The Antidepressant-Like Action of Metabotropic Glutamate 7 Receptor Agonist \(N,N'-\text{Bis}(\text{diphenylmethyl})-1,2-\text{ethanediame}\) (AMN082) Is Serotonin-Dependent

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ABSTRACT

Behavioral studies show that modulation of the glutamatergic system might be an efficient way to achieve antidepressant activity. Among the group III metabotropic glutamate (mGlu) receptors, the mGlu7 receptor subtype seems to be the most promising target for potential antidepressants. It has been shown that a selective, allosteric mGlu7 receptor agonist, \(N,N'-\text{bis}(\text{diphenylmethyl})-1,2-\text{ethanediame}\) (AMN082), induced antidepressant-like action in behavioral tests in mice, although the mechanisms responsible for this action remained unknown. Here, we decided to investigate the possible role of the serotonergic system in the antidepressant-like activity of AMN082 in both the forced swim test (FST) in rats and the tail suspension test (TST) in mice. We found that AMN082 (1–10 mg/kg i.p.) induced a dose-dependent reduction in the immobility of rats and an increase in their swimming behavior, whereas there were not any changes in climbing behavior in the FST in rats. In the TST in mice we found that AMN082 (3 mg/kg i.p.) did not induce an antidepressant-like effect after depletion of serotonin (5-HT) with para-chlorophenylalanine. Moreover, we revealed that citalopram, but not reboxetine, when combined with AMN082 (all compounds used at low subeffective doses), induced a significant antidepressant-like effect in the TST. We also discovered that the 5-HT1A receptor antagonist \(N-[2-[4-(2-\text{methoxyphenyl})-1-piperaziny]l]ethyl]-N-(2-\text{pyridynyl})\) cyclohexane-carboxamide (WAY100635) (0.1 mg/kg s.c.), but not the 5-HT2A/2C receptor antagonist ritalasin (0.5 mg/kg i.p.), blocked the antidepressant-like action of AMN082. Altogether, the results of our studies show that the antidepressant-like action of the mGlu7 receptor-positive modulator AMN082 depends on the activation of the serotonergic system.

Introduction

The glutamatergic system constitutes the main excitatory neurotransmitter system in the mammalian central nervous system (CNS). Two main classes of glutamate receptors have been identified: the ionotropic receptors, i.e., ion channels responsible for fast synaptic transmission, and the metabotropic glutamate (mGlu) receptors, which couple to G proteins to modulate slow synaptic transmission through intracellular second messengers (Nakanishi et al., 1998). mGlu receptors are a family of eight G protein-coupled receptors, which are classified into three groups according to their sequence homology, effector coupling, and pharmacology. Group I mGlu receptors (mGlu1 and mGlu5) are positively coupled to phospholipase C; group II mGlu receptors (mGlu2 and mGlu3), and group III mGlu receptors (mGlu4, mGlu6, mGlu7, and mGlu8) are negatively coupled to adenylyl cyclase (Pin and Duvoisin, 1995).

