The aim of this study was to investigate the role of nitric oxide (NO), a second messenger and/or a neurotransmitter, in convulsions induced by nicotine. We examined the effects of 7-nitroindazole (7-NI), a selective neuronal nitric oxide synthase (NOS) inhibitor, N\(^{\mathrm{G}}\)-nitro-L-arginine (NNA), a non-selective NOS inhibitor, and aminoguanidine, a selective inducible NOS inhibitor, on convulsions induced by intraperitoneally (ip) administered nicotine in mice.

7-NI, at the doses of 50 and 100 mg/kg (ip; 30 min before nicotine), dose-dependently reduced the \(C_D\) of nicotine (the dose of convulsant producing seizures in 50% of mice) from 6.7 to 5.2 (\(p < 0.05\)) and 3.7 (\(p < 0.001\)) mg/kg, respectively. L-arginine (L-Arg), a NO precursor, at a dose of 500 mg/kg (ip), which itself had no effect on the \(C_D\) of nicotine, did not reverse the proconvulsant effect of 7-NI. Lower doses of 7-NI (12.5 and 25 mg/kg) had no effect on convulsions.

NNA, at a dose of 40 mg/kg (ip; 30 min before nicotine), dose-dependently increased the \(C_D\) of nicotine from 6.7 to 9.3 (\(p < 0.001\)) mg/kg. The anticonvulsant effect of NNA was reversed by L-Arg (500 mg/kg) with the nicotine \(C_D\) being 7.1 mg/kg (\(p < 0.001\) vs. NNA given alone). Lower dose of NNA (1 mg/kg) did not change significantly the \(C_D\) value.

Aminoguanidine administered at a dose of 100 mg/kg (ip; 15 and 30 min before nicotine) did not affect convulsions.

The results indicate that in nicotine-induced convulsions: 1) 7-NI is a proconvulsant and its effect does not appear to result only from the impaired NO synthesis, 2) NNA is an anticonvulsant acting most likely via L-Arg-NO pathway, 3) aminoguanidine has no effect on convulsions. This work adds more new data to existing evidence that suggest different action of NOS inhibitors in chemically induced convulsions in animals.

Calcium level must be tightly regulated in time, space and amplitude because cells are able to extract specific information from these parameters. There are different pathways for entry of extracellular calcium into the cell. Many excitable cells express voltage-dependent calcium channels (VDCCs), which translate the action potential into a calcium rise. Ligand-operated calcium channels (LOCCs) are activated by binding an agonist to its specific recognition site close to the channel. Ionotrophic glutamate receptors of NMDA and AMPA subtype may be the example of this group, although the latter one only in specific configurations of GluR2 subunit. Second messenger-operated calcium channels (SMOCC) are regulated by metabotropic receptors and second messengers, like cyclic nucleotides, inositol phosphates, arachidonic acid [Missiaen et al., 2000]. Calcium transport across endoplasmic
reticulum membrane is realized by rianodine and inositol triphosphate receptors [Hofmann et al., 2000]. Elevation of calcium ion level is a functional trigger for a number of cellular processes (e.g. action potential generation, neurotransmitter and hormone release, muscle contraction, neurite outgrowth, synaptogenesis, gene expression and cell death) [Missiaen et al., 2000].

VDCCs are thought to have the heteromeric structure. The most important 1 subunit consists of 4 domains. Each domain contains 6 α-helical regions (S1-6). The loop between S5 and S6 dips into the membrane to form the lining of the pore. This subunit plays also a role of voltage sensor. Associated with the α1 subunit are entirely intracellular β subunit, γ subunit, traversing the membrane four times and the α2δ subunit (extracellular α2 connected with intracellular δ protein). All three regulate the channel kinetics [Randall and Benham, 1999].

Although mutations in calcium channels in humans produce cerebellar ataxia, hemiplegic migraine and stationary night blindness, no seizure phenotype was linked to a calcium channel gene in human pedigrees. In contrast, four spontaneous mutations connected with generalized absence epilepsy and cortical spike-wave discharges were identified in mice (tottering, lethargic, stargazer, and ducky) [Miller, 2001].

