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Influence of LY 300164, an antagonist of AMPA/kainate receptors, on the anticonvulsant activity of clonazepam

Kinga K. Borowicz [a], Jarogniew Łuszczki [a], Marcin Szadkowski [a], Zdzisław Kleinrok [a], Stanisław J. Czuczwar [a,b,*]

[a] *Department of Pharmacology and Toxicology, Lublin Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland*
[b] *Department of Clinical Toxicology, Institute of Agricultural Medicine, Jaczewskiego 2, 20-950 Lublin, Poland*

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Abstract

LY 300164 [7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7*H*-1,3-dioxolo(4,5*H*)-2,3-benzodiazepine], an antagonist of AMPA/kainate receptors, at 5 mg/kg exerted a significant anticonvulsant effect, as regards seizure and afterdischarge durations in amygdala-kindled seizures in rats. At lower doses, LY 300164 did not exert anticonvulsant activity. Clonazepam alone (0.003–0.1 mg/kg) significantly diminished seizure severity, seizure and afterdischarge durations. Coadministration of LY 300164 (2 mg/kg) with clonazepam (0.001 mg/kg) resulted in the significant anticonvulsant activity. Seizure severity score, seizure and afterdischarge durations were reduced from 5 to 4, from 32.6 s to 12.3 s, and 42.7 s to 23.2 s. LY 300164 (2 mg/kg), clonazepam (0.001–0.1 mg/kg) and the combination of clonazepam (0.001 mg/kg) with LY 300164 (2 mg/kg) did not affect long-term memory evaluated in the passive avoidance task in rats. LY 300164 (at the subprotective dose of 2 mg/kg) significantly potentiated the anticonvulsant action of clonazepam against maximal electroshock but not against pentylenetetrazol-induced convulsions in mice. The results indicate that blockade of glutamate-mediated events at AMPA/kainate receptors may differently affect the protection offered by clonazepam, which seems dependent upon the model of experimental seizures.

Keywords: Clonazepam; LY 300164; AMPA/kainate receptor; Amygdala-kindled seizure; Pentylenetetrazol; Electroshock maximal

1. Introduction

Amygdala kindling in rats is believed to represent an animal model of partial seizures with secondary generalization (Löscher and Schmidt, 1988; Löscher et al., 1986). The behavior in initial stages of a kindled seizure is similar to that reported in human cases of complex partial epilepsy while the higher stages resemble the generalization of a focal seizure (Goddard et al., 1986). On the other hand, maximal electroshock has been proposed as a model of generalized clonic–tonic seizures and pentylenetetrazol-induced convulsions — as a model of myoclonic seizures in humans (Löscher and Schmidt, 1988).

Among a variety of antiepileptic drugs, benzodiazepines (diazepam, clonazepam, clobazam) and phenobarbital were reported to be most effective in suppressing the evolution of kindling; the effect of valproate was somewhat less pronounced in this respect, while carbamazepine and diphenylhydantoin even enhanced the development of kindling (Schmutz et al., 1988). Several studies suggested that 1,4-benzodiazepines reduced generalized motor seizures in amygdala-kindled rats and their ED_{50} values were substantially lower than those determined in the maximal electroshock seizure test (Schmutz et al., 1988). The order of potency for forelimb clonus suppression was clonazepam > diazepam > clobazam (1,5-benzodiazepine). Clonazepam showed the most favorable ratio of potency for anticonvulsant vs. motor impairment activity when ataxia rating was considered (Tietz et al., 1989). Long-term clinical usefulness is limited by development of tolerance. Tolerance to the anticonvulsant effect developed most rapidly during clobazam treatment and most slowly during clonazepam treatment. Assay of the amount of drug in brain extracts showed that tolerance was functional, not metabolic (Rosenberg et al., 1989).

