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Anticonvulsive effect of agmatine in mice

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Abstract

The present study was designed to examine the effect of agmatine, the decarboxylated product of L-arginine by L-arginine decarboxylase, on convulsion in the mouse maximal electroshock (MES) test and mouse glutamate-induced convulsant test. MES convulsion and glutamate convulsion were respectively induced by an electrical stimulation (110 V, 0.3 s, 8 Hz) and by intracerebroventricular injection of glutamate (0.5 M, pH 7.4, 5μl). The results were expressed as the tonic and clonic time of convulsion in MES or percentage of mice with tonic hind-limb extension in glutamate-induced convulsant assay. Agmatine given intracerebroventricularly (2–16 mg/kg) or subcutaneously (10–160 mg/kg) significantly shortened the tonic and clonic times of convulsion in a dose-dependent manner in the mouse MES test. Glutamate (0.5 M, 5μl icv per mouse) induced an obvious convulsive response indicated by tonic hind-limb extension in mice, and agmatine (2–16 mg/kg icv) decreased the rate of mice with tonic hind-limb extension like NMDA receptor antagonist MK-801. The anticonvulsive effect of agmatine (80 mg/kg sc) on both the tonic and clonic times of convulsion lasted for more than 4 h after administration in the mouse MES test, which was twice that of barbital. Taken together, the results implicate that agmatine has obvious anticonvulsive effects, and its possible mechanism might be related to the antagonism of the function of NMDA receptors.

Keywords: Agmatine; Convulsion; Maximal electroshock; Glutamate; NMDA receptor

1. Introduction

Epilepsy is a common neurological disorder affecting more than 50 million people in the world, the exact mechanisms of which are not understood clearly. Based on the results available, the mechanisms are related to many receptors, at least including GABA, NMDA and acetylcholine receptors (McIntyre et al., 2002; Dekundy et al., 2001; Eger et al., 2002). Over the years, more and more attention has been focused on the effect of NMDA receptors in the initiation of convulsion. The current available results demonstrate that both glutamate and NMDA are able to induce convulsion (Schoepp et al., 1990; King and Thompson, 1989). NMDA receptor antagonists, such as MK-801, have powerful effects against convulsion induced by kindling or electroshock (Kulkarni and Ticku, 1989), and they enhance the anti-convulsive effect of conventional antiepileptics (Borowicz et al., 2001; Luchowska et al., 2001). Furthermore, the concentration of glutamate and aspartate is increased during the initiation and spread of convulsion (Sherwin et al., 1988). In different kinds of convulsant animal models, NMDA binding sites and glycine binding sites in NMDA receptors are up-regulated significantly (Ekonomou and Angelatou, 1999). All these results suggest the important role of NMDA receptor function in the pathogenesis of convulsion.

Agmatine, which is the decarboxylated product of L-arginine by L-arginine decarboxylase (Reis and Regunathan, 2000), has many pharmacological activities on the central nervous system, which is closely related to NMDA receptors. These actions of agmatine include inhibition of tolerance to and dependence on opioids (Li et al., 1998, 1999a), attenuation of the tremors related to ethanol withdrawal (Uzbay et al., 2000), antidepressant-like (Li et al., in press) and anxiolytic activities (Lavinsky et al., 2003), neuroprotective effects in neurotrauma and in neurodegenerative diseases (Gilad et al., 1996). Recently, agmatine is proven to selectively bind at a special site

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on NMDA receptors, which is different from the binding sites of NMDA, polyamines or glycine, and blocks the channel current of the receptor (Yang and Reis, 1999). Moreover, agmatine is able to protect neuronal cells from death induced by glutamate in vitro (Olmos et al., 1999). These results indicate that some pharmacological actions of agmatine might be related to its inhibition of NMDA receptor functions.

Agmatine is a functional antagonist of NMDA receptors, and the NMDA receptor system plays a very important role in the pathophysiology of convulsion, hence it is reasonable to postulate that agmatine might have anticonvulsive effects through the inhibition of NMDA receptors. In the present study, we investigate the anticonvulsive effects of agmatine in the mouse maximal electroshock test (MES) and mouse glutamate-induced convulsant assay in vivo.

