

## SHORT COMMUNICATION

### INFLUENCE OF LY 300164, AN AMPA/KAINATE RECEPTOR ANTAGONIST UPON THE ANTICONVULSANT ACTION OF ANTIEPILEPTIC DRUGS AGAINST AMINOPHYLLINE-INDUCED SEIZURES IN MICE

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*Influence of LY 300164, an AMPA/kainate receptor antagonist, upon the anticonvulsant action of antiepileptic drugs against aminophylline-induced seizures in mice.* M. ŚWIĄDER, H. KUŹNIAR, Z. KLEINROK, S.J. CZUCZWAR. Pol. J. Pharmacol., 2003, 55, 103–107.

LY 300164 {7-acetyl-3-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxazolo[4,5-h] [2,3]-benzodiazepine}, a novel AMPA/kainate receptor antagonist, administered intraperitoneally protected mice against aminophylline-induced seizures. At doses up to 0.5 mg/kg, which did not significantly affect the convulsant activity of aminophylline, it potentiated the protective activity of diazepam. On the other hand, LY 300164 used at the lowest protective dose of 1.0 mg/kg enhanced anticonvulsant activity of all antiepileptic drugs tested in this seizure model. However, LY 300164 neither alone nor combined with antiepileptic drugs, reduced aminophylline-induced mortality.

**Key words:** antiepileptic drugs, LY 300164, aminophylline-induced seizures

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## INTRODUCTION

The convulsive action of methylxanthines has been known for many years [16, 17]. Clinical data indicate that aminophylline may induce repetitive generalized seizures in asthmatic patients [11, 15]. However, antiepileptic drugs, such as phenobarbital, diphenylhydantoin and diazepam, administered at high doses [18] could partially prevent the convulsions. Furthermore, experimental data indicate that other agents such as carbamazepine, ethosuximide, diphenylhydantoin, acetazolamide, trimethadione, clonidine and amino-oxyacetic acid have been found to be completely ineffective against aminophylline-induced seizures and mortality [7]. On the other hand, aminophylline (and other methylxanthines) sharply reduced the protective efficacy of common antiepileptics after acute administration [5, 7, 8]. Neither an ethylenediamine component of aminophylline nor a pharmacokinetic interaction appeared to be responsible for the methylxanthine-induced impairment [5, 6] of the anticonvulsant action of antiepileptics. Also, the peripheral effects of aminophylline were excluded as a cause of this effect since a peripherally active adenosine antagonist, 8-(p-sulfophenyl)-theophylline, was completely devoid of any effect on the protective potency of antiepileptic drugs [2].

LY 300164 {7-acetyl-3-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxazolo[4,5-h][2,3]-benzodiazepine}, an antagonist of AMPA/kainate receptors, potentiated the anticonvulsant activity of antiepileptic drugs, both against seizures evoked by maximal electroshock in mice [9] or amygdala kindling in rats [1]. Therefore, the aim of the present study was to examine the influence of LY 300164 on the protective effects of diazepam, valproate and carbamazepine against aminophylline-induced seizures and mortality in mice. Any beneficial interaction could be of importance for management of aminophylline overdose.

## MATERIALS and METHODS

### Animals

The experiments were performed on female Swiss mice weighing 22–27 g. The animals were housed in colony cages under standard laboratory conditions with free access to chow pellets (Murigran; Bacutil, Motycz, Poland) and tap water. The experimental temperature was  $21 \pm 1^\circ\text{C}$  and mice

were kept under a natural light-dark cycle. The experimental groups, consisting of 10 animals, were selected by means of a randomized schedule. All experimental procedures were performed between 9.00 a.m. and 2.00 p.m. Each mouse was used only once. The experimental procedures described below were approved by the Ethics Committee of Lublin Medical University.

### Aminophylline-induced convulsions

The mice were injected intraperitoneally (*ip*) with aminophylline (theophylline<sub>2</sub>• ethylenediamine) at the dose of 273 mg/kg, which is the experimentally determined  $\text{CD}_{97}$  value for the induction of clonic convulsions. The animals were then put into individual perspex plexiglas cages ( $25 \times 15 \times 10$  cm) and observed for the occurrence of clonic and tonic seizures for 60 min. The endpoint for the clonic phase was a clonic seizure with a loss of the righting reflex. Immediately at the end of observation and also after 2 and 24 h, numbers of animals, which stayed alive were recorded. The convulsive test was performed in a quiet room. The control animals were injected with aminophylline on each day of the convulsive test, and the results were reproducible.

### Drugs

The following antiepileptic drugs were used: valproate magnesium (Dipromal, Polfa, Rzeszów, Poland), carbamazepine (Amizepin, diazepam (Relanium, both antiepileptics from Polfa, Warszawa, Poland) and LY 300164 {7-acetyl-3-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxazolo[4,5-h][2,3]-benzodiazepine}, a novel antagonist of AMPA/kainate receptor (Lilly Res. Lab., Indianapolis, IN, USA). Valproate and aminophylline were brought into solution with sterile saline whilst diazepam, carbamazepine and LY 300164 were suspended in 1% solution of Tween 80 (Sigma, St. Louis, MO, USA).

