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Psychopharmacological study of agmatine in behavioral tests of schizophrenia in rodents

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ABSTRACT

The effect of agmatine in preclinical behavioral tests of schizophrenia has been examined in rodents. Agmatine at the doses of 40 and 80 mg/kg blocked conditioned avoidance responding, attenuated apomorphine induced climbing, diminished amphetamine and ketamine hyperlocomotor activity and augmented plasma prolactin levels. Pretreatment of animals with 20 mg/kg of agmatine potentiated the inhibitory effect of haloperidol (0.1 mg/kg, ip) and olanzepine (0.5 mg/kg, ip) in conditioned avoidance response test and apomorphine induced climbing. Agmatine alone at the doses tested here did not induce any cataleptic behavior in mice. However significant catalepsy was exhibited when agmatine (80 mg/kg, ip) was injected to mice pretreated with 5-HT1A receptor antagonist, WAY100, 635. These results indicate that agmatine via regulation of brain dopaminergic signaling modulates dopamine mediated behaviors. The alteration in the levels of endogenous agmatine may contribute to the genesis of psychosis and development of drugs that enhance endogenous agmatine content may be better therapeutic approach to treat schizophrenia with low incidences of extra pyramidal side effects.

1. Introduction

Schizophrenia is a neurodevelopmental brain disorder with complicated pathophysiology involving many biochemical abnormalities in brain. Although dysregulation of dopamine (DA) in mesocorticolimbic system has been predominant in schizophrenia, other neurotransmitter systems including glutamate, serotonin, GABA, noradrenaline and acetyl choline have also been investigated extensively (Alsene et al., 2010; Dencker et al., 2011; Meltzer and Massey, 2011; Rotaru et al., 2011). However precise understanding of schizophrenia pathogenesis has remained elusive. Recent preclinical data suggested that agmatine, an endogenous neurotransmitter could be promising mechanism implicated in its pathogenesis (Uzbay, 2009; Uzbay et al., 2010a).

Agmatine is an endogenous amine derived from decarboxylation of L-arginine by arginine decarboxylase. It is considered as a neurotransmitter and/or neuromodulator in mammalian central nervous system being synthesized, stored in synaptic vesicles, accumulated by uptake, released by depolarization and inactivated by agmatinase (Halaris and Plietz, 2007; Reis and Regunathan, 2000). Agmatine activates both I_1/I_2 imidazoline and α_2-adrenergic receptors, blocks N-methyl-D-aspartic acid (NMDA) receptors (Halaris and Plietz, 2007; Reis and Regunathan, 2000; Yang and Reis, 1999) and inhibits all isoforms of enzyme, nitric oxide synthase (NOS) (Auguet et al., 1995). In animal studies injected agmatine possesses neuroprotective (Olmos et al., 1999), antistress (Zhu et al., 2008), antidepressant (Aricioglu and Altunbas, 2003; Zomkowski et al., 2002), antinociceptive (Onal et al., 2004), anxiolytic (Lavinsky et al., 2003), anticonvulsant (Bence et al., 2003) properties and modulates drug addiction processes (Aricioglu-Kartal and Uzbay, 1997; Kotagale et al., 2010; Uzbay et al., 2000).

Agmatine has received considerable attention as possible target for development of new antipsychotics (Palsson et al., 2008; Uzbay et al., 2010a). However, overall these studies have given mixed results in animal models relevant to schizophrenia. For example, although agmatine per se did not alter prepulse inhibition (PPI) response, its pretreatment attenuated the disruptive effect of noncompetitive NMDA antagonist, phencyclidine (PCP) on PPI in mice (Palsson et al., 2008). In contrast, recent study clearly showed disruption of prepulse inhibition of acoustic startle reflex by agmatine in rats (Uzbay et al., 2010a). Stimulation of DA activity is critical for reduction in PPI induced by amphetamine, other dopamine agonists and NMDA antagonists (Swerdlow et al., 1993, 1995). Converging evidence has proposed a link between DA dysfunction and nitric oxide (NO) in psychiatric disorders like schizophrenia (Bernstein et al., 2005). Genetic linkage studies revealed that polymorphisms in the neuronal NOS gene confer increased susceptibility to schizophrenia (Reif et al., 2006). There is also evidence for increased NO in plasma of schizophrenic patients (Yilmaz et al., 2007) and beneficial effect of NOS inhibitors in schizophrenia animal models including PPI (Issy et al., 2011). Owing to ability of agmatine to inhibit all isoforms of NOS (Auguet et al., 1995) it may represent a new approach in pharmacotherapy of schizophrenia. However the issue is

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complicated by experimental (Black et al., 1999) and clinical (Bernstein et al., 2005) data indicating ineffective or adverse effect of NOS inhibitors in schizophrenia. Thus, the implication of agmatine in pathogenesis and/or control of schizophrenia warrants further investigations.

Hence the central purpose of this study was to assess the behavioral profile agmatine in tests like apomorphine induced climbing in mice, conditioned avoidance response in rats, and hyperlocomotion produced of D-amphetamine and ketamine that are widely used - as animal models of schizophrenia. Lastly, agmatine was evaluated in animal model predictive of unwanted side effect such as the catalepsy test in mice which is the homolog of extra pyramidal symptoms (EPS) in humans. Present study may offer more insight into the involvement of agmatine in pathogenesis of schizophrenia relative to new treatment targets.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by Institutional Animal Ethical Committee and carried out under strict compliance with ethical principles and guidelines of Committee for Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests; Government of India; New Delhi. Young healthy male, Sprague–Dawley rats (220–250 g) or Swiss albino male mice (20–25 g) were group housed in separate cages (five per cage) under 12:12 h light/dark cycle (lights on 0700 h) in a temperature controlled (24 ± 1 °C) environment with ad libitum access to food (Hindustan Liver, India) and water.