mGlu receptors are implicated in many diverse functions of the CNS, including regulation of selective and nonselective ion channels, regulation of neurotransmitter release, induction of long-term potentiation and long-term depression, and regulation of the function of the ionotropic receptors (for review see Conn and Pin, 1997; Ferraguti and Shigemoto, 2006). Moreover, mGlu receptors are known to be involved in various CNS diseases, including those that are neuropsychiatric, such as depression. Numerous behavioral studies have shown that action on the mGlu receptors might be an efficient way to achieve antidepressant activity (Pałucha and...
Pilc, 2002, 2005; Palucha, 2006; Pilc et al., 2008). Preclinical studies collected from the last decade have shown that among all mGlu receptor ligands the mGlu5 receptor antagonists and group II mGlu receptor antagonists seem to be the most promising antidepressants (for review see Palucha and Pilc, 2007). However, group III mGlu receptor ligands have been studied less, because of a lack of high-affinity, highly selective, brain-penetrating agents (Lavreyen and Dautzenberg, 2008). Among group III mGlu receptors, the mGlu7 receptor subtype seems to be the most promising target for potential antidepressants. First, mGlu7 receptor KO mice displayed antidepressant-like behavior in both the FST and TST (Cryan et al., 2003). Second, the mGlu7 receptor is known to be widely distributed in the brain areas related to mood disorders (Kinoshta et al., 1998). Finally, our recent study showed that the novel, selective, allosteric mGlu7 receptor agonist \( \text{N}^\{2\}-\text{bis (diphenylmethyl)-1,2-ethanidamine (AMN082)} \) (Mitsukawa et al., 2005) induced a potential antidepressant-like action in both the FST and TST (Palucha et al., 2007), although the mechanisms responsible for the behavioral effects of AMN082 remains unknown. Because activation of the mGlu7 receptor is known to be responsible for the regulation of glutamate and GABA release (Cartmell and Schoepp, 2000), it can be supposed that the mechanism of the antidepressant-like effect of AMN082 might be related to the regulation of glutamatergic or GABAergic neurotransmission. However, activation of mGlu receptors may also be related to the regulation of other neurotransmitters, including those engaged in the mechanism of action of classic antidepressant drugs (ADs), such as 5-HT (Lee and Croucher, 2003; Kawashima et al., 2005; Stachowiicz et al., 2007b, 2009). Furthermore, several studies have shown the structural and functional interaction between the serotonergic and glutamatergic systems and its implication in psychiatric diseases such as psychosis and anxiety (Marek et al., 2000; Kłodzinska et al., 2002; Stachowiicz et al., 2007a, 2007b, 2009; González-Maeso et al., 2008). As such, we decided to investigate the possible role of the serotonergic system in the antidepressant-like activity of the mGlu7 receptor agonist AMN082.

Materials and Methods

Animals and Housing. Male Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) (220–230 g) were used to assess the antidepressant-like activity of AMN082 in the FST and locomotor activity test. Male C57BL/6J mice (23–25 g) were used in the TST and locomotor activity test. The animals were kept under standard laboratory conditions of lighting (light phase 7:00 AM to 7:00 PM) and temperature (19–21°C). Food and water were freely available. Each experimental group consisted of seven to nine animals. All of the subjects were experimentally naive and used only once in each test. Behavioral experiments were performed during the light period (9:00 AM to 2:00 PM) by an observer unaware of the treatment. All procedures were conducted according to the guidelines of the National Institutes of Health Animal Care and Use Committee and were approved by the Ethics Committee of the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Drug Administration. AMN082 (Ascent Scientific, Bristol, UK), ritanserin (Toeris Bioscience, Bristol, UK), and \( \text{N}^\{2\}-\text{bis (diphenylmethyl)-1,2-ethanidamine (AMN082)} \) (Mitsukawa et al., 2005) was dispersed in a suspension of 0.5% methylcellulose, which was used as vehicle. WAY100635 (Sigma-Aldrich) and reboxetine (Pharno) were soluble in sterile water. In the rats, AMN082 was injected intraperitoneally, at a constant volume of 2 ml/kg, 24, 5, and 1 h before the behavioral test. In the mice, AMN082 and ritanserin were administered intraperitoneally 60 min before the experiment. WAY100635 was given subcutaneously 45 min before the test, and citralopram and reboxetine were administered intraperitoneally 30 min before the experiment. The drugs were injected in the mice at a constant volume of 10 ml/kg. Drugs were chosen based on previous experiments (Stachowiacz et al., 2007a,b, 2009). Because lower doses of AMN082 are required in mice than rats to reach equivalent brain levels (Fendt et al., 2008), the doses were adjusted accordingly.

5-HT Depletion. To deplete 5-HT, the mice were pretreated with the tryptophan hydroxylase inhibitor PCPA (300 mg/kg i.p.). Injections were made twice daily (at 9:00 AM and 9:00 PM), for 3 consecutive days. Experiments were done on the fourth day and started at 10:00 AM. The dose and schedule of PCPA treatment were adopted from O’Leary et al. (2007).

Forced Swim Test. The experiments were performed according to the procedure of Porsolt et al. (1977). In brief, the rats were placed individually in glass cylinders (40 cm high, 18 cm in diameter) containing 25 cm of water maintained at 25°C. The water column was deep enough so that the rats could not support themselves by placing their paws on the base of the cylinder. After 15 min, they were removed to a drying room (30°C) for 30 min. They were placed again in the cylinder 24 h later, and the total duration of immobility was measured during a 5-min test. Behavioral scoring was performed according to Detke et al. (1985). Three different behaviors were rated: 1) immobility, rats were judged to be immobile when they remained floating passively in the water; 2) swimming, rats were judged to be swimming if they were making active swimming motions, more than necessary to solely maintain their head above water; and 3) climbing, rats were judged to be climbing when they were making active movements in and out of the water with their forepaws, usually directed against the walls. The time of immobility, swimming, and climbing was measured. AMN082 (1, 5, or 10 mg/kg) was administered as a series of three intraperitoneal injections at 23, 5, and 1 h before the 5-min test on the second day. The first injection was given at the end of a drying period (i.e., 30 min after removal from the water).

