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Effects of the psychotomimetic benzomorphan *N*-allylnormetazocine (SKF 10,047) on prepulse inhibition of startle in mice

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Abstract

N-allylnormetazocine (NANM; SKF 10,047) is a benzomorphan opioid that produces psychotomimetic effects. (+)-NANM is the prototypical agonist for the sigma-1 (σ_1) receptor, and there is a widespread belief that the hallucinogenic effects of NANM and other benzomorphan derivatives are mediated by interactions with σ_1 sites. However, NANM is also an agonist at the κ opioid receptor (KOR) and binds to the PCP site located within the channel pore of the NMDA receptor, interactions that could potentially contribute to the effects of NANM. NMDA receptor antagonists such as phencyclidine (PCP) and ketamine are known to disrupt prepulse inhibition (PPI) of acoustic startle, a measure of sensorimotor gating, in rodents. We recently found that racemic NANM disrupts PPI in rats, but it is not clear whether the effect is mediated by blockade of the NMDA receptor, or alternatively whether interactions with KOR and σ_1 receptors are involved. The present studies examined whether NANM and its stereoisomers alter PPI in C57BL/6J mice, and tested whether the effects on PPI are mediated by KOR or σ_1 receptors. Racemic NANM produced a dose-dependent disruption of PPI (3–30 mg/kg SC). (+)-NANM also disrupted PPI, whereas (–)-NANM was ineffective. Pretreatment with the selective KOR antagonist nor-binaltorphimine (10 mg/kg SC) or the selective σ_1 antagonist NE-100 (1 mg/kg IP) failed to attenuate the reduction in PPI produced by racemic NANM. We also found that the selective KOR agonist (–)-U-50,488H (10–40 mg/kg SC) had no effect on PPI. These findings confirm that NANM reduces sensorimotor gating in rodents, and indicate that the effect is mediated by interactions with the PCP receptor and not by activation of KOR or σ_1 receptors. This observation is consistent with evidence indicating that the σ_1 receptor is not linked to hallucinogenic or psychotomimetic effects.

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Keywords

psychotomimetic; sigma receptor; prepulse inhibition; hallucinogen; dissociative; kappa receptor; mice

INTRODUCTION

Many opioids that act as mixed agonist-antagonists produce psychotomimetic effects that limit their clinical usefulness. It was discovered in the mid-1950s that the morphine antagonist *N*-allylnormorphine (nalorphine) has potent analgesic effects in man (Lasagna and Beecher, 1954; Keats and Telford, 1957), suggesting that it may be possible to separate the analgesic and addictive properties of opiates. Unfortunately, nalorphine was also found to produce disturbing effects such as visual and auditory hallucinations, depersonalization, delusions, and dysphoria (Wikler et al., 1953; Lasagna, 1954; Huggins and Moya, 1955). Antagonists from the benzomorphan structural class were also developed as potential analgesics, but most were found to have nalorphine-like effects. Postoperative patients treated with *N*-allylnormetazocine (NANM, SKF-10,047) experienced profound hallucinogenic and dysphoric effects (Keats and Telford, 1964). Similar effects are induced by other benzomorphans, including pentazocine and cyclazocine (Archer et al., 1962; Lasagna et al., 1964; Haertzen, 1970; Beaver and Feise, 1977; Coursey, 1978; Kumor et al., 1986).

In 1976, Martin and coworkers proposed that the effects of opioid drugs are mediated by three specific types of receptors. They hypothesized that μ receptors (MOR) mediate analgesia, κ receptors (KOR) mediate sedation, and σ receptors mediate the hallucinogenic effects of NANM (Martin et al., 1976). Later it was shown that NANM and other benzomorphans bind to a haloperidol-sensitive σ_1 site in the brain with high affinity (Su, 1982; Tam and Cook, 1984; Largent et al., 1986; De Costa et al., 1989). The dissociative anesthetic phencyclidine (PCP), which acts as an uncompetitive NMDA receptor (NMDA-R) antagonist, also binds to σ_1 sites. Although it was initially proposed that the σ_1 site and the PCP binding site associated with the NMDA-R are identical (Zukin and Zukin, 1981; Itzhak et al., 1985; Mendelsohn et al., 1985), they are now recognized as being discrete entities (Goldman et al., 1985; Tam, 1985; Largent et al., 1986). Cloning of the σ_1 receptor revealed that it contains 223 amino acids and displays sequence homology with a fungal sterol C₈-C₇ isomerase (Hanner et al., 1996; Seth et al., 1997; Mei and Pasternak, 2001).

