

INTERACTION OF GYKI 52466, A SELECTIVE NON-COMPETITIVE ANTAGONIST OF AMPA/KAINATE RECEPTORS, WITH CONVENTIONAL ANTIEPILEPTIC DRUGS IN AMYGDALA-KINDLED SEIZURES IN RATS

*Kinga K. Borowicz*¹, *Andrzej M. Duda*², *Zdzisław Kleinrok*³,
Stanisław J. Czuczwar^{1,4,#}

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

²Janssen-Cilag, Div. Johnson & Johnson Poland, Szyszkowa 20, PL 02-285 Warszawa, Poland,

³Department of Pharmacology and Toxicology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland,

⁴Isotope Laboratory, Institute of Agriculture Medicine, Jaczewskiego 2, PL 20-090 Lublin, Poland

Interaction of GYKI 52466, a selective non-competitive antagonist of AMPA/kainate receptors, with conventional antiepileptic drugs in amygdala-kindled seizures in rats. K.K. BOROWICZ, A.M. DUDA, Z. KLEINROK, S.J. CZUCZWAR. Pol. J. Pharmacol., 2001, 53, 101–108.

GYKI 52466 [1,4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine], a non-competitive AMPA/kainate receptor antagonist, administered *ip* at the dose of 5 mg/kg, exerted a significant anticonvulsant effect, as it decreased seizure and afterdischarge durations, being ineffective at 2 mg/kg. Subsequently, GYKI 52466 (2 mg/kg) was combined with antiepileptic drugs at doses ineffective in fully kindled rats. Co-administration of GYKI 52466 with clonazepam (0.003 mg/kg *ip*) resulted in a significant reduction of seizure severity (by 20%), seizure duration (by 31%) and afterdischarge duration (by 24%). Co-injection of GYKI 52466 with valproate (75 mg/kg *ip*) also resulted in the respective 8%, 16%, and 17% reductions of the three studied seizure parameters. No protection was observed when GYKI 52466 was co-administered with carbamazepine (20 mg/kg *ip*), phenobarbital (20 mg/kg *ip*), or diphenylhydantoin (40 mg/kg *ip*). Combinations of GYKI 52466 with antiepileptic drugs did not cause any significant motor (rotarod test) or long-term memory deficits (passive avoidance task). Only GYKI 52466 administered alone at 5 mg/kg, caused a significant impairment of retention in amygdala-kindled rats. The interaction at a pharmacokinetic level, at least in case of the combination of GYKI 52466 with valproate, can be excluded because GYKI 52466 did not interfere with the free plasma level of valproate. These results give further support to the idea of a potential clinical benefits of the combined treatment of AMPA/kainate receptor antagonists with some antiepileptic drugs.

Keywords: antiepileptics, GYKI 52466, kindling, seizures

[#] correspondence; e-mail: czuczwar@galen.imw.lublin.pl

Abbreviations: AMPA – α -amino-3-hydroxy-5-methylisoxazole-4-propionate, CGP 37849 – *D,L*-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoate, CBZ – carbamazepine, CLO – clonazepam, (\pm)CPP – (\pm)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate, *D*(-)-CPP – *D*(-)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate, CPP-ene – 3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonate, DPH – diphenylhydantoin, ED_{50} – the dose of an antiepileptic necessary to protect 50% of mice against maximal electroshock-induced seizures (effective dose in 50% of the animals), GYKI 52466 – 1,4-aminophenyl-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine, LU-73068 – 4,5-dihydro-1-methyl-4-oxo-7-trifluoromethylimidazo-[1,2a]quinoxaline-2-carbonate, LY 300164 – 7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxolo(4,5H)-2,3-benzodiazepine, MK-801 – dizocilpine maleate, *ip* – intraperitoneally, NBQX – 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline, NMDA – *N*-methyl-*D*-aspartate, PB – phenobarbital sodium, VPA – valproate magnesium