Historically, classification of calcium channels was based on their electrophysiology and pharmacology. L (long-lasting)-type of channels, consisting of α1C, α1D, α1F, α1S, α2δ and β3A subunits, are associated with strong depolarization. This group is sensitive to inhibition by dihydropyridine (DHP) derivatives, calciseptine (from black mamba snake) and calcicludine (from green mamba snake). (DHP) derivatives, calciseptine (from black mamba group is sensitive to inhibition by dihydropyridine [1C, 1D, 1F, 1S, 2 δ] subunit (extracellular α2 connected with intracellular δ protein). All three regulate the channel kinetics [Randall and Benham, 1999].

Although mutations in calcium channels in humans produce cerebellar ataxia, hemiplegic migraine and stationary night blindness, no seizure phenotype was linked to a calcium channel gene in human pedigrees. In contrast, four spontaneous mutations connected with generalized absence epilepsy and cortical spike-wave discharges were identified in mice (tottering, lethargic, stargazer, and ducky) [Miller, 2001].

In the experimental epilepsy, a variety of VDCC antagonists proved antiseizure activity. Substances blocking L- and, to a lesser degree, N-type channels, inhibit epileptogenesis, prevent electrically and chemically evoked convulsions, and potentiate the protective action of several antiepileptic drugs. However, L-type calcium blockers were quite ineffective in cocaine-induced seizures, and aggravated spike-wave discharges in absence epilepsy models. In contrast, drugs exerting antagonistic activity at T-type calcium channels are still the first choice medications in the course of absence epilepsy [Kulak et al., 2003].

Niguldipine, a DHP derivative, is an example of L-type calcium channel antagonists, exerting per se anticonvulsant properties, but attenuating the protective action of some conventional antiepileptics in electrically induced seizures in mice [Borowicz et al., 2002]. On the other hand, amlodipine showed relatively strong antiseizure potential, but produced significant motor impairment. Also clini-
cal trials, based on double blind and crossover studies, revealed poor anticonvulsive efficacy of DHP, PAA and BTZ derivatives [Ku³ak et al., 2003]. However, gabapentin, the antagonist of the regulatory α2δ, may indicate a new avenue in the search of calcium channel blockers with significant antisiezure profile. Under experimental conditions, the drug attenuated pentetrazole-induced seizures and showed synergistic interaction with several antiepileptic drugs in maximal electroshock test in mice [Borowicz et al., 2002]. In clinical practice, the adjunctive or monotherapeutic use of gabapentin led to significant improvement in patients with focal or secondarily generalized partial seizures.

TOPIRAMATE: A FUNCTIONAL POLYTHERAPY OF EPILEPSY

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Monotherapy of epilepsy possesses a number of advantages: no interactions between antiepileptic drugs (AEDs), less adverse effects, better control of therapeutic efficacy. Polytherapy has to be initiated when monotherapy fails. Functional polytherapy combines benefits of mono- and polytherapy and is based upon a concept of using one AED with multiple mechanisms of action.

Topiramate is an AED displaying a number of mechanisms for its anticonvulsant activity. For instance, this AED blocks sodium channels, enhances GABAergic neurotransmission by an increased influx of chloride ions into a neuron and elevated brain GABA concentration. Also, it is an antagonist of a subpopulation of glutamatergic receptors (AMPA/KA), without any activity towards NMDA receptors, blocks L-type calcium channels, and weakly reduces the activity of carbonic anhydrase. Recent evidence points to an effect of this AED upon potassium currents. Specifically, topiramate enhances the membrane permeability for potassium ions.

Multiple mechanisms of topiramate’s anticonvulsant activity probably result in its very high therapeutic efficacy in newly recognized cases of epilepsy. Following a 6-month therapy with this AED, 83% of epileptic patients were seizure free and after one year, still 76% of the patients remained free of epileptic symptoms. After all, undesired effects were less expressed when compared to other clinical trials with the use of polytherapy.

Apart from the topiramate’s high antiepileptic activity, this drug also exerts neuroprotective effects in a number of experimental models of epilepsy [Niebauer and Gruenthal, 1999]. Also, this effect has been recently confirmed in clinical conditions in cases of diabetic neuropathy [Vinik et al., 2003]. This indicates that topiramate may be in fact neuroprotective also in human epilepsy.

