

PHARMACOGENETICS OF ANTIEPILEPTIC DRUGS

Władysława A. Daniel

Department of Pharmacokinetics and Drug Metabolism, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Pharmacological response to drugs acting on the central nervous system (CNS) may be genetically conditioned at the following levels: transportation through the gastrointestinal tract wall, liver metabolism, transportation through the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (B-CSF-B), local metabolism and, finally, polymorphism of pharmacological receptors. Mutations of cytochrome P-450 isoenzyme CYP2C9 (CYP2C6*2, CYP2C6*3, CYP2C6*6) responsible for the metabolism of phenytoin may decrease the elimination of the drug from the body and lead to its accumulation at toxic concentrations. Antiepileptic drugs are substrates for ABC transporters (ATP-binding cassette superfamily), such as P-glycoprotein (PGP) and multidrug resistance-associated proteins (MRPs) distributed in the organs responsible for drug elimination (the liver, kidneys, gastrointestinal tract) and the brain (BBB endothelium, B-CSF-B epithelium, astrocytes, microglia). Those transporters act as active efflux pumps, limiting penetration of antiepileptic drugs into the brain parenchyma. In epileptic patients resistant for pharmacotherapy, PGP and

MRPs (MRP1, MRP2) are overexpressed in the capillary endothelial cells and astrocytes around blood vessels and in displastic neurons (MRP1). The level of expression of those transporters may be affected by epileptic episodes, treatment with antiepileptic drugs and genetic polymorphism of genes coding for PGP and MRP. On the other hand, some mutations of the neuronal nicotinic acetylcholine receptor (nAChR), which are more sensitive to acetylcholine seem to contribute to the autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). ADNFLE patients respond positively to relatively low concentrations of carbamazepine, which is ascribed to the ability of the drug to block the nAChR ion channel and to increase sensitivity of the mutated receptors to carbamazepine. In conclusion, genotyping towards the above-mentioned genes coding for metabolic enzymes, nAChRs and drug transporters, as well as introducing PGP and MRP inhibitors may allow in the future to choose a proper antiepileptic drug, to secure its therapeutic concentration at the site of action and to reduce a number of pharmacoresistant patients.

NEW RESEARCH DIRECTIONS
IN THE PHARMACOTHERAPY OF EPILEPSY*Władysław Lason*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Epilepsy is a common neurological disease, and ca. 30% epileptic patients are resistant to pharmacological treatment. Hence, a search for new antiepileptic medications, in order to treat the drug-resistant form of epilepsy and to improve prognosis after current therapies, with special emphasis on the potential use of neurochemistry and molecular biology achievements, is fully justified. Development of ideas about pathogenesis of seizures and

mechanism of action of antiepileptic drugs reflects remarkable advancement of knowledge of neurotransmission. Blockers of voltage-dependent sodium channels and GABA mimetics are still the most important group of anticonvulsants, and progress in this field is evidenced by many discoveries of still more selective and subtler modes of regulation of their biological targets. Although genetic factors play an essential role in only some rare

forms of epilepsy, nevertheless analysis of receptor and ion channel mutations, which lead to seizures, may provide a valuable information for designing new animal models of epilepsy and antiepileptic drugs. From this perspective, mutations of sodium (SCN1B, SCN1A), potassium (KCNQ2, KCNQ3, KCNA1) and calcium ($\alpha 2\delta 2$) channel subunits deserve special attention. Ionotropic glutamate receptors (NMDA, kainate/AMPA) have been considered to be an attractive target for new anticonvulsants. However, some opinions are now less enthusiastic, since high affinity antagonists of NMDA receptors possess numerous undesired side effects. On the other hand, selective antagonists of NR2B subunit of NMDA receptors, as well as kainate/AMPA receptor antagonists disturb physiological processes to a lesser extent. In search for future directions in the therapy of epilepsy, the emphasis is placed on the significance of molecularly characterized new drug targets, drugs acting in a direct vicinity of seizure foci (focal method of drug

