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Invited review

Phencyclidine-like *in vivo* effects of methoxetamine in mice and rats

Michael D. Berquist [a], William S. Hyatt [a], Jonathan Bauer-Erickson [b], Brenda M. Gannon [a], Andrew P. Norwood [c], William E. Fantegrossi [a, c, *]

[a] Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA
[b] Program in Biochemistry and Molecular Biology, Hendrix College, Conway, AR, USA
[c] Interdisciplinary Biomedical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR, USA

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ABSTRACT

Methoxetamine (MXE) is a novel drug of abuse that is structurally similar to phencyclidine (PCP). In the present study, rats were trained to discriminate PCP from saline and substitution tests were performed with arylcyclohexylamines PCP, eticyclidine (PCE), tenocyclidine (TCP), and MXE. PCP and PCE engendered PCP-lever selection in all subjects, whereas MXE and TCP produced PCP-lever selection in animals that did not display behavioral disruption. Last, the substituted tryptamine dipropyltryptamine (DPT) produced moderate PCP-lever selection and elicited behavioral disruption in all subjects at the highest dose tested. Immediately following the final substitution test in the drug discrimination experiment, the same rats and a separate group of experimentally-naïve rats were implanted with osmotic mini-pumps delivering continuous PCP infusions for 11 days. Consistent with PCP withdrawal, disruption of food-maintained operant responding was observed when the pumps were removed, but cumulative MXE administration dose-dependently reversed this effect. A third group of rats self-administered several unit doses of PCP and MXE. Results of the self-administration tests revealed that MXE was a less effective reinforcer than PCP. Lastly, mice were implanted with radiotelemetry probes to simultaneously monitor thermoregulatory and locomotor responses following injections of PCP, PCE, or MXE. All three arylcyclohexylamines elicited dose-dependent hypothermic effects, but only PCP produced increases in locomotor activity. Together, these findings indicate that MXE elicits PCP-like interoceptive effects, but reduced reinforcing and locomotor stimulant effects *in vivo*.

1. Introduction

Methoxetamine (MXE; 2-(3-methoxyphenyl)-2-(ethylamino)-cyclohexanone) is a novel psychoactive substance first identified in the United Kingdom in September 2010 (EMCDDA, 2013), and its development as a designer drug of abuse is well-documented (Morris, 2011; Morris and Wallach, 2014). Its structure places it in the arylcyclohexylamine chemical class, alongside related compounds PCP and ketamine. Similar to the recent emergence of other designer drugs of abuse, such as the synthetic cathinones, opioids, and cannabinoids, MXE and related arylcyclohexylamines are widely available through internet distributors and local "head shops" (Wood et al., 2012; Corazza et al., 2012; De Paoli et al., 2013). Common motivations for using MXE include ease of access and affordability, a belief that it possesses relatively low hepatoxicity and urotoxicity, and its ketamine- and PCP-like psychotomimetic and anesthetic effects (Hofer et al., 2011; Kjellgren and Jonsson, 2013; Winstock et al., 2016). For review of the history and recreational use of novel arylcyclohexamines, see Morris and Wallach (2014).

MXE and related compounds eticyclidine (PCE; *N*-ethyl-1-phenylcyclohexylamine) and tenocyclidine (TCP; 1-(1-(2-thienyl) cyclohexyl)piperidine) possess an arylcyclohexylamine backbone similar to PCP (Fig. 1). In addition to structural similarities between MXE and PCP, MXE is also a high affinity ligand for the PCP-site on the glutamate *N*-methyl-D-aspartate (NMDA) receptor with an affinity value (pK_i = 6.59 nM) comparable to that of PCP itself (pK_i = 7.23 nM (Roth et al., 2013). Moreover, MXE is a high affinity reuptake inhibitor at monoamine transporters (especially the serotonin transporter [SERT]) as measured *in vitro* (Hondebrink et al., 2017), stimulates the firing rate of dopamine neurons in the ventral tegmental area and increases extracellular dopamine levels in the

* Corresponding author. UAMS Department of Pharmacology and Toxicology, 4301 West Markham, Slot #638, Little Rock, AR 72207, USA.
E-mail address: WEFantegrossi@uams.edu (W.E. Fantegrossi).

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nucleus accumbens of awake, freely-moving Sprague-Dawley rats (Mutti et al., 2016), and increases extracellular dopamine and serotonin concentrations in the medial prefrontal cortex of awake, freely-moving ddY mice (Fuchigami et al., 2015). Similar to MXE, PCP increases extracellular concentrations of dopamine, serotonin, and norepinephrine in the medial prefrontal cortex of Sprague-Dawley and Wistar rats (Quarta and Large, 2011; Etou et al., 1998).

