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The mGluR7 allosteric agonist AMN082 produces antidepressant-like effects by modulating glutamatergic signaling

Stefania Risso Bradley *[,1], Jason M. Uslaner [1], Rose B. Flick, Ariel Lee, Kristina M. Groover, Peter H. Hutson

MRL Department of Neuroscience, Merck & Co., Inc., West Point, PA, USA

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ABSTRACT

Currently prescribed antidepressants affect the reuptake and/or metabolism of biogenic amines. Unfortunately for patients, these treatments require several weeks to produce significant symptom remission. However, recently it has been found that ketamine, a dissociative anesthetic agent that noncompetitively antagonizes NMDA (N-Methyl-D-aspartic acid) receptors, has rapid antidepressant effects at sub-anesthetic doses in clinically depressed patients. These findings indicate that modulation of the glutamatergic system could be an efficient way to achieve antidepressant activity. For this reason, other mechanisms influencing glutamatergic functioning have gained interest. For example, the metabotropic glutamate receptor 7 (mGluR7) allosteric agonist AMN082 (N,N′-dibenzyhydryl-ethane-1,2-diamine dihydrochloride) has been shown to be effective in the forced swim and tail-suspension test, behavioral assays sensitive to antidepressants. Here we extend the characterization of AMN082 by demonstrating its effects on differential reinforcement of low rates of responding (DRL)-30, another assay sensitive to antidepressants. Furthermore, we show the engagement of glutamatergic signaling by demonstrating the ability of the selective AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid) receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione) to reverse the effects of AMN082 in the tail suspension test. In contrast, NBQX failed to reverse the effects of imipramine in the same behavioral test. Finally, we report that behaviorally efficacious doses of AMN082 modulate phosphorylation of AMPA and NMDA receptor subunits in the hippocampus. These results suggest that the antidepressant-like effects of AMN082 are, at least in part, due to modulation of AMPA and NMDA receptor activity. Therefore, our findings confirm the hypothesis that mGluR7 could represent a novel target for treating depression.

1. Introduction

In the search for novel mechanisms to treat depression, the focus has shifted from compounds that influence the monoamines, such as serotonin and norepinephrine, to compounds that modulate other neuronal systems. NMDA receptor antagonists, such as ketamine and MK-801, produce robust antidepressant-like effects in preclinical models (Dubrovina et al., 2007; Molina-Hernandez et al., 2008; Papp and Moryl, 1994) and very rapid and sustained antidepressant effects in patients (Liebrenz et al., 2007; Preskorn et al., 2008). The effects of these compounds appear to be due to their downstream activation of the AMPA receptor, since the AMPA receptor antagonist NBQX blocks the effects of ketamine and MK-801 in rodent assays predictive of antidepressant potential. Furthermore, ketamine decreases hippocampal phosphorylation of the AMPA receptor subunit GluR1 (Maeng et al., 2008). As a consequence of these findings, compounds that modulate glutamatergic signaling and AMPA receptor activation have garnered attention for treating depression (Skolnick et al., 2009).

Given the important role of glutamatergic transmission in alleviating symptoms of depression and also the abuse liability and cognitive disruptive effects of NMDA receptor antagonists, studies examining the therapeutic potential of modulating the glutamatergic transmission acting on the metabotropic glutamate receptors (mGluRs) seems called for. Anatomic evidence demonstrates that mGluR7 has the highest CNS density of all group III mGluR subtypes (Bradley et al., 1998; Shigemoto et al., 1997). Furthermore, immunohistochemical studies at the electron microscopic level demonstrate that mGluR7 is located predominantly on presynaptic terminals of glutamatergic and GABAergic neurons (Bradley et al., 1996; Shigemoto et al., 1997; Cartmell and Schoepp, 2000).

Abbreviations: mGluR7, metabotropic glutamate receptor 7; AMN082, N,N′-dibenzyhydryl-ethane-1,2-diamine dihydrochloride; NMDA, N-Methyl-D-aspartic acid; AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione.