delivery), as well as gene and cell therapy. Considering the latter strategies it is worth mentioning that beneficial effects of ectopic overexpression of an anticonvulsant peptide galanin, and genetically engineered GABA-producing cell transplants or grafts of adenosine-releasing cells in animal model of seizures have been successfully demonstrated. Furthermore, increasing body of evidence indicates that overexpression of multiple drug resistance genes in endothelial cells may be a common cause of refractory epilepsy. Thus, there is a great hope that molecular, genetic and proteomic strategies will help pharmacologists to create new, effective anticonvulsants. However, it seems that a real progress in understanding of the mechanism of epilepsy and the action of antiepileptic drugs will be made when molecular and functional studies are integrated and the obtained data are properly collated and presented, which should instigate comprehensive discussion between specialists in various fields of epileptology.

DRUG RESISTANT EPILEPSY: WHAT CAN WE LEARN FROM EXPERIMENTAL STUDIES?

Stanisław J. Czuczwar^{1,2}

¹Department of Pathophysiology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland,
²Isotope Laboratory, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

Drug resistant epilepsy concerns about 30% of epileptic patients and one possible solution to this problem is polytherapy with antiepileptic drugs, usually in the form of duotherapy [Czuczwar and Borowicz, *Epilepsy Res.*, 2002]. Combinations of antiepileptic drugs (AEDs) should be free from pharmacokinetic interactions and produce the lowest possible adverse effects [Deckers et al., *Epilepsia*, 2000]. A combination of two AEDs may result in synergy, addition or antagonism. Synergy occurs when the final protective effect is greater than the expected protection, based upon the individual anticonvulsant effects of AEDs in a mixture. Addition is observed when the anticonvulsant effect of a mixture of AEDs is not statistically significantly different from the partial protective effects of individual AEDs. Finally, antagonism is defined when the final effect of a mixture is lower than the expected anticonvulsant effect [Borowicz et al., *Epi-*

lepsia, 2002; Deckers et al., *Epilepsia*, 2000]. Certainly, preferentially AED combinations showing synergy with minimal or absent adverse activity may be of clinical relevance [Borowicz et al., *Epilepsia*, 2002; Deckers et al., *Epilepsia*, 2000]. Generally, combinations of antiepileptic drugs showing different mechanisms of action are more likely to result in synergy than drugs sharing similar mechanisms [Deckers et al., *Epilepsia*, 2000]. Isobolographic analysis performed by Shank et al. [*Epilepsia*, 1994] provided evidence that combinations of topiramate with carbamazepine or phenobarbital were synergistic whilst that of topiramate with phenytoin was actually additive. Out of combinations of lamotrigine with conventional or novel AEDs, the best ones were with phenobarbital or topiramate, showing potent synergy [Czuczwar and Borowicz, *Epilepsy Res.*, 2002]. Interestingly, clear-cut antagonism was observed when lamotrigine was combined with car-

bamazepine [Czuczwar and Borowicz, *Epilepsy Res.*, 2002]. Importantly, combinations of gabapentin with conventional AEDs resulted in synergistic interactions [Borowicz et al., *Epilepsia*, 2002].

Experimental data may provide valuable clues as to the choice of rational polytherapy of drug resistant epilepsy in terms of its potent protective efficacy and minimal adverse effects.

DIFFERENT EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITORS ON KAINATE-INDUCED SEIZURES IN MICE

Piotr Tutka, Marian Wielosz

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

Pharmacological strategies for interfering with the nitric oxide (NO) level are being developed as potential interventions for the treatment of seizure disorders [De Sarro et al., *Eur. J. Pharmacol.*, 2000; Tutka et al., *Eur. Neuropsychopharmacol.*, 2002]. However, the modification of NO synthesis exerts contrasting effects upon animal convulsions [Rundfeldt et al., *Eur. J. Pharmacol.*, 1995].

