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Research Report

Effects of the selective kainate receptor antagonist ACET on altered sensorimotor gating in a genetic model of reduced NMDA receptor function

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ABSTRACT

The pathophysiology of schizophrenia may involve reduced NMDA receptor function. Accordingly, experimental models of NMDA receptor hypofunction may be useful for testing potential new antipsychotic agents and for characterizing neurobiological abnormalities relevant to schizophrenia. We demonstrated previously that mice underexpressing the NR1 subunit of the NMDA receptor show supersensitive behavioral responses to kainic acid and that a kainate receptor antagonist normalized altered behaviors in the mutant mice ($NR1^{neo/neo}$). The present work examined effects of another selective kainate receptor antagonist, (S)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxy-5-phenylthiophene-3-yl-methylpyrimidine-2,4-dione (ACET), on altered behavioral phenotypes in the genetic model of NMDA receptor hypofunction. ACET, at a dose of 15 mg/kg, partially reversed the deficits in prepulse inhibition produced by the mutation. The 15 mg/kg dose of ACET was also effective in reversing behavioral effects of the selective kainate agonist ATPA. However, ACET did not significantly reduce the increased locomotor activity and rearing behavior observed in the $NR1^{neo/neo}$ mice. These findings show that a highly selective kainate receptor antagonist can affect the deficits in sensorimotor gating in the $NR1^{neo/neo}$ mice. The results also provide further support for the idea that selective kainate receptor antagonists could be novel therapeutic candidates for schizophrenia.

1. Introduction

Altered glutamate mediated neurotransmission has been suggested to play a role in the pathophysiology of schizophrenia. The most compelling evidence in this regard is the well-documented effects of NMDA receptor antagonists to induce a spectrum of behavioral alterations in healthy humans that mimic positive, cognitive and affective symptoms of schizophrenia (Javitt and Zukin, 1991; Krystal et al., 1994). In addition, NMDA antagonists precipitate symptoms in stabilized schizophrenia patients (Lahti et al., 1995).

Based on the NMDA hypofunction hypothesis of schizophrenia, Mohn et al. (1999) developed a novel mutant mouse model characterized by markedly reduced expression of the

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NR1 subunit of the NMDA receptor. The partial disruption of NR1 subunit expression was produced by insertion of a neomycin resistance cassette into intron 20 of the *NR1* (*Grin1*) locus. This insertion mutation results in a marked under-expression of the NR1 subunit to approximately 10% of wild type values in all regions examined (Mohn et al., 1999; Ramsey et al., 2008). The homozygous *NR1*$^{neo/neo}$ mutant animals are sometimes referred to as *NR1* hypomorphic or *NR1* knock-down, since expression of the gene is reduced, but not eliminated.

The *NR1*$^{neo/neo}$ mice exhibit a number of behavioral phenotypes that support their utility to model certain behavioral characteristics of schizophrenia. These phenotypes include reduced locomotor habituation in a novel environment (Duncan et al., 2002; Mohn et al., 1999) and deficits in prepulse inhibition of acoustic startle (PPI) (Duncan et al., 2004, 2006a, 2006b; Fradley et al., 2005). In addition, the mutant mice show enhanced sensitivity to amphetamine-induced disruption of PPI (Moy et al., 2006). The *NR1*$^{neo/neo}$ mice also show marked deficits in tests of social affiliation and social aggression (Duncan et al., 2004; Halene et al., 2009; Mohn et al., 1999). Behavioral alterations in the *NR1*$^{neo/neo}$ mice are reduced by the administration of typical and atypical antipsychotic drugs, although the atypical drugs exhibit a distinct behavioral profile compared to the typical drugs in the model (Duncan et al., 2006a, 2006b; Mohn et al., 1999).

