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Effect of caffeine on the anticonvulsant effects of oxcarbazepine, lamotrigine and tiagabine in a mouse model of generalized tonic-clonic seizures

Magdalena Chrościńska-Krawczyk[1], Neville Ratnaraj[2], Philip N. Patsalos[2], Stanisław J. Czuczwar[1,3]

[1]Department of Pathophysiology, Medical University of Lublin, Jaczewskiego 8, PL 20-090 Lublin, Poland

[2]Pharmacology and Therapeutics Unit, Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

[3]Department of Physiopathology, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

Correspondence: Stanisław J. Czuczwar, e-mail: czuczwarsj@yahoo.com

Abstract:
Caffeine has been reported to be proconvulsant and to reduce the anticonvulsant efficacy of a variety of antiepileptic drugs (carbamazepine, phenobarbital, phenytoin, valproate and topiramate) in animal models of epilepsy and to increase seizure frequency in patients with epilepsy. Using the mouse maximal electroshock model, the present study was undertaken so as to ascertain whether caffeine affects the anticonvulsant efficacy of the new antiepileptic drugs lamotrigine, oxcarbazepine and tiagabine.
The results indicate that neither acute nor chronic caffeine administration (up to 46.2 mg/kg) affected the ED_{50} values of oxcarbazepine or lamotrigine against maximal electroshock. Similarly, caffeine did not modify the tiagabine electroconvulsive threshold. Furthermore, caffeine had no effect on oxcarbazepine, lamotrigine and tiagabine associated adverse effects such as impairment of motor coordination (measured by the chimney test) or long-term memory (measured by the passive avoidance task). Concurrent plasma concentration measurements revealed no significant effect on lamotrigine and oxcarbazepine concentrations. For tiagabine, however, chronic caffeine (4 mg/kg) administration was associated with an increase in tiagabine concentrations.
In conclusion, caffeine did not impair the anticonvulsant effects of lamotrigine, oxcarbazepine, or tiagabine as assessed by electroconvulsions in mice. Also, caffeine was without effect upon the adverse potential of the studied antiepileptic drugs. Thus caffeine may not necessarily adversely affect the efficacy of all antiepileptic drugs and this is an important observation.

Key words:
antiepileptic drugs, lamotrigine, oxcarbazepine, tiagabine, caffeine, electroconvulsions

Abbreviations: AEDs – antiepileptic drugs, CAF – caffeine, CS_{50} – median current strength, ED_{50} – median effective dose, LTG – lamotrigine, MES – maximal electroshock, OXC – oxcarbazepine, TD_{50} – median toxic dose, TGB – tiagabine

Introduction

Caffeine, a methylxanthine derivative, is probably the most consumed psychoactive substance in the world and it is estimated that in developed countries ~90% of people ingest caffeine on a daily basis [17]. Almost all caffeine originates from dietary sources with coffee, tea and chocolate being the most popular [1, 30]. Caffeine has been reported not only to be proconvulsant in animal models of epilepsy induced by chemical convulsants [8, 9, 16] but also to increase seizure frequency in patients with epilepsy [4, 21]. The mechanism of these effects is not known but may relate to the action of caffeine on adenosine A_1 and A_{2A} receptors [17]. However, neither acute (up to 92.4 mg/kg) nor chronic caffeine (up to 46.2 mg/kg) administration has been shown to affect the threshold for electroconvulsions in mice [11, 18, 19]. Only tea extracts have been documented to increase seizure duration and mortality after electroconvulsions in mice [20]. Clinical data indicate that caffeine may prolong seizure duration in patients undergoing electroconvulsive therapy although the convulsive threshold was not affected [29].

Caffeine has also been documented to interact with antiepileptic drugs (AEDs) and to reduce their anticonvulsant effect in various seizure models. For example, caffeine when administered acutely (11.55–92.4 mg/kg, which is an equivalent to 12.5–100 mg of aminophylline) impairs the protective activity of carbamazepine, phenobarbital, phenytoin, and valproate against electroconvulsions in mice [14]. Furthermore, when caffeine (23.1–46.2 mg/kg) was administered daily over a two week period, no tolerance to this undesirable interaction occurred and the anticonvulsant action of carbamazepine, phenobarbital, phenytoin, and valproate was still compromised [18, 19]. It is noteworthy that with regards to valproate and phenobarbital, the attenuation of their anticonvulsant action by caffeine was actually greater during chronic administration when compared to single-dose acute administration [18].