2.2. Drugs

Agmatine sulfate, apomorphine hydrochloride, amphetamine sulfate, ketamine hydrochloride, WAY100, 635 (Sigma Chemicals, St. Louise, USA), haloperidol, and olanzepine (Sun Pharma, India) were dissolved in saline and administered by intraperitoneal (ip) or subcutaneous (sc) route.

2.3. Conditioned avoidance response (CAR) in rats

Rats were trained individually for one week in a two compartment shuttle box (Techno, India) to move from one compartment to another upon presentation of the buzzer tone (80 dB) for 10 s (conditioned stimulus; CS). If the rat failed to respond to CS, the tone was continued and electric current (0.5 mA) (unconditioned stimulus, UCS) was simultaneously delivered to the grid floor of the chamber for a period of 15 s for foot shock. Each animal was subjected to a daily session of 10 trials separated by a 20 s intertrial interval. The trial terminated once the rat has moved into the other compartment during CS or UCS period. Crossings made by the rat during the CS were recorded as avoidance responses and those made during UCS period were recorded as escape responses. Only those animals characterized by a high level of avoidance responding (>90%) were used for further experiments. The results are expressed as avoidance/10 trials (Ugale et al., 2004; Khisti et al., 2002).

Trained rats were randomly divided into different treatment groups (n = 6). They were injected ip with range of doses of agmatine (20–80 mg/kg, ip) alone or at subeffective doses with haloperidol (0.1 mg/kg) or olanzepine (0.5 mg/kg) or saline 30 min before subjected to CAR test. Numbers of avoidance responses during 10 trials were recorded and expressed as % inhibition of CAR.

2.4. Apomorphine-induced climbing behavior in mice

Administration of apomorphine to mice results in a peculiar climbing behavior characterized initially by rearing and then full climbing activity (Costall et al., 1978). Mice were initially placed individually in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, mesh size 3 mm) for 60 min to acclimatize to the new environment. They were randomly assigned to each drug regimen (n = 6) and received agmatine (20–80 mg/kg, ip) alone or in combination with haloperidol (0.1 mg/kg, ip) or olanzepine (0.5 mg/kg, ip) or vehicle (ip) 30 min before apomorphine (1 mg/kg, sc) injections. Each mouse was placed in wire mesh cages 10 min after apomorphine administration and climbing behavior of individual mouse was scored at 5-min intervals for a period of 20 min. The scoring system used was as: 0 = four paws on the floor, 1 = one paw on the wall of cage, 2 = two paws on the wall of cage, 3 = three paws on the wall of cage, and 4 = four paws on the wall of cage. Climbing scores across each time interval were then summed and expressed as climbing index, thus providing a maximum possible climbing index of 20.

2.5. Amphetamine or ketamine induced motor hyper activity in mice

Mice were transferred from their housing facility to the testing room in their home cages 15 min before any experiment. Animals were placed in actophotometer (20 cm × 20 cm × 10 cm) (Techno, India) equipped with six infrared photo sensors, 2.5 cm apart from each other for 30 min habituation before any testing. After initial habituation each mouse was brought back to home cage where they received drug treatment or saline. Baseline locomotor activity of each mouse was recorded for 20 min as a total count of ambulatory, horizontal and vertical activity (Depoortere et al., 2007; Khisti et al., 2002). Animals were randomly assigned to different treatment regimen (n = 6). Agmatine (20–80 mg/kg, ip) or saline was injected 15 min prior to amphetamine (2 mg/kg, ip) or ketamine (40 mg/kg, ip) or saline administration. The activity count of each mouse was recorded individually at 30 min after amphetamine, ketamine or saline treatment for period of 20 min.

2.6. Catalepsy induction in mice

The severity of catalepsy in individual mice (n = 6) was determined by placing the forepaws of the animal over a wooden bar 0.4 cm in diameter, fixed to a height of 3.5 cm above the tabletop. The time in seconds until mice brought both forepaws down to the tabletop was recorded, with a maximum cut-off time of 300 s (Khisti et al., 2002). Measurement of catalepsy was carried out at 30 min after the administration of saline, agmatine (20–80 mg/kg, ip) or haloperidol (1 mg/kg, ip).

Many atypical antipsychotic drugs in addition to blocking DA D2 receptors also stimulate 5HT1A receptors and thereby offer protection from EPS (Bardin et al., 2006; Depoortere et al., 2007; Kleven and Barret-Grevoz, 2005). In order to investigate whether agmatine effects are associated with 5HT1A receptor component, catalepsy was measured by pretreatment with selective antagonist WAY100, 635 (0.63 mg/kg, sc) or saline 15 min before agmatine (20–80 mg/kg, ip) and 30 min thereafter subjected to bar test.

2.7. Effects of agmatine on plasma prolactin levels in rats

All present antipsychotics are known to elevate plasma prolactin levels due to blockade of DA D2 receptors located on pituitary gland (Ben-jonathan, 1985). Therefore, plasma prolactin levels were measured. Briefly, rats (n = 3) were treated with agmatine (20–80 mg/kg, ip) or saline and 60 min thereafter blood samples (1 ml) were withdrawn from rat tail vein. Prolactin levels were assessed by fluorescence-based enzyme-linked immunosorbent assay as described previously with a commercially available fluorescence linked enzyme assay kit (Cosi et al., 2006).

2.8. Data analysis

Data from the experiments of CAR, apomorphine induced climbing, amphetamine or ketamine induced hyper locomotor activity, plasma prolactin levels and catalepsy was analyzed by one way analysis of variance (ANOVA) with post hoc Newman–Keuls test. $P \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. CAR inhibition by agmatine and antipsychotics in rats

The results presented in Fig. 1, show that agmatine given by ip route caused significant suppression of CAR behavior in rats [$F(3, 23) = 28.99$; $P<0.001$] as indicated by a main effect of drug treatment. Post hoc comparison revealed that agmatine at a dose of 40 mg/kg produced approximately 27% inhibition of avoidance responding ($P<0.01$), whereas at 80 mg/kg abolished CAR by 51% ($P<0.001$). Administration of low dose of agmatine (20 mg/kg) did not change CAR response as compared with saline control group. However, agmatine (20 mg/kg) administration together with haloperidol or olanzepine caused significant inhibition of CAR [$F(5, 35) = 11.22$; $P<0.001$]. Injection of 20 mg/kg, ip agmatine that exerted minimal or no effect potentiated the response to low dose of haloperidol (0.1 mg/kg) [$F(3, 23) = 16.01$; $P<0.001$] and olanzepine (0.5 mg/kg) [$F(3, 23) = 8.81$; $P<0.001$] by 56% ($P<0.001$) and 48% ($P<0.05$) respectively when compared with their respective control. None of the above drug treatment alone or in combination at any dose level used here produced any effect on escape performance.

