Araştırma Makalesi (Research Article)

The Investigation of the Effects of Agmatine in Pentylenetetrazole-induced Epilepsy Model in Mice and the Contribution of Nitric Oxide

Farelerde Pentilenetetrazol ile İndüklenen Epilepsi Modelinde Agmatinin Etkilerinin ve Nitrik Oksitin Katkısının Araştırılması

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Özet

Amaç: Agmatin, endojen katyonik bir amindir ve α2-adrenoseptörler, imidazolin bağlanma yerleri, NMDA reseptörleri ve nitrik oksit (NO) sentaz inhibisyonu aracılığıyla çeşitli nöroterapötik etkileri bildirilmiştir. NO'nun merkezi sinir sisteminde nöromodülatör ve nörotransmiter olarak görev yaptığı, konvülziyon modellerinde prokonvülsan/antikonvülsan aktiviteye sahip olduğu bildirilmiştir. Bu çalışmada, akut agmatin uygulamasının deneysel epilepsi modelindeki etkisi, etkisinin referans antiepileptiklerle karşılaştırılması ve nitrik oksit aracılı mekanizmaların katkısının araştırılması amaçlanmıştır.

Gereç ve yöntemler: Epilepsi nöbetleri, Swiss-albino farelerde tek doz pentilentetrazol (PTZ) (60mg/kg) enjeksiyonu ile indüklendi. Agmatin(10mg/kg), sodyum valproat (150mg/kg), gabapentin (20mg/kg) ve fenitoin (20mg/kg) tek başına veya nitrik oksit prekürsörü N(G)-Nitro-L-arginin-metil-ester (L-NAME,5mg/kg) ve spesifik olmayan NO sentaz inhibitörü L-arginin (L-Arg,60mg/kg) ile kombine edilerek intraperitoneal olarak PTZ'den önce tek doz enjekte edildi. Jeneralize tonik-klonik nöbetler (generalized tonic clonic seizures) (GTCS) ve miyoklonik sıçramalar (myoclonic jerks) (MJ), koruma yüzdesi ve ölüm oranları kaydedildi.

Bulgular: Agmatin, kontrole göre GTCS ve MJ yüzdesini azaltırken koruma yüzdesini anlamlı ölçüde arttırdı. Sodyum valproat sadece GTCS yüzdesini kontrole göre anlamlı ölçüde azaltırken, MJ ve koruma yüzdelerini değiştirmedi. Fenitoin ve gabapentin GTCS, MJ ve koruma yüzdelerini kontrole göre değiştirmedi. L-Arg, agmatinin MJ ve koruma yüzdeleri üzerindeki etkisini anlamlı ölçüde tersine çevirdi. Hem L-Arg hem de L-NAME, sodium valproat ve fenitoinin GTCS, MJ ve koruma yüzdeleri üzerindeki etkisini değiştirmedi. L-Arg, gabapentinin GTCS, MJ ve koruma yüzdeleri üzerindeki etkisini değiştirmedi. L-NAME, gabapentinin MJ yüzdeleri üzerine olan etkisini anlamlı ölçüde iyileştirdi. Ölüm oranları açısından tüm gruplar arasında kontrole göre anlamlı bir farklılık gözlenmedi.

Sonuç: Bu çalışma, agmatinin antikonvülzan etkili olabileceğini ve NO aracılı mekanizmaların agmatin ve gabapentinin etkilerinde rolünün olabileceğini ileri sürmektedir.

Anahtar kelimeler: Agmatin, Fenitoin, Gabapentin, Nitrik oksit, Sodyum valproat

Abstract

Objective: Agmatine is an endogenous cationic amin and has been reported to have several neurotherapeutic effects through α2-adrenoceptors, imidazoline binding sites, inhibition of NMDA receptors and nitric oxide (NO) synthase. NO was reported to act as a neuromodulator and neurotransmitter in central nervous system and has proconvulsant/anticonvulsant activities in convulsion models. We aimed to investigate the effect of agmatine on experimental epilepsy model, compare its effect with reference antiepileptics and the contribution of NO mediated mechanisms.

