Central κ-opioid receptor-mediated antidepressant-like effects of nor-Binaltorphimine: Behavioral and BDNF mRNA expression studies

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Abstract

κ-opioid receptor antagonists such as nor-Binaltorphimine (nor-BNI) have been shown to produce antidepressant-like behavioral effects in animal models of depression. The aim of this study was to investigate further the duration of centrally administered nor-BNI-induced antidepressant-like actions measured by both behavior and brain-derived neurotrophic factor (BDNF) gene expression. In addition, antagonist studies were conducted to determine the role of opioid receptor subtypes and the time course of nor-BNI's pharmacological actions. Antidepressant-like behavioral effects were measured by decreased immobility in the rat forced swim test and BDNF mRNA expression was determined by *in situ* hybridization. Centrally administered nor-BNI (20 μg, i.c.v.) decreased immobility and increased BDNF mRNA expression in the hippocampus on day 1, not on days 3–14, post-administration. Systemic administration of selective μ-, δ- and κ-opioid receptor antagonists did not block nor-BNI-induced antidepressant-like effects. In contrast, i.c.v. administration of nor-BNI 7 or 14 days earlier significantly blocked subsequent nor-BNI-induced decreased immobility and upregulation of BDNF mRNA expression. Although the duration of nor-BNI's antidepressant-like effects did not synchronize with that of its κ-opioid receptor antagonist effects, this study is the first to show that centrally administered nor-BNI, like most clinically used antidepressants, can upregulate BDNF mRNA expression in the rat hippocampus. These findings further demonstrate that central κ-opioid receptor mediates antidepressant-like effects of nor-BNI measured by both behavior and BDNF gene expression.

Keywords: κ-opioid receptor; Depression; Hippocampus; *In situ* hybridization; Neurotrophin

1. Introduction

κ-opioid receptors participate in many physiological functions such as antinociception (Millan, 1989), diuresis (Leander, 1983), hormonal modulation (Fjalland and Christensen, 1990) and neuroprotection (Birch et al., 1991). In addition, several studies have indicated that κ-opioid receptors are involved in mood regulation. For example, systemic administration of κ-opioid receptor agonists such as U-69593 increased immobility in the rat forced swim test and reduced the rewarding impact of the brain stimulation, indicating that κ-opioid receptor agonists elicit prodepressant-like effects (Mague et al., 2003; Todtenkopf et al., 2004; Carlezon et al., 2006). More interesting, central administration of κ-opioid receptor antagonists such as nor-Binaltorphimine (nor-BNI) produced antidepressant-like behavioral effects in animal models of depression including the forced swim test and learned helplessness paradigm (Pliakas et al., 2001; Newton et al., 2002; Mague et al., 2003; Shirayama et al., 2004).

It is well known that a single systemic or central administration of nor-BNI produces long-lasting κ-opioid receptor antagonist

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actions against κ-opioid receptor agonist-evoked responses across different assays and species *in vivo* (Horan et al., 1992; Butelman et al., 1993; Jewett and Woods, 1995; Picker et al., 1996; Ko et al., 1999). For example, central pretreatment with nor-BNI antagonized κ-opioid receptor agonist-induced antinociception for 4 weeks in mice (Horan et al., 1992); systemic nor-BNI blocked decreased food-reinforced responding by κ-opioid receptor agonists for 11 weeks in pigeons (Jewett and Woods, 1995); and central nor-BNI blocked κ-opioid receptor agonist-induced diuresis for 5 months in monkeys (Ko et al., 2003). However, nor-BNI-induced antidepressant-like effects were studied only with 1- or 3-day pretreatment (Pliakas et al., 2001; Mague et al., 2003). Cross-study comparisons of the durations of pharmacological action of nor-BNI could be complicated by several factors including differences in species, measured endpoints, and administration routes. Nevertheless, it is not known how long nor-BNI-induced antidepressant-like effects last and whether prior administration of nor-BNI can block antidepressant-like effects produced by subsequent administration of nor-BNI (*i.e.*, κ-opioid receptor occupancy). It is important to study both issues further to clarify the pharmacological actions of nor-BNI in this context.

