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INFLUENCE OF SEVERAL CONVULSANTS ON THE PROTECTIVE ACTIVITY OF A NON-COMPETITIVE AMPA/KAINATE ANTAGONIST, LY 300164, AND LAMOTRIGINE AGAINST MAXIMAL ELECTROSHOCK IN MICE

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Aminophylline (50-100 mg/kg) and strychnine (0.125-0.5 mg/kg) significantly raised the ED_{50} values of LY 300164 against maximal electroshock in mice, from 4 to 8 mg/kg (aminophylline 100 mg/kg) and from 3.6 to 11.5 mg/kg (strychnine 0.5 mg/kg). Also, aminophylline (25-50 mg/kg) and strychnine (0.125-0.25 mg/kg) increased the ED_{50} value of lamotrigine in this test, for instance from 5.5 to 8.0 mg/kg (aminophylline 50 mg/kg) and from 5.2 to 8.9 mg/kg (strychnine 0.25 mg/kg). Moreover, the ED_{50}s values of aminophylline and strychnine for the reduction of the anticonvulsant effect of LY 300164 (7 mg/kg, the dose equal to its ED_{97} value against maximal electroshock) were 79.9 and 0.2 mg/kg, respectively. The respective ED_{50} values for the inhibition of the antiseizure action of lamotrigine were 40.9 and 0.2 mg/kg. Neither bicuculline nor picrotoxin affected the protective action of LY 300164 or lamotrigine. Strychnine significantly lowered the plasma concentrations of LY 300164 and this my point to a pharmacokinetic mechanism of the observed interaction. Aminophylline did not affect the plasma concentrations of the studied anticonvulsant drugs and strychnine - that of lamotrigine, so a pharmacokinetic interaction does not seem probable. The present results indicate that the potential of aminophylline and strychnine to attenuate the anticonvulsant activity of conventional antiepileptics is extended to LY 300164 and lamotrigine.

Key words: *LY 300164, lamotrigine, aminophylline, strychnine, bicuculline, picrotoxin, electroconvulsions.*

INTRODUCTION

There is increasing evidence that an abnormality of glutamate-mediated neurotransmission may contribute to the epileptic phenomena in various animal and human syndromes (1). One of major possibilities for inhibiting convulsions is blockade of the excitation mediated by excitatory amino acids (glutamate and aspartate) whose important role in the basic mechanisms of epilepsy has been documented (2, 3). The AMPA/kainate receptor antagonists seem to be more advantageous than N-methyl-D-aspartate (NMDA) receptor antagonists when at least considering less expressed or even absent neurotoxic undesired effects (4). On the other hand, deficient inhibitory neurotransmission mediated by gamma-aminobutyric acid (GABA) is suspected to be involved in the pathogenesis of epileptic disorders (5).

It was previously reported that LY 300164 (7-acetyl-5-(-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxolo(4,5H)-2,3-benzodiazepine, a selective non-competitive antagonist of AMPA/kainate receptors), potentiated the anticonvulsant activity of valproate, carbamazepine, diphenylhydantoin and phenobarbital (6). This non-NMDA antagonist also enhanced the protective action of valproate and ethosuximide in pentylenetetrazol convulsions, and increased the antiseizure potency of valproate, diazepam, clonazepam, but not diphenylhydantoin, carbamazepine, phenobarbital or ethosuximide in kindled rats (7). Recently, LY 300164 was introduced to epilepsy treatment as a drug termed Talampanel (8). On the other hand, lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)1,2,4-triazine] is a novel antiepileptic drug, blocking Na+ channels, with a concomitant inhibition of glutamate and aspartate release. The spectrum of action of lamotrigine appears broader than that of either phenytoin or carbamazepine, extending also on absence epilepsy. Results indicate that lamotrigine is also superior to phenytoin and carbamazepine as regards adverse effects (9). However, lamotrigine may affect glutamate release at supratherapeutical concentrations (10).

