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Original Article

Effect of acute and long term potassium bromide administration on spatial working memory in rat

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Abstract

Potassium bromide (KBr), an old antiepileptic agent, is illegally used in pharmaceutical or food industries to improve the product appearance. KBr has been proven to influence several pathways which are important in memory formation. Therefore, the present study aimed to evaluate the effect of KBr on spatial working memory using object recognition task (ORT). Rats received a single dose of KBr (50, 100 or 150 mg/kg), per oral, in acute treatment. KBr long term effects were also studied in animals receiving 50 mg/kg/day of KBr for 28 consecutive days. At the end of treatments, animals underwent two trials of ORT, five min each. In the first trial (T_1), animals encountered with two identical objects for exploration. After 1 h, the animals were exposed to a familiar and an unfamiliar object (T_2). The exploration times for discrimination (D) and recognition (R) as well as the frequency of exploration for any objects were determined. Acute administration of 150 mg/kg of KBr significantly decreased the discrimination and recognition indices (RI and DI) ($P < 0.01$) compared to the control. However, lower doses failed to influence the animals' performance in the test. In addition, long term administration of KBr remarkably diminished the DI and RI and the frequency of exploration ($P < 0.05$). The results of this study indicate that acute doses of KBr as high as 150 mg/kg are required to hamper memory function in ORT. However, cognitive impairment occured with lower doses of KBr when the duration of treatment is extended.

Keywords: Potassium bromide; Acute; Long term; Spatial working memory; Object recognition task

INTRODUCTION

Potassium bromide (KBr) is a chemical compound isolated from the Mediterranean Sea in 1826 (1). KBr and other inorganic bromide salts produce sedative effects (2). Therefore, it was prescribed as an anxiolytic, sedative-hypnotic or antiepileptic agent (2). It is still indicated for the treatment of refractory seizures in children (3). These effects are primarily mediated through the activation of $GABA_A$ receptor. These receptors are ionotropic channels which, upon activation, increase chloride permeation and cause hyperpolarization of neurons (4). This is the main cause of KBr-induced inhibitory effects in the CNS. In this regard, KBr is reported to cause depression, weakness, fatigue, lethargy, coma and other symptoms related to CNS suppression (5). KBr was withdrawn from the pharmaceutical market because of low efficacy, adverse effects, and the discovery of more effective hypnotic and antiepileptic drugs (2). However, it is illegally used for material processing in food industry. For example, oxygenated KBr is added to enhance the volume and mature the flour in bakery. Indeed, it helps oxidize the sulfhydryl groups of the gluten into disulphide bridges, and traps carbon dioxide in the dough. This improves the appearance and elasticity of the dough (6,7). Likewise, potassium bromate is used as a preservative to keep the concentrated fish paste (7). As such, brominated vegetable oil is frequently used as a clouding agent in soft drinks (8). Therefore, people may intake different amount of bromide via food products. Following the ingestion of bromide, it enhances GABA effects in the CNS (4), which can influence neurobehavioral processes like learning and memory (9,10).

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Memory formation and retrieval are complex processes that involve neuronal networks and multiple pre- and post- synaptic events. Several literatures have investigated the molecular mechanisms underlying activity-dependent synaptic changes during memory formation (11,12). GABA is a key mediator in several brain regions involved in memory (13). In addition, drugs stimulating the GABA receptor activity including antiepileptic agents are reported to negatively influence learning and memory (9).

This study aimed to investigate the potential effects of KBr on memory function. In particular, acute and long-term effects of KBr on spatial memory were studied in object recognition task (ORT). ORT is based on the spontaneous behavior of rats to explore a novel object more intensely than a familiar one. It is a non-rewarded, ethologically relevant, relatively simple test. In addition, a large body of evidence have demonstrated that spontaneous exploratory activity in this task can be used as a valid measure of memory function (14,15).

MATERIALS AND METHODS

Animals

The experiments were carried out on male Wistar rats weighing 200 ± 20 g obtained from the animal house of School of Pharmacy and Pharmaceutical Sciences at Isfahan University of Medical Sciences (Isfahan, Iran). The animals had free access to food and water during the experiment and were kept at a constant room temperature (22 ± 1 ºC) under a 12-12 h light/dark cycle. All experimental procedures were conducted during the light phase of the cycle. The study protocol was approved by the Bioethics Committee of Isfahan University of Medical Sciences (Registration No. 394070), and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Apparatus and objects

The apparatus consisted of a circular arena of 83 cm in diameter and walls of 40 cm high which was made of white polyvinyl chloride (16,17). Two different sets of objects consisted of a massive aluminum cube (10 × 5 × 7.5 cm) and a massive aluminum cube with a tapering top (13 × 8 × 8 cm) were used in the task. The objects could not be displaced by rat and each object was available in triplicate (16,15).

Drug administration

Animals were randomly divided into 6 groups of 7 each. In the acute study, rats received a single dose of 50, 100 or 150 mg/kg of KBr per oral. In the long term study, animals were treated with 50 mg/kg of KBr for 28 consecutive days. KBr was dissolved freshly in saline, and the control groups received saline as the vehicle.