Several lines of evidence have suggested that upregulation of brain-derived neurotrophic factor (BDNF) plays an important role in the therapeutic actions of antidepressants (Hashimoto et al., 2004; Duman and Monteggia, 2006; Tardito et al., 2006). BDNF regulates neuronal survival, differentiation, and plasticity (Bramham and Messaoudi, 2005; Tongiorgi et al., 2006). Human studies have linked BDNF with the pathophysiology of various mood disorders. For example, increased hippocampal BDNF immunoreactivity has been found in patients with major depression that had been treated with antidepressants (Chen et al., 2001). Animal studies also showed that chronic treatment with antidepressants could upregulate BDNF mRNA expression in the hippocampus of rats (Nibuya et al., 1995; Russo-Neustadt et al., 2004). In addition, infusion of BDNF into the midbrain or hippocampus produced antidepressant-like effects in rodent models of depression (Siuciak et al., 1997; Shirayama et al., 2002). Given that central administration of κ-opioid receptor antagonists produced antidepressant-like behavioral effect, it is important to know whether central infusion of κ-opioid receptor antagonists can modulate BDNF mRNA expression, showing integration of both behavioral and gene expression changes by κ-opioid receptor antagonists.

The aim of this study was to investigate the time course of centrally administered nor-BNI-induced antidepressant-like effects in the forced swim test, and determine whether nor-BNI-induced changes in BDNF mRNA expression correspond with the duration of its antidepressant-like behavioral effects. BDNF mRNA expression was examined in the brain regions involved in mood regulation including the frontal cortex, CA1, CA3, and dentate gyrus regions of hippocampus, and amygdala, by using *in situ* hybridization (Nibuya et al., 1995; Torregrossa et al., 2004; Zhang et al., 2006). In addition, a series of antagonist studies were performed to verify the role of κ-opioid receptor in both antidepressant-like behavioral effects and BDNF gene expression elicited by centrally administered nor-BNI.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–275 g) were obtained from Harlan Sprague–Dawley (Indianapolis, IN, USA) and were housed in groups of three rats per cage. All animals were allowed *ad libitum* access to food and water, and were maintained on a 12 h light:dark cycle with lights on at 06:30 AM in a room kept at a temperature of 22 ± 1 °C. Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The experimental protocols were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

2.2. Intracerebroventricular (i.c.v.) surgery

Rats were anesthetized by intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic device. Each rat was prepared with a 23-gage stainless steel cannula (Small Parts, Miami Lakes, FL, USA) extending into the right lateral cerebral ventricle (coordinated from bregma, AP: 0.8 mm, ML: 1.5 mm, DV: 4.2 mm; Paxinos and Watson, 1986). The guide cannula was fixed in place with dental cement applied to the surface of the skull. Animals were allowed 6 or 7 days to recover from surgery. Each animal's i.c.v. cannula placement was verified after the experiment by injecting methylene blue and checking for distribution. Only data obtained from animals with appropriate i.c.v. cannula placement were used for data analysis.

2.3. Drug treatment

All drugs were dissolved in sterile water. nor-Binaltorphimine (nor-BNI) 2HCl (10 or 20 μg) (Sigma-Aldrich, St. Louis, MO, USA) was administrated i.c.v. 24 h before the behavioral measurement and *in situ* hybridization histochemistry. In addition, the time course of centrally administered nor-BNI-induced effects was determined by using different pretreatment time points (*i.e.*, 1-, 3-, 7-, or 14-day pretreatment) with a single i.c.v. nor-BNI 20 μg. nor-BNI was administered i.c.v. *via* the guide cannula in volumes of 10 μL using a 25-μL Hamilton syringe attached *via* a polyethylene PE20 tube (Plastics One, Roanoke, VA, USA) to a 30-gage needle (Becton Dickinson, Franklin Lakes, NJ, USA). Solution was administrated over a period of 60 s and the needle was left within the guide cannula for an additional 30 s to prevent reflux. Sterile water was administered as vehicle for the control injection. For the antagonist study, naltrexone HCl and naltrindole HCl (National Institute on Drug Abuse, Bethesda, MD, USA), and nor-BNI were used to verify the involvement of each opioid receptor subtype in i.c.v. nor-BNI-induced antidepressant-like effects. The effects of each opioid receptor antagonist were first tested alone following s.c. administration. Then, sterile water (vehicle, 1 mL/kg), naltrindole (1 mg/kg) or naltrexone (0.1 mg/kg) was administered s.c. 15 min before i.c.v. administration of nor-BNI

20 μg. nor-BNI (10 mg/kg) was administered s.c. 24 h before i.c.v. administration of nor-BNI. The dose and pretreatment time for these opioid receptor antagonists were chosen based on previous studies showing that each antagonist produced selective functional antagonism for μ-, δ- and κ-opioid receptors (Takemori et al., 1988b; Chang et al., 1993; Walker et al., 1994). In addition, the time course of i.c.v. nor-BNI-induced κ-opioid receptors blockade was studied by determining effects of 7- and 14-day pretreatment with a single i.c.v. administration of nor-BNI 20 μg on subsequent i.c.v. nor-BNI (20 μg)-induced antidepressant-like behavioral effects and increases in BDNF mRNA expression.