All drugs were administered *ip* in a volume of 0.01 ml/g of body weight. Valproate magnesium, carbamazepine and LY 300164 were injected 15 min and diazepam – 30 min prior to the test. Doses of valproate refer to its free form.

### Statistics

The data were analyzed by Fischer's exact probability test and  $\text{ED}_{50}$  values of antiepileptics were computed and statistical analysis of the re-

sults was performed by computer probit analysis, according to Litchfield and Wilcoxon [10].

## RESULTS

### Effect of aminophylline at its $CD_{97}$ upon seizure activity

Aminophylline evoked clonic and tonic seizures in a dose-dependent manner, and its  $CD_{97}$  for the induction of clonic phase was 273 mg/kg (data not shown). The latency to the onset of convulsions varied from 15 to 30 min, with a mean  $\pm$  SE of  $25 \pm 4$  min. Mice showed accelerated respiration, occasional tremors, incoordination and mild hyperactivity prior to the onset of seizures. Out of 40 mice injected with aminophylline at  $CD_{97}$ , 38 had clonic

seizures, 36 exhibited the tonic phase and 36 died within the 60 min observation period.

### Influence of LY 300164 on the clonic phase of aminophylline-induced seizures

LY 300164 (up to 0.5 mg/kg) remained without effect against aminophylline (273 mg/kg)-induced clonic seizures in mice. However, LY 300164 (1.0 mg/kg) significantly decreased the number of clonic (Fig. 1) and tonic (result not shown in Figure 1) convulsions in comparison with the control animals. As can be seen from Figure 1, LY 300164 showed a graded dose-response effect on the episodes of clonic convulsions, and its  $ED_{50}$  value against aminophylline-induced seizures was 0.8 (0.5–1.2) mg/kg (Fig. 1).