Material and Methods: Epilepsy seizures were induced by single dose injection of penthylenetetrazole (PTZ) (60mg/kg) in Swiss-albino mice. Single doses of agmatine (10mg/kg), sodium valproate (150mg/kg), gabapentin (20mg/kg) and phenytoin(20mg/kg) were injected intraperitoneally alone or in combination with the precursor of NO, N(G)-Nitro-L-arginine-methyl-ester (L-NAME,5mg/kg) and the non-specific NO synthase inhibitor, L-arginine (L-Arg,60mg/kg) before PTZ injection. Generalized tonic-clonic seizures (GTCS), myoclonic jerks (MJ), protection%, mortality rates were recorded.

Results: Agmatine significantly reduced GTCS%, MJ%, increased protection% compared to control. Sodium valproate reduced GTCS% compared to control but didn't alter MJ% and protection%.Phenytoin and gabapentin didn't alter GTCS%,MJ%,protection% compared to control. L-Arg, significantly reversed the effects of agmatine on MJ% and protection%. Both L-Arg and L-NAME didn't alter the effects of sodium valproate and phenytoin on GTCS%, MJ%, protection%. L-Arg didn't alter the effects of gabapentin on GTCS%, MJ%, protection%. L-NAME significantly enhanced the effects of gabapentin on MJ%. There was no significant difference in mortality rates between control and all groups.

Conclusion: We suggest that agmatine may have anticonvulsant effect and NO mediated mechanisms may contribute to the effects of agmatine and gabapentin.

Keywords: Agmatine, Gabapentin, Nitric oxide, Phenytoin, Sodium valproate

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INTRODUCTION

Epilepsy is a common neurological disorder with complex pathophysiology in the world. The underlying mechanisms of this disorder are still unclear and studies continue to find effective treatment strategies against epilepsy (1). Approximately 70% of epileptic patients use a single antiepileptic drug (AED) and the remaining 30% have refractory or drug-resistant seizure attacks (2) and use combined therapies. These patients need the combinations of AED or alternative treatments (3). Sodium valproate and phenytoin are two of the conventional drugs for the treatment of epileptic seizures. Sodium valproate has been commonly used for over 30 years and phenytoin has also been used for over 50 years as monotherapy for all types including partial, tonic-clonic, generalized tonic-clonic (4). It is considered that sodium valproate is more effective than phenytoin in generalized onset seizures (absence and myoclonus) while phenytoin is more effective in partial onset seizures (simple partial, complex partial, and secondary generalised tonic-clonic seizures) (5) However, there is no randomised controlled trials to support this information (6). Both drugs are reported to have critical side effects, such as teratogenic effect, megaloblastic anemia, fetal hydantoin syndrome and congenital abnormalities (7).

However these agents are still used as conventional drugs in underdeveloped countries (2). Gabapentin is a second generation AED which is used as monotherapy. In general, second generation AEDs were reported to have fewer adverse events such as teratogenic effects and have less drug interactions compared to conventional AEDs (8-10).

Agmatine is a biogenic amine and acts as a neurotransmitter/neuromodulator in the mammalian brain. Agmatine is produced from the decarboxylation of L-Arginine (L-Arg) by arginine decarboxylase and has many biological properties including neuroprotective, cognitive, anxiolytic, anticonvulsant, antinociceptive and antidepressant effects (11). Agmatine was reported to bind and activate imidazoline-1 receptors and α2-adrenoceptors, block N-methyl-D-aspartic acid (NMDA) receptors and other ligand-gated cationic channels, inhibit nitric oxide synthase (NOS) and decrease central noradrenergic activity (12).

Nitric oxide (NO) is known to modulate seizure sensitivity of neurons in the brain (13). In previous studies, NO was reported to have neuromodulator and neurotransmitter functions in central nervous system and to be able to act as proconvulsant (14,15) or anticonvulsant (16) based on the experimental convulsion model. On the other hand, agmatine was shown to be associated with the modulation of the nitric oxide pathway (1). There are some reports suggesting that L-Arg, the precursor of NO, reversed the anticonvulsant effect of agmatine while non-specific nitric oxide synthase inhibitor, N(G)-Nitro-L-arginine-methyl-ester (L-NAME) potentiated this effect in PTZ-induced seizures in mice (11,17).

In this study, we aimed to investigate the effects of acute agmatine administration on pentilentetrazol (PTZ)-induced seizures in mice. We also compared the effects of agmatine with the reference AEDs, sodium valproate, phenytoin and gabapentin. In addition, the contribution of NO in the effects of agmatine and the reference AEDs were also examined.