Despite the widely held view that σ_1 receptor activation can provoke hallucinations, there is actually very little evidence to support this contention. Several findings indicate that the σ receptor does not mediate the dysphoria and psychotomimetic effects produced by benzomorphan derivatives (reviewed by: Musacchio, 1990). The MOR/KOR antagonist naloxone blocks the dysphoria induced by cyclazocine (Jasinski et al., 1968) and pentazocine-induced hallucinations (Jago et al., 1984). Those findings are notable because naloxone does not interact with σ_1 receptors at concentrations up to 100 μ M (Su, 1982; Tam, 1983, 1985; Tam and Cook, 1984). A study comparing the effects of (+)- and (–)-pentazocine reported that the psychotomimetic side effects of pentazocine are mediated by

the (–)-stereoisomer (Forrest et al., 1969). This contrasts with the preference of the σ_1 receptor for (+)-benzomorphans; (+)-pentazocine has approximately 25-fold higher affinity than (–)-pentazocine for σ_1 (De Costa et al., 1989; Carroll et al., 1992). Another clinical trial examined the effects of the benzomorphan MR 2033 in volunteer subjects (Pfeiffer et al., 1986). MR 2033 and its (–)-isomer are selective KOR agonists (Merz and Stockhaus, 1979; Nock et al., 1990); both compounds produced dysphoria, depersonalization, derealization, disorientation, visual hallucinations, and loss of self control, effects that were completely blocked by pretreatment with naloxone (Pfeiffer et al., 1986). By contrast, the KOR-inactive (+)-isomer did not produce any subjective effects, even when administered at a relatively high dose. These findings indicate that KOR may be responsible for the psychotomimetic effects of benzomorphan derivatives. It is well known that KOR activation results in hallucinations and dysphoria. Selective KOR agonists, such as salvinorin A and enadoline, produce profound dissociative effects and hallucinations in humans (Walsh et al., 2001; Johnson et al., 2011; Addy, 2012; Ranganathan et al., 2012; MacLean et al., 2013).

Understanding the mechanism for the effects of NANM is complicated by the fact that the racemic compound is a mixture of two stereoisomers with different pharmacological properties. (+)-NANM has high affinity for σ_1 receptors ($K_i \sim 60$ nM) and low affinity for MOR and KOR. Conversely, (–)-NANM has high affinity for MOR ($K_i = 3$ nM) and KOR ($K_i = 4.7$ nM), but low affinity for σ_1 (Tam, 1985; Largent et al., 1986; Carroll et al., 1992). Both isomers bind to the PCP site, although (+)-NANM ($K_i = 225$ nM) is almost twice as potent as (–)-NANM ($K_i = 504$ nM). Drugs acting on the PCP site produce dissociative effects (Javitt and Zukin, 1991), so interactions with the PCP site could potentially contribute to the psychoactive effects of NANM. Indeed, there is evidence that the behavioral effects of NANM in monkeys and rodents are mediated by interactions with the PCP site, with KOR and σ_1 receptors playing little or no role (Shearman and Herz, 1982; Brady et al., 1982; Balaster, 1989; Holtzmann, 1993).

Uncompetitive NMDA-R antagonists, including PCP, ketamine, methoxetamine, and dizocilpine (MK-801), are known to disrupt prepulse inhibition of startle (PPI) in rodents (Mansbach and Geyer, 1989, 1991; Halberstadt et al., 2016). We recently demonstrated that racemic NANM is also capable of disrupting PPI in rats (Halberstadt et al., 2016). PPI refers to the phenomenon where the startle response is attenuated if the startling stimulus is preceded by a weak prestimulus, and is often used as a cross-species measure of sensorimotor gating. The disruption of PPI by dissociative drugs is believed to have particular relevance to their hallucinogenic effects. PCP and other NMDA-R antagonists are thought to induce hallucinations due to reductions in subcortical gating, which results in sensory flooding (Vollenweider and Geyer, 2001). Hence, the information processing deficits that are responsible for the disruption of PPI by NMDA-R antagonists may also contribute to their hallucinogenic effects.