3.2. Suppression of apomorphine induced climbing in mice

As depicted in Fig. 2, agmatine (40 and 80 mg/kg, ip) exhibited dose dependent antagonism to apomorphine induced climbing behavior in mice, with higher doses producing greater inhibition [$F(3, 23) = 18.87$; $P<0.001$]. No apparent inhibition of climbing behavior was observed at lower dose of agmatine (20 mg/kg, ip). Similarly very low doses of haloperidol (0.1 mg/kg, ip) or olanzepine (0.5 mg/kg, ip) by

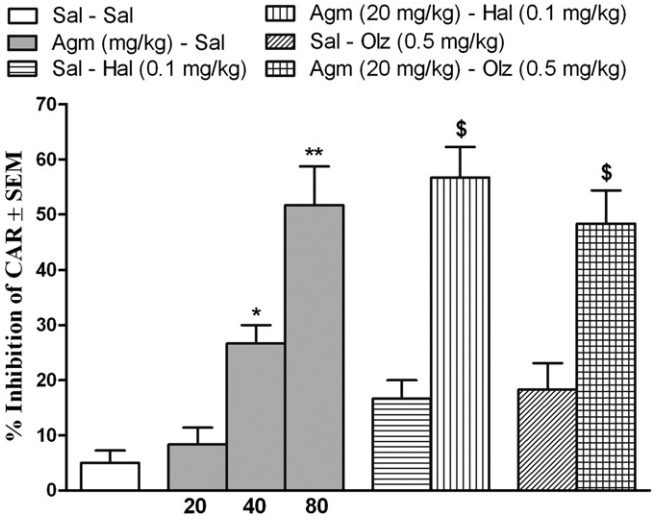

Fig. 1. Effect of agmatine on conditioned avoidance response in rats. Rats were placed individually in the shuttle box for determination of avoidance responses 30 min after agmatine injections (Agm, 20, 40 or 80 mg/kg, ip) or saline (Sal, 1 ml/kg, ip). In separate group, rats were pretreated with agmatine (Agm, 20 mg/kg, ip) or saline 15 min before haloperidol (Hal, 0.1 mg/kg, ip) or olanzepine (Olz, 0.5 mg/kg, ip) or saline (Sal, 1 ml/kg, ip) and placed individually in the shuttle box for the standard ten-trial session. Each column represents the percentage inhibition of conditioned avoidance response ± SEM for a group (n = 6) of rats. *P<0.01, **P<0.001 compared with vehicle-treatment. $P<0.01 compared with agmatine treated group (one way ANOVA post hoc Dunnett/Newman–Keuls mean comparisons).

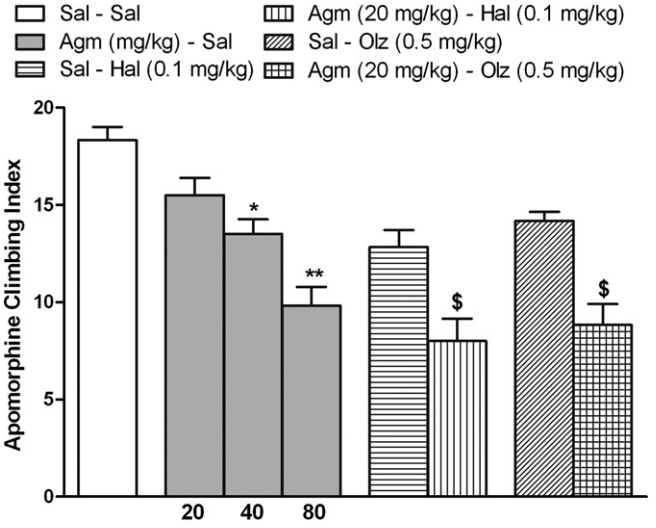

Fig. 2. Effect of agmatine on apomorphine induced climbing behavior in mice. Separate group of mice received agmatine (Agm, 20–80 mg/kg, ip) or saline (Sal, 1 ml/kg, ip) alone or in combination with haloperidol (Hal, 0.1 mg/kg, ip) or olanzepine (Olz, 0.5 mg/kg, ip) or vehicle (ip) 30 min before apomorphine (1 mg/kg, sc) injections and placed individually in the center of wire-mesh cage. Each column represents mean ± SEM (n = 6); total climbing score was assessed at 5 min intervals for 20 min, starting 10 min after apomorphine administration. *P<0.01, **P<0.001 compared with vehicle-treatment. $P<0.01 compared with agmatine treated group (one way ANOVA post hoc Dunnett/Newman–Keuls mean comparisons).

itself did not significantly affect apomorphine induced climbing. However, in agmatine (20 mg/kg, ip) pretreated animals these doses of haloperidol [$F(3, 23) = 9.16$; $P<0.001$] or olanzepine [$F(3, 23) = 8.67$; $P<0.001$] exhibited significant suppression of climbing behavior.

3.3. Attenuation of amphetamine or ketamine induced hyperlocomotor activity in mice

Fig. 3 shows that animals injected with 2 mg/kg, ip D-amphetamine (1114 ± 64.67) or 40 mg/kg, ip ketamine (884 ± 30.13) produced large increase in the number of locomotor counts as compared to saline control (356.8 ± 24.05). However this drug induced hyperlocomotor activity [$F(4, 29) = 23.43$; $P<0.001$] was significantly inhibited in groups pretreated with agmatine (40–80 mg/kg, ip). Lower dose of agmatine (20 mg/kg, ip) did not influence D-amphetamine or ketamine induced locomotor response. Agmatine at any dose level used here (20–80 mg/kg, ip) did not change spontaneous or basal locomotor activity of animals.