Aminophylline, in doses far below its convulsive potential, was shown to block or reduce the anticonvulsive potency of a number of antiepileptic drugs both in pentylenetetrazol-, electroshock-induced and kindled convulsions (11-16). Bicuculline is a well characterized $GABA_A$ receptor antagonist able to evoke generalized clonic-tonic convulsions after systemic administration (17). Surprisingly, bicuculline was not able to affect the protective potential of conventional antiepileptics against electroconvulsions (15). Strychnine is a glycine antagonist, valuable to study seizure mechanisms. It is noteworthy that strychnine-induced convulsions are insensitive to standard antiepileptic therapy, indicating that this model of epilepsy may be used to study drug resistance problem (18). Moreover, strychnine was documented to strongly reduce the protection offered by diphenylhydantoin, phenobarbital and valproate against maximal electroshock-induced seizures in mice (19). Consequently, we decided

to find out whether aminophylline or strychnine tend to influence the anticonvulsant activity of LY 300164 and lamotrigine against maximal electroshock in mice. If these anticonvulsant drugs proved resistant to aminophylline and strychnine then one could assume that they possess additional anticonvulsive mechanisms, not necessarily shared by conventional antiepileptic drugs. We also included two agents inhibiting GABA-ergic transmission, bicuculline and picrotoxin, in order to characterize a possible involvement of GABA-mediated events in the anticonvulsant activity of LY 300164 and lamotrigine.

MATERIALS AND METHODS

General

The experiments were carried out on male Swiss mice weighing 20-25 g purchased from a licensed dealer (T. Górzkowska, Warsaw, Poland). The animals were housed in colony cages with free access to food (chow pellets) and tap water. The experimental temperature was 21 ± 1°C and animals were on a natural light-dark cycle. The experimental groups, consisting of 8-12 mice were chosen by means of a randomized schedule. The use of animals was carried out according to Helsinki declaration.

Drugs

Aminophylline (Polfa, Kraków, Poland), strychnine nitrate, bicuculline, picrotoxin (all three drugs from Sigma, St. Louis, MO, USA), LY 300164 (7-acetyl-5-(4-aminophenyl)8,9-dihydro-8-methyl-7H-1,3-dioxolo-4,5-H-2,3-benzodiazepine hydrochloride; kindly supplied by Eli-Lilly, Indianapolis, IN, USA) and lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine; Glaxo Wellcome] were used in this study. Aminophylline, strychnine and LY 300164 were brought into solution with sterile saline. Bicuculline and picrotoxin were dissolved in a minimal quantity of glacial acetic acid and subsequently made up with saline to the appropriate volume. The final pH was adjusted to 5.0 with 0.2 N NaOH. Lamotrigine was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA). All convulsants were given in a volume of 5 ml/kg, and anticonvulsants in a volume of 10 ml/kg. Convulsant agents were injected up to their highest non-convulsive doses, assessed experimentally in the electroconvulsive threshold test - aminophylline 100 mg/kg and strychnine 0.5 mg/kg. Aminophylline, bicuculline, picrotoxin were injected 30 min and strychnine 10 min before the convulsive test. LY 300164 and lamotrigine were injected 15 and 60 min before electroconvulsions, respectively.

Electroconvulsions

Electroconvulsions were produced with the use of ear-clip electrodes and alternating current (50 Hz) delivered by a Hugo Sachs (Rodent Shocker, Type 221, Freiburg, Germany) generator. The stimulus duration was 0.2 s. Tonic hindlimb extension was taken as the endpoint. The electroconvulsive threshold was evaluated as CS_{50}, which is the current strength (in mA) necessary to produce tonic hindlimb extension in 50% of the animals tested. To estimate the electroconvulsive threshold, at least four groups of mice (8-10 mice per group) were challenged with electroshocks of various intensities. Subsequently, an intensity-response curve was

calculated on the basis of percentage of mice convulsing in experimental groups. The protective activity of LY 300164 and lamotrigine was quantitatively expressed as ED_{50} (with 95% confidence limits) or ED_{97} values and reflected doses of an antiepileptic agent (in mg/kg) predicted to abolish tonic hindlimb extension in 50 % or 97% of mice challenged with the fixed electric stimulus (25 mA; four- to five-fold higher than the CS_{50} value in untreated animals). This electric shock would produce tonic hindlimb extension in 100% of untreated mice. At least four groups of mice, (8-10 animals per group), were used to estimate each ED_{50}/ED_{97} value. When aminophylline or strychnine were given to animals pretreated with an anticonvulsant drug at its ED_{97} then percentages of mice with seizures were taken for the calculation of ED_{50}s for these convulsants.