MATERIALS AND METHODS

Animals

The experimental procedures were performed after approval by the Local Ethical Committee of Eskisehir Osmangazi University for Animal Experimentation (393,17/04/2014). All experiments were performed by using female Swiss-albino mice weighing 30-35 g. The animals were obtained from Medical and Surgical Experimental Research Center of Eskisehir Osmangazi University. Mice were housed under standard laboratory conditions with natural light and dark cycle with free access to food and water ad libitum. Mice were kept under these conditions for 1 week to habituate. The experiments were conducted between 09:00 and 16:00 every day.

Drugs

PTZ, L-Arg, L-NAME were purchased from Sigma-Aldrich (St. Louis, USA). Agmatine sulfate was purchased from Fluka (Buchs, Switzerland). Parenteral preparations of sodium valproate and phenytoin and capsule form of gabapentin were purchased commercially. All the drugs were dissolved in normal saline and injected intraperitoneally (i.p.). The drug solutions were freshly prepared before use.

Experimental design

Total of 105 mice were divided into 15 groups as below:

Group 1: Control (injected with saline)

Group 2: Treated with agmatine 10 mg/kg

Group 3: Treated with sodium valproate 150 mg/kg

Group 4: Treated with phenytoin 20 mg/kg

Group 5: Treated with gabapentin 20 mg/kg

Group 6: Treated with L-Arg 60 mg/kg

Group 7: Treated with L-NAME 5 mg/kg

Group 8: Treated with L-Arg 60 mg/kg+agmatine 10 mg/kg

Group 9: Treated with L-NAME 5 mg/kg+agmatine 10 mg/kg

Group 10: Treated with L-Arg 60 mg/kg+sodium valproate 150 mg/kg

Group 11: Treated with L-NAME 5 mg/kg+ sodium valproate 150 mg/kg

Group 12: Treated with L-Arg 60 mg/kg+phenytoin 20 mg/kg

Group 13: Treated with L-NAME 5 mg/kg+phenytoin 20 mg/kg

Group 14: Treated with L-Arg 60 mg/kg+gabapentin 20 mg/kg

Group 15: Treated with L-NAME 5 mg/kg+gabapentin 20 mg/kg

The doses of agmatine (16,17), sodium valproate (2), phenytoin (2), gabapentin (18), L-Arg (15-17), L-NAME (15,16) and PTZ (19) were determined according to the relevant literatures.

Single doses of agmatine (20,21), sodium valproate (22), gabapentin (2,18), L-Arg (17,21), L-NAME (17,21) were administered 30 minutes before (18,20) the injection of single dose of PTZ 60 mg/kg, while single dose of phenytoin was administered 60 minutes (2) before the injection of single dose of PTZ 60 mg/kg. Control group was treated with normal saline 30 minutes before the single dose injection of PTZ 60 mg/kg. In the combination groups, the drugs were administered with single injections of L-Arg or L-NAME. L-Arg and L-NAME were administered 15 min before the drug injections (16). In all groups, immediately after the injection of PTZ, the convulsive activity was monitored for 30 minutes. Assets of generalized tonic-clonic seizures (GTCS), myoclonic jerks (MJ), mortality of the mice were recorded for each animal (2,18).In addition, protection% was also measured. It was accepted as 'protection against seizures' when the animal had neither GTCS nor MJ. If the animal had one of GTCS or MJ, it was accepted as 'unprotected'. According to these assets; GTCS%, MJ%, protection% and mortality-rate were measured.

Statistical analysis

Statistical analysis was performed by using Pearson Chi-Square test with Monte Carlo significant and Two Proportion Z test with Fisher's exact test. SPSS software package 21.0 and Minitab 16.2.0 were used for statistical analysis and $p<0.05$ was considered as statistically significant.