Although NANM can disrupt PPI, it is not clear whether the effect is mediated by NMDA-R blockade, or alternatively, whether σ_1 and KOR are involved. In the present studies, we examined the effects of racemic NANM, (+)-NANM, and (–)-NANM on PPI in C57BL/6J mice. Pharmacological blockade studies were also conducted to identify the receptor(s) responsible for mediating the effect of NANM on PPI.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice from The Jackson Laboratory (Bar Harbor, ME) aged 6–8 weeks on arrival were housed four per cage in a temperature-controlled (21–22°C) vivarium under a 12-h reverse light/dark cycle (lights off at 0800 hours). The use of reversed light/dark cycles allowed for behavioral testing during the animals' awake phase. Food and water were available *ad libitum*. Animals were acclimatized for approximately 1 week after arrival prior to behavioral testing and maintained in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved facilities that meet all federal and state guidelines. Procedures were approved by the University of California San Diego institutional animal care and use committee. Principles of laboratory animal care were followed as well as specific laws of the USA.

Drugs

Drugs used were as follows: (±)-*N*-allylnormetazocine hydrochloride, (+)-*N*-allylnormetazocine hydrochloride, (–)-*N*-allylnormetazocine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA); 4-methoxy-3-(2-phenylethoxy)-*N,N*-dipropylbenzeneethanamine hydrochloride (NE-100), *trans*-(–)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide hydrochloride ((–)-U-50,488H; Tocris, Minneapolis, MO, USA); and nor-binaltorphimine dihydrochloride (nor-BNI; Abcam Biochemicals, Cambridge, MA, USA). Drug doses are expressed as the salt form. All drugs were dissolved in sterile water. NE-100 was administered by the intraperitoneal route; all other drugs were administered subcutaneously. The injection volume was 5 ml/kg.

Apparatus

Eight startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA) were used to measure startle reactivity (Mansbach et al., 1988). The startle test chambers consisted of a sound-attenuated, lighted, and ventilated enclosure holding a clear nonrestrictive cylindrical Plexiglas stabilimeter, 5 cm in diameter. A high-frequency loudspeaker mounted 33 cm above the Plexiglas cylinder produced all acoustic stimuli. The peak and average amplitudes of the startle response were detected by a piezoelectric accelerometer. At the onset of the startling stimulus, 65 1-ms readings were recorded, and the average amplitude was used to determine the rat startle response. A dynamic calibration system was used to ensure comparable stabilimeter sensitivity across test chambers, and sound levels were measured using the dB(A) scale, as described previously (Mansbach et al., 1988).

Acoustic startle sessions

Acoustic startle test sessions consisted of startle trials (pulse-alone) and prepulse trials (prepulse + pulse). The pulse-alone trial consisted of a 40-ms 120-dB pulse of broadband white noise. Prepulse + pulse trials consisted of a 20-ms acoustic prepulse, an 80-ms delay, and then a 40-ms 120-dB startle pulse (100 ms onset–onset). There was an average of 15 s (range = 9–21 s) between trials. During each inter-trial interval, the movements of the animals were recorded once to measure responding when no stimulus was present (data not

shown). Each startle session began with a 5-min acclimation period to a 65-dB broadband noise that was present continuously throughout the session. One week after arrival, animals were tested in a brief baseline startle/PPI session to create treatment groups matched for levels of startle and PPI. The startle test session contained 12 pulse-alone trials and 30 prepulse + pulse trials (ten prepulses each of 68, 71, and 77 dB) presented in a pseudo-randomized order. Five pulse-alone trials were presented at the beginning and the end of the test session but were not used in the calculation of PPI values.