2.4. Forced swim test

The modified forced swim test (*i.e.*, a one-time, 15-min swim session) was conducted as described by Broom et al. (2002). A series of studies from our laboratory have demonstrated that this procedure can be used to detect the antidepressant potential of compounds (Broom et al., 2002; Torregrossa et al., 2004; Zhang et al., 2006). Rats were placed in a clear cylindrical Plexiglas container (46 cm tall × 20 cm diameter) filled with 30 cm of 25 °C (±1 °C) water for a 15-min swim session. Cylinders were cleaned and fresh water added between each rat. The 15-min swim period was videotaped and behaviors were scored every 5 s by experimenters who were blinded to dosing conditions. Behaviors were classified as immobility, swimming or climbing, as defined by Broom et al. (2002).

2.5. In situ hybridization histochemistry

At each time point, experimentally naïve animals were killed by decapitation, and their brains were rapidly removed and frozen in isopentane at −40 °C, and were stored at −80 °C. Brains were sectioned at 20 μm on a cryostat and were thaw mounted onto poly-L-lysine-subbed slides. Slides were stored at −80 °C until they were processed for *in situ* hybridization, which was performed as described previously (Torregrossa et al., 2004; Zhang et al., 2006). BDNF mRNA expression levels were determined by a double label *in situ* hybridization with a [^{35}S]-labeled BDNF cRNA probe as described previously (Torregrossa et al., 2004; Zhang et al., 2006). The rat BDNF cDNA (Isackson et al., 1991) was donated by Drs. Gall and Lauterborn (University of California, Irvine, CA, USA). The probe was radioactively labeled in a reaction containing 1 μg BDNF antisense linearized plasmid DNA, 5 × transcription buffer, 125 μCi each of [^{35}S]UTP and [^{35}S]CTP, 150 μM each of ATP and GTP, 12.5 mM dithiothreitol, 20 U RNase inhibitor and 6 U of T3 polymerase. The [^{35}S]-labeled BDNF probe was hybridized to brain sections in hybridization buffer containing 1.5 million cpms radiolabeled probe per 80 μL. The slides were exposed on Kodak XAR film (Eastman Kodak, Rochester, NY) for 14 days.

2.6. Quantification of radioactive signal

BDNF mRNA levels were quantified using NIH Image software with standards to convert grey levels to radioactivity

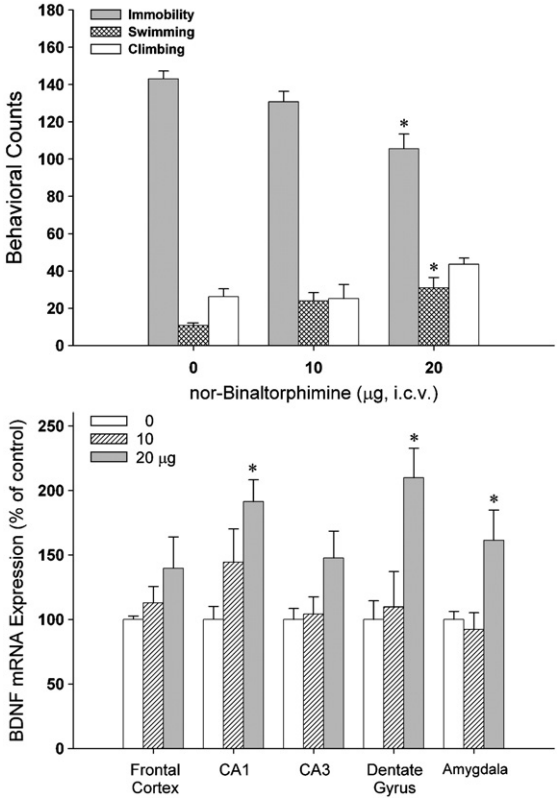

Fig. 1. Effects of central administration of nor-BNI on behavior in the forced swim test and on BDNF mRNA expression in rats. nor-BNI (μg) was administrated i.c.v. 24 h before the behavioral measurement (top panel) and brain collection for *in situ* hybridization (bottom panel). Quantification of BDNF mRNA signals in different brain regions including the frontal cortex, CA1, CA3, and dentate gyrus of hippocampus, and amygdala are represented as mean percent of control (no treatment). Each value represents mean ± S.E.M. (n = 4–6). *p < 0.05 compared with the vehicle condition.

levels (Scion Image, Frederick, MD, USA). BDNF mRNA expression was examined in the frontal cortex, CA1, CA3 and dentate gyrus regions of hippocampus and amygdala. Each brain region was analyzed by creating an outline around the region and measuring both the left and right sides of the brain and from rostral–caudal sections 100–200 μm apart. At least six sections per region per rat were quantified. The signal measurements were corrected for background and were determined as the mean radioactive intensity per pixel for that region. These signal values for each section were then averaged to obtain the mean signal for each region in each rat. These data points were then averaged per group and compared statistically.

