Enhancement of Antidepressant-Like Effects but Not Brain-Derived Neurotrophic Factor mRNA Expression by the Novel N-Methyl-D-aspartate Receptor Antagonist Neramexane in Mice

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ABSTRACT

Improved efficacy in the treatment of depression may be achieved by the combined use of several antidepressants. In the present study, acute administration of the novel N-methyl-D-aspartate (NMDA) receptor antagonist neramexane, as well as the representative antidepressants imipramine, fluoxetine, and venlafaxine, shortened the duration of immobility in the mouse tail suspension test with a minimal effective dose of 5 mg/kg. When tested in combination, the antidepressant-like effects of 5 mg/kg imipramine, 20 mg/kg fluoxetine, and 5 mg/kg venlafaxine were potentiated by neramexane (2.5 mg/kg), a dose that alone did not produce a significant effect on the duration of immobility. These effects seemed to be specific, because they were not accompanied by significant effects on locomotor activity. The enhanced antidepressant-like activity produced with the different combinations was not synergistic as determined by comparing the theoretical and observed ED₅₀ values for each combination. In separate experiments, Northern blot analysis showed that a 14-day treatment with imipramine (10 mg/kg b.i.d.) increased brain-derived neurotrophic factor (BDNF) mRNA expression in the cortex, whereas neramexane (5 mg/kg b.i.d.) decreased it. Combined treatment produced no effect on BDNF mRNA expression. Mice treated with imipramine or neramexane for 14 days and tested shortly after the last dose demonstrated significant shortening of immobility, and the combined treatment produced an even greater antidepressant-like effect. Together, these data support the view that NMDA receptor antagonists enhance the potency of antidepressants, but they leave open the question as to whether enhanced BDNF expression is a necessary feature of the antidepressant-like effect.

Despite several studies on the different approaches for the optimal treatment of major depression as well as the availability of new antidepressant drugs, adequate management of this disorder remains a challenge. The primary reasons for this include the incidence of undesired side effects produced by currently available antidepressants, the apparent delay in achieving measurable therapeutic benefit (Skolnick, 1999; but see Frazer and Morilak, 2005), and a high percentage of treatment-resistant patients (Souery et al., 1999). Thus, there is a compelling need for studies in which novel antidepressant therapies with an improved pharmacological profile are evaluated. Included among the various strategies suggested to improve upon the efficacy of antidepressant treatment are the use of broad-spectrum antidepressants (Skolnick et al., 2003) or the use of combinations of several antidepressants with different mechanisms of action (Frank et al., 2000).

Preclinical studies have demonstrated that the combination of N-methyl-D-aspartate receptor antagonists (NMDAR-As), such as memantine or amantadine, with clinically available antidepressants (e.g., fluoxetine, venlafaxine, and imipramine) enhances their antidepressant-like effects in the forced swim test in rats (Rogoz et al., 2002). Likewise, amantadine was found to potentiate the activity of imipramine in the forced swimming test and in the d-amphetamine-induced locomotor activity in rats (Dzied...
zicka-Wasylewska et al., 2004). Because both memantine and amantadine have demonstrated clinical efficacy and safety for the clinical treatment of Alzheimer’s disease and Parkinson’s disease, respectively (Parsons et al., 1999b), evaluation of this class of compounds (i.e., NMDAR-A) in other neurological and psychiatric conditions may be warranted. Indeed, the positive results from preclinical studies have led to a small, open-label clinical trial, which showed that the combined administration of imipramine and amantadine reduced scores on both the Hamilton Depression Rating Scale and the Beck Depression Inventory both after 3 and 6 weeks of treatment in patients with treatment-resistant unipolar depression (Rogoz et al., 2004).

Neramexane (1-amino-1,3,3,5,5-pentamethyl-cyclohexane) belongs to a recently described group of uncompetitive NMDAR-A as known as the amino-alkyl-cyclohexanes (Danyasz et al., 2002). Like memantine, neramexane exhibits moderate affinity, fast blocking/unblocking kinetics, and strong voltage dependence at the NMDA receptor, attributes that are thought to contribute to the drug’s efficacy and minimal side effect profile (Parsons et al., 1999a).

Thus, the first aim of the present study was to investigate whether the acute administration of neramexane would alone produce a significant antidepressant-like effect as measured by a decrease in the duration of immobility in the mouse tail suspension test (TST). In addition, we determined whether the combined treatment of neramexane with each of three representative antidepressant drugs (fluoxetine, venlafaxine, and imipramine) enhanced their antidepressant-like effects.

