Behavioural Brain Research 312 (2016) 313–320

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Involvement of glutamatergic *N*-methyl-D-aspartate receptors in the expression of increased head-dipping behaviors in the hole-board tests of olfactory bulbectomized mice

Noritaka Hirose, Akiyoshi Saitoh*, Junzo Kamei

Department of Pathophysiology and Therapeutics, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

HIGHLIGHTS

- NMDA receptor antagonist suppressed the head-dipping behaviors in OB mice.
- The glutamate ratios were increased in amygdala and frontal cortex of OB mice.
- The head-dipping behaviors of OB mice were mediated by the NMDA receptors.

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 21 June 2016
Accepted 24 June 2016
Available online 25 June 2016

Keywords:
Anxiety
Animal model
Glutamate
Frontal cortex
Amygdala
Olfactory bulbectomy

ABSTRACT

Olfactory bulbectomized (OB) mice produce agitated anxiety-like behaviors in the hole-board test, which was expressed by an increase in head-dipping counts and a decrease in head-dipping latencies. However, the associated mechanisms remain unclear. In the present study, MK-801 (10, 100 μg/kg), a selective *N*-methyl-D-aspartate (NMDA) receptor antagonist, significantly and dose-dependently suppressed the increased head-dipping behaviors in OB mice, without affecting sham mice. Similar results were obtained with another selective NMDA receptor antagonist D-AP5 treatment in OB mice. On the other hand, muscimol, a selective aminobutyric acid type A ($GABA_A$) receptor agonist produced no effects on these hyperemotional behaviors in OB mice at a dose (100 μg/kg) that produced anxiolytic-like effects in sham mice. Interestingly, glutamine contents and glutamine/glutamate ratios were significantly increased in the amygdala and frontal cortex of OB mice compared to sham mice. Based on these results, we concluded that the glutamatergic NMDA receptors are involved in the expression of increased head-dipping behaviors in the hole-board tests of OB mice. Accordingly, the changes in glutamatergic transmission in frontal cortex and amygdala may play important roles in the expression of these abnormal behaviors in OB mice.

1. Introduction

Following bilateral ablation of olfactory bulbs, rodents exhibit a well-established syndrome of behavioral, physiological, neurochemical, and neuroendocrine changes, resembling those seen in clinical depression [1]. The most consistent behavioral changes caused by olfactory bulbectomy (OB) are hyperemotional responses, such as hyperactivity, agitation, anxiety-like, irritability, impulsive, and aggressive behaviors in novel environments. Because these hyperemotional behaviors are reversed by administering clinically effective anxiolytic and antidepressant drugs, OB in rodents has been proposed as a model with high predictive validity for chronic psychomotor agitation, which is often associated with depression and anxiety [1–3].

We previously found that numbers of head-dips in the hole-board tests of OB mice were significantly greater than those in sham mice [4,5]. Moreover, these abnormal behaviors were completely reversed by pretreatment with diazepam, which is a typical benzodiazepine anxiolytic agent. Furthermore, the selective 5-HT1A-receptor agonist, (±)-8-hydroxy-2-(di-n-propylamino) tetraline hydrobromide (8-OH-DPAT), and the δ-opioid-receptor agonist, (+)-4-[(aR)-a-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethyl

* Corresponding author at: Department of Neuropsychopharmacology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8553, Japan.
E-mail address: asaitoh@ncnp.go.jp (A. Saitoh).

http://dx.doi.org/10.1016/j.bbr.2016.06.045
0166-4328/

benzamide (SNC80), which have a non-benzodiazepine anxiolytic-like effect, also significantly reversed the number of head-dips in OB mice [4,5]. Head-dipping behaviors were also significantly reversed by subcutaneous methylphenidate treatment [5]. Hence, we suggest that hyperemotional behaviors, such as head-dipping in OB mice reflect agitated anxiety-like behaviors in the hole-board tests. However, the associated mechanisms remain unclear.

Olfactory bulbs project excitatory glutamatergic nerve fibers widely through the lateral olfactory tract to the olfactory cortex, including the frontal and amygdaloid complex [1]. Therefore, substantial changes in glutamatergic functions are likely in OB models. In agreement, NMDA receptor densities were defined using binding assays [6] and autoradiographic assays [7] with the selective NMDA receptor antagonist [^{125}I] MK-801, and were lowered in the amygdala and cerebral cortex of OB rats. In contrast, higher NMDA receptor densities were demonstrated in the prefrontal cortex of OB rats using [^{125}I] MK-801 [8]. Previously, Robichaud et al. showed that subcutaneous administration of MK-801 abolished hyper-locomotor activity in open-field (OF) tests of OB rats [7]. Moreover, Ho et al. showed that striatal glutamate release in OB rats was reportedly increased during novelty stress and that local treatment with MK-801 in the amygdala inhibits muricidal behaviors of OB rats [9,10]. And also, OB rats were thought to have substantial changes in GABAergic function. In a previous study cortical $GABA_A$ receptor densities were assessed using [^{3}H]GABA and were significantly increased in OB rats [11]. Indeed, multiple studies report abnormalities of glutamatergic and GABAergic neurons in several brain areas of OB rats.

