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Methoxetamine, a ketamine derivative, produced conditioned place preference and was self-administered by rats: Evidence of its abuse potential

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ABSTRACT

Methoxetamine (MXE) is an N-methyl-D-aspartate (NMDA) receptor antagonist that is chemically and pharmacologically similar to ketamine. Recently, there have been many reports regarding its use/misuse in humans which have resulted in serious or even fatal outcomes. Despite these reports, MXE is not controlled or regulated in many countries which may be partly due to the lack of scientific evidence regarding its abuse potential. Thus, in the present study we evaluated the abuse potential (rewarding and reinforcing effects) of MXE through the conditioned place preference (CPP) and self-administration (SA) tests in Sprague–Dawley rats. In addition, locomotor activity during the conditioning phase of the CPP was also analyzed. Ketamine was used as a reference drug. MXE (2.5 and 5 mg/kg) induced significant CPP in rats, an effect comparable to that of ketamine (5 mg/kg). Interestingly, MXE did not produce any locomotor alterations while ketamine decreased the locomotor activity of rats. In the SA test, rats showed modest self-administration of MXE (0.25, 0.5, 1.0 mg/kg/infusion), while ketamine (0.5 mg/kg/infusion) was robustly self-administered. These results demonstrate that MXE, similar to ketamine, has rewarding and reinforcing effects in rats. The present study strongly suggests that MXE has a potential for human abuse. In addition, the discrepant effects of MXE and ketamine on locomotor activity and rate of self-administration propose that the psychopharmacological effects of these drugs may diverge in some aspects. More importantly, this study advocates the careful monitoring and prompt regulation of MXE and its related substances.

1. Introduction

Methoxetamine (MXE) (2-(3-methoxyphenyl)-2-(ethylamino) cyclohexane) is a new, synthetic, psychoactive drug derived from ketamine (Corazza et al., 2012; Meyer et al., 2013). Similar to ketamine and phencyclidine, it is pharmacologically classified as an N-methyl-D-aspartate (NMDA) receptor antagonist (Corazza et al., 2013; Roth et al., 2013). Initially, MXE was designed in part to prevent the urotoxicity associated with ketamine and to be tested as an antidepressant (EMCDDA, 2014; Meyer et al., 2013). However, since its debut on the internet in 2010, it has become a popular recreational drug especially among adolescents (Morris and Wallach, 2014). Indeed, there has been an increase in the number of reports regarding the abuse of MXE in humans, which resulted in serious or even fatal outcomes (Zawilska, 2014). Accordingly, MXE was included in the list of new psychoactive substances of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), categorized as a ketamine derivative (EMCDDA, 2014). It is believed that MXE is being used as a ketamine substitute, owing to its ability to produce comparable hallucinogenic and dissociative effects (EMCDDA, 2014; Kjellgren and Jonsson, 2013).

Drug substitution is a common practice among drug users/abusers. An important factor behind this practice is drug availability (de la Pena et al., 2013; EMCDDA, 2009). Obtaining a regulated psychoactive drug can be arduous; thus, an addicted individual would seek for alternative ways to obtain their "high" (de la Peña et al., 2014). An emerging trend is the slight modification of the molecular structure of a controlled drug — making a "new" drug that retains/mimics the psychoactive properties of the original drug, but circumvents any existing drug law (Corazza et al., 2012; Fattore and Fratta, 2011). These drugs, aptly called "designer drugs" or "legal highs", are then sold on the internet or black markets (Morris and Wallach, 2014). This practice might also be the case for ketamine and its analog, MXE.

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Despite reports of human misuse, MXE is still not a controlled drug in many countries (EMCDDA, 2014). This may be related to the lack of supporting scientific evidence regarding the abuse potential of this drug. Abuse potential assessment is an integral part in the screening of psychoactive drugs. Thus, the goal of the present study was to characterize the abuse potential of MXE through animal models of drug addiction. Assessment of abuse potential in animals is advantageous because it bypasses the ethical, methodological, and economic constraints associated with human studies. Two of the widely used animal models of drug addiction were employed: the conditioned place preference (CPP) and the self-administration (SA) test. The CPP test evaluates the hedonic value (rewarding or aversive) of a drug, while the SA test measures the motivational/reinforcing effects of the drug (Shippenberg and Koob, 2002). Additionally, locomotor activity (during the conditioning phase of the CPP) was analyzed, based on the findings that euphorigenic effects activate the same neural processes as locomotor activation (Valjent et al., 2010). Ketamine was used as a reference drug.

2. Materials and methods

2.1. Subjects

Male, Sprague–Dawley rats (6 weeks) obtained from Hanlim Animal Laboratory (Korea) were the subjects of this study. The rats were housed in groups of 4 per cage (CPP) or individually (SA) in an animal room with controlled temperature (22 ± 2 °C) and humidity (55 ± 5%) under a 12/12-h light/dark cycle (0700 h–1900 h). The animals were acclimatized to the laboratory setting for five days, before the commencement of any experiments. Water and food were freely available, except during lever training and the SA sessions. All tests were performed in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985) and the Animal Care and Use of Guidelines of Sahmyook University, Korea.