Tail Suspension Test. Immobility was induced by tail suspension according to the procedure of Steru et al. (1985). C57BL/6J mice were individually suspended by their tails by a plastic string positioned horizontally 75 cm above the tabletop by using adhesive tape placed approximately 1 cm from the tip of the tail. The immobility duration was recorded for 6 min. The mice were considered immobile only when they were both hanging down passively and completely motionless.

Locomotor Activity Test in Rats. Spontaneous locomotor activity was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, Columbus, OH) linked on-line to a compatible IBM (White Plains, NY) computer. Each cage (43 × 44 × 25 cm) was surrounded with a 15 × 15 array of photocell beams located 3 cm from the floor surface. Rats were injected in their home cages with a series of three injections of AMN082 given 23, 5, and 1 h before the locomotor activity test. One hour after the last dose of AMN082, the rats were placed individually into locomotor activity chambers. Immediately afterward, locomotor activity was measured for 30 min and recorded every 5 min. Interruptions of photobeams resulted in horizontal activity defined as distance traveled (in centimeters). The data were analyzed with Auto-Track software (Columbus Instruments).

Locomotor Activity Test in Mice. The locomotor activity of mice was measured in Flexiglas locomotor activity chambers (40 × 20 × 15 cm) in a 20-station photobeam activity system (Opto-M3 Activity Meter; Columbus Instruments). The animals were placed there individually, and the total number of ambulations was recorded for 30 min and recorded every 5 min.
Determination of 5-HT Concentration. Thirty minutes after the TST, the mice were sacrificed by decapitation, and their frontal cortices were dissected on an ice-cold plate, then immediately frozen and stored at −80°C for the tissue analysis. 5-HT was assayed in cortical homogenates by high-performance liquid chromatography coupled with coulometric detection. Tissue samples were weighed and homogenized in 0.5 ml of ice-cold 0.1 M perchloric acid containing 0.05 mM ascorbic acid. After centrifugation (10,000g, 10 min), the supernatants were filtered through 0.2-μm cellulose filters (Alltech Associates, Deerfield, IL). Ten-microliter samples were injected into a high-performance liquid chromatography system (Dionex Inc., Sunnyvale, CA) equipped with a Hypersil Gold C18 analytical column (Thermo Fisher Scientific, Waltham, MA) and the Coulochem III detector (ESA Inc., Chelmsford, MA). The mobile phase consisted of 50 mM citrate-phosphate buffer (pH 4.2), 0.25 mM EDTA, 0.25 mM sodium octyl sulfonate, 2.4% methanol, and 1.3% acetonitrile. The flow rate was maintained at 0.8 ml/min. Data were collected and chromatograms were integrated with Chromeleon 6.8 SP3 software (Dionex Inc.). 5-HT was quantified by peak area comparisons with standards, run on the day of analysis. The results are presented as a nanogram of the analyzed compound per gram of weight of the brain tissue.

Statistical Analysis. The data are presented as means ± S.E.M. and were evaluated by one-way ANOVA followed by Dunnett’s post hoc test (FST in rats), two-way ANOVA followed by Bonferroni’s post hoc test (TST in mice), or the repeated-measures ANOVA followed by Dunnett’s test (locomotor activity). Two-way ANOVA was used to analyze 5-HT concentrations in the frontal cortex of the mice. Prism version 4.00 for Windows 2000 (GraphPad Software Inc., San Diego, CA) was used to analyze the data.

Results
Effect of AMN082 on the Behavior of Rats in the FST. One-way ANOVA showed the effects of AMN082 on immobility time \( F(3,24) = 8.755; p = 0.0004 \) and swimming time \( F(3,24) = 4.444; p = 0.0128 \) but no effect on climbing duration \( F(3,24) = 1.257; p = 0.3115 \). Post hoc tests showed that AMN082, given at a dose of 5 mg/kg, significantly decreased the immobility time of rats \((p < 0.05)\), whereas at the dose of 10 mg/kg, AMN082 not only decreased the duration of the immobility \((p < 0.01)\), but it increased swimming time \((p < 0.01; \text{Fig. 1})\).