With the use of kainate as a convulsant (2.5–50 mg/kg *ip* or 0.2–1 nmol *icv*), we compared the effects of N^G-nitro-L-arginine (NNA), a non-selective NO synthase inhibitor, and 7-nitroindazole (7-NI), a selective inhibitor of neuronal NO synthase. As shown in the table, NNA enhanced the seizure susceptibility to *ip* and *icv* kainate, while 7-NI did not influence kainate-induced seizure activity.

In conclusion, an interpretation of the anticonvulsant role of NO in kainate-induced seizures, supported, as for today, by the potentiation of these seizures by NNA [Przegaliński et al., *Neurosci. Lett.*,

1994; Tutka et al., *NeuroReport*, 1996], seems to be complicated by the ineffectiveness of 7-NI. The role of NO in kainate-induced seizures remains still an open question.

Treatment (mg/kg)	CD ₅₀ of kainate <i>ip</i> (mg/kg)	CD ₅₀ of kainate <i>icv</i> (nmol)
NNA	0	32.7 (28.3–37.8)
	0.1	30.7 (26.4–35.9)
	1	23.9 (17.2–33.1)
	10	22.8 (18–28.8)**
	40	7.5 (5.9–9.5)***
7-NI	0	32 (26.4–38.7)
	20	30.2 (27.2–33.4)
	100	29.3 (26.1–32.8)

* p < 0.5, ** p < 0.01, *** p < 0.001 vs. NNA 0; NT –not tested

EFFECT OF ANTIEPILEPTIC DRUGS ON SYNTHESIS OF KYNURENIC ACID IN THE RAT CORTICAL SLICES

Tomasz Kocki, Ewa U. Urbańska, Marian Wielosz, Waldemar A. Turski

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

Epilepsy is a neurological disorder affecting approximately 0.5–1.0% of the world's population. Although antiepileptic drugs (AEDs) are commonly

used to control and prevent seizures, their mechanism(s) of action are still not fully explained. It is widely accepted that their pharmacological effects

involve a variety of mechanisms, including the increased γ -aminobutyric acid (GABA)ergic transmission, blockade of voltage-gated sodium channels and inhibition of excitatory amino acid (EAA) neurotransmission.

Kynurenic acid (KYNA) is an endogenous brain constituent which inhibits the activity at all three ionotropic EAA receptors. KYNA acts most potently at the glycine site of the N-methyl-D-aspartate receptor complex. Cerebral synthesis of KYNA from its bioprecursor L-kynurenine is catalyzed by two distinct kynurenine aminotransferases localized preferentially within astrocytes. The disturbances of KYNA production have been linked to the occurrence of epilepsy. The anticonvulsant and neuroprotective role of KYNA *in vivo* and *in vitro* is well documented.

In the present study, the influence of AEDs: felbamate, vigabatrin, valproate, carbamazepine and

phenobarbital on KYNA synthesis in the rat brain cortex was investigated *in vitro*. KYNA was subjected to HPLC and detected fluorometrically.

Phenobarbital and felbamate at the concentrations of 0.5 and 1 mM significantly increased KYNA production up to 140 and 150% of control values, respectively.

Carbamazepine, valproate and vigabatrin at concentrations of 0.01, 0.1, 0.5, 1 and 3 mM did not influence KYNA production *in vitro*.

Our data showed that some AEDs may enhance KYNA production in the brain tissue. The contribution of this phenomenon to the control of seizures needs to be further investigated.

Supported by grant No 6 P05A 053 21 from the State Committee for Scientific Research, Warszawa, Poland.