2. Materials and methods

2.1. Animals and drugs

Male Kunming mice (18–22 g, supplied by Beijing Animal Center, China) were used in the experiments. The animals were kept in plastic cages at an ambient temperature of 24–25 °C, relative humidity 50–60%, 12:12-h light–dark cycle. Food and water were given ad libitum. All experiments were carried out during the day (between 9:00 and 17:00) to minimize circadian influences on convulsant susceptibility. The animals were randomly distributed into different groups. The experimental protocol was approved by the Institutional Review Committee for the use of Animals.

Agmatine sulfate, MK-801 and glutamate were obtained from Sigma (St. Louis, MO, USA). Barbital sodium was produced by the Beijing Pharmaceutical Plant. Agmatine, MK-801 and barbital were all dissolved in distilled water. Agmatine was administered subcutaneously or intracerebroventricularly. MK-801 was intracerebroventricularly injected. Glutamate was dissolved in 1 N NaOH, neutralized with 1 N HCl to pH 7.4 and was intracerebroventricularly injected. Barbital was injected intraperitoneally. The volume of intracerebroventricular injections was 5 μl per animal.

The intracerebroventricular injection was performed through the following procedure. Each conscious mouse was grasped firmly by the loose skin behind the head, and its snout was gently pushed into the mouth of a 1.5-ml Eppendorf tube, which was horizontally fixed on the edge of a table. The cone of the Eppendorf tube was cut off to secure ventilation. The animal was injected at the bregma with a 10-μl Hamilton syringe, fitted with a 26-gauge needle, which was adjusted to be inserted 2.4 mm deep. The intracerebroventricular injection volume was 5 μl, and injection sites were verified by injecting the same volume of 1% methylene blue into the site and then observing the distribution of the injected dye in the ventricular space. The dye injected intracerebroventricularly was found to be distributed in the ventricular spaces and ventral surface of the brain and in the upper cervical portion of the spinal cord.

2.2. MES-induced convulsion

An electroshock was evoked by ear-clip electrodes to induce convulsion. Only those mice that reacted with tonic hind-limb extension in a pretest before the experiment were used. The stimulus parameters were 110 V, 0.3 s and 8 Hz. The mice were intracerebroventricularly injected with normal saline or different doses of agmatine (2, 4, 8, 12 and 16 mg/kg) at a volume of 5 μl. After 5 min, electrical stimulation was performed, and the number of mice with convulsion was observed. The results were expressed as percentage of mice with convulsion. Groups of 20 mice per treatment were used, and each animal was used for only one treatment.

To further determine the anticonvulsive effect in the electroshock test, agmatine was administered by subcutaneous injection. In this experiment, the mice were subcutaneously injected with normal saline or different doses of agmatine (10, 20, 40, 80, 160 mg/kg). After 30 min, electrical stimulation was performed, and the tonic and clonic times of the convulsion were determined. Groups of 20 mice per treatment were used, and each animal was used for only one treatment.

To evaluate the anticonvulsive duration of action of agmatine, the effects of agmatine (80 mg/kg sc) were observed at 0.5, 2 and 4 h after drug administration in mouse MES-induced convulsion. Barbital sodium (30 mg/kg ip) was used as positive control. Groups of 10 mice per treatment were used, and each animal was used for only one treatment.

2.3. Glutamate-induced convulsion

The mice were intracerebroventricularly injected with normal saline or different doses of agmatine (2, 4, 6, 8, 12 and 16 mg/kg), at a volume of 5 μl. After 5 min, glutamate (0.5 M, pH 7.4) was injected into the lateral ventricles at a volume of 5 μl. The number of mice with convulsion was observed. MK-801 (0.1 mg/kg icv) was used as positive control. Groups of 20 mice per treatment were used, and each animal was used for only one treatment.

2.4. Data analysis

The tonic and clonic times of convulsion were expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) was used to analyze the data followed by Dunnett's t test. The rate of mice with convulsion was analyzed by grouped chi-square test. A P value less than .05 was the critical criterion for statistical significance.

3. Results

3.1. Effect of agmatine on MES-induced convulsion

In the normal saline-treated group, 100% of the mice exhibited convulsions indicated by tonic hind-limb extension and, generally, clonic seizure. Agmatine given intracerebroventricularly reduced the rate of convulsion in a dose-dependent manner. In the presence of 16 mg/kg agmatine, the rate of mice with tonic hind-limb extension induced by electroshock was only 15% (Fig. 1). The difference between the saline and agmatine group was quite significant [$\chi^2(5)=44.03$, $P<.0001$]. The ED_{50} for the anticonvulsive effect of agmatine was 8.59 mg/kg (7.09–10.63 mg/kg).

