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# Enhancement of brain kynurenic acid production by anticonvulsants—Novel mechanism of antiepileptic activity?

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**Abstract**

In this study, we describe the effect of antiepileptic drugs on the production of kynurenic acid in rat cortical slices, and on the activity of kynurenic acid biosynthetic enzymes, kynurenine aminotransferases (KATs I and II) in the brain tissue. Phenobarbital, felbamate, phenytoin and lamotrigine (all at 0.5–3.0 mM) enhanced kynurenic acid production in vitro, and stimulated the activity of KAT I. In contrast, vigabatrin, gabapentin and tiagabine inhibited kynurenic acid synthesis in cortical slices with $IC_{50}$ of 3.9 (2.8–7.9), 3.7 (2.5–5.4) and 7.5 (3.5–14.3) mM, respectively. Vigabatrin, gabapentin and tiagabine reduced also the activity of KAT I with $IC_{50}$ of 1.6 (1.1–2.4), 0.1 (0.01–0.15), 0.9 (0.7–1.2) mM, and the activity of KAT II with $IC_{50}$ values of 6.0 (4.8–7.5), 0.2 (0.1–0.3) and 2.0 (1.5–2.6) mM, respectively. In conclusion, the enhancement of kynurenic acid formation displayed by carbamazepine, phenytoin, phenobarbital, felbamate and lamotrigine seems to be a novel mechanism, synergistic with other actions of these drugs, and potentially valuable in terms of better control of epilepsy.


*Keywords:* Kynurenic acid; Kynurenine aminotransferase; Antiepileptic drug; Epilepsy; In vitro; Brain

## 1. Introduction

Epileptic disorders affect approximately 0.5–1% of human population. Over 2/3 of cases is idiopathic, the rest being symptomatic, associated with alcohol withdrawal, head trauma, genetic predisposition or epileptogenic drugs (Delorenzo et al., 2005). Among complex neurobiological abnormalities involved in the pathogenesis of seizures, the reduced inhibitory neurotransmission and an excessive excitatory input have emerged as the most important (Meldrum, 1995; Chapman, 1998; Ben-Ari and Holmes, 2005). Aberrant glutamate-mediated neurotransmission may initiate a cascade of changes leading to the initiation and propagation of convulsions and to the subsequent neuronal loss. The peripheral or intracerebral application of excitatory amino acid receptor agonists is considered a classical way of inducing seizures in rodents (Urbanska et al., 1998). Conversely, the experimental use of various antagonists of ionotropic glutamate receptors prevents seizures in different experimental models, including genetically determined, chemically- or electrically-evoked convulsions (Chapman, 1998; Urbanska et al., 1998).

Molecular targets of currently used antiepileptic drugs comprise voltage-activated sodium and calcium channels, $GABA_A$ receptors, ionotropic glutamate receptors, GABA transporters, GABA transaminase and others (Rogawski and Löscher, 2004). However, despite theoretically optimal regimen of drug treatment, about one out of three epileptic patients remains refractory to the pharmacological therapy (Schmidt and Löscher, 2005). Therefore, further identification of a novel and more efficient ways to limit the seizure activity remains a scientific challenge.

Kynurenic acid is the only known endogenous antagonist of excitatory amino acid receptors and of α7 nicotinic receptors (Stone, 2000; Hilmas et al., 2001). Brain kynurenic acid reaches nanomolar to micromolar concentrations and displays high affinity for the glycine site of *N*-methyl-D-aspartate (NMDA)

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receptor complex (Stone, 2000; Turski et al., 1989). In the central nervous system, kynurenic acid is synthesized via an irreversible transamination of its bioprecursor, L-kynurenine, by kynurenine aminotransferases (KATs) I and II, enzymes found in glial and neuronal cells (Roberts et al., 1992). Inappropriate formation of brain kynurenic acid was suggested as a factor which may contribute to the epileptogenesis. Indeed, a nonspecific aminotransferase inhibitor, aminooxyacetic acid, increasing GABA levels at low doses, and inhibiting kynurenic acid synthesis at higher doses, was shown to evoke neuronal loss and seizures in rodents (Urbanska et al., 1991; Turski et al., 1991). Aminooxyacetic acid acts also as a proconvulsant, lowering the threshold for seizures e.g. induced by the mitochondrial toxin, 3-nitropropionic acid (Haberek et al., 2000). On the other hand, kynurenic acid displays antiepileptic and neuroprotective effects, under in vitro and in vivo conditions (Stone, 2000). Kynurenic acid prevents the development of seizures and neuronal loss caused by aminooxyacetic acid, quinolinic acid – an endogenous NMDA receptor agonist, NMDA itself or bicuculline – $GABA_A$ receptor antagonist (Foster et al., 1984; Chiamulera et al., 1990; Turski et al., 1990, 1991). Clinical studies have shown that in some forms of human epilepsy such as West syndrome or infantile spasm, the level of kynurenic acid in the cerebrospinal fluid is reduced (Yamamoto et al., 1994, 1995). Moreover, hippocampal kynurenate rises within hours after experimentally evoked seizures, possibly as a regulatory defense mechanism (Wu and Schwarcz, 1996).