The second aim of the present study was to investigate whether chronic administration of neramexane produced alterations at the molecular level, which may be associated with an antidepressant-like effect. Generally, significant measurable improvement of depressive symptoms is observed after some period of continuous antidepressant drug treatment (Frazier and Morilak, 2005). Thus, it has been suggested that it is those neurochemical and molecular effects occurring after repeated drug administration that may be related, at least partially, to the therapeutic action of the antidepressant treatment (Vetulani and Sulser, 1975; Duman et al., 1994; Skolnick et al., 1996). In this regard, long-term adaptations that result from the treatment with antidepressants may involve alterations in structural neuroplasticity (Kitayama et al., 1994). It has been suggested that brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain, may be one such target, because it is known to regulate neuronal survival (Ghosh et al., 1994) and differentiation and plasticity (Thoenen, 1995). However, although chronic treatment with antidepressants has been shown to increase BDNF mRNA and protein expression within several brain regions, including the neocortex and hippocampus, this effect may not be common to all antidepressants (Nibuya et al., 1995; Dias et al., 2003). Therefore, the present study examined whether chronic treatment with neramexane, imipramine, and the combination significantly affected BDNF mRNA expression in the mouse cortex and hippocampus.

Materials and Methods

Subjects

For all experiments, male C57BL/6J Han mice were obtained from IMP (Lodz, Poland) and weighed ~25 g at the start of the experiment. Mice were group-housed in standard laboratory cages and kept in a temperature-controlled colony room (21 ± 2°C) with a 12-h light/dark cycle (lights on at 7:00 AM). Commercial food and tap water were available ad libitum. In those experiments where the acute treatment with neramexane and representative antidepressants were evaluated, the mice that were used for the tail suspension test were again used 1 week later to evaluate the effects of the different drug treatments on the locomotor activity. Otherwise, all subjects were used once.

All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (revised 1996) and were approved by the Institute of Pharmacology, Polish Academy of Sciences in Krakow Animal Care and Use Bioethics Commission.

Behavioral Studies

All behavioral studies were conducted by personnel blinded to the treatment conditions.

Tail Suspension Test. Mice were transferred from the housing room to the testing area in their home cages and allowed at least a 1-h adaptation period to the new environment before drug treatment. Behavioral despair was induced by tail suspension according to the procedure of Steru et al. (1985). Mice were attached individually 75 cm above the flat surface of the tabletop with a 1-cm piece of paper adhesive tape placed ~1 cm from the tip of the tail. Animals were suspended for 6 min, and the duration of immobility was recorded using the Porsolt data collection program (Infallible Software, Rockville, MD). Mice were considered immobile only when they were completely motionless.

For the acute drug treatment, compounds were administered i.p. 40 min (vehicle or neramexane) and 30 min (vehicle or antidepressants) before the test. In the chronic studies, mice were treated for 2 weeks with twice-daily i.p. injections of vehicle, imipramine (10 mg/kg), neramexane (6 mg/kg), and their combination, and then they were tested in the TST in a pseudo-Latin square design. Thus, on the 1st test day, one-half of the mice were tested 30 to 40 min after the last dose, and the other one-half were tested 16 to 17 h after the last injections. Two days later (during which the mice received continued drug treatment), the second TST assessment was performed, in which the interval between the last treatment and the test was interchanged. This way, chronically treated mice were tested twice in a manner allowing investigation of whether repeated TST affects the behavioral response.

Locomotor Activity. At least 1 h before the start of the experiment, mice were transferred to the experimental room for acclimation. The spontaneous locomotor activity was measured in custom-made circular aluminum actometers (30 cm of diameter, 10 cm of height with two light sources and two photoresistors, which beam cross at the center) (Kos and Popik, 2005). The design of the apparatus limited the data collection to only the gross movements of the animals. Mice were injected 40 min (vehicle or neramexane) and 30 min (vehicle or antidepressants) before the test and placed individually in an actometer for 6 min of measurement. As with the tail suspension test, this procedure did not allow the mice to habituate to the test apparatus before experimentation (see above).

Molecular Studies

A separate set of mice was randomly divided into four groups and treated twice daily for 2 weeks (i.p.) with either vehicle (physiological saline), imipramine (10 mg/kg), neramexane (5 mg/kg), or their combination. The brains were removed 16 to 17 h after the last dose, and the frontal cortex, neocortex, and hip-
TABLE 1

Effect of neramexane, imipramine, fluoxetine, and venlafaxine on the duration of immobility and locomotor activity following acute treatment: dose-response analysis