MES (110 V, 0.3 s, 8 Hz) was able to induce convulsions in 100% of the mice in a preexperiment. The convulsions consisted of two phases, tonic phase and clonic phase. Agmatine given subcutaneously exerted the same anticonvulsive effect as that observed intracerebroventricularly (Table 1). In the normal saline-treated group, the tonic time of convulsion was 14.8 s and the time of clonic phase was 23.7 s. Agmatine significantly shortened both the tonic and clonic times of convulsion induced by electroshock in a dose-dependent manner [ANOVA, $F(5,114)=5.39$, $P<.01$]. In the presence of agmatine (160 mg/kg), the time of tonic phase was decreased by 60% and the clonic phase by 63% compared with the saline group.

3.2. Duration of the effect of agmatine in MES-induced convulsion

In the normal saline group, the time of tonic phase was 14.6 s, and the time of clonic phase was 21.1 s (Fig. 2).

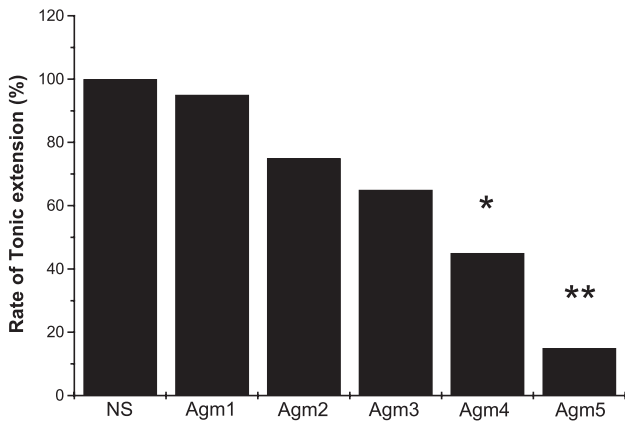

Fig. 1. Inhibition of agmatine on electroshock-induced convulsion in mice. The mice were intracerebroventricularly administrated with saline (NS) or different doses of agmatine (Agm1 = 2, Agm2 = 4, Agm3 = 8, Agm4 = 12 and Agm5 = 16 mg/kg) at a volume of 5 μl. After 5 min, electroshock was performed to evaluate the antiseizure effect. The number of mice with tonic hind-limb extension was recorded. Groups of 20 mice per treatment were used. $*P<.05$, $**P<.01$ compared with the NS group. Grouped chi-square test: $\chi^2(5)=44.03$.

Table 1
Inhibition of agmatine on electroshock-induced convulsion in mice

Drugs	Dose (mg/kg)	Tonic time (s)	Clonic time (s)
Saline	–	14.8 ± 0.4	23.7 ± 2.5
Agmatine	10	14.5 ± 0.9	24.9 ± 2.6
	20	11.7 ± 0.9	18.9 ± 1.5
	40	11.6 ± 1.2	18.9 ± 2.3
	80	8.7 ± 1.8 *	13.9 ± 3.3 *
	160	5.9 ± 2.7 *	8.8 ± 3.9 *

The mice were subcutaneously administrated saline or different doses of agmatine. After 30 min, electroshock was performed to evaluate the antiseizure effect. The times of the tonic and clonic phases of mice were recorded. Each value represents the mean ± S.E.M. of 20 mice in each group.

* $P<.01$ compared with the NS group. One-way ANOVA followed by Dunnett's t test [$F(5,114)=5.39$].

Barbital sodium (30 mg/kg) produced a strong anticonvulsive effect 30 min after intraperitoneal injection; the time of the tonic and clonic phases were inhibited by 49% and 37%,