Summing up, functional polytherapy with topiramate may offer a number of benefits: high antiepileptic efficacy, broad spectrum of activity, no drug interactions, less adverse effects, and possibly neuroprotective activity.
ELECTROPHYSIOLOGY OF EPILEPTOGENESIS – IN VITRO MODELS

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The rodent in vitro brain slice preparations have been widely used to study activity of neuronal populations which resembles that observed during or between epileptic seizures in the patients. This review focuses on the mechanisms that account for epileptiform activity patterns which could be produced in the hippocampal slices. While the hyperexcitability of neurons could be induced by raising extracellular K\(^+\) concentration or the blockade of certain K\(^+\) channels, excessive discharge in a population of interconnected neurons could also be induced by an enhancement of glutamatergic excitatory synaptic transmission, mediated mainly through NMDA receptors or a blockade of inhibitory GABAergic transmission. Recent work has demonstrated the role of excitatory action of GABA\(_A\) receptors, mediated through bicarbonate inward currents, and the role of calcium release from intracellular stores in epileptiform discharges. Experimental evidence has also implicated the non-synaptic mechanisms in the synchronization of neuronal activity, among which the excitatory effects of the electrical field and gap-junctional communication predominate. While the involvement of these mechanisms in naturally occurring epilepsy remains to be established, genetically determined alterations in ion channels have been found in certain human epileptic patients.

INTERACTIONS BETWEEN LOSIGAMONE AND CONVENTIONAL ANTIEPILEPTIC DRUGS: AN ISOBIOLOGraphic ANALYSIS

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Losigamone (LSG) is one of new antiepileptic drugs (AEDs) exerting considerable anticonvulsive activity in both in vitro and in vivo studies. The exact mechanism of its action remains unclear. The overall antiseizure activity of LSG may be due to inhibition of Na\(^+\) and/or Ca\(^{2+}\) inward currents. Activation of K\(^+\) channels and GABA\(_A\) receptors is also considered. At high concentrations, LSG inhibited glutamate/aspartate release and adenosine uptake from mouse cortex slices. Apart from anticonvulsive activity, LSG was found to exert anxiolytic, antidepressant and memory enhancing effects in a variety of animal models. Pharmacokinetics of LSG occurred linear in both healthy volunteers and epileptic patients. Cytochrome CYP2A6 appears to be the main isoenzyme responsible for the metabolism of LSG. No harmful undesired effects or teratogenic risk were observed in either animal or human studies. In clinical trials, the drug exhibited effectiveness in the treatment of highly refractory partial seizures (with or without secondary generalized seizures) [Stein, 1995; Stein et al., 1991].

The aim of the presented study was the isobioGraphic evaluation of pharmacodynamic interactions between LSG and conventional AEDs, valproate, carbamazepine, diphenylhydantoin and phenobarbital, against maximal electroshock-induced convulsions in mice. IsobioGraphic analysis is considered to be the optimal method to detect synergy (supra-additivity), additive interaction, or an-
tagonism (infra-additivity) in animal models of epilepsy. To perform the isobolographic analysis, the mixtures of LSG with an AED were co-administered at three fixed dose ratios of 1:3, 1:1 and 3:1.

Results evidently indicate that LSG acts synergistically with valproate. Moreover, the combinations between LSG and carbamazepine or diphenylhydantoin proved pure additive interactions, whereas the combinations of LSG with phenobarbital were either additive (for dose ratios of 1:3 and 1:1), or antagonistic (for the 3:1 proportion).

Moderate motor impairment (evaluated in the chimney test) was noted only in the case of valproate given at its ED50. However, valproate did not affect long-term memory (evaluated in the passive-avoidance task). LSG, carbamazepine, diphenylhydantoin and phenobarbital (applied at doses equal to their ED50 values), as well as the combinations between LSG and AEDs (including valproate) did not produce any significant undesired effects in tested animals.

In immunofluorescence assays, LSG did not affect brain levels of valproate and phenobarbital. However, it elevated brain concentrations of carbamazepine and diphenylhydantoin.

The obtained data suggest that co-administration of LSG with valproate might be promising from an experimental standpoint for further clinical evaluation. As far as the experimental data could be extrapolated into clinical practice, such combination might provide the adequate seizure control in refractory epilepsy. On the contrary, combined LSG/phenobarbital treatment should be avoided in epileptic patients, particularly in the case of LSG prevalence.

