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Evidences for the agmatine involvement in antidepressant like effect of bupropion in mouse forced swim test

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ABSTRACT

Although bupropion has been widely used in the treatment of depression, the precise mechanism of its therapeutic actions is not fully understood. The present study investigated the role of agmatine in an antidepressant like effect of bupropion in mouse forced swim test.

The antidepressant like effect of bupropion was potentiated by pretreatment with agmatine (10–20 mg/kg, ip) and by the drugs known to increase endogenous agmatine levels in brain viz., L-arginine (40 μg/mouse, icv), an agmatine biosynthetic precursor, ornithine decarboxylase inhibitor, DL-α-difluoromethyl ornithine hydrochloride, DFMO (12.5 μg/mouse, icv), diamine oxidase inhibitor, aminoguanidine (6.5 μg/mouse, icv) and agmatinase inhibitor, arcaine (50 μg/mouse, icv) as well as imidazoline I_1 receptor agonists, moxonidine (0.25 mg/kg, ip) and clonidine (0.015 mg/kg, ip) and imidazoline I_2 receptor agonist, 2-(2-benzofuranyl)-2-imidazoline hydrochloride, 2-BFI (5 mg/kg, ip). Conversely, prior administration of I_1 receptor antagonist, efaroxan (1 mg/kg, ip) and I_2 receptor antagonist, idazoxan (0.25 mg/kg, ip) blocked the antidepressant like effect of bupropion and its synergistic combination with agmatine. These results demonstrate involvement of agmatine in the antidepressant like effect of bupropion and suggest agmatine and imidazoline receptors as a potential therapeutic target for the treatment of depressive disorders.

1. Introduction

Bupropion is categorized as an atypical antidepressant because its neurotransmitter effects differ from all other antidepressants (Piacentini et al., 2003). Bupropion acts via dual inhibition of norepinephrine (NE) and dopamine (DA) uptake and is devoid of clinically significant serotonergic effects or direct effects on postsynaptic receptors (Kavoussi et al., 1997). However, bupropion is as effective as other antidepressants including the selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressant (TCAs) (Chouinard, 1983; Ferris and Cooper, 1993) without causing common antidepressant associated side effects. Therefore bupropion is considered as an antidepressant with unique pharmacologic properties with distinct tolerability (Stahl et al., 2004). However, the precise mechanism as to how bupropion exerts antidepressant activity is still uncertain. In recent years, central agmatine has gained much attention as potential therapeutic target for CNS disorders including neurological and psychiatric disorders (Reis and Regunathan, 2000; Halaris and Piletz, 2001). Agmatine, a cationic amine, is recognized as an important neuromodulator and neurotransmitter in brain region that mediates emotional behaviors and response to stress (Reis and Regunathan, 2000; Zhu et al., 2008). Agmatine acts on α_2-adrenoceptor and I_1/I_2 imidazoline receptors, blocks N-methyl-D-aspartate (NMDA) receptors as well as other ligand gated ion channels and inhibits NOS, responsible for NO formation in brain (Yang and Reis, 1999; Reis and Regunathan, 2000; Raasch et al., 2001). Exogenously administered agmatine in rodents not only produce antidepressant like effect (Zomkowski et al., 2002; Li et al., 2003; Jiang et al., 2008) but also a range of central effects (Onal et al., 2004; Lavinsky et al., 2003; Bence et al., 2003; Olmos et al., 1999; Kolesnikov et al., 1996; Aricioglu-Kartal and Uzbay, 1997; Li et al., 1998; Wu et al., 2007). Physiological roles in CNS and therapeutic potential of agmatine have been recently reviewed in a comprehensive paper (Molderings and Haenisch, 2011; Uzbay, 2012). Several studies have shown that major depression is associated with dysregulation of agmatine binding imidazoline recognition sites in the human brain (Garcia-Sevilla et al., 1996, 1998; Halaris and Piletz, 2001; Piletz et al., 2008; Sastre et al., 1995; Piletz et al., 1994, 2000). Moreover, Bernstein et al. (2012) have shown the upregulation of agmatine-degrading enzyme, agmatinase in patients with unipolar and bipolar depression suggesting that reduction of the endogenous brain agmatine plays a central role in the pathogenesis of the mood disorders. These evidences strongly support the role of

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agmatine as a relevant endogenous antidepressant in mammalian brain (Aricioglu and Altunbas, 2003). Clinical studies have demonstrated normalized plasma agmatine levels and I_1 binding sites in platelets of depressed patients chronically treated with bupropion (Piletz et al., 1996a,b; Garcia-Sevilla et al., 1996; Zhu et al., 1999; Halaris et al., 2002).

In light of these reports we expect that agmatine by an interaction with imidazoline receptors might also influence antidepressant like effect of bupropion. Therefore we tested whether exogenously administered agmatine or selective modulation of agmatine homeostasis as well as imidazoline receptors influence the antidepressant like effect of bupropion in mouse forced swim test (FST).

