**Pharmacological Reports**
2005, 57, 787–794
ISSN 1734-1140



# 2-Chloro-$N^6$-cyclopentyladenosine enhances the anticonvulsant action of carbamazepine in the mouse maximal electroshock-induced seizure model[A]

Jarogniew J. Łuszczki[1,*], Maria Kozicka[2], Mariusz J. Świąder[2], Stanisław J. Czuczwar[1,3]

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

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

[3]Department of Physiopathology, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950, Lublin, Poland

**Correspondence:** Jarogniew J. Łuszczki, e-mail: jarogniew.luszczki@am.lublin.pl
* Recipient of the Fellowship for Young Researchers from the Foundation for Polish Science.

**Abstract:**
This study examines the anticonvulsant profile of interactions between 2-chloro-$N^6$-cyclopentyladenosine (CCPA, a selective adenosine $A_1$ receptor agonist) and four conventional antiepileptic drugs (AEDs: carbamazepine – CBZ, phenobarbital, phenytoin and valproate) in the mouse maximal electroshock seizure (MES) model. Acute adverse effects produced by AEDs in combination with CCPA were determined in the chimney test (motor performance) and passive avoidance task (long-term memory).
Results indicate that CCPA administered alone at 0.25 and 0.5 mg/kg significantly elevated the electroconvulsive threshold in mice. Additionally, the agent at a sub-threshold dose of 0.125 mg/kg potentiated the anticonvulsant activity of CBZ by reducing its $ED_{50}$ in the MES test from 11.2 to 7.7 mg/kg ($p < 0.01$). In contrast, 8-cyclopentyl-1,3-dimethylxanthine (DPCPX, a selective adenosine $A_1$ receptor antagonist at 5 mg/kg) abolished the enhanced anticonvulsant effects offered by the combination of CBZ with CCPA (0.125 mg/kg). Moreover, CCPA (0.125 mg/kg) co-administered with other tested AEDs had no significant impact on their antiseizure properties in the MES test in mice. Neither CCPA (0.125 mg/kg) administered singly, nor in combinations with conventional AEDs (at their $ED_{50}$s) affected motor performance in the chimney test and long-term memory in the passive avoidance task. No pharmacokinetic alterations in brain CBZ concentrations were observed after administration of CCPA at 0.125 mg/kg.
It may be concluded that CCPA, acting selectively on adenosine $A_1$ receptors, enhances pharmacodynamically the antiseizure effect of CBZ in the MES test.

**Key words:**
2-Chloro-$N^6$-cyclopentyladenosine, carbamazepine, maximal electroshock, adenosine $A_1$ receptors, antiepileptic drugs

**Abbreviations:** AED – antiepileptic drug, CBZ – carbamazepine, CCPA – 2-chloro-$N^6$-cyclopentyladenosine, CNS – central nervous system, DPCPX – 8-cyclopentyl-1,3-dipropylxanthine, GABA – γ-aminobutyric acid, MES – maximal electroshock, MEST – maximal electroshock seizure threshold, NMDA – N-methyl-D-aspartate, PB – phenobarbital, PHT – phenytoin, VPA – valproate.

## Introduction

Overwhelming evidence indicates that endogenous adenosine and its analogues possess the anticonvulsant activity and reduce convulsions induced by a variety of chemical and electrical stimuli in various *in vivo* and *in vitro* models of epilepsy [8, 11, 15, 16, 18,

[A] The results of this study were presented in part at "*15th International Congress of the Polish Pharmacological Society" held in Poznań, Poland, on September 12–14, 2004 (abstract available in Pol J Pharmacol, 2004, 56, Suppl, 71–72).*

57]. To date, four adenosine receptor subtypes, named $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$, have been identified and cloned from mammalian tissues [20]. Electrophysiological and neurochemical studies revealed that activation of adenosine $A_1$ receptors inhibited the release of excitatory amino acids [9], which then contributed to the suppression of seizure and neuroprotection [2, 9, 16, 17, 19]. In the central nervous system (CNS), the adenosine $A_1$ receptors are mainly localized in the cerebral cortex, cerebellum and in the hippocampus [20, 42] and their activation results in opening $K^+$ channels, which induces hyperpolarizing effects and reduction in excitability of postsynaptic neurons [20, 50].

Experimental evidence indicates that 2-chloro-$N^6$-cyclopentyladenosine (CCPA – the most selective adenosine $A_1$ receptor agonist) reduces convulsions in various models of epilepsy. For instance, it has been reported that CCPA, administered both singly and repeatedly (twice daily for 7 days), possesses the antiseizure activity, protecting genetically-prone DBA/2 mice against sound-induced seizures [12]. Moreover, the protective effects of CCPA have been shown in pentetrazole-induced seizures [1] and against pilocarpine-induced convulsions [26] in rats. Relatively recently, CCPA has been found to suppress seizures in the rat model of audiogenic brainstem epilepsy [24], as well as in the mouse model of pharmacoresistant epilepsy induced by intrahippocampal injection of kainic acid [22].

