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Pharmacokinetic and pharmacodynamic interactions of aminophylline and topiramate in the mouse maximal electroshock-induced seizure model

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Abstract

The aim of this study was to determine the influence of acute (single) and chronic (twice daily for 14 consecutive days) treatments with aminophylline (theophylline$_2$·ethylenediamine) on the anticonvulsant potential of topiramate (a broad-spectrum antiepileptic drug) in the mouse maximal electroshock-induced seizure model. Additionally, the effects of acute and chronic administration of aminophylline on the adverse effect potential of topiramate were assessed in the chimney test (motor performance). To evaluate pharmacokinetic characteristics of interaction between topiramate and aminophylline, total brain concentrations of topiramate and theophylline were estimated with fluorescence polarization immunoassay technique. Results indicate that aminophylline in non-convulsive doses of 50 and 100 mg/kg (i.p.), both in acute and chronic experiments, markedly attenuated the anticonvulsant potential of topiramate by raising its ED_{50} value against maximal electroconvulsions. Aminophylline at a lower dose of 25 mg/kg did not affect significantly the ED_{50} value of topiramate in the acute experiment, but the drug markedly increased the ED_{50} value of topiramate during the chronic treatment in mice. Only, aminophylline at 12.5 mg/kg, in both acute and chronic experiments, did not affect the antielectroshock action of topiramate in mice. Moreover, aminophylline at a dose of 100 mg/kg had no impact on the adverse effect potential of topiramate in the chimney test. Pharmacokinetic evaluation of total brain concentrations of topiramate and theophylline revealed that topiramate significantly increased total brain theophylline concentrations following both acute and chronic applications of aminophylline. Conversely, aminophylline did not alter total brain concentrations of topiramate in mice. Based on this preclinical study, one can conclude that aminophylline attenuated the antiseizure action of topiramate in the mouse maximal electroshock-induced seizure model and the observed interaction between drugs was both pharmacokinetic and pharmacodynamic in nature.

Keywords: Aminophylline; Topiramate; Maximal electroshock; Pharmacokinetic interaction; Pharmacodynamic interaction; Chimney test

1. Introduction

Overwhelming evidence indicates that some methylxanthine derivatives (i.e., caffeine and aminophylline [theophylline$_2$·ethylenediamine]) possess strong convulsive potential in experimental studies on animals (Ault et al., 1987; Chu, 1981; Czuczwar et al., 1987b,c). Moreover, severe seizure activity was reported clinically upon the intensive treatment of status asthmaticus and obturatory pulmonary disease with aminophylline or theophylline (Nakada et al., 1983; Yarnell and Chu, 1975; Zwillich et al., 1975). Similarly, caffeine was responsible for the reduction in seizure threshold and increased seizure frequency in epileptic patients treated with conventional antiepileptic drugs (Bonilha and Li, 2004; Kaufman and Sachdeo, 2003). Furthermore, aminophylline attenuated the anticonvulsant potential of conventional antiepileptic drugs and aminophylline-induced seizure activity was relatively resistant to standard antiepileptic drug therapy (Czuczwar et al., 1986, 1987a,b,c, 1989, Wlaz et al., 1992, 1993). Considering the fact that epileptic patients may be exposed to methylxanthine medication for other than epilepsy reasons (such as obturatory pulmonary disease or status asthmaticus) or may ingest dietary

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methylxanthines in the form of caffeinated beverages, the problem of reduction of the anticonvulsant potential of commonly used antiepileptic drugs is of pivotal importance for epileptic patients.