Because caffeine may compromise the effectiveness of AED therapy in patients with epilepsy [21], the present study was undertaken to ascertain whether caffeine attenuates the anticonvulsant effect of some of the newer AEDs: oxcarbazepine (OXC), lamotrigine (LTG), and tiagabine (TGB). The mouse maximal electroshock (MES)-induced seizure model was used and adverse effects were ascertained by measurement of long-term memory (the step-through passive avoidance task) and motor coordination (chimney test). Finally, total plasma AED concentrations were also measured to determine any pharmacokinetic contribution to the observed effects.

Materials and Methods

Animals

The experiments were conducted on male Swiss mice, weighing 22–27 g. Experimental groups, consisting of 8–10 animals, were chosen randomly. The animals were housed in colony cages, under standard laboratory conditions, with free access to food (chow pellets, Bacutil, Motycz, Poland) and tap water. All mice were maintained at an ambient temperature of 20 ± 1°C and on the natural light-dark cycle. All the procedures undertaken in this study were approved by the Bioethical Committee at the Medical University of Lublin.

Drugs

The following AEDs were used in this study using marketed tablet formulations: lamotrigine (LTG; Lamictal, Glaxo Wellcome, Kent, Great Britain), tiagabine (TGB; Gabitril, Sanofi Winthrop, Gentilly, France), and oxcarbazepine (OXC, Trileptal, Novartis Pharma, Basel, Switzerland). All drugs were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA). Caffeine (Coffeinum Natrium benzoicum, Pliva, Kraków, Poland) was available in a sterile saline solution, which was subsequently made up to the appropriate volume with saline and administered *ip.* All drug doses refer to their free forms.

Experimental schedule

Caffeine or saline were administered to the mice by *ip* injected on a twice a day (at 7.00 a.m. and 7.00 p.m) basis as follows:
Group 1: control group – saline administered for 14 days followed by a final injection on day 15.
Group 2: acute caffeine group – saline administered for 14 days followed by a single dose of caffeine on day 15.
Group 3: chronic caffeine group – caffeine administered for 14 days followed by a final dose of caffeine on day 15.

On day 15th, mice from all groups were also administered with LTG, TGB (4 and 6 mg/kg) or OXC. At least four different doses of LTG and OXC were administered so as to enable calculation of their respective ED_{50} or TD_{50} values in the MES and chimney tests. LTG was administered at 60 min, TGB at 15 min and OXC at 30 min prior to induction of electroconvulsions. Caffeine was administered 30 min before the tests. Caffeine doses were 23.1 and 46.2 mg/kg and were based on doses previously studied [18, 19]. Dose ranges for LTG and OXC were taken from Łuszczki and Czuczwar [24] and doses of TGB from Łuszczki et al. [26]. Treatment times to provide maximum anticonvulsant effects were based on previous studies [24, 26].

Maximal electroshock test (MES)

Electroconvulsions were produced with the use of auricular electrodes and alternating current (50 Hz; 25 mA) delivered by a Hugo Sachs generator (rodent Shocker, type 221, Freiburg, Germany). The stimulus duration was 0.2 s. The end point was the tonic extension of the hind limbs. The protective efficacy of OXC and LTG was determined as their ability to protect 50% of mice against the MES-induced tonic hind limb extension and expressed as their respective median effective dose (ED_{50}) values.

Determination of the convulsive threshold

Because TGB is not fully effective in the mouse MES test, its effect on the threshold for electroconvulsions was estimated. Electroconvulsions were induced by an alternating current (50 Hz) delivered *via* ear-clip electrodes, by a Hugo Sachs generator. The experimental groups were challenged with electroshocks of various intensities and a duration of 0.2 s. The current intensity applied in the control group oscillated between 5 and 8 mA, and in the TGB group between 6 and 8 mA. Tonic hind limb extension was taken as the criterion for the occurrence of seizure activity. From the current intensity-effect curve the convulsive threshold as CS_{50} values (50% current strength) was determined. Each CS_{50} value represents the current strength (mA) necessary to induce tonic hind limb extension in 50% of the animals tested.