The present study was aimed at determining the influence of CCPA on the anticonvulsant activity of four conventional antiepileptic drugs (AEDs: carbamazepine – CBZ, phenobarbital – PB, phenytoin – PHT, and valproate – VPA) against maximal electroshock-induced seizures (MES) in mice. Additionally, the effects of CCPA and its combinations with conventional AEDs in relation to motor impairment and long-term memory alteration were evaluated by the use of the chimney test and step-through passive avoidance task, respectively. Finally, total brain CBZ concentrations were measured so as to ascertain whether the observed effects were consequent to a pharmacodynamic and/or a pharmacokinetic interaction.

## Materials and Methods

### Animals and experimental conditions

All experiments were performed on adult male albino Swiss mice weighing 22–26 g. The mice were kept in colony cages with free access to food and tap water *ad libitum*, under standardized housing conditions (12 h of a light-dark cycle, temperature was 21 ± 1°C, relative humidity of 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of eight mice. Each mouse was used only in one experiment. All tests were performed between 9.00 a.m. and 3.00 p.m. Procedures involving animals and their care were conducted in conformity with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this manuscript were approved by the Local Ethics Committee at the Medical University of Lublin.

### Drugs

The following AEDs and substances were used in this study: CBZ (gift from Polfa, Starogard, Poland); CCPA [2-chloro-$N^6$-cyclopentyladenosine] and DPCPX [8-cyclopentyl-1,3-dipropylxanthine] (both from RBI, Natick, MA, USA), PB (Polfa, Kraków, Poland), PHT (Polfa, Warszawa, Poland) and VPA (gift from ICN-Polfa, Rzeszów, Poland). All drugs (except for VPA) were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline, whereas VPA was dissolved in sterile saline. All drugs were administered systemically by *ip* injection in a volume of 0.005 ml/g. Fresh drug solutions of conventional AEDs, CCPA and DPCPX were prepared *ex tempore* on each day of experimentation and administered as follows: PHT – 120 min, DPCPX and PB – 60 min, CBZ, CCPA and VPA – 30 min before electroconvulsions, motor coordination, long-term memory tests, as well as, before brain sampling for the measurement of CBZ concentrations. These pretreatment times were chosen based upon information about their biological activity from the literature and from our previous study [4].

### Maximal electroshock seizure threshold (MEST)-test

Electroconvulsions were produced by means of an alternating current (0.2 s stimulus duration, 50 Hz) delivered *via* ear-clip electrodes by a Hugo Sachs stimulator (Rodent Shocker, Type 221, Freiburg, Germany). The electrical system of the stimulator was

self-adjustable so that changes in impedance did not result in alterations of current intensity (i.e. the system provides constant current stimulation). The criterion for the occurrence of seizure activity was the tonic hindlimb extension (i.e. the hind limbs of animals outstretched 180° to the plane of the body axis). In order to evaluate the threshold for electroconvulsions, at least 4 groups of mice, consisting of 8 animals per group, were challenged with electroshocks of various intensities to yield 10–30%, 30–50%, 50–70%, and 70–90% of animals with seizures. Then, a current intensity-effect curve was constructed, according to a log-probit method by Litchfield and Wilcoxon [29], from which a $CS_{50}$ (median current strength in mA with 95% confidence limits) was estimated. Each $CS_{50}$ value represents the current intensity required to induce tonic hindlimb extension in 50% of the mice challenged. The threshold for electroconvulsions was denoted for 3 different doses of CCPA, as follows: 0.125, 0.25 and 0.5 mg/kg. The experimental procedure has been described in more detail in our earlier studies [32, 33, 44].

### Maximal electroshock seizure (MES)-test

The anticonvulsant activities of conventional AEDs administered alone and in combination with CCPA were evaluated as their median effective doses ($ED_{50}$s in mg/kg) against maximal electroconvulsions (fixed current intensity of 25 mA, maximum stimulation voltage of 500 V). The animals were administered with different AED doses to obtain a variable percentage of protection against MES-induced seizures, allowing the construction of a dose-effect line. Subsequently, the $ED_{50}$s with their 95% confidence limits were calculated according to the log-probit method by Litchfield and Wilcoxon [29]. The experimental procedure has been described in more detail in our earlier studies [32, 34, 44].

### Measurement of total brain CBZ concentrations

Brain CBZ concentrations were determined in mice that were administered CBZ + vehicle and the combination of CBZ + CCPA. Mice were killed by decapitation at times chosen to coincide with that scheduled for the MES test and whole brains were removed from skulls, weighed, and homogenized using Abbott buffer (2:1 vol/weight; Abbott Laboratories, North Chicago, IL, USA) in an Ultra-Turrax T8 homogenizer (Staufen, Germany). The homogenates were centrifuged at 10,000 × g for 10 min and the supernatant samples (75 μl) were analyzed by fluorescence polarization immunoassay using a TDx analyzer and reagents exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). The total brain CBZ concentrations were expressed in μg/ml of brain supernatants as the means ± SD of at least eight separate brain preparations.