3.4. Catalepsy measurement in mice

Agmatine at any dose (20–80 mg/kg, ip) tested here did not cause any significant change in descent latency of mice as compared to that of vehicle treated groups [$F(3, 23) = 1.38$; $P>0.05$]. However, its 80 mg/kg, ip (but not 20–40 mg/kg, ip) administration to animals pretreated with 5-HT1A receptor antagonist WAY100, 635 (0.63 mg/kg, sc) resulted in an increased amount of time spent by animals in a cataleptic position [$F(7, 47) = 31.99$; $P<0.001$]. Further, we have employed haloperidol (1 mg/kg, ip) as a standard cataleptic agent which exhibited catalepsy in mice as compared to control group ($P<0.001$) (Fig. 4).

3.5. Effects on plasma prolactin levels in rats

Agmatine (40 and 80 mg/kg, ip) injections produced a significant increase with mean of 0.47 ± 0.023 ($P<0.05$) and 0.67 ± 0.038 ng/ml ($P<0.001$) in plasma prolactin levels respectively as compared to saline (0.32 ± 0.035 ng/ml) treated animals [$F(3, 11) = 26.82$, $P<0.001$, one way ANOVA post hoc Dunnett mean comparisons). However plasma

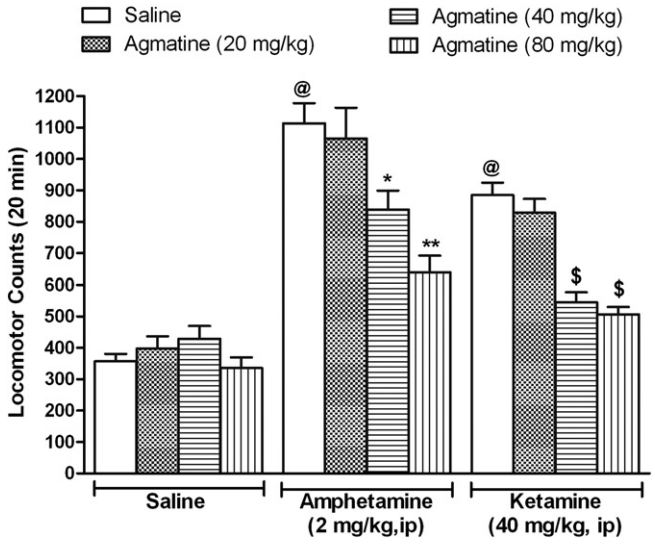

Fig. 3. Effect of agmatine treatment on (a) D-amphetamine- or (b) ketamine-induced hyperlocomotor activity in mice. Animals were injected with agmatine (20–80 mg/kg, ip) or saline, 20 min before D-amphetamine (2 mg/kg, ip) or ketamine (40 mg/kg, ip) or saline. Each bar represents the mean locomotor counts ± SEM recorded for 20 min. $^{@}P<0.001$ compared with the control animals. $^{\$,*}P<0.01$, $^{**}P<0.001$ compared with amphetamine or ketamine treated group (one-way ANOVA post hoc Newman–Keuls mean comparison).

prolactin levels in the animals treated with 20 mg/kg remained unaltered.

4. Discussion

We demonstrated in the present study that acute ip injections of agmatine blocked conditioned avoidance response; attenuated apomorphine induced climbing and diminished amphetamine and ketamine hyperlocomotor activity. There has been considerable investigation of suppression of these behaviors by both typical and atypical antipsychotics (Reynolds and Czudek, 1995; Seeman, 1992; Ugale et al., 2004; Wadenberg et al., 1993). Numerous studies have proposed hyperdopaminergia in the mesolimbic DA system and hypodopaminergia in mesocortical system of schizophrenic patients. Although the direct interaction of agmatine and D2 receptors is yet to be reported, it diminished dopamine levels in ventral tegmental area evoked by morphine withdrawal (Wei et al., 2007), aggravated the symptoms of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced neurotoxicity (Gilad et al., 2005) and modulated drug addictive processes (Kotagale et al., 2010; Taksande et al., 2010). The data of present study therefore suggest that regulation of brain dopaminergic signaling possibly through DA D2 receptors by agmatine is critical for attenuation of behavioral profile relevant to schizophrenia. This hypothesis further supported by the fact that agmatine augmented plasma prolactin levels, a classical effect resulting from blockade at DA D2 receptors located on pituitary gland (prolactin release is tonically inhibited by dopamine acting on D2 receptors: Ben-jonathan, 1985). Interestingly most effective antipsychotics exhibit this effect (Cosi et al., 2006). It must be noted that acute amphetamine induced locomotor stimulation is said to be related to euphoric and abusing effect of drug rather its psychosis inducing action. In order to evaluate antipsychotic profile further studies are needed using chronic administration of amphetamine or amphetamine induced locomotor sensitization model. It is important to note that agmatine possesses multireceptorial affinity. It activates α_2-adrenergic and imidazoline receptors (Halaris and Plietz, 2007; Reis and Regunathan, 2000) and antagonizes N-methyl-D-aspartic acid (NMDA) receptors (Yang and Reis, 1999). In addition, agmatine has an important role in regulating metabolic pathways of L-arginine. It inhibits all isoforms of enzyme, NOS which synthesize NO from L-arginine (Auguet et al., 1995). Recent evidence suggests a link between NO and dopamine in psychiatric disorders like schizophrenia (Klamer et al., 2004a). Genetic studies examining a large population of schizophrenic patients revealed that polymorphisms in the neuronal NOS gene may confer increased susceptibility to schizophrenia (Reif et al., 2006). NO is increased in plasma of schizophrenic patients (Yilmaz et al., 2007) and NOS inhibitors like L-NAME or L-NNA could block several symptoms in animal models of schizophrenia like apomorphine induced climbing, PPI and amphetamine, ketamine or phencyclidine induced hyperlocomotion (Issy et al., 2011; Klamer et al., 2004b) etc., comparable to that of conventional antipsychotics like haloperidol and clozapine (Issy et al., 2009). Moreover, potent antipsychotics are reported to decrease NO synthesis in brain (Hu et al., 1994). Subsequent studies have shown an inverse relation between NO levels and release of prolactin (Andric et al., 2003; Duvilanski et al., 1995). In fact NOS inhibitors increase the prolactin secretion in rodents (Duvilanski et al., 1995). This consideration indicates that NOS inhibition by agmatine may be the basis for ability to antagonize ketamine induced hyperlocomotor activity as well as dopamine mediated behaviors relevant to schizophrenia. Alternately besides NMDA receptors, contribution of other receptor system like imidazoline, α_2-adrenergic in agmatine induced behavior can not be completely ruled out.