Chimney test

The effects of OXC, LTG and TGB alone or in combination with caffeine on motor performance were quantified with the chimney test of Boissier et al. [3]. In this test, animals had to climb backwards up a plastic tube (25-cm length, 3-cm inner diameter) with a rough inner surface. Motor impairment was indicated by the inability of the animals to perform the test within 60 s. Results were expressed as a percentage of mice unable to perform this test. The neurotoxic effects of these AEDs were expressed as their TD_{50} values, representing the doses at which the AEDs impaired motor coordination in 50% of the animals.

Passive avoidance task

According to Venault et al. [33] the step-through passive avoidance task may be regarded as a measure of long-term memory acquisition. The animals were placed separately in an illuminated box (10 × 13 × 15 cm), connected to a larger (25 × 20 × 15 cm) dark compartment equipped with an electric grid floor. The entry into the dark compartment was punished by an electric footshock (0.6 mA for 2 s; facilitation of acquisition).

On the first day of the test, the animals (following administration of AEDs alone or in combination with caffeine) were placed separately in the illuminated box and those that would not enter the dark compartment within 180 s were excluded from the study. On the following day (24 h later), the same animals (without any drug administration) were again placed in the illuminated box. Animals avoiding the dark compartment for longer than 180 s were regarded as remembering the task.

Plasma TGB, LTG and OXC concentrations

Total plasma concentrations of TGB, OXC and LTG were determined by high-pressure liquid chromatography at the peak of the anticonvulsant activity of each AED. For LTG and OXC the method described by Borowicz et al. [5] and Łuszczki and Czuczwar [24], respectively, was used. For TGB the method described by Wang et al. [34] was used.

Statistical analysis

The CS_{50} and ED_{50} values were calculated by probit analysis, according to Litchfield and Wilcoxon [23] and evaluated statistically according to Łuszczki and Czuczwar [25].

The chimney test data were compared by using Fischer's exact probability test. The passive avoidance task data were calculated as medians with 25 and 75 percentiles. The Kruskal-Wallis test (non-parametric ANOVA) followed by the subsequent Dunn's test were applied for statistical evaluation of medians and plasma concentrations of AEDs.

Results

Acute or chronic caffeine and the convulsive threshold for electroconvulsions

Neither acute nor chronic caffeine administration, up to 46.2 mg/kg, affected the convulsive threshold. Its control value of 6.4 mA ranged insignificantly from 6.2 to 7.4 mA (results not shown).

Tab. 1. Effect of acute and chronic caffeine (CAF) administration on the protective action of oxcarbazepine (OXC) and lamotrigine (LTG) against maximal electroshock-induced convulsions

Treatment (mg/kg)	ED_{50} (mg/kg) OXC	ED_{50} (mg/kg) LTG
Vehicle	13 (12.2–13.9)	7.5 (6.1–9.2)
CAF (23.1 acute)	15.6 (14.2–17)	8.2 (7–9.6)
CAF (23.1 chronic)	14.2 (13.1–15.4)	7.6 (6.4–9)
CAF (46.2 acute)	15.3 (14.1–16)	8.2 (7–9.6)
CAF (46.2 chronic)	14.5 (13–16.2)	7.4 (5.8–9.5)

Values in parenthesis indicate 95% confidence intervals. OXC was administered at 60 min; LTG and CAF at 30 min prior to the test. Statistical analysis was performed according to Łuszczki and Czuczwar [25]

Influence of caffeine upon the protective activity of LTG and OXC against MES

Caffeine (up to 46.2 mg/kg) administered acutely or chronically was without effect on the protective effects of LTG against MES. The ED_{50} values for LTG ranged 7.4–7.6 mg/kg (for chronic caffeine administration) and the control ED_{50} for LTG was 7.5 mg/kg (Tab. 1). Similarly, caffeine administered either acutely or chronically, was without effect upon the anticonvulsant activity of OXC in the MES test (Tab. 1).