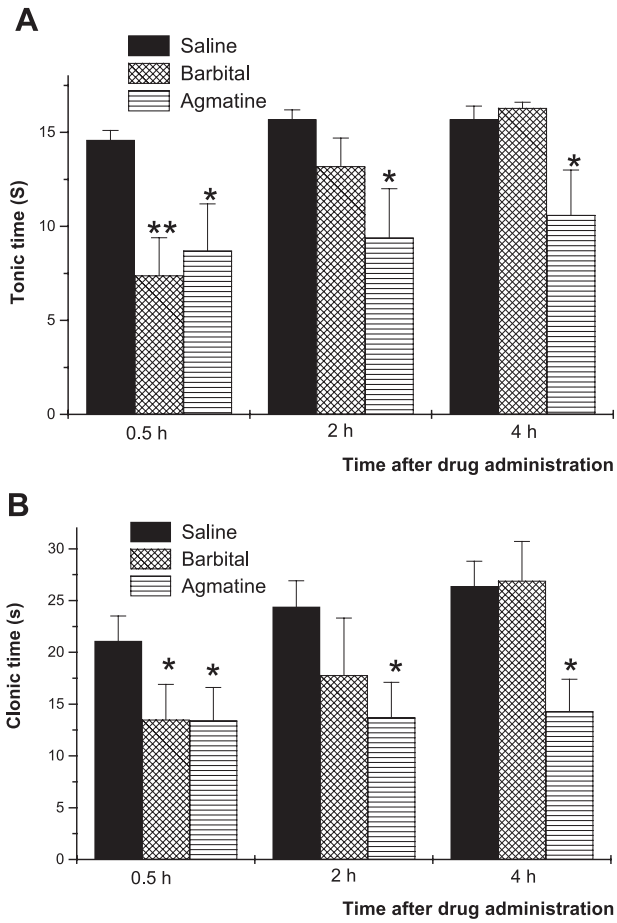

Fig. 2. Time causes of agmatine on times of tonic phase (A) and clonic phase (B) of MES-induced convulsion in mice. The mice were subcutaneously administrated with saline, agmatine (80 mg/kg) or intraperitoneally injected with barbital (30 mg/kg). Electroshock was performed 0.5, 2 and 4 h after drug administration, and the times of the tonic and clonic phase were observed. Each value represents the mean ± S.E.M. of 10 mice in each group. $*P<.01$, $**P<.01$ compared with the NS group. One-way ANOVA was followed by Dunnett's t test.

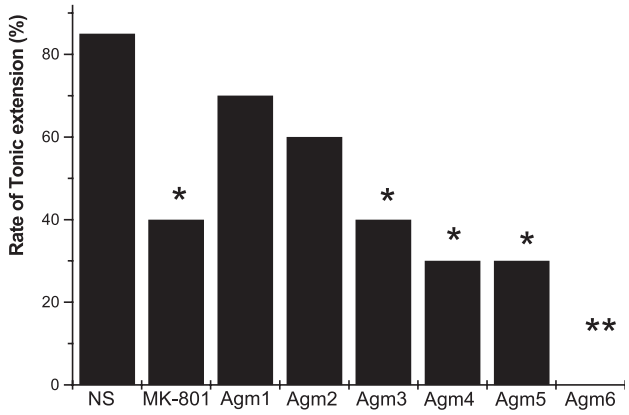

Fig. 3. Inhibition of agmatine on glutamate-induced convulsion in mice. The mice were intracerebroventricularly injected with saline, MK-801 (2 mg/kg) or different doses of agmatine (Agm1 = 2, Agm2 = 4, Agm3 = 6, Agm4 = 8, Agm5 = 12 and Agm6 = 16 mg/kg) at a volume of 5 μl. After 5 min, glutamate (0.5 M, pH 7.4) was injected intracerebroventricularly at a volume of 5 μl to evaluate the anticonvulsion effect. The number of mice with tonic hind-limb extension was recorded. Groups of 20 mice per treatment were used. $*P<.05$, $**P<.01$ compared with NS group. Grouped chi-square test: $\chi^2(7)=28.47$.

respectively ($P<.05$, $n=10$). The effect of barbital sodium disappeared 2 h after injection. Agmatine (80 mg/kg sc) significantly reduced the times of the tonic and clonic phases of convulsion induced by electroshock ($P<.01$, $n=10$). The effects of agmatine lasted for 4 h after subcutaneous injection, which was twice as long as that of barbital sodium.

3.3. Anticonvulsive effect of agmatine in the glutamate-induced mouse model

Glutamate (0.5 M, 5 μl icv) induced 17 of 20 mice to exhibit tonic hind-limb extension in the normal saline group (Fig. 3). MK-801 (0.1 mg/kg) significantly reduced the rate of tonic hind-limb extension. Agmatine (intracerebroventricular) reduced the rate of tonic hind-limb extension in a dose dependent manner [$\chi^2(7)=28.47$, $P<.001$]. In the presence of agmatine (16 mg/kg), no mice exhibited tonic hind-limb extension after glutamate injection ($P<.01$, $n=20$). The ED_{50} for the anticonvulsive effect of agmatine was 5.72 mg/kg (3.98–7.51 mg/kg).

