European Journal of Pharmacology 732 (2014) 26–31

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural pharmacology

Imidazoline binding sites mediates anticompulsive-like effect of agmatine in marble-burying behavior in mice

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ARTICLE INFO

Article history:
Received 13 August 2013
Received in revised form
4 February 2014
Accepted 23 February 2014
Available online 19 March 2014

Keywords:
Agmatine
Marble burying behavior
Imidazoline binding sites
Obsessive compulsive disorder

ABSTRACT

Agmatine is a cationic amine formed by decarboxylation of L-arginine by the mitochondrial enzyme arginine decarboxylase and widely distributed in mammalian brain. Although the precise function of endogenous agmatine has been largely remained unclear, its exogenous administration demonstrated beneficial effects in several neurological and psychiatric disorders. This study was planned to examine the role of imidazoline binding sites in the anticompulsive-like effect of agmatine on marble-burying behavior. Agmatine (20 and 40 mg/kg, ip), mixed imidazoline I_1/α_2 agonists clonidine (60 μg/kg, ip) and moxonidine (0.25 mg/kg, ip), and imidazoline I_2 agonist 2- BFI (10 mg/kg, ip) showed significant inhibition of marble burying behavior in mice. In combination studies, the anticompulsive-like effect of agmatine (10 mg/kg, ip) was significantly potentiated by prior administration of moxonidine (0.25 mg/kg, ip) or clonidine (30 μg/kg,) or 2-BFI (5 mg/kg, ip). Conversely, efaroxan (1 mg/kg, ip), an I_1 antagonist and idazoxan (0.25 mg/kg, ip), an I_2 antagonist completely blocked the anticompulsive-like effect of agmatine (10 mg/kg, ip). These drugs at doses used here did not influence the basal locomotor activity in experimental animals. These results clearly indicated the involvement of imidazoline binding sites in anti-compulsive-like effect of agmatine. Thus, imidazoline binding sites can be explored further as novel therapeutic target for treatment of anxiety and obsessive compulsive disorders.

1. Introduction

Obsessive compulsive disorder (OCD) is characterized by recurrent and persistent thoughts, impulses or images (obsessions), and/or repetitive, seemingly purposeful behaviors (compulsions), e.g., doubting, checking and washing (Rasmussen and Eisen, 1992; Sasson et al., 1997). Although OCD is classified as an anxiety disorder, patients with OCD demonstrate a high incidence of comorbid depression (Sasson et al., 1997). The first line therapy of OCD includes selective serotonin reuptake inhibitors (SSRIs) to which 40–60% of the patients did not respond satisfactorily (Pallanti and Quercioli, 2006). Refractory patients, however respond to antidopaminergics and N-methyl-D-aspartate (NMDA) receptor antagonists (Denys, 2006), suggesting that multiple neurotransmitters are probably involved in the regulation of compulsive behavior.

Agmatine [4- (amino butyl) guanidine] is an endogenous amine, widely present in mammalian brain and proposed as a novel neurotransmitter in the central nervous system (Li et al., 1994; Reis and Regunathan, 2000). It is a metabolite of L-arginine via arginine decarboxylase and hydrolyzed to putrescine and urea by agmatinase (Reis and Regunathan, 2000; Halaris and Piletz, 2007). Besides its function to regulate formation of intracellular polyamines, agmatine has been ascribed roles in several biological processes like neuroprotection (Olmos et al., 1999), chronic pain (Onal et al., 2004; Kotagale et al., 2013), epilepsy (Bence et al., 2003), stress (Zhu et al., 2008), depression (Zomkowski et al., 2002), schizophrenia (Kotagale et al., 2012) and modulation of addictive behavior (Kotagale et al., 2010; Taksande et al., 2010). The localization of agmatine like immunoreactivity has been demonstrated in several brain regions implicated in the regulation of anxiety-like behavior including amygdala (Otake et al., 1998). Moreover, numerous studies have demonstrated its anxiolytic profile in rodents (Lavinsky et al., 2003; Gong et al., 2006). Likewise, agmatine was also effective in the marble-burying paradigm and decreased the number of marbles buried (Krass et al., 2010). However, the exact mechanism of its anxiolytic action has largely remained elusive.

Agmatine is a biologically active substance and considered as an endogenous ligand at I_1/I_2 imidazoline binding sites. Brain regions that regulate endocrine and affective functions have abundant imidazoline binding sites and their endogenous ligands

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http://dx.doi.org/10.1016/j.ejphar.2014.02.045
0014-2999/

are colocalized at these sites (De Vos et al., 1994; Raasch et al., 1995). Imidazoline binding sites are the unique non-adrenergic high affinity binding sites that exist in three major subclasses (I_1, I_2 and I_3) based upon their ligand selectivity, subcellular distribution and physiological functions (Bousquet et al., 2000; Santos et al., 2005). In human brain, I_1 sites are distributed in a regional manner with highest density in hippocampus, amygdala and susbstantia nigra (De Vos et al., 1994). The imidazoline I_2 binding sites (I_{2A} and I_{2B}) are allosteric, widely distributed in brain and located on monoamine oxidase (MAO) (Raddatz et al., 1997; Eglen et al., 1998). It is important to note that several of behavioral effects of agmatine are said to be mediated through its interaction with imidazoline binding sites (Zeidan et al., 2007). In present investigation, we attempted to examine the role of imidazoline binding sites agents in the anticompulsive-like effect of agmatine on marble-burying behavior (MBB).

