Phencyclidine and Dizocilpine Induced Behaviors Reduced by N-acetylaspartylglutamate Peptidase Inhibition via Metabotropic Glutamate Receptors

Rafal T. Olszewski, Marta M. Wegorzewska, Ana C. Monteiro, Kristyn A. Krolikowski, Jia Zhou, Alan P. Kozikowski, Katrice Long, John Mastropaolo, Stephen I. Deutsch, and Joseph H. Neale

Background: *N*-methyl-D-aspartate (NMDA) receptor open channel blockers phencyclidine (PCP) and dizocilpine (MK-801) elicit schizophrenia-like symptoms in humans and in animal models. Group II metabotropic glutamate receptor agonists reverse the behavioral effects of PCP and MK-801 in animal models. *N*-acetylaspartylglutamate (NAAG), the third most prevalent neurotransmitter in the mammalian nervous system, is a selective group II metabotropic glutamate receptor agonist. We previously reported that ZJ43, a potent inhibitor of the enzymes that inactivate synaptically released NAAG, reduced motor and stereotypic effects of PCP in the rat.

Methods: To confirm the efficacy of NAAG peptidase inhibition in decreasing motor behaviors induced by PCP and MK-801, ZJ43 was tested in additional schizophrenia models.

Results: ZJ43 reduced MK-801-induced motor activation in a mouse model that has been used to characterize the efficacy of a wide range of pharmacotherapies for this human disorder. In a second mouse strain, the peptidase inhibitor reduced PCP-induced stereotypic movements. ZJ43 also reduced PCP-induced negative symptoms in a resident-intruder assay. The group II metabotropic glutamate receptor antagonist, LY341495, blocked the effect of NAAG peptidase inhibition in these mouse models of positive and negative PCP- and MK-801-induced behaviors. Additionally, LY341495 alone increased some PCP-induced behaviors suggesting that normal levels of NAAG act to moderate the effect of PCP via a group II mGluR.

Conclusions: These data support the proposal that NAAG peptidase inhibition and elevation of synaptic NAAG levels represent a new therapeutic approach to treating the positive and negative symptoms of schizophrenia that are modeled by open channel NMDA receptor antagonists.

Key Words: Group II metabotropic glutamate receptor, LY341495, mGluR3, MK-801, NAAG, N-acetylaspartylglutamate, NMDA receptors, phencyclidine, schizophrenia

Nearly one percent of humans express symptoms of schizophrenia. The efficacy of dopamine D_2 antagonists, haloperidol and chlorpromazine, in treating schizophrenia supports the view that dopaminergic neurons contribute to the expression of this disorder (1), while studies using drugs that affect *N*-methyl-D-aspartate (NMDA) receptors suggest that glutamatergic pathways also are involved in schizophrenia (2–8). For example, open channel NMDA receptor antagonists phencyclidine (PCP), ketamine and dizocilpine (MK-801) induce schizophrenia-like positive, negative, and cognitive symptoms in humans and behaviors in animals. Drugs that are useful in treating schizophrenic patients moderate these PCP- and MK-801-induced behaviors. Group II metabotropic glutamate receptor (mGluR2 and mGluR3) agonists reduce these symptoms in humans and animal models (9–13). Further, polymorphisms in the human mGluR3 gene meet the criterion of association with risk of schizophrenia in three independent studies (reviewed in 14). Consistent with a role for glutamate and NMDA receptors in expression of schizophrenia, D-serine, D-alanine, and D-cycloserine, positive modulators of NMDA receptors show promise as adjuvant therapy for this disorder (15,16). Additionally, NMDA receptor deficits have been identified in vivo in medication-free schizophrenic patients (17).

The peptide neurotransmitter N-acetylaspartylglutamate (NAAG) is widely distributed in the central and peripheral nervous systems at millimolar concentrations (18–20). NAAG is a mGluR3 selective group II mGluR agonist (21,22) and is codistributed with different small amine transmitters including glutamate and GABA (reviewed in 23,24). One function of NAAG is the activation of presynaptic mGluRs to inhibit transmitter release (reviewed in 25). Synaptically released NAAG is inactivated by two extracellular peptidases, glutamate carboxypeptidase II and III (26–29). Inhibition of these peptidases reduces symptoms in animal models of glutamate-mediated clinical conditions including stroke, inflammatory and neuropathic pain, and traumatic brain injury (25,30).