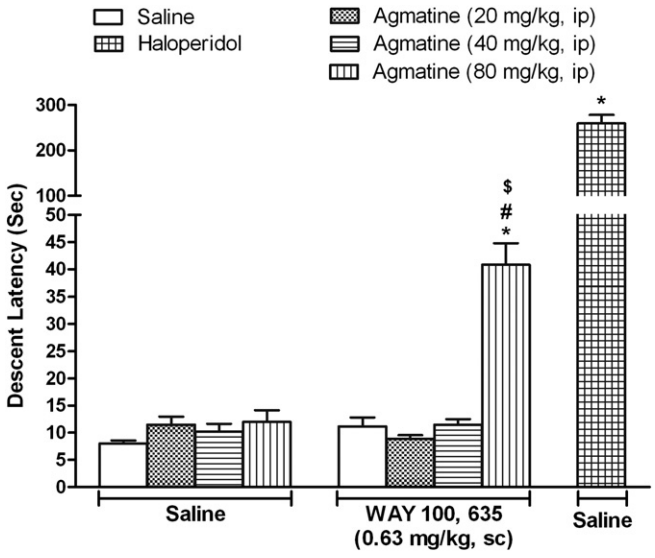

Fig. 4. Effect of saline (1 ml/kg, ip), agmatine (20, 40 or 80 mg/kg, ip) or haloperidol (1 mg/kg, ip) in bar test. In separate group, mice were pretreated with 5HT1A antagonist, WAY100, 635 (0.63 mg/kg, ip) 15 min before agmatine (20, 40 or 80 mg/kg, ip) and 30 min thereafter animals were tested for descent latency in bar test. Each bar represents mean descent latency (Sec.) ± SEM (n = 6). $^{*}P<0.001$ compared with control group, $^{\$}P<0.001$ compared with agmatine treated group and $^{\#}P<0.001$ compared with WAY100, 635 treated group (one way ANOVA post hoc Newman–Keuls mean comparison).

In the CAR test, agmatine suppressed conditioned avoidance responding without causing escape failure suggesting reduced propensity to produce EPS (Arnt, 1982). In fact newer antipsychotic compared to classical first generation is more potent in blocking DA agonist induced hyperactivity or CAR than in causing catalepsy (Moore et al., 1993). The induction of catalepsy in rodent is considered to be predictive of EPS liability of antipsychotic drugs (Hoffman and Donovan, 1995). Neuroleptic induced catalepsy is linked to disruption of nigrostriatal DA transmission (Calderon et al., 1988; Sanberg, 1980) or blockade of DA D2 receptors (Baik et al., 1995; Farde et al., 1992; Kapur et al., 1995, 2000). In the present study, despite DA D2 receptor blockade like behavioral profile, agmatine did not produce notable cataleptic effect in mice. On the other hand, in other behavioral tests combination of low dose of agmatine augmented the effect of antipsychotic drugs haloperidol (D2 antagonist) and olanzepine (D2/5HT2 antagonist). Thus it could be

expected that if agmatine has D2 antagonistic profile it should had induced catalepsy. However our findings are not conclusive as we have not assessed the effect of prolong agmatine administration or acute injections of much higher doses. However, agmatine has affinity for 5-HT1A receptors (Zomkowski et al., 2004), and its agonist, 8-OH DPAT potentiated antidepressant like effects of agmatine (Zomkowski et al., 2004). Moreover 5-HT1A receptor activation by agonist reduces the cataleptic effect of DA D2 receptor antagonist (Broekkamp et al., 1988; Invernizzi et al., 1988). Interestingly, co-administration of agmatine and 5-HT1A receptor antagonist WAY100, 635 induced catalepsy in mice. This is in agreement with previous interaction studies of dopamine D2/5-HT1A antipsychotics and WAY100, 635 (Bardin et al., 2006; Kleven and Barret-Grevoz, 2005). Thus lack of catalepsy may be due to its affinity for 5-HT1A receptor. Notably, clozapine, a $5HT_{1A}$ agonist/dopamine D2 antagonist possesses potent antipsychotic activity with very low EPS in human and catalepsy in rodents (Auclair et al., 2009; Iqbal et al., 2003; Leucht et al., 2003). Thus, blockade of dopamine D2 receptors and activation 5-HT1A receptors by agmatine might be attributed to its favorable profile and low side effect liability in rodent models of schizophrenia. Further, although agmatine inhibits the hyperlocomotor activity of amphetamine or ketamine, it did not influence the spontaneous locomotor activity even at much higher doses than required for suppression of CAR, apomorphine induced climbing and amphetamine and ketamine hyperlocomotor activity. This is very fascinating aspect of agmatine as most of the antipsychotic, both typical and atypical themselves causes reduction in locomotor activity at moderate to higher doses (Costall and Naylor, 1974). In fact, earlier studies from our laboratory and results of other clearly demonstrated an inhibitory effect of agmatine on hyperlocomotor activity induced by substances of abuse like alcohol, caffeine, morphine and nicotine without altering spontaneous locomotor activity (Kotagale et al., 2010; Ozden et al., 2011; Uzbay et al., 2010b). There is no correlation between the ability of antipsychotics to antagonize ketamine induced hyperlocomotion and their affinity at dopamine D2 receptors (Bardin et al., 2007; Depoortere et al., 2007), suggesting involvement of other biological targets of agmatine. The neurochemical mechanism for this specific selectivity although remains to be elucidated, targeting endogenous agmatine may provide additional benefit to the patients of schizophrenia. Furthermore, exogenous agmatine administrations have shown several intriguing neurally relevant functions of potential therapeutic implication in schizophrenia. Agmatine possesses antidepressant, anxiolytic, neuroprotective properties and also facilitates memory processing (Lavinsky et al., 2003; Lu et al., 2010; Olmos et al., 1999; Zomkowski et al., 2002). These biological properties of agmatine further extend its therapeutic importance in amelioration of deleterious negative symptoms of schizophrenia.