2.7. Statistical analysis

Behavioral data from the forced swim test were expressed as mean ± S.E.M. BDNF signals from *in situ* hybridization were expressed as mean percent of control (*i.e.*, no treatment) ± S.E.M. Statistical analysis was performed using one-way ANOVA with Dunnett's *post hoc* test to compare differences between groups where $p < 0.05$ was considered significant.

3. Results

3.1. Dose-response of i.c.v. nor-BNI on the forced swim test and brain BDNF mRNA expression

Earlier studies have shown that κ-opioid receptor antagonist activity of nor-BNI reached its peak effect at 24 h after i.c.v. administration (Jones and Holtzman, 1992; Spanagel et al., 1994). Therefore 24 h pretreatment with nor-BNI was selected to conduct the dose-response study. Fig. 1 shows that i.c.v. nor-BNI at 24 h post-administration decreased immobility and increased swimming and climbing in a dose-dependent manner (top panel). *Post hoc* comparisons indicated that nor-BNI 20 μg significantly decreased immobility and increased swimming, but did not alter climbing significantly. *In situ* hybridization results showed that the baseline levels for BDNF mRNA in control groups in different areas were 7.4±0.3 (front cortex), 5.9±0.2 (CA1), 18.1±1.5 (CA3), 16.8±2.8 (dentate gyrus), and 9.0±1.2 (amygdala) respectively. In order to compare the results between conditions, the BDNF mRNA expressions in this study were expressed as mean percent of control. Fig. 1 shows that nor-BNI 20 μg significantly increased BDNF mRNA expression in the CA1 and dentate gyrus of hippocampus and amygdala when compared with the vehicle condition (bottom panel).

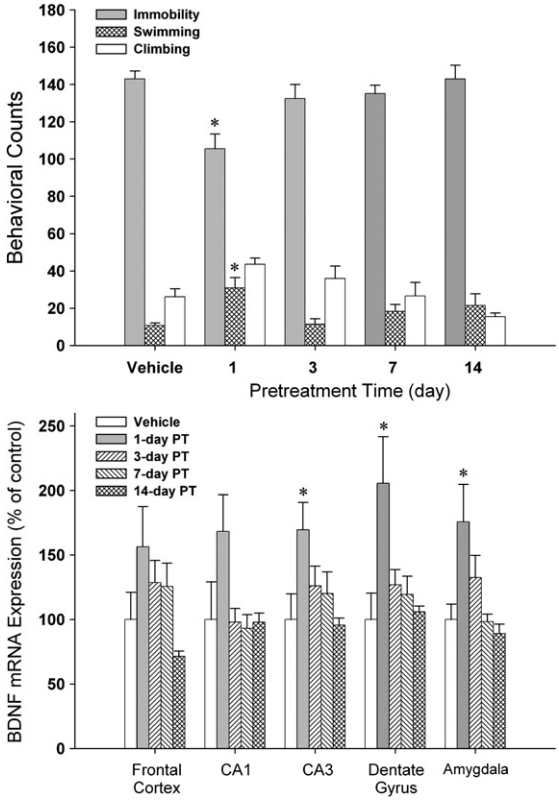

Fig. 2. Time course of centrally administered nor-BNI-induced antidepressant-like effects and increases of BDNF mRNA expression. Effects of i.c.v. nor-BNI 20 μg on behavior in the forced swim test and on BDNF mRNA expression were measured. nor-BNI was administered 1, 3, 7, or 14 days before the behavioral measurement (top panel) and brain collection for *in situ* hybridization (bottom panel) in different groups of rats. Each value represents mean±S.E.M. (n=4–6). *p<0.05 compared with the vehicle condition. See Fig. 1 for other details.