In the previous report, an enhancement of brain kynurenic acid production evoked by antiepileptic drug, carbamazepine, has been demonstrated (Kocki et al., 2004). Here, we have assessed the effects of various antiepileptics on the central production of kynurenic acid and on the activity of kynurenic acid biosynthetic enzymes.

## 2. Materials and methods

### 2.1. Animals and experimental conditions

Male Wistar rats, weighing 200–220 g, were used throughout the experiments. Animals were housed under standard laboratory conditions, at 20 °C environmental temperature, with food and water available ad libitum. Experimental procedures have been approved by the Local Ethical Committee in Lublin and are in agreement with European Communities Council Directive on the use of animals in experimental studies.

### 2.2. Drugs

Phenobarbital (sodium salt), felbamate, phenytoin, lamotrigine, valproate (sodium salt), diazepam, midazolam, vigabatrin, gabapentin, tiagabine and cellulose membrane dialysis tubing were purchased from Sigma–Aldrich (St. Louis, MO), whereas all the high-performance liquid chromatography (HPLC) reagents were obtained from J.T. Baker Laboratory Chemicals. Other chemicals were purchased from Sigma–Aldrich (St. Louis, MO). All of the compounds used during experimental procedures were dissolved in water and their stock solutions were adjusted to pH 7.4, when necessary.

### 2.3. Kynurenic acid synthesis in cortical slices

Kynurenic acid production in vitro was investigated according to the method of Turski et al. (1989). Briefly, animals were killed by decapitation and their brains rapidly removed from the skull. For each experiment the cortical slices (1 × 1 mm base) obtained from three animals were prepared with McIlwain tissue chopper and randomly placed in culture wells (8 per each well) containing the oxygenated Krebs–Ringer buffer, pH 7.4. Every experiment was repeated at least twice. The incubation (37 °C, 2 h) was carried out in the presence of 10 μM L-kynurenine and the solutions of tested compounds (added 10 min before L-kynurenine), in a final volume of 1 ml. Blanks contained all the components of incubation buffer except for the brain tissue. Each antiepileptic drug was analyzed during a separate experiment performed with an independent control. The incubation was ended by a rapid transfer of culture wells to the ice-cold environment and a quick separation of media from the tissue. Subsequently, the media were acidified with 0.1 ml of 1 N HCl and 14 μl of 50% trichloroacetic acid (wt:vol), and centrifuged. Supernatant was applied on the cation-exchange resin (Dowex 50 $W^+$). Eluted kynurenic acid was subjected to the HPLC and quantified fluorimetrically (Varian HPLC system; ESA catecholamine HR-80, 3 μm, $C_{18}$ reverse-phase column).

### 2.4. Determination of kynurenine aminotransferases (KATs) I and II activities

The activities of KAT I and KAT II were assayed as previously described (Kocki et al., 2004). Briefly, the animals not subjected to any pharmacological treatment were decapitated and their brains rapidly removed from the skull. The cortical tissue was subsequently homogenized (1:10; wt:vol) in 5 mM Tris-acetate buffer, pH 8.0, containing 50 μM pyridoxal-phosphate and 10 mM 2-mercaptoethanol. The resulting homogenate was dialyzed overnight at 8 °C, against 4 L of the buffer composed as above. The enzyme preparation was incubated in the reaction mixture containing 2 μM L-kynurenine, 1 mM pyruvate, 70 μM pyridoxal-5′-phosphate, 150 mM Tris-acetate buffer (all concentrations final), and the solutions of tested compounds, at pH of 7.0 or 9.5, for KAT II or KAT I, respectively. Six replicates were used for each concentration and each experiment was repeated twice. Glutamine (final concentration 2 mM), the inhibitor of KAT I, was added to the samples assaying KAT II activity. Blanks contained the enzyme preparation that was heat-deactivated (100 °C, 10 min). The incubation (37 °C, 2 h) was terminated by the rapid transfer of samples to an ice-bath and by subsequent addition of 50% trichloroacetic acid and 0.1 N HCl. Denaturated protein was removed by centrifugation and the supernatant was applied to a Dowex 50 $W^+$ column. Further procedures were performed as described above.