Data are means ± S.E.M. Immobility scores in the TST (seconds/6 min) and the distance traveled (arbitrary units/6 min) are summarized. The calculated ED50 values for each compound in the TST are also presented. Immobility scores were converted to the percentage maximal possible effect so that for each ED50 calculation, the vehicle produced an effect of 0%, and the maximal effect of the compound was 100%. For example, because the vehicle score was 185.5 s and the neramexane score at 2.5 mg/kg was 182.1 s, this dose of neramexane produced 1.83% of the maximum anti-immobility effect. These maximal possible effect values were used to calculate ED50 values and confidence limits using linear regression.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>TST ± S.E.M.</th>
<th>n</th>
<th>Locomotor Activity ± S.E.M.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neramexane</td>
<td>0</td>
<td>185.5 ± 5.1</td>
<td>12</td>
<td>91.3 ± 6.1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>182.1 ± 5.5</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>151.2 ± 6.1**</td>
<td>11</td>
<td>126.0 ± 8.7**</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100.7 ± 9.5***</td>
<td>12</td>
<td>135.5 ± 14.8**</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>45.3 ± 8.1***</td>
<td>12</td>
<td>152.0 ± 12.1***</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>F(4,52) = 67.34; P &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ED50</td>
<td>10.65 (7.92–16.27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>0</td>
<td>178.7 ± 6.1</td>
<td>11</td>
<td>118.3 ± 10.4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>144.5 ± 12.5*</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>134.4 ± 9.5*</td>
<td>12</td>
<td>91.5 ± 6.6*</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>74.6 ± 9.5**</td>
<td>12</td>
<td>95.3 ± 5.9*</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>F(3,44) = 17.94; P &lt; 0.001</td>
<td></td>
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<tr>
<td></td>
<td>ED50</td>
<td>17.65 (5.56–17.22)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fluoxetine</td>
<td>0</td>
<td>178.1 ± 13.3</td>
<td>10</td>
<td>108.4 ± 7.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>134.1 ± 14.2*</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>120.3 ± 8.4**</td>
<td>10</td>
<td>125.2 ± 9.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>56.5 ± 11.8***</td>
<td>10</td>
<td>122.2 ± 5.6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>F(3,36) = 17.19; P &lt; 0.001</td>
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</tr>
<tr>
<td></td>
<td>ED50</td>
<td>12.97 (5.55–17.57)</td>
<td></td>
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<tr>
<td>Venlafaxine</td>
<td>0</td>
<td>169.3 ± 8.7</td>
<td>10</td>
<td>106.0 ± 5.6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>128.1 ± 16.2*</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>95.4 ± 12.2***</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>51.1 ± 11.1***</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>9.2 ± 2.3***</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F(4,44) = 30.02; P &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ED50</td>
<td>11.16 (9.46–12.93)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.S., not significant.
* P < 0.05, ** P < 0.01, and *** P < 0.001 compared with the corresponding vehicle (Dunnett’s test followed by one-way ANOVAs).

pocampus were dissected on ice. All structures were frozen immediately on dry ice and stored at −80°C. The procedure for determination of BDNF mRNA levels was performed according to Legutko et al. (2001).

RNA Extraction. Total RNA was extracted using TRIzol reagent (Life Technologies, Grand Island, NY) following the protocol supplied by the manufacturer. The final RNA pellet was resuspended in diethyl pyrocarbonate-treated H2O, quantified (absorbance at 260 nm), and used for Northern blot.

Northern Blot. Northern blot analysis was performed with 10 µg of total RNA, separated on 1% denaturing agarose-formaldehyde gel, transferred to nylon membranes (Nytran; Schleicher and Schuell Dassel, Germany), and immobilized by UV radiation. A probe for rat BDNF was generated by polymerase chain reaction from cDNA using the primers 5'-ACT-CTG-GAG-AGC-GTG-AAT-GG-3' and 5'-CAG-CCT-TCC-TTC-GTG-TAA-CC-3'. The 470-base pair fragment was subcloned into the TA cloning vector pCRII and cut out with EcoRI. The insert was randomly primer-labeled with [α-32P]c(3 cTP and purified (Prime-It RmT; Stratagene, Cedar Creek, TX). Hybridization was conducted overnight in Church's buffer (1% bovine serum albumin, 1 mM EDTA, 0.25 M Na2HPO4, and 7% SDS) at 65°C with radiolabeled cDNA probe. After hybridization, the filters were washed for 30 min in 2× saline sodium citrate (SSC) buffer/0.1% SDS at room temperature followed by a 30-min wash in 0.1× SSC/0.1% SDS at 55°C. After exposure, filters were stripped off the BNDF probe (washed three times in 0.1× SSC/0.1% SDS at 100°C for 10 min) and rehybridized for the β-actin cDNA probe (Clontech, Mountain View, CA) to normalize RNA loading. Northern blots were quantified using digitized autoradiographs (Fuji phosphorimager, with Image Gauge 4.0; Fujifilm, Tokyo, Japan). The values for the −4.0- and 1.7-kilobase BDNF transcripts were combined for analysis.

