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Research Article

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Dopaminergic Effects of Isobutyl Nitrite, Isoamyl Nitrite and Butyl Nitrite

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ABSTRACT

Alkyl nitrites have been problematic worldwide due to their recreational use. Although there is a lack of scientific information on their action on the central nervous system (CNS), many anecdotal reports and case studies have inferred the CNS actions of alkyl nitrites. The objective of this study was to investigate the effects of alkyl nitrites on the CNS, especially focusing on the dopaminergic system. Locomotor activity was measured in rats after treatment with three representative alkyl nitrites (isobutyl nitrite, isoamyl nitrite, and butyl nitrite) at several doses to determine the psychostimulating dose of the test substances. Then, a dopamine receptor antagonist, haloperidol, was administered to the animals before a psychostimulating dose of the test substances in order to evaluate the involvement of the dopaminergic system in the locomotor effects of alkyl nitrites. Locomotor activity was significantly increased (to approximately 1.5-2.5-fold that of the control group) in rats treated with 5 mg/kg of the test substances. Stimulated locomotor activity was significantly inhibited (approximately 1.5-2.5-fold reduction relative to the haloperidol-free group) following pre-treatment with haloperidol (0.1 mg/kg). The locomotor effects of the three alkyl nitrites are likely mediated by dopamine receptors, implying that alkyl nitrites increase dopamine levels in the CNS. Our results provide additional scientific evidence in support of controlling alkyl nitrites as psychoactive substances.

Keywords: Alkyl nitrites; Central nervous system; Dopamine; Haloperidol; Locomotor activity

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INTRODUCTION

Alkyl nitrites are a group of chemical compounds based upon the molecular structure R-NO_2. Formally, they are alkyl esters of nitrous acid, such as butyl nitrite ($C_5H_9NO_2$), amyl nitrite ($C_4H_7NO_2$), and so on. They act on the body through release of the nitrite ion, relaxing smooth muscle, including that of blood vessels, thus reducing peripheral vascular resistance, in turn leading to decreased systemic blood pressure [1]. Alkyl nitrites were introduced during the 1800s as therapeutic agents for relief from acute attacks of angina pectoris because they could abate the discomfort associated with this disease by lowering arterial tension [2]. However, alkyl nitrites are notorious for their growing popularity as recreational substances. The reasons reported to motivate alkyl nitrite abuse include altering one's state of consciousness, stimulating dancing, and intensifying sexual experiences [3]. The availability and high popularity of these substances among adolescents define alkyl nitrites as potential 'gateway drugs'. Widespread recreational use of amyl nitrite is thought to have started in the 1960s. After consequent restrictions on its production and use, various other related alkyl nitrites appeared, such as isobutyl nitrite, butyl nitrite, and isopropyl nitrite. Some users are addicted to these substances for the euphoria and feelings of excitement they elicit. Still, alkyl nitrites are popular street drugs, commonly referred to as "Poppers", "Rush", "Snappers", "TNT", "Liquid gold", "Boppers", etc., and are easy to obtain. The effects begin within 30 seconds of inhalation and last for 2-3 minutes, although the users may re-dose to prolong the experience. It is known that these substances raise the heart rate, meaning more oxygen-rich blood reaches the brain to produce a "rush" sensation [4]. The effects these substances have on blood pressure and blood vessels in the brain may be the cause of some of the negative side effects experienced by users, such as emesis, dyspnea, syncope, and dizziness [5]. Moreover, there have been several reports of eye damage, such as maculopathy [6]

and loss of vision [7] associated with the use of alkyl nitrites. According to a previous report, users with glaucoma take additional risks, as alkyl nitrites increase intraocular pressure [8]. Aside from ophthalmological effects, other toxic effects of alkyl nitrites have been reported by previous rodent studies. In humans, alkyl nitrites decrease blood pressure, increase heart rate [9], and increase the weight of some organs, such as the spleen, lungs, and kidneys, in rats [10], affect T cell proliferative responses [11], and form methemoglobin, reducing the availability of oxygen to the tissues [12].

