

Adenosine receptor agonists differentially affect the anticonvulsant action of carbamazepine and valproate against maximal electroshock test-induced seizures in mice

Mirosław Jasiński¹, Magdalena Chrościńska-Krawczyk^{1,2}, Stanisław J. Czuczwar^{2,3}

¹ Clinic of Pediatrics, Endocrinology and Neurology,
Medical University of Lublin, Poland

² Department of Pathophysiology,
Medical University of Lublin, Poland

³ Department of Physiopathology,
Institute of Rural Health, Lublin, Poland

Received November 30, 2017

Accepted for publication on-line December 15, 2017

Published on-line January 15, 2018

Correspondence

Stanisław J. Czuczwar
Department of Pathophysiology, Medical University of Lublin
Jaczewskiego 8b, 20-090 Lublin, Poland
e-mail: czuczwar@yaho.com

SUMMARY

Background. Adenosine is regarded as an endogenous anticonvulsant and its agonists have been proved to affect the anticonvulsant activity of a number of antiepileptic drugs (AEDs) in animal models of seizures.

Aim. To evaluate effects of adenosine agonists on carbamazepine (CBZ) and valproate (VPA) in mouse model of generalized tonic-clonic convulsions.

Methods. The following adenosine receptor agonists were used: A₁– cyclohexyladenosine, A_{2A}– CGS 21 680, A₃– N⁶-benzyl-NECA and A₁ (preferentially) and A₂– 2-chloroadenosine. Their possible anticonvulsant effects were studied in a threshold electroconvulsive test for maximal electroconvulsions. The protective activity of AEDs alone or in combinations with adenosine agonists was evaluated in the form of their respective ED₅₀ values necessary to protect 50% of mice against tonic extension of the hind limbs, following maximal electroshock, delivered through ear electrodes. The specificity of interactions between AEDs and adenosine agonists was challenged with an adenosine receptor A₁ and A₂ antagonist, aminophylline (5 mg/kg). The effects of AEDs alone or with adenosine agonists were tested for the occurrence of adverse effects (AE) (impairment of motor coordination) in a chimney test. All combinations with an enhancement the protective activity of CBZ or VPA were verified with the free plasma or brain concentration of these AED.

Results. Adenosine receptor agonists (cyclohexyladenosine up to 4 mg/kg; CGS 21 680 – 8 mg/kg; N⁶-benzyl-NECA – 1 mg/kg; 2-chloroadenosine – 2 mg/kg) did not significantly affect the threshold for maximal electroconvulsions. Cyclohexyladenosine (1 mg/kg), N⁶-benzyl-NECA (0.5 and 1 mg/kg) and 2-chloroadenosine (1 mg/kg) potentiated the anticonvulsant activity of CBZ. Valproate's protective action was enhanced by one adenosine agonist – cyclohexyladenosine (1 mg/kg). Only the combination of CBZ + N⁶-benzyl-NECA (1 mg/kg) was resistant to aminophylline (5 mg/kg). Pharmacokinetic interactions were evident in case of the combination of CBZ + N⁶-benzyl-NECA (1 mg/kg) and resulted in an increased free plasma concentration of this CBZ. Interestingly, total brain concentration of CBZ confirmed the pharmacokinetic interaction as regards CBZ + N⁶-benzyl-NECA (1 mg/kg).

Conclusion. The best profile was shown by the combination of CBZ + 2-chloroadenosine which involved no AE or a pharmacokinetic interaction. The remaining positive combinations in terms of anticonvulsant activity were associated with general profound AE and pharmacokinetic interactions in some of them.

Key words: adenosine receptor agonists • aminophylline • carbamazepine • valproate • electroconvulsions • mice

BACKGROUND

Epilepsy affects more than 50 million people around the world and in given populations the percentage of patients with epilepsy may reach ca 1–3% (Shorvon, 1996). It is widely accepted that a deficit of GABA-ergic and enhancement of glutamatergic neurotransmission may be associated with the pathophysiology of this serious neurologic disease. In addition to the altered processes of inhibition and excitation in the central nervous system (CNS), the disturbed activity of ion channels for sodium, calcium and potassium cations seems of particular importance. Also, other neurotransmitter systems have been considered, including noradrenergic, serotonergic, cholinergic and purinergic ones and the latter has been proved as an endogenous inhibitor of seizure spread (Schwartzkroin, 1993; Löscher, 1998; Czapiński et al., 2005).