RESULTS

The effects of agmatine alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection% and mortality rate

Agmatine alone significantly reduced GTCS% and MJ% compared to control ($p<0.05$) **(Table 1)**. L-Arg alone also significantly reduced GTCS% and MJ% compared to control ($p<0.05$) **(Table 1)**. L-NAME alone only reduced GTCS% compared to control ($p<0.05$) **(Table 1)**. On the other hand, when agmatine administered with L-Arg, both GTCS% and MJ% were increased compared to agmatine alone administration. This incease was statistically significant on MJ% ($p<0.05$) **(Table 1)**. When agmatine administered with L-NAME, there was no significant alteration on GTCS% and MJ% compared to agmatine alone administration ($p>0.05$) **(Table 1).**

Agmatine significantly increased protection% compared to control ($p<0.01$) **(Table 1)**. L-Arg alone also significantly increased protection% compared to control ($p<0.01$) while L-NAME alone did not significantly alter protection% compared to control ($p>0.05$) **(Table 1)**. When agmatine was administered with L-Arg, the protection% was significantly reduced compared to agmatine alone or L-Arg alone administrations ($p<0.05$) **(Table 1)**. There was no significant alteration on protection% when agmatine was given in combination with L-NAME compared to agmatine alone administration ($p>0.05$) **(Table 1).**

There was no significant difference on mortality rate between agmatine alone administration and its combined administrations with L-Arg or L-NAME ($p>0.05$) **(Table 1).**

The effects of sodium valproate alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection% and mortality rate

Sodium valproate alone significantly reduced GTCS% compared to control ($p<0.05$) while did not alter MJ% compared to control ($p>0.05$) **(Table 2)**. When sodium valproate administered with L-Arg or L-NAME, there was no significant alteration on GTCS% and MJ% compared to sodium valproate alone administration ($p>0.05$) **(Table 2)**.

Sodium valproate alone did not significantly change protection% compared to control ($p>0.05$) **(Table 2)**. There was an increase in protection% when sodium valproate was combined with L-Arg or L-NAME compared to sodium valproate alone administration, however this was not statistically significant ($p>0.05$) **(Table 2)**. On the other hand, the protection% was significantly increased in sodium valproate and L-NAME combination compared to control ($p<0.01$) **(Table 2)**. There was no significant difference on mortality

Table 1. The effects of agmatine alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection % and mortality rate

	GTCS %	MJ %	Protection %	Mortality rate %
Control	85.7	85.7	0	28.6
L-Arg	14.3*	14.3*†	85.7**†	0
L-NAME	14.3*	57.1	42.9	0
Agmatine	14.3*	14.3*†	85.7**†	14.3
L-Arg + Agmatine	42.9	85.7	14.3	0
L-NAME + Agmatine	14.3*	57.1	42.9	0

GTCS: generalized tonic-clonic seizures, MJ: myoclonic jerks, L-Arg: L-Arginine, L-NAME: N(G)-Nitro-L-arginine-methyl-ester.
*p<0.05, **p<0.01: compared to control. †p<0.05: compared to L-Arg+Agmatine.

Table 2. The effects of sodium valproate alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection % and mortality rate

	GTCS%	MJ%	Protection%	Mortality rate %
Control	85.7	85.7	0	28.6
L-Arg	14.3*	14.3*	85.7**	0
L-NAME	14.3*	57.1	42.9	0
Sodium Valproate	14.3*	57.1	42.9	0
L-Arg + Sodium Valproate	28.6	42.9	57.1	14.3
L-NAME + Sodium Valproate	0**	14.3*	85.7**	0

GTCS: generalized tonic-clonic seizures, MJ: myoclonic jerks, L-Arg: L-Arginine, L-NAME: N(G)-Nitro-L-arginine-methyl-ester.*p<0.05, **p<0.01: compared to control.

Table 3. The effects of phenytoin alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection % and mortality rate

	GTCS%	MJ%	Protection%	Mortality rate%
Control	85.7	85.7	0	28.6
L-Arg	14.3*	14.3*	85.7**	0
L-NAME	14.3*	57.1	42.9	0
Phenytoin	57.1	71.4	28.6	28.6
L-Arg + Phenytoin	42.9	85.7+	14.3+	14.3
L-NAME + Phenytoin	57.1	71.4	28.6	0

GTCS: generalized tonic-clonic seizures, MJ: myoclonic jerks, L-Arg: L-Arginine, L-NAME: N(G)-Nitro-L-arginine-methyl-ester.*p<0.05, **p<0.01: compared to control. +p<0.05: compared to L-Arg.

