Pharmacological Reports
2009, 61, 252–260
ISSN 1734-1140

Influence of agmatine on the protective action of numerous antiepileptic drugs against pentetrazole-induced seizures in mice[A]

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Abstract:
The aim of this study was to assess the influence of agmatine (an endogenous neuromodulator/neurotransmitter in the brain) on the protective action of numerous classical and second-generation antiepileptic drugs (clonazepam, ethosuximide, gabapentin, phenobarbital, tiagabine, vigabatrin, and valproate) in the mouse pentetrazole-induced clonic seizure model.
The results indicate that agmatine (up to 100 mg/kg, *ip*, 45 min before the test) did not alter the threshold for pentetrazole-induced clonic seizures in mice. However, agmatine (100 mg/kg, *ip*) significantly attenuated the anticonvulsant effects of vigabatrin against pentetrazole-induced clonic seizures by elevating the ED_{50} value of vigabatrin from 517.5 to 790.3 mg/kg ($p < 0.01$). In contrast, agmatine at a dose of 50 mg/kg did not significantly affect the anticonvulsant action of vigabatrin, although an increase in the ED_{50} value of the antiepileptic drug from 517.5 to 629.1 mg/kg was documented. Moreover, agmatine at doses of 50 and 100 mg/kg (*ip*) had no significant impact on the anticonvulsant action of clonazepam, ethosuximide, gabapentin, phenobarbital, tiagabine, or valproate in pentetrazole-induced seizures in mice.
In conclusion, the combination of agmatine with vigabatrin seems to be unfavorable due to the reduction of the anticonvulsant effect of vigabatrin after concomitant administration of agmatine in the pentetrazole-induced seizure model. Therefore, the utmost caution is advised when combining agmatine with vigabatrin in further clinical settings.

Key words:
agmatine, antiepileptic drugs, drug interactions, pentetrazole-induced seizures, vigabatrin

Introduction

Agmatine (1-amino-4-guanidinobutane) is an endogenous amine synthesized by the decarboxylation of L-arginine by arginine decarboxylase (EC 4.1.1.19), and hydrolyzed by agmatinase (EC 3.5.3.11) to putrescine and urea [33, 37]. Agmatine has been detected in various mammalian organs, especially in the brain, where it acts as a novel neurotransmitter/neuromodulator [33, 35]. Although the physiological roles of agmatine are still unknown and under intensive investigation, a number of experimental studies have reported that agmatine: (a) binds with high affinity to imidazoline I_1 receptors and α_2-adrenoceptors [19,

[A] Results from this study were presented in part at the "49th Annual Meeting of the German Society of Pharmacology and Toxicology", Mainz, Germany, March 11–13, 2008 [abstract in Naunyn-Schmiedeberg's Arch Pharmacol, 2008, 377, Suppl 1, 51 (abstract no. 236)].