Effect of caffeine on the protective activity of TGB against electroconvulsions

Whilst TGB (4 mg/kg) raised the convulsive threshold for electroconvulsions from 4.9 (4.4–5.5) to 6.1 (5.4–6.9) mA, the effect was not statistically significant. In contrast, 6 mg/kg TGB was associated with

Tab. 2. Effect of acute and chronic administration of caffeine (CAF) on tiagabine (TGB) associated threshold for electroconvulsions

Treatment (mg/kg)	Control	CAF (mg/kg)				
		Acute			Chronic	
		0	23.1	46.2	23.1	46.2
TGB (4)	4.9 (4.4–5.5)	6.1 (5.3–7.0)	6.9 (6.0–8.0)	6.7 (6.0–7.5)	6.9 (6.0–8.0)	6.4 (5.6–7.3)
TGB (6)	4.9 (4.4–5.5)	6.4 (5.7–7.2)*	6.2 (5.5–7.0)	6.0 (5.3–6.8)	6.3 (5.4–7.4)	6.1 (5.3–7.0)

Data are CS_{50} values in mA (with 95% confidence intervals in parentheses). Control animals received saline and a 1% solution of Tween 80. TGB was administered at 15 min and CAF at 30 min before the electroconvulsive test. Statistical comparisons were made according to Łuszczki and Czuczwar [25]. * $p < 0.05$ *vs* control group

Tab. 3. Influence of acute and chronic administration of caffeine (CAF) on the neurotoxic effects of lamotrigine (LTG), oxcarbazepine (OXC) and tiagabine (TGB) in the chimney test

CAF (mg/kg)	TD_{50} (mg/kg)		
	LTG	OXC	TGB
Vehicle	35 (32–38)	73.5 (65.5–82.5)	7.1 (5.3–9.5)
Acute 46.2	34 (32–38)	70 (62–79.5)	5.6 (3.8–8.2)
Chronic 23.1	28 (22–34)	67 (62–73)	4.2 (2.7–6.3)
Chronic 46.2	24 (17–33)	71 (66–78)	5.9 (5.1–6.8)

TD_{50} values (with 95% confidence intervals in parentheses), representing the doses at which the various antiepileptic drugs impair motor coordination in 50% of the animals. Statistical evaluation of the data was undertaken according to Łuszczki and Czuczwar [25]. For drug pretreatment times see footnotes of Tabs. 1 and 2

a significant increase ($p < 0.05$) in threshold to 6.4 (5.7–7.2) mA (Tab. 2). Regardless, however, neither acute or chronic caffeine (up to 46.2 mg/kg) administration was associated with an effect on TGB (4 or 6 mg/kg) associated electroconvulsive threshold effects (Tab. 2).

Effect of AEDs singly and in combination with caffeine on motor performance in the chimney test

Both, acute and chronic caffeine (up to 46.2 mg/kg) administered alone was without significant effect on the performance of mice in the chimney test. Chronic caffeine disturbed motor coordination in 1 out of 10 mice, whilst acute caffeine was without effect (results not shown). Interestingly, it has been previously reported that acute caffeine (92.4 mg/kg) impaired only 10% of mice in the chimney test [27].

Neither acute nor chronic caffeine (up to 46.2 mg/kg) administration affected the neurotoxic effects of the studied AEDs, evaluated in the chimney test. Thus TD_{50} values of the AEDs administered alone for the impairment of motor coordination did not differ significantly from values obtained in the presence of caffeine (Tab. 3).

Effect of AEDs singly and in combination with caffeine on the passive avoidance task test