Purinergic system mediates its effects via two types of receptors: P1 (or adenosine receptors) and P2. Adenosine receptors comprise four subclasses: A_1 , A_{2A} , A_{2B} and A_3 which are associated with the G protein (Ralevic, Burnstock, 1998). As for A_1 receptors, their stimulation results in the reduced activity of adenylate cyclase which in turn inhibits cyclic adenosine monophosphate (cAMP) production. These receptors, when activated by adenosine, facilitate the influx of potassium ions through potassium channels and thus lead to hyperpolarization of neuronal membranes (van Calcar et al., 1978). A different susceptibility to respective antagonists led to identification of two subtypes of adenosine A_2 receptors: A_{2A} and A_{2B} (Daly et al., 1983), both positively coupled to adenylate cyclase and responsible for the activation of CNS (Sebastiao, Ribeiro, 1996). Finally, activation of A_3 adenosine receptors results in the rise of phospholipase C followed by increased 1,4,5-triphosphoinositol (IP3) synthesis which eventually induces mobilization of intraneuronal calcium from the endoplasmatic reticulum (Abbracchio et al., 1995). Similarly to A_1 receptors, stimulation of A_3 receptors results in adenylate cyclase inhibition (Zhou et al., 1992).

As already mentioned, adenosine may produce anti-convulsant effects and a deficit in adenosinergic neurotransmission seems responsible for seizure activity (Dragunov, 1986; Zhang et al., 1993; Young, Dragunov, 1994; Świąder et al., 2014). Moreover, adenosine agonists, preferably stimulating A_1 receptors but there are also positive data as for A_{2A} and A_3 receptor agonists, were documented to raise the convulsive thresh-

old in a number of animal models of seizures (Malhotra, Gupta, 1997; Świąder et al., 2014).

Bearing in mind that about 30% of patients with epilepsy are resistant to currently available antiepileptic drugs (Miziak et al., 2013), there is an intensive search for the efficient methods of treatment. A possibility arises that the combined treatment of antiepileptic drugs (AEDs) with adenosine agonists might be an effective way for the enhancement of their protective activity.

AIM

Consequently, the effects of a number of various adenosine receptor agonists on the anticonvulsant activity of two conventional AEDs, (CBZ) and valproate (VPA), were evaluated in the maximal electroshock (MES) test in mice, which is an accepted model of human generalized tonic-clonic seizures (Löscher, Schmidt, 1988). It is noteworthy that none of the adenosine receptor agonists (CHA up to 4 mg/kg; CGS 21 680 – 8 mg/kg; N^6 -benzyl-NECA – 1 mg/kg; 2-chloroadenosine – 2 mg/kg) significantly affected the convulsive threshold for maximal electroconvulsions in mice. The respective data may be found in Jasiński et al. (2011).

MATERIALS AND METHODS

The experiments were undertaken using adult male Swiss mice, weighing 22–28 g. Experimental groups, consisting of 8–10 animals, were randomly completed. The total number of mice used reached 610 animals. Mice were assigned to Plexiglas perspex colony cages and were kept under standard laboratory conditions and on the natural light-dark cycle, food (chow pellets) and tap water being available ad libitum.

