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An in vivo evaluation of the antiseizure activity and acute neurotoxicity of agmatine

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Abstract

Agmatine, an endogenous cationic amine, exerts a wide range of biological effects, including modulation of glutamate-activated *N*-methyl-D-aspartate (NMDA) receptor function in the central nervous system (CNS). Since glutamate and the NMDA receptor have been implicated in the initiation and spread of seizure activity, the capacity of agmatine to inhibit seizure spread was evaluated in vivo. Orally administered agmatine (30 mg/kg) protected against maximal electroshock seizure (MES)-induced seizure spread in rats as rapidly as 15 min and for as long as 6 h after administration. Inhibition of MES-induced seizure spread was also observed when agmatine was administered intraperitoneally. Agmatine's antiseizure activity did not appear to be dose-dependent. An in vivo neurotoxicity screen indicated that agmatine was devoid of any acute neurological toxicity at the doses tested. These preliminary data suggest that agmatine has promising anticonvulsant activity.

Keywords: Agmatine; Seizure; Epilepsy; Acute neurotoxicity; Maximal electroshock; Anticonvulsant activity

1. Introduction

Epilepsy and related electroconvulsive disorders affect millions of people worldwide and over 2.5 million individuals in the United States. The etiology of these disorders is complex, as epileptic syndromes may be of genetic, developmental, or acquired origin. Although their specific causes vary, the interaction of glutamate with ionotropic and metabotropic receptors appears to be a factor in the initiation and spread of some types of seizure activity (Meldrum et al., 1999). The *N*-methyl-D-aspartate (NMDA) receptor is one of the ionotropic glutamate receptors that has been implicated in the generalized initiation of epileptic seizures. Anticonvulsant properties have been observed in all classes of NMDA antagonists, including competitive NMDA antagonists, antagonists that bind in the receptor-associated ion channel, glycine site antagonists, and polyamine site antagonists (Chapman, 2000). The development and pharmacological evaluation of NMDA antagonists may lead to novel, clinically useful antiepileptic agents.

Agmatine (1-amino-4-guanidinobutane), a biogenic amine, is formed from the decarboxylation of L-arginine by arginine decarboxylase (ADC). Agmatine has been detected in nearly all of the organs of the rat (Raasch et al., 1995a,b) and also has been found in the brain (Li et al., 1994; Otake et al., 1998; Reis et al., 1998). In rat, the levels of agmatine in brain is approximately 20-fold less than those in the small intestine and 3-fold less than the levels of agmatine in the adrenal gland (Raasch et al., 2001). Agmatine exerts a wide range of biological activities in several organ systems, including the central nervous system (CNS), where it has been proposed to act as a neurotransmitter (Reis and Regunathan, 2000). Agmatine interacts with the I_1-binding site (I_1-BS), α_2-adrenoceptor (α_2-R), NMDA, nicotinic cholinergic (NIC), and 5-HT_3 (via the sigma-2 binding site) receptors (reviewed by Raasch et al., 2001). Agmatine has been reported to have analgesic properties (Onal and Soykan, 2001), to play a role in depression (Halaris et al., 1999), to impair specific types of learning and memory (McKay et al., 2002), and to attenuate the tremors associated with ethanol withdrawal (Uzbay et al., 2000).

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One area of particular interest is the interaction of agmatine with the NMDA receptor. Until recently, there was only inferential evidence to suggest that agmatine could modulate the actions of L-glutamate and the NMDA receptor in the brain (Anis et al., 1990; Kolesnikov et al., 1996). The association between agmatine and NMDA receptor function has now been confirmed with the discovery that agmatine selectively modulates the NMDA subclass of glutamate receptors in rat hippocampal neurons via an interaction between the guanidine group of agmatine and the NMDA channel pore (Yang and Reis, 1999).

In light of the importance of glutamate in seizure activity and the known glutamate- and NMDA-modulating properties of agmatine in the CNS, this study investigated the effect of systemically administered agmatine on epileptic seizures in rats. Concurrent neurotoxicological studies were also conducted in order to evaluate the acute neurotoxicity of systemic agmatine administration.

2. Materials and methods

2.1. Animals and laboratory

Male Sprague–Dawley rats (105–130 g) age 28–38 days obtained from Simonsen (Gilroy, CA) and male albino CF#1 mice (20.5–25.5 g) from Charles River (Wilmington, MA) were used in the neurotoxicity and anticonvulsant evaluation of agmatine. Male animals were used for these experiments to avoid potential variation of animals in their biological responses due to estrus cycles of female animals. All rats were prescreened 24 h prior to drug testing to confirm they were capable of responding to electrical seizure stimulations. Seizure activity in mice is highly predictive (99%), thus, routine testing on every batch was not done. Still, animals were periodically examined to determine if there were any changes in response at different times of the year. Any nonresponders were excluded from the actual drug experimentation. The weight and age range of the animals were chosen because it has been determined that animals in the chosen range are the most sensitive to electroshock seizures. Animal age has previously been demonstrated to have a significant effect on response to electroshock seizures (Petty and Karler, 1965). In addition, none of the experimental animals were genetically prone to seizures.