Determination of LY 300164 and lamotrigine plasma levels

Blood samples of approximately 1 ml (obtained from mice after decapitation) were collected into Eppendorf tubes. The plasma levels of LY 300164 were measured by high-pressure liquid chromatography (HPLC). The chromatograph (from Laboratorij Pristroje, Praha, Czech Republic) was equipped with a 305 micropump (LCP 3001) and an ultraviolet (UV) detector (HP 1050) with a sensitivity setting of 0.1 AUFS (absorbance units full scale) and a time constant of 0.1 s. The Rheodyne 7125 injector valve with a 100 µl sample loop was used for sample injection. For HPLC, a stainless-steel HP ODS column (200 x 4.6 mm) was used at an ambient temperature of 22°C. The mobile phase was methanol: acetonitrile: 0.05 M C_2H_5COOH buffer; 20:20:60 vol/vol (BAKER HPLC grade). The mobile phase flow rate was 1 ml/min. At the times of electroconvulsive seizures, animals were killed and plasma samples of 200 µl were added to 200 µl of water, 100 µl of methanol:water solution; 1:1, and 50 µl of MeOH:water solution; 1:1 buffer. The solutions were evaporated to dryness under a vacuum system and redissolved in 1 ml of tertbuthyl-methyl ether (HPLC, Aldrich), and again evaporated to dryness under a vacuum system. The remains were redissolved in 100 µl mobile phase; samples of 20 µl were then injected into the chromatograph. LY 300164 concentrations were calculated according to the external standard method using the original Gilson 715 software. The amount of LY 300164 (expressed in micrograms per milliliter of blood) was determined by comparing their peak area with the peak area of the external standards. Stock solutions of LY 300164 (µg/ml) serving as external standards (0.2: 0.6: 1.2: 2.4: 4.8 µg), were prepared in mobile phase. They were placed at the beginning and end of each measurement sequence.

The plasma levels of lamotrigine were evaluated in a similar way. The main difference was that the mobile phase consisted with methanol: acetonitrile: 20 mM citric acid/ 40 mM sodium citrate buffer; 330: 90: 580 vol/vol. Moreover, the evaporated solutions were redissolved in 4 ml of tertbuthyl-methyl ether, and samples of 50 µl were injected into the chromatograph.

Statistics

Evaluation of the CD_{97}, and CS_{50}, with 95% confidence limits was performed according to the method of Litchfield and Wilcoxon (20), modified in that a dose-effect curve was constructed on a computer. The results concerning the influence of either aminophylline or strychnine, administered in a wide dose range, on the anticonvulsive effects of LY 300164 or lamotrigine, were shown as number of animals convulsing/total number of mice per an experimental group. Fisher's exact probability test was used to compare these data. Plasma levels of LY 300164 and lamotrigine were statistically verified by Student's t-test for unpaired data.

RESULTS

Estimation of the ED_{97} values for LY 300164 and lamotrigine

Both anticonvulsants were injected in a wide range of doses to provide a different degree of protection against maximal electroshock-induced tonic hindlimb extension. The calculated ED_{97} values, according to Litchfield and Wilcoxon (20), for LY 300164 and lamotrigine were 7 and 7.4-7.6 mg/kg, respectively.

Influence of convulsants upon the electroconvulsive threshold

None of the tested convulsants affected the threshold for electroconvulsions which was 6.9 (6.2-7.8) mA. Specifically, the threshold after aminophylline (100 mg/kg) was 7.0 (6.4-8.2) mA, after strychnine (0.5 mg/kg) 7.2 (6.4-8.4) mA, after bicuculline (2 mg/kg) 6.8 (6.2-7.7) mA and after picrotoxin (3 mg/kg) 7.4 (6.5-8.6) mA.

