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Antidepressant like effect of selective serotonin reuptake inhibitors involve modulation of imidazoline receptors by agmatine

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ABSTRACT

Recent findings demonstrated the dysregulation of imidazoline receptor binding sites in major depression and their normalization by chronic treatment with antidepressants including selective serotonin reuptake inhibitors (SSRIs). Present study investigated the role of agmatine and imidazoline receptors in antidepressant like effect of SSRIs and imipramine in mouse forced swimming test (FST) paradigm. The antidepressant like effect of fluoxetine or paroxetine was potentiated by imidazoline I_1/I_2 receptor agonist agmatine (5–10 mg/kg, ip), imidazoline I_1 receptor agonists, moxonidine (0.25–0.5 mg/kg, ip) and clonidine (0.015–0.03 mg/kg, ip), imidazoline I_2 receptor agonist, 2-(2-benzofuranyl)-2-imidazoline (5–10 mg/kg, ip) as well as by the drugs known to increase endogenous agmatine levels in brain viz., L-arginine, an agmatine biosynthetic precursor (40 μg/mouse, icv), ornithine decarboxylase inhibitor, difluoromethyl ornithine (12.5 μg/mouse, icv), diamine oxidase inhibitor, aminoguanidine (6.5 μg/mouse, icv) and agmatinase inhibitor, arcaine (50 μg/mouse, icv). Conversely, prior administration of I_1 receptor antagonist, efaroxan (1 mg/kg, ip), I_2 receptor antagonist, idazoxan (0.25 mg/kg, ip) and arginine decarboxylase inhibitor, D-arginine (100 mg/kg, ip) blocked the antidepressant like effect of paroxetine (10 mg/kg, ip) and fluoxetine (20 mg/kg, ip). On the other hand, antidepressant like effect of imipramine was neither augmented nor attenuated by any of the above drugs. Mice pretreated with SSRIs but not imipramine and exposed to FST showed higher concentration of agmatine in brain as compared to saline control. This effect of SSRIs on agmatine levels was completely blocked by arginine decarboxylase inhibitor D-arginine but not by imidazoline receptor antagonists, efaroxan or idazoxan. These results demonstrate that modulation of imidazoline receptors by agmatine are implicated in the antidepressant like effect of SSRIs and may be projected as a potential therapeutic target for the treatment of depressive disorders.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, paroxetine and citalopram are widely used in the treatment of psychiatric disorders including depression, obsessive compulsive disorder, panic disorder and several other conditions (Eriksson et al., 1995; Steiner et al., 1995; Su et al., 1997; Masand et al., 2005; Duman et al., 2006). SSRIs act by inhibiting brain serotonin transporters (SERT) leading to enhanced serotonergic transmission in the several subcortical regions (Stanford, 1996; Gourion et al., 2004). However, the mechanism through which they exert antidepressant effect remains uncertain (Gourion et al., 2004). Indeed, it has been argued that antidepressive efficacy of SSRIs may involve modulation of other neurotransmitters and/or receptor systems along with their direct effect on serotonergic signaling (Stanford, 1996; Tunnicliff et al., 1999; Gourion et al., 2004). There is accumulative evidence that favors the involvement of imidazoline receptor binding sites in the pathophysiology of depression (Garcia-Sevilla et al., 1996, 1998; Piletz et al., 1996a, 2008; Zhu et al., 1999; Halaris and Piletz, 2001; Holt, 2003) and these sites have been proposed as an novel therapeutic targets for the treatment of depressive disorders (Holt, 2003).

Imidazoline receptors 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 (Michel and Insel, 1989; Michel and Ernsberger, 1992; Parini et al., 1996; Eglen et al., 1998; Bousquet et al., 2000; Santos et al., 2005). In human brain, I_1 sites are distributed in a regional manner with highest density in striatum, pallidum and gyrus dentatus of 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