In addition to recent characterizations of MXE's neuropharmacological effects, previous studies have reported that MXE produces abuse-related behavioral effects that are similar to those of ketamine. For example, rats initially trained to self-administer ketamine also self-administered MXE during substitution trials, although the reinforcing effects of MXE were less robust than those of ketamine (Botanas et al., 2015; Mutti et al., 2016). However, MXE produced a conditioned place preference comparable to that observed with ketamine (Botanas et al., 2015), and substituted for 7.5 mg/kg ketamine in a drug discrimination procedure (Chiamulera et al., 2016). Nevertheless, there is currently only a single study that has directly compared the *in vivo* effects of MXE to PCP (Halberstadt et al., 2016). Thus, several rodent procedures were used in the present study in an effort to further characterize the behavioral and thermoregulatory effects of MXE and related arylcyclohexylamines. A drug discrimination procedure was used to characterize the interoceptive effects of MXE, PCE, and TCP in rats trained to discriminate PCP from saline. A rodent model of drug withdrawal was used to evaluate whether MXE would attenuate PCP-withdrawal-related reductions in an operant responding. The reinforcing effects of MXE were also investigated in rats using intravenous self-administration procedures. Finally, the locomotor and thermoregulatory effects of MXE, PCE, and PCP were compared in mice. Together, these experiments indicate that MXE has some PCP-like *in vivo* effects, which may support its abuse liability observed in human users.

2. Materials and methods

2.1. Drugs

All drugs were dissolved in 0.9% physiological saline. For mice, injections were administered intraperitoneally (IP) in a volume of 0.01 ml/g. For rats in drug discrimination and PCP withdrawal studies, injections were administered IP in a volume of 1.0 ml/kg. For rats in intravenous self-administration experiments, drug solutions were delivered intravenously (IV) by a single-speed infusion pump at a flow rate of 0.055 ml/s for a duration of 2 s through 3 French (0.036 inch) heparin-coated polyurethane round tip catheters. PCP, TCP, and PCE were obtained through the NIDA Drug Supply Program as hydrochloride salts. Because only limited quantities of TCP and PCE were available, the *in vivo* effects of these compounds were tested in mice only. Purified MXE HCl (molecular weight = 283.79) was authenticated via single proton nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy and gas chromatography–mass spectrometry, tested to be a 99.26± 0.20% pure via quantitative NMR, and provided as a generous gift by the Drug Enforcement Administration Special Testing & Research Laboratory Reference Materials Program (Dulles, VA). All other laboratory reagents and supplies were obtained from standard commercial sources.

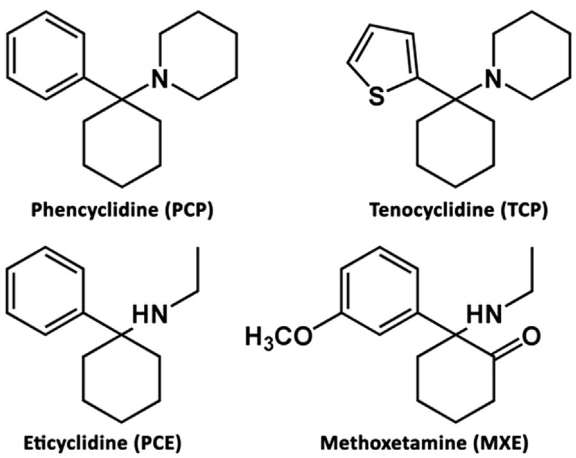

Fig. 1. Chemical structures of the drugs used in these studies: PCP, TCP, PCE, and MXE.

2.2. Animals

Fourteen male Sprague-Dawley rats (Harlan Sprague-Dawley) weighing 220–240 g on delivery were housed two animals per cage (23.75 × 45.40 × 17.78 cm^3), and fourteen male NIH Swiss mice (Harlan Sprague-Dawley) weighing 20–25 g on delivery were housed three animals per cage (15.24 × 25.40 × 12.70 cm). All animals were housed in a temperature- and humidity-controlled vivarium in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility. Room conditions were maintained at 22 ± 2 °C and 45–50% humidity, with lights set to a 12 h/12 h light/dark cycle. During experimental testing, rats were maintained at 340–360 g by food presentations earned during the experiments (45 mg chocolate flavor Dustless Precision Pellets no. F0299, Bio-Serv, Frenchtown, NJ, USA) and supplemental feeding following experimental sessions (Lab Diet rodent chow Laboratory Rodent Diet no. 5001, PMI Feeds, St Louis, MO, USA). Mice were free-fed throughout testing (Lab Diet rodent chow Laboratory Rodent Diet no. 5001, PMI Feeds, St Louis, MO, USA). For all animal subjects, water was available *ad libitum* in the home cages throughout testing, and all subjects were drug naïve (with the exception of surgical anesthetics, where applicable) before testing. Aside from the 24 h radiotelemetry and locomotor testing sessions (see below), all experiments were conducted during the animals' light cycle. All studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals (2013). All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

2.3. Drug discrimination

Rats ($n = 5$) were trained in two-lever operant chambers (Model ENV-008-VP; Med Associates) that were enclosed in light- and sound-attenuating cabinets (Model G7211, Gerbrands) equipped with an exhaust fan for air circulation and masking of ambient laboratory noise. The operant chambers were equipped with stimulus lights over the retractable levers and a house light located near the chamber ceiling. A pellet dispenser delivered 45 mg chocolate flavor pellets (Dustless Precision Pellets no. F0299, Bio-Serv, Frenchtown, NJ) into a food magazine centered between the levers. All programming and recording were controlled through a MED Associates interface with MED-PC software (V4, Fairfax VT, USA) by a computer located in an adjacent room.

Lever press training: Responding on extended levers was initially reinforced under a fixed ratio 1 (FR1) schedule in daily sessions that lasted for 60 min or until 60 reinforcers were earned, whichever occurred first. During lever press training sessions, the house light and stimulus lights were illuminated to signal reinforcer availability. Every 20th reinforcer earned incremented the response requirement by 2, and the final FR value carried across

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