Experimental procedure

The animals underwent the ORT 90 min post-treatment in either acute or chronic experiments. ORT consisted of three defined phases, (T_1) a training session or first trial; and a training-test interval, and (T_2) a test session or second trial.

These phases last for 5, 60 and 5 min, respectively (18). During the T_1 phase, animals encountered with two identical objects (A_1 and A_2) which were placed in a symmetrical position about 10 cm away from the wall. In the T_2 trial, they faced with one identical and one novel object (A and B) to explore. Exploration was defined as directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose, and sitting on the object was not considered an exploratory behavior.

The exploration time (s) for each object was recorded and the following memory indicating factors were calculated. e_1, is the total exploration time for both objects in the first trial (eA_1+ eA_2); e_2, is the total exploration time for both objects in the second trial (eA + eB); and DI is (eB - eA)/(eB + eA). In this set, DI (discrimination index) indicates the discrimination between new and familiar objects. Its' values vary between +1 and -1, where a positive score indicates more time spent with the novel object, a negative score indicates more time spent with the familiar object, and a zero score shows a null preference.

F, is the frequency of the object exploration. Another measure of the ORT is the recognition index (RI) which is the time spent to explore the novel object relative to the both object exploration time and calculated as,

$RI = eB/ (eB + eA)$

In every section, animals who explored less than 10 s in the second trial ($e_2 < 10$) were excluded from the study (15-17,19).

Statistical analysis

The data are presented as mean ± SEM. The values were analyzed using t-test or one-way analysis of variance (ANOVA) according to the number of comparable groups. Multiple comparisons were accomplished using Tukey post-hoc test and $P < 0.05$ was considered as statistically significant. Statistical analysis was conducted using Graph pad prism V.5.

RESULTS

Acute effects of KBr on exploratory behavior in T_2 trial

A single dose of 150 mg/kg of KBr administered 90 min prior to the T_1 trial significantly decreased the DI compared to the control group ($P < 0.01$) (Fig. 1A). However, lower doses of KBr (50 or 100 mg/kg) did not alter this index (Fig. 1A).

In addition, a single dose of 100 ($P < 0.05$) or 150 ($P < 0.01$) mg/kg of KBr remarkably decreased RI in the T_2 trial in comparison to the control group (Fig. 1B), whereas, KBr at 50 mg/kg failed to produce a significant effect on RI (Fig. 1B).

Acute treatment with KBr at doses as high as 150 mg/kg did not change the frequency of novel object exploration in the T_2 trial compared to the control group (Fig. 2A).

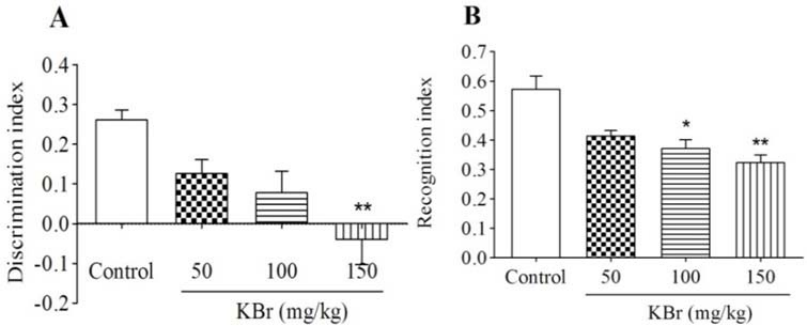

Fig. 1. Acute effects of KBr on discrimination and recognition indices in T_2 trial. (A) A single dose of KBr (150 mg/kg) significantly decreased the discrimination index but 50 or 100 mg/kg failed to produce a considerable change compared to the control group. (B) A single dose of 50 mg/kg KBr did not change the recognition index while 100 or 150 mg/kg of KBr significantly decreased this index in the T_2 trial. Data are presented as mean ± SEM. (**$P < 0.01$, *$P < 0.05$). KBr, potassium bromide.

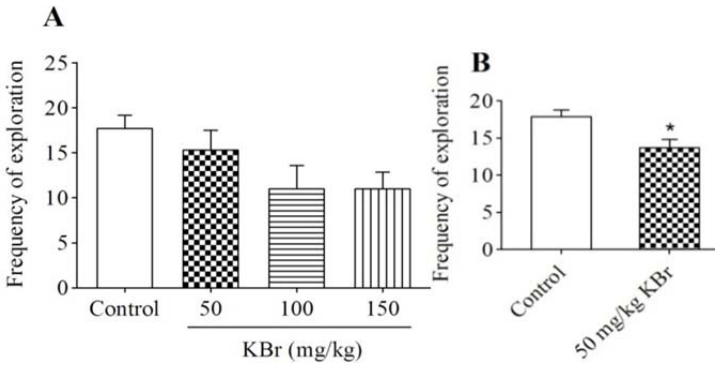

Fig. 2. Acute and long-term effects of KBr on the frequency of exploration in T_2 trial. (A) A single dose of KBr (50, 100 or 150 mg/kg) did not alter the frequency of novel object exploration in the T_2 trial compared to the control group. (B) Repeated doses of 50 mg/kg/ day for 28 days led to a considerable reduction in the frequency of exploration. Data are presented as mean ± SEM. (*$P < 0.05$). KBr, potassium bromide.