Regarding the mechanisms by which alkyl nitrites affect the central nervous system (CNS), not enough scientific information is available. There is research on alkyl nitrites suggesting administration of these substances to impair motor coordination and learning/memory ability [13], and potentially cause dependence [14]. Despite this research, the CNS effects of alkyl nitrites have received little attention. Furthermore, in Korea, CNS action and dependence liability are the most crucial criteria informing the regulation of psychoactive substances. The present study therefore focused on this gap in the literature, especially with regard to the involvement of dopamine receptors, because dopamine is strongly associated with substance dependence. The psychostimulant effects of three alkyl nitrites (isobutyl nitrite, isoamyl nitrite, and butyl nitrite) were measured through the use of a locomotor activity test, while the dopamine-receptor-mediated effects of these substances were investigated using a dopamine receptor antagonist, haloperidol.

EXPERIMENTAL SECTION

Animals and Substances

Adult male (250~350 g) Sprague-Dawley rats (n = 6) were kept under constant conditions of temperature (23 ± 1℃) and humidity (55 ± 5%), and on a 12-hour light/12-hour dark cycle (light on at 7:00 h) with food and water *ad libitum*. All experiments were performed at the same time of day during the light period. All 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).

Alkyl nitrites (isobutyl nitrite [IBN], isoamyl nitrite [IAN], and butyl nitrite [BN]), cocaine, and haloperidol were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in 0.9% sodium chloride (normal saline solution). The alkyl nitrites were administered at dosages of 5 and 50 mg/kg, and the haloperidol was administered at the dosage of 0.1 mg/kg. Cocaine (30 mg/kg) was used as a positive control, and saline was used as a negative control. All substances were administered intraperitoneally (i.p.) at a volume of 2 mL/kg of body weight. The drug stocks were freshly prepared each day.

Apparatus

Locomotor activity was measured with Plexiglas chambers (ENV520, Med Associates Inc., Latham, NY, USA) that had eight automated photocell cages (43.2 cm × 43.2 cm × 30.5 cm) equipped with infrared beams located 1.5 cm above the chamber floor and spaced 2.5 cm apart to detect horizontal activity. Each cage had sixteen evenly-spaced infrared transmitters and receivers on each of its four sides, which detected the animal's position in three dimensions (x, y, and z). Software (Activity Monitor 5.0, Med Associates Inc., Latham, NY,) recorded the status of the infrared beams every 50 ms, effectively generating a spatiotemporal map of the animal's movement throughout a testing session.

Methods

Locomotor activity test:

The acutely administered group received a single dose only, while the chronically administered group received the test substances every other day for 10 days. Experiments were performed immediately after the last injection. The rats were randomly assigned (n = 6 for each group) and tested in a random order. They were habituated to the locomotor activity chamber for 30 min, and monitored for 60 min after their last injection. To evaluate effects on the dopaminergic system, haloperidol was injected into rats 30 min before alkyl nitrite administration. Locomotor activity was recorded as the total distance traveled (cm) over the course of each trial (60 min). Testing took place under bright ambient light conditions (15-20 lux).

Statistical analysis:

Data are expressed as mean ± standard error, and statistical significance was assessed by one-way analysis of variance (ANOVA) followed by the post-hoc Holm-Sidak method. $P < 0.05$ was considered to be significant. The statistical software package Sigma Stat 3.5 (Systat Software Inc., San Jose, CA, USA), was used for these analyses.

RESULTS AND DISCUSSION

Determination of Psychostimulating Dose of Alkyl Nitrites

Changes in the locomotor activity of the rats for the 60 min following alkyl nitrite treatment are shown in Figures 1 and 2. Cocaine (30 mg/kg) was used as a positive control (Figure 3), and vehicle (saline) was used as a negative control. In the acutely administered group, total distance traveled was significantly increased by treatment with 5 mg/kg alkyl nitrites compared to the negative control group (IBN: $p < 0.001$, IAN: $p < 0.001$, BN: $p = 0.001$). When 50 mg/kg of the tested substances were administered, no significant changes in locomotor activity were observed. In the chronically administered group, locomotor activity was significantly increased when 5 mg/kg of the tested alkyl nitrites were administered (IBN: $p < 0.05$, IAN: $p < 0.05$, BN: $p = <0.01$). However, compared to saline-administered animals, 50 mg/kg of the test substances did not significantly affect the total distance traveled by the animals. Consequently, the following experiments were performed with 5 mg/kg of the tested alkyl nitrites.