Consistent with the efficacy of group II mGluR agonists in moderating the schizophrenia-like behaviors elicited by PCP and MK-801, inhibition of NAAG peptidases by a novel NAAG analogue, ZJ43, also is effective in reducing PCP-evoked motor and stereotypic movements in rats (31). The present study tests the hypothesis that inhibition of NAAG peptidase and the consequent increase in NAAG activation of group II mGluRs, is effective across different models of positive and negative symptoms elicited by PCP and MK-801.

Methods and Materials

Animals

The experimental protocols used in this research were approved by the Georgetown University Animal Care and Use

From the Departments of Biology (RTO, MMW, ACM, KAK, JHN) and Psychiatry (SID), Georgetown University; Mental Health Service Line (KL, JM, SID), Department of Veterans Affairs Medical Center, Washington D.C.; Acenta Discovery Inc. (JZ, APK), Tucson, Arizona; Department of Medicinal Chemistry and Pharmacognosy (APK), University of Illinois at Chicago, Illinois.

Address reprint requests to Joseph H. Neale, Ph.D., Department of Biology, Georgetown University, Washington, D.C. 20057; E-mail: nealej@georgetown.edu.

Received March 13, 2007; revised April 13, 2007; accepted April 13, 2007.

0006-3223/08/$34.00
doi:10.1016/j.biopsych.2007.04.016

Committee consistent with guidelines of the United States National Institutes of Health (NIH).

Adult C57BL/6J mice and NIH Swiss mice (National Cancer Institute, Frederick Research Center, Frederick, Maryland) and DAB/2 mice (Taconic Farms, Rockville, Maryland) were maintained on a 12:12 hour light-dark cycle. Food and water were available ad libitium. Mice were housed 5 to a cage, except as noted for the resident-intruder assay. Behavioral testing was performed between 10 AM and 4 PM. Animals were weighed prior to drug administration and observation.

Drugs

ZJ43 was synthesized by Acenta Discovery, Inc., as described in Olszewski *et al.*, (31), PCP and MK-801 ([+]-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine maleate; dizocilpine) were from Sigma Aldrich (St. Louis, Missouri). LY341495 was from Tocris Cookson Ltd. (Bristol, United Kingdom). LY341495 is a highly selective group II mGluR antagonist (32). All compounds were dissolved in saline for intraperitoneal (IP) injection.

Open Field Stereotypic Movement Assessment

Adult male DBA/2 male mice, 25-30 g in weight, were placed individually in a Med Associates (St. Albans, Vermont) ENV-515 open field chamber (43 × 43 cm) with evenly spaced infrared beams and detectors. After a 10 min habituation interval, each animal was injected IP first time with saline or ZJ43 (10-200 mg/kg) or LY341495 (1 or 3 mg/kg) alone or ZJ43 plus LY341495, and placed back into the chamber for another 10 min. Mice then were given a second IP injection with PCP (6 mg/kg) or saline. The motor behavior was continuously scored for 15 min starting 10 min after the second injection. Stereotypic movements were assessed automatically by the infrared sensors. Stereotypic index or counts were defined as the total number of vertical or horizontal sensor breaks within a predefined "Box" within the open field. A "Box Size" of 4 beams (defined in Med Associates Activity Monitor 5 open field chamber and software) was selected to maximize the detection of stereotypic head weaving and discriminate these movements from ambulatory movements across boxes within the open field.

MK-801 Induced Jumping

Adult male NIH-Swiss mice were habituated for 5 min in a clear plastic open field chamber (18 cm × 29 cm × 12 cm high) that was identical in size to their home cage.

Mice then were injected IP with either saline or LY341495 (1 mg/kg) or ZJ43 (200 mg/kg) with or without LY341495 and placed back into the chamber. After another 5 min, mice were injected with saline or MK-801 (1 mg/kg). Mice were then continuously observed and jumping episodes were scored during the next 30 min by an observer who was naive with respect to the drug treatments. A single jumping episode consisted of a series of continuous uncontrolled vertical movements lasting from 1-5 sec. Data are reported as the total jumping episodes observed over the 30 min interval.