The amplitude of acoustic startle response is decreased if the startle stimulus is preceded by nonstartle eliciting stimulus. This sensorimotor gating phenomenon known as PPI is diminished or impaired in schizophrenic individuals. The dopamine agonists disrupt PPI and classical antipsychotics reverse this disruption. However, recent studies have demonstrated an interesting profile of agmatine. Pretreatment with low dose (20 mg/kg, ip) of agmatine attenuated disruptive effect of phencyclidine on PPI in mice (Palsson et al., 2008). On the other hand its very high dose (160 mg/kg, ip) not only disrupted PPI of acoustic startle reflex in rats but also increased the disruptive effect of apomorphine (Uzbay et al., 2010a). The agmatine induced disruption of PPI was resistant to classical as well as atypical antipsychotics including haloperidol, clozapine and quetiapine which mainly act through DA D2 or 5-HT receptors (Uzbay et al., 2010a). Together these finding suggest that agmatine may disrupt PPI by other than dopaminergic mechanisms like NMDA antagonism (Uzbay et al., 2010a) or accumulation of agmatine metabolites spermine and spermidine due to very high doses of agmatine (Andrews, 1985; Gilad et al., 1995; Ramchand et al., 1994; Richardson-Andrews, 1983). It is noteworthy that PPI deficits are not unique to schizophrenia spectrum disorders but also observed in patients with other psychiatric conditions (Ludewig et al., 2002; Swerdlow et al., 1993, 1995; Tam et al., 1998) and even some atypical antipsychotics with marked agonist activity at 5-HT1A receptors (Auclair et al., 2009) like sarizotan, SSR181507, bifeprunox were found to have robust PPI-disrupting effects of their own. Since schizophrenic patients usually show disrupted basal PPI levels (Swerdlow et al., 1993) such an effect induced by any antipsychotic can be problematic. Therefore further studies should be performed to characterize the effect of agmatine in PPI disruption.

In conclusion, our results show interesting pharmacological profile of agmatine in modulation of dopaminergic responses in animal models of schizophrenia. These preclinical observations suggest a crucial role of agmatine in pathogenesis of schizophrenia. Although further investigations are needed to surmount agmatine based therapeutic strategies, development of drugs that increases its endogenous content may represent a highly promising approach to treat schizophrenia with low incidences of extra pyramidal side effects.

Abbreviations

DA	Dopamine
5-HT	5-hydroxy tryptamine
NOS	Nitric oxide synthase
PPI	Prepulse inhibition
NMDA	N-methyl-D-aspartate
EPS	Extra pyramidal side effects
NO	Nitric oxide
CAR	Conditioned avoidance response
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
GABA	Gamma amino butyric acid
PCP	Phencyclidine

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References

Alsene KM, Fallace K, Bakshi VP. Ventral striatal noradrenergic mechanisms contribute to sensorimotor gating deficits induced by amphetamine. Neuropsychopharmacology 2010;35(12):2346–56.
Andrews RC. The side effects of antimalarial drugs indicate a polyamine involvement in both schizophrenia and depression. Med Hypotheses 1985;18(1):11–8.
Andric SA, Gonzalez-Iglesias AE, Goor FV, et al. Nitric oxide inhibits prolactin secretion in pituitary cells downstream of voltage-gated calcium influx. Endocrinol 2003;144:2912–21.
Aricioglu F, Altunbas H. Is agmatine an endogenous anxiolytic/anti-depressant agent? Ann NY Acad Sci 2003;1009:136–40.
Aricioglu-Kartal F, Uzbay IT. Inhibitory effect of agmatine on naloxone-precipitated abstinence syndrome in morphine dependent rats. Life Sci 1997;61:1775–81.
Arnt J. Pharmacological specificity of conditioned avoidance response inhibition in rats: inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta Pharmacol Toxicol 1982;51(4):321–9.
Auclair AL, Kleven MS, Barret-Grevoz C, et al. Differences among conventional, atypical and novel putative D2/5-HT1A antipsychotics on catalepsy-associated behaviour in cynomolgus monkeys. Behav Brain Res 2009;203:288–95.
Auguet M, Viossat I, Marin JG, et al. Selective inhibition of inducible nitric oxide synthase by agmatine. Jpn J Pharmacol 1995;69:285–7.
Baik JH, Picetti R, Saiardi A, et al. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 1995;377(6548):424–8.
Bardin L, Kleven MS, Barret-Grevoz C, et al. Antipsychotic-like vs cataleptogenic actions in mice of novel antipsychotics having D2 antagonist and 5-HT1A agonist properties. Neuropsychopharmacol 2006;31:1869–79.
Bardin L, Auclair A, Kleven MS, et al. Pharmacological profiles in rats of novel antipsychotics with combined dopamine D2/serotonin 5-HT1A activity: comparison with typical and atypical conventional antipsychotics. Behav Pharmacol 2007;18(2):103–18.
Bence K, David R, Stables JP, et al. An in vivo evaluation of the antiseizure activity and acute neurotoxicity of agmatine. Pharmacol Biochem Behav 2003;74:771–5.
Ben-Jonathan N. Dopamine: a prolactin inhibiting hormone. Endocr Rev 1985;6:564–89.
Bernstein H, Bogerts B, Keilhoff G. The many faces of nitric oxide in schizophrenia. A review. Schizophrenia Res 2005;78:69–86.