INTRODUCTION

Glutamate is the principal excitatory neurotransmitter in the brain and, as such, it plays a role in the initiation and spread of seizure activity. It also plays a critical role in epileptogenesis [22]. The involvement of *N*-methyl-*D*-aspartate (NMDA) receptors in synaptic transmission may underlie the long-lasting changes in neuronal function induced by kindling [24]. NMDA and non-NMDA (α -amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate; AMPA/kainate) receptor antagonists were identified as powerful anticonvulsants in various models of experimental epilepsy [6, 7, 19, 31]. However, both high-affinity competitive and non-competitive NMDA receptor antagonists are of limited usefulness against kindled seizures in rats and (on the basis of preliminary evidence) in patients with drug-refractory complex partial seizures, since they produce serious cognitive adverse effects in epileptic subjects [18, 22]. Moreover, although 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP) was quite effective against electroconvulsions [3], it did not reduce kindled seizures and evoked ataxia accompanied by a reduced muscle tone in rats [21]. On the other hand, another competitive NMDA receptor antagonist, *DL*-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP

37849), inhibited amygdala-kindled seizures in rats, but this effect was paralleled by a marked phencyclidine-like behavioral syndrome (ataxia, hyperlocomotion, stereotypies), comparable with the adverse effects following the administration of the non-competitive NMDA receptor antagonist, dizocilpine maleate (MK-801) [16]. Low doses of NMDA receptor antagonists potently enhanced the protective action of AMPA/kainate receptor antagonists in amygdala-kindled rats [20]. Memantine, a low-affinity channel blocker of NMDA receptors, was ineffective in the kindling model of epilepsy. Co-administration of memantine with 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline (NBQX, a competitive antagonist of AMPA/kainate receptors) resulted in supra-additive anticonvulsant effect, without overt adverse effects [17]. Furthermore, 4,5-dihydro-1-methyl-4-oxo-7-trifluoromethylimidazo[1,2a]quinoxaline-2-carbonate (LU 73068, the novel glutamate receptor antagonist with high affinity for both NMDA and AMPA receptor, significantly reduced kindled seizures in rats [27]. Two potent AMPA/kainate receptor antagonists, NBQX and 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) displayed marked anticonvulsant activity against amygdala-kindling, with no serious undesired effects [23]. Finally, GYKI 52466 considerably enhanced the protection offered by conventional antiepileptic drugs against maximal electroshock-induced seizures in mice [1], a widely recognized model of generalized clonic-tonic convulsions in man [21].

This prompted us to examine the effects of GYKI 52466 on the protection provided by conventional antiepileptics in amygdala-kindled seizures in rats, a predictive animal model of complex partial seizures with secondary generalization [21]. The adverse effects were evaluated in the rotarod test (motor coordination) and the passive avoidance task (long-term memory). A possibility of an involvement of a pharmacokinetic mechanism in the interaction between GYKI 52466 and antiepileptic drugs was also considered.

MATERIALS and METHODS

Animals and experimental conditions

Male Wistar rats (200–250 g) were used throughout the experiments after at least one week of acclimatization. They were housed in plastic perspex

cages under controlled conditions (ambient temperature of $22 \pm 1^\circ\text{C}$, natural light-dark cycle). Standard laboratory chow pellets and tap water were freely available. All experiments were done at the same time of day (between 9.00 a.m. and 12.00 a.m.) to minimize circadian influences on seizure susceptibility. The experimental groups consisted of 8 rats.

Surgery and kindling procedure

The rats were anesthetized with pentobarbital (Abbott, USA; 50 mg/kg *ip*) and received stereotaxic implantation of one bipolar electrode in the right basolateral amygdala, according to Fifkova and Marsala [13]. Coordinates for electrode implantation were AP = -1.5, L = 4.4, V = 8.5. All coordinates were measured from bregma. Skull screws served as the indifferent reference electrode. The electrode assembly was attached to the skull by dental acrylic cement (P-10, 3M, USA). After electrode implantation the animals were treated locally with an antibiotic for one week to prevent infection.

After a post-operative period of two 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 with the use of a stimulator (type 215/S), stimulus isolation unit (type 261), constant current unit (type 251; all equipment from Hugo Sachs Elektronik, Freiburg, Germany) 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 severity was assessed according to a modified Racine's scale [28]: 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 (stage 1–2) and motor seizures (stage 3–5). Afterdischarges were defined as spikes with a frequency of at least 1 Hz and 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.