Table 1
Influence of antiepileptic drugs on the kynurenic acid production in vitro and on the brain activity of KAT I and KAT II

| | Kynurenic acid production (pmol of kynurenic acid/well/1 h) | KAT I activity (pmol of kynurenic acid/mg of tissue/1 h) | KAT II activity (pmol of kynurenic acid/mg of tissue/1 h) |
|---|---|---|---|
| Control | 17.51±0.81 | 8.43±0.32 | 4.91±0.76 |
| Phenobarbital | 19.55±1.51 | 9.32±0.30 | 5.02±0.94 |
| 0.01 | | | |
| 0.1 | 21.56±1.01[b] | 10.7±0.80[b] | 5.2±0.72 |
| 1.0 | 27.51±0.93[b] | 12.61±1.51[c] | 4.86±0.70 |
| 3.0 | 31.52±1.12[c] | 13.84±0.93[c] | 4.32±0.94 |
| 5.0 | 34.33±0.75[c] | 16.92±1.54[c] | 5.13±0.22 |
| Control | 14.72±1.36 | 6.84±0.92 | 3.83±0.17 |
| Felbamate 0.01 | 14.29±1.93 | 6.7±0.85 | 3.7±0.4 |
| 0.1 | 16.42±2.40 | 8.6±0.83[a] | 3.53±0.32 |
| 0.5 | 20.31±1.84[a] | 9.85±0.91[c] | 3.87±0.29 |
| 1.0 | 21.97±2.03[c] | 10.42±1.15[c] | 3.83±0.27 |
| 3.0 | 25.96±1.22[c] | 10.56±0.93[c] | 3.72±0.34 |
| 5.0 | 25.73±1.84[c] | 10.93±0.94[c] | 4.06±0.26 |
| Control | 16.9±0.84 | 7.37±0.62 | 4.23±0.23 |
| Phenytoin 0.01 | 16.02±1.25 | 6.94±0.20 | 4.55±0.40 |
| 0.1 | 17.36±0.62 | 7.22±0.10 | 4.93±0.18 |
| 1.0 | 20.83±1.95[b] | 9.03±0.63[a] | 4.26±0.27 |
| 3.0 | 23.34±1.32[c] | 11.36±0.54[c] | 4.42±0.53 |
| 5.0 | 24.56±1.75[c] | 12.34±0.75[c] | 4.76±0.25 |
| Control | 18.69±0.89 | 6.95±0.18 | 3.86±0.13 |
| Lamotrigine | | | |
| 0.01 | 18.27±1.67 | 6.88±0.97 | 3.75±0.2 |
| 0.1 | 18.92±0.75 | 7.25±0.5 | 4.03±0.38 |
| 1.0 | 26.43±2.14[b] | 8.68±0.63[b] | 3.36±0.15 |
| 3.0 | 27.36±1.83[c] | 9.13±0.36[c] | 3.85±0.18 |
| 5.0 | 28.03±0.68[c] | 9.44±0.35[c] | 3.77±0.37 |
| Control | 14.43±1.30 | 7.34±0.43 | 2.90±0.13 |
| Valproate 0.01 | 14.9±0.92 | 7.12±0.56 | 3.02±0.33 |
| 0.1 | 13.72±1.24 | 6.71±0.65 | 2.73±0.55 |
| 1.0 | 14.26±1.05 | 7.81±0.73 | 3.18±0.22 |
| 3.0 | 13.65±0.45 | 6.71±0.65 | 3.11±0.14 |
| 5.0 | 13.62±0.98 | 7.12±0.56 | 3.26±0.81 |
| Control | 13.51±1.83 | 6.49±0.13 | 3.47±0.13 |
| Diazepam 0.01 | 13.91±0.45 | 6.22±0.64 | 3.15±0.15 |
| 0.1 | 13.75±0.31 | 6.34±0.26 | 2.93±0.18 |
| 1.0 | 12.24±1.14 | 6.83±0.8 | 3.22±0.23 |
| 3.0 | 13.32±0.42 | 6.57±0.20 | 3.48±0.37 |
| 5.0 | 13.61±1.92 | 7.05±0.72 | 3.25±0.85 |
| Control | 15.31±1.25 | 5.93±0.16 | 3.21±0.14 |
| Midazolam 0.01 | 15.63±1.63 | 5.63±0.54 | 2.72±0.63 |
| 0.1 | 14.56±1.15 | 5.11±0.65 | 2.95±0.15 |
| 1.0 | 14.22±1.57 | 5.86±0.7 | 2.97±0.36 |
| 3.0 | 15.63±0.92 | 5.92±0.3 | 3.34±0.11 |
| 5.0 | 14.51±1.43 | 5.25±0.31 | 3.03±0.23 |
| Control | 19.33±2.22 | 5.61±0.12 | 3.55±0.24 |
| Vigabatrin 0.001 | 19.42±2.21 | 5.73±0.94 | 3.45±0.43 |
| 0.01 | 20.12±1.73 | 5.42±0.42 | 3.63±0.4 |
| 0.1 | 19.83±2.15 | 3.96±0.26[b] | 3.35±0.3 |
| 1.0 | 19.25±2.51 | 3.08±0.35[c] | 3.77±0.32 |
| 3.0 | 13.91±1.82[b] | 2.68±0.2[c] | 2.52±0.24[a] |
| 5.0 | 10.16±2.44[c] | 2.04±0.11[c] | 1.93±0.52[c] |
| Control | 19.32±1.91 | 5.82±0.14 | 3.5±0.21 |
| Gabapentin | | | |
| 0.001 | 19.52±1.52 | 5.42±0.43 | 3.24±0.12 |
| 0.01 | 20.11±1.76 | 3.83±0.21[a] | 2.94±0.34 |
| 0.1 | 14.03±2.12[a] | 2.62±0.51[c] | 1.57±0.23[c] |
| 1.0 | 11.02±1.53[c] | 3.15±0.32[c] | 0.63±0.31[c] |
| 3.0 | 10.45±1.85[c] | 2.61±0.21[c] | 0.63±0.23[c] |
| 5.0 | 8.83±1.46[c] | 2.13±0.15[c] | 0.61±0.12[c] |
| Control | 14.42±2.02 | 8.43±0.65 | 4.78±0.32 |
| Tiagabine 0.001 | 14.77±1.58 | 8.94±0.78 | 4.63±0.52 |
| 0.01 | 12.04±0.26[b] | 7.16±0.62[a] | 4.34±0.21 |
| 0.1 | 10.50±1.41[c] | 5.05±0.72[c] | 3.96±0.53[a] |
| 1.0 | 8.29±1.25[c] | 3.28±0.09[c] | 2.48±0.24[c] |
| 3.0 | 8.26±1.22[c] | 2.61±0.13[c] | 1.92±0.13[c] |
| 5.0 | 7.84±1.36[c] | 2.44±0.43[c] | 1.86±0.06[c] |