MOLECULAR MECHANISMS OF ANTIEPILEPTIC DRUGS

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It is a quite common opinion that new antiepileptic drugs show broad spectrum of action, beneficial pharmacokinetics, do not interact with other drugs and are devoid of severe undesired effects. However, at least 25% of epileptic patients remain drug resistant. Moreover, one should be aware that progress in the field of anticonvulsants within the last three decades has been largely limited to more selective regulations of long known neurochemical mechanisms. Indeed, the blockers of voltage-dependent sodium channels and drugs enhancing GABA A receptor activity are still the most widely used group of anticonvulsants. Drugs which bind directly to GABA recognizing sites on GABA A receptor complex proved disappointing, whereas positive modulators of that receptors, e.g. benzodiazepines, are clinically useful. Moreover, some partial agonists of benzodiazepine receptors, are thought to possess anticonvulant activity without producing tolerance. Modulators of iono(NMDA, kainate/AMPA)- and metabotropic excitatory amino acid receptors are considered to be an attractive target in the search for new anticonvulsants, however, some of them possess numerous unwanted effects. It has been suggested that high efficacy partial agonists of glycine B receptor and competitive NMDA receptor antagonists may have advantages over noncompetitive NMDA antagonists and glycine B receptor antagonists as potential antiepileptic drugs (Wlaź, Brain Res. Bull., 1998, 6, 535–540). On the other hand, analysis of some receptor and ion channel mutations which lead to seizures, e.g. sodium (SCN1B, SCN1A), potassium (KCNQ2, KCNQ3, KCNA1) and calcium (α6,δ2) channel subunits, may provide a valuable information for designing new anticonvulsants. Looking for future directions in the pharmacotherapy of epilepsy, one has to stress the necessity to characterize molecular targets for antiepileptic drugs with unknown mechanism of action discovered as a result of screening studies. Thus, there is a great hope that recent development of genetic and proteomic strategies will help to create new, effective anticonvulsants.
SOME SELECTED TWO-DRUG COMBINATIONS OF
ANTIEPILEPTIC DRUGS IN EXPERIMENTAL AND CLINICAL
STUDIES

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Epilepsy is a common serious neurological disorder affecting 1% of human population worldwide. In spite of a progress in the understanding of epileptogenesis and profound knowledge concerning the mechanism(s) of action of available antiepileptic drugs (AEDs), there are still around 30% of patients inadequately medicated with current frontline AEDs in monotherapy. Thus, about 120,000 of epileptic patients in Poland are refractory to the applied monotherapy. In some multicenter randomized clinical studies, it has been proved that the addition of a second or a third AED to unsuccessful monotherapy may provide 14% of patients with full protection against seizures [Kwan and Brodie, 2000]. Hence, polytherapy may be effective in approximately 56,000 patients with refractory seizures in Poland [Majkowski, 1996].

In clinical practice, the evaluation of efficacy of AED combinations is drastically limited due to some ethical reasons related with combining the AEDs in humans. Generally, it is ethically unacceptable to test primarily some combinations of AEDs directly in humans without previous studies on animals, because of a possibility of appearance of unexpected side-effects or enhanced AEDs’ toxicity. Moreover, with the advent of some novel AEDs (lately introduced into the therapy of epilepsy) the number of possible combinations increases extensively. This number of AED combinations is described by the equation as follows:

\[ C = \binom{n}{k} = \frac{n!}{(n-k)!k!} \]

where C is a total number of AED combinations, \( n \) – total number of available AEDs, \( k \) – number of drugs in each combination (usually, two AEDs in combination), and ! is the factorial. For instance, 10 AEDs give only 45 various two-drug combinations, whereas 20 different AEDs generate 190 two-drug combinations, which may occur advantageous in clinical practice. In such a situation, only experimental studies on animals can provide a full analysis of the efficacy of possible combinations among AEDs. In animal models of epilepsy (i.e. maximal electroshock- or pentetrazole-induced seizure tests), it is quite easily to assess the interactions between two AEDs, especially when an isobolographic analysis is applied. From a preclinical point of view, only combinations displaying synergy in animals should be clinically verified in patients with drug resistant epilepsy. Other combinations, especially these showing antagonism in animal models of epilepsy need not have been tested in humans.