Drugs

Diphenylhydantoin, carbamazepine (both drugs purchased from Sigma, St. Louis, MO, USA), valproate magnesium (Polfa, Rzeszów, Poland), phenobarbital sodium and clonazepam (both drugs from Polfa, Kraków, Poland) and GYKI 52466 (Institute for Drug Research, Budapest, Hungary) were used in this study. Diphenylhydantoin, carbamazepine and clonazepam were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA). Valproate, phenobarbital and GYKI 52466 were dissolved in sterile saline. All drugs were administered *ip*, in a volume of 3 ml/kg. Treatment times were established according to the maximal protective activity of respective drugs against kindled seizures in rats. Actually, diphenylhydantoin was administered 120 min, phenobarbital 60 min, valproate, carbamazepine, clonazepam, 30 min and GYKI 52466 15 min before the tests.

Rotarod test

Motor coordination was assessed with the use of the rotarod test [11]. Each rat was placed on a rod 8 cm long rotating at 3 rounds/min. The time to falling off the rod was noted and the test was run up to 120 s.

Passive avoidance task

The pretreated rats were placed in an illuminated box ($40 \times 40 \times 30$ cm) connected to a dark box ($40 \times 40 \times 30$ cm), which was equipped with an metal grid floor. Entrance to the dark box was punished by an electric footshock (0.7 mA for 2 s; facilitation of acquisition). The animals that did not enter the dark compartment were excluded from the experiment. On the next day (24 h later), the same animals (without any treatment) were put into the illuminated box and observed up to 180 s. Time elapsing to rat's entrance to the dark box was subsequently recorded and the medians with 25 and 75 percentiles were calculated. According to Venault et al. [32], the step-through passive avoidance task may be recognized as a measure of long-term memory.

Estimation of the plasma levels of valproate

The animals were administered vehicle + valproate or GYKI 52466 with this antiepileptic drug. Blood samples of approximately 1 ml (obtained from the puncture of the saphenous vein in immobilized non-anesthetized rats) were collected into Eppendorf tubes. Samples of blood were centrifuged at 10 000 r.p.m. (Abbott centrifuge; Irving, TX, USA) for 3 min and plasma samples were pipetted into a micropartition system MPS-1 (Amicon, Danvers, MA, USA) for separation of free from protein bound microsolute. The MPS-1 tubes were then centrifuged at 3 000 r.p.m. (MPW-360 centrifuge; Mechanika Precyzyjna, Warszawa, Poland) for 10 min and the filtrate samples of 50 μ l were transferred to Abbott system cartridges. Valproate free plasma levels were measured by immunofluorescence, with the use of an Abbott TDx analyzer (Abbott, Irving, TX, USA). Plasma levels of this antiepileptic were expressed in μ g/ml of plasma as means \pm SD of at least 6 determinations.

Statistics

The statistical significances between seizure scores, seizure and afterdischarge durations in the same group of animals were calculated by the Wilcoxon signed rank test. The results from the passive avoidance and rotarod tests were compared statistically using the Kruskal-Wallis test followed by Dunn's post-hoc test. Plasma levels of antiepilep-

tics alone or in combination with GYKI 52466 were evaluated with Student's *t* test.

RESULTS

Effect of GYKI 52466 on fully kindled seizures in rats

GYKI 52466 (2 mg/kg) was devoid of any significant action upon each seizure parameter studied. GYKI 52466 administered at a dose of 5 mg/kg exerted a protective effect, considerably reducing seizure severity, seizure duration and afterdischarge duration (Tab. 1).

Effects of antiepileptic drugs on amygdala-kindled seizures in rats

Carbamazepine (20 mg/kg) did not influence seizure parameters, but at the higher dose of 30 mg/kg it significantly shortened seizure and afterdischarge durations. Phenobarbital at 30 mg/kg, valproate (100 mg/kg), diphenylhydantoin (50 mg/kg) and clonazepam (0.005 mg/kg), but not at lower doses, exerted a similar effect (Tab. 1 and 2). Also, carbamazepine (30 mg/kg), clonazepam (0.005 mg/kg), phenobarbital (30 mg/kg), and valproate (100 mg/kg), but not diphenylhydantoin (50 mg/kg), significantly reduced the seizure score.