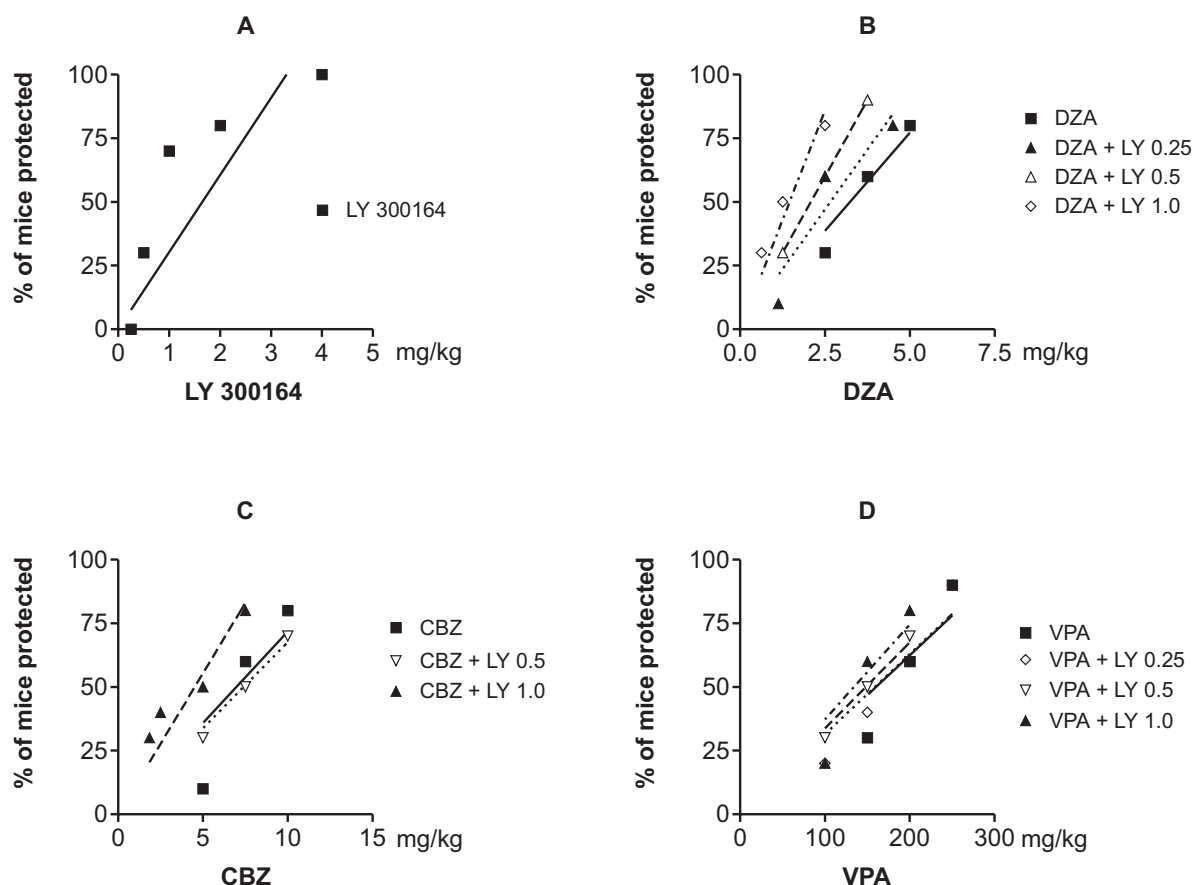


Fig. 1. Effect of LY 300164 upon the protective activity of antiepileptic drugs, was expressed as a percentage of mice protected against aminophylline-induced seizures. The control groups of animals were injected with aminophylline on each day of the convulsive test. Aminophylline was administered at its  $CD_{97}$  dose (the dose of drug necessary to induce clonic seizures in 97% of animals), which was 273 mg/kg. DZA – diazepam; CBZ – carbamazepine; VPA – valproate magnesium. All drugs were administered *ip* in volume of 0.01 ml/g of body weight, VPA, CBZ and LY 300164 – 15 min, DZA – 30 min prior to the test. The  $ED_{50}$ s of DZA + LY 300164 (0.5 mg/kg), DZA + LY 300164 (1.0 mg/kg), VPA + LY 300164 (1.0 mg/kg) were statistically significantly different vs. the respective  $ED_{50}$ s of DZA, CBZ, and VPA at least at  $p < 0.05$

## Influence of LY 300164 upon the protective activity of antiepileptic drugs against clonic phase of aminophylline-induced seizures and postconvulsive mortality

LY 300164 (0.5 mg/kg), when applied together with diazepam, significantly reduced its ED<sub>50</sub> value from 3.3 (2.5–4.2) to 1.8 (1.2–2.7) mg/kg. Moreover, LY 300164 (1 mg/kg) co-administered with diazepam and carbamazepine also potentiated their activity [ED<sub>50</sub> values were reduced from 3.3 to 1.1 (0.7–1.9) mg/kg and from 7.4 (6.0–9.0) to 3.6 (2.1–5.9) mg/kg, respectively]. Also, LY 300164 at the dose of 1 mg/kg reduced the ED<sub>50</sub> value of valproate from 185 (160–215) to 139 (113–171) mg/kg, being without effect at lower doses (Fig. 1).

Interestingly, the AMPA/kainate receptor antagonist and antiepileptic drugs reduced the mortality rate, evaluated after the first and second hour following the injection of the convulsant. Neither antiepileptics or the AMPA/KA receptor antagonist alone nor their combinations influenced this parameter when assessed after 24 h (results not shown).

## DISCUSSION

This study has shown that LY 300164 protected mice from clonic seizures induced by aminophylline. Moreover, at the highest dose of 1.0 mg/kg, it significantly enhanced the protective activity of the studied antiepileptics against the clonic phase of aminophylline-induced convulsions. The AMPA/KA receptor antagonist reduced ED<sub>50</sub>s of carbamazepine and diazepam by over 50% or valproate by about 25%. Similarly, Czuczwar et al. [7] indicated that some NMDA receptor antagonists afforded protection against aminophylline-induced clonic seizure activity. Neither antiepileptic drug tested nor LY 300164, administered alone or combined with antiepileptics, reduced mortality assessed after 24 h.

The previous studies indicated poor efficacy of conventional antiepileptic drugs against aminophylline-induced convulsions in mice [12]. Also, Czuczwar et al. [7] proved that ethosuximide, diphenylhydantoin, trimethadione and carbamazepine were totally ineffective in aminophylline-induced clonic seizures. However, the present results show that carbamazepine protected against aminophylline-induced convulsions, but at lower doses. Carbamazepine used at higher doses (20–50 mg/kg) was totally ineffective against aminophylline-induced

seizures in mice [7]. Moreover, novel antiepileptic drugs (e.g. lamotrigine, vigabatrine) did not protect mice against aminophylline seizures and some of them could even shorten the latency period to onset of convulsions [13].

Aminophylline has been shown to produce decreased cerebral blood flow, respiratory alkalosis, systemic hypotension, and cardiac tachyarrhythmia. Such systemic effects undoubtedly are not the only factors playing an important role in mice mortality following aminophylline administration. In fact, it might be due to the combined effects of direct action of methylxanthine on the central nervous system (the earlier onset of seizures or mortality in the control groups as trigger mechanism) and the accompanying metabolic and cardiovascular abnormalities (occurring after some hours) [3]. Interestingly, a combination of some conventional antiepileptics and a  $\beta$ -blocker produced a significant protection against aminophylline-induced mortality [4, 14].

In conclusion, our study indicates that in aminophylline intoxication, the basic problem is not to stop seizure activity, but to overcome long-term mortality. Common antiepileptics and LY 300164 diminished seizures, however, they were completely ineffective against aminophylline-induced mortality. Therefore, further studies are needed to fully elucidate the mechanisms of aminophylline epileptogenicity and to establish an algorithm for clinical treatment of the patients suffering from aminophylline intoxication.

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