It is widely accepted that glutamate is involved in the initiation of seizures and their propagation. Unfortunately,

* Corresponding author. Department of Pharmacology and Toxicology, Lublin Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland. Tel.: +48-81-7478924; fax: +48-81-5328903; e-mail: czuczwar@galen.imw.lublin.pl

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competitive and non-competitive NMDA receptor antagonists in patients with complex partial epilepsy, induced severe neurotoxic effects in doses which were well-tolerated in healthy volunteers (Löscher, 1998). It is noteworthy that the kindling model of seizures was the only animal model which predicted the unfavorable clinical activity of competitive NMDA receptor antagonists, indicating that this model may be of particular importance in the search for new antiepileptic drugs (Löscher, 1998).

It was previously reported that the AMPA/kainate receptor antagonist, 1-(4-aminophenyl)-4-methoxy-7, 8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride (GYKI 52466), reduced seizure score and afterdischarge duration in kindled rats, but remained without effect upon the development of kindling (Dürmüller et al., 1994). Moreover, GYKI 52466 was active in pentylenetetrazol-evoked convulsions (Löscher and Hönack, 1994) and potentiated the protective action of valproate, carbamazepine, diphenylhydantoin, but not phenobarbital against maximal electroshock in mice (Borowicz et al., 1995). Also, the derivative of GYKI 52466, 7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7*H*-1,3-dioxolo-4, 5*H*-2, 3-benzodiazepine hydrochloride (LY 300164), enhanced the anticonvulsant potency of conventional antiepileptics against maximal electroshock and increased the protective efficacy of valproate and ethosuximide against pentylenetetrazol-induced convulsions (Czuczwar et al., 1998a,b).

Consequently, we decided to study the effects of the selective non-competitive antagonist of AMPA/kainate receptors, LY 300164, on the protection provided by clonazepam against maximal electroshock, pentylenetetrazol-evoked seizures in mice and in amygdala-kindled rats. The adverse effects were evaluated in the passive avoidance test (evaluating long-term memory).

2. Materials and methods

2.1. Animals and experimental conditions

Male Wistar rats (200–250 g) and male Swiss mice (20–25 g) were used throughout the experiments after at least 1 week of acclimatization. They were housed in plastic perspex cages under standard conditions (ambient temperature of 22 ± 1°C, natural light–dark cycle). Chow pellets and tap water were freely available. All experiments were done at the same period of time (between 0900 h and 1200 h) to minimize circadian influences on seizure susceptibility. The experimental groups consisted of eight rats or mice.

2.2. Surgery and kindling procedure

The rats were anesthetized with pentobarbital (50 mg/kg i.p.) and received stereotaxic implantation of one bipolar electrode in the right basolateral amygdala. The following stereotaxic coordinates for electrode implantation were used: AP −1.5, L −4.4, V −8.5, according to the brain atlas of Paxinos and Watson (1986). All coordinates were measured from bregma. Scull screws served as the indifferent reference electrode. The electrode assembly was attached to the scull by dental acrylic cement. After electrode implantation, the animals were treated with an antibiotic for 1 week to prevent infection.

After a post-operative period of 2 weeks, the stimulation of amygdala was initiated. Each stimulus consisted of a 1 s train of 50 Hz, 1 ms biphasic square-wave pulses, with pulse amplitude of 500 μA, and was delivered every 24 h, until at least 10 sequential fully kindled stage 5 was elicited. The afterdischarges from the amygdala were recorded prior to and after the stimulation. The seizure activity was assessed according to a modified system of Racine (1972): 0 = no seizure response, 1 = immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus, 2 = head nodding associated with more severe facial clonus, 3 = clonus of one forelimb, 3.5 = bilateral forelimb clonus without rearing, 4 = bilateral forelimb clonus with rearing, 4.5 = falling on a side (without rearing), loss of righting reflex accompanied by generalized clonic seizures, 5 = rearing and falling on the back accompanied by generalized clonic seizures. Seizure duration was the duration of limbic seizures (stages 1–2) and motor seizures (stages 3–5). Afterdischarges were defined as spikes with a frequency of at least 1 Hz and an amplitude at least twice greater than the pre-stimulation baseline present in the EEG recorded from the site of stimulation. Control readings were made 2 days before and 2 days after respective treatments.