Table 1. Effects of combined treatment with GYKI 52466 and CBZ or PB upon fully kindled seizures in rats

Treatment (mg/kg)	Seizure severity		Seizure duration (s)		Afterdischarge duration (s)	
	Control	Treated	Control	Treated	Control	Treated
GYKI (2)	5 (5; 5)	5 (5; 5)	32.4 \pm 0.7	31.1 \pm 1.0	44.1 \pm 1.1	42.0 \pm 0.8
GYKI (5)	5 (5; 5)	4 (3; 4.5) ^a	31.0 \pm 2.0	24.3 \pm 1.4 ^b	45.2 \pm 1.3	39.2 \pm 1.5 ^b
CBZ (30)	5 (5; 5)	4 (3.5; 4.5) ^a	29.3 \pm 1.3	22.7 \pm 1.0 ^a	42.0 \pm 1.5	33.9 \pm 1.6 ^b
CBZ (20)	5 (5; 5)	4.5 (4; 5)	25.2 \pm 1.3	25.2 \pm 1.0	41.7 \pm 1.6	39.2 \pm 1.2
CBZ (20) + GYKI (2)	5 (5; 5)	4.5 (4; 5)	26.7 \pm 1.2	25.6 \pm 1.0	42.5 \pm 1.4	41.1 \pm 1.5
PB (30)	5 (5; 5)	4 (3; 4.5) ^a	30.6 \pm 1.2	24.9 \pm 0.8 ^b	44.7 \pm 1.3	39.1 \pm 1.1 ^b
PB (20)	5 (5; 5)	4.5 (4; 5)	27.9 \pm 1.1	26.8 \pm 1.2	41.2 \pm 1.4	40.7 \pm 1.3
PB (20) + GYKI (2)	5 (5; 5)	5 (4.5; 5)	28.4 \pm 1.3	25.5 \pm 1.0	40.9 \pm 1.5	39.2 \pm 1.7

Table data represent medians with 25 and 75 percentiles (for seizure severity) or means \pm SE of 6 rats per group. Control readings were made 2 days before and after the respective treatments. PB was given 60 min, CBZ – carbamazepine 30 min, and GYKI 52466 (expressed as GYKI) 15 min prior to the test. ^a *p* < 0.05, ^b *p* < 0.01, ^c *p* < 0.001 versus respective controls [Wilcoxon signed rank test]. PB – phenobarbital; CBZ – carbamazepine

Table 2. Effects of combinations of GYKI 52466 with VPA, CLO or DPH on amygdala-kindled seizures

Treatment (mg/kg)	Seizure severity		Seizure duration (s)		Afterdischarge duration (s)	
	Control	Treated	Control	Treated	Control	Treated
VPA (100)	5 (5; 5)	4 (3; 4) ^b	33.9 ± 0.9	28.3 ± 0.8 ^b	45.1 ± 1.0	38.2 ± 1.1 ^c
VPA (75)	5 (5; 5)	4.5 (4; 5)	33.2 ± 0.7	32.7 ± 0.6	43.5 ± 1.0	43.0 ± 1.3
VPA (75) + GYKI (2)	5 (5; 5)	4.5 (4; 5)	32.4 ± 0.7	27.3 ± 0.6 ^c	44.6 ± 1.1	36.8 ± 1.2 ^b
VPA (50) + GYKI (2)	5 (5; 5)	5 (5; 5)	33.7 ± 0.8	31.5 ± 0.7	43.4 ± 0.9	40.6 ± 0.8
CLO (0.005)	5 (5; 5)	4 (3.5; 4.5) ^a	32.9 ± 1.3	24.6 ± 1.0 ^c	43.2 ± 1.7	36.1 ± 1.4 ^c
CLO (0.003)	5 (5; 5)	4.5 (4.5; 5)	29.4 ± 1.2	27.8 ± 1.5	43.1 ± 1.5	42.3 ± 1.6
CLO (0.003) + GYKI (2)	5 (5; 5)	3.5 (3; 4) ^b	24.9 ± 1.2	17.1 ± 1.0 ^c	42.2 ± 1.4	32.0 ± 1.3 ^a
CLO (0.001) + GYKI (2)	5 (5; 5)	4 (3.5; 4.5) ^a	31.6 ± 1.7	23.5 ± 2.0 ^b	43.9 ± 1.6	36.1 ± 1.5 ^b
CLO (0.0005) + GYKI (2)	5 (5; 5)	4.5 (4; 5)	29.4 ± 1.6	27.0 ± 1.9	43.5 ± 1.7	40.6 ± 1.6
DPH (50)	5 (5; 5)	5 (5; 5)	32.4 ± 1.1	27.4 ± 1.2 ^a	45.1 ± 1.2	42.6 ± 1.3 ^a
DPH (40)	5 (5; 5)	5 (4; 5)	32.0 ± 0.9	31.9 ± 0.7	44.2 ± 1.2	46.1 ± 1.4
DPH (40) + GYKI (2)	5 (5; 5)	5 (4.5; 5)	31.1 ± 1.0	30.4 ± 0.9	45.1 ± 1.2	41.7 ± 1.2