Previously, it has been documented that both acute (single) and chronic (twice daily for 14 consecutive days) administration of aminophylline at non-convulsive doses of 50 mg/kg or lower considerably reduced the antiseizure potentials of conventional antiepileptic drugs (carbamazepine, phenytoin, diazepam, phenobarbital and valproate) in the maximal electroshock-induced seizure test in mice (Czuczwar et al., 1986, 1987b,c, 1989, Wlaz et al., 1992, 1993). Only, felbamate — a newer (second-generation) antiepileptic drug was partly resistant to the aminophylline-induced reduction of the antiseizure effects in the maximal electroshock in mice. It has been found that aminophylline administered singly at doses of 50 and 75 mg/kg did not affect the anticonvulsive action of felbamate, but aminophylline at 100 mg/kg significantly diminished the antiseizure potential of felbamate in the maximal electroshock seizures (Gasior et al., 1998). Experimental evidence indicates that the attenuation of the antiseizure effects of conventional antiepileptic drugs was due to theophylline, because the ethylenediamine component of aminophylline had no effect on the anticonvulsant potential of examined antiepileptic drugs in mice (Czuczwar et al., 1986). Moreover, aminophylline administered acutely and chronically up to 100 mg/kg had no effect on the threshold for electroconvulsions in mice (Czuczwar et al., 1987c; Wlaz et al., 1992, 1993, 1994).

With the advent of ten newer (second-generation) antiepileptic drugs, lately introduced into the therapy of epilepsy, there has appeared a wishful hope of finding an antiepileptic drug, which could be resistant to the hazardous action produced by methylxanthines. Among these antiepileptic drugs, topiramate seemed to fulfill expectations related to the resistance to the methylxanthine-induced attenuation of the antiseizure potential of antiepileptic drugs. In the clinical settings, topiramate is recommended for patients with partial convulsions with or without secondary generalization and tonic–clonic seizures (Brodie and Schachter, 2001). Additionally, the drug can be used for reducing drop attacks in children with Lennox–Gastaut syndrome (French et al., 2004). Topiramate possesses a number of potential mechanisms of action, which may account for its broad-spectrum antiseizure effects in various experimental models of epilepsy and clinical settings (Brodie and Schachter, 2001).

Considering the fact that aminophylline diminished the antiseizure effects of a number of conventional antiepileptic drugs, it was of pivotal importance to evaluate the effects of acute and chronic pretreatments with aminophylline on the antielectroshock action of topiramate in mice. Therefore, the present study was undertaken to find out whether aminophylline administered acutely and chronically affected the anticonvulsant activity of topiramate in the mouse maximal electroshock-induced seizure model. Generally, the maximal electroshock-induced seizure test is thought as an experimental model of tonic–clonic seizures and, to a certain extent, of partial epilepsy in man (Löscher and Schmidt, 1988). Additionally, we investigated the combination of aminophylline with topiramate in relation to motor impairment by the use of the chimney test. Finally, total brain topiramate and theophylline concentrations were measured in order to ascertain whether any observed effects were consequent to a pharmacodynamic and/or a pharmacokinetic interaction.

2. Materials and methods

2.1. 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, under standardized housing conditions (natural light–dark cycle, temperature of 23±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 comprising of 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 15.00 h. 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 and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs

The following drugs were used in this study: topiramate (Topamax, Janssen-Cilag International N.V., Beerse, Belgium) and aminophylline ([theophylline$_2$·ethylenediamine]; Sigma, St. Louis, MO, USA). Topiramate was suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA), whereas aminophylline was dissolved in sterile saline and both drugs were administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight. Fresh drug solutions were prepared on each day of experimentation.

2.3. Treatment protocol

This study consisted of two experiments associated with acute (single) and chronic (twice daily for 14 days) administration of aminophylline, as it has been documented previously (Wlaz et al., 1992, 1993).

In the chronic experiment, animals were injected twice daily at 08.00 and 20.00 h with: a) saline for 14 days and b) aminophylline for 14 days at doses of 12.5, 25, 50, and 100 mg/kg. On the 15th day, mice from all groups received topiramate (60 min, before the test) and the respective doses of aminophylline (30 min, before the test). Subsequently, the animals were challenged either with the maximal electroshock-induced seizures, chimney test or brain sampling. Control animals received i.p. injections of saline twice daily at respective times. All treatments were started on day 1 at 20.00 h.

In the acute experiment, animals received topiramate (60 min, prior to the test) and aminophylline at doses of 12.5, 25, 50 and 100 mg/kg (30 min, before the test). Next, the animals were subjected to the maximal electroshock-induced seizures, chimney test and brain sampling.