33, 35]; (b) blocks N-metyl-D-aspartic acid (NMDA) receptors and other ligand-gated cationic channels, such as serotonin (5-HT_3) and nicotinic acetylcholine receptors [35, 44]; (c) inhibits nitric oxide synthase (NOS) activity [2, 8, 11]; (d) blocks voltage-gated calcium channels [43]; (e) stimulates the release of norepinephrine from vascular nerve terminals and vasopressin from neurohypophysis [16]. Thus, agmatine possesses several biological functions, of which the neuroprotective, anticonvulsant, and antinociceptive properties are the most important [3, 9, 15, 29, 39]. For instance, agmatine is capable of protecting neurons from glutamate-induced death *in vitro* [29] as a result of its inhibition of NMDA receptor functions, but not the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainic acid receptors in rat hippocampal neurons [44]. Moreover, agmatine has been reported to block the increase in extracellular glutamate during pentetrazole-induced seizures [10]. Agmatine also reduces the potentiating effects of polyamines by competitive antagonism at a specific site on the NMDA receptor complex [14]. It is worth noting that the activation of NMDA receptors in the brain increases intracellular calcium, which activates neuronal NOS to produce NO from L-arginine [12, 40]. Experimental evidence indicates that the activation of NMDA receptors is involved in and responsible for the development of seizures in both preclinical studies on animals and clinical conditions in humans [21]. In contrast, the reduction of NMDA receptor-mediated activity in the brain exerts an anticonvulsant effect, contributing to the suppression of seizures in both *in vivo* and *in vitro* experiments [21, 42]. Agmatine affects the synthesis of NO by activating the endothelial NOS [28], while inhibiting the inducible NOS [2, 11] and neuronal NOS [8]. Agmatine also suppresses NO production in the microglia of rats [1]. Again, it is worth noting that NO is involved in the modulation of seizure susceptibility of neurons in the brain [12, 40]. Therefore, it is very likely that the anticonvulsant effect of agmatine is related to modulation of the L-arginine/NO pathway, as L-arginine pretreatment partially reversed the antiseizure effect of agmatine in pentetrazole-induced seizures [9]. In contrast, N^G-nitro-L-arginine methyl ester (a NOS inhibitor) potentiated the anticonvulsant effect of agmatine in pentetrazole-induced seizures in mice [9]. Interestingly, agmatine was inactive in the mouse maximal electroshock-induced seizure model after intraperitoneal (*ip*) administration of the drug, however, when the agent was administered orally (*po*), it significantly suppressed maximal electroshock-induced seizures in rats [3]. Agmatine administered intracerebroventricularly (*icv*) and subcutaneously (*sc*) significantly shortened the tonic and clonic phases of electrically evoked convulsions [39]. On the other hand, it has been found that yohimbine, an α_2-adrenoceptor antagonist, completely inhibited the anticonvulsant activity of agmatine in pentetrazole-induced seizures, which supports the hypothesis about the involvement of α_2-adrenoceptors in this effect [9].

Relatively recently, it was found that agmatine significantly enhanced the anticonvulsant activity of phenobarbital and valproate, but not of carbamazepine, phenytoin, oxcarbazepine, topiramate, or lamotrigine in the mouse maximal electroshock-induced seizure model [23]. Moreover, agmatine combined with phenobarbital and valproate displayed favorable interactions. The nature of the interactions was pharmacodynamic because the total brain phenobarbital and valproate concentrations were not altered after concomitant administration with agmatine [23].

When considering the anticonvulsant properties of agmatine in experimental studies, it was of pivotal importance to evaluate the effect of agmatine on the threshold for pentetrazole-induced seizures, and to assess the influence of agmatine on the protective activity of numerous classical and second-generation antiepileptic drugs (clonazepam, ethosuximide, gabapentin, phenobarbital, tiagabine, valproate, and vigabatrin) in the mouse pentetrazole-induced seizure test, which is thought to be an experimental model of myoclonic seizures in humans [22]. It is significant that in this experimental test one can readily assess the antiseizure potential of agents and compounds possessing the anticonvulsant properties, as well as determining their influence on classical and second-generation antiepileptic drugs [22]. Therefore, it was appropriate to use the pentetrazole-induced seizure model in order to evaluate the effect of agmatine on the protective action of the various antiepileptic drugs in preclinical study.

Materials and Methods

Animals and experimental conditions

Adult male Swiss mice (weighing 22–26 g) that were kept in colony cages with free access to food and tap

water and under standardized housing conditions (natural light-dark cycle, temperature of 22 ± 1°C, relative humidity of 55 ± 5%) were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups, each comprised of 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 15.00 hours. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this manuscript were approved by the Local Ethics Committee at the Medical University of Lublin (License No.: 538/2005/577/2005) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