Effect of AMN082 on the Locomotor Activity of Rats. In rats, AMN082 at a dose of 5 mg/kg did not induce changes in locomotor activity \( F(1,65) = 0.008385; p = 0.9284 \), although a significant effect of time on the parameter was observed \( F(5,65) = 40.54; p < 0.0001 \) (Fig. 2). AMN082 at a dose of 10 mg/kg significantly decreased the locomotor activity of rats (the curves were significantly different) \( F(1,65) = 25.06; p = 0.0002 \). Furthermore, there was a significant influence of time on locomotor activity \( F(5,65) = 58.97; p < 0.0001 \) and time-dose interaction \( F(5,65) = 28.50; p < 0.0001 \). Post hoc analysis revealed that AMN082 at a dose of 10 mg/kg decreased locomotor activity at 5 min \((p < 0.001)\) and 10 min \((p < 0.05)\) (Fig. 2).

Effect of 5-HT Depletion on the Antidepressant-Like Activity of AMN082 in the TST. AMN082, given at a dose of 3 mg/kg, significantly decreased the immobility time of vehicle-treated mice in the TST \( F(1,26) = 14.88; p = 0.0007 \). Two-way ANOVA also revealed significant interaction between PCPA and AMN082 \( F(1,26) = 4.373; p = 0.0464 \), showing that AMN082 was not active in the TST in 5-HT-depleted mice (Fig. 3).

Effect of AMN082 on the Locomotor Activity of 5-HT-Depleted and Control Mice. Two-way ANOVA showed that PCPA-pretreated mice were less active in the locomotor activity test than vehicle-treated animals (i.e., the curves were significantly different) \( F(1,70) = 10.34; p = 0.0062 \). No significant differences between groups treated with vehicle/AMN082 (3 mg/kg) and PCPA/AMN082 (3 mg/kg) were observed \( F(1,70) = 1.642; p = 0.2209 \). Statistical analysis of the behavior of mice at 5 min revealed that AMN082 decreased basal locomotor activity \((p = 0.0327)\) (Fig. 4, inset),
nonactive dose of 2 mg/kg, coadministered with a nonactive dose of AMN082 (0.3 mg/kg), did not change the behavior of the mice in this test \( F(1,27) = 0.09886; p < 0.7556 \) (Fig. 5B). Subeffectives doses of citalopram, reboxetine, and AMN082 were defined on the basis of dose-response curves, performed in the preceding set of experiments (data not shown).

**Effects of Citalopram or Reboxetine and AMN082 on the Locomotor Activity of Mice.** AMN082, at a dose of 0.3 mg/kg, did not induce changes in the locomotor activity of mice \( F(1,60) = 0.4494; p = 0.5153 \). Citalopram, used at a dose of 5 mg/kg, also did not influence this parameter \( F(1,60) = 0.006506; p = 0.9370 \). The locomotor activity of the mice injected with AMN082 (0.3 mg/kg) and citalopram (5 mg/kg) was comparable with that of the control animals (the curves were not significantly different) \( F(1,60) = 2.254; p = 0.1591 \). No changes in the locomotor activity between all of the groups of mice at the first time point of 5 min \( p > 0.05 \) were revealed, suggesting no influence of used compounds on the exploratory activity (Fig. 6A).

Likewise, reboxetine (2 mg/kg) \( F(1,60) = 0.6506; p = 0.6621 \) or reboxetine (2 mg/kg), coadministered with AMN082 (0.3 mg/kg) \( F(1,60) = 0.3830; p = 0.8585 \), did not change the locomotor activity of mice. Moreover, statistical analysis of all of the groups of animals did not reveal any changes at the first time point of 5 min \( p > 0.05 \) (Fig. 6B).

**Effects of Antagonists of 5-HT Receptors on the Antidepressant-Like Activity of AMN082 in the TST.** Two-way ANOVA showed that AMN082, administered at a dose of 3 mg/kg, significantly decreased the immobility time of the C57BL/6J mice in the TST \( F(1,28) = 19.58; p = 0.0001 \). An antagonist of the 5-HT1A receptor, WAY100635 (0.1 mg/kg), when given by itself, did not change the behavior of the animals in this test, although it antagonized a AMN082 (3 mg/kg)-induced decrease in the immobility time of mice \( F(1,28) = 6.781; p = 0.0146 \) (Fig. 7A).