Acute and chronic caffeine alone (up to 46.2 mg/kg) did not significantly shorten the retention time which was 180 s (26, 180; chronic caffeine at 23.1 mg/kg) and 125 s (23, 180) for chronic caffeine at 46.2 mg/kg. All vehicle-treated animals did not enter the dark compartment within 180 s. Similarly, as in the chimney test, caffeine (up to 46.2 mg/kg) given acutely or chronically with AEDs, did not significantly affect the performance of mice in the passive avoidance task. A tendency to reduce the retention time as compared to the vehicle group was observed in the case of OXC (14.5 mg/kg) for both acute and chronic caffeine (46.2 mg/kg) administration – the respective retention times were 90 s (33, 180) and 120 s (20, 180). Similarly, 120 s (49, 180) was observed for LTG (7.4 mg/kg) + chronic caffeine (46.2 mg/kg). The combination of TGB (4 mg/kg) with chronic caffeine (46.2 mg/kg) resulted in a retention time of 100 s (51, 180). The retention times for LTG (7.4 mg/kg), OXC (14.5 mg/kg), and TGB (4 mg/kg) were: 180 s (85, 180), 180 s (38, 180), and 180 s (90, 180), respectively. For all the other studied combinations the mean retention times were > 125 s.

Effect of caffeine on the plasma AED concentrations

LTG and OXC total plasma concentrations were unaffected by acute or chronic caffeine (up to 46.2 mg/kg) administration. In contrast, caffeine (46.2 mg/kg) administered chronically significantly elevated TGB total plasma concentrations in the group administered 4 mg/kg TGB (Tab. 4).

Discussion

There is considerable evidence to indicate that both acute and chronic caffeine administration can impair

Tab. 4. Effect of acute or chronic caffeine (CAF) on lamotrigine (LTG), oxcarbazepine (OXC), and tiagabine (TGB) total plasma concentrations

Treatment (mg/kg)	Vehicle	CAF acute (46.2 mg/kg)	Vehicle	CAF chronic (46.2 mg/kg)
LTG (8.2)	6.0 ± 1.7	8.5 ± 3.3	ND	ND
LTG (7.4)	ND	ND	4.9 ± 1.1	6.5 ±1.9
OXC (15.3)	5.3 ± 1.1	4.4 ± 1.2	ND	ND
OXC (14.5)	ND	ND	4.9 ± 1.3	3.9 ± 1.2
TGB (4.0)	1.55 ± 0.11	1.58 ± 0.089	ND	2.5 ± 0.23**

Data are shown in μg/ml as the means ± SDs of at least 8 determinations. Kruskal-Wallis non-parametric ANOVA with subsequent Dunn's test were used. LTG and OXC were given at their respective ED_{50}s taken from respective combinations with CAF in the maximal electroshock test. For drug pretreatment times see footnotes of Tabs. 1 and 2. ND – not determined. ** $p < 0.01$ *vs.* TGB (4 mg/kg)-treated group

the anticonvulsant activity of various classical AEDs (carbamazepine, phenobarbital, phenytoin, valproate) and that of the new AED topiramate, in animal models of epilepsy [7, 14, 18, 19]. Additionally, since chronic caffeine administration (15 days, twice daily) was associated with an even more profound effect compared to acute administration, particularly with regards to phenobarbital and valproate, it would suggest that tolerance does not develop to caffeine's undesirable effects [18]. Interestingly, caffeine (acute and chronic administration) is associated with a significant reduction in topiramate anticonvulsant activity in the mouse MES-induced convulsions model [7], even though topiramate has multiple mechanisms of action. These include: enhancement of GABA-mediated inhibition, reduction of glutamatergic neurotransmission *via* AMPA/KA receptors, and inhibition of voltage-operated sodium and L-calcium channels [10, 31]. Valproate which also is associated with multiple mechanisms of action (e.g. blockade of sodium and calcium channels, enhancement of GABA-mediated events) [10, 31] is particularly sensitive to the effects of caffeine administration [11, 14, 18]. Limited clinical data based on case reports confirm these experimental findings in that seizure frequency is reported to increase significantly in patients ingesting caffeine-rich tea or coffee [2, 4, 21]. Additionally, when such patients discontinued their caffeine ingestion their seizure frequency returned to pre-caffeine levels [2, 4, 21].

From the present study, it would appear that these effects of caffeine do not necessarily occur with all AEDs since neither acute nor chronic caffeine administration was associated with any significant reduction on the anticonvulsant effects of OXC, LTG and TGB as ascertained in the mouse MES (OXC, LTG) and the threshold for electroconvulsions (TGB) tests (Tabs. 1 and 2). Additionally, caffeine co-administration was not associated with any enhancement of AED associated neurotoxicity (motor or memory impairment) as assessed by the chimney and passive avoidance tests, respectively.