2.4. Maximal electroshock-induced seizure test

The experimental procedure has been described in more detail in our earlier studies (Luszczki et al., 2003a, 2006b). Briefly, electroconvulsions were produced by means of an alternating current (0.2 s; 25 mA; 500 V; 50 Hz) delivered via ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The electrical system of the stimulator was self-adjustable so that changes in impedance did not result in alterations of current intensity (i.e., the system provides constant current stimulation). The criterion for the occurrence of seizure activity was the tonic hindlimb extension (i.e., the hind limbs of animals outstretched 180° to the plane of the body axis). Protective activities of topiramate administered alone and in combination with various doses of aminophylline against maximal electroshock-induced seizures were evaluated as its median effective doses (ED_{50} values in mg/kg; with their 95% confidence limits) using log-probit analysis according to Litchfield and Wilcoxon (1949). This experimental procedure has been described in detail in our earlier studies (Luszczki et al., 2003a, 2006b). Topiramate in the maximal electroshock-induced seizure test was administered i.p. at doses ranged between 60–180 mg/kg.

2.5. Chimney test

The chimney test of Boissier et al. (1960) was used to quantify the adverse effect potential of topiramate administered singly or in combination with aminophylline on motor performance in mice. In this test, the animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length), and motor performance impairment was indicated by the inability of the mice to climb backward up the transparent tube within 60 s. The adverse effect potential of topiramate administered alone and in combination with aminophylline was determined as its median toxic doses (TD_{50} values, in mg/kg; with their 95% confidence limits), representing the doses of topiramate, which impaired motor coordination in 50% of the animals tested. This experimental procedure has been described in detail in our earlier studies (Luszczki et al., 2003a, 2006b). Topiramate in the chimney test was administered i.p. at doses ranged between 400–800 mg/kg.

2.6. Measurement of total brain theophylline and topiramate concentrations

Since aminophylline chemically consists of 2 molecules of theophylline combined with ethylenediamine, it was appropriate to evaluate total brain theophylline concentrations, which adequately and precisely correspond to the aminophylline content in the brain tissue. Total brain concentrations of theophylline and topiramate were determined in mice that received either a single aminophylline (100 mg/kg) injection or aminophylline twice daily 100 mg/kg for 14 consecutive days. Topiramate was administered at doses corresponding to its ED_{50} values as determined in the maximal electroshock-induced seizure test. Mice were killed by decapitation at times chosen to coincide with that scheduled for the maximal electroshock test and whole brains were removed from skulls, weighed, and homogenized using Abbott buffer (2:1 vol/weight; Abbott Laboratories, North Chicago, IL, USA) in an Ultra-Turrax T8 homogenizer (IKA Werke, Staufen, Germany). The homogenates were centrifuged at 10,000 ×*g* for 10 min and the supernatant samples (75 μl) were analyzed by fluorescence polarization immunoassay technique using a TDx analyzer and reagents (theophylline and topiramate) exactly as described by the manufacturers (Abbott Laboratories, North Chicago, IL, USA for theophylline and Seradyn Inc., Indianapolis, IN, USA for topiramate). Total brain concentrations were expressed in μg/ml of brain supernatants as means ± S.D. of at least 8 separate brain preparations.

2.7. Statistics

Both, ED_{50} and TD_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon (1949). Subsequently, the respective 95% confidence limits were transformed into S.E.M. as described previously (Luszczki et al., 2003a, 2006b). Statistical analysis of data was performed with one-way analysis of variance (ANOVA) followed by the post-hoc Tukey/Kramer test for multiple comparisons (Luszczki and Czuczwar, 2005a). Total brain concentrations of theophylline and topiramate were statistically compared using the unpaired Student's *t*-test.