### Chimney test

The effects of conventional AEDs, CCPA and their combinations on motor performance were quantified with the chimney test of Boissier et al. [3]. In this test, animals had to climb backwards up a plastic tube (3 cm in inner diameter, 25 cm long), and motor impairment was indicated by the inability of the animals to climb backward up the transparent tube within 60 s.

### Light-dark, step-through passive avoidance task

Animals were administered conventional AEDs either singly or in combination with CCPA on the first day before training. The time before the commencement of the training session (after the drug administration) was identical to that for the MES test. Subsequently, the animals were placed in an illuminated box (10 × 13 × 15 cm) connected to a larger dark box (25 × 20 × 15 cm) equipped with an electric grid floor. The animals entering the dark box were punished by an adequate electric footshock (0.6 mA for 2 s). The mice that did not enter the dark compartment were excluded from the experiment. On the next day (24 h later), the pre-trained animals (without treatment) were placed again into the illuminated box and observed up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The time that elapsed before the mice entered the dark box, was recorded and subsequently, the median latencies with 25th and 75th percentiles were calculated. The step-through passive avoidance task gives information about ability to acquire the task (learning) and to recall the task (retrieval). Therefore, it may be regarded as a measure of long-term memory [56]. This experimental procedure has been described in detail in our earlier study [35, 36].

### Statistical analysis

Both median current strengths ($CS_{50}$s) and median effective doses ($ED_{50}$s) with their 95% confidence limits were calculated and statistically analyzed by computer log-probit analysis according to Litchfield and Wilcoxon [29]. Total brain CBZ concentrations were analyzed using the unpaired Student's *t*-test. Qualitative variables from the chimney test were compared with Fisher's exact probability test, whereas, the results obtained in the step-through passive avoidance task were statistically evaluated using the Kruskal-Wallis nonparametric ANOVA test followed by Dunn's multiple comparisons test.

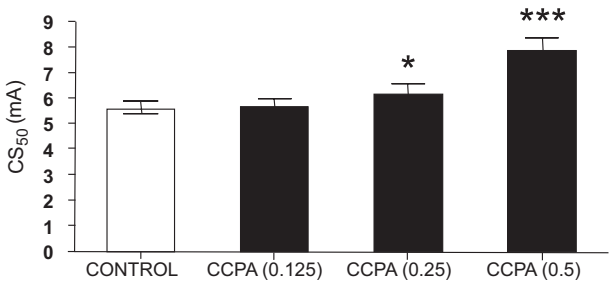


**Fig. 1.** Effect of 2-chloro-$N^6$-cyclopentyladenosine (CCPA) on the electroconvulsive threshold in mice. Data are presented as median current strengths ($CS_{50}$s with 95% confidence limits as the error bars) required to evoke tonic hindlimb extension in 50% of animals subjected to the maximal electroshock seizure threshold (MEST)-test. CCPA (2-chloro-$N^6$-cyclopentyladenosine) was administered *ip* 30 min before the MEST-test. Statistical analysis of data was performed using log-probit method [29]; * $p < 0.05$; *** $p < 0.001$ *vs.* control (vehicle-treated) animals

## Results

### Effect of CCPA on the electroconvulsive threshold

CCPA (administered *ip*, 30 min prior to the test) raised the threshold for electroconvulsions in a dose-dependent manner. It was found experimentally that CCPA at 0.25 mg/kg significantly elevated the electroconvulsive threshold in mice from 5.6 (5.4–5.9) to 6.2 (5.8–6.7) mA ($p < 0.05$; Fig. 1). Similarly, the $A_1$ adenosine receptor agonist – CCPA, administered at 0.5 mg/kg, significantly increased its $CS_{50}$ value from 5.6 (5.4–5.9) to 7.9 (7.3–8.5) mA ($p < 0.001$; Fig. 1). In contrast, CCPA administered at a lower dose of 0.125 mg/kg had no impact on the electroconvulsive threshold in mice (Fig. 1).

**Tab. 1.** Effect of 2-chloro-$N^6$-cyclopentyladenosine (CCPA) on the anticonvulsant action of carbamazepine (CBZ) in the mouse maximal electroshock-induced seizure model

| Treatment (mg/kg) | $ED_{50}$ (mg/kg) |
|---|---|
| CBZ + vehicle | 11.2 (9.8–12.9) |
| CBZ + CCPA (0.0675) | 9.7 (8.1–11.8) |
| CBZ + CCPA (0.125) | 7.7 (6.0–9.8)** |
| CBZ + CCPA (0.125) + DPCPX (5) | 10.4 (8.7–12.5) |