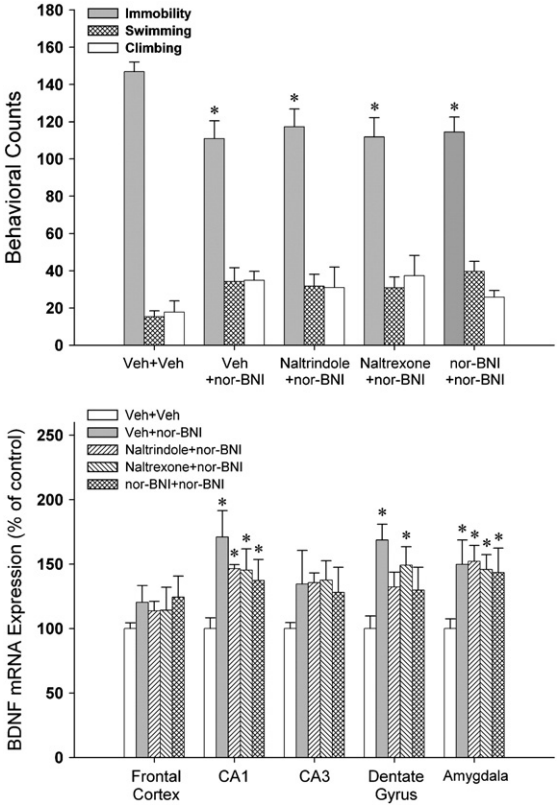

Fig. 3. Effects of systemic opioid receptor antagonists on centrally administered nor-BNI-induced effects. nor-BNI (20 μg) decreased immobility in the forced swim test and increased BDNF mRNA expression 24 h following i.c.v. administration. Sterile water (1 mL/kg, Veh), naltrindole (1 mg/kg) or naltrexone (0.1 mg/kg) was administered s.c. 15 min before i.c.v. administration of nor-BNI (*i.e.*, Veh+nor-BNI, naltrindole+nor-BNI, and naltrexone+nor-BNI). nor-BNI (10 mg/kg) was administered s.c. 24 h before i.c.v. administration of nor-BNI (*i.e.*, nor-BNI+nor-BNI). Each value represents mean±S.E.M. (n=4–6). *p<0.05 compared with the Veh+Veh condition. See Fig. 1 for other details.

3.2. Time course of effects of i.c.v. nor-BNI on the forced swim test and brain BDNF mRNA expression

The duration of the antidepressant-like effects of nor-BNI 20 μg was further studied by using longer pretreatment time points including 1-, 3-, 7-, and 14-day pretreatment. Fig. 2 shows that only at day 1 post-administration i.c.v. nor-BNI was effective at decreasing immobility and increasing BDNF mRNA expression in the CA3 and dentate gyrus of hippocampus. Pretreatment with nor-BNI 3-, 7-, and 14 days before did not change behavioral responses in the forced swim test or brain BDNF mRNA expression.

3.3. Effects of s.c. opioid receptor antagonists on i.c.v. nor-BNI-induced activity in the forced swim test and brain BDNF mRNA expression

In order to investigate whether μ-, δ-, and κ-opioid receptors are involved in effects of nor-BNI, a selective δ-opioid receptor antagonist, naltrindole, or a μ-opioid receptor antagonist, naltrexone, was administrated 15 min before i.c.v. administration of nor-BNI 20 μg. In addition, 10 mg/kg of nor-BNI was administered s.c.

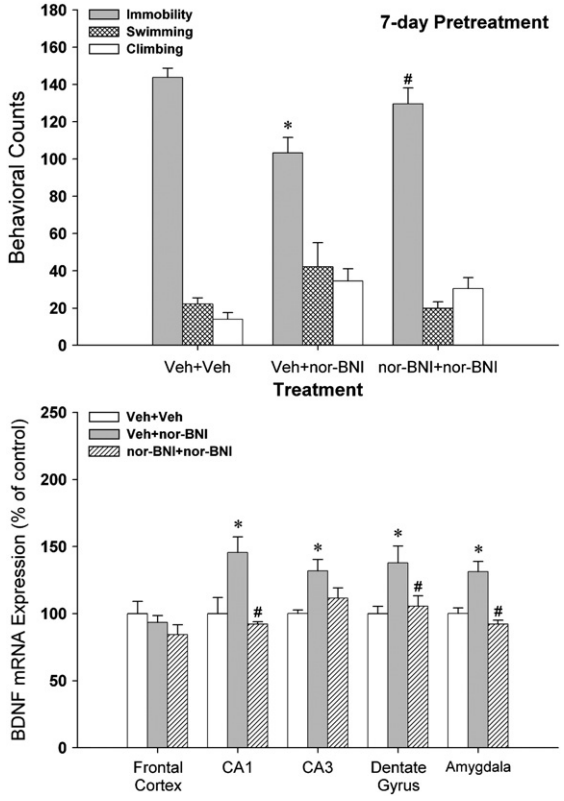

Fig. 4. Effects of 7-day central pretreatment with nor-BNI on i.c.v. nor-BNI-induced antidepressant-like effects and increases brain BDNF mRNA expression. Sterile water (vehicle, 10 μL) or nor-BNI (20 μg) was administrated i.c.v. 7 days prior to i.c.v. administration of nor-BNI 20 μg (*i.e.*, Veh+nor-BNI and nor-BNI+nor-BNI). Last nor-BNI was administered 24 h before the behavioral measurement (top panel) and brain collection for *in situ* hybridization (bottom panel). Each value represents mean±S.E.M. (n=4–6). *p<0.05 compared with the Veh+Veh condition; $^{\#}p$<0.05 compared with the Veh+nor-BNI condition. See Figs. 1 and 3 for other details.

