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Research article

Abuse potential and dopaminergic effect of alkyl nitrites

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HIGHLIGHTS

- The abuse of alkyl nitrites is common among adolescents and young adults worldwide.
- Mice treated with alkyl nitrites showed greater drug-paired place preference.
- Isobutyl nitrite induced greater dopamine release from striatal synaptosomes.
- Alkyl nitrites could lead to dependence and dopaminergic effects.
- This provides evidence for controlling alkyl nitrites as psychoactive substances.

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ABSTRACT

The abuse of alkyl nitrites is common among adolescents and young adults worldwide. However, the information regarding the effects of alkyl nitrites on the central nervous system and the associated psychological abuse potential is scarce. The abuse potential of 3 representative alkyl nitrites – isobutyl nitrite, isoamyl nitrite, and butyl nitrite – was evaluated in mice using conditioned place preference tests with an unbiased method. The dopamine levels released by synaptosomes extracted from the striatal region were measured using high performance liquid chromatography. Mice treated with the test substances (50 mg/kg, i.p.) exhibited a significantly increased drug-paired place preference. Moreover, greater levels of dopamine were released by striatal region synaptosomes in response to isobutyl nitrite treatment in mice. Thus, our findings suggest that alkyl nitrites could lead to psychological dependence and dopaminergic effects. Furthermore, these results provide scientific evidence to support the regulation of alkyl nitrites as psychoactive substances in the future.

1. Introduction

Alkyl nitrite – generally known as popper – is a flammable liquid chemical that is commonly sold as room air fresheners or deodorizers in some clubs, headshops, and online [1]. In addition, poppers are used for recreational purposes as they quickly induce euphoria and sexual arousal [2,3]. Amyl nitrite has also been previously used to manage medical conditions such as angina pectoris and cyanide poisoning [4], as it results in blood vessel dilation. However, nausea, disorientation, and maculopathy were found to be the adverse effects of this medication, and its use in the medical setting was consequently discontinued [5,6]. Nevertheless, certain alkyl nitrites are still being marketed as "rush," "poppers," "snappers," and other names, particularly for aphrodisiac purposes. This constitutes a major problem as they are easily available to young people [7], and their use is associated with attempted suicide [8].

The decision to control alkyl nitrite use as a psychoactive substance has been controversial in many countries. In France, alkyl nitrites were controlled under Decree No. 2007-1636 on November 20, 2007, "on products containing aliphatic alkyl nitrites, cyclic, heterocyclic or their isomers for their isomers for the consumer and not benefiting from a marketing authorization," which was abolished in 2009 due to trade-related problems. Nevertheless, certain articles have emphasized on the need to control these substances [9].

Abbreviations: CPP, conditioned place preference; CNS, central nervous system; HPLC, high performance liquid chromatography; HRP, horseradish peroxidase; ECD, electrochemical detector.

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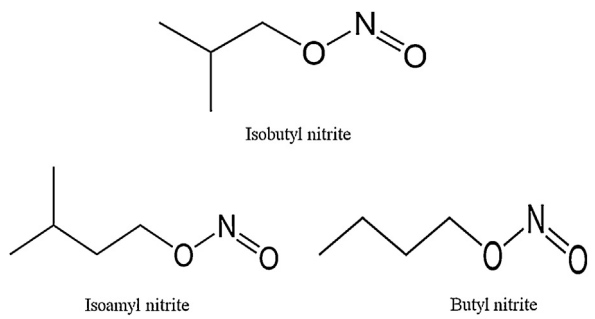

Fig. 1. Chemical structures of isobutyl nitrite, isoamyl nitrite, and butyl nitrite.

In Japan, 6 alkyl nitrites (isobutyl nitrite, isopropyl nitrite, isoamyl nitrite, tertiarybutyl nitrite, cyclohexyl nitrite, and butyl nitrite) are controlled as "designated substances" under the "Pharmaceutical Affairs Law." In some countries such as Korea, it is vital to first elucidate the action on the central nervous system (CNS) and the dependence potential of a drug before it can be controlled as a psychoactive substance. Thus, the findings of the present study could serve as a legal basis for controlling the tested substance as a psychoactive substance, under the "Act on Narcotics Control".

Several reports have provided evidence of the toxicity induced by alkyl nitrite inhalation. In particular, ophthalmological toxicity is one of the well-known adverse effects. Some reports have described cases of alkyl nitrite-induced maculopathy [10–12] or visual loss [13]. Other toxic effects such as immunotoxicity [14], hepatotoxicity [15], or cardiovascular toxicity [16] have also been reported in animals. Although the toxic effects of alkyl nitrites have been reported in the literature, the information on the abuse potential is scarce and is only briefly described in certain anecdotal reports [9].