Black MD, Selk DE, Hitchcock JM, Wettstein JG, Sorensen SM. On the effect of neonatal nitric oxide synthase inhibition in rats: a potential neurodevelopmental model of schizophrenia. Neuropharmacology 1999;38:1299–306.
Broekkamp CLE, Oosterloo SK, Berendsen HHG, van Delft AML. Effect of metergoline, fenfluramine, and 8-OHDPAT on catalepsy induced by haloperidol or morphine. Arch Pharmacol 1988;338:191–5.
Calderon SF, Sanberg PR, Norman AB. Quinolinic acid lesions of rat striatum abolish D1- and D2-dopamine receptor-mediated catalepsy. Brain Res 1988;450:403–7.
Costall B, Naylor RJ, Nohria V. Climbing behavior induced by apomorphine in mice: a potential model for the detection of neuroleptic activity. Eur J Pharmacol 1978;50:39–50.
Costall B, Naylor RJ. Mesolimbic involvement with behavioural effects indicating antipsychotic activity. Eur J Pharmacol 1974;27:46–58.
Cosi C, Carilla-Durand E, Assié MB, Ormiere AM, Maraval M, Leduc N, Newman-Tancredi A. Partial agonist properties of the antipsychotics SSR181507, aripiprazole and bifeprunox at dopamine D2 receptors: G protein activation and prolactin release. Eur J Pharmacol 2006;535:135–44.
Dencker D, Wörtwein G, Weikop P, et al. Involvement of a subpopulation of neuronal M4 muscarinic acetylcholine receptors in the antipsychotic-like effects of the M1/M4 preferring muscarinic receptor agonist xanomeline. J Neurosci 2011;31 (16):5905–8.
Depoortere R, Bardin L, Auclair AL, et al. F15063, a compound with D2/D3 antagonist, 5-HT1A agonist and D4 partial agonist properties: (II) activity in models of positive symptoms of schizophrenia. Br J Pharmacol 2007;151:253–65.
Duvilanski BH, Zambruno C, Seilicovich A, et al. Role of nitric oxide in control of prolactin release by the adenohypophysis. Proc Natl Acad Sci 1995;92:170–4.
Farde L, Nordstrom AL, Wiesel FA, et al. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Arch Gen Psychiatry 1992;49:538–44.
Gilad GM, Gilad VH, Finberg JPM, et al. Neurochemical evidence for agmatine modulation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity. Neurochem Res 2005;30:713–9.
Gilad GM, Gilad VH, Casanova MF, et al. Polyamines and their metabolizing enzymes in human frontal cortex and hippocampus: preliminary measurements in affective disorders. Biol Psychiatry 1995;38(4):227–34.
Halaris A, Plietz J. Agmatine metabolic pathway and spectrum of activity in brain. CNS Drugs 2007;21(11):885–900.
Hoffman DC, Donovan H. Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. Psychopharmacol 1995;120(2):128–33.
Hu J, Lee JH, El-Fakahany EE. Inhibition of neuronal nitric oxide synthase by antipsychotic drugs. Psychopharmacol 1994;114(1):161–6.
Invernizzi RW, Cervo L, Samanin R. 8-Hydroxy-2-(di-N-propylamino) tetralin, a selective serotonin1A receptor agonist, blocks haloperidol-induced catalepsy by an action on raphe nuclei medianus and dorsalis. Neuropharmacology 1988;27:515–8.
Iqbal MM, Rahman A, Husain Z, et al. Clozapine: a clinical review of adverse effects and management. Ann Clin Psychiatry 2003;15(1):33–48.
Issy AC, Lazzarini M, Szawka RE, et al. Nitric oxide synthase inhibitors improve prepulse inhibition responses of Wistar rats. Behav Brain Res 2011;217:416–23.
Issy AC, Salum C, Del Bel EA. Nitric oxide modulation of methylphenidate-induced disruption of prepulse inhibition in Swiss mice. Behav Brain Res 2009;205:475–81.
Kapur S, Remington G, Zipursky RB, et al. The D2 dopamine receptor occupancy of risperidone and its relationship to extrapyramidal symptoms: a PET study. Life Sci 1995;57(10):103–7.
Kapur S, Zipursky R, Jones C, et al. A positron emission tomography study of quetiapine in schizophrenia. Arch Gen Psychiatry 2000;57:553–9.
Khisti RT, Deshpande LS, Chopde CT. The neurosteroid 3 alpha-hydroxy-5 alpha-pregnan-20-one affects dopamine-mediated behavior in rodents. Psychopharmacol 2002;161(2):120–8.
Klamer D, Engel JA, Svensson L. The neuronal selective nitric oxide synthase inhibitor, N N-propyl-L-arginine, blocks the effects of phencyclidine on prepulse inhibition and locomotor activity in mice. Eur J Pharmacol 2004a;503:103–7.
Klamer D, Palsson E, Revesz A, et al. Habituation of acoustic startle is disrupted by psychotomimetic drugs: differential dependence on dopaminergic and nitric oxide modulatory mechanisms. Psychopharmacol 2004b;176:440–50.
Kleven MS, Barret-Grevoz C, Bruins Slot LB, et al. Novel antipsychotic agents with 5-HT (1A) agonist properties: role of 5-HT(1A) receptor activation in attenuation of catalepsy induction in rats. Neuropharmacol 2005;49:135–43.
Kotagale NR, Taksande BG, Gahane AY, et al. Repeated agmatine treatment attenuates nicotine sensitization in mice: modulation by α_2-adrenoceptors. Behav Brain Res 2010;213:161–74.
Lavinsky D, Arteni NS, Netto CA. Agmatine induces anxiolysis in the elevated plus maze task in adult rats. Behav Brain Res 2003;141(1):19–24.
Leucht S, Wahlbeck K, Hamann J, et al. New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. Lancet 2003;361:1581–9.