The following adenosine agonists were used: CHA (N^6 -cyclohexyladenosine; an A_1 receptor agonist); 2-CADO (2-chloroadenosine; a preferential A_1 agonist also stimulating A_2 receptors); CGS (21680 – 2-[p-(2-carbonyl-ethyl)-phenylethylamine]-5'-N-ethylcarboxyamidadenosine; an A_{2A} receptor agonist), N^6 -benzyl-NECA (N^6 -benzyl-5'-N-ethylcarboxyamidadenosine; an A_3 receptor agonist), all substances from RBI, Natick, MA, USA). Aminophylline (theophylline 2-ethylenediamine; Aminophyllinum, Polfa, Kraków) was administered as a non-specific adenosine receptor antagonists to verify whether the interaction of AEDs with adenosine agonists is associated with adenosine receptor-mediated events. AEDs used in this study were carbamazepine (CBZ, Amizepin; Polfa, Warsaw, Poland) and valpro-

ate (VPA, Dipromal; ICN Polfa Rzeszów, Poland). Adenosine receptor agonists and CBZ were suspended in a 1% water solution of Tween 81 (Loba Chemie, Vienna, Austria) whilst VPA and aminophylline were sufficiently water soluble. All drugs and substances were administered intraperitoneally in a volume of 5 ml/kg, 30 min prior to MES or behavioral testing.

MES test

MES was delivered through ear-clip electrodes and alternating current (50 Hz; 25 mA; 0.2 s duration) delivered by a Hugo Sachs generator (rodent Shocker, type 221, Freiburg, Germany). The end point for the occurring seizure activity was the tonic extension of the hind limbs. The protective action of CBZ or VPA (alone or in combination with adenosine agonists) was evaluated as their respective ED_{50} values, i.e. doses of these AEDs necessary to protect 50% of mice against MES-induced convulsions. The doses of AEDs were chosen in order to obtain protection against MES-induced convulsions in the range of 10–90% and they correspond to the former studies with the use of MES (Borowicz et al., 1999). Subsequently, dose-effect curves were constructed and the ED_{50} values were calculated according to Litchfield and Wilcoxon (1949). At least 32 mice were used to calculate each ED_{50} value.

Chimney test

Motor performance of mice was tested in the chimney test. The mice had to climb backwards up a plastic tube (25-cm length, 3-cm inner diameter) within 60 s. Animals unable to perform this task were considered impaired. Motor impairment in groups pretreated with AEDs alone or combined with adenosine agonists was expressed as a percentage of mice failing to perform the task.

Determination of the free plasma and total brain concentration of AEDs

Blood samples of ca 0.5–1 ml were obtained from decapitated mice and transferred to heparinized Eppendorf tubes at times of the maximal anticonvulsant effects of AEDs. Following centrifugation at 10,000 rpm ($10,286 \times g$; with an Abbott centrifuge – Abbott, Irving, TX, USA), plasma samples of 75 μ l were pipetted to a micropartition system, MPS-1 (Amicon, Danvers, MA, USA) and centrifuged at 3,000 rpm ($1,462 \times g$; MPW-360 centrifuge; Mechanika Precyzyjna, Warsaw, Poland) for separation of free microsolutes. Subsequently,

the filtrate samples were pipetted into Abbott cartridges which were placed into a 20 sample carousel. Control free plasma samples, provided by the manufacturer (Abbott, Irving, TX, USA), were also included at the beginning and end of the carousel in order to verify the calibration. Free plasma levels of AEDs were estimated by immunofluorescence using an Abbott TDx analyzer and Abbott reagents (Abbott, Irving, TX, USA). The concentration of AEDs was expressed in μ g/ml.

Mice were sacrificed at times for the evaluation of the anticonvulsant effects of AEDs alone or combined with adenosine agonists. The brains were dissected at 40°C and all subsequent procedures were brought about according to Borowicz et al. (1999). Briefly, brains were homogenized in TDx buffer (Abbott, Irving, TX, USA) in a volume/weight proportion of 2:1. Homogenates were centrifuged and the brain concentration of AEDs in supernatants was determined by immunofluorescence as indicated above. The concentration of AEDs was expressed in μ g/g of wet brain tissue.

