Behavioural Brain Research 312 (2016) 64–76

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Agmatine attenuates reserpine-induced oral dyskinesia in mice: Role of oxidative stress, nitric oxide and glutamate NMDA receptors

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HIGHLIGHTS

- Orofacial antidyskinetic effects of agmatine in reserpine-treated mice.
- Reserpine increased nitrite and nitrate levels in cerebral cortex of mice.
- Involvement of NMDAR and NO in the orofacial antidyskinetic effects of agmatine.
- Agmatine reversed the reserpine-induced decrease of striatal dopamine and NPSH levels.

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 3 June 2016
Accepted 7 June 2016
Available online 13 June 2016

Keywords:
Agmatine
Reserpine
Orofacial dyskinesia
Oxidative stress
Nitric oxide
NMDA receptors
Dopaminergic system

ABSTRACT

Dyskinesia consists in a series of trunk, limbs and orofacial involuntary movements that can be observed following long-term pharmacological treatment in some psychotic and neurological disorders such as schizophrenia and Parkinson's disease, respectively. Agmatine is an endogenous arginine metabolite that emerges as neuromodulator and a promising agent to manage diverse central nervous system disorders by modulating nitric oxide (NO) pathway, glutamate NMDA receptors and oxidative stress. Herein, we investigated the effects of a single intraperitoneal (i.p.) administration of different agmatine doses (10, 30 or 100 mg/kg) against the orofacial dyskinesia induced by reserpine (1 mg/kg, s.c.) in mice by measuring the vacuous chewing movements and tongue protusion frequencies, and the duration of facial twitching. The results showed an orofacial antidyskinetic effect of agmatine (30 mg/kg, i.p.) or the combined administration of sub-effective doses of agmatine (10 mg/kg, i.p.) with the NMDA receptor antagonists amantadine (1 mg/kg, i.p.) and MK801 (0.01 mg/kg, i.p.) or the neuronal nitric oxide synthase (NOS) inhibitor 7-nitroindazole (7-NI; 0.1 mg/kg, i.p.). Reserpine-treated mice displayed locomotor activity deficits in the open field and agmatine had no effect on this response. Reserpine increased nitrite and nitrate levels in cerebral cortex, but agmatine did not reverse it. Remarkably, agmatine reversed the decrease of dopamine and non-protein thiols (NPSH) levels caused by reserpine in the striatum. However, no changes were observed in striatal immunocontent of proteins related to the dopaminergic system including tyrosine hydroxylase, dopamine transporter, vesicular monoamine transporter type 2, pDARPP-32[Thr75], dopamine D_1 and D_2 receptors. These results indicate that the blockade of NO pathway, NMDAR and oxidative stress are possible mechanisms associated with the protective effects of agmatine against the orofacial dyskinesia induced by reserpine in mice.

1. Introduction

Dyskinesia consists in a series of trunk, limbs and orofacial involuntary movements. This disabling condition could be induced by long-term use of some drugs acting on the dopaminergic system.

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http://dx.doi.org/10.1016/j.bbr.2016.06.014
0166-4328/

For instance, drug-induced dyskinesia can be observed in several psychotic and neurological disorders, such as schizophrenia and Parkinson's disease (PD), following the prolonged use of antipsychotics and levodopa (L-DOPA), respectively [1–3]. Dopaminergic and non-dopaminergic mechanisms are involved in drug-induced dyskinesia with the development of abnormal neuronal plasticity in the striatum and prefrontal cortex [4,5]. In this context, the reserpine-induced orofacial dyskinesia model in rodents has been extensively used in the investigation of behavioral alterations, neurochemical and molecular mechanisms of orofacial dyskinesia [5–8]. This model is characterized by an increase in vacuous chewing movements (VCM), facial twitching (FT), and tongue protrusion (TP) [9,10].

Reserpine presents high affinity for the vesicular monoamine transporter type 2 (VMAT-2) and blocks monoamine binding to its site, inhibiting its vesicular storage and preventing the release of monoamine into the synaptic cleft [11]. Additionally, reserpine promotes dopamine autoxidation and oxidative catabolism by monoamine oxidase (MAO) and these events are related to the oxidative stress process in the brain [12]. Accordingly, the development of orofacial dyskinesia has been associated with oxidative stress, being attenuated by antioxidant compounds [6,7,13,14] and potentiated by pro-oxidant drugs [15]. Moreover, an increase in striatal levels of nitric oxide (NO) also seems to contribute to the development of VCM [15,16]. The NO signaling plays a crucial role in the integration of information transmitted via corticostriatal pathway [17]. The activation of glutamate *N*-methyl-D-aspartate receptor (NMDAR) increases intracellular Ca^{2+} levels and subsequently promotes Ca^{2+}-dependent enzymes activation such as nitric oxide synthase (NOS) [17]. In this context, a previous study observed that MK801, an NMDAR antagonist, reduced the VCM frequency in reserpine-treated mice [18]. Furthermore, a recent study [5] demonstrated that VCM induced by reserpine (1 mg/kg) in mice is associated with alterations in dopaminergic system proteins, including a significant decrease in tyrosine hydroxylase (TH) immunoreactivity and in the activity of MAO-A and MAO-B in the striatum, with no changes in the immunocontent of striatal dopamine transporter (DAT).