INFLUENCE OF TOPIRAMATE ON THE ANTICONVULSANT ACTIVITY OF CONVENTIONAL ANTIEPILEPTIC DRUGS

Andrzej M. Duda¹, Kinga K. Borowicz², Jarogniew Łuszczki², Stanisław J. Czuczwar^{2,3}

¹Janssen-Cilag, Div. Johnson@Johnson Poland, Szyszkowa 20, PL 02-285 Warszawa, Poland,

²Department of Pathophysiology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland,

³Isotope Laboratory, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

In this study, the interactions between the novel antiepileptic drug (AED), topiramate, with conventional AEDs were evaluated in amygdala-kindled seizures, a model of complex partial seizures in humans. The results are expected to provide experimental clues for the rational treatment of epilepsy, since around 30% of epileptic patients are not satisfactorily treated [for review refer to Czuczwar and Borowicz, *Epilepsy Res.*, 2002]. Experiments were conducted on fully kindled rats. Adverse effects were measured in the chimney test (motor coordination) and passive avoidance task (long-term memory). The plasma concentrations of AEDs were measured by immunofluorescence to exclude a possibility of pharmacokinetic interactions. Topiramate at 20 mg/kg inhibited amygdala-kindled convulsions as evidenced by shortening

of seizure and afterdischarge durations, while its lower doses were ineffective. Combined treatment of topiramate (10 mg/kg) with valproate (at a sub-effective dose of 50 mg/kg) resulted in distinct reductions of seizure and afterdischarge durations. Furthermore, topiramate (10 mg/kg) co-administered with carbamazepine (at a non-effective dose of 15 mg/kg) significantly increased afterdischarge threshold, reducing the remaining seizure parameters (duration or severity of seizures and afterdischarge duration). Topiramate (10 mg/kg) combined with phenobarbital (15 mg/kg) very potently decreased seizure severity and shortened seizure and afterdischarge durations. Combinations of topiramate with diphenylhydantoin were ineffective in alleviating seizures. When co-administered with val-

proate or phenobarbital, topiramate did not affect their plasma levels. In contrast, its combination with carbamazepine resulted in an increased free plasma carbamazepine concentration. Neither topiramate (10 and 20 mg/kg) alone nor its combinations with conventional AEDs affected motor coordination or

long-term memory, evaluated in the chimney and passive avoidance tests in rats, respectively.

The results of this study indicate that except for diphenylhydantoin, the combinations of topiramate with conventional AEDs are potentially beneficial from clinical point of view.

INFLUENCE OF SOME CONVULSANTS ON THE ANTICONVULSANT ACTIVITY OF NOVEL ANTIEPILEPTIC DRUGS AGAINST MAXIMAL ELECTROSHOCK-INDUCED SEIZURES IN MICE

Mariusz Świąder¹, Marian Wielosz¹, Stanisław J. Czuczwar^{2,3}

¹Department of Pharmacology and Toxicology, ²Department of Pathophysiology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, ³Isotope Laboratory, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

Occasionally, severe seizures may be encountered in patients receiving high dose of aminophylline for pulmonary reasons and this seizure activity is relatively resistant to standard antiepileptic drugs (AEDs). Moreover, aminophylline at doses far below its convulsive potential was shown to block or reduce the anticonvulsive potency of a number of AEDs both in pentetrazole-, electroshock-induced and kindled convulsions [Czuczwar et al., *Epilepsia*, 1985; Czuczwar et al., *Epilepsy Res.*, 1987; Dragunov et al., *Epilepsia*, 1985; Skerrit et al., *Epilepsia*, 1983]. Strychnine, a selective antagonist of glycine site at strychnine-sensitive glycinergic receptors, produced seizures and at doses below its convulsive potential impaired the anticonvulsive effects of conventional AEDs against maximal electroshock (MES)-induced seizures. It is noteworthy that strychnine-induced convulsions are generally insensitive to standard antiepileptic therapy, indicating that this model of epilepsy may be used to study the problem of drug resistance [Löscher and Schmidt, *Epilepsy Res.*, 1988]. Bicuculline is a well characterized GABA_A receptor antagonist able to evoke generalized clonic-tonic convulsions after systemic administration [Meldrum, *Int. Rev. Neurobiol.*, 1975]. Similar type of seizures is produced by picrotoxin,

an antagonist of Cl⁻ channel at the GABA/benzodiazepine receptor complex.