Influence of aminophylline or strychnine on the anticonvulsant activity of LY 300164 or lamotrigine against maximal electroshock in mice

Aminophylline (50-100 mg/kg) and strychnine (0.125-0.5 mg/kg) decreased the protective activity of LY 300164 and lamotrigine, significantly increasing their ED_{50} values. For instance, aminophylline at 50 mg/kg elevated the ED_{50}s of LY 300164 and lamotrigine from 4 (3.2-5) to 6.5 (6.1-7.0) mg/kg and from 5.5 (4.9-6.1) to 8.0 (7.0-9.3) mg/kg, respectively. Strychnine (0.25 mg/kg) increased the ED_{50}s of LY 300164 and lamotrigine from 3.6 (2.8-4.7) to 8.1 (7.1-9.4) mg/kg and from 5.2 (4.3-6.3) to 8.9 (7.0-11.3) mg/kg, respectively (*Table 1*).

Also the protective activity of both anticonvulsants administered at their ED_{97}s against maximal electroshock, was impaired by aminophylline and strychnine (*Tables 2,3,4*).

Influence of bicuculline and picrotoxin upon the protective action of LY 300164 and lamotrigine

Neither bicuculline (2 mg/kg) nor picrotoxin (3 mg/kg) affected the protective action of LY 300164 and lamotrigine (*Table 1*).

Effect of strychnine and aminophylline upon the plasma levels of LY 300164 and lamotrigine

Strychnine (0.2 mg/kg) significantly decreased the plasma concentration of LY 300164 (7 mg/kg) but not that of lamotrigine (7.6 mg/kg) as measured by high-pressure liquid chromatography. In contrast, aminophylline (79.9 mg/kg) did not affect the plasma concentration of LY 300164 (7 mg/kg), and

Table 1. Influence of aminophylline, strychnine, bicuculline or picrotoxin upon the protective action of LY 300164 and lamotrigine in mice

Treatment (mg/kg)	ED_{50} (mg/kg)
LY 300164 + saline	4.0 (3.2-5.0)
LY 300164 + AMI (100)	8.0 (6.4-9.9)[c]
LY 300164 + AMI (75)	6.8 (6.1-7.5)c
LY 300164 + AMI (50)	6.5 (6.1-7.0)[c]
LY 300164 + AMI (25)	4.9 (4.2-5.8)
LY 300164 + AMI (12.5)	4.5 (3.6-5.6)
LY 300164 + saline	3.6 (2.8-4.7)
LY 300164 + STR (0.5)	11.5 (10.2-13.1)[c]
LY 300164 + STR (0.25)	8.1 (7.1-9.4)[c]
LY 300164 + STR (0.125)	5.7 (4.7-6.9)[b]
LY 300164 + STR (0.0625)	4.7 (4.1-5.4)
LY 300164 + saline	3.9 (3.3-4.7)
LY 300164 + BIC (2)	4.7 (4.1-5.5)
LY 300164 + PIC (3)	4.2 (3.6-4.8)
Lamotrigine + saline	5.5 (4.9-6.1)
Lamotrigine + AMI (50)	8.0 (7.0-9.3)[c]
Lamotrigine + AMI (25)	7.9 (6.5-9.6)[b]
Lamotrigine + AMI (12.5)	6.5 (5.6-7.5)
Lamotrigine + saline	5.2 (4.3-6.3)
Lamotrigine + STR (0.25)	8.9 (7.0-11.3)[c]
Lamotrigine + STR (0.125)	7.2 (6.0-8.8)[a]
Lamotrigine + STR (0.06125)	5.7 (4.3-7.5)
Lamotrigine + saline	5.6 (4.7-6.6)
Lamotrigine + BIC (2)	6.9 (6.3-7.6)
Lamotrigine + BIC (1)	6.1 (5.4-6.9)
Lamotrigine + PIC (3)	6.5 (6.0-7.1)

Both anticonvulsant drugs were administered i.p., LY 300164 15 min, lamotrigine 60 min before the test. Aminophylline (AMI), bicuculline (BIC), picrotoxin (PIC) were injected 30 min and strychnine (STR) 10 min before the electroconvulsions. The data are ED_{50} values (in mg/kg) with 95% confidence limits in parentheses. ED50 values and statistical analysis of the data were calculated according to Litchfield and Wilcoxon (1949).
[a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ vs. vehicle.