Experimental design

Animals were placed in the startle chambers 120 min after treatment with nor-BNI, 25 min after treatment with NE-100, and 15 min after treatment with NANM or (–)-U-50488. In experiment 1, mice (n = 14–15, 57 total) were treated with vehicle, 3, 10, or 30 mg/kg (±)-NANM. In experiment 2, mice (n = 11–12, 46 total) were treated with vehicle, 3, 10, or 30 mg/kg (+)-NANM. In experiment 3, mice (n = 11–13, 48 total) were treated with vehicle, 3, 10, or 30 mg/kg (–)-NANM. In experiment 4, mice (n = 10–11, 42 total) were treated with NE-100 (vehicle or 1 mg/kg) 10 min before administration of (±)-NANM (vehicle or 10 mg/kg). A 1 mg/kg dose of NE-100 was chosen because previous studies have shown that σ_1 receptor-mediated responses can be blocked by 0.5–1 mg/kg NE-100 (Reddy et al., 1998; Hiramatsu and Hoshino, 2005; Hashimoto et al., 2007). In experiment 5, mice (n = 12, 48 total) were treated with nor-BNI (vehicle or 10 mg/kg) 120 min before administration of (±)-NANM (vehicle or 10 mg/kg). Administration of 10 mg/kg nor-BNI produces profound blockade of KOR-mediated responses in mice (Takemori et al., 1988; Zhang et al., 2005; Munro et al., 2012; Patkar et al., 2013) and reduces [³H]U69,593 binding to KOR by ~50% in brain homogenates *ex vivo* (Patkar et al., 2013). In experiment 6, mice (n = 12, 48 total) were treated with vehicle, 10, 20, or 40 mg/kg (–)-U-50488.

Data analysis

The amount of PPI was calculated as a percentage score for each prepulse + pulse trial type: %PPI = 100 – {[(startle response for prepulse + pulse trial) / (startle response for pulse-alone trial)] × 100}. Startle magnitude was calculated as the average response to all of the pulse-alone trials. PPI data were analyzed by two- or three-factor analysis of variance (ANOVA) with pretreatment and/or treatment as between-subjects factors and trial type (prepulse intensity) as a repeated measure. For experiments in which there was no significant interaction between drug and prepulse intensity, PPI data were collapsed across prepulse intensity and the average PPI was used as the main dependent measure. To compare effects on PPI across experiments, average PPI values from each mouse were normalized relative to the mean of the vehicle control group; the normalized PPI data were analyzed by two-way ANOVA. Startle magnitude data were analyzed by one- or two-factor (pretreatment and/or treatment) ANOVA. Post-hoc analyses were carried out using Tukey's test. The alpha level was set at 0.05.

RESULTS

Effect of (±)-NANM

Similar to our findings in rats (Halberstadt et al., 2016), (±)-NANM significantly reduced PPI in mice ($F(3,53) = 14.58$, $p < 0.0001$; Fig. 1A). 10 mg/kg (±)-NANM disrupted PPI at all three prepulse intensities ($p < 0.05$, 0.01, Tukey's test), whereas 30 mg/kg (±)-NANM significantly decreased PPI only at the 71- and 77-dB prepulse intensities (6 and 12 dB over background; $p < 0.01$, Tukey's test). (±)-NANM had a main effect on startle amplitude ($F(3,53) = 5.79$, $p < 0.002$), although this was not confirmed by post hoc analysis. Large changes in startle magnitude can potentially impair measurement of PPI due to floor or ceiling effects. To verify that the effect on PPI is independent of changes in startle magnitude, we examined the effect of (±)-NANM on PPI in subgroups of mice ($n = 10$ –14/group) that were closely matched for startle magnitude (mean $\pm$ SEM = 68.0 ± 6.5 , 69.3 ± 8.5 , 53.5 ± 4.2 , and 50.2 ± 8.6). In those subgroups, (±)-NANM reduced PPI ($F(3,43) = 8.98$, $p = 0.0001$), even though it had absolutely no effect on startle magnitude ($F(3,43) = 2.03$, *NS*).

Effect of (+)-NANM

(+)-NANM altered PPI with an inverted U-shaped dose-response function (Drug effect: $F(4,42) = 5.26$, $p < 0.004$; Fig. 1B). PPI was significantly reduced by 10 mg/kg (+)-NANM at the 68- and 71-dB prepulse intensities ($p < 0.05$, 0.01, Tukey's test), whereas lower (3 mg/kg) and higher (30 mg/kg) doses had no effect. As was found with the racemic compound (see above), there was a main effect of (+)-NANM on startle magnitude ($F(3,42) = 4.02$, $p < 0.02$; Table 1). Importantly, pairwise comparisons revealed that 10 mg/kg (+)-NANM did not significantly reduce startle responding, indicating that the effect on PPI was not due to floor effects on startle. Moreover, PPI was still significantly reduced by (+)-NANM ($F(3,37) = 5.08$, $p < 0.005$) in a subset of the mice ($n = 41$) with overlapping startle values ($F(3,37) = 1.61$, *NS*).