2.2. Drugs

Methoxetamine in crystallized white powder was provided by the Laboratory of Medicinal Chemistry of Kyunghee University (Seoul, Korea). Ketamine was purchased from Bayer Animal Health Co. (Suwon, Korea). All drugs were diluted in normal saline (0.9% w/v of NaCl) and administered either intraperitoneally (CPP) or intravenously (SA). The dosages used in the present study were based on previous CPP and SA studies with NMDA receptor antagonists (de la Peña et al., 2012; De Luca and Badiani, 2011; Trujillo et al., 2011).

2.3. Conditioned place preference test

2.3.1. Apparatus

The CPP apparatus was a two-compartment, polyvinylchloride, boxes measuring 47 × 47 × 47 cm. Each compartment had unique visual and tactile cues: one section had black walls with smooth black floor while the other section had white painted dotted walls with rough black floor. A guillotine door separated the compartments during the conditioning phase. A software package (Ethovision, Noldus IT BV, Wageningen, Netherlands) was used to record animal behaviors.

2.3.2. Procedure

The CPP test was performed as previously described (de la Peña et al., 2012). Each test was composed of three phases: (1) habituation and preconditioning, (2) conditioning, and (3) post-conditioning. For the first two days, the rats were allowed to access both compartments for 15 min, once a day (habituation). On the third day, preconditioning followed where the time spent (seconds) on each compartment was measured (Ethovision), to determine the preferred and non-preferred compartment of each rat. Then, the guillotine door was closed in preparation for the conditioning phase. During this period, the rats were injected intraperitoneally with MXE (1.25, 2.5 or 5 mg/kg) or ketamine (5 mg/kg) and confined to their non-preferred compartment. On alternate days, they received saline and were placed on their preferred compartment. These treatments were repeated for three cycles (6 days). The control group received saline every day. Locomotor activity was also recorded during this phase. The post-conditioning followed where the guillotine door was opened and, as in the pre-conditioning phase, drug-free rats were allowed access to both compartments.

2.4. Self-administration test

2.4.1. Apparatus

SA tests were performed in standard operant chambers (Coulbourn Instruments, Allentown, Pennsylvania, USA) placed inside sound-attenuating boxes with ventilation fans, to further mask external noise. Each operant chamber has a food pellet dispenser, two 4.5 cm wide response levers (left and right), a stimulus light located 6 cm above the left lever, and a centrally positioned house light (2.5 W, 24 V) at the top of the chamber. A downward pressure (approximately 25 g) on the levers would result in a programmed consequence. Located beside the operant chamber was a motor-driven syringe pump that delivered solutions at a rate of 0.01 ml/s. Solutions flowed through Teflon tubing connected to a swivel, which was mounted on a counterbalanced arm at the top of the chamber that allowed free movement of the animals. The tubing was connected to the animal's intravenous catheter system. The Graphic State Notation software (Coulbourn Instruments) controlled experimental parameters and collected data.

2.4.2. Procedure

After acclimatization, food access was limited until the rats were reduced to 85% of their free-feeding body weight. Then, they were trained to press a lever (30 min/day for 3 days) for a contingent food pellet reward on a continuous schedule of reinforcement. Only rats that earned at least 80 pellets on the last session of training were prepared for surgery. Detailed description of the surgical and post-surgical procedures was outlined in our previous studies (de la Peña et al., 2012). After recovery, the rats were subjected to a two-hour daily SA session under a fixed-ratio (FR) 1 schedule for 7 days. During the SA sessions, both levers were present and a press on the left lever (active lever) would result in an infusion of 0.1 ml of MXE (0.25, 0.5, or 1.0 mg/kg/infusion), ketamine (0.5 mg/kg/infusion), or saline. Simultaneously, the house light was switched off, while the stimulus light was illuminated which remained lit for another 20 s after the end of the infusion (time-out period). Lever presses during time-out periods were recorded but did not have any corresponding effects. As a control for general activity, presses on the right lever (inactive lever) were recorded but not reinforced. A significant difference between active and inactive lever responses was considered to reflect self-administration. To prevent possible intoxication, the rats were only allowed 30 drug infusions per session, although lever presses were still recorded until the end of the session. A day before and on the last day of the SA test, catheters were injected with 0.1 ml of thiopental sodium (10 mg/kg) to assess catheter patency. The rats that did not lose muscle tone within 3–5 s were excluded from the experiment.

2.5. Data analysis

All data were presented as means and standard error of the mean (SEM). CPP results were expressed as the difference in time spent in the drug-paired compartment during the pre- and post-conditioning phases. One-way analysis of variance (ANOVA) was used to determine the variation between groups, followed by a Dunnett's posttest to compare each group to the control group. Locomotor activity was presented as the distance moved (cm) and movement duration (s) during the conditioning phase of CPP. Two-way ANOVA was employed to determine the effects of drug, day, or interaction between these factors, followed