Drugs

Neramexane HCl (Merz Pharmaceuticals GmbH, Frankfurt/Main, Germany), imipramine HCl (Sigma-Aldrich, Steinheim, Germany), fluoxetine HCl (from Dr. J. Kuncio, ICN Polfa, Rzeszow, Poland), and venlafaxine HCl (Forest Research Institute, Jersey City, NJ; manufactured by Cipla Mumbai, India) were dissolved in sterile physiological saline (vehicle) by sonication. All drugs were administered by i.p. route using a volume of 10 ml/kg (0.2 ml/mouse); the doses refer to the salt formulations. The choice of doses for the chronic study was based on the observations indicating that administration of neramexane at a dose of 5 mg/kg leads to free extracellular brain concentrations of −0.8 µM, sufficient to inhibit NMDA receptors (Danyssz et al., 2002). The dose of 10 mg/kg b.i.d. of imipramine has been frequently used in the chronic studies in rats (e.g., Papp and Moryl, 1994).

Statistics

The data fulfilled the criteria for normal distribution. Therefore, to evaluate the dose-effect of test compounds in the TST and locomotor activity test, one-way ANOVAs followed by the Duncan’s test were used.

In addition, the anti-immobility ED50 effects for each treatment were calculated. Immobility scores were converted to the %maximal possible effect according to the formula 100 × (vehicle score – compound score)/vehicle score, so that for each ED50 calculation, the respective vehicle produced an effect of 0%, and the maximal effect of the compound was 100%. The maximal possible effect values were used to calculate the ED50 with confidence limits derived by linear regression analysis.

To test the interaction between the effects of different doses of neramexane and the effects of different doses of the test antidepressants.
The duration of immobility in the TST (seconds/6 min) and the distance traveled (arbitrary units/6 min) are presented as the mean ± S.E.M. For each drug combination, a two-way ANOVA was calculated, and the results are shown for the dose of the antidepressant, the neramexane dose, and the combination (interaction). Following the analysis using two-way ANOVAs, the data were analyzed using the Dunnett's post hoc test.

<table>
<thead>
<tr>
<th>Antidepressant Dose</th>
<th>Neramexane Dose</th>
<th>TST ± S.E.M.</th>
<th>n</th>
<th>Locomotor Activity ± S.E.M.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/kg</td>
<td>0 mg/kg</td>
<td>165.8 ± 7.7</td>
<td>12</td>
<td>102.2 ± 5.5</td>
<td>11</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>5 mg/kg</td>
<td>163.5 ± 7.5</td>
<td>12</td>
<td>92.5 ± 7.6</td>
<td>11</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>20 mg/kg</td>
<td>49.2 ± 8.0</td>
<td>12</td>
<td>77.6 ± 5.6*</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0 mg/kg</td>
<td>162.0 ± 10.5</td>
<td>12</td>
<td>114.8 ± 5.2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg</td>
<td>186.0 ± 6.9*</td>
<td>12</td>
<td>97.8 ± 3.6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg</td>
<td>42.1 ± 6.4*</td>
<td>12</td>
<td>93.2 ± 9.6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0 mg/kg</td>
<td>33.4 ± 6.7</td>
<td>12</td>
<td>188.6 ± 10.2*</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg</td>
<td>11.8 ± 3.3*</td>
<td>12</td>
<td>133.5 ± 7.6*</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg</td>
<td>11.6 ± 2.8*</td>
<td>12</td>
<td>80.7 ± 7.0</td>
<td>11</td>
</tr>
</tbody>
</table>

\[ \text{Interaction } F(2,99) = 22.24*** \]

\[ \text{ Ner dose } F(2,99) = 20.5*** \]

\[ \text{ Interaction } F(4,99) = 5.53*** \]

\[ \text{ Venlafaxine + vehicle } \]

\[ \text{ 0 mg/kg } \]

\[ 149.0 ± 9.1 \]

\[ 10.57 ± 5.2 \]

\[ 11.14 ± 10.2 \]

\[ 118.3 ± 4.7 \]

\[ 112.4 ± 5.4 \]

\[ 148.6 ± 11.8* \]

\[ 156.6 ± 9.3* \]

\[ 161.9 ± 12.3* \]

\[ \text{ Venlafaxine + low dose of neramexane } \]

\[ 2.5 \]

\[ 170.0 ± 7.6e \]

\[ 111.4 ± 10.2 \]

\[ 118.3 ± 4.7 \]

\[ 112.4 ± 5.4 \]

\[ 148.6 ± 11.8* \]

\[ 156.6 ± 9.3* \]

\[ 161.9 ± 12.3* \]

\[ \text{ Venlafaxine + high dose of neramexane } \]

\[ 0 mg/kg \]

\[ 20 \]

\[ 42.8 ± 4.5* \]

\[ 11.90 ± 7.6* \]

\[ 156.6 ± 12.8* \]

\[ \text{ Venlafaxine + low dose of neramexane } \]

\[ 0 mg/kg \]

\[ 2.5 \]

