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Typical and atypical antipsychotic drug effects on locomotor hyperactivity and deficits in sensorimotor gating in a genetic model of NMDA receptor hypofunction

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Abstract

Psychotomimetic effects of NMDA antagonists in humans suggest that NMDA receptor hypofunction could contribute to the pathophysiology of schizophrenia. A mouse line that expresses low levels of the NMDA R1 subunit (NR1) of the NMDA receptor was generated to model endogenous NMDA hypofunction. These mutant mice show increased locomotor activity, increased acoustic startle reactivity and deficits in prepulse inhibition (PPI) of acoustic startle. The present study examined effects of a typical antipsychotic drug, haloperidol, and two atypical antipsychotic drugs (olanzapine and risperidone) on behavioral alterations in the NR1 hypomorphic (NR1−/−) mice. Haloperidol significantly reduced activity in the wild type controls at each dose tested (0.05, 0.1, and 0.2 mg/kg). The NR1−/− mice were less sensitive to the haloperidol-induced locomotor inhibition in comparison to the NR1+/+ mice. In contrast to haloperidol, olanzapine reduced the hyperactivity in the NR1−/− mice at a dose that produced minimal effects on locomotor activity in the wild type mice. These data suggest that non-dopaminergic blocking properties of olanzapine contribute to the drug's ability to reduce hyperactivity in the NR1 deficient mice. In the PPI paradigm, haloperidol (0.5 mg/kg) did not affect the increased startle reactivity in the NR1−/− mice, but did reduce startle amplitude in the NR1+/+ mice. Haloperidol increased PPI in both the mutant and wild type strains. Unlike haloperidol, risperidone (0.3 mg/kg) and olanzapine (3 mg/kg) reduced startle magnitude in both NR1+/+ and NR1−/− mice. Like haloperidol, risperidone and olanzapine increased PPI in both NR1+/+ and NR1−/− mice. The similar effects of these atypical antipsychotic drugs in wild type mice and mice with markedly reduced NR1 expression suggest that the drugs were not working by a NMDA receptor-dependent mechanism to increase PPI. Since both haloperidol and the atypical drugs increased PPI, it is likely that D_2 dopamine receptor blockade is responsible for the drug effects on sensorimotor gating.

Keywords: Locomotor activity; Prepulse inhibition; Acoustic startle; Haloperidol; Olanzapine; Risperidone; NMDA receptor; Animal model; Schizophrenia

1. Introduction

NMDA receptor antagonists such as ketamine and phencyclidine (PCP) induce behaviors in healthy humans that mimic positive, negative and cognitive symptoms of schizophrenia (Cohen et al., 1962; Krystal et al., 1994; Lahti et al., 2001; Luby et al., 1959; Malhotra et al., 1996). In addition, schizophrenia patients challenged with ketamine have symptom exacerbation similar to that experienced during active phases of their illness (Lahti et al., 1995a,b; Malhotra et al., 1997). These data provide support for the hypothesis that reduced NMDA receptor function could contribute to the pathophysiology of schizophrenia.

If reduced function of NMDA receptors is involved in schizophrenia, investigating neurobiological and behavioral consequences of experimentally-induced NMDA receptor hypofunction could contribute to understanding the disease. To study the effects of endogenous NMDA receptor hypofunction, a mouse genetic model of reduced NMDA receptor expression was generated in which the NMDA R1 (NR1) subunit of the NMDA receptor is reduced markedly (Mohn

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et al., 1999). These mice are characterized as NR1 hypomorphic, since the genetic alteration (insertion of a neomycin resistance gene into intron 20 of the *NR1* locus) results in dramatic under-expression, but not elimination, of the NR1 gene. Western blot analysis of cerebral cortical homogenates indicated almost 90% reduction in the expression of the NR1 protein in the mutant mice (Mohn et al., 1999). Autoradiographic analysis of ^{3}H-MK-801 binding demonstrated global reduction in ligand binding with decreases of 60–85% binding found among different brain regions. The hippocampal formation exhibited the greatest reduction in ^{3}H-MK-801 binding in the mutant mice.

The NR1 hypomorphic mice (NR1−/−) exhibit altered phenotypes that include increased locomotor activity (Mohn et al., 1999), reduced metabolic activity in the medial prefrontal cortex, anterior cingulate cortex, and the hippocampus (Duncan et al., 2002), deficits in social interactions (Duncan et al., 2004; Mohn et al., 1999), and deficits in prepulse inhibition of acoustic startle (PPI) (Duncan et al., 2006, 2004; Fradley et al., 2005). In addition, the NR1−/− mice show enhanced sensitivity to amphetamine-induced PPI disruption (Moy et al., 2006), but not to the locomotor stimulatory or Fos-inducing effects of amphetamine (Miyamoto et al., 2004).