Physical or psychological dependence is the state of tolerance to and withdrawal from a drug after a prolonged period of use [17]. Several experimental approaches have involved behavioral pharmacological techniques. One such method is the conditioned place preference (CPP) test, which specifically evaluates the rewarding effects of unknown substances [18–20]. Moreover, it is well known that substance dependence is related to the dopaminergic pathway of the CNS. Hence, the measurement of dopamine levels in the CNS can be useful for predicting the abuse potential of a substance. Several investigations of the CNS using brain slices or synaptosomes have indicated that neurotransmitters may be regulated via additional mechanisms [21,22].

In the present study, the CPP paradigm was adopted to evaluate the abuse potential of 3 representative alkyl nitrites – isobutyl nitrite, isoamyl nitrite, and butyl nitrite. Moreover, the changes in the dopamine levels induced by alkyl nitrites were measured via high performance liquid chromatography (HPLC) to elucidate the mechanism of action of alkyl nitrites.

2. Materials and methods

2.1. Animals and substances

Male ICR mice (age: 8–12 weeks; weight: 30–37 g) were obtained from Samtacobio Korea (Osan, Korea). The mice were maintained in a temperature- (23 ± 1 °C) and humidity-controlled ($55\% \pm 5\%$) room with a 12-h light/dark cycle (lights on from 07:00 to 19:00); laboratory mouse chow and water were provided ad libitum. Handling occurred only during the light cycle. Alkyl nitrites (isobutyl nitrite, isoamyl nitrite, and butyl nitrite; Fig. 1), cocaine, and methamphetamine HCl were purchased from Sigma Aldrich (St. Louis, MO, USA). For the CPP test, animals received intraperitoneal (i.p.) injections of vehicle (saline) or cocaine (20 mg/kg) and 2 doses of the test substances (isobutyl nitrite [5 and 50 mg/kg], isoamyl nitrite [5 and 50 mg/kg], and butyl nitrite [5 and 50 mg/kg]). For HPLC analyses, 3 doses of methamphetamine (1, 10, and 100 μM) and 3 doses of isobutyl nitrite (0.3, 3, and 30 μM) were administered to the synaptosomes in the striatal regions of the brain in the mice. All the animal experiments in the present study were approved by the National Institute of Food and Drug Safety Evaluation/Ministry of Food and Drug Safety Animal Ethics Board (Approval number: 1501MFDS04).

2.2. CPP apparatus

The CPP test box and chamber is manufactured by Saeronbio Inc. (Korea) and consists of 2 distinct compartments (black and white) separated by guillotine doors. The dimensions of each compartment of the white and black box are $15 \times 17 \times 15.5$ cm. The duration of time spent by the animals in each box for conditioning was recorded based on infrared detection via a sensor controller.

2.3. The CPP test

The test consists of 5 phases: (1) Habituation: For 2 days (day 1 and 2), the mice were allowed free access to both compartments of the apparatus for 15 min on each day. (2) Pre-conditioning: The mice were drug-free and were allowed unrestricted access to both compartments of the apparatus for 15 min. The time spent by the mice in each compartment was recorded, and these values served as a baseline. Accordingly, the mice showed a preference for either the black or white compartment, and the preference was recorded within the scope of the mean (15–20%); based on these results, the mice were selected for further experiments and were assigned to 8 groups. (3) Conditioning: On day 4, one group of the selected mice was treated with drugs, and was maintained in the white compartment for 40 min. On the next day (day 5), the group of mice was treated with saline, and was maintained in the black compartment for 40 min. This protocol was repeated for 4 cycles (8 days). During the drug- and saline-paired sessions, the compartments were closed using a guillotine door. (4) Post-conditioning: On day 12, the mice were drug-free and were allowed free access to both compartments of the apparatus for 15 min. The time spent by the mice in each compartment was recorded, and these results were used as test values. (5) Scoring: The results were calculated based on the difference in post-conditioning (test values) and pre-conditioning (baseline values). During the 40-min pairing sessions, the mice were intraperitoneally injected with 5 or 50 mg/kg of alkyl nitrites in either the black or white compartment; the sessions were conducted on alternate days and alternated with saline administration, thus achieving a total of 4 pairings for each treatment.

2.4. Preparation of synaptosomal fractions

Synaptosomes were prepared as previously described [23–26], with slight modifications. Untreated male ICR mice were killed by cervical dislocation and decapitation, and the striatum region of their brain was quickly removed (n = 3). The striatum was homogenized in 10 volumes of ice-cold 0.32 M sucrose by using a Dounce tissue grinder (Kontes, USA). The lysates were centrifuged for 10 min/3000*g* at 4 °C. The supernatant (S1), containing the crude synaptosomal fraction, was transferred to a new tube. The S1 lysate was added and diluted in a 1:1 fashion with Krebe-Hepes buffer pH 7.4 (117 mM NaCl, 4.8 mM KCl, 2.5 mM $MgCl_2$, and 25 mM Hepes), and then centrifuged for 20 min/10,000*g* at 4 °C to obtain the pellet (P1).