DPH was administered 120 min whilst CLO and VPA – 30 min before amygdala-kindling. GYKI 52466 was given at 2 mg/kg. VPA – valproate; CLO – clonazepam; DPH – diphenylhydantoin. For further details see Table 1

Table 3. Influence of GYKI 52466, antiepileptics and the combinations of GYKI 52466 with antiepileptics on motor coordination and long-term memory in kindled rats

Treatment (mg/kg)	Rotarod test (s)	Long-term memory (s)
Saline	120 (120; 120)	180 (180; 180)
GYKI 52466 (2)	120 (120; 120)	154 (65; 180)
GYKI 52466 (5)	108 (36; 120)	109 (67; 180) ^a
VPA (100)	120 (120; 120)	180 (120; 180)
VPA (75)	120 (120; 120)	180 (180; 180)
VPA (75) + GYKI 52466 (2)	120 (120; 120)	180 (42; 180)
CLO (0.005)	120 (120; 120)	180 (180; 180)
CLO (0.003)	120 (120; 120)	180 (180; 180)
CLO (0.003) + GYKI 52466 (2)	120 (120; 120)	180 (64; 180)
CLO (0.001)	120 (120; 120)	180 (180; 180)
CLO (0.001) + GYKI 52466 (2)	120 (120; 120)	161 (111; 180)

Table data are medians (with 25, 75 percentiles) of 6 determinations. Motor impairment was indicated when the animals were unable to perform the rotarod task within 120 s. Long-term memory impairment was considered when the animal did not avoid the dark compartment within 180 s (see Materials and Methods). Kruskal-Wallis test with Dunn's post-hoc modification was used for statistical analysis of the data. For more details see also the legends of Tables 1 and 2

Effect of the combinations of GYKI 52466 with antiepileptic drugs on amygdala-kindled seizures in rats

The combined treatment of GYKI 52466, at the subprotective dose of 2 mg/kg, with valproate (75 mg/kg), resulted in the significant reduction of all seizure parameters. The combination of GYKI 52466 (2 mg/kg) with clonazepam (0.001–0.003 mg/kg), also resulted in shortening both seizure parameters. No protection against kindled seizures was observed, when the AMPA/kainate receptor antagonist (at 2 mg/kg) was co-administered with carbamazepine, phenobarbital, or diphenylhydantoin (Tab. 1 and 2).

Rotarod test and passive avoidance task

GYKI 52466 (2 mg/kg), valproate (75 and 100 mg/kg) and clonazepam (0.003 and 0.005 mg/kg) administered alone, did not cause adverse effects in amygdala-kindled rats. Also, the combinations of GYKI 52466 (2 mg/kg) with valproate (75 mg/kg) or clonazepam (0.003 mg/kg), did not produce motor or memory impairment. GYKI 52466 (5 mg/kg) did not produce motor deficits, but worsened retention in rats, evaluated in the passive avoidance task (Tab. 3).

Influence of GYKI 52466 upon the plasma levels of valproate

GYKI 52466 (2 mg/kg) did not significantly affect the free plasma level of valproate (75 mg/kg, Tab. 4).

Table 4. Influence of GYKI 52466 upon the free plasma levels of VPA

Treatment (mg/kg)	Free plasma level
VPA (75)	104.47 ± 5.72
VPA (75) + GYKI 52466 (2)	107.25 ± 4.88

Presented values are the means (in g/ml of plasma) of 6 determinations ± SD. Unpaired Student's *t*-test was used for statistical analysis of the data. For abbreviations see Table 2

DISCUSSION

Our results demonstrate that co-administration of GYKI 52466 (at the subprotective dose of 2 mg/kg) with valproate or clonazepam (at non-effective doses), resulted in a clear-cut anticonvulsant effect against amygdala-kindled seizures in rats. Any interaction at the pharmacokinetic level, at least in the case of valproate, can be excluded, because GYKI 52466 did not interfere with valproate free plasma level. Clonazepam was used in this study at a dose too low to estimate its plasma concentration by immunofluorescence. However, an involvement of the pharmacokinetic mechanism in the interaction between GYKI 52466 and clonazepam does not seem probable since 5-fold lower doses of this antiepileptic were still effective when combined with the AMPA/kainate receptor antagonist.