2.3. Electroconvulsions

Electroconvulsions in mice were produced with the help of ear-clip electrodes and alternating current delivered by a Hugo Sachs (Type 221, Freiburg, Germany) generator. The stimulus duration was 0.2 s. Full tonic extension of both hind limbs was taken as the endpoint. The convulsive threshold was evaluated as CS_{50}, which is the current strength (in mA) required to produce tonic hindlimb extension in 50% of the animals tested. To calculate the convulsive threshold, at least three groups of mice (consisting of at least eight animals per group) were challenged with electroshocks of various intensities. An intensity–response curve was calculated with a computer, taking into consideration the percentage of animals convulsing in experimental groups. To calculate the ED_{50} value for clonazepam, the mice were challenged with maximal electroshock (25 mA). At least four groups (eight animals per group) were used to estimate the ED_{50} value.

2.4. Pentylenetetrazol-induced seizures

At least four groups of mice (eight mice per group) were injected intraperitoneally with various doses of clon-

azepam and subcutaneously with pentylenetetrazol in a dose of 85 mg/kg, which was its CD_{97} (convulsive dose 97% for the clonic phase). Following the injection, mice were placed separately in transparent Plexiglass cages (25 cm × 15 cm × 10 cm) and observed for 30 min for the occurrence of clonic and tonic seizures. Clonic seizure activity was defined as clonus of whole body lasting over 3 s, with an accompanying loss of righting reflex, whereas the tonic extension of the hind limbs was considered as the endpoint for tonic seizures. To estimate the ED_{50} value for clonazepam, an intensity–response curve was calculated on the basis of the percentage of mice with clonic convulsions.

2.5. Drugs

Clonazepam (Polfa, Warsaw, Poland) was suspended in a 1% solution of Tween-80 (Sigma, St. Louis, MO, USA). LY 300164, kindly supplied by Eli-Lilly, Indianapolis, IN, USA) and pentylenetetrazol (Sigma, St. Louis, MO, USA) were dissolved in sterile saline. Clonazepam and LY 300164 were administered i.p., in a volume of 3 ml/kg (in rats) and 10 ml/kg (in mice), clonazepam being injected 30 min, while LY 300164 — 15 min before the tests.

2.6. Passive avoidance task

The rats were placed in an illuminated box (40 cm × 40 cm × 30 cm) connected to a dark box (40 cm × 40 cm × 30 cm), which was equipped with an electric grid floor. The respective dimensions of the boxes for mice were 10 cm × 13 cm × 15 cm and 25 cm × 20 cm × 15 cm. Entrance to the dark box was punished by an electric footshock of 2 s duration (0.7 mA for rats and 0.6 mA for mice). The animals that did not enter the dark compartment were excluded from the experiment. On the next day (24 h later), the same animals were put into the illuminated box and observed up to 180 s. The time period an animal entered the dark box was subsequently noted and the medians with 25 and 75 percentiles were calculated. According to Venault et al. (1986), the step-through passive avoidance task is recognized as a measure of long-term memory.

2.7. Evaluation of ataxia

Ataxia was evaluated according to the six-point scoring system (according to Löscher and Hönack, 1991): (1) slight ataxia (tottering of hind limbs), (2) more pronounced ataxia (dragging of hind limbs), (3) further increase of ataxia (more pronounced dragging of hind limbs), (4) marked ataxia (animals only occasionally lose balance during forward locomotion), and (6) animals despite attempts, are not able to move forward.

