Behavioural Brain Research 215 (2010) 141–145

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Influence of olfactory bulbectomy on maternal behavior and dopaminergic function in nucleus accumbens in mice

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ARTICLE INFO

Article history:
Received 7 June 2010
Received in revised form 9 July 2010
Accepted 10 July 2010
Available online 16 July 2010

Keywords:
Olfactory bulbectomy
Maternal behavior
Dopamine
Tyrosine hydroxylase
Depression
Autoradiography

ABSTRACT

Olfactory bulbectomy (OBX) induces behavioral, physiological, and neurochemical alterations resembling clinical depression and is widely used as an animal model of depression. It has been reported that depression is a critical cause of child abuse and neglect and that maternal behavior involves dopaminergic neurons of the mesolimbic pathway. In a previous study we found that OBX mice show maternal behavior deficits which are improved by administration of apomorphine, a non-selective dopamine agonist. Therefore, in this study, we investigated the effect of L-3,4-dihydroxyphenylalanine (L-DOPA) on maternal behavior deficits to examine the influence of pre-synaptic dopaminergic function in OBX mice. Furthermore, we measured tyrosine hydroxylase (TH) levels using microphotometry and quantified dopamine D1- and D2-like receptors using autoradiography in the nucleus accumbens (NAc). As a result, 25 mg/kg L-DOPA with 12.5 mg/kg benserazide improved disrupted maternal behavior in OBX mice and there are no changes in TH levels or number of D1- and D2-like receptors between sham and OBX mothers. The behavioral data support the hypothesis that changed dopaminergic function may contribute to maternal behavior deficits in OBX mice. However, our findings concerning dopaminergic function suggest that the deficits in OBX mice are not simply due to changes in TH levels or dopamine receptor number in the NAc.

1. Introduction

Bilateral olfactory bulbectomized (OBX) rodents are one of the most commonly used animal models of depression. They express behavioral, physiological and neurochemical alterations similar to clinical depression symptoms and these changes are reversed by chronic, but not acute, antidepressant treatment (for review, see [38]). Specifically, OBX produces neurodegeneration in the locus coeruleus or dorsal raphe that causes dysfunction of the noradrenergic or serotonergic system, respectively [16,17]. In addition, OBX animals show varied behavioral changes including impairment of cognitive function and an increase in nocturnal locomotor activity and exploratory behavior [11,13,25,26,41,46]. Recently, it has been reported that abnormal maternal behavior is expressed in various animal models of depression. Flinders Sensitive Line rats, a genetic animal model of depression, show reduced nursing and licking behaviors compared with Sprague–Dawley control rats [4,23]. Furthermore, rat mothers that have acquired learned helplessness display decreased licking and arched back nursing behavior [21]. In addition, OBX has been reported to induce deficits in maternal responding in mice [9,10], and we observed maternal behavior deficits in OBX mice in a previous study [35].

Maternal behavior is strongly related to the mesolimbic dopaminergic system which is a major component of the brain reward system (for review, see [20,31]). Dysfunction of the reward system causes anhedonia [24] which is one of the core symptoms of major depression, and it is also observed in OBX animals [5,34,35,39]. It is known that disruption of the mesolimbic dopaminergic system leads to maternal behavior deficits, for example, destruction of the ventral tegmental area (VTA) which projects dopaminergic nerves to the mesolimbic area or by administration of a dopamine antagonist or 6-hydroxydopamine to the nucleus accumbens (NAc) disrupted maternal behavior [8,12,19,29,37]. Furthermore, it has been reported that maternal interaction with their pups increases dopamine release into the mother's NAc [6,22]. Previously, we found that disruption of maternal behavior in OBX mice was ameliorated by administration of apomorphine, a non-

Abbreviations: ANOVA, analysis of variance; L-DOPA, L-3,4-dihydroxyphenylalanine; MPOA, medial preoptic area; NAc, nucleus accumbens; NAcC, nucleus accumbens core; NAcSh, nucleus accumbens shell; NGS, normal goat serum; OB, olfactory bulb; OBX, olfactory bulbectomy; PBS, phosphate-buffered saline; PND, postnatal day; SEM, standard error of the mean; SSRI, selective serotonin reuptake inhibitor; TH, tyrosine hydroxylase; vBST, ventral bed nucleus of the stria terminalis; VP, ventral pallidum; VTA, ventral tegmental area.

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doi:10.1016/j.bbr.2010.07.012

selective dopamine agonist [35]. This result suggests that the disruption of maternal behavior in OBX mice is related to dopaminergic function in the central nervous system.