Basing upon several multicenter randomized clinical studies, a list of 10 most effective two-AED combinations has been established [Stephen and Brodie, 2000]. Among them, combinations of Phenobarbital (PB) + Phenytoine (DPH); Carbamazepine (CBZ) + Valporate (VPA); DPH + CBZ; PB + CBZ; VPA + Lamotrigine (LTG); LTG + Topiramate (TPM); Gabapentine (GBP) + CBZ; TPM + CBZ; CBZ + Vigabatrine (VGB) and LTG + CBZ, have provided the patients with drug resistant epilepsy with a status of seizure-free over 1 year on polytherapy [Stephen and Brodie, 2000].

Isobolographic experiments have displayed that combinations of VPA + LTG; LTG + TPM; GBP + CBZ and TPM + CBZ were synergistic against the maximal electroconvulsions in mice. Moreover, the combinations between conventional AEDs (i.e. PB + DPH; CBZ + VPA; DPH + CBZ; and PB + CBZ) were barely additive in isobolography. The observed interactions and their effectiveness in humans have been confirmed in animal models of epilepsy. In contrast, the combination of LTG + CBZ was antagonistic in the maximal electroshock test in mice [Łuszczki et al., 2003]. Thorough review of medical literature, concerning the patients on add-on therapy with LTG and CBZ, has revealed that this combination in some patients had worsened seizures. In patients receiving CBZ in
monotherapy, it was observed, that the addition of
LTG aggravated seizures, increasing epileptic at-
tacks by over 50% [JóŸwiak and Terczyñski, 2000].
Therefore, considering both, human clinical studies
and isobolographic animal experiments, it seems
that combination of LTG+CBZ should be rather
avoided in clinical practice, albeit it was initially
classified as the advantageous combination. This
fact proves the exactitude of isobolographic analy-
sis in the detection of a character of interactions
among AEDs. With isobolography, one can easily
preselect the combinations of AEDs in vivo, in ani-
mal models of epilepsy and precisely choose some
combination with synergic interactions, offering
full protection against seizures. The results from
isobolographic studies are quite similar to the inter-
actions observed in humans.
Summing up, it is worth noting that using the iso-
bolographic analysis, a character of observed inter-
actions, perfectly correlating with clinical practice, may
be assessed. Hence, by comparing the animal and
clinical studies, the exactness of this method in
screening and searching for the advantageous combi-
nations, worth further clinical recommendation, has
been proven. No doubt that the experiments on ani-
mals give full insight into a character of the observed
interactions, which may very likely appear in clinical
practice. Additionally, the use of animals for testing
of some promising combinations with isobolo-
graphic analysis has been evidently substantiated.

ISOBOLOGIC ANALYSIS OF ZONISAMIDE COMBINED
WITH CONVENTIONAL ANTIEPILEPTIC DRUGS IN MICE

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Although the significant progress in pharma-
cotherapy of epilepsy during last decade was achieved,
about one third of patients are resistant to the current
treatment. When the monotherapy is not efficient, the
polytherapy should be applied. One of the new antie-
pileptic drugs (AEDs) used in refractory epilepsy
treatment is zonisamide (1,2-benzisoxazole-3-me-
thanesulfonamide; Zonegran; Excegran, ZNS),
a sulfonamide derivative available in Japan for
over a decade and marketed in USA from 2000. Its
efficacy in various types of seizures was confirmed
in different animal models of epilepsy as well as in
clinical conditions. ZNS exerts a broad spectrum of
antiepileptic activity and is effective in the treat-
ment of generalized and partial refractory seizures.
Mechanism of action of the drug is complex. ZNS in-
hbits voltage-dependent Na⁺ channels and Ca²⁺
channels of T-type. Another postulated mechanism is
associated with the blockade of K⁺- evoked
 glutamate-mediated synaptic excitation. The drug in-
fluences also monoamine neurotransmission, has the
inhibitory effect on the excessive nitric oxide pro-
duction and is characterized by free radicals scav-
enging properties. These effects are responsible not
only for its antiepileptic, but also neuroprotective
activity. ZNS was also described as an inhibitor of
carbonic anhydrase. Although some authors hy-
pothesized that central benzodiazepine receptors
are specific for ZNS binding, it was evidenced that
ZNS does not potentiate GABA_A receptor-related
events. The drug presents favorable pharmacokinetic
profile with excellent oral bioavailability, great
blood-brain barrier penetration and, moreover, it does
not influence liver enzymes. Furthermore, ZNS treat-
ment, comparable to other anticonvulsants, is rela-
tively safe and well tolerated. Since ZNS is often
used in polytherapy, its interactions with other AEDs
seem to be of particular importance. However, the ex-
perimental data are rather inconsistent. In the re-
search, the isobolographic analysis of interactions of
new AED: ZNS and conventional AEDs: valproate
(VPA), diphenylhydantoin (DPH), carbamazepine
(CBZ) and phenobarbital (PB) in maximal electroshock (MES) in mice was performed. The aim of the work was to determine the type of pharmacodynamic interactions between ZNS and conventional AEDs, to estimate the undesired effects of ZNS and its combinations with conventional AEDs and also to detect the pharmacokinetic interactions of the drug combinations.