Effect of (–)-NANM

In contrast to the effect of (±)-NANM and its (+)-stereoisomer, (–)-NANM did not significantly alter PPI ($F(3,44) = 2.06$, *NS*; Fig. 1C) or startle responding ($F(3,44) = 1.02$, *NS*).

Comparison of the effects of (±)-NANM, (–)-NANM, and (+)-NANM on PPI

To compare the effects of (±)-NANM and its two stereoisomers on PPI, average PPI values were normalized to vehicle PPI levels for each experiment. The normalization procedure confirmed that PPI was reduced by (±)-NANM ($F(3,53) = 14.59$, $p < 0.0001$) and (+)-NANM ($F(3,43) = 4.80$, $p < 0.006$), but not by (–)-NANM ($F(3,44) = 2.07$, *NS*). Comparison of the effects of the three compounds by two-way ANOVA showed there was a main effect of dose ($F(2,106) = 13.98$, $p < 0.0001$) and revealed an interaction between dose and drug that approached significance ($F(4,106) = 2.36$, $p < 0.06$). Specific comparisons confirmed that 10 mg/kg (+)-NANM was more effective than 10 mg/kg (–)-NANM at disrupting PPI ($p < 0.05$,

Tukey's test; Figure 2). The maximal reduction of normalized PPI was 60.9% for 10 mg/kg (+)-NANM, 50.7% for 30 mg/kg (±)-NANM, and 30.0% for 30 mg/kg (-)-NANM.

Effect of σ_1 blockade with NE-100 on the response to (±)-NANM

As expected, 10 mg/kg (±)-NANM significantly reduced PPI ($F(1,38) = 40.51, p < 0.0001$). There was, however, no interaction between pretreatment and treatment, and 1 mg/kg NE-100 did not attenuate the PPI disruption produced by (±)-NANM (Fig. 3). NE-100 alone had no effect on PPI. There was no effect of pretreatment or treatment on startle magnitude, nor was there an interaction between pretreatment and treatment for that measure (Table 1).

Effect of KOR blockade with nor-BNI on the response to (±)-NANM

Nor-BNI did not block the effect of (±)-NANM on PPI (pretreatment $\times$ treatment: $F(1,45) = 0.02, NS$; Fig. 4). Treatment with (±)-NANM significantly reduced PPI ($F(1,44) = 12.59, p = 0.0009$), but pretreatment with nor-BNI did not influence PPI. There was a main effect of (±)-NANM on startle magnitude ($F(1,44) = 7.21, p < 0.02$), and a trend toward an effect of nor-BNI ($F(1,44) = 3.15, p < 0.09$), but there was no pretreatment $\times$ treatment interaction.

Effect of the selective KOR agonist (-)-U-50,488H

As shown in Figure 5, treatment with the selective KOR agonist (-)-U-50,488H did not alter PPI in mice (Main effect: $F(3,44) = 1.67, NS$). The magnitude of the startle response was significantly reduced ($p < 0.01$, Tukey's test) by 10, 20, and 40 mg/kg (-)-U-50,488H (Main effect: $F(3,44) = 8.08, p = 0.0002$; Table 1).

DISCUSSION

The present investigation demonstrates that the psychotomimetic opioid NANM produces a dose-dependent reduction of PPI, a measure of sensorimotor gating, in mice. The effect of NANM on PPI was stereospecific; (±)-NANM and (+)-NANM produced a significant disruption of PPI, whereas (-)-NANM did not alter PPI at doses up to 30 mg/kg. It is unlikely that KOR or σ_1 receptors play a significant role in mediating the PPI disruption produced by (±)-NANM because nor-BNI and NE-100 failed to block the response. In addition, tests with (-)-U-50,488 confirmed that KOR activation does not alter PPI in mice. These results indicate that the binding of NANM to the PCP receptor may be responsible for the effect on PPI.