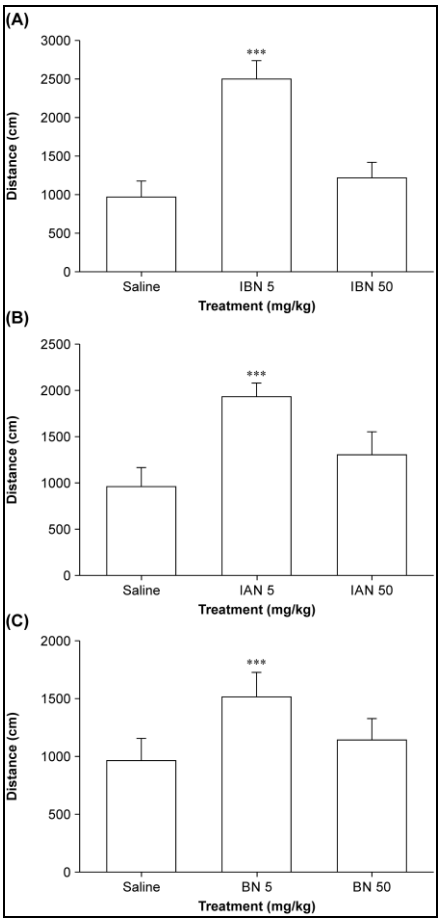

Figure 1: Effects of alkyl nitrites on locomotor activities in acute group. (A) Isobutyl nitrite, (B) Isoamyl nitrite, (C) Butyl nitrite. The rats received the test substances one time, and were immediately monitored up for 60 min. The total distance was significantly increased in 5 mg/kg-treated groups compared to the control group. The activity of 50 mg/kg group was increased but it did not produce significant changes. (One-way ANOVA followed by Holm-Sidak post-hoc test, $^{***}p < 0.001$ vs. saline-treated group)

Effects of Haloperidol on Locomotor Activity

To determine the appropriate dosage of haloperidol, 0.1 or 0.3 mg/kg of the dopamine receptor antagonist were administered 30 min before a saline injection. As shown in Figure 4, the total distance traveled tended to decrease in a dose-dependent manner, however, haloperidol did not produce statistically significant changes compared to the negative control. In light of these results, 0.1 mg/kg of haloperidol was deemed to be the appropriate dose for administration to evaluate the effects of alkyl nitrites on dopamine receptors.

Effect of Haloperidol on Alkyl-Nitrite-Induced Locomotor Activity Increase

In order to evaluate the effects of alkyl nitrites on dopamine receptors, animals were pre-treated with 0.1 mg/kg of haloperidol before administration of the test substances. The increased locomotor activity following administration of 5 mg/kg of one of the three alkyl nitrites decreased significantly after haloperidol injection (IBN: $p < 0.001$, IAN: $p = 0.002$, BN: $p < 0.001$). These results are depicted in Figure 5.

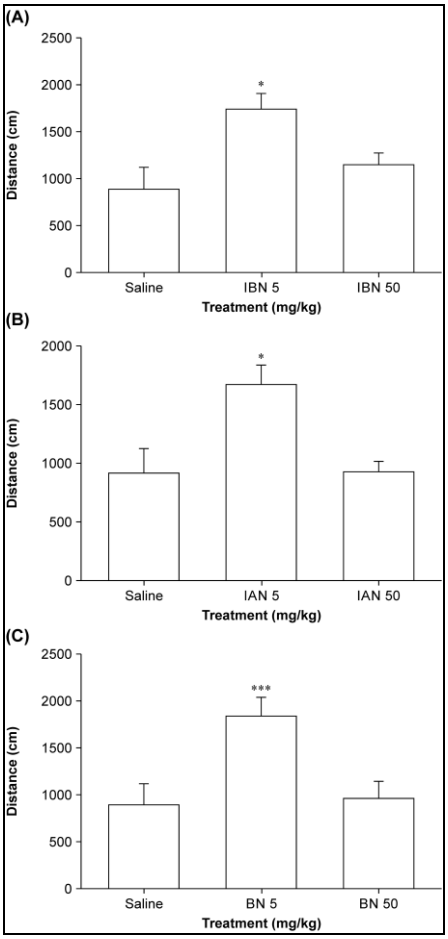

Figure 2: Effects of alkyl nitrites on locomotor activities in chronic group. (A) Isobutyl nitrite, (B) Isoamyl nitrite, (C) Butyl nitrite. The rats received the test substances every other day for 10 days, and were immediately monitored for 60 min after the last injection. The total distance was significantly increased in 5 mg/kg-treated groups compared to the control group. The activity of 50 mg/kg group was increased but was not significant. (One-way ANOVA followed by Holm-Sidak post-hoc test, $^{**}p < 0.05$, $^{***}p < 0.001$ vs. saline-treated group)