3. Results

3.1. Effects of acute and chronic administrations of aminophylline on the anticonvulsant potential of topiramate in the maximal electroshock-induced seizures

Topiramate administered alone (i.p., 60 min before the test) exerted a clear-cut anticonvulsant effect against maximal electroconvulsions and its ED_{50} value is presented in Table 1. With one-way ANOVA ($F(4,123)=20.21$, $P<0.0001$) followed by the post-hoc Tukey/Kramer test, it was found that the acute administration of aminophylline (i.p., 30 min, before the test), at doses of 50 and 100 mg/kg, attenuated significantly the antielectroshock potential of topiramate in mice by raising the ED_{50} value of topiramate from 88.9 mg/kg (control) to 116.1 mg/kg ($P<0.001$), and 149.3 mg/kg ($P<0.001$), respectively (Table 1). In contrast, acute treatment with aminophylline at lower doses of 12.5 and 25 mg/kg had no significant effect on the antiseizure action of topiramate in mice, although its ED_{50} values were slightly elevated from 88.9 mg/kg (control) to 95.5 mg/kg, and 104.4 mg/kg, respectively (Table 1). Similarly, one-way ANOVA ($F(4,123)=19.46$, $P<0.0001$) followed by the post-hoc Tukey/Kramer test revealed that the chronic administration of aminophylline (twice daily for 14 consecutive days and

Table 1
Influence of acute and chronic administration of aminophylline on the anticonvulsant activity of topiramate in the maximal electroshock-induced seizures in mice

Treatment (mg/kg)	ED_{50} (mg/kg)	S.E.M.	N
Acute experiment			
Topiramate + vehicle	88.9 (80.0–98.8)	4.79	32
Topiramate + aminophylline (12.5)	95.5 (87.8–103.8)	4.07	24
Topiramate + aminophylline (25)	104.4 (95.6–114.0)	4.67	16
Topiramate + aminophylline (50)	116.1 (104.6–129.0)[a]	6.20	32
Topiramate + aminophylline (100)	149.3 (140.1–159.2)[a]	4.86	24
$F(4,123)=20.21$; $P<0.0001$			
Chronic experiment			
Topiramate + vehicle	88.9 (80.0–98.8)	4.79	32
Topiramate + aminophylline (12.5)	97.8 (88.2–108.5)	5.16	24
Topiramate + aminophylline (25)	109.2 (100.0–119.3)[b]	4.93	24
Topiramate + aminophylline (50)	126.2 (114.7–139.9)[a]	6.16	32
Topiramate + aminophylline (100)	155.7 (147.1–164.9)[a]	4.52	16
$F(4,123)=19.46$; $P<0.0001$			

Results are presented as median effective doses (ED_{50} values in mg/kg; with 95% confidence limits in parentheses) of topiramate administered singly and in combination with aminophylline against maximal electroshock-induced seizures in mice. The ED_{50} values were calculated by using log-probit method according to Litchfield and Wilcoxon (1949). Statistical evaluation of data was performed with one-way ANOVA followed by the post-hoc Tukey/Kramer test for multiple comparisons. In the acute experiment, the drugs were administered systemically (i.p.), as follows: topiramate at 60 min and aminophylline at 30 min before the test. In the chronic experiment, topiramate was administered singly at 60 min, whereas aminophylline was administered twice daily for 14 days and on the 15th day of experimentation the drug was administered at 30 min prior to the test. S.E.M. — standard error of the mean of ED_{50} values; *N* — total number of animals used at doses whose expected anticonvulsant effects ranged between 4 and 6 probits. [a]$P<0.001$ and [b]$P<0.05$ vs. control group (topiramate + vehicle-treated animals).

on the 15th day, 30 min before the test) at doses of 25, 50 and 100 mg/kg markedly reduced the antielectroshock potential of topiramate by increasing its ED_{50} values in the mouse maximal electroshock-induced seizure model from 88.9 mg/kg to 109.2 mg/kg ($P<0.05$), 126.2 mg/kg ($P<0.001$), and 155.7 mg/kg ($P<0.001$), respectively (Table 1). Only, aminophylline administered chronically at a dose of 12.5 mg/kg did not affect significantly the antiseizure action of topiramate against maximal electroconvulsions in mice, although a slight increase in the ED_{50} value of topiramate (from 88.9 mg/kg to 97.8 mg/kg) was observed (Table 1).