The effect of NANM on PPI is probably not mediated by KOR receptors. We observed that PPI is disrupted by (+)-NANM but not by (-)-NANM. Because the levo isomer of NANM is more potent than the dextro isomer at KOR and MOR (Tang and Code, 1983; Tam, 1985; Carroll et al., 1992), it is reasonable to conclude that opiate receptors are not responsible for the effect on PPI. This conclusion is supported by two additional findings. First, in mice, the effect of (±)-NANM on PPI was not blocked by nor-BNI. We have confirmed that the reduction of PPI produced by 5 mg/kg (±)-NANM in rats is not antagonized by pretreatment with nor-BNI (data not shown). There is the possibility that a higher dose of nor-BNI would have blocked the effects of NANM on PPI; however, because 10 mg/kg nor-BNI was previously shown to block the behavioral effects of selective KOR agonists in mice

(Takemori et al., 1988; Zhang et al., 2005; Munro et al., 2012; Patkar et al., 2013), this dose was likely high enough to block KOR effects of NANM in the current study. Second, the selective KOR agonist (–)-U-50,488H had no effect on PPI in mice. Although it was previously reported that (–)-U-50,488H reduces PPI in Sprague-Dawley rats (Bortolato et al., 2005), another group observed that U-50,488H and salvinorin A have absolutely no effect on PPI in that rat strain (Tejeda et al., 2010). We have now verified that KOR activation does not alter sensorimotor gating in mice.

(+)-NANM has high affinity for σ_1 receptors (Tam et al., 1985; Largent et al., 1986; Carroll et al., 1992), and therefore the stereoselectivity of the effect of NANM on PPI is consistent with a role for σ_1 receptor activation. However, the selective σ_1 antagonist NE-100 failed to block the effect of NANM on PPI. Previous studies have shown that NE-100 antagonizes σ_1 receptor-mediated responses when administered to mice at 0.5–1 mg/kg (Reddy et al., 1998; Hiramatsu and Hoshino, 2005; Hashimoto et al., 2007). Although we cannot discount the possibility that a higher dose of NE-100 would have blocked the response to NANM, it is important to note that the σ_1 agonist (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP) did not significantly alter PPI when tested in mice (Curzon and Decker, 1998). Likewise, the selective σ_1 ligands NPC 16377 and MS-377 had no effect on PPI in rats (Clissold et al., 1993; Yamada et al., 2000). Given those findings, it does not seem likely that NANM would reduce sensorimotor gating by activating the σ_1 receptor.

The dose-dependence of NANM binding to σ_1 sites is another factor indicating that NANM does not disrupt PPI via effects on σ_1 . According to *ex vivo* studies, (+)-NANM and (–)-NANM (both administered IP) displace the binding of (+)-[³H]NANM to σ_1 sites in mouse brain homogenates with ED₅₀ values of 0.03 mg/kg and 1.8 mg/kg, respectively (Ferris et al., 1988). By contrast, in the present investigation, (+)-NANM had no effect on PPI when tested at 3 mg/kg (see Fig. 1). It is therefore unlikely that interactions with σ_1 are responsible for the effects of NANM on PPI in mice. Alternatively, the potency of NANM interactions with NMDA-R *in vivo* is much more compatible with the dose-dependence of the effects on PPI; for (+)-, (±)-, and (–)-NANM, the ED₅₀ values for displacement of [³H]TCP binding to the PCP site in mouse hippocampus are 8.22, 11.5, and 29.7 mg/kg, respectively (Maurice and Vignon, 1990).

The conclusion that the PCP receptor is likely responsible for the NANM-induced reduction of PPI is consistent with a large body of evidence showing NANM influences behavior in rodents through a PCP-like mechanism. It is well established that noncompetitive and competitive NMDA-R antagonists can disrupt PPI in mice and rats (Mansbach and Geyer, 1989, 1991; Curzon and Decker, 1998; Bakashi et al., 1999; Halberstadt et al., 2016). (±)-NANM is a NMDA-R antagonist *in vitro* (Wong et al., 1986) and both (+)-NANM and (–)-NANM protect mice from seizures induced by administration of NMDA (Singh et al., 1990). NANM produces complete substitution in laboratory animals trained to discriminate PCP or MK-801 from saline (Brady et al., 1982; Shannon et al., 1982; McMillan et al., 1988; Sanger and Zivkovic, 1989; Geter et al., 1997). Likewise, the discriminative stimulus effects of (+)-NANM and (±)-NANM generalize completely to PCP and MK-801 but not to a variety of ligands acting at KOR or σ_1 receptors (Shearman and Herz, 1982; Balster, 1989; Singh et al., 1990; Holtzman, 1993). NANM and PCP produce similar behavioral effects in rodents,

including locomotor hyperactivity, ataxia, and stereotypy (Greenberg and Segal, 1986). Cross-tolerance also occurs between the behavioral effects of (+)-NANM and PCP (Stafford et al., 1983; Lu et al., 1992).