Excitatory glutamatergic NMDA receptor and/or inhibitory GABAergic $GABA_A$ receptor-mediated functions play pivotal roles in the regulation of emotional behaviors in rodents. Therefore, we hypothesized that these changes are associated with the expression of OB-induced hyperemotional behaviors. Thus, in the present study, we examined the effects of the selective NMDA receptor antagonists MK-801, D-AP5 and the selective $GABA_A$ receptor agonist muscimol on hyperemotional behaviors of OB mice using the hole-board tests. In addition, we clarified the influence of glutamatergic and GABAergic neuron functions after OB, and evaluated glutamate, glutamine and GABA contents in the amygdala and frontal cortex, which play important roles in the control of emotional behaviors of rodents.

2. Method and methods

2.1. Animals

Experiments were conducted using 4-week-old male ICR mice (Tokyo Laboratory Animals Science, Tokyo) weighing 18–23 g. 112 animals were used in experiments. Animals were given food and water ad libitum and were housed in an animal room maintained at 24 ± 1 °C under a 12-h light cycle/12-h dark cycle. This study was performed in accordance with the Declaration of Helsinki and the guidelines for the use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

2.2. Drugs

The drugs (+)-MK-801 hydrogen maleate, D-AP5 and muscimol (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in saline and were injected 30 min prior to the hole-board tests.

2.3. Olfactory bulbectomy

Olfactory bulbectomy was performed in accordance with our previous report [4]. Briefly, 4-week-old ICR mice were subjected to olfactory bulbectomy. Prior to surgery, mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) diluted in 0.9% saline. Subsequently, the part of the skull covering the bulbs was exposed by making a skin incision and drilling a burr hole (2.0 mm prior to the bregma, 1 mm lateral to the midline), and both olfactory bulbs were removed by suction through the hole. Sham operations were performed in an identical manner, but the skull and bulbs were left intact. After completing behavioral experiments, mice were decapitated, and the histological effects of bulbectomy were visually inspected. Data from animals that suffered incomplete removal of bulbs or frontal cortex damage were discarded. Following removal of olfactory bulbs, OB mice were immediately housed in individual Plexiglas cages (15 × 10 × 12.5 cm) for 14 days. Sham operations were performed in the same way, but with olfactory bulbs left intact.

2.4. Hole-board tests

The hole-board test is performed in 7–8 week-old mice following 2 weeks after having surgery. The results of the hole-board tests were automatically determined as described previously [12,13]. The hole-board apparatus comprises of a grey wooden box (50 × 50 × 50 cm) with four 3-cm diameter holes that are equally spaced on the floor. The box was placed in indirect light (40 lx). An infrared beam sensor was installed on the wall to detect the number of rearing and head-dipping actions.

2.5. Intracerebroventricular (i.c.v.) administration

D-AP5 was less able to cross the blood brain barrier. Therefore, we selected an i.c.v. administration route. The i.c.v. administration was performed following the method described by Haley and McCormick [14] using a 10 μL Hamilton syringe. Briefly, 24 h before drug treatment, the mice were anesthetized with ether and a 2-mm double-needle (tip: 27 gauge × 2 mm and base: 22G × 10 mm, Natsume Seisakusyo, Tokyo, Japan) attached to a 25-μL Hamilton microsyringe was inserted into the unilateral injection site (1.5 mm lateral from the midline, 0 mm anterior from the bregma and 3.0 mm deep from the surface of the skull), and a simple hole for the injection was made in the skull. In the test day, the drugs were injected through the hole with the mice unanesthetized, and the volume for i.c.v. injection was 5 μL. The i.c.v. treatment with drugs was performed 30 min before the hole-board test. The dose of D-AP5 was performed following the previous report by Mathis [15].

2.6. Analysis of glutamate, glutamine, and GABA concentrations

Mice were decapitated after behavioral experiments and the success of bulbectomy was assessed after removing brains. Subsequently, frontal cortices and amygdala were quickly dissected and placed on an ice-cold glass plate and brain tissues were stored at −80 °C until use. Glutamate, glutamine, and GABA levels were then analyzed using HPLC with an electrochemical detector (HTEC-700, EICOM, Kyoto, Japan) after derivatization with *o*-phthalaldehyde (OPA; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The derivatizing reagent contained 27 mg of OPA dissolved in 1 mL of ethanol, 9 mL of 500 mM carbonate buffer, and 20 μL of 2-mercaptoethanol. One volume (4 μL) of derivatizing reagent was added to three volumes (12 μL) of sample and the reaction proceeded for 2.5 min at room temperature under the control of an autosampler (M-500, EICOM, Kyoto, Japan). Glutamate was separated from samples using a reverse-phase column (Eicompack FA-3ODS, ϕ3 × 75 mm, EICOM, Kyoto, Japan) at 40 °C. The potential of the glassy carbon electrode (WE-GC, EICOM, Kyoto, Japan) was set at +0.6 V (vs. Ag/AgCl), and glutamate was eluted using a