The following drugs were used: agmatine (sulfate salt – Sigma, St. Louis, MO, USA), clonazepam (Polfa, Warszawa, Poland), ethosuximide (Sigma, St. Louis, MO, USA), gabapentin (Neurontin – Parke-Davis GmbH, Freiburg, Germany), phenobarbital (Polfa, Kraków, Poland), tiagabine (Gabitril – Sanofi Winthrop, Gentilly, France), vigabatrin (Sabril – Marion Merrell S.A., Puteaux, France), and valproate (magnesium salt – kindly donated by ICN-Polfa S.A., Rzeszów, Poland). All drugs, except for agmatine and valproate, were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water, while agmatine and valproate were directly dissolved in distilled water. All drugs were administered *ip* as a single injection, in a volume of 5 ml/kg body weight. Fresh drug solutions were prepared on each day of experimentation and administered as follows: vigabatrin was administered 240 min; gabapentin and phenobarbital – 60 min; agmatine and ethosuximide – 45 min; valproate – 30 min; clonazepam and tiagabine – 15 min before initiation of pentetrazole-induced seizures. The pretreatment times before testing of the antiepileptic drugs were based upon information about their biological activity from the literature and our previous experiments [5, 6, 23–27]. The route of *ip* administration of agmatine and the pretreatment time before testing of its threshold for anticonvulsant action were based upon information from the literature [9, 32]. Pentetrazole (Sigma, St. Louis, MO, USA) was dissolved in sterile saline and administered *sc* into a loose fold of skin in the midline of the neck in a volume of 5 ml/kg body weight.

Threshold for pentetrazole-induced clonic seizures

The threshold for clonic convulsions was determined in control (vehicle-treated) mice by *sc* administration of pentetrazole at doses ranging from 50 to 100 mg/kg. Following the injection of pentetrazole, mice were placed separately into transparent Plexiglas cages (25 × 15 × 10 cm) and observed for 30 min for the occurrence of clonic seizures. Clonic seizure activity was defined as clonus of the whole body lasting for more than 3 s, with an accompanying loss of righting reflex. The number of animals convulsing out of the total number of mice tested was noted for each treatment condition. The convulsive action of pentetrazole was evaluated as the CD_{50} (median convulsive dose, i.e., the dose of pentetrazole that produced clonic seizures in 50% of the mice tested). To determine the CD_{50} value for control animals, 5 varying doses of pentetrazole were tested (8 mice per group). Subsequently, a dose-response curve was determined from the percentage of mice convulsing according to the log-probit method of Litchfield and Wilcoxon [20]. Afterwards, the CD_{50} value for control animals was calculated from the equation of dose-response curve for pentetrazole. The influence of agmatine (administered at doses of 50 and 100 mg/kg) on the CD_{50} value of pentetrazole was determined as in the control animals. At least 4 groups of animals (8 mice per group) were used to estimate each CD_{50} value for the combination of pentetrazole and agmatine. Subsequently, from the respective dose-response curves, the CD_{50} values for pentetrazole were calculated according to Litchfield and Wilcoxon [20].

Pentetrazole-induced seizures

The anticonvulsant activity of classical and second-generation antiepileptic drugs administered alone and in combination with agmatine was determined in the pentetrazole-induced clonic seizure test in mice. Clonic convulsions were induced in mice by the *sc* administration of pentetrazole at a dose of 100 mg/kg, the predetermined CD_{97} (the dose necessary to induce clonic seizures in 97% of animals tested) of the com-

pound. Following the administration of pentetrazole, mice were placed separately into transparent Plexiglas cages (25 × 15 × 10 cm) and observed for 30 min for the occurrence of clonic seizures. The clonic seizures were defined as clonus of the whole body lasting for more than 3 s, with an accompanying loss of righting reflex. The number of mice convulsing out of the total number of mice tested was noted for each treatment regimen. The anticonvulsant action of each antiepileptic drug alone or in combination with agmatine was evaluated as its ED_{50} (median effective dose; i.e., the dose of an antiepileptic drug that protected 50% of mice against pentetrazole-induced convulsions). Each ED_{50} value was calculated from the respective dose-response curve according to the log-probit method of Litchfield and Wilcoxon [20].