2.8. Estimation of the free plasma levels of antiepileptic drugs

The animals were given clonazepam + saline or clonazepam + LY 300164 (2 mg/kg). The mice were decapitated at times scheduled for the convulsive test and blood samples of approximately 1 ml were collected into Eppendorf tubes. Samples of blood were centrifuged at 10 000 rpm (Abbott centrifuge, Irving, TX, USA) for 3 min and plasma samples of 200 μl were pipetted into a micropartition system, MPS-1 (Amicon, Danvers, MA, USA), for separation of free from protein-bound microsolutes. Then, the MPS-1 tubes were centrifuged at 3000 rpm (MPW-360 centrifuge; Mechanika Precyzyjna, Warsaw, Poland) for 10 min and 50 μl filtrate samples were pipetted into original Abbott system cartridges which were subsequently put into a carousel for up to 20 samples. Control plasma samples of an antiepileptic were placed at the beginning and end of each carousel for verification of the correctness of calibra-

Table 1
Effects of LY 300164 and clonazepam alone upon fully kindled seizures in rats
Table data represent means ± S.E. of eight rats per group. Control readings were made 2 days before and after the respective treatments. CLO was administered 30 min, LY 300164 15 min prior to the test. CLO, clonazepam; SSv, seizure severity; SD, seizure duration; AD, afterdischarge duration.

Treatment (mg/kg)	SSv		SD (s)		AD (s)	
	Control	Treated	Control	Treated	Control	Treated
LY 300164 (1)	5	5	35.7 ± 1.6	33.7 ± 2.3	54.7 ± 2.3	55.2 ± 4.4
LY 300164 (2)	5	5	33.4 ± 2.0	31.8 ± 1.3	55.6 ± 5.5	43.4 ± 3.1
LY 300164 (3.5)	5	4.7 ± 0.2	35.7 ± 2.1	35.9 ± 2.4	51.5 ± 4.3	50.7 ± 4.3
LY 300164 (5)	5	4.6 ± 0.1	34.2 ± 2.3	23.2 ± 2.1[b]	52.0 ± 3.4	35.8 ± 3.9[b]
CLO (0.003)	5	4.5 ± 0.2	31.2 ± 1.1	27.7 ± 0.7[a]	44.7 ± 1.3	40.9 ± 1.7[a]
CLO (0.005)	5	4.4 ± 0.2[a]	33.9 ± 0.4	28.6 ± 0.7[c]	46.6 ± 0.9	41.5 ± 0.9[c]
CLO (0.01)	5	3.8 ± 0.1[c]	33.7 ± 0.5	24.5 ± 0.5[c]	48.9 ± 0.6	35.4 ± 0.6[c]
CLO (0.05)	5	2.8 ± 0.2[c]	34.1 ± 0.5	14.5 ± 0.5[c]	47.4 ± 0.9	26.0 ± 0.6[c]
CLO (0.1)	5	1.7 ± 0.2[c]	32.2 ± 0.1	10.2 ± 1.0[c]	45.4 ± 1.0	15.2 ± 1.1[c]

[a] $P < 0.05$ vs. respective controls (Wilcoxon signed rank test).
[b] $P < 0.01$ vs. respective controls (Wilcoxon signed rank test).
[c] $P < 0.001$ vs. respective controls (Wilcoxon signed rank test).

tion. The free plasma levels of clonazepam were estimated by immunofluorescence, with an Abbott TDx analyzer (Abbott, Irving, TX, USA). Plasma levels were expressed in ng/ml as means ± S.D. of eight determinations.

2.9. Statistics

The statistical significances of differences between seizure scores, seizure and afterdischarge durations were calculated by the Wilcoxon signed rank test. Mann–Whitney *U*-test was used to analyze the results obtained in the passive avoidance task. ED_{50} values and statistical analysis of the results obtained in the electroconvulsive and pentylenetetrazol tests were calculated by computer probit analysis, according to Litchfield and Wilcoxon (1949). Plasma levels of clonazepam alone or in combination with LY 300164 were evaluated with Student's *t*-test.