The 5-HT2A/2C receptor antagonist ritanserin (0.5 mg/kg) was not active in the TST when given by itself and did not influence the AMN082 (3 mg/kg)-induced attenuation of the antidepressant-like activity of mice in the TST \( F(1,28) = 4.016; p = 0.0548 \) (Fig. 7B).

AMN082 was not active in the TST at a dose of 0.3 mg/kg.
Two-way ANOVA revealed that WAY100635 (0.1 mg/kg) showed the tendency to enhance the nonactive dose of AMN082 (0.3 mg/kg), although the effect was not statistically significant \(F(1,28) = 3.056; p = 0.0914\) (Fig. 8).

**Effects of Antagonists of 5-HT Receptors and AMN082 on the Locomotor Activity of Mice.** AMN082, at a dose of 3 mg/kg, decreased the locomotor activity of mice \(F(1,60) = 32.81, p < 0.0001\), and a significant effect of time on the parameter \(F(5,60) = 9.920, p < 0.0001\) was observed. WAY100635, at a dose of 0.1 mg/kg, did not influence the basal locomotor activity of mice \(F(1,60) = 0.0505\). Mice injected with AMN082 coadministered with WAY100635 were statistically less active in the locomotor activity test than the control animals (the curves were significantly different) \(F(1,60) = 16.52, p = 0.0016\), although their activity did not change compared with the group of mice injected with AMN082 (the curves were not significantly different) \(F(1,60) = 2.533, p = 0.1374\) (Fig. 9).

Statistical analysis also revealed that AMN082 decreased basal locomotor activity at the first time point of 5 min \(p = 0.0209\) (Fig. 9, inset), suggesting decreased exploratory activity of the mice. Two-way ANOVA showed that WAY100635 did not significantly influence AMN082-induced changes in the locomotor activity of the mice in the first 5 min of the locomotor activity test \(F(1,24) = 3.329, p = 0.0805\) (Fig. 9, inset).

Similar effects were observed in the group of animals ad-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5-HT Content</th>
<th>Change</th>
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<tbody>
<tr>
<td></td>
<td>ng/g tissue</td>
<td>%</td>
</tr>
<tr>
<td>Vehicle</td>
<td>592.3 ± 18.44</td>
<td>-77.0</td>
</tr>
<tr>
<td>AMN082</td>
<td>135.3 ± 10.90</td>
<td>-75.4</td>
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**Fig. 5.** The effects of citalopram (Cit) or reboxetine (Reb) and AMN082 (AMN) on the immobility time of mice in the TST. AMN082 (0.3 mg/kg i.p.) was administered 60 min before the behavioral test. Citalopram (5 mg/kg i.p.) and reboxetine (2 mg/kg i.p.) were given 30 min before the test. Values expressed as means ± S.E.M. were analyzed by two-way ANOVA. **Fig. 6.** The effects of citalopram (Cit; 5 mg/kg) (A) or reboxetine (Reb; 2 mg/kg) (B) and AMN082 (AMN; 0.3 mg/kg) on the locomotor activity of mice. AMN082 was administered intraperitoneally 60 min before the behavioral test, and citalopram and reboxetine were given intraperitoneally 30 min before the test. Values expressed as the means ± S.E.M. were evaluated by repeated-measures ANOVA.
ministered AMN082 and ritanserin. Locomotor activity of mice coadministered ritanserin and AMN082 was not significantly changed, compared with the AMN082 group of mice \( F(1,60) = 3.057; p = 0.1059 \) (Fig. 10). Moreover, ritanserin did not influence AMN082-induced changes in the exploratory locomotor activity measured at the first time point of 5 min \( (p = 0.7778) \) (Fig. 10, inset).

Discussion

The FST in rats is one of the most widely used animal models for estimating antidepressant-like activity of new potential ADs (Porsolt et al., 1977; Cryan et al., 2002). Here, we used the modified version of the FST, originally introduced by Detke et al. (1995), in which three specific types of behavior, i.e., immobility, climbing, and swimming, were measured. It has been proposed that climbing is sensitive to catecholamine-based ADs, whereas swimming is modified by ADs, acting via modulation of the serotonergic system, including SSRIs (Detke et al., 1995; Page et al., 1999). In this study, conducted for the first time on rats, it was demonstrated that AMN082 induced a dose-dependent reduction in their immobility and an increase in their swimming behavior, whereas there were no changes in their climbing behavior. This profile of action may suggest that the mechanism of the antidepressant-like activity of AMN082 is related to the modulation of the serotonergic system.