It was previously reported that GYKI 52466 (10 mg/kg), reduced seizure score and afterdischarge duration in kindled rats, but remained without effect upon the development of kindling [12]. Moreover, GYKI 52466 (5 mg/kg) was active in pentetrazole-evoked convulsions [18] and potentiated the protective action of valproate, carbamazepine, diphenylhydantoin, but not phenobarbital, against maximal electroshock in mice [1]. Also the derivative of GYKI 52466, LY 300164 (2 mg/kg), enhanced the anticonvulsive potency of valproate, carbamazepine, diphenylhydantoin and phenobarbital against maximal electroshock [9], increased the protective efficacy of valproate against pentetrazole-induced convulsions, and potentiated the antiseizure activity of valproate, diazepam and clo-

nazepam in amygdala-kindled rats [2,4,5,8]. Another competitive AMPA/kainate receptor antagonist, NBQX, suppressed the development of kindling [25], exerted the antiseizure activity in pentetrazole-induced seizures [18] and potentiated the protective action of valproate, carbamazepine, diphenylhydantoin and phenobarbital against maximal electroshock [33].

There are electrophysiological data indicating the involvement of NMDA-mediated component in the mechanism of action of carbamazepine, diphenylhydantoin and valproate [29]. Thus, the NMDA component may be responsible for beneficial interactions of antiepileptics with AMPA/kainate receptor antagonists. In fact, NBQX and LY 300164 had a broader spectrum of activity against maximal electroshock than GYKI 52466, since they enhanced the protective action of not only carbamazepine, diphenylhydantoin, and valproate, but also that of phenobarbital [9,33]. On the other hand, both GYKI 52466 and LY 300164 [5] were much less effective in the kindling model of epilepsy, as regards their ability to potentiate the efficacy of antiepileptic drugs. The beneficial interactions were observed only between AMPA/kainate receptor antagonists and valproate or benzodiazepines. According to Tarnawa et al. [30], GYKI 52466 has some affinity for central benzodiazepine receptors, although it is several orders of magnitude weaker than that of diazepam. It might be hypothesized that the effect of AMPA/kainate receptor antagonists upon the anticonvulsant activity of benzodiazepines could result from their action at different binding sites within the GABA_A receptor complex.

The combination of GYKI 52466 with antiepileptics did not produce serious adverse effects. In fact, AMPA/kainate receptors do not seem to be involved in the induction of long-term potentiation, which underlies memory processes [26].

In conclusion, our data indicate that, because of their higher benefit/risk index, AMPA/kainate receptor antagonists seem to be more useful for the combined treatment with conventional antiepileptics than high-affinity NMDA receptor antagonists. Only low-affinity NMDA receptor antagonists may be candidates for potential antiepileptic drugs [7]. Summing up, the results of this study clearly indicate that the blockade of AMPA/kainate receptors may be associated with the increased efficacy of some antiepileptic drugs, particularly benzodiazepines and valproate. Consequently, the obtained

data provide an experimental background for a successful treatment of partial seizures or status epilepticus. One has to consider that there are about 30% of epileptic patients and 35% of epileptic patients with status epilepticus who cannot receive effective pharmacological treatment [10]. Recently, anticonvulsant compounds have been encountered among metabotropic glutamate receptor ligands, which easily enter the brain after peripheral injections [14]. Interestingly, a group II metabotropic glutamate receptor agonist also potently enhanced the protective effects of diazepam against pentetrazole-induced convulsions in mice [15] with practically no observable adverse effects. This may create a new approach to the treatment of epilepsy, too.

Acknowledgments. The generous gifts of GYKI 52466 from Laboratory for Drug Research, Budapest, Hungary (courtesy of Dr. I. Tarnawa) is greatly appreciated. This study was supported by a grant from Lublin Medical University. The financial support from Janssen-Cilag (Div. Johnson & Johnson Poland) is also appreciated.