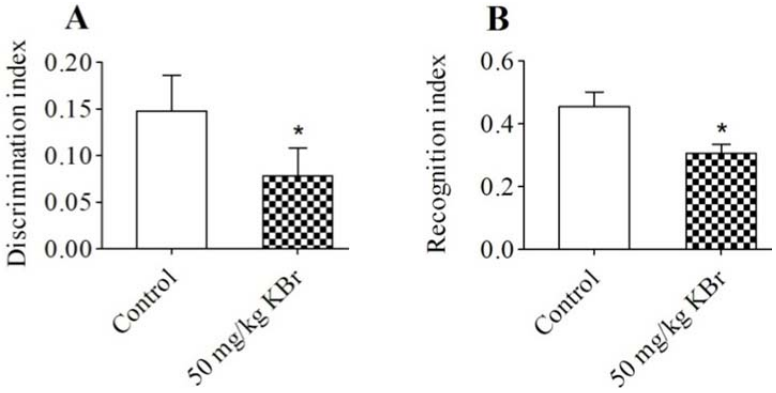

Fig. 3. Long-term effects of KBr on discrimination and recognition indices in T_2 trial. Long term administration of KBr (50 mg/kg for 28 days) considerably reduced the (A) discrimination index as well as (B) recognition index in the T_2 trial in comparison with the control group. Data are presented as mean ± SEM. (*P < 0.05). KBr, potassium bromide.

Long-term effects of KBr on exploratory behavior in T_2 trial

Daily administration of 50 mg/kg KBr for 28 consecutive days before ORT considerably (P < 0.05) reduced the DI and RI in T_2 trial compared to the control (Fig. 3A, 3B). As such, it significantly decreased the frequency of exploration of new object in comparison with the control group (P < 0.05) (Fig. 2B).

DISCUSSION

In the present study, the effect of KBr, as an illegal additive of bakery products, in a single or repeated dose/s was evaluated on the spatial memory using ORT. The ORT is a non-rewarded, stress free task which could study the spatial memory of rodent correctly (14,15).

The results showed that single doses of 50 or 100 mg/kg of KBr did not disrupt the memory in rats. However, single dose of 150 mg/kg KBr significantly hampered memory formation compared to the control group. Furthermore, long term administration of KBr at the dose of 50 mg/kg/day for 28 days led to the cognitive impairment in rats. In addition, we observed that the memory impairment induced by KBr is a dose related effect. Specifically, doses of 50 or 100 mg/kg of KBr in the acute treatment failed to influence cognitive function. Our findings are in agreement with studies demonstrating the disruptive effects of different antiepileptic drugs on memory. For instance, Tsutsumi, *et al*. reported cognitive impairment in rat offspring exposed prenatally to phenytoin during 7th to 18th day of gestation (20). Likewise, exposure to phenobarbital, carbamazepine, valproate, and topiramate are associated with impairment in learning and memory (21-24).

KBr could easily reach CNS, and influence the physiological function of neurotransmitters such as GABA. So, it can potentially alter neurological processes as well as behavioral activities. KBr is proven to stimulate the $GABA_A$ receptor which is a chloride channel. Upon activation, it leads to the hyperpolarization of neurons and induces an inhibitory effect in the brain (4). Memory impairment in this study can be related to KBr-induced activation of GABA receptor (25). Based on a great number of literatures, increase of GABA concentration or receptor activity results in memory impairment (9).

It has been fully demonstrated that the cholinergic system plays a key role in learning and memory processes (26). In particular, disruption of cholinergic activity impairs cognitive function both in animal and human subjects. On the other hand, enhancement of cholinergic function is meant to improve cognitive function in dementia related disorders (27). In this regard, bromide decreases acetylcholine release from presynaptic cells (28). This inhibitory effect can explain, to some extent, the memory impairment observed in rats treated with KBr.

Alteration of serotonergic signaling may also play a role in bromide-induced cognitive

dysfunction. In this regard, *in vitro* studies have demonstrated that bromide inhibits serotonin release (28). In addition, serotonin plays an important role in a great variety of behaviors such as learning and memory, particularly by interacting with cholinergic, glutamatergic, dopaminergic or GABAergic systems (29-31). Therefore, bromide inhibition of serotonergic function can translate to modification of other neurotransmitters, and finally result in memory dysfunction. This may also explain KBr-induced memory impairment in this research, and requires further investigation.

CONCLUSION

Taken together, we demonstrated that single high dose as well as multiple low dose administration of KBr can cause memory impairment in ORT. The stimulation of GABAergic transmission as well as inhibition of cholinergic or serotonergic activity could possibly explain KBr-induced cognitive dysfunction.

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