The stereospecificity of the effect of NANM on PPI is another factor that is consistent with a mechanism involving the PCP receptor. As noted above in the introduction, the PCP binding site within NMDA-R channel displays some degree of selectivity for (+)-NANM (Largent et al., 1986; Carroll et al., 1992). (+)-NANM has consistently been shown to be more potent and effective than (–)-NANM as a PCP-mimetic (Brady et al., 1982; Shannon et al., 1982; Iwamoto, 1986; McMillan et al., 1988; Sanger and Zivkovic, 1989). The withdrawal syndrome induced by repeated administration of PCP to rats is completely suppressed by (±)-NANM and (+)-NANM but not by (–)-NANM (Stafford et al., 1983). Furthermore, Slifer and Dykstra (1987) have demonstrated that it is possible to train rats to discriminate between PCP and the KOR agonist ethylketocyclazocine (EKC) in a two-lever operant task. They found that (+)-NANM produced PCP-like responding whereas (–)-NANM produced EKC-appropriate responding. Therefore, the fact that PPI was significantly disrupted by (±)-NANM and (+)-NANM but not by (–)-NANM supports our contention that the effect is mediated by the PCP receptor.

One issue that remains unresolved is the degree to which the present findings are relevant to humans. In contrast to the effect of NANM on PPI in mice, most of the available evidence indicates that benzomorphans induce hallucinations by activating KOR. The hallucinogenic effects of pentazocine are attributable to the (–)-stereoisomer (Forrest et al., 1969), which has high affinity for the κ_1 KOR subtype (Chien and Pasternak, 1995) and low affinity for the PCP binding site (Carroll et al., 1992). Similarly, naloxone blocks the dysphoria induced by cyclazocine (Jasinski et al., 1968) and the hallucinogenic effects of (±)-MR 2033 and (–)-MR 2033 (Pfeiffer et al., 1986). Therefore, it does not appear likely that the PCP receptor is responsible for the hallucinogenic effects of NANM in humans. One possible explanation is that there may be differences in the selectivity of NANM for PCP sites and KOR in rodents vs. humans, and therefore interactions with KOR may make a greater contribution to the effects of NANM in humans compared with rodents. Nevertheless, the fact that NANM disrupts PPI in rats and mice is consistent with reports that it has hallucinogenic effects in humans. PPI is a cross-species behavioral paradigm, and there is often homology between human and animal measures of PPI. LSD decreases PPI in rats (Halberstadt and Geyer, 2010) and it was recently confirmed that LSD disrupts PPI in human volunteers (Yasmin et al., 2015).

Despite the fact that it is still unclear whether the hallucinogenic effects of NANM are mediated by the PCP binding site or by KOR, it is clear that the response is not mediated by the σ_1 receptor. Indeed, there is a dearth of evidence directly linking the σ_1 receptor to psychotomimetic effects. The hypothesis that the σ_1 receptor is involved in hallucinogenesis was based primarily on the observation that certain ligands that target σ_1 induce psychotomimetic effects, whereas other σ_1 ligands act as antipsychotics and ameliorate psychosis (Su, 1982; Tam and Cook, 1984). It is now recognized that the hallucinogenic effects of drugs such as PCP and benzomorphan opioids are mediated by receptors other than σ_1 (Musacchio, 1990). In addition, many ligands that bind to σ_1 do not induce

hallucinations; examples include fluvoxamine (Narita et al., 1996) and neurosteroids such as progesterone (Su et al., 1988; Hayashi and Su, 2001; Su and Hayashi, 2003). Nalorphine, the prototypical psychotomimetic opiate, does not actually bind to σ_1 , and induces hallucinogenic effects through a naloxone-sensitive mechanism (Jasinski et al., 1968). Although many aspects of σ_1 signaling and function are unresolved, the present findings provide additional support for the conclusion that σ_1 is not responsible for generating hallucinogenic effects.