3. Results

3.1. Effects of clonazepam and LY 300164 on amygdala-kindled seizures in rats

Clonazepam (0.001 mg/kg) did not influence kindling parameters, but when administered at higher doses (0.003–0.1 mg/kg), it significantly reduced the seizure severity and shortened seizure and afterdischarge durations (Table 1). LY 300164 (1 mg/kg, 2 mg/kg and 3.5 mg/kg) was devoid of any significant action upon each kindling parameter studied. LY 300164 administered at the dose of 5 mg/kg exerted a protective effect, considerably reducing both the seizure and afterdischarge durations (Table 1).

3.2. Effect of LY 300164 on the protective activity of clonazepam in kindled rats

Co-administration of LY 300164 at the subprotective dose of 2 mg/kg with clonazepam (0.0005–0.001 mg/kg), resulted in the clear-cut anticonvulsant activity, comparable to that exerted by clonazepam (0.1 mg/kg) alone. It is noteworthy that a combination of clonazepam (0.0005 mg/kg) with LY 300164 (2 mg/kg) still significantly inhibited amygdala-kindled seizures. When the dose of clonazepam was reduced to 0.0001 mg/kg, the combined treatment with LY 300164 (2 mg/kg) was no longer effective (Table 2).

Table 3
Influence of LY 300164 on the anticonvulsant activity of clonazepam against maximal electroshock and pentylenetetrazol-induced seizures
The data are 50% effective doses (ED_{50} values; 95% confidence limits) for the protection against maximal electroshock (MES) or pentylenetetrazol(PTZ)-induced seizures. ND, not determined. For more details see also legend of Table 1.

Treatment (mg/kg)	ED_{50} (MES)	ED_{50} (PTZ)
Clonazepam + saline	14.4 (10.8–19.3)	0.028 (0.011–0.068)
Clonazepam + LY 300164 (0.25)	11.6 (9.1–14.8)	ND
Clonazepam + LY 300164 (0.5)	7.0 (5.5–8.9)[a]	ND
Clonazepam + LY 300164 (1)	3.8 (2.9–4.8)[a]	ND
Clonazepam + LY 300164 (2)	1.8 (1.1–3.0)[a]	0.026 (0.013–0.052)

[a] $P < 0.001$ vs. respective control group.

3.3. Effects of LY 300164 on the protective activity of clonazepam against maximal electroshock- and pentylenetetrazol-induced seizures

LY 300164 (2 mg/kg) did not influence the threshold for electroconvulsions and pentylenetetrazol-induced seizures in mice (results not shown in Tables), which is in agreement with previously reported data (Czuczwar et al., 1998a,b). The AMPA/kainate receptor antagonist (2 mg/kg) did not affect the ED_{50} of clonazepam in pentylenetetrazol-induced seizures. However, LY 300164 (0.5 mg/kg, 1 mg/kg, and 2 mg/kg) significantly reduced the ED_{50} of the benzodiazepine against maximal electroshock from 14.4 mg/kg to 7 mg/kg, 3.8 mg/kg, and 1.8 mg/kg, respectively (Table 3).

3.4. Passive-avoidance task

The saline-treated animals did not enter the dark box within the observation time limit. LY 300164 (2 mg/kg) did not produce any significant impairment of long-term memory in rats or mice. Clonazepam alone, administered at its ED_{50} value against maximal electroshock of 14.4 mg/kg, significantly reduced retention in mice. However, clonazepam (1.8 mg/kg) alone or in combination with LY 300164 (2 mg/kg) did not disturb long-term memory in mice. Also, clonazepam (0.1 mg/kg) alone or in combina-

Table 2
Influence of the combined treatment of clonazepam with LY 300164 upon fully kindled seizures in rats
For details see legend of Table 1.