Considering the mechanisms of action of the studied AEDs, TGB selectively elevates the concentration of GABA in synapses *via* blockade of GAT-1 – an active transporter of GABA from the synaptic cleft into nerve endings and glial cells [10, 15]. OXC is a potent inhibitor of voltage dependent sodium and calcium P- and T-type channels whilst LTG is also a sodium and calcium (N, P/Q, R, and T-type) channel blocker, probably also inhibiting glutamate release from nerve endings and blocking AMPA/KA glutamate receptors [10, 22, 31]. With regards to the mechanisms of action of those AEDs whose anticonvulsant effects were reduced by caffeine, they act by: blocking sodium channels (carbamazepine, phenytoin, valproate), blocking L- and T-type calcium channels (carbamazepine, topiramate, and valproate), enhancing GABA-mediated inhibition (phenobarbital, topiramate, valproate), and blocking AMPA/KA receptors (topiramate) [10, 15, 31]. Thus, the differences in mechanism of action between the previously studied AEDs and those AEDs evaluated in the present study relates to blockade of other types of calcium channels (N, P/Q, and R – OXC and LTG) and a considerably more pronounced synaptic GABA increase – TGB is much more potent than valproate in this respect [31]. These differences may be, in fact, responsible for the lack of an effect by caffeine on the anticonvulsant effect of LTG and OXC. Furthermore, with regards to TGB, a pharmacokinetic contribution may also be involved, since TGB plasma concentration were significantly in-

creased when caffeine was chronically administered with TGB (4 mg/kg). In contrast, caffeine administered acutely was without effect on the pharmacokinetics of TGB which would suggest that the potent elevation by TGB of synaptic GABA concentrations may be of importance as regards to the lack of the attenuation of the anticonvulsant effect of TGB by caffeine.

Caffeine may exert its pharmacological effect *via* release calcium ions from the endoplasmic reticulum [28] *via* ryanodine receptors [6] although only at micromolar concentrations [30]. Indeed, caffeine (10 mM) has been associated with epileptiform electrical activity in rat hippocampal slices by this mechanism [28] and interestingly, clonazepam, carbamazepine, and valproate reduced the epileptiform potential of caffeine, with clonazepam being the most potent in this respect [28]. These data would suggest that the reduction by AEDs of caffeine-induced calcium release from intracellular stores does not prevent their susceptibility to the adverse effect of caffeine, since the anticonvulsant activity of these drugs was significantly decreased by caffeine or aminophylline against electroconvulsions [14]. However, this mechanism (reduction of caffeine-induced calcium release from intracellular stores) may be of importance in pentylenetetrazole-induced convulsions in mice because in this seizure model caffeine even at the high dose of 92.4 mg/kg was without effect upon the anticonvulsant efficacy of clonazepam and valproate [27]. The interaction of methylxanthine derivatives with AEDs may be even more complex since acute aminophylline at 50 mg/kg reduced the anticonvulsant action of LTG against maximal electroshock-induced seizures in mice [5]. However, the blockade of adenosine receptors by caffeine [32] does not seem to be involved in the interaction between the methylxanthine and classical AEDs (carbamazepine, phenobarbital, phenytoin, and valproate) or topiramate [for discussion see 7, 12, 14]. Also, an interaction of the nonxanthine adenosine receptor antagonist, CGS 15943A, has revealed that up to 1 mg/kg, this agent was unable to affect the protective activity of carbamazepine, diazepam, phenobarbital, phenytoin, and valproate against MES in mice [13].. However, CGS 15943A (1 mg/kg) attenuated 2-chloroadenosine and valproate-induced reductions of locomotor activity [13]. Only at 5 mg/kg, did the adenosine receptor antagonist moderately decreased the protection offered by phenytoin against MES which may point to a partial involvement of adenosine-mediated events in the anticonvulsant effects of this AED [13]. Acute or chronic caffeine administration at 23.1 mg/kg (119 μmol/kg) to mice resulted in the plasma concentration of the methylxanthine in the range of 20 μg/ml (ca 100 μmol) [18]. This indicates that the higher dose of caffeine (46.2 mg/kg) is not expected to produce millimolar plasma concentrations of the methylxanthine. In this context, the possibility that caffeine may reduce the protective potential of classical AEDs or topiramate *via* mobilization of calcium from intracellular stores or inhibiton of phosphodiesterase activity, which require millimolar caffeine concentrations [30], seems unlikely. Caffeine in micromolar concentrations, however, was sufficient to reduce the anticonvulsant effects of classical AEDs [11, 18, 19] but apparently not sufficient enough to affect the protective activity of LTG, OXC, and TGB.