* Corresponding author at: Merck Sharp & Dohme Corp., 770 Sumneytown Pike, WP26-283A, West Point, PA 19486, USA. Tel.: +1 215 652 2021.

E-mail addresses: Stefania_bradley@merck.com (S.R. Bradley), Jason_uslaner@merck.com (J.M. Uslaner), Rosemarie_flick@merck.com (R.B. Flick), Ariel_lee@merck.com (A. Lee), Kristina_groover@merck.com (K.M. Groover), Peter_hutson@merck.com (P.H. Hutson).

[1] Equal contributors.

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AMN082 is an allosteric mGluR7 agonist (Mitsukawa et al., 2005) that has been found to produce antidepressant-like effects in preclinical tests such as the tail-suspension and forced swim tests (Palucha et al., 2007; Palucha-Poniewiera et al., 2010). The antidepressant-like effects of AMN082 are absent in mGluR7 knockout mice, suggesting that its effects are indeed due to mGluR7 activation, rather than off-target activity. Nevertheless, AMN082 was has weak binding affinity for the norepinephrine, dopamine and serotonin transporters. Furthermore, the principle rat metabolite of AMN082 met-1 was significantly weaker at mGluR7 receptors but had moderate affinity (Ki ~ 300 nM) for the serotonin transporter and demonstrated antidepressant activity in rodents; (Sukoff Rizzo et al., 2011). Furthermore, it was recently shown that the antidepressant-like effects of AMN082 are dependent on serotonergic functioning, as depletion of serotonin, as well as 5-HT1a receptor antagonism, blocked the antidepressant-like effects of AMN082 (Palucha-Poniewiera et al., 2010). It is unclear, however, whether the influence of AMN082 on serotonergic transmission is a direct effect, or a downstream consequence of increasing glutamate release (Li et al., 2008) and subsequent AMPA receptor activation, as has been found for other compounds that directly modulate glutamatergic transmission (Karasawa et al., 2005).

The present experiments were designed to further characterize the antidepressant-like effects of AMN082 and its mechanism of action. First using the AMPA receptor antagonist NBQX, we examined the importance of AMPA receptor activation on the ability of AMN082 to produce antidepressant-like effects in the tail suspension test. Second, we measured the influence of AMN082 on GluR1, NR1, and NR2b phosphorylation in the hippocampus. Finally, we extended the behavioral characterization of AMN082 to an additional assay sensitive to antidepressants, the differential reinforcement of low rates of responding (DRL).

2. Materials and methods

2.1. Animals

All protocols were approved by Merck & Co. Institutional Animal Care and Use Committee and in accordance with the National Institute of Health's Guide for Care and Use of Laboratory Animals. Adult C57Bl6 male mice weighing 20–30 g were used for the tail suspension test. Mice were housed in groups of four and were maintained on a 12 h light/dark cycle (lights on 7:00 am) with food and water available ad libitum. Male Sprague–Dawley rats (250–300 g) purchased from Charles River Laboratories were used for the differential reinforcement tasks. Animals were single-housed in a 12/12 h reverse light/dark cycle (lights on at 19:00). Water and food were available ad libitum until the training phase of the experiment. Subsequently, rats were fed 16 g of rat chow per day following the daily operant session. Temperature and relative humidity were maintained at 22–24 °C and 50–55%, respectively.