Data are presented as median effective doses ($ED_{50}$s with 95% confidence limits in parentheses) protecting 50% of animals tested against MES-induced seizures. CBZ and CCPA were administered 30 min, whereas DPCPX (8-cyclopentyl-1,3-dipropylxantine) was given 60 min prior to the maximal electroconvulsions. Statistical analysis was performed using log-probit method according to Litchfield and Wilcoxon [29]. ** $p < 0.01$ *vs.* control ([CBZ + vehicle]-treated) animals

### Effect of CCPA in combinations with conventional AEDs on MES-induced seizures

CCPA (at a sub-threshold dose of 0.125 mg/kg) potentiated the anticonvulsant effects of CBZ by reducing its $ED_{50}$ value from 11.2 (9.8–12.9) to 7.7 (6.0–9.8) mg/kg ($p < 0.01$; Tab. 1). The agent, administered at a lower dose of 0.0625 mg/kg did not enhance the protective action of CBZ against MES-induced seizures, leaving the $ED_{50}$ of the latter drug almost intact (Tab. 1). To prove a specific adenosine $A_1$ receptor-mediated potentiation of the antiseizure

**Tab. 2.** Influence of 2-chloro-$N^6$-cyclopentyladenosine (CCPA) on the anticonvulsant action of phenobarbital (PB), phenytoin (PHT) and valproate (VPA) against maximal electroconvulsions in mice

| Treatment (mg/kg) | $ED_{50}$ (mg/kg) |
|---|---|
| PB + vehicle | 26.1 (22.4–30.5) |
| PB + CCPA (0.125) | 21.7 (17.5–26.8) |
| PHT + vehicle | 11.1 (9.4–13.2) |
| PHT + CCPA (0.125) | 10.7 (9.0–12.7) |
| VPA + vehicle | 281.3 (255.4–309.9) |
| VPA + CCPA (0.125) | 257.5 (217.9–304.4) |

Data are presented as median effective doses ($ED_{50}$s with 95% confidence limits in parentheses) protecting 50% of animals tested against MES-induced seizures. All AEDs and CCPA were administered *ip* at the times corresponding to their time of peak drug effect, as follows: PHT at 120 min, PB – 60 min, VPA and CCPA – 30 min prior to the MES test. Statistical evaluation of the data was performed using log-probit method according to Litchfield and Wilcoxon [29]

effects of CBZ by CCPA, the animals were co-administered with CBZ, CCPA (0.125 mg/kg) and DPCPX (5 mg/kg). In such cases, the anticonvulsant effects offered by the combination of CBZ and CCPA were reversed by DPCPX (Tab. 1).

In contrast, CCPA (0.125 mg/kg) co-administered with the remaining AEDs (PB, PHT and VPA) did not enhance their antiseizure properties in the MES test in mice. The experimentally-derived $ED_{50}$s for AEDs administered alone and in combination with CCPA (0.125 mg/kg) are presented in Table 2.

### Motor coordination impairment and long-term memory testing

The combinations of CCPA (0.125 mg/kg) and AEDs (at their $ED_{50}$s) had no impact on motor coordination in the chimney test in mice (Tab. 3). Likewise, the co-administered drugs did not alter long-term memory in animals challenged with the step-through passive avoidance task (Tab. 3).

**Tab. 3.** Influence of 2-chloro-$N^6$-cyclopentyladenosine (CCPA) in combination with conventional antiepileptic drugs on motor performance and long-term memory in mice

| Treatment (mg/kg) | Motor deficits (%) | Retention time (s) |
|---|---|---|
| Control (vehicle) | 0 | 180 (180–180) |
| CCPA (0.125) + vehicle | 0 | 180 (180–180) |
| CBZ (7.7) + vehicle | 0 | 180 (180–180) |
| CBZ (7.7) + CCPA (0.125) | 0 | 180 (180–180) |
| PHT (10.7) + vehicle | 0 | 180 (180–180) |
| PHT (10.7) + CCPA (0.125) | 0 | 180 (180–180) |
| PB (21.7) + vehicle | 0 | 180 (180–180) |
| PB (21.7) + CCPA (0.125) | 0 | 180 (180–180) |
| VPA (257.5) + vehicle | 20 | 180 (165.5–180) |
| VPA (257.5) + CCPA (0.125) | 30 | 175.5 (120–180) |

Data are presented either as percentage of mice showing motor impairment or as median retention time (in s; with 25th and 75th percentiles in parentheses) of 10 animals challenged with the chimney and step-through passive avoidance tests, respectively. Motor deficits in mice in the chimney test were expressed as the inability of the animals to climb backwards up the plastic tube within 60 s. In the passive avoidance task, the mice should avoid the entrance into the darkened compartment within 180 s. Impairment of long-term memory is evident if the mice enter the dark box before this cutoff time. Statistical analysis of the data was performed using either the Fisher's exact probability test (chimney test) or nonparametric Kruskal-Wallis ANOVA followed by *post-hoc* Dunn's test (passive avoidance task)

### Brain CBZ concentrations

Total brain concentration of CBZ administered singly at 7.7 mg/kg was 3.72 ± 0.46 μg/ml and did not differ statistically significantly from that observed for the combination of CBZ (7.7 mg/kg) with CCPA (0.125 mg/kg), which amounted to 4.04 ± 0.52 μg/ml.