\[ 162.0 ± 6.1* \]

\[ 113.1 ± 6.1 \]

\[ 128.0 ± 9.8 \]

\[ 156.6 ± 12.8* \]

\[ \text{ Venlafaxine + high dose of neramexane } \]

\[ 0 mg/kg \]

\[ 20 \]

\[ 25.9 ± 4.3* \]

\[ 163.2 ± 12.3* \]

\[ \text{ Venlafaxine + low dose of neramexane } \]

\[ 0 mg/kg \]

\[ 2.5 \]

\[ 112.4 ± 9.4* \]

\[ 111.3 ± 6.1 \]

\[ 128.0 ± 9.8 \]

\[ 156.6 ± 12.8* \]

\[ \text{ Venlafaxine + high dose of neramexane } \]

\[ 0 mg/kg \]

\[ 20 \]

\[ 5.7 ± 0.8* \]

\[ 221.8 ± 19.1* \]

\[ \text{ Venlafaxine + low dose of neramexane } \]

\[ 0 mg/kg \]

\[ 2.5 \]

\[ 42.3 ± 5.4* \]

\[ 163.7 ± 10.1* \]

\[ \text{ Venlafaxine + high dose of neramexane } \]

\[ 0 mg/kg \]

\[ 20 \]

\[ 24.4 ± 7.0* \]

\[ 199.1 ± 16.0* \]

\[ \text{ Venlafaxine + low dose of neramexane } \]

\[ 0 mg/kg \]

\[ 2.5 \]

\[ 42.3 ± 5.4* \]

\[ 163.7 ± 10.1* \]

\[ \text{ Venlafaxine + high dose of neramexane } \]

\[ 0 mg/kg \]

\[ 20 \]

\[ 5.7 ± 0.8* \]

\[ 221.8 ± 19.1* \]
TABLE 3

Theoretical and observed ED_{50} values of the acute antidepressant-like effect of neramexane coadministered with three different antidepressants

<table>
<thead>
<tr>
<th>Drug Combination</th>
<th>Theoretical ED_{50}</th>
<th>Observed ED_{50}</th>
<th>Calculated/tabular F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipramine + neramexane</td>
<td>13.87 (3.87–21.68)</td>
<td>13.67 (0.99–23.37)</td>
<td>1.29/6.94, N.S.</td>
</tr>
<tr>
<td>Fluoxetine + neramexane</td>
<td>11.93 (8.12–15.06)</td>
<td>20.48 (14.24–55.32)</td>
<td>1.42/6.94, N.S.</td>
</tr>
<tr>
<td>Venlafaxine + neramexane</td>
<td>10.95 (9.94–11.90)</td>
<td>9.77 (5.94–12.88)</td>
<td>1.98/6.94, N.S.</td>
</tr>
</tbody>
</table>

N.S., not significant.

Results

Effects of a Single Administration of Neramexane, Imipramine, Fluoxetine, and Venlafaxine in the TST and on Locomotor Activity: Dose-Response Analysis (Table 1)

Each of the four compounds, when administered alone, significantly reduced the duration of immobility in the TST in a dose-dependent manner with a minimal effective dose of 5 mg/kg. The same compounds, however, produced different effects on spontaneous locomotor activity. At the doses tested in this set of experiments, neramexane (5, 10, and 20 mg/kg) and venlafaxine (40 mg/kg) increased, whereas imipramine (10 and 20 mg/kg) decreased this behavior. Fluoxetine did not alter locomotor activity.

Effects of a Single Administration of Neramexane Given in Combination with Imipramine, Fluoxetine, or Venlafaxine (Table 2)

Interaction with Imipramine. The combined administration of neramexane (2.5 mg/kg) and imipramine (5 mg/kg) at doses that alone did not exhibit antidepressant-like activity produced a significant decrease in the duration of immobility without producing significant effects on the locomotor activity. Although the combination of neramexane (2.5 mg/kg) with imipramine (20 mg/kg) reduced the duration of immobility without altering locomotor activity, this antidepressant-like effect was not significantly different compared with the corresponding dose of imipramine alone because of the “floor” effect of imipramine administered at 20 mg/kg. The high dose of neramexane (20 mg/kg) potentiated the antidepressant-like activity of both doses of imipramine; however, for the low-dose imipramine (5 mg/kg) group, the combination also produced a significant increase in the locomotor activity compared with vehicle-only treated animals.

Interaction with Venlafaxine. The combined administration of neramexane (2.5 mg/kg) and venlafaxine (5 mg/kg) produced a significant reduction in the duration of immobility compared with vehicle-treated animals and with animals treated with each drug administered alone without significantly affecting locomotor activity. Neramexane (2.5 mg/kg) also enhanced the antidepressant-like effect of venlafaxine (20 mg/kg); however, the locomotor activity was significantly greater compared with vehicle. Likewise, coadministration of the high dose of neramexane (20 mg/kg) with venlafaxine (5 and 20 mg/kg) produced a greater reduction in the duration of immobility compared with venlafaxine alone but with an apparent enhanced effect on the locomotor activity.