aminophylline (40.9 mg/kg) did not influence the plasma level of lamotrigine (7.6 mg/kg; *Table 6*). Both chemoconvulsants were administered at doses equal to their ED_{50} values to inhibit the anticonvulsant activity of either LY 300164 or

Table 2. Influence of strychnine on the protective activity of LY 300164 and lamotrigine against maximal electroshock in mice

Treatment (mg/kg) Strychnine (mg/kg)	0	0.06126	0.125	0.25	0.5
LY 300164 (7)	0/12	NT	2/12	6/12[b]	12/12[c]
Lamotrigine (7.6)	0/12	2/8	4/8[a]	6/8[b]	NT

Data represent number of animals convulsing/total number of animals per an experimental group. NT not tested. Anticonvulsants were administered i.p. at their CD_{97}s against maximal electroshock. For more details see also the legend of *Table 1.* [a]P<0.05, [b]P<0.01, [c]P<0.001 vs. respective control groups.

Table 3. Influence of aminophylline on the protective activity of LY 300164 and lamotrigine against maximal electroshock in mice

Treatment (mg/kg) Aminophylline	0	25	50	75	100
LY 300164 (7)	0/12	NT	1/12	5/12[a]	9/12[c]
Lamotrigine (7.6)	0/8	2/8	4/8[a]	7/8[c]	NT

[a]P<0.05, [c]P<0.001 vs. respective control groups.
See also legends of *Tables 1* and *2*.

Table 4. ED50 values of aminophylline and strychnine for the reversal of the anticonvulsive action of LY 300164 and lamotrigine against maximal electroshock-induced seizures in mice

Treatment (mg/kg)	ED_{50} of Aminophylline	ED_{50} of strychnine
Lamotrigine (7.5)	40.9 (28.8-58.0)	0.2 (0.1-0.3)
LY 300164 (7)	79.9 (66.2-96.4)	0.2 (0.1-0.3)

Table data are ED50 (in mg/kg; with 95% confidence limits) for the reversal of the protective activity of lamotrigine and LY 300164 against maximal electroshock. The calculation of the ED_{50}s was based upon the method of Litchfield and Wilcoxon (1949), modified in that the dose-effect curve was calculated on a computer. See also legends of *Table 1.*

lamotrigine, and anticonvulsants at doses equal to their ED_{97} values against maximal electroshock in mice.

DISCUSSION

Our study has revealed that both aminophylline and strychnine reduced the anticonvulsant potential of LY 300164 and lamotrigine in the maximal electroshock test. Generally, a pharmacokinetic interaction does not seem probable (to the degree reflected by the plasma levels of the studied anticonvulsants) and only strychnine (0.2 mg/kg) elevated the plasma concentration of LY 300164.

Table 5. Influence of STR and AMI upon plasma levels of LY 300164 and lamotrigine in mice

Treatment (mg/kg)	Plasma levels (μg/ml)
LY 300164 (7) + saline	9.64 ± 1.74
LY 300164 (7) + STR (0.2)	7.44 ± 1.03[a]
LY 300164 (7) + saline	9.64 ± 1.74
LY 300164 (7) + AMI (79.9)	9.85 ± 2.34
Lamotrigine (7.6) + saline	2.33 ± 0.19
Lamotrigine (7.6) + STR (0.2)	2.45 ± 0.23
Lamotrigine (7.6) + saline	2.33 ± 0.19
Lamotrigine (7.6) + AMI (40.9)	2.43 ± 0.33

Presented values are means ± S.E. of at least eight determinations. Statistical analysis was performed by Student's t test.
[a]$P<0.01$ vs. the respective control group.
See also legend of *Table 1*.