4. Discussion

In the current experiments, agmatine, given subcutaneously or intracerebroventricularly, significantly shortened the times of the tonic and clonic phases of mouse convulsion induced by MES in a dose-dependent manner. Moreover, intracerebroventricular agmatine also dose-dependently inhibited glutamate-induced convulsion. The anticonvulsive effect of agmatine lasted for at least 4 h after subcutaneous injection in the mouse MES-induced convulsion assay. These results indicate that agmatine has anticonvulsive action and that the mechanisms of action might be related to inhibition of NMDA receptor functions.

Both MES- and glutamate-induced convulsions are reported to be related to the stimulation of NMDA receptors and result in tonic and clonic convulsions in mice characterized by tonic hind-limb extension and generalized clonic convulsion, respectively (Kulkarni and Ticku, 1989; Ekonomou and Angelatou, 1999). The activation of NMDA receptors increases the concentration of calcium in plasma promoted via activation of nitric oxide synthase (NOS) activity and increases the concentration of nitric oxide (NO). In recent years, NO has also been proven to play an important role in convulsion. Excessive release of excitatory amino acid transmitter results in over stimulation of glutamate receptors and enhances NO formation in the brain. It has been reported that NO concentration in the central nervous system is increased fourfold in convulsive animals induced by MES (Lapouble et al., 2002; Kata et al., 1998). Furthermore, NOS inhibitors have been proven to have anticonvulsive effects in some convulsion models (Baran et al., 1997; Przegalinski et al., 1996; Borowicz et al., 2000). Based on this mechanism, NMDA antagonists, calcium channel blockers and NOS inhibitors might have neuroprotective and anticonvulsive effects at the same time. Agmatine has neuroprotective effects (Gilad et al., 1996), antagonizes NMDA receptor current (Yang and Reis, 1999), blocks calcium channels (Weng et al., 2003) and inhibits NOS activity (Li et al., 1999b); all these properties infer its possible anticonvulsive effects. On the other hand, agmatine was reported to block nicotinic acetylcholine receptor currents (Santos et al., 2001), and this may also be relevant to its anticonvulsive effect. However, the effect of agmatine on glutamate-induced convulsion further indicates the mechanism of action of agmatine might be related to the inhibition of NMDA receptor function.

Bence et al. (2003) reported on the anticonvulsive effect of agmatine in rats. In the present study, we proved the anticonvulsive effects of agmatine in MES- and glutamate-induced seizure models in mice, and provided further evidence for the participation of NMDA receptors in this process. Agmatine exhibited a long duration for anticonvulsive effect. The effect lasted for at least 4 h after subcutaneous injection, which is consistent with those obtained in rats (Bence et al., 2003).

In conclusion, our present results indicate that agmatine has anticonvulsive effects on MES- and glutamate-induced convulsions and its mechanisms might relate to inhibition of NMDA receptors and their signal transduction process. The inhibition of agmatine on both the tonic and clonic phase of seizure and its long duration in preventing the onset of convulsion indicate its possible utilization in the therapy of epileptic seizures.

References

Baran L, Siwanowicz J, Przegalinski E. Effect of nitric oxide synthase inhibitors and molsidomine on the anticonvulsant activity of some antiepileptic drugs. Pol J Pharmacol 1997;49:363–8.

Bence AK, Worthen DR, Stables JP, Crooks PA. An in vivo evaluation of the antiseizure activity and acute neurotoxicity of agmatine. Pharmacol Biochem Behav 2003;74:771–5.

Borowicz KK, Kleinrok Z, Czuczwar SJ. 7-Nitroindazole differentially affects the anticonvulsant activity of antiepileptic drugs against amygdala-kindled seizures in rats. Epilepsia 2000;41:1112–8.

Borowicz KK, Kleinrok Z, Czuczwar SJ. Glutamate receptor antagonists differentially affect the protective activity of conventional antiepileptics against amygdala-kindled seizures in rats. Eur Neuropsychopharmacol 2001;11:61–8.

Dekundy A, Blaszczak P, Kaminski R, Turski WA. On the interactions between antimuscarinic atropine and NMDA receptor antagonists in anticholinesterase-treated mice. Arch Toxicol 2001;74:702–8.

Eger II EI, Gong D, Xing Y, Raines DE, Flood P. Acetylcholine receptors and thresholds for convulsions from flurothyl and 1,2-dichlorohexafluorocyclobutane. Anesth Analg 2002;95:1611–5.