Therefore, we examined the effect of L-3,4-dihydroxyphenylalanine (L-DOPA) on maternal behavior and tyrosine hydroxylase (TH) levels, the biosynthetic rate-controlling enzyme of catecholamines such as dopamine, in the NAc to investigate pre-synaptic function of dopaminergic nerves in OBX mothers in present study. We quantified D1- and D2-like dopamine receptors in the NAc using autoradiography, because it is reported that roles of dopamine receptors in maternal behavior vary according to subtype [29]. As OBX mice have depressive symptoms this study also investigated whether the antidepressive effect of a selective serotonin reuptake inhibitor (SSRI) can improve the maternal behavior deficits in OBX mice. In this study, we selected fluvoxamine as a SSRI, because it has been reported that the drug has a relatively low reproductive toxicity compared to other SSRIs [42].

2. Materials and methods

2.1. Animals

Eight-week-old ddY virgin female mice were obtained from Nippon SLC (Hamamatsu, Japan). Animals were housed in polypropylene cages (13 cm height × 21 cm width × 31 cm length) with a stainless steel wire lid and wood shavings as bedding material (Beta chip, Charles River Laboratory International, Inc., USA). The animals had free access to food and water. Temperature (22 ± 2 °C) and humidity (55 ± 10%) were maintained within a narrow range and the animals were on a 12-h light/dark cycle (light phase; 7:00–19:00 h). In the behavioral study, 45 sham and 36 OBX mice were used. A further 12 (sham, $n = 6$; OBX, $n = 6$) and 10 mice (sham, $n = 5$; OBX, $n = 5$) were used to examine TH levels and dopamine receptors in the NAc, respectively. All experiments were performed according to the Guide for Care and Use of Laboratory Animals at Tohoku Pharmaceutical University.

2.2. Drugs

L-DOPA (12.5 and 25 mg/kg, Sigma–Aldrich, Inc., St. Louis, MO, USA) was dissolved in physiological saline and benserazide (12.5 mg/kg, Sigma–Aldrich, Inc.) was suspended in 0.5% Tween 80. L-DOPA was intraperitoneally administered 15 min before and benserazide was administered 45 min before maternal behavior observation began to prevent peripheral metabolism of L-DOPA. The time of administration of these drugs was based on a previous report by Tayarani-Binazir et al. [40]. Fluvoxamine dissolved in distilled water (60 mg/kg) was administered perorally from the end of mating until delivery (14–21 days). On the day of testing fluvoxamine was administered 60 min before behavior observation began. The dosage of fluvoxamine was determined accorded to the report of Ichimaru et al. [14]. Drugs were administered at a dose of 0.1 mL/10 g of mouse body weight.

2.3. Surgery

Mice anesthetized with pentobarbital Na (50 mg/kg, i.p.; Dainippon Sumitomo Pharma, Osaka, Japan) were placed in a stereotaxic frame. The scalp was incised, two holes were drilled to expose the olfactory bulb (OB), which was then bilaterally aspirated using a suction pump. All animals were sacrificed at the end of the experiment and the lesions were verified visually. It was confirmed that at least two thirds of the OB had been removed and that some parts of the olfactory nuclei also had also been lesioned. If the lesion was either not extensive enough or extended to the cortex, data from these animals were excluded. Sham operations were performed in the same manner but without the removal of the OB.

2.4. Mating and maternal behavior observation

Two or three females were allowed to mate with a male of the same strain for 7 days, 2 weeks post surgery. Pregnant mice were transferred to another cage of the same type and housed individually and were checked daily for parturition. Postnatal day (PND) 0 is defined as the day when delivery is complete by 12:00. Based on the report of Champagne et al. [7], maternal behavior observation was conducted for 75 min on PND 0 and 4 between 12:00 and 17:00. The observed maternal behaviors comprise one of the following two types that were shown to improve following administration of apomorphine in a previous study [35]: (1) Mother off pups (duration when mother was apart from her pups) and (2) arched back posture (mother is arching her back with rigid limbs and blankets the pups attached to her nipples).