Statistics

Both the CD_{50} and ED_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [20]. Subsequently, the respective 95% confidence limits were transformed into SE as described previously [24]. Statistical analysis of data from the pentetrazole-induced clonic seizures was performed with one-way analysis of variance (ANOVA), followed by the *post-hoc* Tukey-Kramer test for multiple comparisons. Differences among the values were considered statistically significant if $p < 0.05$. All statistical tests were performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Influence of agmatine upon the threshold for pentetrazole-induced clonic seizures

Agmatine administered systemically (*ip*, 45 min prior to the test) at doses of 50 and 100 mg/kg did not affect the threshold for pentetrazole-induced clonic seizures in mice. In this case, the experimentally derived CD_{50} values of pentetrazole for animals receiving agmatine at increasing doses did not significantly differ from that value determined for control animals (Tab. 1).

Tab. 1. Effect of agmatine on the threshold for pentetrazole-induced clonic seizures in mice

Treatment (mg/kg)	CD_{50} (mg/kg)	n	SE
Control	68.54 (59.57–78.85)	24	4.90
Agmatine (50)	73.89 (64.56–84.57)	32	5.09
Agmatine (100)	79.03 (69.73–89.57)	24	5.04
F (2; 77) = 0.960; p = 0.388			

Data are presented as the median doses of pentetrazole (CD_{50} values with 95% confidence limits in parentheses) required to produce clonic seizures in 50% of the animals tested. Agmatine was administered *intraperitoneally* 45 min. before the test. Statistical evaluation of the data was performed with the log-probit method [20], followed by one-way ANOVA. n – number of animals tested at those doses of pentetrazole, whose seizure effects ranged from 16% to 84%. F – F-statistics from one-way ANOVA; p – probability value from one-way ANOVA

Effects of agmatine on the protective action of classical and second-generation antiepileptic drugs in the mouse pentetrazole-induced clonic seizure model

All investigated classical and second-generation antiepileptic drugs (clonazepam, ethosuximide, gabapentin, phenobarbital, tiagabine, valproate, and vigabatrin) administered alone exhibited a definite anticonvulsant activity in the pentetrazole-induced clonic seizure test in mice and their ED_{50} values are presented in Table 2. When agmatine at a dose of 100 mg/kg was co-administered with vigabatrin, it significantly reduced the anticonvulsant effect of the latter drug against pentetrazole-induced clonic seizures by elevating its ED_{50} value from 517.5 to 790.3 mg/kg ($p < 0.01$; Tab. 2). In contrast, agmatine at the lower dose of 50 mg/kg did not significantly diminish the antiseizure activity of vigabatrin in the pentetrazole-induced seizure test, although a slight increase in the ED_{50} of vigabatrin from 517.5 to 629.1 mg/kg was observed (Tab. 2). With regard to the remaining antiepileptic drugs tested (i.e., clonazepam, ethosuximide, gabapentin, phenobarbital, tiagabine, and valproate), agmatine at doses of 50 and 100 mg/kg had no significant impact on the anticonvulsant activity of these antiepileptic drugs against pentetrazole-induced clonic seizures (Tab. 2).

Tab. 2. Effect of agmatine on the protective activity of the various antiepileptic drugs in pentetrazole-induced seizures in mice