## Discussion

Results presented in this study indicated that CCPA (administered at a sub-threshold dose of 0.125 mg/kg) potentiated the anticonvulsant activity of CBZ against maximal electroconvulsions, leaving the antiseizure effects offered by other AEDs (PB, PHT, and VPA) almost unchanged. The enhancement of CBZ anticonvulsant action by CCPA resembles that previously reported for the combination of 2-chloroadenosine (a non-selective adenosine $A_1/A_3$ receptor agonist) with CBZ in the MES-induced seizures [4]. In contrast, the preferential adenosine $A_1$ receptor agonist L-phenylisopropyladenosine had no effect on the anticonvulsant property of CBZ against electroconvulsions in mice [10]. Noteworthy, the observed enhancement of anticonvulsant effects of CBZ by CCPA was reversed by systemic administration of DPCPX, a selective adenosine $A_1$ receptor antagonist, indicating the involvement of adenosine $A_1$ receptor-mediated events in CCPA-induced enhancement of CBZ anticonvulsant activity.

One can try to explain the selective enhancement of antielectroshock effect of CBZ by CCPA considering molecular mechanisms of action of the studied AED. Accumulating evidence indicates that CBZ acts by preventing sustained high-frequency repetitive firing of action potential in depolarized neurons *via* use-, time- and voltage-dependent blockade of $Na^+$ channels [37]. The drug produces the blockade of $Na^+$ channels through the binding to inactive state and by slowing the rate of recovery of these channels from inactivation [37]. CBZ reduces also N-methyl-D-aspartate (NMDA)-activated currents in cultured spinal cord neurons [27] and, at relatively low concentrations, the drug limits NMDA-induced elevation of intracellular $Ca^{2+}$ concentration in primary cultures of cerebellar granule neurons [6, 23]. At therapeutic concentrations, CBZ inhibited the binding of the adeno-

sine analogues [$^3$H]L-phenylisopropyladenosine and [$^3$H]cyclohexyladenosine to synaptosomal membranes prepared from rat brain [40, 52]. The drug inhibited also second messenger-responses mediated by adenosine $A_1$ receptors, but not those mediated by high-affinity adenosine $A_{2A}$ receptors [55]. Moreover, CBZ reduced the adenosine $A_{2B}$ receptor-mediated increase in cAMP content in cultured astroglia cels [55]. To date, there is still no consensus whether CBZ is an adenosine receptor agonist or antagonist. Some authors have classified CBZ as a partial agonist [40, 52, 58], whereas the others have claimed that the drug acts as an antagonist at adenosine receptors [21, 46].

With regard to molecular mechanisms of action of PB, experimental evidence indicates that PB exerts its anticonvulsant effect by facilitating γ-aminobutyric acid (GABA)-mediated inhibition through the allosteric modulation of neuronal postsynaptic $GABA_A$ receptors [49]. The drug enhances the activation of $GABA_A$ receptors by increasing the mean channel open duration, having no impact on open frequency and channel conductance [39]. Moreover, PB is able to directly activate the $GABA_A$ receptors in the absence of GABA [48].

In case of PHT, the drug at therapeutic concentrations produces a use-dependent blockade of voltage-gated $Na^+$ channels [38]. PHT regulates $Na^+$-$K^+$ ATP-ase activity and thus, the drug has a neuronal stabilizing effect on excitable neurons [13]. The drug limits neurotransmitter release from synaptosomes during the action potential by minimizing and limiting $Ca^{2+}$ entry [14]. PHT inhibits also L- and T-type $Ca^{2+}$ channels in neurons [51, 54], as well as it reduces uptake and sequestration of $Ca^{2+}$ in the nerve terminal after $Ca^{2+}$ entry [47]. Moreover, PHT was shown to inhibit adenosine uptake into presynaptic terminals which resulted in an increase in the extracellular level of this neurotransmitter [46].

As for the molecular mechanisms of action of VPA, the drug increases GABA in whole brain and nerve terminals, although its precise mechanisms of action are still unknown [30]. VPA inhibits enzymes involved in GABA degradation including GABA-transaminase and succinic semialdehyde dehydrogenase [28, 30, 45]. In addition, VPA increases the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis [31, 43, 45] and blocks $Na^+$ channels in a voltage-dependent manner [41]. In both *in vivo* and *in vitro* experiments VPA suppressed NMDA-induced excitation in rat neocortical neurons [59]. The drug reduced the level and release of the excitatory amino acid aspartate in rat and mouse brains [7, 30]. Additionally, VPA (at high concentrations) activates $K^+$ conductance and blocks low-threshold T-type $Ca^{2+}$ channels in peripheral ganglion neurons [25].