24 h before i.c.v. administration of nor-BNI. Subcutaneous administration of naltrindole (1 mg/kg), naltrexone (0.1 mg/kg) and nor-BNI (10 mg/kg) alone had no effect in the forced swim test and BDNF mRNA expression (data not shown). Fig. 3 illustrates that i.c.v. nor-BNI (*i.e.*, the Veh+nor-BNI group) significantly produced the reduction of immobility and upregulation of BDNF mRNA expression, when compared with the Veh+Veh group, at day 1 post-administration. Pretreatment with s.c. naltrindole, naltrexone, or nor-BNI did not significantly alter subsequent i.c.v. nor-BNI-induced decreases in immobility and increases in BDNF mRNA expression in the sub-region of hippocampus and amygdala when compared with the vehicle pretreatment (*i.e.* Veh+nor-BNI). In other words, most i.c.v. nor-BNI's effects with s.c. naltrindole, naltrexone, or nor-BNI pretreatment showed significant reduction of immobility and increase of BDNF mRNA expression when compared with the Veh+Veh group.

3.4. Effects of i.c.v. nor-BNI pretreatment on i.c.v. nor-BNI-induced activity in the forced swim test and brain BDNF mRNA expression

Because our study showed that i.c.v. nor-BNI given 7 or 14 days earlier had no effect in the forced swim test and on brain BDNF mRNA expression (Fig. 2), both pretreatment time points were used to determine whether central pretreatment with nor-BNI 20 μg could block antidepressant-like effects produced by a subsequent i.c.v. administration of nor-BNI 20 μg. Fig. 4 shows that 7-day central pretreatment with nor-BNI significantly blocked subsequent i.c.v. nor-BNI-induced reduction in immobility and increases in BDNF mRNA expression in the regions of hippocampus and amygdala, when compared with the vehicle pretreatment group (*i.e.*, Veh+nor-BNI). Likewise, Fig. 5 shows that 14-day central pretreatment with nor-BNI significantly blocked subsequent nor-BNI-induced antidepressant-like effects measured by the behavioral responses and BDNF mRNA expression, when compared with the vehicle pretreatment group (Veh+nor-BNI).

4. Discussion

The behavioral study showed that i.c.v. administration of nor-BNI decreased immobility in the rat forced swim test. This finding is consistent with previous reports and supports the notion that κ-opioid receptor antagonists may have therapeutic potential as antidepressants (Pliakas et al., 2001; Mague et al., 2003). More

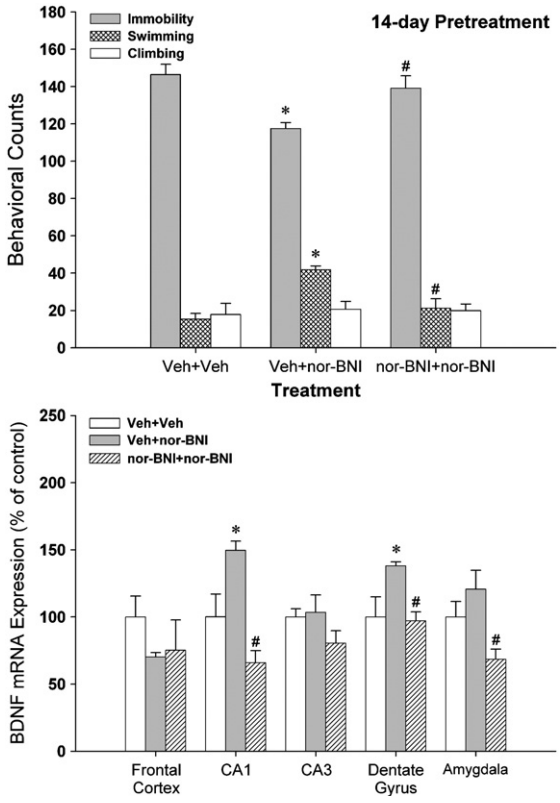

Fig. 5. Effects of 14-day central pretreatment with nor-BNI on i.c.v. nor-BNI-induced antidepressant-like effects and increases brain BDNF mRNA expression. Sterile water (vehicle, 10 μL) or nor-BNI (20 μg) was administrated i.c.v. 14 days prior to i.c.v. administration of nor-BNI 20 μg (*i.e.*, Veh+nor-BNI and nor-BNI+nor-BNI). Last nor-BNI was administered 24 h before the behavioral measurement (top panel) and brain collection for *in situ* hybridization (bottom panel). Each value represents mean±S.E.M. (n=4–6). *p<0.05 compared with the Veh+Veh condition; $^{\#}p$<0.05 compared with the Veh+nor-BNI condition. See Figs. 1 and 4 for other details.