Treatment (mg/kg)	ED_{50} (mg/kg)	n	SE
Clonazepam + vehicle	0.018 (0.009–0.033)	16	0.0057
Clonazepam + Agmatine (50)	0.025 (0.014–0.045)	24	0.0074
Clonazepam + Agmatine (100)	0.041 (0.024–0.069)	16	0.0110
F (2; 53) = 1.782; p = 0.178			
Ethosuximide + vehicle	145.0 (129.9–161.8)	16	8.10
Ethosuximide + Agmatine (50)	135.1 (116.0–157.4)	24	10.52
Ethosuximide + Agmatine (100)	131.8 (110.3–157.5)	32	11.98
F (2; 69) = 0.296; p = 0.745			
Gabapentin + vehicle	485.7 (287.7–820.0)	16	129.70
Gabapentin + Agmatine (50)	399.8 (263.5–606.7)	16	85.02
Gabapentin + Agmatine (100)	363.4 (226.9–582.1)	24	87.30
F (2; 53) = 0.386; p = 0.682			
Phenobarbital + vehicle	11.55 (8.45–15.78)	32	1.84
Phenobarbital + Agmatine (50)	9.13 (7.31–11.40)	40	1.31
Phenobarbital + Agmatine (100)	8.11 (5.48–12.0)	32	1.62
F (2; 101) = 1.177; p = 0.312			
Tiagabine + vehicle	0.90 (0.50–1.60)	24	0.27
Tiagabine + Agmatine (50)	0.73 (0.45–1.16)	24	0.18
Tiagabine + Agmatine (100)	0.62 (0.32–1.20)	16	0.21
F (2; 61) = 0.357; p = 0.702			
Valproate + vehicle	157.1 (123.8–199.3)	24	19.06
Valproate + Agmatine (50)	148.1 (112.2–195.5)	32	20.97
Valproate + Agmatine (100)	144.3 (126.8–164.1)	16	9.49
F (2; 69) = 0.087; p = 0.917			
Vigabatrin + vehicle	517.5 (411.6–650.6)	24	60.41
Vigabatrin + Agmatine (50)	629.1 (548.5–721.4)	16	43.94
Vigabatrin + Agmatine (100)	790.3 (697.3–895.7)**	24	50.44
F (2; 61) = 7.074; p = 0.0017			

Results are presented as the median effective doses (ED_{50} in mg/kg, with 95% confidence limits in parentheses) of antiepileptic drugs protecting 50% of the animals tested against pentetrazole-induced clonic seizures. All drugs were administered *intraperitoneally* at the indicated number of minutes before the convulsive test: vigabatrin – 240 min; phenobarbital and gabapentin – 60 min; ethosuximide and agmatine – 45 min; valproate – 30 min; clonazepam and tiagabine – 15 min. The pentetrazole-induced clonic seizures were produced by the *subcutaneous*-injection of pentetrazole at its CD_{97} (100 mg/kg). Statistical analysis of data was performed with one-way ANOVA, followed by the *post-hoc* Tukey-Kramer test for multiple comparisons. n – total number of animals used at those doses, whose anticonvulsant effects ranged from 4 to 6 probits; F – F-statistics from one-way ANOVA; p – probability value from one-way ANOVA. ** $p < 0.01$ *vs.* the respective control group (vigabatrin + vehicle-treated animals)

Discussion

The results presented in this study demonstrate that agmatine administered systemically (*ip*) at doses up to 100 mg/kg did not significantly affect the threshold for pentetrazole-induced clonic seizures in mice. Our findings, then, are in contrast to those reported earlier that agmatine, in a dose dependent manner, protected animals at the threshold for pentetrazole-induced clonic seizures [9, 36]. This apparent discrepancy between the findings presented in the current study and those documented by Demehri et al. [9] and Riazi et al. [36] probably results from the different animal models used to determine the threshold for pentetrazole-induced clonic seizures. In our study, pentetrazole was administered *sc* at increasing doses ranging from 50–100 mg/kg, which allowed us to calculate the CD_{50} value, i.e., the dose of pentetrazole producing clonic convulsions in 50% of the animals tested. In contrast, in the studies by Demehri et al. [9] and Riazi et al. [36], pentetrazole was infused intravenously (*iv*) into the tail vein of freely moving mice as a 1% solution and the threshold for pentetrazole-induced clonic seizures was determined by calculating a total dose of pentetrazole required to induce the clonus in experimental animals. Generally, the threshold for pentetrazole-induced clonic seizures evoked by *iv* infusion of pentetrazole is considerably lower than that determined after *sc* administration of pentetrazole [22]. This is the reason why the threshold dose of pentetrazole required to produce clonic seizures in control (vehicle-treated) animals in our study was determined to be 68.54 mg/kg, whereas that dose in the studies by Demehri et al. [9] and Riazi et al. [36] was approx. 2-fold lower, 38 mg/kg. On the other hand, it was found in the present study that agmatine administered alone produced no protective action against pentetrazole-induced clonic seizures because the CD_{50} values of pentetrazole for animals receiving agmatine (50 and 100 mg/kg) did not differ significantly from that of the control (vehicle-treated) animals.