In conclusion, caffeine is a widely used stimulant and ingestion of 2–3 cups of coffee (equivalent to a dose of 200 mg) a day causes visible behavioral effects [30]. It is thus not unexpected that patients with epilepsy will invariably ingest caffeine and this has been associated by increased seizure frequency consequent to a reduction in AED anticonvulsant efficacy *per se* [2, 4, 21]. The data reported in the present study would suggest that this may not necessarily be the case for all AEDs in that the anticonvulsant effects LTG , OXC, and TGB were not affected by caffeine co-ingestion. However, these data need to be confirmed in the clinical setting.

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References:

1. Bernstein GA, Carroll ME, Thuras PD, Cosgrove KP, Roth ME: Caffeine dependence in teenagers. Drug Alcohol Depend, 2002, 66, 1–6.
2. Błaszczyk B: Caffeine and management of epilepsy – a clinical evidence. Pharmacol Rep, 2007, 59, 116.
3. Boissier JR, Tardy J, Divierres JC: Une nouvelle methode simple pour explorer l'action „tranquillisante": le test de la cheminee. Med Exp, 1960, 3, 81–84.
4. Bonilha L, Li M.: Heavy coffee drinking and epilepsy. Seizure, 2004, 13, 284–285.
5. Borowicz KK, Świąder M, Zgrajka W, Sawulski C, Turski WA, Czuczwar SJ: Influence of several convulsants