The aim of this study was to evaluate the effects of aminophylline, strychnine, bicuculline and picrotoxin on the anticonvulsant action of the following novel AEDs: LY 300164 (Talampanel®; a non-NMDA antagonist), lamotrigine and felbamate. Aminophylline (up to 100 mg/kg), strychnine (up to 0.5 mg/kg), bicuculline (up to 2 mg/kg) and picrotoxin (3 mg/kg) did not affect the seizure threshold. When combined with LY 300164 or lamotrigine, aminophylline (50–100 mg/kg) and strychnine (0.125–0.5 mg/kg) impaired their protective activity against MES, while bicuculline (2 mg/kg) and picrotoxin (3 mg/kg) were ineffective. Aminophylline (100 mg/kg), strychnine (0.25–0.5 mg/kg) and picrotoxin (3 mg/kg) impaired the protective activity of felbamate against MES. It may be concluded, that contrary to LY 300164 and lamotrigine, also GABAergic mechanisms contribute to the anticonvulsant activity of felbamate in this test. Similarly to conventional AEDs, novel AEDs are also sensitive to aminophylline, which indicates that this antiasthmatic drug needs to be used with caution in epileptic patients.

ISOBOLOGRAPHIC ASSESSMENT OF INTERACTIONS BETWEEN OXCARBAZEPINE AND CONVENTIONAL ANTIPILEPTIC DRUGS IN TWO EXPERIMENTAL MODELS OF EPILEPSY

Jarogniew J. Łuszczki¹, Kinga K. Borowicz¹, Stanisław J. Czuczwar^{1,2}

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

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

Although monotherapy of epilepsy is efficacious in about 70% of epileptic patients, the remaining 30% require to be intensively treated with two or more antiepileptic drugs (AEDs) [Brodie M.J., *Epilepsy Res.*, 2001]. In these clinically difficult situations, rational polytherapy offers some advantages and the patients are given combinations of AEDs, specifically selected for providing the most effective therapy [Deckers C.L.P. et al., *Epilepsia*, 2000]. Fundamental rules underlying the rational polytherapy and combining drugs are based either on theoretical presumptions (i.e. mechanisms of action of commonly applied AEDs) or on experimental studies on animal models of epilepsy [Czuczwar S.J. and Borowicz K.K., *Epilepsy Res.*, 2002]. There is an urgent need to test all promising drug combinations (theoretically presumed to be effective, on the basis of their supplementary mechanisms of action) in animal studies in order to discover the best ones, i.e. combinations showing pharmacological synergy [Czuczwar S.J., *Epileptologia*, 1998]. It is generally accepted that only these AED combinations which show synergy in animal experiments are recommended to be used in clinical practice for the treatment of intractable seizures [Bourgeois B.F.D., *J. Pharmacol. Exp. Ther.*, 1988].

Better understanding of pathophysiological processes related with epileptogenesis and seizure propagation, allowed for creation of some novel AEDs with specific mechanisms of action and much less toxic than conventional AEDs. These novel AEDs, make possible to efficaciously treat some uncontrolled seizures in humans, contributing to improvement of patients' quality of life [Brodie M.J. and Schachter S.C., *Epilepsy*, 2nd edn., 2001]. The last decade of the XXth century was abundant in novel AED registrations. Among novel AEDs, oxcarbazepine (OXC – chemically modified carbamazepine molecule) has been approved to the add-on therapy of patients with partial refractory or gener-

alized tonic-clonic seizures [Barcs G. et al., *Epilepsia*, 2000].