The research was performed on Swiss mice using the model of MES. The mixtures of the drugs at three ratios: 3:1, 1:1, 1:3 were administered intraperitoneally in the peak time of their anticonvulsant action. The undesired effects were assessed by the passive avoidance test and chimney test. To evaluate the pharmacokinetic interactions, the immunofluorescence estimation of brain concentration of conventional AEDs was performed. The precise determination of interaction type was possible by using the isobolographic analysis.

The anticonvulsant activity of the studied drugs was proven in MES test. The synergistic interactions were observed between ZNS and VPA at a ratio 1:1 as also ZNS and DPH at a ratio 1:1. The interactions of other studied combinations (ZNS and VPA at ratios 3:1, 1:3, ZNS and DPH at ratios 3:1, 1:3, ZNS and CBZ at ratios 3:1, 1:1, 1:3, ZNS and PB 3:1, 1:1, 1:3) proved to be additive. The pharmacokinetic interactions dependent on ZNS influence on brain concentrations of conventional AEDs were excluded. Conventional AEDs and ZNS and also their combinations at the ED$_{50}$ dose did not cause disorders of motor coordination and long-term memory in mice.

Experimental research with isobolographic analysis of interactions between AEDs used in combination allow for development of the new strategy of rational polytherapy of epilepsy and better control of seizures. The results of this work suggest that synergism of combination of ZNS and VPA (at a ratio 1:1) as also ZNS and DPH (at a ratio 1:1) should be confirmed in clinical research concerning efficacy of drugs used in epilepsy polytherapy. Differences in interaction type of the same drug combination depend on drug dose ratio, that underlines the significance of exact dose ratio selection and allows to identify right drug dose in drug combinations.

**EFFECT OF ADENOSINE NEUROMODULATION ON THE EFFICACY OF ANTICONVULSANT DRUGS AGAINST 3-NITROPROPIONIC ACID-INDUCED SEIZURES IN MICE**

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3-Nitropropionic acid (3-NPA), a mitochondrial toxin found in numerous plants and fungi, irreversibly inhibits the activity of the succinate dehydrogenase, what leads to the disruption of mitochondrial oxidative phosphorylation [Ludolph et al., 1991]. Increasing evidence suggests that neuronal mitochondrial dysfunction might contribute to the altered cellular functions, and finally cause neuronal injury or death [Urbańska et al., 1998]. Chronic peripheral administration of relatively low doses of 3-NPA, or its local intrastriatal application were demonstrated to evoke selective neuronal loss within striatum. These 3-NPA-induced changes show striking similarities to the neuropathologic and neurochemical features of Huntington’s disease [Borlongan et al., 1997]. Increasing body of evidence suggests that the impaired energy metabolism initiates also a cascade of metabolic events leading to the initiation and propagation of convulsions and to the seizure-related neuronal loss [Lees, 1993].

Adenosine is a potent neuromodulator exerting depressant effects on neuronal excitability [Dunvidia and Worth, 1982]. Adenosine and its analogues
interacting with specific membrane receptors of A<sub>1</sub>-type may influence the release of excitatory amino acids and other neurotransmitters or directly inhibit spontaneous neuronal firing and synaptic transmission [Fredholm, 1997]. The protective effects of adenosine and its analogues were demonstrated in various seizure models, e.g. those determined genetically, generated by electric current and induced chemically, by bicuculline, pentetetrazole, N-methyl-D-aspartate, kainate or pilocarpine [De Mendoca et al., 2000].