3.2. Effects of acute and chronic treatments with aminophylline on the adverse effect potential of topiramate in the chimney test

Topiramate administered alone (i.p., 60 min, before the chimney test) produced clear-cut adverse effects manifesting in form of motor coordination impairment. The TD_{50} value for topiramate administered separately was 678.0 (615.8–746.5) mg/kg. The acute administration of aminophylline at a maximally tested dose of 100 mg/kg had no effect on topiramate-induced impairment of motor coordination in the chimney test and the TD_{50} value for the drug was 714.9 (629.2–812.3) mg/kg. Likewise, the chronic treatment with aminophylline at 100 mg/kg did not alter the adverse effect potential of topiramate, as determined in the chimney test. In this case, the TD_{50} value was 595.3 (500.0–708.7) and did not differ significantly from that for control group (topiramate alone-treated animals).

Table 2
Influence of topiramate on total brain concentrations of theophylline in mice

Treatment (mg/kg)	Brain concentrations (μg/ml)[a]
Acute experiment	
Aminophylline (100) + vehicle	30.68±2.75
Aminophylline (100) + topiramate (149.3)	36.14±2.47[b]
Chronic experiment	
Aminophylline (100) + vehicle	23.56±3.61
Aminophylline (100) + topiramate (155.7)	35.80±4.31[b]

Data are presented as means ± S.D. of at least 8 separate determinations. Statistical evaluation of data was performed by use of the unpaired Student's *t*-test. Topiramate was administered in combination with aminophylline (100 mg/kg) at doses corresponding to its ED_{50} values from the maximal electroshock test. Acute experiment was performed after a single administration of aminophylline at a dose of 100 mg/kg. Chronic experiment was performed after 14 days of administration (twice daily) of aminophylline at 100 mg/kg, and on the 15th day, topiramate was administered 60 min, whereas aminophylline (100 mg/kg) was administered at 30 min before the brain sampling for the measurement of theophylline concentrations.

[a] Since aminophylline comprises of 2 molecules of theophylline and ethylenediamine, it was appropriate to estimate theophylline concentrations in experimental animals with fluorescence polarization immunoassay technique. For more detail see "Materials and methods" section.

[b] $P<0.001$ vs. aminophylline-treated animals.

3.3. Total brain concentrations of theophylline and topiramate

In the acute experiment (after administration of aminophylline in a single dose of 100 mg/kg), topiramate (at 149.3 mg/kg) significantly increased (by 18%) total brain concentrations of theophylline (Table 2). Likewise, in the chronic experiment, topiramate (155.7 mg/kg) combined with aminophylline (twice daily 100 mg/kg for 14 days) markedly increased (by 52%) total brain concentrations of theophylline in mice (Table 2). In contrast, aminophylline (at 100 mg/kg), in both acute and chronic experiments, did not alter significantly total brain concentrations of topiramate in mice (Table 3).

Table 3
Influence of aminophylline on total brain concentrations of topiramate in mice

Treatment (mg/kg)	Brain concentrations (μg/ml)
Acute experiment	
Topiramate (149.3) + vehicle	26.87±2.53
Topiramate (149.3) + aminophylline (100)	27.00±1.98
Chronic experiment	
Topiramate (155.7) + vehicle	27.56±2.45
Topiramate (155.7) + aminophylline (100)	26.76±3.05

Data are presented as means ± S.D. of at least 8 separate determinations. Statistical evaluation of data was performed by use of the unpaired Student's *t*-test. Topiramate was administered in combination with aminophylline (100 mg/kg) at doses corresponding to its ED_{50} values from the maximal electroshock-induced seizures. For more details see the legend to Table 2.