Our findings also indicate that agmatine at the dose of 100 mg/kg significantly reduced the antiseizure action of vigabatrin by increasing its ED_{50} value against pentetrazole-induced seizures in mice. In contrast, agmatine at the dose of 100 mg/kg had no significant impact on the anticonvulsant action of the remaining antiepileptic drugs tested (i.e., clonazepam, ethosuximide, phenobarbital, gabapentin, tiagabine, and val-

proate) against pentetrazole-induced clonic seizures in mice. The observed attenuation of the antiseizure effect of vigabatrin against pentetrazole-induced seizures is in opposition to our recent findings documenting that agmatine at 100 mg/kg significantly enhanced the antiseizure action of phenobarbital and valproate by reducing their ED_{50} values in the maximal electroshock-induced seizure test, while having had no impact on the antielectroshock action of carbamazepine, phenytoin, topiramate, oxcarbazepine, and lamotrigine in mice [23]. Interestingly, as reported earlier, agmatine enhanced the anticonvulsant action of phenobarbital and valproate in the maximal electroshock-induced seizures, but showed no significant effect when combined with the same drugs (phenobarbital and valproate) in pentetrazole-induced clonic seizures in mice. A similar situation has been observed for the combination of phenobarbital or valproate with loreclezole in the maximal electroshock- and pentetrazole-induced seizure tests [24, 25]. It has been documented that the combination of phenobarbital or valproate with loreclezole in the pentetrazole test produced a barely additive interaction, whereas the same combinations synergistically interacted in the maximal electroshock-induced seizure test in mice [24, 25]. Thus, one can ascertain that the characteristics of interactions of agmatine with antiepileptic drugs may change depending on the seizure models used to determine the anticonvulsant action of the antiepileptic drugs in preclinical study. The diverse characteristics of interactions between phenobarbital, valproate and agmatine or loreclezole confirmed the hypothesis that the same drugs may interact in a completely different manner, depending on the experimental model used [24, 25].

On the other hand, the results from the pentetrazole-induced seizure test, which show a significant decrease in the anticonvulsant action of vigabatrin after concomitant administration of agmatine, are similar to those documented earlier for the combination of vigabatrin with N^G-nitro-L-arginine (a non-selective NOS inhibitor, administered *ip*, at 40 mg/kg), where the ED_{50} value of vigabatrin was significantly elevated after concomitant administration of N^G-nitro-L-arginine in the pentetrazole-induced seizure test in mice [26]. Moreover, it has been reported that N^G-nitro-L-arginine (at 40 mg/kg) also attenuated the anticonvulsant action of ethosuximide and oxcarbazepine against pentetrazole-induced clonic seizures in mice [6, 26]. However, in the present study, agmatine did not significantly alter the antiseizure action of ethosuximide in the pentetrazole test in mice. With regard to 7-nitroindazole (a preferential neuronal NOS inhibitor), preclinical studies revealed that the agent administered *ip*, at a dose of 50 mg/kg, significantly enhanced the antiseizure action of clonazepam and ethosuximide, but not that of phenobarbital and valproate in the pentetrazole-induced seizure test in mice [5]. Moreover, 7-nitroindazole had no impact on the anticonvulsant action of gabapentin, oxcarbazepine, tiagabine, and vigabatrin in the pentetrazole-induced seizure test in mice (unpublished data). Since agmatine interacted with classical and second-generation antiepileptic drugs in contrast to N^G-nitro-L-arginine and 7-nitroindazole, it seems that agmatine may not exert its antiseizure effect in mice by the inhibition of neuronal NOS activity. Thus, one can indirectly conclude that the molecular mechanism(s) of the interaction of agmatine with vigabatrin is(are) not related to the inhibition of NOS activity in experimental animals.