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oxidase (MAO) (Tesson et al., 1991; Raddatz et al., 1997, 2000; Eglen et al., 1998) an enzyme that causes oxidative deamination of neurotransmitters like serotonin (5-hydroxy tryptamine, 5-HT), noradrenaline, dopamine and exogenous amines. Several imidazoline I_2 ligands have been shown to inhibit MAO activity (Carpene et al., 1995; Raasch et al., 1999; Jones et al., 2007) and chronic treatment with MAO inhibitors (like clorgyline and pargyline) downregulate I_2 receptor density (Alemany et al., 1995). Previous studies have shown that major depression is associated with dysregulation of imidazoline receptors (Garcia-Sevilla et al., 1996, 1998; Halaris and Piletz, 2001; Piletz et al., 2008, 1994; Sastre et al., 1995) in human brain. Meanwhile, some studies in human have demonstrated lowered or normalized I_1 binding sites in platelets of depressed patients treated with either of antidepressants imipramine, desipramine, fluoxetine, citalopram, clomipramine (Piletz et al., 1996a,b; Garcia-Sevilla et al., 1996) or bupropion (Zhu et al., 1999; Halaris et al., 2002). However, none of these antidepressants interact directly with I_1 imidazoline receptors at therapeutic concentration (Holt, 2003). Subsequently, numerous imidazoline I_1/I_2 ligands like agmatine, BU-224 (2-(4, 5-dihydroimidazole-2-yl) quinoline hydrochloride), harmane and β-carboline have been reported to exert antidepressant like activity in rodents (Abramets and Dolzhenko, 1986; Adell et al., 1996; Finn et al., 2003; Farzin and Mansouri, 2006; Zomkowski et al., 2002). Recently, agmatine a novel neurotransmitter has gained attention in depressive disorders. Agmatine is synthesized following decarboxylation of L-arginine by arginine decarboxylase (ADC) in brain and other tissues. L-arginine is also converted into ornithine and nitric oxide by enzyme arginase and nitric oxide synthase, respectively (Reis and Regunathan, 2000). Ornithine subsequently converted into putrescine by L-ornithine decarboxylase. Agmatine is also metabolized to putrescine and guanido-butanoic acid by an enzyme agmatinase and diamine oxidase respectively (Reis and Regunathan, 2000; Lu et al., 2003; Huang et al., 2003; Regunathan, 2006).

Besides imidazoline receptors, agmatine also binds to α_2-adrenoceptors, N-methyl D-aspartate (NMDA) receptors as well as 5-HT receptors with lower affinity and inhibit nitric oxide synthase (NOS) in brain as well (Yang and Reis, 1999; Reis and Regunathan, 2000; Raasch et al., 2001). Exogenous administration of agmatine have been shown to produce not only antidepressant effect (Zomkowski et al., 2002; Li et al., 2003) but also possesses antinociceptive (Onal et al., 2004), anxiolytic (Lavinsky et al., 2003), anticonvulsant (Bence et al., 2003), antiinflammatory (Satriano et al., 2001), antiproliferative (Regunathan and Reis, 1997), neuroprotective (Olmos et al., 1999) properties and modulates morphine tolerance or dependence (Kolesnikov et al., 1996; Aricioglu-Kartal and Uzbay, 1997; Li et al., 1998; Wu et al., 2007). It is worth noting that agmatine induced effects including antidepressant action are said to be mediated through its selective interaction with imidazoline receptors (Zeidan et al., 2007).

Thus, it is a matter of investigation whether antidepressant like effect of SSRIs involve imidazoline receptors. It is speculated that SSRIs may modulate release of some endogenous mediators to interact with imidazoline I_1 and I_2 receptors. In this study, we determined the effect of imidazoline receptor agents and drugs that alter the brain levels of agmatine on the antidepressant like effect of SSRIs (fluoxetine and paroxetine) and prototype typical antidepressant (imipramine) using mouse forced swimming 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. Every possible effort was made to reduce the suffering of animals.

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, L-arginine monohydrochloride and D-arginine monohydrochloride were purchased from Sigma–Aldrich Co., USA. 2-(2-benzofuranyl)-2-imidazoline hydrochloride (2-BFI) was purchased from Tocris Biosciences, UK. Fluoxetine, imipramine (Sun Pharmaceuticals, Vadodara, India) and paroxetine (Dr. Reddy's Laboratory, Hyderabad, India) were received as gift samples.

Agmatine, moxonidine, clonidine, 2-(2-benzofuranyl)-2-imidazoline, efaroxan, idazoxan and D-arginine were dissolved in 0.9% 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) of following 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 (Cervo and Samanin, 1991; Lu et al., 2003; Su et al., 2003; MacInnes and Handley, 2003; Inan et al., 2004; Gentili et al., 2006; Zeidan et al., 2007; Velloso et al., 2008).

2.3. Intracerebroventricular administration

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 (Hirani et al., 2002; Ugale et al., 2004). 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 used 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 ($n = 6$–8) and injections (5 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 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 (<20%), 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 swimming test (FST)

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 upto 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 (6-8 animals/group) and treated with either a 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 antidepressant like effect of the drug. Forced swimming tests were conducted 30 min after the administration of drugs.