Ekonomou A, Angelatou F. Upregulation of NMDA receptors in hippocampus and cortex in the pentylenetetrazol-induced "kindling" model of epilepsy. Neurochem Res 1999;24:1515–22.

Gilad GM, Salame K, Rabey JM, Gilad VH. Agmatine treatment is neuroprotective in rodent brain injury models. Life Sci 1996;58:PL41–6.

Kata M, Baran L, Siwanowicz J, Dziedzicka-Wasylewska M. Effect of kainic acid and pentetrazole administration on the activity of nitric oxide synthase in the mouse brain. Pol J Pharmacol 1998;50:259–64.

King AE, Thompson SW. Characterization of deep dorsal horn neurones in the rat spinal cord in vitro: synaptic and excitatory amino acid induced excitations. Comp Biochem Physiol, A 1989;93:171–5.

Kulkarni SK, Ticku MK. Interaction between GABAergic anticonvulsants and the NMDA receptor antagonist MK 801 against MES- and picrotoxin-induced convulsions in rats. Life Sci 1989;44:1317–23.

Lapouble E, Montecot C, Sevestre A, Pichon J. Phosphinothricin induces epileptic activity via nitric oxide production through NMDA receptor activation in adult mice. Brain Res 2002;957:46–52.

Lavinsky D, Arteni NS, Netto CA. Agmatine induces anxiolysis in the elevated plus maze task in adult rats. Behav Brain Res 2003;141:19–24.

Li J, Li X, Pei G, Qin BY. Inhibitory effects of agmatine on tolerance to and substance dependence on morphine in guinea pig ileum in vitro. Acta Pharmacol Sin 1998;19:564–9.

Li J, Li X, Pei G, Qin BY. Effects of agmatine on tolerance to and substance dependence on morphine in mice. Acta Pharmacol Sin 1999a;20:232–8.

Li J, Li X, Pei G, Qin BY. Correlation between inhibitions of morphine withdrawal and nitric oxide synthase by agmatine. Acta Pharmacol Sin 1999b;20:375–80.

Li YF, Gong ZH, Luo ZP, Li J. Antidepressant-like effect of agmatine and its possible mechanism. Eur J Pharmacol [in press].

Luchowska E, Luchowski P, Wielosz M, Kleinrok Z, Urbanska EM. beta-Adrenoceptor blockade enhances the anticonvulsant effect of glutamate receptor antagonists against maximal electroshock. Eur J Pharmacol 2001;431:209–14.

McIntyre DC, Hutcheon B, Schwabe K, Poulter MO. Divergent GABA(A) receptor-mediated synaptic transmission in genetically seizure-prone and seizure-resistant rats. J Neurosci 2002;22:9922–31.

Olmos G, DeGregorio-Rocasolano N, Paz Regalado M, Gasull T, Assumpcio Boronat M, Trullas R, et al. Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. Br J Pharmacol 1999;127:1317–26.

Przegalinski E, Baran L, Siwanowicz J. The role of nitric oxide in chemically- and electrically-induced seizures in mice. Neurosci Lett 1996;217:145–8.

Reis DJ, Regunathan S. Is agmatine a novel neurotransmitter in brain? Trends Pharmacol Sci 2000;21:187–93.

Santos WC, Hernandez-Guijo JM, Ruiz-Nuno A, Olivares R, Jurkiewicz A, Gandia L, et al. Blockade by agmatine of catecholamine release from chromaffin cells is unrelated to imidazoline receptors. Eur J Pharmacol 2001;417:99–109.

Schoepp DD, Gamble AY, Salhoff CR, Johnson BG, Ornstein PL. Excitatory amino acid-induced convulsions in neonatal rats mediated by distinct receptor subtypes. Eur J Pharmacol 1990;182:421–7.

Sherwin A, Robitaille Y, Quesney F, Olivier A, Villemure J, Leblanc R, et al. Excitatory amino acids are elevated in human epileptic cerebral cortex. Neurology 1988;38:920–3.

Uzbay IT, Yesilyurt O, Celik T, Ergun H, Isimer A. Effects of agmatine on ethanol withdrawal syndrome in rats. Behav Brain Res 2000;107:153–9.

Weng XC, Gai XD, Zheng JQ, Li J. Agmatine blocked voltage-gated calcium channel in cultured rat hippocampal neurons. Acta Pharmacol Sin 2003;24:746–50.

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.