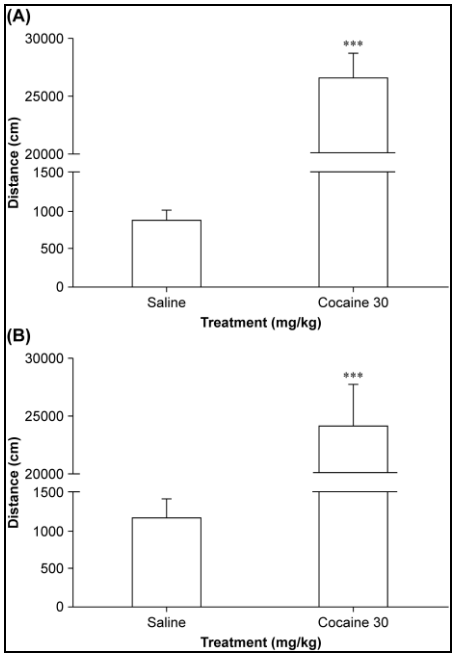

Figure 3: Cocaine (30 mg/kg) was used as a positive control and vehicle (saline) was used as a negative control

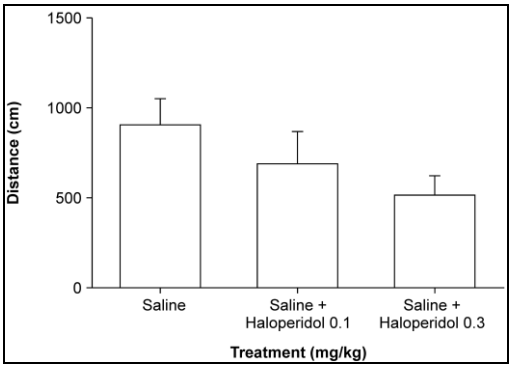

Figure 4: Effects of haloperidol on locomotor activities in the rats. The total distance was decreased dose-dependently by haloperidol. However, it did not produce a significant change compared to the control group

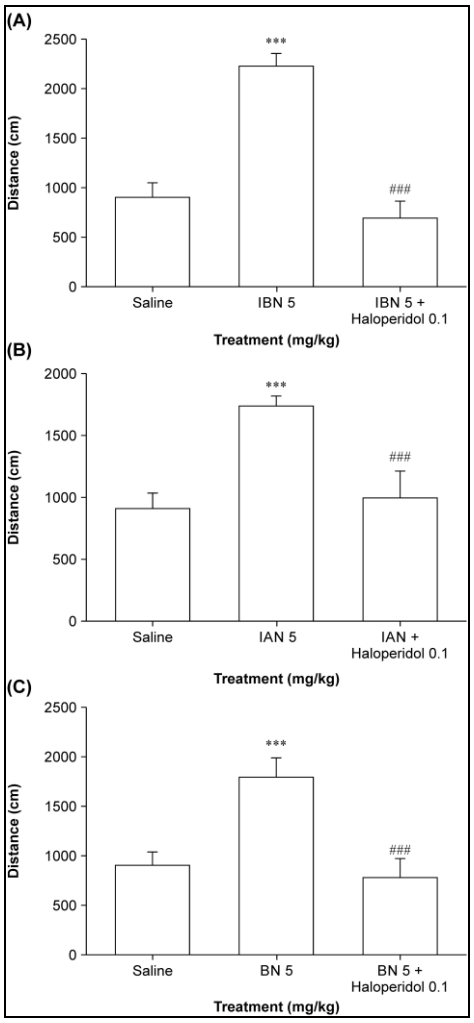

Figure 5: Effects of haloperidol on alkyl nitrite-induced locomotor activities in the rat. (A) Isobutyl nitrite, (B) Isoamyl nitrite, (C) Butyl nitrite. Haloperidol (0.1 mg/kg) was administered 30 min before administration of alkyl nitrites. Pretreatment with haloperidol produced significant changes in the alkyl nitrites-induced locomotor activities. (One-way ANOVA followed by Holm-Sidak post-hoc test, $^{***}p < 0.001$ vs. saline-treated group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. alkyl nitrite-treated group)