4. Discussion

Results presented herein indicate that aminophylline administered both acutely (singly) and chronically (twice daily for 14 days), at doses of 50 and 100 mg/kg, attenuated the anticonvulsant effects of topiramate against maximal electroshock-induced seizures in mice. In contrast, the acute and chronic applications of aminophylline, at a maximally tested dose of 100 mg/kg, had no effect on topiramate-induced impairment of motor coordination in mice challenged with the chimney test. Generally, the results of this study are consistent with previous experimental data, reporting that aminophylline administrated acutely and chronically (for 14 days) considerably alleviated the anticonvulsant action of conventional antiepileptic drugs in the maximal electroshock-induced seizure test in mice (Czuczwar et al., 1985, 1986, 1987c, 1989; Wlaz et al., 1992, 1993). However, this study indicated for the first time that topiramate increased significantly total brain theophylline concentrations after both acute and chronic administration of aminophylline. In contrast, aminophylline did not affect total brain topiramate concentrations in mice. It is important to note that in this study we evaluated total brain theophylline concentrations instead of aminophylline used. As already mentioned in the Materials and methods, aminophylline chemically consists of 2 molecules of theophylline combined with ethylenediamine, therefore, it was appropriate to estimate theophylline concentrations, which adequately reflect any changes evoked by aminophylline in vivo.

Noteworthy, pharmacokinetic studies performed previously for conventional antiepileptic drugs co-administered with aminophylline have revealed no significant changes in total plasma concentrations of theophylline and conventional antiepileptic drugs (Czuczwar et al., 1987a,c, 1989; Wlaz et al., 1992, 1993). It is important to note that pharmacokinetic interactions at the plasma level may influence brain concentrations of antiepileptic drugs. However, experimental evidence indicates that there may be independent pharmacokinetic interactions at the central (brain) compartment in the absence of interactions at the plasma level and, in contrast, there may be pharmacokinetic interactions at the plasma level without any significant changes in brain antiepileptic drug concentrations. For instance, it has recently been documented that 2-phosphonomethyl-pentanedioic acid significantly increased total brain concentration of valproate, having had no impact on free plasma valproate concentration in mice (Luszczki et al., 2006a). In contrast, loreclezole markedly increased free plasma concentration of valproate, without any significant effect on total brain valproate concentration (Luszczki et al., 2006b). Considering the fact that antiepileptic drugs influence brain functioning and exert their effects on neurons, the estimation of antiepileptic drug concentrations in the central (brain) compartment seems to be more suitable for the evaluation of pharmacokinetic interactions between drugs in experimental studies on animals than the estimation of antiepileptic drug concentrations at plasma level (for more detail see Cadart et al., 2002; Luszczki et al., 2003b). This is why in this study we determined both topiramate and theophylline concentrations in the brain tissue in mice.

The question arises, whether the effects observed earlier for the combinations of aminophylline with a number of conventional antiepileptic drugs in the maximal electroshock-induced seizures (associated with the reduction of antielectroshock potential of conventional antiepileptic drugs) were also related to an increase in total brain theophylline concentrations, despite no changes documented in total plasma concentrations of the examined drugs in experimental animals. To confirm or reject this hypothesis, suggesting the increased brain theophylline concentrations in experimental animals, more advanced pharmacokinetic studies are required.

Previously, it has been documented that some antiepileptic drugs markedly potentiated their acute adverse effects producing motor coordination impairment in experimental animals in the chimney test. Such a situation has been observed after adding tiagabine to valproate (Luszczki et al., 2003b). Moreover, the isobolographic evaluation of acute adverse-effect profiles of antiepileptic drugs in combination revealed the existence of synergistic interactions between oxcarbazepine and lamotrigine (Luszczki and Czuczwar, 2004), felbamate and clonazepam (Borowicz et al., 2004), loreclezole and tiagabine (Luszczki and Czuczwar, 2005b), loreclezole and clonazepam (Luszczki et al., 2005), and stiripentol and clonazepam (Luszczki et al., 2006c), in terms of motor coordination impairment in the chimney test. Generally, the impairment of motor coordination in experimental animals closely corresponds to ataxia and other coordination disturbances seen in humans (Löscher et al., 1991). Therefore, it was of importance to determine in this study whether aminophylline potentiated acute adverse effects produced by topirmate in the chimney test in mice. Results obtained herein indicated that aminophylline administered acutely and chronically did not alter the acute neurotoxic effects produced by topiramate in terms of motor coordination impairment as assessed in the chimney test.