The recommendations in the National Research Council Publication, *Guide for the Care and Use of Laboratory Animals* were followed in maintaining the environment, housing, and management of all of the animals. All animals were euthanized in a manner consistent with the Institute of Laboratory Resources policies on the humane care of laboratory animals.

Animals were fed S/L Custom Lab Diet-7 and received food and water ad libitum except during the experimental procedures. For all animals, the experiments were conducted between 0800 and 1700 h and insecticides capable of altering the activity of hepatic drug metabolism enzymes were not used in the animal facilities.

2.2. Drugs

Agmatine sulfate was purchased from Sigma (St. Louis, MO). On the day of the test, agmatine sulfate was dissolved in 0.5% methylcellulose and administered to the animals orally or via intraperitoneal injection.

2.3. Maximal electroshock seizure (MES) test

The MES test is an experimental model for generalized tonic–clonic seizures that identifies compounds that prevent MES-induced seizure spread. An advantage of this model is that the behavioral and electrographic seizures are consistent with those observed in humans (White et al., 1995b). Additionally, the MES model is normally highly reproducible and has a consistent endpoint. This model was used in both mouse and rat as a measure of anticonvulsant protection.

In the MES test, the animal received an electrical stimulus through corneal electrodes primed with an electrolyte solution consisting of 0.5% w/v tetracaine hydrochloride in 0.9% w/v saline and applied to the eyes of each animal prior to placement of the corneal electrodes. The 0.2-s stimulation was generated with 150 mA at 60 Hz in rats and 50 mA in mice. Rats received this electrical stimulus 0.25, 0.5, 1, 2, 4, or 6 h after oral or intraperitoneal administration of agmatine (30, 60, or 120 mg/kg), while mice received the electrical stimulus 0.5 and 4 h after intraperitoneal administration of agmatine at doses of 30, 100, or 300 mg/kg. Initial time points of 0.5 and 4 h were evaluated based on the standard procedure used in the assessment of compounds in the NIH Anticonvulsant Screening Program (Stables and Kupferberg, 1997). Additional time points were evaluated based on the initially observed activity responses. Routinely, four animals were used for each time point, however, up to 20 animals were used at certain time points to confirm and validate the results. Overall, a total of 230 animals were used in the assessment of seizure activity. Control animals received the electrical stimulus without the administration of agmatine.

The MES test endpoint was the measurement of hindlimb tonic extension, a convulsive action that is one of the recognized components of a maximal seizure (White et al., 1995b). Inhibition of hindlimb tonic extension indicated that the test compound was able to prevent MES-induced convulsive behavior associated with seizure spread (White et al., 1995a,b).

2.4. Minimal neurotoxicity

Toxicity in rats was assessed using the positional sense and gait tests (White et al., 1995b). The rats were tested at 0.25, 0.5, 1, 2, 4, 6, and 8 h after oral (30 mg/kg) or

intraperitoneal administration (30, 60, or 120 mg/kg) of agmatine. These time points paralleled those in which the anticonvulsive activity of agmatine was measured. Additionally, oral doses of up to 480 mg/kg were evaluated at 0.25, 0.5, and 1.0 h after agmatine administration ($n = 8$ at each timepoint). In the positional sense test, one hind leg was gently lowered over the edge of a table. Control rats can quickly return to a normal position. If the rat experienced neurological toxicity, it was unable to quickly lift its leg back onto the table. In the gait and stance test, a circular or zigzag gait after administration of the test compound indicated neurotoxicity. In addition, neurotoxicity was also indicated by ataxia, abnormal spread of the legs, abnormal posture, tremor hyperactivity, lack of exploratory behavior, somnolence, stupor, or catalepsy. Two to eight animals were used at each timepoint for each dose and a total of 168 animals were assessed for neurotoxicity. Neurotoxicity was indicated if, at any time point or after any agmatine dose, the animals displayed any standardized signs of neurological impairment.

Toxicity in mice was assessed with the standardized rotorod test (Dunham and Miya, 1957). Mice not receiving agmatine could maintain their equilibrium for an indefinite period when placed on a rotating (6 rpm) rod. Animals were considered neurologically impaired if they could not maintain equilibrium for 1 min in each of three successive trials after agmatine (30–300 mg/kg) was administered via intraperitoneal injection. The mice were tested at 0.5 and 2 h after agmatine administration. These time points paralleled the initial time points evaluated for the antiseizure activity.