In the present study, the CNS effects of alkyl nitrites were investigated, specifically, those mediated by dopamine receptors. Locomotor activity is a commonly used behavioral measure for the evaluation of psychoactivity, and is prevalently used in assessments of dopaminergic activity [15,16]. The increased locomotor activity observed at 5 mg/kg dose of the tested alkyl nitrites indicates a psychostimulating effect. Interestingly, locomotor activities did not increase at 50 mg/kg dose, suggesting the induction of adverse effects (i.e. toxicity) rather than psychostimulation. This notion is supported by the previous study of Cha et al., which showed that 50 mg/kg of alkyl nitrites induce toxicity and affect motor coordination and learning/memory ability [13].

Regarding toxicity of alkyl nitrites, most of the studies are anecdotal or case reports. Anecdotally, the users reporting adverse effects all showed similar symptoms: high pulse rate, low blood pressure, and blue coloring of the skin (cyanosis). Some even reported methemoglobinemia, a potentially fatal condition. Aside from these case reports, many scientific reports evidencing the various toxicities of alkyl nitrites have been published, describing ophthalmological, immunological, cardiovascular, and hematological impairments following alkyl nitrite use [6,7]. Additionally, recently reported CNS-related issues associated with alkyl nitrites include neurotoxicity [13].

Considering the liability of dependence or CNS effects of alkyl nitrites, Nutt et al. suggested alkyl nitrites to be, as a group of compounds, minimally addictive and harmful compared to the twenty most commonly abused drugs, such as cocaine, amphetamine, and alcohol ([17]. Moreover, Jeon et al. showed that striatal dopamine levels were increased by administration of alkyl nitrites [14], supporting our conclusion of the dopaminergic effect of alkyl nitrites in the CNS. One such study suggested that motor activity may be considered a test of nervous system function, as it reflects the integrated output of the sensory, motor, and associative processes of the nervous system [18]. Other reports have shown that dopamine affects locomotor activity; many dopamine agonists, such as apomorphine [19], 3-PPP [20], 7-OH-DPAT [21] and quinpirol [22] increase locomotor activity. Additionally, the work of Anden et al. suggests that locomotor activity is reduced by dopamine-receptor-blocking drugs [23].

Haloperidol, a non-specific dopamine receptor antagonist, induced no significant effects at the dosages of 0.1 mg/kg and 0.3 mg/kg tested in the present study (Figure 4). The appropriate dosage (0.1 mg/kg) of haloperidol in this study was determined after considering in light of these results and those of a previous report which found 0.5 mg/kg to have a sedative effect [24]. Regarding the putative dopaminergic effects of alkyl nitrites, the present data show that increased locomotor activity due to alkyl nitrites significantly decreased after treatment with haloperidol, suggesting alkyl nitrites to be of agonistic effect on dopamine receptors. Additionally, the aforementioned study by Jeon et al. revealed that total dopamine levels increased following the administration of alkyl nitrites [14], though it was not clarified whether this was due to increased dopamine release or inhibition of reuptake. Our results, indicating that alkyl nitrites elicit dopamine receptor agonism, could be just the evidence required to substantiate the hypothesis that alkyl nitrites increase dopamine levels via agonizing dopamine receptors and boosting dopamine release.

CONCLUSION

In conclusion, the present study suggests that alkyl nitrites possibly affect the CNS via dopaminergic pathways stimulated through alterations of dopamine receptor activation. However, our results do not adequately demonstrate the precise means by which alkyl nitrites alter dopaminergic activity. There is a lack of data regarding the dopaminergic activity of alkyl nitrites *in vitro*, and the data presented here do not demonstrate the mechanisms of other nitrite substrates such as isopropyl nitrite and cyclohexyl nitrite. The mechanism of action of alkyl nitrites in relation to dopamine and other neurotransmitters should be elucidated in greater detail in future studies.

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