To explain the observed reduction in the anticonvulsant effects of vigabatrin after concomitant administration of agmatine, one should consider the molecular mechanisms of action of the examined drugs. With respect to vigabatrin, experimental evidence indicates that the antiepileptic drug binds to neuronal and glial GABA-transaminase and irreversibly inhibits the enzyme responsible for GABA turnover, leading to an increase in GABA concentration in the brain [18]. As mentioned in the Introduction, agmatine has various molecular mechanisms of action, of which the interaction with imidazoline I_1 receptors and α_2-adrenoceptors [19, 33], the blockade of NMDA receptors and voltage-gated calcium channels [43, 44], as well as the inhibition of inducible and neuronal NOS activity [2, 8, 11] may play an important role in seizure suppression in experimental studies. Agmatine competitively antagonizes the potentiating effect evoked by polyamines acting on NMDA receptors [14]. Additionally, spermine/spermidine, the *in vivo* metabolites of agmatine, are both inhibitors of NOS [4]. Recently, it has been found that systemic (*ip*) injection of agmatine rapidly crosses the blood-brain barrier in mice [32]. However, an experimental neurochemical study has indicated that about 1% of injected (*ip*) agmatine reached the brain due to the rapid metabolism of the compound in peripheral tissues (like kidney and liver), where agmatine is oxidized by diamine oxidase [17] or hydrolyzed by agmatinase [37]. Thus, considering the above-mentioned facts, it is impossible at

present to unequivocally ascertain which mechanism(s) of action of agmatine is(are) responsible for this selective reduction in the antiseizure activity of vigabatrin in the pentetrazole-induced seizure test. It cannot be excluded that several various mechanisms of action may be responsible for the observed effects of agmatine in combination with classical and second-generation antiepileptic drugs in pentetrazole-induced seizures; their final effects may produce a selective reduction in the antiseizure action of vigabatrin, but not in clonazepam, ethosuximide, gabapentin, phenobarbital, tiagabine, and valproate in mice.

It is important to note that in the present study, we did not evaluate the total brain concentrations of vigabatrin in experimental animals. However, the appearance of pharmacokinetic interactions between agmatine and vigabatrin is, at least in part, unlikely because vigabatrin does not bind to plasma protein, does not activate or inhibit microsomal cytochrome P450 isozymes, does not undergo metabolic transformation in the liver, and is almost entirely eliminated *via* the kidneys as unchanged drug [30, 34]. Additionally, in our previous study, no pharmacokinetic alterations in the total brain phenobarbital and valproate concentrations were observed in mice after concomitant administration of agmatine [23]. Thus, one can ascertain that pharmacokinetic interactions between agmatine and vigabatrin are unlikely, but not completely ruled out.

Accumulating clinical evidence indicates that vigabatrin, gabapentin, and tiagabine are contraindicated for patients with myoclonic epilepsy because these antiepileptic drugs can aggravate myoclonic seizures or even induce non-convulsive status epilepticus [7, 13, 31, 38, 41]. However, in the present study, agmatine reduced only the anticonvulsant action of vigabatrin, but not that of gabapentin or tiagabine. Thus, one can ascertain that the modulation of agmatine content in the brain by application of exogenous agmatine in patients receiving vigabatrin may be unfavorable from a therapeutic point of view, and the utmost caution is advised during the combined administration of agmatine with vigabatrin in patients with myoclonic seizures.

Conclusions

Based on this preclinical study, it can be concluded that the combination of agmatine with vigabatrin is unfavorable in the pentetrazole-induced seizure model. More advanced molecular, neurochemical, and electrophysiological studies are required to elucidate the exact mechanism(s) responsible for the reduction of the anticonvulsant action of vigabatrin in this seizure test in mice. If the results from this study could be confirmed in other experimental models of epilepsy, agmatine would not be concomitantly administered with vigabatrin in further clinical trials.

Acknowledgments:
This study was supported by a grant from the Medical University of Lublin (DS 475/2006-2008). The authors are grateful for the generous gift of valproate from ICN-Polfa S.A. (Rzeszów, Poland).

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Received:
June 30, 2008; in revised form: February 18, 2009.