In this context, we hypothesized that agmatine may exhibit antidyskinetic effects on orofacial dyskinesia induced by reserpine. This endogenous polyamine is synthesized from L-arginine decarboxylation by arginine decarboxylase (ADC) and is degraded by agmatinase and diamine oxidase enzymes [19]. Considered as a neuromodulator in the central nervous system (CNS), agmatine interacts with many molecular targets, acting as NMDAR antagonist [20–22] and NOS inhibitor [23,24]. The ability of agmatine to act as reactive oxygen species (ROS) scavenger, protecting against oxidative stress-induced mitochondrial dysfunction and apoptosis [25,26] is also remarkable. Finally, agmatine emerges as a potential agent to manage diverse CNS disorders (for review, see [27]), including PD [28–31].

Therefore, the aim of the present study was to investigate possible influence of agmatine on the reserpine-induced orofacial dyskinesia in mice, considering the modulatory action of agmatine on NO synthesis, NMDAR and oxidative stress.

2. Materials and methods

2.1. Animals

Male *Swiss* mice (10–12 weeks, 40–50 g) acquired from the central animal facility of Federal University of Santa Catarina (UFSC) were kept in cages of 49 × 34 × 16 cm (15 animals per cage) and maintained in a room under controlled temperature (23 ± 2 °C) and 12 h light-dark cycle (light phase at 7:00 h to 19:00 h) with free access to water and food. All experimental procedures were carefully conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Ethics Committee on Animal Use of UFSC (CEUA PP830/2012 – UFSC).

2.2. Drugs

Reserpine, agmatine sulfate, amantadine hydrochloride and 7-nitroindazole (7-NI) were obtained from Sigma Chemical Co. (USA), and (+)-MK801 (dizocilpine maleate) was purchased from Research Biochemicals (USA). Reserpine was dissolved in vehicle consisting in a glacial acetic acid solution 0.5%, whereas 7-NI was dissolved in dimethyl sulfoxide (DMSO) 1%. The vehicle used for other drugs was NaCl 0.9%.

2.3. Experimental design

In all experiments, orofacial dyskinesia was induced in mice through two subcutaneous (s.c.) reserpine injections (1 mg/kg) administered with an interval of 48 h. Twenty-four hours after the last reserpine administration, mice were treated by intraperitoneal (i.p.) route with agmatine alone or in combination with amantadine, 7-NI or MK801 and, 30 min after the drug treatments, orofacial dyskinesia was evaluated (Fig. 1).

Firstly, a possible agmatine orofacial antidyskinetic effect was assessed at doses of 10, 30 and 100 mg/kg (i.p.) by measuring the VCM and TP frequencies, and the duration of FT. In order to investigate the role of NMDAR in the agmatine orofacial antidyskinetic effect, the efficacy of different doses of the NMDAR antagonists amantadine (1, 10, 40 and 60 mg/kg, i.p.) and MK801 (0.01, 0.03, 0.1 and 0.3 mg/kg, i.p.) in reducing VCM frequency was evaluated. Then, sub-effective doses of amantadine (1 mg/kg) and MK801 (0.01 mg/kg) were administered alone or in combination with a sub-effective dose of agmatine (10 mg/kg).

The involvement of nitrergic system in the agmatine orofacial antidyskinetic effect was also investigated. Initially, we evaluated the effects of different doses of 7-NI (0.1, 1 and 10 mg/kg, i.p.) on the VCM frequency. Thereafter, a sub-effective dose of 7-NI (0.1 mg/kg) was administered alone or in combination with a sub-effective dose of agmatine (10 mg/kg) 30 min before VCM frequency evaluation.

Additional experiments addressing putative changes on locomotor activity of mice induced by reserpine (1 mg/kg, s.c.) and/or agmatine (30 mg/kg, i.p.) were also performed in the open field test immediately after the VCM frequency evaluation, followed by brain dissection for biochemical analysis. The animals were killed by decapitation immediately after the behavioral tests. Prefrontal cortex, cerebral cortex and/or striatum were dissected in order to evaluate oxidative stress parameters and nitrite and nitrate levels using the Griess assay, which indirectly measures the amount of NO in these brain structures. In the striatum, the evaluation of the levels of dopamine was performed by ultra performance liquid chromatography-mass spectrometry (UPLC–MS) and the immunocontent of proteins related to the dopaminergic system was assessed by western blot.

2.4. Behavioral tests

2.4.1. Orofacial dyskinesia

Orofacial dyskinesia parameters were evaluated as described by Burger et al. [13] with some modifications. The evaluation of VCM frequency consists in a manual counting of continuous single mouth openings in a vertical plane, not directed to a physical material. The TP frequency is defined as the number of an individual movement of visible extension followed by tongue retraction outside of the mouth and not directed at anything. If TP occurred during