2.5. Statistic analysis

A two-tailed Fisher's Exact Test was used to determine statistically significant differences between the control and agmatine-treated groups. $P < .05$ was considered significant.

3. Results

3.1. MES test

Agmatine was evaluated in the MES seizure test for its capacity to inhibit convulsive behavior associated with seizure spread in rats and mice. Agmatine was not active in the mouse MES model at intraperitoneal doses of up to 300 mg/kg. This is in contrast with the protection observed by the oral route in the rat model. As summarized in Fig. 1, orally administered agmatine (30 mg/kg) prevented seizure spread in 8.3% to 16.7% of the rats that received the MES shock 15 min to 2 h after oral agmatine administration (30 mg/kg). At 4 h, seizure spread was prevented in 50% of the rats tested ($P < .001$). At higher doses, the observed protection was fairly consistent at 1 h after administration of 30 (8%, 1/12), 60 (17%, 2/12), and 120 mg/kg (12%, 1/8). Interestingly, activity levels were not consistent at 4 h after oral administration, where MES-induced seizure spread was inhibited in 50% (6/12, $P < .001$), 17% (2/12, $P < .05$), and 25% (2/8, $P < .02$) of rats treated with oral doses of 30, 60, and 120 mg/kg, respectively. Protection was also inconsistent between the 30- and 60-mg/kg dose at time points before 1 h. At 30 mg/kg, protection was observed in 15% (3/20, $P < .02$) and 8% (1/12, $P = .20$) of rats at 0.25 and 0.5 h, respectively. In contrast, at 60 mg/kg, protection was observed in 0% (0/10) and 20% (2/10, $P < .03$) of rats at 0.25 and 0.5 h, respectively. In hindlimb tonic extension experiments, reproducibility occurred in 99% of control rats.

As a result of the inconsistent results obtained at oral doses greater than 30 mg/kg, the capacity of agmatine to inhibit seizure spread in rats after intraperitoneal administration was also evaluated (Table 1). Three different doses were examined and four rats were evaluated at each indicated timepoint. While 25% of the rats were protected from seizure at 2, 4, and 6 h after administration, the results do

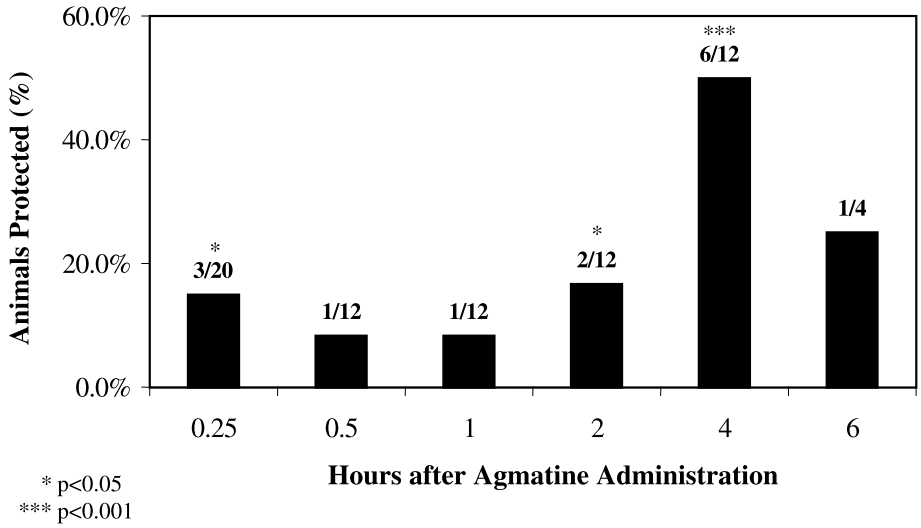

Fig. 1. Inhibitory effect of oral doses of agmatine (30 mg/kg) on MES-induced convulsive activity in rats. Results are expressed as the ratio and percentage of rats protected from hindlimb tonic extension.

Table 1
Effect of intraperitoneal doses of agmatine (30, 60, or 120 mg/kg) on MES-induced convulsive activity in rats

Hours after agmatine administration	30 mg/kg	60 mg/kg	120 mg/kg
0.25	0/4	1/6	ND
0.5	0/4	0/4	ND
1	0/4	0/4	ND
2	0/4	1/4	1/4
4	2/4	1/4	0/4
6	ND	1/4	1/4

The results are expressed as the ratio of rats protected from tonic hindlimb convulsions. ND = not determined.

not appear to be dose-dependent and with the exception of the 30-mg/kg dose administered 4 h prior to the electrical stimulus, the results are not significantly different from control.