Statistical analysis of the results

ED_{50} values with 95% confidence limits were calculated on a computer according to the log-probit method of Litchfield and Wilcoxon (1949). Subsequently, ED_{50} s were transformed to decimal logarithms and their respective 95% confidence limits to standard errors (SEs), as per procedure described by Łuszczki et al. (2005). The statistical evaluation of ED_{50} s was performed with the use of one-way ANOVA followed by the post-hoc Bonferroni's multiple comparisons test.

Results obtained in the chimney test were statistically verified with Fisher's exact probability test. Free plasma and brain AEDs' concentration were compared by using unpaired t-Student test.

Ethics Committee

The experimental procedures running in this study were approved by the Lublin Local Ethical Committee.

RESULTS

Influence of adenosine receptor agonists on the protective activity of CBZ against MES

N^6 -benzyl-NECA (0.5 and 1.0 mg/kg) significantly potentiated the anticonvulsant activity of CBZ, Aminophylline (5.0 mg/kg) did not reverse the enhancing effect of N^6 -benzyl-NECA (at 1.0 mg/kg; Table 1). 2-CADO (1.0 mg/kg) decreased the ED_{50} of CBZ from

Table 1. Influence of adenosine agonists on the protective activity of CBZ against MES-induced convulsions in mice

Treatment (mg/kg)	ED ₅₀ (mg/kg)	SE
CBZ + vehicle	16.1 (14.4–17.9)	0.897
CBZ + N ⁶ -benzyl-NECA (0.125)	13.2 (11.8–14.8)	0.765
CBZ + N ⁶ -benzyl-NECA (0.5)	10.7 (9.5–12.1) ^{***}	0.644
CBZ + N ⁶ -benzyl-NECA (1.0)	10.3 (8.8–12.2) ^{***}	0.856
CBZ + N ⁶ -benzyl-NECA (1.0) +AMI (5.0)	10.7 (9.5–12.1) ^{***}	0.644
CBZ + vehicle	11.9 (10.3–13.7)	0.862
CBZ + 2-CADO (0.25)	11.2 (9.8–12.8)	0.767
CBZ + 2-CADO (0.5)	9.6 (7.9–11.8)	0.989
CBZ + 2-CADO (1.0)	7.4 (6.2–8.8) ^{**}	0.649
CBZ + 2-CADO (1.0) +AMI (5.0)	9.9 (8.3–11.7)	0.868

ED₅₀ values are given with 95% confidence limits in parentheses.

SE – standard error, CBZ – carbamazepine, 2-CADO – 2-chloroadenosine, AMI – aminophylline

Experimental groups comprised 8 mice and at least 32 animals were used to calculate each ED₅₀ value according to Litchfield and Wilcoxon (1949). SEs were calculated according to Łuszczki, Czuczwar (2005). One-way ANOVA followed by the post-hoc Bonferroni's multiple comparisons test was used for statistical evaluation – ^{**}P < 0.01, ^{***}P < 0.001 vs CBZ + vehicle. For comparisons to the first vehicle group (CBZ – 16.1 mg/kg), F (4,75) = 10.298 and to the second (CBZ – 11.9 mg/kg) - F (4,75) = 4.298.

Table 2. Effect of CHA upon the anticonvulsant action of VPA against MES-induced seizures in mice

Treatment (mg/kg)	ED ₅₀ (mg/kg)	SE
CBZ + vehicle	299 (279–321)	10.80
VPA + CHA (0.25)	273 (240–298)	15.70
VPA + CHA (0.5)	258 (225–296)	18.09
VPA + CHA (1.0)	229 (204–255) ^{**}	12.92
VPA + CHA (1.0) + AMI (5.0)	265 (225–312)	22.12

VPA – valproate, CHA – cycloheksyladenosine. ^{**}P < 0.01 vs VPA + vehicle-treated group; F (3,60) = 3.989

For further explanations, see the legend of Table 1.