Resident-Intruder Assay

Male DBA/2 mice, 30-45 days old, were housed as either "resident" or "intruder" mice in the same animal room. Resident mice were isolated in individual cages for 7-14 days and intruder mice were continuously housed in groups of five. On the test day, resident mice were injected with either saline or ZJ43 (150 mg/kg, IP) or LY341495 (1 mg/kg, IP) or ZJ43 plus LY341495 and returned to the cage for 10 min prior to injection with saline or PCP (6 mg/kg, IP). These resident mice were then returned to their home cages and 5 min later an intruder mouse was introduced into the resident's home cage. The resident mouse's behavior was video recorded for 10 min. The videos of resident behaviors were scored separately by two observers who were blinded to the treatments. A series of behaviors were scored including the following: crouch defense, when experimental mouse crouched when in contact with the intruder mouse; following, when experimental mouse walked after the intruder mouse; flight, when experimental mouse ran away from the intruder mouse; leap, when experimental mouse jumped off the ground in response to the presence of the intruder.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS software 11.0 (SPSS Inc., Chicago, Illinois) with significant differences noted with $p < .05$. When ANOVA was significant, the post hoc comparisons using Student-Newman-Keul test identified pair-wise group differences.

Results

DBA/2 mice treated with PCP (6 mg/kg, IP) exhibited a two-fold increase in stereotypic motor activity in the open field assay system (Figure 1) compared to mice injected with saline, saline with ZJ43 or saline with the group II mGluR antagonist LY341495 (1 or 3 mg/kg, IP). ZJ43 (150 mg/kg, IP) given 10 min before PCP significantly reduced this behavior. LY341495 at 3 mg/kg, but not 1 mg/kg, blocked the effect of ZJ43. The group II antagonist at 3 mg/kg also significantly increased the effect of PCP in the absence of ZJ43, an effect taken to suggest that endogenous NAAG or glutamate activation of group II mGluR reduces the effects of PCP even in the absence of peptidase inhibitor. ZJ43 reduced the effect of PCP in a dose- dependent manner when tested between 10 and 150 mg/kg (IP) (Figure 2).

In order to determine if the effects of NAAG peptidase inhibition were specific for PCP or generalized to other NMDA open channel

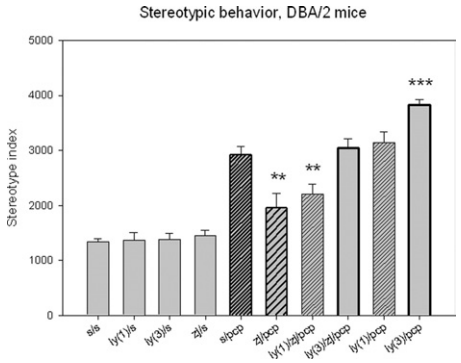

Figure 1. N-acetylaspartylglutamate (NAAG) peptidase inhibition reduces phencyclidine (PCP)-induced stereotypic movements in DBA/2 mice. Mice (10 to 15 mice per group) were given two intraperitoneal [IP] injections 10 min apart and their activity monitored in an open field chamber 10 min after the last injection. Injection with the group II antagonist LY341495 (1 or 3 mg/kg) or the NAAG peptidase inhibitor, ZJ43 (150 mg/kg), followed by saline had the same effect as two injections of saline. Saline followed by 6 mg/kg of PCP induced a significant increase in stereotypic behavior versus each of the other saline groups ($p < .001$). ZJ43 (150 mg/kg) significantly decreased the effects of PCP on stereotypic behavior and this action of ZJ43 was reversed by coinjection of 3 mg/kg LY341495 with ZJ43 10 min prior to injection of PCP. Preinjection of mice with 3 mg/kg LY341495 alone prior to PCP injection significantly increased the stereotypy above that obtained with saline PCP. * $p < .05$; ** $p < .01$; *** $p < .001$.