For the in vitro experiments freshly prepared cortical slices (1×1 mm base; $N=6$ per well) randomly obtained form three animals were incubated in 0.1 M Krebs–Ringer buffer, pH=7.4, containing 10 μM L-kynurenine and the solutions of tested compounds (added 10 min before L-kynurenine), in a final volume of 1 ml. Each experiment was repeated at least twice. The enzymatic activity was assessed using freshly obtained cortical tissue ($N=6$) (homogenized 1:10 in 5 mM Tris-acetate buffer, pH 8.0, containing 50 μM pyridoxal-phosphate and 10 mM 2-mercaptoethanol, and further dialysed against 4 L of the buffer composed as above). Dialysate was incubated for 2 h, at 37 °C, in the presence of various concentrations of tested compounds, 2 μM L-kynurenine, 1 mM pyruvic acid and 70 μM pyridoxal-5′-phosphate in 150 mM Tris-acetate buffer, pH 7.0 for KAT II and pH 9.5 for KAT I analysis. Glutamine (final concentration 2 mM), the inhibitor of KAT I, was added to samples assaying KAT II activity. Blanks contained the enzyme preparation that was heat-deactivated at 100 °C for 10 min. Each experiment was repeated at least twice. Newly synthesized kynurenic acid was quantified fluorimetrically, with HPLC. Data are mean values±S.D. of six determinations. [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$ vs control (ANOVA with Bonferroni post-hoc test).