2. Materials and methods

2.1. Subjects

Male Swiss albino mice (20–25 g) were group housed in perspex cages (five per cage) maintained on a 12 h light/ dark cycle (lights on at 07.00 h) in a room at controlled temperature (24 ± 1 °C) with free access to food pellets (Hindustan Lever Ltd., Mumbai) and water. Animals were handled and acclimatized to laboratory conditions at least 12 h prior to experiment. All experiments were conducted between 0900 and 1500 h. The experiments were executed in strict accordance with the ethical principles and guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Govt. of India and approved by the Institutional Animal Ethical Committee.

2.2. Drug solutions and administration

Agmatine sulfate, moxonidine hydrochloride, clonidine hydrochloride, efaroxan hydrochloride and idazoxan hydrochloride were purchased from Sigma-Aldrich Co., USA. While 2-(2-Benzofuranyl)-2-imidazoline hydrochloride (2-BFI) was purchased from Tocris Biosciences, UK. All the drugs were dissolved in 0.9% w/v saline and administered by intraperitoneal (ip) route.

2.3. Marble-burying behavior (MBB)

MBB is considered to be a potential model of OCD based on behavioral similarity (Njunge and Handley, 1991; Ichimaru et al., 1995; Londei et al., 1998). The MBB test was performed as described previously (Witkin, 2008). Briefly, mice were placed individually in plastic cages (17 cm × 28 cm × 12 cm) containing 5 cm thick sawdust bedding. Twenty blue glass marbles were arranged on the bedding, evenly spaced in 4 rows of 5 each. Mice were exposed to marbles for 30 min without food and water; thereafter, the unburied marbles were counted. A marble was considered 'buried' if two-thirds of its size was covered with sawdust. The number of marbles buried was considered as an index of compulsive behavior. The number of marbles buried during 30 min was analyzed by observers who were blind to treatment groups. All the drugs were given intraperitoneally 30 min before the test; agonist or antagonists were given 15 min before agmatine and 30 min thereafter individual mouse was subjected MBB test.

2.4. Dose specific effects of agmatine and imidazoline agents on MBB

This experiment examined the dose dependent effect of agmatine, moxonidine, clonidine, 2BFI, efaroxan and idazoxan on MBB. Different groups of mice ($n=6$) were administered with agmatine (10–40 mg/kg, ip) or moxonidine (0.25–1.0 mg/kg, ip), clonidine (15–60 μg/kg, ip), 2-BFI (5–15 mg/kg, ip), efaroxan (1–2 mg/kg, ip) and idazoxan (0.25–1.0 mg/kg, ip) or saline (1 ml/kg, ip). 30 min after drug treatment each mouse was evaluated for MBB for 30 min.

2.5. Modulation of anticompulsive-like effect of agmatine by imidazoline stimulation or blockade

Different groups of mice were pretreated with imidazoline I_1 agonist, moxonidine (0.25 mg/kg, ip) or clonidine (30 μg/kg, ip) or imidazoline I_2 agonist, 2-BFI (10 mg/kg, ip) or saline (1 ml/kg, ip) 15 min prior to subeffective dose of agmatine (10 mg/kg, ip) or saline (1 ml/kg, ip) and the anticompulsive-like effect was determined for 30 min test session in MBB. Similarly in another set of experiment, mice were injected with imidazoline I_2 antagonist, efaroxan (2 mg/kg, ip) or imidazoline I_2 antagonist, idazoxan (1 mg/kg, ip) or saline (1 ml/kg, ip) 15 min prior to administration of effective dose of agmatine (20 mg/kg, ip) and 30 min thereafter animals were subjected to MBB test. The doses of different agents used in the present study were selected from available literature (Krass et al., 2010; Taksande et al., 2009) and based upon our preliminary experiments.

2.6. Locomotor activity in mice

This test was performed to assess whether drug effect on MBB was associated with changes in motor activity. Locomotor activity was measured using actophotometer (20 cm × 20 cm × 10 cm) (Techno, India) equipped with six infrared photo sensors, 2.5 cm apart from each other. Mice were habituated to the actophotometer chamber for 30 min before any testing. Baseline locomotor activity of each mouse was recorded for 20 min as a total count of ambulatory, horizontal and vertical activity 30 min after drug administration. Animals were used only once and after each test the actophotometer grid floor was carefully cleaned. The doses and treatment schedules were identified as described earlier.

2.7. Data analysis

Data presented here was analyzed by one way analysis of variance (ANOVA) followed by post hoc Dunnett or Bonferroni multiple comparison test. Data was expressed as a mean ± S.E.M and value of $P < 0.05$ was considered to be statistically significant in all the cases.

3. Results

3.1. Anticompulsive-like effect of agmatine on marble burying behavior

Administration of agmatine (20–40 mg/kg, ip) to mice (Fig. 1) produced dose dependent anticompulsive-like effect as evident by significant decrease in the marble burying behavior as compared to saline treated animals [F (3, 23) = 13.62, $P < 0.001$]. Post hoc Dunnett mean comparison revealed that agmatine 20 mg/kg ($P < 0.05$) and 40 mg/kg ($P < 0.001$) had significant effect on marble burying behavior. However its lower dose (10 mg/kg) failed to alter MBB in mice.