important, the present study is the first to show that i.c.v. nor-BNI upregulated BDNF mRNA expression in some sub-regions of hippocampus and that the duration of nor-BNI-induced antidepressant-like behavioral effects synchronized with nor-BNI-mediated increase in BDNF mRNA expression (Fig. 2). Although other brain regions such as frontal cortex and amygdala have been examined, increased BDNF mRNA expression by i.c.v. nor-BNI has been consistently observed in the dentate gyrus of the hippocampus throughout the study. The hippocampus is an important brain region in the pathophysiology of depression as well as in antidepressant treatments (Sheline et al., 2003; Duman, 2004). Most antidepressants and electroconvulsive shock therapy have been reported to increase BDNF mRNA expression especially in the hippocampus (Nibuya et al., 1995; Russo-Neustadt et al., 2004). In addition, microinfusion of BDNF into the hippocampus produces antidepressant-like effects in animal models of depression (Siuciak et al., 1997; Shirayama et al., 2002). Given that reduction of BDNF mRNA expression induced by the acute immobilization stress has been observed in the major subfields of the hippocampus (Smith et al., 1995; Nibuya et al., 1995), the ability of nor-BNI to increase BDNF mRNA expression in the hippocampus indicates that this is an important anatomical basis for its antidepressant-like actions. It is worth nothing that moderate increases in BDNF mRNA expression found in this study are similar to those induced by other pharmacological agents including clinically used antidepressants (Nibuya et al., 1995; Russo-Neustadt et al., 2004; Torregrossa et al., 2004). Previous studies have found that antidepressant-induced modulation of mRNA and protein expression may not always correlate (Lesch and Manji, 1992; Jacobsen and Mork, 2004). It is important to further investigate whether i.c.v. nor-BNI increases the BDNF protein level.

It is well known that nor-BNI has a slow-onset, long-lasting κ-opioid receptor antagonist effect. nor-BNI produces peak and selective κ-opioid receptor antagonist effects 24 h after administration and its κ-opioid receptor antagonist effects continue for weeks or months when it is given centrally (Horan et al., 1992; Jewett and Woods, 1995; Ko et al., 2003). Nevertheless, the present time course study showed that antidepressant-like effects of centrally administered nor-BNI can be detected only at day 1 post-administration, not at day 3, 7, or 14 post-administration. The duration of central nor-BNI-induced antidepressant-like actions clearly differs from reported durations of central nor-BNI-induced κ-opioid receptor antagonist effects. This distinction in nor-BNI's duration of actions raises a possibility that nor-BNI may not act as a neutral κ-opioid receptor antagonist (*i.e.*, zero efficacy) when it displays antidepressant-like actions. Given that κ-opioid receptor agonists increased immobility (*i.e.*, positive efficacy) (Mague et al., 2003; Carlezon et al., 2006) and nor-BNI decreased immobility (*i.e.*, negative efficacy) in the forced swim test (Pliakas et al., 2001; Mague et al., 2003), the antidepressant-like effects of nor-BNI (*i.e.*, opposite effects to those of κ-opioid receptor agonists) can be considered as actions of an *inverse* agonist under these experimental conditions (Milligan et al., 1995; de Ligt et al., 2000).