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Highlights

- NANM disrupts prepulse inhibition of startle (PPI) in C57BL/6J mice
- The effect of NANM on PPI is stereoselective ((+)-NANM > (±)-NANM > (-)-NANM)
- The PPI disruption induced by NANM is not antagonized by blockade of σ_1 receptors
- NANM-induced PPI disruption is not blocked by a kappa opioid receptor antagonist
- It is likely that NANM disrupts PPI by blocking the NMDA receptor

Figure 1.

Effects of (A) (±)-N-allylnormetazocine, (B) (+)-N-allylnormetazocine, and (C) (-)-N-allylnormetazocine on prepulse inhibition (PPI). *Left panels* Effects on PPI are shown for the three prepulse intensities (68, 71, and 78 dB). *Right panels* Effects on PPI averaged across the three prepulse intensities. Values represent mean ± SEM for each group. Drug doses are in milligram per kilogram. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, significantly different from vehicle control.

Figure 2.

Comparison of the effects of (±)-*N*-allylnormetazocine, (+)-*N*-allylnormetazocine, and (–)-*N*-allylnormetazocine on normalized prepulse inhibition (PPI) values in mice. Effects on PPI were averaged across the three prepulse intensities and then normalized to baseline PPI levels in vehicle control groups. Values represent mean ± SEM for each group. Drug doses are in milligram per kilogram. * $p < 0.05$, significantly different from (–)-*N*-allylnormetazocine.

Figure 3.

Pretreatment with the selective σ_1 receptor antagonist NE-100 does not block the disruption of PPI induced by (±)-*N*-allylnormetazocine (NANM) in mice. Effects on PPI were averaged across the three prepulse intensities. Values represent mean $\pm$ SEM for each group.

** $p < 0.01$, significantly different from vehicle control.

Figure 4.

Pretreatment with the selective κ opioid receptor antagonist nor-binaltorphimine (nor-BNI) does not block the disruption of PPI induced by (±)-*N*-allylnormetazocine (NANM) in mice. Effects on PPI were averaged across the three prepulse intensities. Values represent mean $\pm$ SEM for each group.

Figure 5.

Effects of (-)-U-50,488H on prepulse inhibition (PPI) in mice. Values represent mean $\pm$ SEM for each group. Drug doses are in milligram per kilogram. Values represent mean $\pm$ SEM for each group.

Table 1

Effect of drug treatment on startle magnitude.

Pretreatment (doses in mg/kg)	Treatment (doses in mg/kg)	Startle Magnitude ($\pm$ SEM)
	Vehicle	74.0 $\pm$ 8.5
	($\pm$)-NANM 3	97.9 $\pm$ 13.4
	($\pm$)-NANM 10	53.5 $\pm$ 4.2
	($\pm$)-NANM 30	48.8 $\pm$ 8.4
	Vehicle	176.4.0 $\pm$ 20.5
	(+)-NANM 3	179.2 $\pm$ 14.7
	(+)-NANM 10	135.4 $\pm$ 20.5
	(+)-NANM 30	103.6 $\pm$ 17.2 *
	Vehicle	164.2.0 $\pm$ 33.8
	(-)-NANM 3	163.0 $\pm$ 19.0
	(-)-NANM 10	129.8 $\pm$ 18.6
	(-)-NANM 30	115.9 $\pm$ 23.8
Vehicle	Vehicle	119.9.0 $\pm$ 12.8
Vehicle	($\pm$)-NANM 10	125.8 $\pm$ 21.2
NE-100 1	Vehicle	141.1 $\pm$ 31.0
NE-100 1	($\pm$)-NANM 10	88.4 $\pm$ 9.0
Vehicle	Vehicle	172.3 $\pm$ 19.4
Vehicle	($\pm$)-NANM 10	114.5 $\pm$ 20.7
Nor-BNI 10	Vehicle	130.4 $\pm$ 15.0
Nor-BNI 10	($\pm$)-NANM 10	94.6 $\pm$ 13.7 *
	Vehicle	87.0 $\pm$ 9.8
	(-)-U-50488 10	41.6 $\pm$ 7.3
	(-)-U-50488 20	29.8 $\pm$ 8.2
	(-)-U-50488 40	39.7 $\pm$ 10.2

*
 $p < 0.05$ versus respective vehicle or vehicle-vehicle control.