Interaction with Fluoxetine. Coadministration of neramexane (2.5 mg/kg) with fluoxetine (20 mg/kg) significantly reduced the duration of immobility compared with vehicle and produced a greater antidepressant-like effect compared with the effect produced when each drug was administered alone at the corresponding dose. The antidepressant-like effect produced by this combination was specific, because it did not significantly alter the locomotor activity. The high dose of neramexane (20 mg/kg) enhanced the antidepressant-like activity of both doses of fluoxetine (5 and 20 mg/kg). However, the locomotor activity was also significantly increased when fluoxetine was combined with the high dose of neramexane.
served interaction between neramexane and fluoxetine (Table 3, indicated in bold) yielded a higher ED$_{50}$ value than the theoretical interaction, suggesting a subadditive effect, this comparison was not significant.

**Effects of Chronic Treatment with Neramexane and Imipramine on BDNF mRNA Expression**

**Neocortex.** Imipramine (10 mg/kg), given chronically, increased BDNF mRNA expression in the mouse neocortex. By contrast, chronic neramexane (5 mg/kg) treatment produced a decrease in neocortical BDNF mRNA expression. Combined treatment of these compounds produced no change in BDNF mRNA expression compared with control (Fig. 1).

**Frontal Cortex.** Similar to that observed in the neocortex, frontal cortical BDNF mRNA expression was increased by imipramine and decreased by neramexane. Chronic treatment with the combination of imipramine and neramexane produced no change in the expression of BDNF mRNA compared with control (Fig. 2).

**Hippocampus.** Chronic treatment with imipramine did not significantly change the expression of BDNF mRNA in the mouse hippocampus. However, as observed in the cortex, neramexane significantly decreased BDNF mRNA expression in the hippocampus. Similar to that observed in the other brain regions studied, the combination did not significantly change the expression of BDNF mRNA compared with control (Fig. 3).

**Behavioral Effects of Chronic Administration of Imipramine with Neramexane**

Although chronic administration of imipramine produced an increase in BDNF mRNA in the cortex, neramexane produced a decrease in its expression in each of the three brain regions evaluated. Because an increase in BDNF mRNA/protein expression is thought to be associated with the antidepressant-like effect of various antidepressant drugs, this result was somewhat unexpected, considering that neramexane exhibited antidepressant-like activity in behavioral tests. To determine whether chronic treatment may have altered behavioral response to neramexane, and thus its effects on BDNF mRNA expression, mice were treated for 14 days and tested immediately after the last dose and again 16 to 17 h later (the latter time point was designed to mimic the time frame of the molecular studies). The order of testing (first immediate then delayed/first delayed then immediate) did not affect the results, suggesting that repeated testing in the tail suspension test may produce reliable results.

Mice treated twice daily for 14 days with imipramine, neramexane, or the combination were tested immediately (30–40 min) after the last dose. Each treatment resulted in a significant decrease in the duration of immobility with the combined treatment producing a greater antidepressant-like effect compared with that of imipramine (10 mg/kg) or neramexane (5 mg/kg) administered alone (Table 4). By contrast, when the mice were tested 16 to 17 h (delayed) after the last dose, there was no significant difference of any drug treatment on the duration of immobility compared with vehicle-treated animals.

**Discussion**

In the current study, we tested whether the acute administration of NMDA receptor antagonist neramexane could potentiate the behavioral response of three representative antidepressant drugs when administered in combination. In 

![Fig. 2. Effect of chronic treatment with neramexane and imipramine on BDNF mRNA expression in mouse frontal cortex using Northern blot analysis. ANOVA: F(3,35) = 8.441; P < 0.001. *, P < 0.05 versus vehicle; see Fig. 1 legend for details.](image1)

![Fig. 3. Effect of chronic treatment with neramexane and imipramine on BDNF mRNA expression in mouse hippocampus using Northern blot analysis. ANOVA: F(3,34) = 6.967; P < 0.001. **, P < 0.01 versus vehicle; see Fig. 1 legend for details.](image2)
a separate set of experiments, we determined whether changes in the expression of BDNF mRNA were associated with the antidepressant-like response after chronic (14-day) treatment. Together, the data demonstrate that although neramexane seems to augment the acute and long-term antidepressant-like response of classic antidepressants, its behavioral actions do not seem to be associated with significant changes in BDNF mRNA expression in the cortex or hippocampus.