Conceptually, it seems reasonable to assume that the antidepressant-like actions of nor-BNI are due to blockade of actions of endogenous κ-opioid receptor peptides such as dynorphins during the stressful context. However, animals with 3-, 7-, or 14-day pretreatment with nor-BNI did not show reduced immobility when they were exposed to the forced swim test (Fig. 2). In order to verify whether nor-BNI-induced κ-opioid receptor antagonist effects still exist between 3- and 14-day post-administration, experiments were conducted to examine whether central 7- and 14-day pretreatment with nor-BNI (*i.e.*, 1st dosing) could block antidepressant-like effects elicited by a subsequent i.c.v. nor-BNI (*i.e.*, 2nd dosing). Interestingly, our findings show that 7-day or 14-day central pretreatment with nor-BNI not only blocked subsequent i.c.v. nor-BNI-induced decreased immobility, but also blocked subsequent nor-BNI-induced upregulation of BDNF mRNA expression (Figs. 4 and 5). Due to the short life of i.c.v. cannula (~3 weeks), κ-opioid receptor antagonist effects of central nor-BNI with longer pretreatment time could not be examined. Nevertheless, these results suggest that nor-BNI displayed different pharmacological actions through central κ-opioid receptor by first showing acute antidepressant-like effects (*i.e.*, inverse agonist actions), followed by long-term κ-opioid receptor antagonist effects. The unique pharmacological actions of nor-BNI may be because nor-BNI is resistant to metabolism or/and that nor-BNI induces conformational changes in κ-opioid receptor. There are other compounds that also show changing pharmacological actions over time. For example, Buprenorphine produced acute analgesia (*i.e.*, μ-opioid agonist action) followed by long-term μ-opioid receptor antagonist effects (Cowan et al., 1977; Walker et al., 1995).

In the present study, s.c. administration of nor-BNI 10 mg/kg did not produce antidepressant-like effects (Fig. 3). What could contribute to the distinct effects between systemic and central administration of nor-BNI in producing antidepressant-like effects is not clear. Doses of nor-BNI studied herein (*i.e.*, s.c. 10 mg/kg and i.c.v. 20 μg) are sufficient to occupy κ-opioid receptor as indicated by κ-opioid receptor antagonist effects in other behavioral assays (Takemori et al., 1988b; Jones and Holtzman, 1992). However, there may be different distribution and bioavailability between systemic and central nor-BNI. Mague et al. (2003) have reported that another κ-opioid receptor antagonist, GNTI, was effective in reducing immobility in the forced swim test following i.c.v., but not s.c. administration. Interestingly, a more lipophilic κ-opioid receptor antagonist, ANTI, reduced immobility following i.p. administration (Mague et al., 2003). In addition, the present antagonist studies show that the antidepressant-like effects produced by i.c.v. nor-BNI can be blocked by prior i.c.v. administration of nor-BNI, not by s.c. nor-BNI administration (Fig. 3 and 4), suggesting that site of actions for i.c.v. and s.c. administration of nor-BNI may be different. It is possible that the κ-opioid receptor population and anatomy required for nor-BNI to produce antidepressant-like effects are different from those required to produce other behavioral effects. It will be valuable to characterize the time course of κ-opioid receptor changes in a specific neural substrate (*e.g.*, hippocampus) following central and systemic administration of nor-BNI by using brain imaging techniques.

Although nor-BNI is characterized as a selective κ-opioid receptor antagonist based on *in vitro* binding assays (Takemori et al., 1988a), some studies have suggested that nor-BNI may have antagonist actions *in vivo* on other opioid receptor subtypes such as μ-opioid receptors (Spanagel et al., 1994; Endoh et al., 1992). In contrast, a recent study indicated that there is a tonic balance between μ-opioid receptors and κ-opioid receptors in the homeostatic maintenance of body temperature (Chen et al., 2005). CTAP, a μ-opioid receptor antagonist, could antagonize the increased temperature induced by i.c.v. administration of nor-BNI, suggesting that endogenous μ-opioid receptor activation is involved in nor-BNI-induced hyperthermia (Chen et al., 2005). In order to examine whether antidepressant-like effects of nor-BNI on day 1 post-administration were associated with other opioid receptor subtypes, selective μ- and δ-opioid receptor antagonists, naltrexone and naltrindole, were used. In particular, a previous study has shown that systemic administration of naltrexone and naltrindole at the same doses used in this study could block effects of centrally administered μ- and δ-opioid receptor agonists in the forced swim test and brain BDNF mRNA expression (Zhang et al., 2006). However, both naltrexone and naltrindole failed to block the antidepressant-like effects of nor-BNI (Fig. 3), indicating that both μ- and δ-opioid receptors play a minimal role in nor-BNI-elicited antidepressant-like effects.

The present study demonstrates that i.c.v. administration of nor-BNI decreases the immobility in the forced swim test and increases BDNF mRNA expression in hippocampus of rats, suggesting nor-BNI's therapeutic potential as an antidepressant. Antagonist studies confirm that central κ-opioid receptors mainly mediate the antidepressant-like effects of nor-BNI measured by both behavior and BDNF gene expression. Although the duration of nor-BNI's antidepressant-like actions does not synchronize with that of its κ-opioid receptor antagonist effects, development of more lipophilic κ-opioid receptor antagonists will facilitate exploring their therapeutic potential as antidepressants.

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