2.2. mGluR7 cAMP assay

CHO-NFAT cells were stably transfected to constitutively express human mGluR7a and Gα15. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 20 mM HEPES, 2 mM L-glutamine, 100 mM penicillin/streptomycin, 1× non-essential amino acids, 1 mM sodium pyrvuate, 55 μM B-mercaptoethanol, 250 μg/ml zeocin, 5 μg/ml puromycin and 1 mg/ml G418 (Invitrogen, Carlsbad, CA) at 37 °C in the presence of 5% CO_2. On the day of the assay, growth medium was replaced by starvation medium (DMEM, 10% dialyzed fetal bovine serum, 100 mM penicillin/streptomycin) and incubated at 37 °C in the presence of 5% CO_2 for 4 h before use. Cells were incubated with either agonists (glutamate (Sigma), LAP4 (Tocris), AMN082 (Tocris)) or antagonists MMPIP (Tocris) in assay media (glutamate free, serum free DMEM, Invitrogen) at a density of 20,000 cells/well, in white opaque 384 well plates (Perkin Elmer) for 30 min at 37 °C. Cyclic AMP formation was stimulated by incubation with 5 μM Forskolin (Sigma) for an additional 30 min at 37 °C. Following incubation antibody reagent was added and cells were lysed with ED reagent/Lysis buffer/CL substrate reagent combination (cAMP XS HitHunter Assay Kit, DiscoveRx) and incubated for 1 h at room temperature. After a subsequent incubation with EA Reagent (cAMP XS HitHunter Assay Kit, DiscoveRx) for 2 h at RT cAMP concentrations were determined by measuring luminescence on the Envision Microplate Luminometer (Perkin Elmer). Compounds were titrated in quadruplicate, in an 11 point titration, with 3-fold increments. EC_{50} values were calculated from concentration response curves using Prism software (GraphPad, San Diego, CA).

2.3. Behavioral studies

2.3.1. Tail suspension test (TST)

Three experiments were conducted in order to characterize the effects of AMN082 on the TST and determine its mechanism of action. The first experiment was conducted to determine the dose-effect relationship of AMN082 on the TST. Animals were given vehicle (0.5% Methylcellulose) or AMN082 (1, 3, or 10 mg/kg) via an IP injection and 40 min later the animals were attached to the tail suspension apparatus and immobility was assessed for 6 min. The second experiment was designed to determine whether the effects of AMN082 on TST are mediated by AMPA receptor activation. Mice were dosed via an IP injection with vehicle (0.5% Methylcellulose), NBQX (10 mg/kg), AMN082 (10 mg/kg), or both compounds and 40 min later animals were examined on the TST. Finally, in order to determine the specificity of the effects of NBQX, we examined its ability to influence the ability of imipramine to decrease immobility on the TST. Mice were dosed with vehicle, NBQX (10 mg/kg), imipramine (30 mg/kg), or both compounds and 40 min later animals were examined in TST.

2.3.2. Differential reinforcement of low rates of responding (DRL)

Sixteen standard rat operant boxes (Med Associates) were employed for training and testing. Each box contained two retractable levers with a stimulus light located above each lever. A food hopper in which 45 mg sucrose pellets (BioServ) could be delivered was centered between the left and right levers.

After seven days of habituation to the colony room, each animal was given ~40 sucrose pellets in their home cage to prevent neophagia from occurring during training. On the following day, animals were placed in the operant boxes (with both levers retracted) and given sucrose pellets on a variable interval 30 s schedule to train animals to obtain sucrose pellets from the food hopper.

On the next day, rats were trained on a fixed ratio (FR1) schedule. Both levers were extended into the operant box, but only one of the levers was active. Pressing on the active lever resulted in the delivery of a sucrose pellet and activation of the stimulus light above the lever. Pressing the inactive lever had no consequences, but was recorded. Rats were considered to have successfully completed the FR1 schedule after obtaining >100 reinforcers in the 60 min session.

Following FR1 completion, rats were transferred to a DRL-5 schedule in which animals were rewarded for pressing the active lever at least 5 s following the previous lever press. Premature responses were not reinforced and reset the timer back to 0 s. Rats spent 2 days on DRL-5, 3 days on DRL-10, 2 days on DRL-20, and then were moved to a DRL-30 schedule until they achieved a stable baseline. Performance was considered stable when the SEM of the last three sessions was <1 for reinforcers earned. Each DRL session lasted 15 min and reinforcers earned, responses per minute, and response efficiency were recorded.

On the day of testing, animals received either vehicle (0.5% methylcellulose), AMN082 (10 mg/kg; i.p.), or desipramine (5 mg/kg