Considering the above-mentioned mechanisms of action of conventional AEDs one can easily ascertain that only CBZ is able to interact directly and specifically with adenosine receptors in the brain. Thus, CCPA through the activation of $A_1$ receptors may cooperate with CBZ in reduction of seizure activity in the MES-test in mice. Noteworthy, PB, PHT and VPA did not enhance adenosine receptor-mediated events in the brain and, therefore, CCPA did not affect their anticonvulsant activities in the MES-test in mice.

Additionally, our findings support evidence that the enhancement of antiseizure effect offered by combination of CBZ with CCPA may allow for the reduction of CBZ dose. Noticeably, the decreased dose of CBZ in combination with CCPA did not interfere with the normal behavior of animals, since neither motor coordination nor long-term memory were altered. This fact may be of pivotal importance for further clinical practice, especially, since it became evident that the adenosine agonists cannot be used separately as anticonvulsant agents because of their cardiovascular side effect [5, 53]. Moreover, it was found experimentally that CCPA did not affect the total brain CBZ concentrations, and thus, the observed potentiation of antiseizure effect of CBZ by CCPA was pharmacodynamic in nature.

Summing up, the enhancement of anticonvulsant action of CBZ by CCPA against maximal electroconvulsions, the lack of potential harmful adverse effects and no pharmacokinetic interaction within the brain make the examined combination of CBZ with CCPA of pivotal importance from clinical point of view. Considering that adenosine is a recognized endogenous anticonvulsant agent, a concept of combining AEDs with adenosine receptor agonists deserves special attention.

**Acknowledgment:**
This work was supported by a grant from the Medical University of Lublin (PW 430/2002–2004). The kind gifts of valproate sodium from ICN-Polfa Rzeszów S.A. (Poland) and carbamazepine from Polfa, Starogard (Poland) are greatly appreciated.