Table 4. The effects of gabapentin alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection % and mortality rate

	GTCS%	MJ%	Protection%	Mortality rate%
Control	85.7	85.7	0	28.6
L-Arg	14.3*	14.3*	85.7**	0
L-NAME	14.3*	57.1	42.9	0
Gabapentin	57.1	71.4	28.6	0
L-Arg + Gabapentin	71.4	71.4	28.6	0
L-NAME + Gabapentin	0**†¥	0**†¥	100***	0

GTCS: generalized tonic-clonic seizures, MJ: myoclonic jerks, L-Arg: L-Arginine, L-NAME: N(G)-Nitro-L-arginine-methyl-ester.*p< 0.05, **p< 0.01, ***p<0.001: compared to control. †p<0.05: compared to L-Arg+Gabapentin. ¥p<0.05: compared to Gabapentin.

rate between sodium valproate alone administration and its combined administrations with L-Arg or L-NAME (p>0.05) **(Table 2).**

The effects of phenytoin alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection% and mortality rate

Phenytoin alone did not significantly change GTCS%, MJ%, protection% and mortality rate compared to control (p>0.05) **(Table 3)**. There was no significant alteration on GTCS%, MJ%, protection% and mortality rate when phenytoin was given in combination with L-Arg or L-NAME compared to phenytoin alone administration (p>0.05) **(Table 3).**

The effects of gabapentin alone or in combination with L-Arg and L-NAME on GTCS%, MJ%, protection% and mortality rate.

Gabapentin alone did not significantly change GTCS%, MJ%, protection% and mortality rate compared to control (p>0.05) **(Table 4).**

When gabapentin administered with L-Arg, there was no significant alteration on GTCS%, MJ%, protection% and mortality rate compared to sodium valproate alone administration (p>0.05) **(Table 4)**. On the other hand, when gabapentin administered with L-NAME, GTCS% and MJ% reduced compared to gabapentin alone administration and this redu-

ction was statistically significant on MJ% ($p<0.05$) **(Table 4)**. Protection% was also increased in gabapentin and L-NAME combination compared to gabapentin alone administration, however this was not statistically significant ($p>0.05$) **(Table 4)**. On the other hand, Protection% was significantly increased in gabapentin and L-NAME combination compared to control ($p>0.001$) **(Table 4)**. There was no significant difference on mortality rate between gabapentin alone administration and its combined administrations with L-Arg or L-NAME ($p>0.05$) **(Table 4)**.

DISCUSSION

In this study, we examined the acute effects of agmatine, as well as the reference AEDs sodium valproate, phenytoin, gabapentin on PTZ-induced seizures in mice and the contribution of NO. We observed that agmatine significantly prevented but reference AEDs did not prevent the PTZ-induced seizures. In addition, L-Arg reversed the preventive effect of agmatine. L-Arg or L-NAME did not alter the effects of sodium valproate and phenytoin. L-NAME increased the preventive activity of gabapentin on seizures.

PTZ can induce acute seizures with a single dose of administration (23). PTZ can affect the functions of certain neurotransmitters, such as GABA, adenosine and glutamate (24,25). PTZ blocks the GABA-mediated inhibition by binding to the GABA receptor complex on the picrotoxin site and therefore it leads to disinhibition. The activation of NMDA receptors (26), the inhibition of GABA and/or adenosine seem to be responsible for the initiation and generalization of PTZ-induced seizures. Studies reported that NO levels were increased in mice brain in PTZ-induced epilepsy (27-29). NO plays an important role in various animal models of epilepsy. There is a contradiction in the literature on the role of NO in epileptogenesis. The data considered NO as both anticonvulsant or pro-convulsant (30). Some of the evidences show that the effects of NO on GABA can be biphasic (31,32). It was reported that low concentrations of NO can inhibit GABA-ergic transmission while high concentrations of NO stimulates the release of GABA It was also demonstrated that there is a significant interaction between GABA-ergic, glutamatergic and NO: cGMP pathways (34). NMDA receptor activation leads to the formation of NO and increase cGMP. It was suggested that the increase of cGMP in brain leads to induce the epileptic activity (35). The inhibition of guanylate cyclase by methylene blue was also shown to have the anticonvulsant activity (33,36).