To explain the decrease in the antiseizure potential of topiramate following the systemic (i.p.) administration of aminophylline, molecular mechanisms of action of both drugs should be borne in mind. Neurochemical studies have revealed that aminophylline blocks adenosine receptors (Chu, 1981), impairs adenosine synthesis (Jensen et al., 1984), mobilizes Ca^{2+} from intracellular stores (Neering and McBurney, 1984), and enhances excitatory amino acids release from neurons (Corradetti et al., 1984). Moreover, aminophylline inhibits phosphodiesterase activity, although this action was observed after administration of the methylxanthine derivative at higher toxic doses not used in clinical studies (Persson et al., 1986). On the other hand, the antagonistic effects of aminophylline on adenosine neurotransmitter system, as the mechanisms of action, which could be responsible for convulsant properties of aminophylline in experimental models of epilepsy, are questionable (Czuczwar and Kleinrok, 1990; Hornfeldt and Larson, 1994). Quite recently, there has appeared a suggestion that the excessive production of reactive oxygen and nitrogen species, and thus, imbalance between the oxidative/anti-oxidative state in neurons, may be involved in the generation of seizure activity after systemic administration of

aminophylline (Gulati et al., 2005). Noteworthy, one of the major metabolites of theophylline undergoes metabolic degradation via xanthine–xanthine oxidase system, which is involved in the generation of reactive oxygen species in vivo (Lohmann and Miech, 1976). Moreover, experimental evidence indicates that pretreatment with melatonin and *N*-acetylcysteine (two potent anti-oxidants), as well as, with 7-nitroindazole and N^G-nitro-L-arginine methyl ester (two nitric oxide synthase inhibitors) considerably attenuated aminophylline-induced seizures in mice (Gulati et al., 2005). In contrast, it has been documented that N^G-nitro-L-arginine (another nitric oxide synthase inhibitor), administered i.p. up to 40 mg/kg significantly enhanced the convulsive properties of aminophylline (Urbanska et al., 1996). Thus, experimental evidence indicates that N^G-nitro-L-arginine potentiates, whereas N^G-nitro-L-arginine methyl ester attenuates aminophylline-induced seizures in mice. The explanation of these contradictory results is, at present, impossible. Nevertheless, the involvement of free radical formation (reactive oxygen and nitrogen species) in the convulsant action of aminophylline has been postulated (Gulati et al., 2005). With respect to topiramate, at therapeutically relevant concentrations, the drug: 1) inhibits voltage-sensitive Na^+ channels (Taverna et al., 1999); 2) potentiates γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission through binding to a novel site on the $GABA_A$-receptor complex (White et al., 2000); 3) blocks excitatory neurotransmission through a negative modulatory effect on Ca^{2+}-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate subtypes of glutamate receptors (Gibbs et al., 2000); 4) inhibits neuronal L-type high-voltage-activated Ca^{2+}channels (Zhang et al., 2000); 5) inhibits $GABA_A$-receptor mediated depolarizing responses by enhancing simultaneously the conductance of some types of K^+ channels (Herrero et al., 2002); 6) weakly inhibits the carbonic anhydrase isoenzymes CA II and CA IV, and through the modulation of pH, the drug influences voltage- and receptor-gated ion channels (Dodgson et al., 2000) and 7) selectively inhibits GluR5 kainate receptors (Gryder and Rogawski, 2003). Moreover, TPM binds to phosphorylation sites on AMPA/kainate receptors and thereby exerts an allosteric modulatory effect on channel conductance (Angehagen et al., 2004; Shank et al., 2000). Considering molecular mechanisms of action of both drugs, it is impossible, at present, to unequivocally ascertain which mechanism(s) is (are) responsible for such an antagonistic interaction between aminophylline and topiramate in the maximal electroshock test in mice. Perhaps, other unknown as yet mechanisms of action of aminophylline (theophylline) attenuated the antielectroshock action of topiramate in mice.

In light of the above-mentioned facts and results presented herein, one can conclude that the administration of aminophylline should be avoided in patients treated with topiramate because of the reduction of its antiseizure potential. Moreover, for the first time it was documented that the antagonistic interaction between topiramate and aminophylline in the maximal electroshock-induced seizures test resulted in pharmacokinetic interaction between drugs in the brain compartment.

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