Treatment (mg/kg)	SSv		SD (s)		AD (s)	
	Control	Treated	Control	Treated	Control	Treated
CLO (0.001)	5	4.6 ± 0.2	33.6 ± 0.5	32.7 ± 0.6	46.5 ± 0.7	45.5 ± 0.7
CLO (0.0001) + LY 300164 (2)	5	4.9 ± 0.2	34.3 ± 0.9	33.6 ± 1.0	45.2 ± 0.9	44.0 ± 0.9
CLO (0.0005) + LY 300164 (2)	5	4.4 ± 0.2[a]	32.3 ± 0.9	22.2 ± 1.2[b]	43.3 ± 0.9	34.3 ± 1.2[b]
CLO (0.001) + LY 300164 (2)	5	4.0 ± 0.3[b]	32.7 ± 0.8	12.3 ± 0.7[c]	42.7 ± 0.8	23.2 ± 1.4[c]

Table 4
Influence of LY 300164 and clonazepam on long-term memory in mice and kindled rats
Table data are medians (with 25 and 75 percentiles in parentheses) of eight determinations. Control animals avoided the dark compartment for at least 180 s. m — experiments on mice; r — experiments on kindled rats. Mann–Whitney *U*-test was used for statistical analysis of the data.

Treatment (mg/kg)	Retention time (s)
Vehicle-r	180 (180, 180)
LY 300164 (2)-r	180 (135, 180)
CLO (0.1)-r	180 (124, 180)
CLO (0.001)-r	180 (180, 180)
CLO (0.001) + LY 300164 (2)-r	180 (119, 180)
Vehicle-m	180 (180, 180)
LY 300164 (2)-m	180 (159, 180)
CLO (14.4)-m	47.5 (35, 177.5)[a]
CLO (1.8)-m	142.5 (55, 180)
CLO (1.8) + LY 300164 (2)-m	143.5 (59.5, 180)

[a] $P < 0.01$ vs. respective control group.

tion (0.001 mg/kg) with LY 300164 (2 mg/kg) did not produce memory deficits in rats (Table 4).

3.5. Behavioral effects of LY 300164 and clonazepam in mice or kindled rats

LY 300164 (2 mg/kg) did not produce visible ataxia either in rats or mice. Similarly, no ataxia was observed when rats were injected with clonazepam (0.001–0.1 mg/kg) or the combination of clonazepam (0.001 mg/kg) with LY 300164 (2 mg/kg). On the contrary, mice injected with clonazepam (14.4 mg/kg) showed marked ataxia (around score 4), while clonazepam (1.8 mg/kg) alone or in combination with LY 300164 (2 mg/kg) produced slight ataxia (around score 1; results not shown in Tables).

3.6. Effect of LY 300164 on the free plasma levels of clonazepam

Free plasma level of clonazepam (1.8 mg/kg) was 93.3 ± 10.4 ng/ml in mice. When clonazepam (1.8 mg/kg) was combined with LY 300164 (2 mg/kg), the free plasma level of the benzodiazepine reached 97.3 ± 10.0 ng/ml and did not differ significantly from the control level. The immunofluorescent method was not sensitive enough to measure the plasma level of clonazepam (0.001 mg/kg) in rats.

4. Discussion

Our results demonstrate that LY 300164, at the subprotective dose of 2 mg/kg, potentiated the anticonvulsant action of clonazepam against maximal electroshock, but not against pentylenetetrazol-induced seizures in mice. Any interaction at the pharmacokinetic level can be excluded, because LY 300164 did not interfere with the free plasma level of clonazepam. Also, co-administration of extremely low doses of clonazepam with the subprotective dose of LY 300164 (2 mg/kg) resulted in the inhibition of amygdala-kindled seizures in rats. Although, the possibility of a pharmacokinetic interaction has to be considered, it is unlikely that LY 300164 could produce such a dramatic increase in the plasma level of clonazepam (0.001 mg/kg) that could account for the comparable seizure protection of the benzodiazepine produced at the dose of 0.1 mg/kg, at least in terms of seizure duration. It is worth recalling that clonazepam alone (0.1 mg/kg) reduced seizure severity, seizure and afterdischarge durations from 5 to 1.7, 32.2 s to 10.2 s and from 45.4 s to 15.2 s, respectively. While the combination of LY 300164 (2 mg/kg) with clonazepam (0.001 mg/kg) reduced all three kindling parameters from 5 to 4, 32.7 s to 12.3 s and from 42.8 s to 23.2 s. On the other hand, the combined treatment of clonazepam (0.001 mg/kg) with GYKI 52466 (a structurally closely related agent to LY 300164), proved slightly less effective in kindled rats, since the respective parameters were changed from 5 to 4.4, 31.6 s to 23.5 s and from 43.9 s to 36.1 s (unpublished data).