### 2.5. Statistics

Data are expressed as the mean±S.D. The concentration of the drug necessary to induce 50% inhibition of kynurenic acid production ($IC_{50}$) (with confidential limits) was calculated using the computerized linear regression analysis of quantal log dose-probit function. The statistical comparisons were made using one-way analysis of variance (ANOVA), with the post-hoc Bonferroni test.

## 3. Results

### 3.1. Production of kynurenic acid in cortical slices

Phenobarbital at 0.1–5.0 mM, felbamate used at 0.5–5.0 mM, phenytoin at 1.0–5.0 mM and lamotrigine used at 0.5–5.0 mM concentration significantly increased kynurenic acid production in vitro (Table 1). Valproate, diazepam and midazolam (at ≤3.0 mM concentration) have not affected kynurenic acid production in cortical slices (Table 1). Vigabatrin (3.0–5.0 mM), gabapentin (0.1–5.0 mM) and tiagabine (0.01–5.0 mM) significantly reduced the synthesis of kynurenic acid in vitro with $IC_{50}$ values of 3.9 (2.8–7.9), 3.7 (2.5–5.4) and 7.5 (3.5–14.3) mM, respectively (Table 1).

### 3.2. Effect of antiepileptic drugs on the activity of kynurenine aminotransferase I and II (KAT I and II)

Phenobarbital at 0.1–5.0 mM, felbamate at 0.1–5.0 mM, phenytoin at 1.0–5.0 mM, and lamotrigine at 1.0–5.0 mM

concentration enhanced KAT I activity (Table 1). Valproate, diazepam and midazolam did not influence the activity of KAT I (Table 1). Vigabatrin (0.1–5.0 mM), gabapentin (0.01–5.0 mM) and tiagabine (0.01–5.0 mM) inhibited the activity of KAT I with $IC_{50}$ values of 1.6 (1.1–2.4), 0.1 (0.01–0.15), 0.9 (0.7–1.2) mM, respectively (Table 1).

Phenobarbital, felbamate, phenytoin, lamotrigine, valproate, diazepam and midazolam (all up to 5.0 mM concentration) did not influence the activity of KAT II (Table 1). Vigabatrin (3.0–5.0 mM), gabapentin (0.1–5.0 mM) and tiagabine (0.1–5.0 mM) inhibited the activity of KAT II with $IC_{50}$ values of 6.0 (4.8–7.5), 0.2 (0.1–0.3) and 2.0 (1.5–2.6) mM, respectively (Table 1).

## 4. Discussion

An increased production of kynurenic acid seems to be a novel, additional mechanism involved in the action of some antiepileptic agents, such as carbamazepine (Kocki et al., 2004), phenytoin, phenobarbital, felbamate and lamotrigine. Among other studied here antiepileptics, valproate, diazepam and midazolam, do not affect kynurenic acid production, whereas vigabatrin, gabapentin and tiagabine reduce its formation. Stimulation of kynurenic acid production in cortical slices was displayed by the drugs which increase the activity of KAT I. Conversely, compounds which diminished the activity of KAT I or KAT II, reduced also the formation of kynurenic acid in vitro.

Among studied here drugs found to increase the production of kynurenic acid, phenobarbital and felbamate, but not phenytoin or lamotrigine, acted at the concentrations falling within the clinical ranges reported in the literature. Therapeutic levels of phenobarbital and felbamate usually do not exceed 200 and 600 μM, respectively (Williams et al., 2003; Jannuzzi et al., 2000). The lowest concentration of the above drugs affecting the production of kynurenic acid reached 100 and 500 μM, respectively. Therefore, an increased formation of kynurenic acid might partially contribute to the displayed in vivo antiepileptic action of phenobarbital and felbamate. Here, the effective concentrations of phenytoin and lamotrigine exceeded their therapeutic serum levels (Williams et al., 2003; Jannuzzi et al., 2000), but it cannot be excluded that they might reach in the brain concentrations high enough to affect production of kynurenic acid.