on the protective activity of a non-competitive AMPA/kainate antagonist, LY 300164, and lamotrigine against maximal electroshock in mice. J Physiol Pharmacol, 2002, 53, 859–869.
6. Butanda-Ochoa A, Höjer G, Morales-Tlalpan V, Diaz-Muńoz M: Recognition and activation of ryanodine receptors by purines. Curr Med Chem, 2006, 13, 647–657.
7. Chrościńska M., Jargiełło M., Czuczwar S.J.: Influence of caffeine on the protective action of some conventional and novel antiepileptic drugs. Pharmacol Rep, 2007, 59, 118.
8. Chu N-S: Caffeine- and aminophylline-induced seizures. Epilepsia, 1981, 22, 85–94.
9. Cutrufo C, Bortot L., Giachetti A, Manzini S: Different effects of various xanthines on pentylenetetrazole-induced seizures in rats: An EEG and behavioural study. Eur J Pharmacol, 1992, 222, 1–6.
10. Czapiński P, Błaszczyk B, Czuczwar SJ: Mechanisms of action of antiepileptic drugs. Curr Top Med Chem, 2005, 5, 3–14.
11. Czuczwar SJ, Gasior M, Szczepanik B, Janusz W, Włodarczyk D, Kleinrok Z: Influence of different methylxanthines on the anticonvulsant action of common antiepileptic drugs in mice. Epilepsia, 1990, 31, 318–323.
12. Czuczwar SJ, Ikonomidou C, Kleinrok Z, Turski L, Turski WA: Effect of aminophylline on the protective action of common antiepileptic drugs against electroconvulsions in mice. Epilepsia, 1986, 27, 204–208.
13. Czuczwar SJ, Janusz W, Szczepanik B, Kleinrok Z: Influence of CGS 15943A (a nonxanthine adenosine antagonist) on the protection offered by a variety of antiepileptic drugs against maximal electroshock-induced seizures in mice. J Neural Transm, 1991, 86, 127–134.
14. Czuczwar SJ, Kleinrok Z: Modulation of the protective efficacy of common antiepileptic drugs by xanthine derivatives: implications for the clinical use of xanthines in epileptic patients. Pharmacol Res, 1990, 22, 661–665.
15. Czuczwar SJ, Patsalos PN: The new generation of GABA enhancers. Potential in the treatment of epilepsy. CNS Drugs, 2001, 15, 339–350.
16. De Sarro A, Grasso S, Zappala M, Nava F, De Sarro G: Convulsant effects of some xanthine derivatives in genetically epilepsy-prone rats. Naunyn-Schmiedebergs Arch Pharmacol, 1997, 356, 48–55.
17. Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE: Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev, 1999, 51, 83–133.
18. Gasior M, Borowicz K, Buszewicz G, Kleinrok Z, Czuczwar SJ: Anticonvulsant activity of phenobarbital and valproate against maximal electroshock in mice during chronic treatment with caffeine and caffeine discontinuation. Epilepsia, 1996, 37, 262–268.
19. Gasior M, Borowicz K, Kleinrok Z, Czuczwar SJ: Chronic caffeine and the anticonvulsant potency of antiepileptic drugs against maximal electroshock. Pharmacol Biochem Behav, 1996, 54, 639–644.
20. Gomes A, Das M, Vedasiromoni JR, Ganguly DK: Proconvulsive effect of tea (Camellia sinensis) in mice. Phytotherapy Res, 1999, 13, 376–379.
21. Kaufman K, Sachdeo R: Caffeinated beverages and decreased seizure control. Seizure, 2003, 12, 519–521.
22. Lee C-Y, Fu W-M, Chen C-C, Su M-J, Liou H-H: Lamotrigine inhibits postsynaptic AMPA receptor and glutamate release in the dentate gyrus. Epilepsia, 2008, 49, 888–897.
23. Litchfield LT, Wilcoxon F: A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther, 1949, 96, 99–113
24. Łuszczki JJ, Czuczwar SJ: Preclinical profile of combinations of some second-generation antiepileptic drugs: an isobolographic analysis. Epilepsia, 2004, 45, 894–907.
25. Łuszczki JJ, Czuczwar SJ: How significant is the difference between drug doses influencing the threshold for electroconvulsions? Pharmacol Rep, 2005, 57, 782–786.
26. Łuszczki JJ, Świąder M, Parada-Turska J, Czuczwar SJ: Tiagabine synergistically interacts with gabapentin in the electroconvulsive threshold test in mice. Neuropsychopharmacology, 2003, 28, 1817–1830.
27. Łuszczki JJ, Zuchora M, Sawicka KM, Kozińska J, Czuczwar SJ: Acute exposure to caffeine decreases the anticonvulsant action of ethosuximide, but not clonazepam, phenobarbital and valproate against pentetrazole-induced seizures in mice. Pharmacol Rep, 2006, 58, 652–659.
28. Margineau DG, Klitgaard H: Caffeine-induced epileptiform field potentials in rat hippocampal slices: a pharmacological characterization. Neuropharmacology, 2004, 47, 926–934.
29. McCall WV, Reid S, Rosenquist P, Foreman A, Kiesow-Webb N: A reappraisal of the role of caffeine in ECT. Am J Psychiat, 1993, 150, 1543–1545.
30. Nehlig A, Daval JL, Debry G: Caffeine and the central nervous system: Mechanism of action, biochemical, metabolic and psychostimulant effects. Brain Res Rev, 1992, 17, 139–170.
31. Perucca E: An introduction to antiepileptic drugs. Epilepsia, 2005, 46, Suppl 4, 31–37.
32. Schwabe U, Ukena D, Lohse MJ: Xanthines derivatives as antagonists at A_1 and A_2 adenosine receptors. Naunyn-Schmiedebergs Arch Pharmacol, 1985, 330, 212–221.
33. Venault P, Chapouthier G, de Carvalho LP, Simiand J, Morre M, Dodd RH, Rossier J: Benzodiazepines impair and beta-carbolines enhance performance in learning and memory tasks. Nature, 1986, 321, 864–866.
34. Wang X, Ratnaraj N, Patsalos PN: The pharmacokinetic inter-relationship of tiagabine in blood, cerebrospinal fluid and brain extracellular fluid (frontal cortex and hippocampus). Seizure, 2004, 13, 574–581.

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