Behavioral Studies. In the mouse tail suspension test, three clinically available antidepressant drugs—imipramine, fluoxetine, and venlafaxine—produced a decrease in the duration of immobility at doses between 5 and 20 mg/kg, which is in agreement with previous findings (Steru et al., 1985). The effect of these treatments seemed to be specific because, with the exception of venlafaxine at 40 mg/kg, we observed no stimulation of locomotor activity.

Inhibitors of glutamatergic neurotransmission (for review, see Kugaya and Sanacora, 2005) and, particularly, antagonists of the glutamatergic ionotropic NMDA receptor have been shown to exhibit antidepressant-like activity in the forced swim test in mice (Trullas and Skolnick, 1990). For the NMDA receptor channel blockers memantine, neramexane, and amantadine, antidepressant-like activity was reported in the rat forced swim test (Rogoz et al., 2002). The present study extends these observations to the mouse tail suspension test, where the acute administration of the uncompetitive NMDAR-A neramexane reduced the duration of immobility with an efficacy comparable with that of imipramine, fluoxetine, and venlafaxine. However, the anti-immobility effects of neramexane seemed less specific, because those doses, which decreased immobility, also increased the general activity of the mouse.

The drug interaction studies demonstrated that neramexane, at a dose that itself did not exhibit antidepressant-like activity (2.5 mg/kg), significantly augmented the behavioral response produced by the low (5-mg/kg) doses of imipramine and venlafaxine. Likewise, the combination of the low dose of neramexane with a high dose of fluoxetine (20 mg/kg) produced a significantly greater antidepressant-like response compared with either drug given alone. In each case, the potentiation was not due to an increase in locomotor activity, indicating that the enhanced antidepressant-like response was not due to a nonspecific effect of drug treatment. Our results resemble similar enhancement of antidepressant potency by the α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid receptor potentiator LY392098 reported by Li et al. (2003) and by NMDAR-As such as memantine or amantadine (see Introduction).

The design of the present study also allowed us to examine whether the antidepressant-like effect produced by the acute administration of the high dose of each antidepressant was maximal or whether it could be potentiated by cotreatment with neramexane. This seemed to be the case for each of the three antidepressants tested, since the combination of 20 mg/kg neramexane with 20 mg/kg each antidepressant produced a greater reduction in the duration of immobility compared with administration of the respective antidepressant alone. However, with many of the drug combinations, the apparent increase in the antidepressant-like effect was associated with a corresponding increase in locomotor activity. Nevertheless, it is important to emphasize that an ineffective dose of neramexane (2.5 mg/kg) was shown to potentiate the antidepressant-like action of all three antidepressants without affecting the locomotor response.

The mechanism(s) by which NMDAR-As, including neramexane, enhance the antidepressant-like response of classic antidepressants is currently unknown. Owen and Whitten (2005) reported recently that amantadine and bupidine (the weak NMDAR-As) given acutely with known antidepressants elevated extracellular serotonin concentration in the frontal cortices of rats and facilitated and potentiated this effect after prolonged treatment. These authors suggested that effects on serotonin concentrations could be due to a number of reasons, including alteration of glutamatergic tone at NMDARs that could facilitate serotonin neurotransmission as well as bupidine and amantadine effects on monoamine metabolism. Although similar effects of other NMDAR-As, including neramexane await investigation, the “serotonergic” hypothesis of an enhancement of antidepressant potency seems attractive.

Because neramexane has been shown to be an uncompeti-

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**TABLE 4**

Effect of chronic (14-day) treatment with neramexane and imipramine on the duration of immobility when measured 30 to 40 min and 16 to 17 h after the last dose.

<table>
<thead>
<tr>
<th>Time between Last Dose and Test</th>
<th>Chronic Treatment</th>
<th>TST ± S.E.M.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–40 min (immediate)</td>
<td>Vehicle</td>
<td>142.2 ± 15.6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Imipramine, 10</td>
<td>65.8 ± 11.5*</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Neramexane, 5</td>
<td>87.0 ± 12.0**</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Neramexane, 5 + imipramine, 10</td>
<td>27.7 ± 3.7**</td>
<td>12</td>
</tr>
<tr>
<td>16–17 h (delayed)</td>
<td>Vehicle</td>
<td>121.7 ± 15.9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Imipramine, 10</td>
<td>127.2 ± 14.1†</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Neramexane, 5</td>
<td>114.3 ± 8.3§</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Neramexane, 5 + imipramine, 10</td>
<td>117.7 ± 12.7§</td>
<td>12</td>
</tr>
</tbody>
</table>

Three-way ANOVA

<table>
<thead>
<tr>
<th>Order of testing (1) F(1,40) = 0.001, N.S.</th>
<th>Treatment (2) F(3,40) = 5.57**</th>
<th>Repeated factor (3) F(1,40) = 44.93***</th>
<th>Interaction of 2 and 3 F(3,40) = 16.22***</th>
</tr>
</thead>
</table>

N.S., not significant.