We recently discovered that the *NR1*$^{neo/neo}$ mice exhibit an increase in sensitivity to seizure producing effects of systemically administered kainic acid, an agonist of a kainate subtype of glutamate receptors (Duncan et al., 2010). To test the hypothesis that increased kainate receptor sensitivity to endogenous glutamate could contribute to the abnormal behavioral phenotypes of the *NR1*$^{neo/neo}$ mice, a highly selective kainate receptor antagonist, LY382884, was given to the mice before assessment of prepulse inhibition of acoustic startle and activity in an open field (Duncan et al., 2010). The drug completely normalized the exaggerated baseline startle response of the mutant mice but had no effect on startle amplitude in the controls. LY382884 increased PPI in both wild type and control mice. The drug also reduced the hyperactivity in horizontal locomotion and rearing behavior in the *NR1*$^{neo/neo}$ mice, without affecting activity in the wild type mice. It is of interest that the behavioral profile of LY382884 in the mutant mice is similar to that of several atypical antipsychotic drugs tested in the model (Duncan et al., 2006a, 2006b). LY382884 has relatively low potency as a kainate antagonist and penetrates the brain poorly. However, these initial studies open an interesting door of possibilities: that kainate antagonists could have efficacy in the treatment of schizophrenia. In order to further pursue this exciting prospect, the present work tested another highly selective and more potent kainate antagonist in the NR1 hypomorphic model, (S)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxy-5-phenylthiophene-3-yl-methylpyrimidine-2,4-dione, abbreviated ACET (Dargan et al., 2009; Jane et al., 2009).

2. Results

2.1. ACET blockade of ATPA-induced immobility

Optimal doses for ACET efficacy in behavioral assays were determined by an initial screen, using only wild type mice, against the characteristic immobility following i.p. administration of ATPA, the *tert*-butyl analogue of AMPA. In previous studies, ATPA was identified as an AMPA agonist (Ornstein et al., 1996), but was later discovered to be a selective agonist of the GluK1 (formerly GluR5) kainate receptor (Hoo et al., 1999; Jane et al., 2009). In the present study, ATPA (30 mg/kg, i.p.) consistently induced a state of complete immobility and flat body posture in all mice tested. However, if the mice were picked up they would exhibit typical activation and when returned to the cage would move briefly, but then become immobile with the flat body posture. Pretreatment with a low dose of ACET (7.5 mg/kg, s.c.), given 1 h before ATPA, failed to attenuate immobility. However, a higher dose of ACET (15 mg/kg) antagonized ATPA-induced behavioral suppression and all 5 mice tested at this dose demonstrated normal ambulatory activity. Therefore, we utilized 7.5 mg/kg and 15 mg/kg as low and moderate doses of ACET for the acoustic startle and activity tests (described below).

2.2. Receptor-binding profile of ACET at non-glutamate receptors

Although the specificity of ACET for the kainate subtype of glutamate receptors is well documented, there is no information available for potential off-target non-glutamatergic receptor selectivity. The ability of ACET to bind to a wide range of receptors was examined and results are reported in the supplementary data. The receptors examined included subtypes of serotonin, norepinephrine, dopamine, histamine, acetylcholine, and monoamine transporters. Results of this screening process showed no appreciable binding affinity of ACET for any receptor tested, with Ki values ranging from 3000 nM to >10,000 nM (Table 1, supplemental).

2.3. Effects of ACET on acoustic startle and PPI in wild type and NR1$^{neo/neo}$ mice

The *NR1* hypomorphic mice showed the expected increased startle responses and reduced PPI, in comparison to the wild type subjects. Overall repeated measures analyses revealed significant interactions between genotype, treatment, and decibel level for amplitude [$F(5,345)=3.9$, $p=0.0019$] and ppi [$F(4,276)=3.21$, $p=0.0135$]. The analyses also revealed significant effects of ACET dose for each measure [amplitude, interaction between genotype, dose, and decibel level, $F(5,345)=3.72$, $p=0.0027$; PPI, treatment × dose interaction, $F(1,69)=9.81$, $p=0.0025$]. Separate analyses were then conducted to determine treatment effects at each ACET dose (7.5 or 15 mg/kg).

In the cohort of mice tested with the lower dose of ACET (7.5 mg/kg), repeated measures ANOVAs indicated highly significant main effects of genotype on startle amplitude [$F(1,31)=11.69$, $p=0.0018$] and PPI [$F(1,31)=20.74$, $p<0.0001$], but no significant main effects or interactions for ACET treatment (Figs. 1 and 2). In contrast, the higher dose of ACET (15 mg/kg) had significant effects on startle amplitude (Fig. 3) and on PPI (Fig. 4), dependent upon genotype. Similar to the cohort of mice used to assess the 7.5 mg/kg dose of ACET, in the cohort used to test the 15 mg/kg dose the overall repeated measures ANOVAs revealed significant main effects of genotype for startle amplitudes [$F(1,38)=26.93$, $p<0.0001$] and PPI [$F(1,38)=29.33$,