## References:

1. Adami M, Bertorelli R, Ferri N, Foddi MC, Ongini E: Effects of repeated administration of selective adenosine

A1 and A2A receptor agonists on pentylenetetrazole-induced convulsions in the rat. Eur J Pharmacol, 1995, 294, 383–389.
2. Ault B, Wang CM: Adenosine inhibits epileptiform activity arising in hippocampal area CA3. Br J Pharmacol, 1986, 87, 695–703.
3. Boissier JR, Tardy J, Diverres JC: Une nouvelle methode simple pour explorer l'action «tranquilisante»: le test de la cheminee. Med Exp (Basel), 1960, 3, 81–84.
4. Borowicz KK, Łuszczki J, Czuczwar SJ: 2-Chloroadenosine, a preferential agonist of adenosine A1 receptors, enhances the anticonvulsant activity of carbamazepine and clonazepam in mice. Eur Neuropsychopharmacol, 2002, 12, 173–179.
5. Bueters TJ, IJzerman AP, van Helden HP, Danhof M: Characterization of the pharmacokinetics, brain distribution, and therapeutic efficacy of the adenosine A1 receptor partial agonist 2'-deoxy-N6-cyclopentyladenosine in sarin-poisoned rats. Toxicol Appl Pharmacol, 2003, 192, 86–94.
6. Cai Z, McCaslin PP: Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainate- and N-methyl-D-aspartate-induced intracellular $Ca^{2+}$ levels in neuronal culture. Eur J Pharmacol, 1992, 219, 53–57.
7. Chapman AG, Croucher MJ, Meldrum BS: Anticonvulsant activity of intracerebroventricularly administered valproate and valproate analogues. A dose-dependent correlation with changes in brain aspartate and GABA levels in DBA/2 mice. Biochem Pharmacol, 1984, 33, 1459–1463.
8. Concas A, Santoro G, Mascia MP, Maciocco E, Dazzi L, Ongini E, Biggio G: Anticonvulsant doses of 2-chloro-N6-cyclopentyladenosine, an adenosine A1 receptor agonist, reduce GABAergic transmission in different areas of the mouse brain. J Pharmacol Exp Ther, 1993, 267, 844–851.
9. Corradetti R, Lo Conte G, Moroni F, Passani MB, Pepeu G: Adenosine decreases aspartate and glutamate release from rat hippocampal slices. Eur J Pharmacol, 1984, 104, 19–26.
10. Czuczwar SJ, Szczepanik B, Wamil A, Janusz W, Kleinrok Z: Differential effects of agents enhancing purinergic transmission upon the antielectroshock efficacy of carbamazepine, diphenylhydantoin, diazepam, phenobarbital, and valproate in mice. J Neural Transm Gen Sect, 1990, 81, 153–166.
11. De Sarro G, De Sarro A, Meldrum BS: Anticonvulsant action of 2-chloroadenosine injected focally into the inferior colliculus and substantia nigra. Eur J Pharmacol, 1991, 194, 145–152.
12. De Sarro G, Donato Di Paola E, Falconi U, Ferreri G, De Sarro A: Repeated treatment with adenosine A1 receptor agonist and antagonist modifies the anticonvulsant properties of CPPene. Eur J Pharmacol, 1996, 317, 239–245.
13. Delgado-Escueta AV, Horan MP: Brain synaptosomes in epilepsy: organization of ionic channels and the $Na^{+}$-$K^{+}$ pump. Adv Neurol, 1980, 27, 85–126.
14. DeLorenzo RJ, Sun DA: Phenytoin and other hydantoins. Mechanisms of action. In: Antiepileptic Drugs, 5th edn. Ed. Levy RH, Mattson RH, Meldrum BS, Perucca E, Lippincott Williams & Wilkins, Philadelphia, 2002, 551–565.
15. Dragunow M, Goddard GV, Laverty R: Is adenosine an endogenous anticonvulsant? Epilepsia, 1985, 26, 480–487.
16. Dunwiddie TV: The physiological role of adenosine in the central nervous system. Int Rev Neurobiol, 1985, 27, 63–139.
17. Dunwiddie TV, Masino SA: The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci, 2001, 24, 31–55.
18. Dunwiddie TV, Worth T: Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J Pharmacol Exp Ther, 1982, 220, 70–76.
19. Dunwiddie TV, Hoffer BJ, Fredholm BB: Alkylxanthines elevate hippocampal excitability. Evidence for a role of endogenous adenosine. Naunyn Schmiedebergs Arch Pharmacol, 1981, 316, 326–330.
20. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J: International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev, 2001, 53, 527–552.
21. Fujiwara Y, Sato M, Otsuki S: Interaction of carbamazepine and other drugs with adenosine (A1 and A2) receptors. Psychopharmacology (Berl), 1986, 90, 332–335.
22. Gouder N, Fritschy JM, Boison D: Seizure suppression by adenosine A1 receptor activation in a mouse model of pharmacoresistant epilepsy. Epilepsia, 2003, 44, 877–885.
23. Hough CJ, Irwin RP, Gao XM, Rogawski MA, Chuang DM: Carbamazepine inhibition of N-methyl-D-aspartate-evoked calcium influx in rat cerebellar granule cells. J Pharmacol Exp Ther, 1996, 276, 143–149.
24. Huber A, Guttinger M, Mohler H, Boison D: Seizure suppression by adenosine A(2A) receptor activation in a rat model of audiogenic brainstem epilepsy. Neurosci Lett, 2002, 329, 289–292.
25. Kelly KM, Gross RA, Macdonald RL: Valproic acid selectively reduces the low-threshold (T) calcium current in rat nodose neurons. Neurosci Lett, 1990, 116, 233–238.
26. Khan GM, Smolders I, Ebinger G, Michotte Y: 2-Chloro-N(6)-cyclopentyladenosine-elicited attenuation of evoked glutamate release is not sufficient to give complete protection against pilocarpine-induced seizures in rats. Neuropharmacology, 2001, 40, 657–667.
27. Lampe H, Bigalke H: Carbamazepine blocks NMDA-activated currents in cultured spinal cord neurons. Neuroreport, 1990, 1, 26–28.
28. Larsson OM, Gram L, Schousboe I, Schousboe A: Differential effect of gamma-vinyl GABA and valproate on GABA-transaminase from cultured neurones and astrocytes. Neuropharmacology, 1986, 25, 617–625.
29. Litchfield JT, Wilcoxon F: A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther, 1949, 96, 99–113.
30. Löscher W: Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs, 2002, 16, 669–694.
31. Luder AS, Parks JK, Frerman F, Parker WD Jr: Inactivation of beef brain alpha-ketoglutarate dehydrogenase complex by valproic acid and valproic acid metabolites. Possible mechanism of anticonvulsant and toxic actions. J Clin Invest, 1990, 86, 1574–1581.