2. Materials and methods

2.1. Subjects

Male Swiss albino mice (20–25 g body weight) 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, DL-α-difluoromethyl ornithine hydrochloride (DFMO), aminoguanidine hemisulfate, arcaine sulfate, efaroxan hydrochloride, idazoxan hydrochloride, and L-arginine monohydrochloride were purchased from Sigma-Aldrich Co., USA. 2-(2-Benzofuranyl)-2-imidazoline hydrochloride (2-BFI) was purchased from Tocris Biosciences, UK. Bupropion was received as gift sample from Sun Pharmaceuticals, Vadodara, India. Agmatine, moxonidine, clonidine, 2-(2-benzofuranyl)-2-imidazoline, efaroxan, and idazoxan were dissolved in 0.9% w/v saline and administered by intraperitoneal (ip) route. DFMO, arcaine, aminoguanidine and L-arginine were injected by intracerebroventricular (icv) route to alter the levels of brain agmatine and avoid peripheral effects. For icv administration of drugs, dilutions were made with artificial cerebrospinal fluid (aCSF) (composition — 0.2 M NaCl, 0.02 M NaH_2CO_3, 2 mM KCl, 0.5 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.8 mM $MgCl_2$, 0.5 mM Na_2SO_4 and 5.8 mM D-glucose. Doses employed in the protocols were selected on the basis of our preliminary experiments and available literature (Taksande et al., 2009, 2010; Kotagale et al., 2010).

2.3. Intracerebroventricular cannulations and verification

For icv administration of drugs, mice were anaesthetized with pentobarbital sodium (60 mg/kg, ip) and unilateral cannula was implanted stereotaxically (David Kopf Instruments, CA, USA) as described earlier (Taksande et al., 2009). Briefly, 28 gauge stainless steel guide cannula (C315 G/Spc, Plastic One Inc., Virginia, USA) was implanted into right lateral ventricle [coordinates: AP 0.22 mm; ML 1 mm and DV 2.5 mm; relative to bregma, Paxinos and Franklin, 1997] and secured in place by dental cement (Dental Products of India, Mumbai) affixed to two stainless steel screws. A stainless steel dummy cannula was inserted to occlude the guide cannula when not in use. The animals were then housed individually and allowed to recover for 1 week, before being tested in FST. Oxytetracycline injection (25 mg/kg, im, Pfizer Ltd., Chennai) was given and neosporin ointment (Burroughs Wellcome Ltd., Mumbai) was applied topically for 3 days post surgery to avoid infection. During this period animals were habituated to the experimental protocols to minimize nonspecific stress.

Mice were then assigned to different treatment groups and injections (2 μl/mouse) were made into right lateral ventricle over 1 min period with microliter syringe (Hamilton, Nevada, USA) connected to 30 gauge internal cannula (C315 I/spc) by polyethylene tubing. The injection cannula was left in place for further 1 min before being slowly withdrawn to avoid back flow. At the end of all icv experiments, dilute India ink (5 μl/mouse) was injected by icv route and animals were euthanized by an overdose of pentobarbital sodium. Immediately, the brain was dissected out and the cannula placement was verified histologically by distribution of dilute India ink in the ventricle. In some animals (<15%), guide cannula was found to be placed incorrectly and hence excluded from the study. Data from only those animals showing uniform distribution of ink into lateral ventricle was used for statistical analysis.

2.4. Forced swim test

The procedure was quite similar to that described by Porsolt et al. (1977) except that mice were subjected to a “pretest session” to maintain consistency in the basal immobility time between different groups. Briefly, mice were placed individually in plexiglass cylinders (21 cm height 12 cm internal diameter) containing fresh water up to a height of 9 cm at 25 ± 1 °C and forced to swim for 15 min. Twenty four hours later, the animals were randomly divided into different groups and treated with either drug (test group) or vehicle (control group). Each mouse was again forced to swim in a similar environment for the period of 6 min in a “test session” and immobility time was measured by the trained observer blind to the treatment. A mouse was judged to be immobile when it remained floating motionless in the water, making only necessary movements to keep its head above water. Each mouse was used only once in test session. Reduction in the duration of immobility was considered as an antidepressant like effect of the drug. Forced swim tests were conducted 30 min after the administration of drugs.

2.5. Dose specific effects of bupropion, agmatine and their combination in FST

This experiment examined the dose dependent effect of bupropion and agmatine on immobility time in FST. Different groups of mice (n = 6) were administered with bupropion (5–20 mg/kg, ip) or agmatine (5–20 mg/kg, ip) or saline. In combination studies subeffective dose of agmatine (5 mg/kg, ip) was administered 15 min before bupropion (5 mg/kg, ip) or saline (1 ml/kg, ip).

2.6. Effect of agmatine modulators on antidepressant like effect of bupropion

We examined the influence of drugs which either stimulate agmatine synthesis or prevent its catabolism leading to increased brain agmatine levels. Different groups of mice (n = 6) were injected with L-arginine (40 μg/mouse, icv); biosynthetic precursor of agmatine or aminoguanidine (6.25 μg/mouse, icv), a diamine oxidase inhibitor (DAO); arcaine (50 μg/mouse, icv), an agmatinase inhibitor or DFMO (12.5 μg/mouse, icv), ornithine decarboxylase inhibitor or aCSF (2 μl/mouse, icv) 15 min before subeffective dose of bupropion (5 mg/kg, ip) or saline (1 ml/kg, ip). Thirty minutes later each mouse was subjected to FST for 6 min to monitor immobility duration.