3.2. Toxicity

Concurrent neurotoxicological assessment of potential anticonvulsants is an important component of the early drug development process. Accordingly, the acute neurological toxicity of agmatine was evaluated. All of the 168 animals tested in the neurological toxicity component of this study remained free of the characteristic symptoms of acute neurotoxicity. Rats were tested after receiving oral or intraperitoneal agmatine and were evaluated using the positional sense and gait tests for up to 6 h after receiving the agmatine dose. Indeed, oral doses of 480 mg/kg and intraperitoneal doses of 120 mg/kg were administered with no observed toxicity. Agmatine's apparent lack of acute neurotoxicity was also evidenced in mice. The mice received serial doses of agmatine (30, 100, or 300 mg/kg) delivered intraperitoneally. The animals were then evaluated using the rotorod test for symptoms of neurotoxicity at 30 min and 2 h after agmatine administration. All of the animals remained free of acute neurotoxic symptoms after receiving agmatine.

4. Discussion

These studies clearly demonstrate that agmatine exerts anticonvulsant activity when administered at nontoxic doses. The precise mechanism underlying this activity has yet to be elucidated. In addition to its direct action on the NMDA receptor, the anticonvulsant properties of agmatine may result from its interaction at several other CNS receptor sites. Agmatine binds with high affinity to all subclasses of α_2-R (Tabor and Tabor, 1984; Pinthong et al., 1995). It is not known whether agmatine is an agonist or antagonist of α_2-R in the CNS. In the periphery, agmatine appears to act as an agonist at several presynaptic neuronal α_2-adrenergic sites (Molderings and Gothert, 1995; Gonzalez et al., 1996).

Agmatine also has been identified as a regulatory agent in the production of polyamines. Agmatine induces antizyme (Satriano et al., 1998), which inhibits ornithine decarboxylase (ODC)—a key enzyme in the synthesis of polyamines. Although agmatine is a putative precursor of the polyamines via hydrolysis to putrescine by agmatinase (Sastre et al., 1996), this regulatory function may ultimately result in decreased levels of polyamines. Additionally, in hepatocyte cultures, agmatine increased the activity of spermidine/spermine acetyltransferase resulting in a decrease in spermidine and spermine, but an increase in putrescine levels (Vargiu et al., 1999). Since ODC induction and polyamine regulation and distribution may have a role in seizure and epileptic activity (Baudry et al., 1986; Hayashi et al., 1993; Laschet et al., 1999), the capacity of agmatine to regulate polyamine levels may contribute to its observed anticonvulsant activity.

The data from these experiments indicate that the antiseizure activity of agmatine has a rapid onset of action after oral administration. Protection was observed at 15 min after agmatine administration and remained consistent until 2 h after agmatine administration. The peak effect was observed at 4 h. This antiseizure protection profile indicates that there may be both an acute and an extended phase to the antiseizure activity of orally administered agmatine. These data allow the speculation that either multiple mechanisms may be contributing to the observed antiseizure activity, that there may be delayed penetration of agmatine into the CNS, or that agmatine is converted to one or more active metabolites, which may be responsible for the extended duration of activity.

The results obtained after intraperitoneal administration and after oral doses of greater than 30 mg/kg were inconsistent, with minimal protection observed at timepoints greater than 2 h. The response was nonlinear, did not appear to be dose dependent, and was not significantly different from control. These results may arise as a consequence of poor systemic absorption, recycling from primary sites of absorption, the saturation of sites responsible for transport of the drug into systemic circulation, or a drug delivery problem. Additionally, the systemic absorption of intraperitoneal agmatine may be delayed and its antiseizure activity not consistently detectable by the MES-screen, which is an acute screen. The data indicate that oral administration of agmatine is more effective in inhibiting MES-induced seizure spread in rats and therefore, may be the preferred route of administration. Despite these variable effects, it is clear that agmatine exerts significant reproducible antiseizure activity in rats. Notably, no acute neurological toxicity was observed in any animal, at any dose level, or at any timepoint tested.

The novel observation that agmatine possesses anticonvulsant activity in vivo suggests that agmatine may be useful in the therapy of epileptic seizures or other electroconvulsive disorders. The pharmaceutical properties of this compound, including its mechanism of action, metabolism, and pharmacokinetics, must be further characterized in order to develop it into a useful antiepileptic drug. Still, this

research demonstrates the antiseizure activity of agmatine, which might be improved upon with additional chemical synthetic work and structure activity studies. Acute neurotoxicity screens in rats and mice indicate that agmatine may be devoid of untoward neurotoxicity up to the fairly high dose of 480 mg/kg, further underscoring the potential of this and related compounds as novel pharmacophores for the development of new anticonvulsant drugs. Studies aimed at identifying the precise mechanisms underlying the antiseizure activity of agmatine, and the design of even more effective agmatine analogues, are currently underway in our laboratories.

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