Clonazepam had the most advantageous profile in kindled seizures (the lowest effective dose was 0.003 mg/kg), while the ED_{50} value for this drug in maximal electroshock was 14.4 mg/kg. However, the anticonvulsant action of clonazepam was markedly potentiated by LY 300164 in both models of seizures, which reduced its ED_{50} in maximal electroshock to 1.8 mg/kg and diminished its lowest effective dose in kindling to 0.0005 mg/kg. It is noteworthy that clonazepam in combinations with LY 300164 was devoid of adverse effects as regards long-term memory. The benzodiazepine, however, at its ED_{50} of 14.4 mg/kg caused the significant deficits of memory in mice.

The mechanism of action of clonazepam is closely related to its agonism at the specific benzodiazepine recognition site within the γ-amino-butyric$_A$ ($GABA_A$) receptor complex (Olsen and Trobin, 1990). On the other hand, these authors suggest that at least a part of the action of another benzodiazepine, diazepam, upon tonic electroconvulsions is independent of central benzodiazepine receptors (Löscher and Hönack, 1994). In addition to the effect of benzodiazepines on the $GABA_A$ receptors, they also reduce repetitive firing of action potentials in neurones, possibly by direct influence on calcium and sodium conductances, which can be involved in the protection against generalized tonic–clonic seizures and status epilepticus in humans (DeLorenzo, 1988). Interestingly, the blockade of glutamate-mediated events via the AMPA/kainate receptors, led to the potentiation of the anticonvulsant activity of clonazepam in two out of three studied models of seizures. However, a question arises whether clonazepam, co-administered in extremely low doses with LY 300164 in rats also would produce tolerance upon chronic administration, as regards anticonvulsant effects? The lack of tolerance would be a considerable benefit of the combination therapy.

Summing up, the advantageous interaction between clonazepam and LY 300164 in two animal models of seizures might be the result of different receptor processes, e.g., synergistic action of these two substances on two different subunits of $GABA_A$ receptor, or concomitant action on AMPA/kainate, $GABA_A$ receptors and sodium or/and calcium channels. Seizure susceptible limbic areas such as amygdala and hippocampus, which are sensitive to the anticonvulsant activity of the benzodiazepines, have correspondingly high densities of benzodiazepine receptors (Tietz et al., 1984). It was also reported that the number of ^{3}H-diazepam binding sites (B_{max}) in hippocampus significantly increased in fully kindled rats (Tietz et al., 1984). Anyway, the beneficial interaction between clonazepam and LY 300164 in mice and kindled rats seems to be profitable from the clinical point of view and may create a new approach in the treatment of drug-resistant complex partial seizures or status epilepticus in humans. So far, available data have indicated that AMPA/kainate receptor antagonists enhance the protection offered by conventional antiepileptics, including diazepam, against maximal electroshock in mice (Żarnowski et al., 1993; Borowicz et al., 1995; Czuczwar et al., 1998a). In contrast, the simultaneous administration of a benzodiazepine and AMPA/kainate receptor antagonist seems of no benefit against myoclonic seizures, although the combinations of other antiepileptic drugs with LY 300164 may be of importance (Czuczwar et al., 1998b).

Although a relatively great body of evidence points to the potentiation by *N*-methyl-D-aspartate (NMDA) receptor antagonists of the protective activity of conventional antiepileptic drugs against maximal electroshock-induced seizures in mice (for review see Czuczwar et al., 1996a,b), this problem has not been yet evaluated in the amygdala-kindled rats.

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