REFERENCES

- Borowicz K.K., Gašior M., Kleinrok Z., Czuczwar S.J.: The non-competitive AMPA/KA receptor antagonist, GYKI 52466, potentiates the anticonvulsant activity of conventional antiepileptics. *Eur. J. Pharmacol.*, 1995, 281, 319–326.
- Borowicz K.K., Łuszczki J., Szadkowski M., Kleinrok Z., Czuczwar S.J.: Influence of LY 300164, an antagonist of AMPA/kainate receptors, on the anticonvulsant activity of clonazepam. *Eur. J. Pharmacol.*, 1999, 380, 67–72.
- Borowicz K.K., Kleinrok Z., Czuczwar S.J.: Influence of D(–)-CPP upon the protective action of conventional antiepileptic drugs against electroconvulsions in mice. *Pol. J. Pharmacol.*, 2000, 52, 431–439.
- Borowicz K.K., Kleinrok Z., Czuczwar S.J.: The AMPA/kainate receptor antagonist, LY 300164, increases the anticonvulsant action of diazepam. *Naunyn-Schmied. Arch. Pharmacol.* 2000, 361, 629–635.
- Borowicz K.K., Kleinrok Z., Czuczwar S.J.: Glutamate antagonists differentially affect the protective activity of conventional antiepileptics against amygdala-kindled seizures in rats. *Eur. Neuropsychopharmacol.*, 2001, in press.
- Chapman A.G.: Glutamate receptors in epilepsy. *Prog. Brain Res.*, 1998, 116, 371–383.
- Czuczwar S.J.: Perspectives for the use of excitatory amino acid ionotropic receptor antagonists as antiepileptic drugs. *Pol. J. Pharmacol.*, 2000, 52, 67–70.
- Czuczwar S.J., Gašior M., Kamiński R., Kleinrok Z., Kozicka M., Ossowska G., Pietrasiewicz T.: GYKI 52466 [1-(4-aminophenyl)-4-methoxy-7,8-methylene-dioxy-5H-2,3-benzodiazepine hydrochloride] and the anticonvulsive activity of conventional antiepileptics against pentetrazole in mice. *Mol. Chem. Neuropharmacol.*, 1998, 33, 149–162.
- Czuczwar S.J., Świąder M., Kuźniar H., Gašior M., Kleinrok Z.: LY 300164, a novel antagonist of AMPA/kainate receptors, potentiates the anticonvulsive activity of antiepileptic drugs. *Eur. J. Pharmacol.*, 1998, 359, 103–109.
- Deckers C.L.P., Czuczwar S.J., Hekster Y.A., Keyser A., Kubova H., Meinardi H., Patsalos P.N., Renier W.O., Van Rijn C.M.: Selection of antiepileptic drug polytherapy based on mechanisms of action: the evidence reviewed. *Epilepsia*, 2000, 41, 1364–1374.
- Dunham N.W., Miya T.S.: A note on a simple approach for determining neurological deficits in rats and mice. *J. Amer. Pharmacol. Assoc.*, 1957, 46, 208.
- Dürmüller N., Craggs M., Meldrum B.S.: The effect of the non-NMDA receptor antagonists GYKI 52466 and NBQX and the competitive NMDA receptor antagonist D-CPPene on the development of amygdala kindling and on amygdala-kindled seizures. *Epilepsy Res.*, 1994, 17, 167–174.
- Fifkova E., Marsala J.: Stereotaxic atlases for the cat, rabbit and rat. In: *Electrophysiological Methods in Biological Research*, Eds. Bures J., Petran M., Zachar J., Academic Press, New York, 1967, 653–695.
- Kłodzińska A., Chojnacka-Wójcik E., Pilec A.: Selective group II glutamate metabotropic receptor agonist LY354740 attenuates pentetrazole- and picrotoxin-induced seizures. *Pol. J. Pharmacol.*, 1999, 51, 543–545.
- Kłodzińska A., Bijak M., Chojnacka-Wójcik E., Krocza B., Świąder M., Czuczwar S.J., Pilec A.: Roles of group II glutamate metabotropic receptors in modulation of seizure activity. *Naunyn-Schmied. Arch. Pharmacol.*, 2000, 361, 283–288.
- Löscher W., Hönack D.: Effects of the competitive NMDA receptor antagonist, CGP 37849, on anticonvulsant activity and adverse effects of valproate in amygdala-kindled rats. *Eur. J. Pharmacol.*, 1993, 234, 237–245.
- Löscher W., Hönack D.: Over-additive anticonvulsant effect of memantine and NBQX in kindled rats. *Eur. J. Pharmacol.*, 1994, 259, R3–R5.
- Löscher W., Hönack D.: Effects of the non-NMDA antagonists NBQX and the 2,3-benzodiazepine GYKI 52466 on different seizure types in mice: comparison with diazepam and interactions with flumazenil. *Brit. J. Pharmacol.*, 1994, 113, 1349–1357.
- Löscher W., Nolting B., Hönack D.: Evaluation of CPP, a selective NMDA antagonist, in various rodent models of epilepsy. Comparison with other NMDA antagonists, and with diazepam and phenobarbital. *Eur. J. Pharmacol.*, 1988, 152, 9–17.
- Löscher W., Rundfeldt C., Hönack D.: Low doses of NMDA receptor antagonists synergically increase the anticonvulsant effect of AMPA receptor antagonist