According to previous reports, competitive and non-competitive NMDA antagonists inhibited both bicuculline- and picrotoxin-induced convulsions (21), suggesting that their convulsive effects may also involve NMDA $Ca2^{+}$ ion complex. Also conventional antiepileptics (diphenylhydantoin, carbamazepine, phenobarbital, diazepam) inhibited the convulsions produced by bicuculline and picrotoxin (21). Therefore, the anticonvulsant effects of these drugs may involve an activation of $GABA_A$ receptors. On the contrary, the present study revealed that neither picrotoxin nor bicuculline interfered with the protective action of LY 300164 and lamotrigine. It is worth mentioning that bicuculline (0.5 mg/kg) and picrotoxin (1-2 mg/kg) were able to attenuate GABA- and aminooxyacetic acid-induced increases in the electroconvulsive threshold in mice (22).

As it was previously reported, aminophylline and strychnine impaired the anticonvulsant action of conventional antiepileptics against maximal electroshock in mice (11-13, 19, 23). It should be stressed that any pharmacokinetic interactions, which could be responsible for these effects, were not determined (19, 24). Moreover, aminophylline (30 mg/kg) attenuated the protective activity of phenobarbital (20 mg/kg; 12), carbamazepine (30 mg/kg) and valproate (100 mg/kg; data not published) in kindled rats. This effect of aminophylline is suggested to result from the influence on central nervous system, because 8-(p-sulfophenyl)theophylline (a theophylline derivative unable to cross blood-brain barrier, did not affect the protective activity of antiepileptics (25). Nevertheless, the aminophylline (50 mg/kg)-induced impairment of the protective activity of conventional antiepileptic drugs against maximal electroshock is probably not associated with the blockade of adenosine A_1 receptors (26). In short, aminophylline at 5 mg/kg did not affect the protection

provided by antiepileptics in maximal electroshock (24) but was able to prevent adenosine receptor agonists from potentiation of the protective activity of conventional antiepileptic drugs (27).

The sensitivity of LY 300164 and lamotrigine to the convulsive action of strychnine, may indicate that strychnine-sensitive glycinergic transmission may play a significant role in the protective activity of these anticonvulsants against maximal electroshock. Although, the pharmacokinetic component is evident in case of LY 300164, the magnitude of this interaction points that also the pharmacodynamic component may be significant. As already mentioned, strychnine very potently reduced the anticonvulsant activity of major antiepileptic drugs and chlormethiazole against maximal electroshock-induced convulsions in mice and any pharmacokinetic interactions were excluded (15, 19). This may indicate that many antiepileptic drugs may share a specific mechanism of action reversible by strychnine-sensitive glycinergic receptors. An intriguing hypothesis on the existence of an endogenous strychnine-like neuromediator or modulator needs to be verified (28, 29).

In our previous study, we also reported that GYKI 52466 (another selective AMPA/kainate antagonist, structurally closely related to LY 300164) was completely resistant to the proconvulsive action of aminophylline (30). This clinically advantageous effect may indicate that any future antiepileptic drugs derived from AMPA/kainate antagonists might be recommended to epileptic patients being also on antiasthmatic therapy (30). On the other hand, the protective action of GYKI 52466 was significantly diminished by strychnine (30), similarly to our present results. The origin of discrepancy between GYKI 52466 and LY 300164 is difficult to explain at present. There are data, however, indicating subtle differences between these two AMPA/kainate antagonists. Actually, GYKI 52466 potentiated the protective action of valproate, carbamazepine, diphenylhydantoin, but not that of phenobarbital against maximal electroshock (31). Unlike GYKI 52466, LY 300164 also potentiated the anticonvulsant efficacy of phenobarbital (6).

In conclusion, the results of our study may indicate the necessity to search for new alternative medications to overcome the problem of asthma in epileptic patients. Among xanthine derivatives there are some agents, which do not antagonize the anticonvulsant activity of antiepileptic drugs. So far, enprofylline (3-propylxanthine) and 8-(p-sulfophenyl)-theophylline seem to be the appropriate examples (12, 13, 25). Also there are anticonvulsant drugs, completely resistant to aminophylline (GYKI 52466; 30) or showing very low propensity for interaction with methylxanthines (felbamate; 32). The potent interaction between strychnine and antiepileptic drugs and anticonvulsant agents needs to be also underlined. Whether cases of pharmacoresistance in epileptic patients may be associated with abnormally high concentrations of an endogenous strychnine-like modulator (29) remains an open question.

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