* P < 0.05 versus 30- to 40-min vehicle.

† P < 0.05 versus 16- to 17-h vehicle.

§ P < 0.05 versus 30- to 40-min imipramine as well as versus 30- to 40-min neramexane.

‡ P < 0.05 versus respective drug or drug combination treatment tested 30 to 40 min after last dose.
5-hydroxytryptamine3 receptor antagonists produce antidepressant-like effects in animal models and also potentiate the antidepressant-like effects of several antidepressants (Martin et al., 1992; Kos et al., 2006), other affected receptor mechanisms cannot be excluded.

Pharmacokinetic factors should also be considered when interpreting drug interaction experiments. However, at clinically relevant concentrations neramexane neither induces nor inhibits cytochrome P-450 isoenzymes. This compound also does not induce, inhibit, or serve as a substrate of the p-glycoprotein (Merz Pharmaceuticals, unpublished data). Last, since neramexane produced similar behavioral effects when combined with three structurally unrelated antidepressants that are differently metabolized, pharmacokinetic factors are not likely to have contributed to the present findings; however, direct testing of this would need to be done to exclude this possibility.

**Molecular Studies.** It is well known that electroconvulsive shock as well as chronic (but not acute) treatment with antidepressants can produce an increase in the expression of BDNF mRNA and its receptor TrkB in the hippocampus and cortex of rats (Nibuya et al., 1995; Dias et al., 2003). Moreover, BDNF protein levels have been shown to be increased in the brains of depressed patients who have been treated with antidepressants (Chen et al., 2001). Our results are generally consistent with previous reports demonstrating that chronic antidepressant treatment increases cortical BDNF expression. Although treatment with imipramine increased cortical BDNF mRNA expression, it did not alter its expression in the mouse hippocampus. These observations support the data of Vinet et al. (2004) who reported that treatment with fluoxetine and desipramine did not significantly alter mouse hippocampal BDNF expression.

In contrast to the effects of imipramine, chronic administration with neramexane produced a decrease in BDNF mRNA expression in all three brain areas examined. Because increases in the levels of BDNF mRNA in cortical and hippocampal regions have often been associated with antidepressant-like activity in behavioral tests, this result was somewhat surprising given that NMDAR-A have been consistently shown to produce antidepressant-like effects using acute screening procedures (see Introduction) and also in a chronic animal model of depression (Papp and Moryl, 1994). Although Marvanova et al. (2001) showed that the structurally related NMDAR-A memantine produced an increase in BDNF mRNA expression in various brain areas, this effect was assessed after acute administration. Similar increases in BDNF expression have been reported for other NMDAR-A, including MK-801 and ifenprodil after acute administration (Linden et al., 2000; Matsu ki et al., 2001; Toyomoto et al., 2005). The observed decrease in BDNF mRNA expression after the 14-day administration of neramexane, seems to contradict the hypothesis that antidepressant-like responses are associated with an increase in BDNF levels. The effect of neramexane on BDNF mRNA expression does not seem to be the result of tolerance to the antidepressant-like effect of the drug, because neramexane given over a 14-day period continued to produce a robust decrease in the duration of immobility when tested 30 to 40 min after the last dose. That the BDNF mRNA levels were measured 16 to 17 h after the last dose (when the behavioral response was no longer apparent) is not likely to be the reason for the observed decrease. This is because changes in mRNA levels after chronic drug administration would probably reflect a long-lasting adaptive effect of the drug treatment. Possibly, an increase in BDNF expression may occur early during chronic administration, which was not detected at later time points because of other adaptive mechanisms. However, this would not necessarily explain the observed apparent decrease. An alternative hypothesis is that changes in BDNF expression may not be directly associated with antidepressant-like effects seen in behavioral tests. Indeed, recent analyses indicate that an increase in BDNF expression after chronic antidepressant treatment may not be a uniformly reported phenomenon. For example, Alt et al. (2006) and Tardito et al. (2006) list a number of studies showing either no change or even a decrease of BDNF expression because of the chronic treatment with antidepressants. In addition, Frazer and Morilak (2005) discuss controversial data demonstrating acute behavioral antidepressant-like effects of BDNF and its increased expression seen only after chronic treatment with antidepressants.

Taken together, the present data confirm and extend previous findings showing that NMDA receptor antagonists exhibit antidepressant-like activity in different animal models and may potentiate the behavioral response of currently available antidepressant drugs. Although the reports published to date suggest that treatment with NMDAR channel blocker ketamine alleviates symptoms of major depression (Berman et al., 2000) (but see a negative data with a relatively low dose of memantine, Zarate et al., 2006), systematic evaluation of a combined treatment has not yet been accomplished.

**References**


Kos T, Pepik P, Pietraszek M, Sacher D, Danysz W, Dravolina O, Blokhina E,