Lu W, Dong HJ, Gong ZH, et al. Agmatine inhibits morphine-induced memory impairment in the mouse step-down inhibitory avoidance task. Pharmacol Biochem Behav 2010;97(2):256–61.
Ludewig S, Ludewig K, Geyer MA, et al. Prepulse inhibition deficits in patients with panic disorder. Depress Anxiety 2002;15(2):55–60.
Meltzer H, Massey B. The role of serotonin receptors in the action of atypical antipsychotic drugs. Curr Opin Pharmacol 2011 Febb;11(1):59–67.
Moore NA, Calligaro DO, Wong TD, et al. The pharmacology of olanzapine and other new antipsychotic agents. Curr Opin Invest Drugs 1993;2:281–93.
Olmos G, DeGregorio-Rocasolano N, Paz Regalado M, et al. Protection by imidazoline drugs and agmatine of glutamate induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. Br J Pharmacol 1999;127: 1317–26.
Onal A, Delen Y, Ulker S, et al. Agmatine attenuates neuropathic pain in rats: possible mediation of nitric oxide and noradrenergic activity in the brainstem and cerebellum. Life Sci 2004;73:413–28.
Ozden O, Kayir H, Ozturk Y, Uzbay T. Agmatine blocks ethanol-induced locomotor hyperactivity in male mice. Eur J Pharmacol 2011;659:26–9.
Palsson E, Fejgin K, Wass C, et al. Agmatine attenuates the disruptive effects of phencyclidine on prepulse inhibition. Eur J Pharmacol 2008;590:212–6.
Ramchand CN, Das I, Gliddon A, et al. Role of polyamines in the membrane pathology of schizophrenia. A study using fibroblasts from schizophrenic patients and normal controls. Schizophr Res 1994;13(3):249–53.
Reif A, Herterich S, Strobel A, et al. A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function. Mol Psychiatry 2006;11:286–300.
Reis DJ, Regunathan S. Is agmatine a novel neurotransmitter in brain? Trends in Pharmacological Sci 2000;21:187–93.
Reynolds GP, Czudek C. New approaches to the drug treatment of schizophrenia. Adv Pharmacol 1995;32:461–503.
Richardson-Andrews RC. A central role for the polyamines in the aetiology of schizophrenia. Med Hypotheses 1983;11(2):157–66.
Rotaru DC, Yoshino H, Lewis DA, et al. Glutamate receptor subtypes mediating synaptic activation of prefrontal cortex neurons: relevance for schizophrenia. J Neurosci 2011;31(1):142–56.
Sanberg PR. Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature 1980;284(5755):472–3.
Seeman P. Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4. Neuropsychopharmacol 1992;7:261–83.
Swerdlow NR, Paulsen J, Braff DL, et al. Impaired prepulse inhibition of acoustic and tactile startle response in patients with Huntington's disease. J Neurol Neurosurgery Psychiatry 1995;58:192–200.
Swerdlow NR, Benbow CH, Zisook S, et al. A preliminary assessment of sensorimotor gating in patients with obsessive compulsive disorder. Biol Psychiatry 1993;33 (4):298–301.
Taksande BG, Kotagale NR, Patel MR, et al. Agmatine, an endogenous imidazoline receptor ligand modulates ethanol anxiolysis and withdrawal anxiety in rats. Eur J Pharmacol 2010;637:89-101.
Tam WC, Sewell KW, Deng HC. Information processing in schizophrenia and bipolar disorder: a discriminant analysis. J Nerv Ment Dis 1998;186(10):597–603.
Ugale RR, Hirani K, Morelli M, et al. Role of neuroactive steroid allopregnanolone in antipsychotic-like action of olanzapine in rodents. Neuropsychopharmacol 2004;29: 1597–609.
Uzbay IT. New pharmacological approaches to the treatment of schizophrenia. Turk Psikiyatri Derg 2009;20(2):175–82.
Uzbay T, Kayir H, Goktalay G, et al. Agmatine disrupts prepulse inhibition of acoustic startle reflex in rats. J Psychopharmacol 2010a;24:923–9.
Uzbay T, Kose A, Kayir H, et al. Sex-related effects of agmatine on caffeine-induced locomotor activity in Swiss Webster mice. Eur J Pharmacol 2010b;630:69–73.
Uzbay T, Yesilyurt O, Celik T, Isimer A. Effect of agmatine on ethanol withdrawal syndrome in rats. Behav Brain Res 2000;107:153–9.
Wadenberg ML, Ahlenius S, Svensson TH. Potency mismatches for behavioral and biochemical effects by dopamine receptor antagonists: implications for the mechanism of action of clozapine. Psychopharmacol 1993;110:273–9.
Wei XL, Su RB, Wu N, Lu XQ, Zheng JQ, Li J. Agmatine inhibits morphine-induced locomotion sensitization and morphine-induced changes in striatal dopamine and metabolites in rats. Eur Neuropsychopharmacol 2007;17:790–9.
Yang XC, Reis DJ. Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J Pharmacol Exp Ther 1999;288:544–9.
Yilmaz N, Herken H, Cicek HK, et al. Increased levels of nitric oxide, cortisol and adrenomedullin in patients with chronic schizophrenia. Med Princ Pract 2007;16: 137–41.
Zhu MY, Wang WP, Cai ZW, et al. Exogenous agmatine has neuroprotective effects against restraint-induced structural changes in the rat brain. Eur J Neurosci 2008;27(6):1320–32.
Zomkowski ADE, Hammes L, Lin J, et al. Agmatine produces antidepressant-like effects in two models of depression in mice. Neuroscience Report 2002;13:387–91.
Zomkowski E, Dias A, Rosaa AO, et al. Evidence for serotonin receptor subtypes involvement in agmatine antidepressant like-effect in the mouse forced swimming test. Brain Res 2004;1023:253–63.