domains in ASDs, including qualitative impairments in social interaction and communication and "restricted repetitive and stereotyped patterns of behavior" [34]. MPEP significantly reduced repetitive self-grooming behavior in BTBR mice at doses that did not affect locomotor activity in an open field or measures of sociability. The current study explored the effect of MPEP (30 mg/kg, intraperitoneally) on sociability and spontaneously occurring stereotypic behaviors in the inbred Balb/c strain, another mouse model of ASDs, and the outbred comparator Swiss Webster strain [5,7,15,16,24,33] in a standard paradigm; spontaneous stereotypic behaviors were measured while Balb/c and Swiss Webster mice were allowed to interact freely with a social stimulus mouse in this paradigm. Importantly, stereotypies emerging spontaneously during social interaction can be a disabling symptom in persons with ASDs, competing or interfering with the salience of social stimuli [8,21]. The data confirm a beneficial effect of MPEP on spontaneously occurring stereotypic behaviors in the context of a social interaction; however, there were complex effects on sociability in the two test strains, including worsening of at least some measures of sociability.

2. Methods

2.1. Subjects

Experimentally-naïve, 8-week old male, outbred Swiss Webster and genetically inbred Balb/c "test" mice (Harlan Laboratories, Frederick, MD) were housed 2 per cage, in hanging clear Plexiglas cages with free access to food and water, and maintained on a 12 h light/dark cycle. The "stimulus" mice were 4-week old male ICR mice, housed 4 per cage. Housing conditions were adopted from prior literature [33]. All animal procedures were approved by the Eastern Virginia Medical School Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Group sizes in each of the experimental treatment conditions were 20 animals per group with the exception of the saline-treated Swiss Webster mice, whose group size was equal to 22 animals.

2.2. Drugs

MPEP (2-methyl-6-(phenylethynyl)-pyridine; Sigma–Aldrich Co., St. Louis, MO) was dissolved in 0.9% saline and prepared each day of the experiment. MPEP (30 mg/kg or the saline vehicle) was injected intraperitoneally in a volume of 0.01 ml/g of body weight 20 min prior to testing sociability. The dose of MPEP was selected based on its ability to reduce repetitive self-grooming in BTBR mice and modulate the stress-induced reduction of the antiseizure efficacy of MK-801 (dizocilpine) in Balb/c mice [15], [34]).

2.3. Apparatus

The three-compartment testing apparatus consisted of a black Plexiglas rectangular box (52.07 cm × 25.40 cm × 22.86 cm), without a top or bottom. The center compartment was slightly smaller (12.07 cm × 25.40 cm) than the two end compartments that were of equal size (19.05 cm × 25.40 cm). Inverted wire cups (Galaxy Cup, Kitchen Plus, http://www.kitchen-plus.com) were placed in each side of the end compartments during sessions I and II (discussed below) and housed the stimulus mouse. 500 ml glass bottles were placed on top of the inverted wire cups to prevent climbing during testing. After each test mouse was studied in the sociability paradigm, the apparatus and wire cups were thoroughly cleaned with Quatricide PV solution.

2.4. Sociability procedure

The laboratory adopted an established mouse behavioral procedure for the quantitative assessment of sociability [5,7,12,13,15,16,24,30,33,34]. Briefly, in the first session, a test mouse is placed in the middle compartment and allowed to acclimate to the sociability apparatus for 5 min. In the second 5-min session, a stimulus mouse is enclosed in an inverted wire cup in the side designated as the social compartment, and an empty inverted wire cup is placed in the side designated as the nonsocial compartment. The side designated for the location of the enclosed stimulus mouse is randomly assigned in a counterbalanced fashion throughout the experiment. In the third 5-min session, the stimulus mouse is released from the inverted wire cup, and the test and stimulus mice are allowed to interact freely with each other. All sessions were conducted in dim lighting and videotaped using a Panasonic SDR-S26 SD Video Camera (Panasonic Corp., Osaka, Japan) for future viewing and data collection.

The amount of time test mice spend in the social and nonsocial compartments, the amount of time test mice explore (sniffing) within a 2-cm vicinity of the social and nonsocial inverted cups and locomotor activity (i.e., transitions between compartments) is measured in the second 5-min session. The following measures of sociability, stereotypic behaviors and locomotor activity were reliably obtained in the third 5-min session of free interaction between test and stimulus mice and analysed in this report: discrete episodes of social approach; time spent engaged in social pursuit; discrete episodes of rearing; discrete episodes of wall climbing and time spent engaged in self-grooming [23,34]. Social approach is defined as a discrete episode of initiation of sniffing the social stimulus mouse by the test mouse within at least a two-cm vicinity of each other. Social pursuit is defined as the amount of time the test mouse is engaged in following or chasing the social stimulus mouse from initiation of the encounter until both mice separate by a distance of at least two cm. Rearing is defined as a discrete episode of raising forelimbs and standing on hindlimbs. Wall climbing is defined as a discrete episode of raising forelimbs and placing front paws on walls of the sociability apparatus. Grooming is defined as the amount of time the test mouse is engaged in licking and rubbing of fur with forelimbs during the 5-min period of free social interaction (i.e., third 5-min session). A transition between compartments is defined as the number of times all four extremities cross between compartments.

2.5. Statistics

A two-way ANOVA was used to examine effects of strain (Balb/c vs. Swiss Webster), treatment condition (MPEP vs. saline), and their interaction in discrete episodes of social approach; discrete episodes of rearing; discrete episodes of wall climbing; and time spent engaged in self-grooming. Fisher's LSD Multiple-Comparison post-hoc tests were applied, where appropriate. An inferential statistical technique (e.g., ANOVA) was not used to analyse the data obtained for time engaged in social pursuit due to a floor effect for this dependent variable in one of the cells (i.e., for Balb/c mice treated with MPEP, the time they engaged in social pursuit was 0 s for all cases). Thus, a Pearson Chi-Square test was used to compare the proportion of mice that spent either 0 s or greater than or equal to 5 s engaged in social pursuit as a function of treatment condition (i.e., saline and MPEP). Paired *t* tests were used for within strain comparisons examining the ability of MPEP to influence the salience of a social stimulus, relative to saline-treated test mice. Independent Samples *t*-tests were used to conduct exploratory analyses of differences in the salience of the social stimulus between Balb/c and Swiss Web-