In order to develop the rationale for polytherapy with OXC, the combinations of this drug with conventional AEDs were evaluated in the maximal electroshock (MES) and pentetrazole (PTZ)-induced seizure tests in mice. The MES test is considered as an experimental model of generalized tonic-clonic seizures and to a certain extent of partial convulsions in humans, whilst the PTZ-evoked convulsions are an animal model of myoclonic seizures and to a certain degree of absence attacks in humans [Löscher W. and Schmidt D., *Epilepsy Res.*, 1988]. To determine the exact type of pharmacodynamic interactions between OXC and AEDs, an isobolographic analysis was performed as an eligible method allowing for detection of synergy, additivity or antagonism between the tested AEDs [Tallarida R.J. et al., *Life Sci.*, 1997].

The experiments were carried out on adult male Swiss mice weighing 20–26 g. All experimental procedures applied in this study were approved by Local Ethics Committee of the Medical University in Lublin. The MES-induced seizures were produced by an alternating current (25 mA, 50 Hz, 0.2 s of stimulus duration, the hindlimb extension taken as the endpoint) using auricular electrodes. Convulsions in the PTZ-test were evoked by subcutaneous administration of PTZ at a dose of 100 mg/kg (i.e. which is CD₉₇, i.e. the dose of PTZ, inducing the clonic phase of seizures in 97% of the tested animals). Anticonvulsant properties of conventional AEDs (diphenylhydantoin, valproate, phenobarbital, clonazepam, carbamazepine in the MES test and valproate, phenobarbital, clonazepam and ethosuximide in the PTZ test) co-administered with OXC (at fixed ratios of 1:3, 1:1 and 3:1) were evaluated isobolographically and expressed in the form of ED₅₀s (i.e. median effective dose of a drug mixture, which protects 50% of animals against

electrically or chemically evoked convulsions). Furthermore, adverse effects of the drug combinations, in terms of the impairment of motor coordination, were also evaluated by the use of isobolographic analysis in the chimney test in mice, which allowed for establishing the TD_{50} values (median toxic dose, inducing impairment of motor coordination in 50% of the tested mice).

Results evidently indicate that OXC administered alone is much more effective in the MES test (ED_{50} was 10.6 mg/kg) than in the PTZ-induced convulsions in mice (ED_{50} was 20.1 mg/kg). Isobolographic analysis of interactions in the MES test revealed that OXC combined with diphenylhydantoin, at the fixed ratio of 1:1, exerted an antagonistic type of interaction, whereas other combinations of the drugs (1:3 and 3:1) showed additive effect. OXC combined with phenobarbital, valproate or carbamazepine (for all fixed ratios were 1:3, 1:1 and 3:1) were merely additive in the MES. For the first time, a phenomenon of transition of interaction from antagonism to synergy was observed for the combination of OXC with clonazepam (biphasic character of this interaction was dependent on the applied drug doses in a mixture). When low doses of OXC were combined with high doses of clonazepam (at the fixed ratio of 1:3), the antagonism

was evident, whereas the drugs combined at the fixed ratios of 1:1 and 3:1 exhibited synergistic action in the MES test.

It should be stressed that OXC specifically interacted with clonazepam in the PTZ-test in mice, evoking synergistic interaction for the fixed ratio of 1:1. In isobolography, OXC co-administered with ethosuximide (at the fixed-ratio of 3:1) exerted antagonistic type of interaction. All remaining tested combinations of OXC and conventional AEDs were additive in the PTZ-induced convulsions. Furthermore, adverse effects related with AEDs in combinations (evaluated isobolographically in the chimney test) were additive.

Summing up, the results obtained in the present study suggest that the combined treatment of epilepsy with OXC + diphenylhydantoin, or OXC + ethosuximide cannot be recommended for use in clinical practice. In addition, experimental evidence indicates that the patients with intractable seizures should not be medicated with the combination of OXC and clonazepam because of the biphasic interaction, which can change from synergy to antagonism. Combinations of OXC with phenobarbital or valproate offered merely additive interactions when examined in two animal models of epilepsy.