Based on their molecular targets, the antiepileptics used in this study can be classified into three groups. Phenytoin, carbamazepine and lamotrigine block predominantly sodium channels. Lamotrigine might also antagonize high voltage-activated calcium channels (Rogawski and Löscher, 2004). Valproate, felbamate, gabapentin and phenobarbital display mixed or not fully understood actions. Valproate seems to stimulate GABA synthesis and turnover; it also blocks calcium channels of T-type and reduces the activation of sodium channels (Rogawski and Löscher, 2004). Felbamate appears to act as a functional antagonist of the NMDA receptor–ionophore complex, blocker of sodium and calcium channels, and, to a certain degree, as a positive modulator of $GABA_A$ receptor. Gabapentin increases GABA synthesis and turnover, and may block calcium channels. Phenobarbital allosterically modulates $GABA_A$ receptors, leading to an increased GABA-mediated transmission and displays some activity against calcium and sodium channels (Rogawski and Löscher, 2004). Vigabatrin, tiagabine, diazepam and midazolam act mainly via and enhancement of GABAergic neurotransmission. Benzodiazepines positively modulate $GABA_A$ receptor complex. Vigabatrin diminishes the breakdown of GABA via interference with GABA-transaminase but, paradoxically, does not enhance $GABA_A$ receptor-mediated responses (Rogawski and Löscher, 2004). Tiagabine, a potent inhibitor of GABA transporter, impairs the presynaptic uptake of GABA (Rogawski and Löscher, 2004). Interestingly, all of the antiepileptics enhancing kynurenic acid production are known to block voltage-dependent sodium channels. This action might partially explain presented data, considering the fact that in a low-sodium milieu the in vitro production of kynurenic acid increases (Turski et al., 1989).

Additionally, phenytoin, phenobarbital, felbamate and lamotrigine directly enhanced the activity of KAT I, what seems to be another mechanism underlying the observed increase of kynurenic acid formation. KAT I is an enzyme synthesizing presumably a minor portion of kynurenic acid under physiological conditions (Guidetti et al., 1997). However, recent data indicate that it can efficiently convert L-kynurenine to kynurenic acid also at physiological pH (Han et al., 2004). Thus, activation of KAT I may yield substantial quantities of newly formed kynurenic acid, sufficient to impair the glutamate-mediated neurotransmission and to limit seizure activity. Indeed, in vivo studies demonstrated that the brain synthesis of kynurenic acid increases following convulsive episode, what may constitute a central response aimed to counteract seizure activity (Wu and Schwarcz, 1996).

As demonstrated, gabapentin, vigabatrin, and tiagabine reduce the formation of kynurenic acid in vitro and potently inhibit the activity of KAT I and KAT II. Among these three drugs, gabapentin acted already at 100 μM concentration i.e. within therapeutic range reported in the serum (up to 180 μM) (Williams et al., 2003; Jannuzzi et al., 2000), however, it cannot be excluded that the other two drugs may attain brain levels high enough to reduce the production of kynurenic acid. Gabapentin and vigabatrin may precipitate myoclonus, tiagabine as an add-on drug was implicated in the development of non-convulsive status epilepticus, and vigabatrin may worsen absence and generalized tonic–clonic seizures (Sazgar and Bourgeois, 2005). Numerous evidence indicates that the absence seizures result from the aberrant GABA-mediated transmission and excessive calcium currents via T-type channels (Futatsugi and Riviello, 1998), whereas myoclonic epilepsies are associated with various genetically determined biochemical alterations (Leppik, 2003). Considering the fact that NMDA receptor antagonists suppress genetically determined recurrent absence seizures in rats (Koerner et al., 1996), and myoclonic seizures in baboons (Smith and Meldrum, 1992), an enhanced glutamate-mediated events might also contribute to these types of seizures. Therefore, it cannot be excluded that the reduced availability of an endogenous glutamate receptor antagonist may be partially

responsible for the paradoxical exacerbation of absence or myoclonic seizures, as sometimes observed clinically after the administration of gabapentin, tiagabine or vigabatrin.

In conclusion, the enhancement of kynurenic acid formation displayed by carbamazepine, phenytoin, phenobarbital, felbamate and lamotrigine seems to be a novel mechanism, synergistic with the other actions of these drugs, and potentially valuable in terms of better control of epilepsy. The appropriate in vivo studies are on the way.

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