Agmatine is an endogenous polyamine produced from L-Arg by arginine decarboxylase (37). Studies showed that agmatine was found in the brain and acts as a neurotransmitter or a co-transmitter (38). It can bind to α2-adrenoceptors, imidazoline and glutamate NMDA receptors (37,39). Agmatine is known to inhibit nitric oxide synthase (NOS) isoenzymes in central nervous system (38). It is suggested that agmatine exerts antiseizure activity by the inhibition of NO production, the blockade of glutamate NMDA receptors (37,40), lowering extracellular glutamate levels (41) and stimulation of α2-adrenoceptors (20). It was reported that systemic administration of agmatine diminished chemically and electrically induced acute convulsions (37). Similarly, in this study, we observed that agmatine prevented PTZ-induced seizures. In addition, we observed that L-Arg, the precursor of NO, alleviated the antiseizure effect of agmatine in line with the existing data (16). This reflects that NO mediated mechanisms may be involved in the preventive effect of agmatine against PTZ-induced seizures. We may suggest that agmatine showed antiseizure effect by decreasing NO levels and this is in accordance with the NOS inhibitory action of agmatine. On the other hand, we observed that non-specific NOS inhibitor L-NAME did not make an alteration in the antiseizure effect of agmatine. This is not in accordance with the study which reported that L-NAME with higher doses significantly enhanced anticonvulsant effect of agmatine (20). In the above mentioned study, 30 mg/kg doses of L-NAME was used in PTZ-induced epilepsy in mice. However, we used L-NAME at a dose of 5 mg/kg and this dose did not affect the activity of agmatine but increased the anticonvulsant activity of gabapentin and sodium valproate.

The conventional antiepileptic drugs phenytoin and sodium valproate is commonly used in the treatment of epilepsy (4). Phenytoin is known as a sodium channel blocker and sodium valproate has multiple therapeutic targets such as GABA potentiation, glutamate (NMDA) inhibition, sodium channel and T-type calcium channel blockade. The second generation antiepileptic agent gabapentin acts as a calcium channel blocker (α2δ subunit) (7). In our study, single dose of phenytoin did not exert preventive effect against PTZ-induced seizures. We also observed that neither L-Arg nor L-NAME altered the effect of phenytoin. Similar to our results, in a study in mice, chronic administration of phenytoin did not affect or had lower anticonvulsant activity (27,42) and also levels of NO did not change in the brain in PTZ-induced seizures (27). The researchers of that study suggested that high levels of PTZ-induced lipid peroxidation that lead to greater production of free radical generation can be correlated with high seizure activity in phenytoin-treated mice (27).

In our study, we observed that sodium valproate did not show preventive effect against PTZ-induced seizures but ameliorated generalized tonic-clonic seizures. It did not affect myoclonic jerks. In a study, 150 mg/kg dose of sodium valproate with combination of aspirin 100 mg/kg showed reduction in generalized clonic seizures, but did not show the same effect when administered alone (22).

In this study, we also observed that gabapentin had no significant preventive action against PTZ-induced seizures. Differently from our results, in a study, gabapentin 20 mg/kg administered alone increased seizure threshold in PTZ-induced epilepsy in Laca mice. (18). However, it seems that there is a role of NO in the anticonvulsant activity of gabapentin because L-NAME significantly increased the activity of gabapentin. Our results showed that a significant antiseizure

activity was produced when gabapentin and L-NAME were given in combination. NOS inhibitors were suggested either protective or dual proconvulsive/anticonvulsive effects (30). It was reported that L-NAME had anticonvulsant activity against tonic convulsions in the PTZ test at high doses and L-Arg reversed this effect (43). In another study, L-NAME enhanced the activity of benzodiazepines against PTZ-induced seizures (30).

As a conclusion, agmatine prevented PTZ-induced seizures in mice by reducing both GTCS and MJ and exerted a significant protection. We suggest that agmatine possess antiseizure activity and may be considered as a potential antiepileptic agent. We also suggest that NO mediated mechanisms may contribute to the antiseizure activity of agmatine and NOS inhibitory action may have a role in this effect. In addition, the reference AED sodium valproate also prevented GTCS, however did not exert a significant protection as it could not prevent MJ. The other reference AEDs phenytoin and gabapentin also did not prevent GTCS and MJ and could not produce a protective effect. L-Arg and L-NAME did not make any alteration on the effects of sodium valproate, phenytoin and gabapentin. On the other hand, a potentialization was observed with L-NAME and gabapentin combination and it seems that there is an interaction through NO associated mechanism between them.

Further studies are needed to elucidate the exact antiseizure mechanisms of action of agmatine and we plan to support the behavioral antiepileptic effects of agmatine with histological and molecular studies.

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