Our experiments showing antidepressant-like effects of AMN082 yield paradoxical results to the data describing the antidepressant-like phenotype of mGlu7 receptor KO mice (Cryan et al., 2003). However, recently reported profound changes in GABAergic system in mGlu7 KO mice (Wieron ska et al., 2010) may explain the antidepressant-like phenotype of mGlu7 knockouts and resolve that discrepancy. Moreover, the recent data demonstrating that pharmacological blockade of mGlu7 receptors by 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonolo[4,5-c]pyridin-4(5H)-one (MMPIP) did not cause antidepressant effects (Hikichi et al., 2010) support the view that it is the stimulation of mGlu7 receptors that brings about antidepressant-like activity. The possibility that AMN082 may act via additional mechanisms not related to the stimulation of mGlu7 receptors also has to be considered. AMN082 causes internalization of mGlu7 receptors (Pelkey et al., 2007) and is active in mGlu7 KO mice, producing a decrease in locomotor activity (Palucha et al., 2007). However, its antidepressant-like action is absent in mGlu7 KO mice, showing that it is related to mGlu7 receptor stimulation.

Compounds that increase locomotor activity can often provide false positive results in the FST. We observed that AMN082 at a dose of 5 mg/kg had no effect on the locomotor activity of rats and there was a significant decrease of this parameter after an injection of AMN082 at a dose of 10 mg/kg, adding validity to the antidepressant-like activity of the drug in the FST. These results confirm our previously published data showing AMN082-induced decreases in the locomotor activity of mice at doses active in the FST (Palucha et al., 2007).

To further investigate the potential serotonergic-dependent mechanism of the antidepressant-like activity of
AMN082, we used the TST in the C57BL/6J mice. In this test, mice hung by the tail develop an immobile posture and acutely given ADs decrease the time of immobility. The TST detects the antidepressant-like activity of not only typical ADs drugs but also atypical and new potential ADs, such as the mGlu receptor ligands (Cryan et al., 2005), including mGlu7 receptor agonist (Pałucha et al., 2007).

To investigate the role of the serotonergic system in the antidepressant-like activity of AMN082 in the TST, we used mice after the pharmacological depletion of 5-HT by a selective inhibitor of tryptophan hydroxylase, PCPA (Koe and Weissman, 1966). We adopted a schedule of treatment with PCPA from O’Leary et al. (2007) obtaining identical 77% depletion of 5-HT levels in frontal cortices. The antidepressant-like effect of AMN082 was abolished by PCPA administration, suggesting that a regular level of 5-HT in the brain is essential to the antidepressant-like action of AMN082 in the TST. The lack of antidepressant-like efficacy of AMN082 in serotonin-depleted rats resembles the results of O’Leary et al. (2007), who demonstrated that SSRIIs were not active in the TST in the PCPA-pretreated mice, and may suggest similar mechanisms of the antidepressant efficacy of both groups of compounds.

Locomotor activity studies showed that PCPA did not significantly influence AMN082-induced changes in motility, suggesting that the results of the TST studies did not result from possible hypolocomotion of mice treated with PCPA and AMN082.

Our experiments have shown that a selective inhibitor of the 5-HT transporter, citalopram, combined with AMN082 (both used at low, subeffective doses) induced a significant antidepressant-like effect in the TST. However, a selective inhibitor of the noradrenaline transporter, reboxetine, given at a nonactive dose, together with a nonactive dose of AMN082, did not induce any changes in the immobility time of mice. These results suggest a synergistic action of the SSRI and the mGlu7 receptor agonist, indicating that both agents act at different targets, stimulation of which leads to the same final results. This further indicates that the serotonergic system is engaged in the mechanism of action of AMN082, whereas the noradrenergic system is not.

Locomotor activity studies showed that citalopram, rebox-
etine, and AMN082, given alone or in combination at doses nonactive in the TST, did not change the locomotion of mice, ensuring the specificity of antidepressant-like effect of combination of AMN082 with citalopram.