2.5. Immunohistochemical procedure

The preparation of brains for the measurement of TH levels was conducted within 24 h following delivery. Lactating female mice were anesthetized with pentobarbital Na (50 mg/kg, i.p.) and perfused through the heart with ice-cold phosphate-buffered saline (PBS, pH 7.4), immediately followed by a fixative containing 4% paraformaldehyde (Sigma–Aldrich, Inc., St. Louis, MO, USA) and 0.2% glutaraldehyde (Nacalai Tesque, Osaka, Japan) in PBS. The brain was then postfixed with the same fixative solution at 4 °C for 1 h and then placed in a 10% sucrose-buffered solution at 4 °C for 12 h. The tissue was frozen on dry ice and cut into 20 μm-thick coronal sections (NAc; Bregma, 1.10 mm) on a cryostat (Leitz, Stuttgart, Germany). The immunohistochemical staining procedure was carried out as previously described [27]. Briefly, the rabbit anti-TH antibody (diluted 1:100 with PBS including 0.1% Triton X-100 and 0.05% normal goat serum (NGS); Millipore corporation, Billerica, MA, USA) was applied to each brain slice, which was then incubated at 4 °C for 12 h. The polyclonal antibody to bovine brain TH was produced in rabbit. This TH antibody cross-reacted immunohistochemically with TH from the brains of several species including mice, rats, ferrets and feline. The secondary antibody consisted of FITC-labeled anti-rabbit IgG goat serum (diluted 1:200 with PBS; Millipore corporation), and was allowed to react in the dark at room temperature for 3 h. The stained sections were mounted in 10% glycerin-PBS, and kept at 4 °C in a dark room until measurements were carried out. The distribution of TH immunofluorescence intensities was quantitatively analyzed using a modified brain mapping analyzer system (Yamato Scientific Co., Inc., Tokyo, Japan). The background value, including non-specific fluorescence originating from glutaraldehyde, was subtracted photometrically from the total fluorescence intensity value at each point measured. Immunohistochemical fluorescence intensities obtained for the various regions are relative to that of standard 1 mM quinine sulphate.

2.6. Quantitative receptor autoradiography

Sham and OBX mice were sacrificed by decapitation within 24 h after delivery and their brains were removed and frozen in isopentane at −40 °C and stored at −80 °C. Using a cryostat (Leitz, Stuttgart, Germany), 20 μm-thick coronal sections from the NAc were collected on gelatin-coated slides and stored at −80 °C until use. Dopamine D1- and D2-like receptor binding sites were visualized using [^{3}H]SCH23390 (85.0 Ci/mmol) and [^{3}H]raclopride (62.2 Ci/mmol) respectively (PerkinElmer Life and Analytical Sciences, Boston, MA, USA). The protocol was carried out as previously described [1] with minor modifications. Briefly, sections were preincubated for 15 min in Tris–HCl buffer [Tris 50 (for D1) or 170 (for D2) mM, NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, pH 7.4] at room temperature prior to a 60 min incubation in the same buffer containing either 1 nM [^{3}H]SCH23390 or 3 nM [^{3}H]raclopride. Non-specific binding was determined by inclusion of 1 μM SCH23390 (Sigma–Aldrich, Inc.) or 300 μM sulpiride (Sigma–Aldrich, Inc.) in the assay buffer containing 1 nM [^{3}H]SCH23390 or 3 nM [^{3}H]raclopride, respectively. At the end of the incubation, sections were rinsed four times, 2 min each in ice-cold buffer, then dipped in deionized water to remove salts and rapidly dried. All slides were exposed to a tritium sensitive imaging plate (Fujifilm Corporation, Tokyo, Japan) together with [^{3}H]microscale standards (GE Healthcare UK Ltd., Buckinghamshire, UK) for 10 days for both bindings. Specific labeling was quantified (nCi/mg wet weight) using a computer-assisted Image Reader BAS 5000 ver. 1.12 (Fujifilm Corporation).

2.7. Data analysis

The significance of differences was determined using the Student's t-test for two-group comparison of TH levels and dopamine D1 and D2 receptor on PND 0. Two-way analysis of variance (ANOVA) was used for comparison of the effect of L-DOPA or fluvoxamine on maternal behavior. Post hoc analysis was performed using the Fisher's PLSD test. The criterion of significance was set at $p < 0.05$. All results are expressed as mean ± standard error of the mean (SEM).

3. Results

3.1. Effect of L-DOPA on maternal behavior

A two-way ANOVA revealed a significant effect of group in time mothers spent off pups [group: $F_{(1,60)} = 7.359$, $p = 0.0087$; treatment: $F_{(2,60)} = 2.096$, $p = 0.1318$; group × treatment: $F_{(2,60)} = 2.242$, $p = 0.1151$]. A post hoc test revealed that the duration of time mothers spent off pups in OBX mice was significantly increased compared with the sham group ($p = 0.0022$) and it was reduced following administration of L-DOPA 0.25 mg/kg ($p = 0.0070$) (Fig. 1A). Regarding arched back posture, an ANOVA revealed a significant effect of group and group × treatment [group: $F_{(1,60)} = 9.882$, $p = 0.0026$; treatment: $F_{(2,60)} = 0.831$, $p = 0.4404$;