The aim of the present study was to evaluate the effect of adenosinergic neurotransmission on seizures evoked by mitochondrial toxin, 3-NPA. The studies were carried out on male Albino-Swiss mice. Convulsions were evoked by intraperitoneal (ip) administration of 3-NPA at the dose of 210 mg/kg or intracerebroventricular (icv) application of 3-NPA at the dose of 3 μmol in mice.

The obtained results indicate that a nonselective A<sub>1</sub>/A<sub>2</sub> receptor agonist, 2-CADO, displayed dose-dependent anticonvulsant activity against 3-NPA-induced seizures with ED<sub>50</sub> of 3.5 (1.7–7.1) mg/kg. Similarly, a selective A<sub>1</sub> adenosine agonist, R-PIA, protected animals from 3-NPA-evoked convulsions displaying ED<sub>50</sub> of 1.5 (0.7–3.3) mg/kg. Both agonists prolonged the latency to the onset of 3-NPA-evoked seizures and reduced the mortality caused by 3-NPA. icv administration of A<sub>1</sub>/A<sub>2</sub> receptor agonist, 5'-N-ethylcarboxamidoadenosine (NECA), prevented mice from the development of seizures induced by the intracerebral injection of 3-NPA with ED<sub>50</sub> of 0.3 (0.03–2.4) nmol and this effect was reversed by co-administration of an adenosine receptor antagonists, 8-(p-sulfophenyl)theophilline.

A nonselective adenosine receptor antagonist, aminophylline, as well as a selective adenosine A<sub>1</sub> receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), given ip at the non-convulsive dose simultaneously with 3-NPA, reversed the protective action of adenosine receptor agonists, 2-CADO and R-PIA, in seizures evoked by systemic 3-NPA administration. The ED<sub>50</sub> value of R-PIA increased from 1.5 (0.7–3.3) to 10.7 (5.3–21.5) (p < 0.01) and to 7.0 (3.5–21.5) (p < 0.001) mg/kg, respectively. In contrast, A<sub>1</sub>/A<sub>2</sub> adenosine receptor antagonist 8-(p-sulphophenyl)theophilline, which does not cross blood-brain barrier, did not alter protective effects of 2-CADO and R-PIA.

Moreover, non-selective adenosine receptor antagonist, aminophylline and DPCPX, but not 8-(p-sulphophenyl)theophilline, were found to attenuate anticonvulsive activity of diazepam, phenobarbital, valproic acid and gabapentin in seizures caused by systemic application of 3-NPA. Aminophylline diminished the anticonvulsive activity of diazepam, phenobarbital, valproate and gabapentin against 3-NPA-evoked seizures increasing their ED<sub>50</sub> values from 4.9 (3.1–7.6), 28.5 (19.7–41.3), 315.6 (224.1–444.5) and 270.2 (207.4–352.1) to 14.1 (10.5–18.8) (p < 0.01), 63.9 (55.5–73.5) (p < 0.001), 516.9 (445.0–600.4) (p < 0.01) and 581.7 (493.3–598.7) (p < 0.001) mg/kg, respectively. Similarly, selective A<sub>1</sub> adenosine receptor antagonist, DPCPX, attenuated the anticonvulsive activity of diazepam, phenobarbital, valproate and gabapentin against 3-NPA-evoked seizures increasing their ED<sub>50</sub> values from 5.6 (3.3–9.7), 34.8 (19.6–61.6), 339.7 (265.4–434.8), 270.2 (207.4–352.1) to 10.3 (7.3–14.6) (p < 0.05), 60.5 (51.9–70.5) (p < 0.05), 594.3 (517.0–683.1) (p < 0.001), 734.7 (646.9–834.5) (p < 0.001) mg/kg, respectively.

The obtained results indicate that impairment of the central adenosinergic modulation seems to be one of the mechanisms underlying the anticonvulsant action of drugs and substances effective in the model of seizures evoked by the mitochondrial toxin, 3-NPA. In the view of the presented data, it might be concluded that protective effects of diazepam, phenobarbital, valproic acid and gabapentin may depend on the central stimulation of adenosine A<sub>1</sub> receptors. Moreover, when these anticonvulsants are administered together with aminophylline, their clinical antiepileptic efficacy might be reduced, especially among patients suffering from seizures related to the disturbances of mitochondrial respiratory chain.