To further investigate the role of the serotonergic system in the mechanism of the antidepressant-like activity of AMN082, we studied the engagement of specific 5-HT receptors in the action of this drug in the TST. 5-HT1A and 5-HT2A receptors have been shown to play a crucial role in the regulation of serotonergic neurotransmission and the mechanism of action of several ADs, including SSRIs, monoamine oxidase inhibitors, and tricyclics (Middlemis et al., 2002; Hensler, 2006). Furthermore, pretreatment with antagonists of 5-HT1A and 5-HT2A receptors has been shown to antagonize the action of the SSRIs in the TST in mice (Miyata et al., 2004). Thus, we examined the influence of the pretreatment of mice with the 5-HT1A receptor antagonist WAY100635 (Forster et al., 1995) and the 5-HT2A/2C receptor antagonist ritanserin (Hoyer et al., 1994) by using TST. The action of AMN082 was inhibited by WAY100635, but not by ritanserin, indicating the involvement of 5-HT1A but not the 5-HT2/2C receptors in the antidepressant-like action of AMN082. Direct influence of AMN082 on the serotonergic receptors can be excluded, because no significant binding interaction of 1 μM AMN082 with the serotonergic receptors was observed (Mitsukawa et al., 2005).

Locomotor activity studies showed that WAY100635 or ritanserin, given by itself, did not change the locomotion of mice and did not significantly change AMN082-induced decreases in this parameter, confirming the specificity of the observed effects.

5-HT1A receptors are localized presynaptically on serotonergic neurons in the raphé, where they play the role of autoreceptors and postsynaptically mainly on nonserotonergic neurons, including glutamatergic ones (Santana et al., 2004). Generally, postsynaptic 5-HT1A receptor exists in limbic structures, including the hippocampus, frontal cortex, entorhinal cortex, and amygdalar complex (Hensler, 2006). Numerous data show that 5-HT1A receptor antagonists, used at subeffective doses, enhance the antidepressant-like activity of several ADs (Artigas et al., 1996). It is supposed that such effects resulted probably from a blockade of 5-HT1A autoreceptors and a subsequent increase in 5-HT concentration in the serotonergic synapse in the dorsal raphé nucleus. Thus, the antagonists of 5-HT1A receptors synergistically augment the 5-HT level, increased by ADs acting as inhibitors of 5-HT transporters (Romero et al., 1996). In the present study, we found that the 5-HT1A receptor antagonist WAY100635, and the mGlu7 receptor antagonist, AMN082, both used in the TST at subactive doses, did not induce an antidepressant-like effect, although a nonsignificant tendency was observed. These data may suggest that postsynaptic 5-HT1A receptors, rather then presynaptic ones, are engaged in the mechanism of the antidepressant-like activity of AMN082.

A large amount of data shows that an increased function of the glutamatergic system is observed in depression (see Palucha and Pilec, 2007; Sanacora et al., 2008; Wieronska and Pilec, 2009). Limbic structures, including the prefrontal cortex, are believed to be related to emotional processes and depressive states. Thus, it may be supposed that the activation of glutamatergic cerebral pyramidal neurons might play an important role in depression. Therefore, AMN082, acting at presynaptic mGlu7 receptors, may induce its antidepressant-like effect by inhibition of glutamate release. On the other hand, prefrontal pyramidal neurons have been shown to be inhibited by 5-HT via activation of the inhibitory 5-HT1A receptors (Amargós-Bosch et al., 2004). Therefore, a selective blockade of 5-HT1A receptors may antagonize the inhibitory effects of 5-HT on pyramidal neurons, thus inducing an increased activity of these cells. This mechanism may account for a WAY100635-induced blockade of the antidepressant-like effect of AMN082 in the TST. Similar data were reported earlier, when an anxiolytic effect of ACPT-I [(1S,3R,4S)-1-amino cyclopentane-1,3,4-tricarboxylic acid], a group III mGlu receptor agonist, was antagonized by WAY100635 (Stachowicz et al., 2009). This indicates that an interaction between group III mGlu receptors and 5-HT1A receptors might be a general phenomenon involved not only in depression but also anxiety processes.

Altogether, the results of our studies show that the antidepressant-like action of the mGlu7 receptor-positive modulator AMN082 depends on the serotonergic system activation. There is a functional interaction between mGlu7 receptor and serotonergic receptors (possibly 5-HT1A receptor), which may account for the behavioral effects observed in our experiments.

References


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