Pharmacology, Biochemistry and Behavior 148 (2016) 69–75

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

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

Adam L. Halberstadt [a,b,*], James Hyun [a], Michael A. Ruderman [a], Susan B. Powell [a,b]

[a] Department of Psychiatry, University of California San Diego, La Jolla, CA, United States
[b] Research Service, VA San Diego Healthcare System, San Diego, CA, United States

ARTICLE INFO

Article history:
Received 16 January 2016
Received in revised form 12 May 2016
Accepted 23 May 2016
Available online 26 May 2016

Keywords:
Psychotomimetic
Sigma receptor
Prepulse inhibition
Hallucinogen
Dissociative
Kappa receptor
Mice

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.

1. 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 Beeeher, 1954; Keats and Telford, 1957), suggesting that it may be possible to separate the analgesic and addictive properties of opioids. 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 Moyer, 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. According to their hypothesis, 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 C_8-C_7 isomerase (Hanner et al., 1996; Seth et al., 1997; Mei and Pasternak, 2001).

* Corresponding author at: University of California San Diego, Department of Psychiatry, 9500 Gilman Drive, La Jolla, CA 92093-0804, United States.
E-mail address: ahalbers@ucsd.edu (A.L. Halberstadt).

http://dx.doi.org/10.1016/j.pbb.2016.05.009
0091-3057/

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 μM (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 ~ 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; Balster, 1989; Holtzman, 1993).

Uncompetitive NMDA-R antagonists, including PCP, ketamine, methoxetamine, diphenidine, and dizocilpine (MK-801), are known to disrupt prepulse inhibition of startle (PPI) in rodents (Mansbach and Geyer, 1989, 1991; Wiley et al., 2003; Halberstadt et al., 2016; Wallach 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.

2. Materials and methods

2.1. 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 h). 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.

2.2. 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 (IP) route; all other drugs were administered subcutaneously. The injection volume was 5 mL/kg.

2.3. 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).

2.4. 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