32. Łuszczki JJ, Czuczwar SJ: Isobolographic and subthreshold methods in the detection of interactions between oxcarbazepine and conventional antiepileptics – a comparative study. Epilepsy Res, 2003, 56, 27–42.
33. Łuszczki JJ, Czuczwar SJ: Isobolographic profile of interactions between tiagabine and gabapentin: a preclinical study. Naunyn Schmiedebergs Arch Pharmacol, 2004, 369, 434–446.
34. Łuszczki JJ, Czuczwar SJ: Interaction between lamotrigine and felbamate in the maximal electroshock-induced seizures in mice: an isobolographic analysis. Eur Neuropsychopharmacol, 2005, 15, 133–142.
35. Łuszczki JJ, Świąder M, Parada-Turska J, Czuczwar SJ: Tiagabine synergistically interacts with gabapentin in the electroconvulsive threshold test in mice. Neuropsychopharmacology, 2003, 28, 1817–1830.
36. Łuszczki JJ, Wójcik-Ćwikła J, Andres MM, Czuczwar SJ: Pharmacological and behavioral characteristics of interactions between vigabatrin and conventional antiepileptic drugs in pentylenetetrazole-induced seizures in mice: an isobolographic analysis. Neuropsychopharmacology, 2005, 30, 958–973.
37. Macdonald RL: Carbamazepine. Mechanisms of action. In: Antiepileptic Drugs, 5th edn. Ed. Levy RH, Mattson RH, Meldrum BS, Perucca E, Lippincott Williams & Wilkins, Philadelphia, 2002, 227–235.
38. Macdonald RL, Kelly KM: Antiepileptic drug mechanisms of action. Epilepsia, 1995, 36, Suppl 2, S2–S12.
39. Macdonald RL, Rogers CJ, Twyman RE: Barbiturate regulation of kinetic properties of the GABAA receptor channel of mouse spinal neurones in culture. J Physiol, 1989, 417, 483–500.
40. Marangos PJ, Post RM, Patel J, Zander K, Parma A, Weiss S: Specific and potent interactions of carbamazepine with brain adenosine receptors. Eur J Pharmacol, 1983, 93, 175–182.
41. McLean MJ, Macdonald RL: Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther, 1986, 237, 1001–1011.
42. Murphy KM, Snyder SH: Heterogeneity of adenosine A1 receptor binding in brain tissue. Mol Pharmacol, 1982, 22, 250–257.
43. Nau H, Löscher W: Valproic acid: brain and plasma levels of the drug and its metabolites, anticonvulsant effects and gamma-aminobutyric acid (GABA) metabolism in the mouse. J Pharmacol Exp Ther, 1982, 220, 654–659.
44. Parada-Turska J, Czuczwar M, Kiś J, Czuczwar P, Cioczek A, Łuszczki J, Czuczwar SJ: Allopurinol does not affect the anticonvulsant activity of carbamazepine and valproate in maximal electroshock-induced convulsions in mice. Pol J Pharmacol, 2004, 56, 67–72.
45. Phillips NI, Fowler LJ: The effects of sodium valproate on gamma-aminobutyrate metabolism and behavior in naive and ethanolamine-O-sulphate pretreated rats and mice. Biochem Pharmacol, 1982, 31, 2257–2261.
46. Phillis JW: Interactions of the anticonvulsants diphenylhydantoin and carbamazepine with adenosine on cerebral cortical neurons. Epilepsia, 1984, 25, 765–772.
47. Rampe D, Ferrante J, Triggle DJ: The actions of diazepam and diphenylhydantoin on fast and slow $Ca^{2+}$ uptake processes in guinea pig cerebral cortex synaptosomes. Can J Physiol Pharmacol, 1987, 65, 538–543.
48. Rho JM, Donevan SD, Rogawski MA: Direct activation of GABAA receptors by barbiturates in cultured rat hippocampal neurons. J Physiol, 1996, 497(Pt 2), 509–522.
49. Rogawski MA, Porter RJ: Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol Rev, 1990, 42, 223–286.
50. Rudolphi KA, Schubert P, Parkinson FE, Fredholm BB: Neuroprotective role of adenosine in cerebral ischaemia. Trends Pharmacol Sci, 1992, 13, 439–445.
51. Schumacher TB, Beck H, Steinhauser C, Schramm J, Elger CE: Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal granule cells from patients with temporal lobe epilepsy. Epilepsia, 1998, 39, 355–363.
52. Skerritt JH, Davies LP, Johnston GA: Interactions of the anticonvulsant carbamazepine with adenosine receptors. 1. Neurochemical studies. Epilepsia, 1983, 24, 634–642.
53. Stella L, Berrino L, Maione S, de Novellis V, Rossi F: Cardiovascular effects of adenosine and its analogs in anaesthetized rats. Life Sci, 1993, 53, 755–763.
54. Todorovic SM, Lingle CJ: Pharmacological properties of T-type $Ca^{2+}$ current in adult rat sensory neurons: effects of anticonvulsant and anesthetic agents. J Neurophysiol, 1998, 79, 240–252.
55. Van Calker D, Steber R, Klotz KN, Greil W: Carbamazepine distinguishes between adenosine receptors that mediate different second messenger responses. Eur J Pharmacol, 1991, 206, 285–290.
56. Venault P, Chapouthier G, de Carvalho LP, Simiand J, Morre M, Dodd RH, Rossier J: Benzodiazepine impairs and beta-carboline enhances performance in learning and memory tasks. Nature, 1986, 321, 864–866.
57. Von Lubitz DK, Paul IA, Carter M, Jacobson KA: Effects of N6-cyclopentyl adenosine and 8-cyclopentyl-1,3-dipropylxanthine on N-methyl-D-aspartate induced seizures in mice. Eur J Pharmacol, 1993, 249, 265–270.
58. Weir RL, Padgett W, Daly JW, Anderson SM: Interaction of anticonvulsant drugs with adenosine receptors in the central nervous system. Epilepsia, 1984, 25, 492–498.
59. Zeise ML, Kasparow S, Zieglgansberger W: Valproate suppresses N-methyl-D-aspartate-evoked, transient depolarizations in the rat neocortex *in vitro*. Brain Res, 1991, 544, 345–348.

**Received:**
May 4, 2005; in revised form: August 23, 2005.