Schizophrenia patients show deficits in sensorimotor gating in acoustic startle and tactile startle PPI paradigms (Braff et al., 2001). NMDA antagonists disrupt PPI in rodents, and the prototypical "atypical" antipsychotic drug clozapine is more effective than the typical antipsychotic drug haloperidol in blocking the consequence of this pharmacologically-induced NMDA receptor hypofunction (Bakshi et al., 1994; Keith et al., 1991). Some of the newer "atypical" or second generation antipsychotic drugs (e.g. olanzapine, quetiapine) also block NMDA antagonist-induced deficits in PPI (Bakshi and Geyer, 1995; Swerdlow et al., 1996). In a previous study, we found that clozapine and quetiapine, but not haloperidol, reduced the acoustic startle responses in the NR1 hypomorphic mice (Duncan et al., 2006). However, all three of the antipsychotics tested reduced PPI in the mutant mice. The present study compared effects of haloperidol with two other atypical antipsychotic drugs, olanzapine and risperidone, on deficits in PPI in the NR1 hypomorphic mice, to determine if these atypical antipsychotics exhibit profiles similar to clozapine and quetiapine in the model. In addition, effects of haloperidol and olanzapine were compared on locomotor activity in the NR1+/+ and NR1−/− mice.

2. Methods

2.1. Generation of F1 hybrid NR1 hypomorphic mice

The NR1-deficient mice were created initially on a mixed genetic background consisting of alleles derived from 129/SvEv, C57BL/6, and DBA/2 (Mohn et al., 1999). It is clear that modifier alleles present in various inbred mouse lines can dramatically alter the impact of primary genetic lesions. Therefore, it was important to obtain populations of mice that differ genetically only at the NR1 locus. A strategy was devised to produce NR1 hypomorphs and genetically identical wild type populations by generating F1 hybrid mice from C57BL/6 and 129/ SvEv inbred strains. C57BL/6 heterozygous (NR1+/−) animals were intercrossed with 129/SvEv heterozygous animals. All of the F1 offspring of these litters are genetically identical at all loci except at the *NR1* gene. This approach was taken because the NR1-/- homozygotes do not breed and the NR1-/- mice must be bred from heterozygote NR1+/- mice to obtain NR1-/- progeny. When attempts were made to breed NR1+/- mice from parents of the same inbred strain, the mutation produced stunted growth and lower than expected yield of the NR1-/- genotype. These problems were not found for the F1 hybrid NR1−/− mice. The NR1 mutation was maintained on the C57BL/6 and 129/ SvEv genetic backgrounds by breeding heterozygous animals to commercially available stock (Jackson Laboratories). Resulting heterozygous offspring from these crosses were used to maintain the lines and to provide heterozygous breeders for the generation of the F1 hybrid homozygous mice (NR1−/−) and their control populations (NR1+/+).

All procedures in the present study were conducted in strict compliance with the policies on animal welfare of the National Institutes of Health and the University of North Carolina (stated in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, 1996 edition), and approved by the University of North Carolina Institutional Animal Care and Use Committee.

2.2. Drugs

Drugs were administered IP either immediately before testing, for the activity measure, or 30 min before testing, for the PPI measure. Doses of haloperidol (Pharmaceutical Associates, Inc.) were derived by diluting an oral solution of haloperidol lactate with 0.9% saline. Risperidone (Sigma) was suspended in a 23% (w/v) solution of cyclodextrin (Sigma). Olanzapine (Eli Lilly) was dissolved in vehicle containing 5 μl of 20% acetic acid/ml 0.9% saline.

2.3. Assessment of locomotor activity and drug administration

Locomotor activity was assessed in photocell-based activity chambers under standardized environmental conditions, using a TruScan activity monitor (Coulbourn Instruments, Allentown, PA) with a 25.8×25.8 cm Plexiglas chamber and a beam spacing of 1.52 cm. Mice were acclimated to the room in which testing was carried out for at least at least 7 days before testing. Mice were injected with vehicle, haloperidol (0.05–0.2 mg/kg), or olanzapine (0.625 or 1.25 mg/kg) immediately prior to placement in the activity chambers. Separate cohorts of mice were used for each dose of each drug examined. Activity data were collected for each mouse over a 180 min time course, beginning when the mouse was first placed in the testing chamber. Data were collected in five-minute intervals. The distance traveled in each five-minute interval was measured as the total of all vectored *X*–*Y* coordinate changes. For each group of mice, the mean±SEM was calculated for each five-minute time interval.