- NBQX in the kindling model of epilepsy. *Eur. J. Neurosci.*, 1993, 5, 1545–1550.
21. Löscher W., Schmidt D.: Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res.*, 1988, 2, 145–181.
 22. Meldrum B.S.: The role of glutamate in epilepsy and other CNS disorders. *Neurology*, 1994, 44, S14–S23.
 23. Meldrum B.S., Craggs M.D., Dürmüller N., Smith S.E., Chapman A.G.: The effects of AMPA receptor antagonists on kindled seizures and on reflex epilepsy in rodents and primates. *Epilepsy Res.*, 1992, 9, Suppl., 307–311.
 24. Mody I., Heinemann U.: NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. *Nature*, 1987, 326, 701–704.
 25. Namba T., Morimoto K., Sato K., Yamata N., Kuroda S.: Antiepileptogenic and anticonvulsant effects of NBQX, a selective AMPA receptor antagonist, in the rat kindling model of epilepsy. *Brain Res.*, 1994, 638, 36–44.
 26. Parada J., Czuczwar S.J., Turski W.A.: NBQX does not affect learning and memory tasks in mice: a comparison with D-CPP-ene and ifenprodil. *Cognitive Brain Res.*, 1992, 1, 67–71.
 27. Potschka H., Löscher W., Wlaź P., Behl B., Hofmann H.P., Treiber H.J., Szabo L.: LU 73068, a new non-NMDA and glycine/NMDA receptor antagonist: pharmacological characterization and comparison with NBQX and L-701,324 in the kindling model of epilepsy. *Brit J. Pharmacol.*, 1998, 125, 1258–1266.
 28. Racine R.J.: Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr. Clin. Neurophysiol.*, 1972, 32, 281–294.
 29. Steppuhn K.G., Turski L.: Modulation of the seizure threshold for excitatory amino acid in mice by antiepileptic drugs and chemoconvulsants. *J. Pharmacol. Exp. Ther.*, 1993, 265, 1063–1070.
 30. Tarnawa I., Farkas S., Berzsenyi P., Pataki A., Andrási F.: Electrophysiological studies with a 2,3-benzodiazepine muscle relaxant: GYKI 52466. *Eur. J. Pharmacol.*, 1989, 213, 151–153.
 31. Urbańska E., Czuczwar S.J., Kleinrok Z., Turski W.A.: Excitatory amino acids in epilepsy. *Restor. Neurol. Neurosci.*, 1998, 13, 25–39.
 32. Venault P., Chapoutier G., De Carvalho L.P., Simiand J., Morre M., Dodd R.H., Rossier J.: Benzodiazepines impair and beta-carbolines enhance performance in learning and memory tasks. *Nature*, 1986, 321, 864–866.
 33. Żarnowski T., Kleinrok Z., Turski W.A., Czuczwar S.J.: 2,3-Dihydroxy-6-nitro-7-sulfamoylbenzo-(F)quinoline enhances the protective activity of common antiepileptic drugs against maximal electroshock-induced seizures in mice. *Neuropharmacology*, 1993, 32, 895–900.

Received: February 19, 2001; in revised form: April 10, 2001.