11.9 to 7.4 mg/kg, being without effect in lower doses of 0.25 and 0.5 mg/kg and aminophylline (5.0 mg/kg) only partially reversed the interaction of CBZ + 2-CADO (1.0 mg/kg; Table 1). CGS 21 680 (4.0 mg/kg) did not affect the protective action of CBZ because its ED₅₀ value was reduced insignificantly from 16.1 (14.4–17.9) to 15.9 (14.3–17.7) mg/kg. CHA (0.25 and 1.0 mg/kg) did not modify the anticonvulsant activity of CBZ insignificantly reducing the ED₅₀ of CBZ from 13.2 (11.8–14.8) to 13.0 (11.4–14.9) and 10.0 (8.4–11.9), respectively (results not shown).

Effects of adenosine agonists on the protective action of VPA against MES in mice

Only CHA at 1.0 mg/kg was able to potentiate the anticonvulsant efficacy of VPA against MES, reducing its control ED₅₀ from 299 to 228 mg/kg. This positive effect was partially reversed by aminophylline (5.0 mg/kg; Table 2). However, CGS 21 680 (4.0 mg/kg) in-

significantly raised its control ED₅₀ value to 319 (294–345) mg/kg (result not shown). Similarly, 2-CADO (at 1.0 and 2.0 mg/kg) insignificantly modified the VPA's control ED₅₀ value of 275 (253–299) mg/kg, calculated in another set of experiments, to 251 (225–280) and 250 (212–295) mg/kg, respectively. Also, N⁶-benzyl-NECA (at 0.5 and 1.0 mg/kg) remained ineffective upon the anticonvulsant activity of VPA whose respective ED₅₀ values were 260 (228–285) and 262 (232–296) mg/kg (results not shown).

Influence of combined treatment with AEDs and adenosine agonists upon the motor coordination in the chimney test

Only combinations with the enhanced anticonvulsant activity of CBZ by adenosine agonists were verified in the chimney test. CBZ (10.3 mg/kg) combined with N⁶-benzyl-NECA (1.0 mg/kg) produced motor impairment in 90% of mice which was highly signifi-

Table 3. Influence of the combined treatment with CBZ with adenosine agonists on the motor coordination of mice in the chimney test.

Treatment (mg/kg)	% of animals impaired
Vehicle + vehicle	0
CBZ (7.4 or 11.9) + vehicle	0
Vehicle + 2-CADO (1.0)	30
CBZ (7.4) + 2-CADO (1.0)	30
CBZ (16.1) + vehicle	20
CBZ (10.3) + vehicle	0
Vehicle + N ⁶ -Benzyl-NECA (1.0)	90 ^{***}
CBZ (10.3) + N ⁶ -Benzyl-NECA (1.0)	90 ^{***,a}

In each case, in the vehicle + vehicle groups no animals were impaired, the result being shown only once. *P < 0.05; ***P < 0.001 vs vehicle + vehicle; ^aP < 0.01 vs CBZ (10.3) group (Fisher's exact probability test). For abbreviations, see the legend of Table 1.

Table 4. Effect of combined treatment with VPA and CHA on the motor performance of mice in the chimney test

Treatment	% of mice impaired
Vehicle + vehicle	0
VPA (299) + vehicle	50
VPA (299) + vehicle	0
Vehicle + CHA (1.0)	30
VPA (229) + CHA (1.0)	100 ^{***,a}

*P < 0.05; ***P < 0.001 vs vehicle + vehicle group; ^aP < 0.001 vs VPA (229) group (Fisher's exact probability test). For abbreviations, see the legend of Table 2.

cant against CBZ alone at 10.3 mg/kg inducing no motor impairment. Interestingly, N⁶-benzyl-NECA alone (1.0 mg/kg) – pretreated mice also exhibited profound neurotoxic effect (90% of animals were not able to complete the task; Table 3).

As regards 2-CADO (1.0 mg/kg) alone or its combination with CBZ (7.4 mg/kg), both treatments resulted in insignificant motor impairment (30% impaired mice) whilst CBZ (7.4 mg/kg) did not affect motor coordination at all (Table 3).

VPA, at its ED₅₀ value of 299 mg/kg against MES, significantly impaired motor coordination in 50% of mice. CHA (1.0 mg/kg) produced a non-significant effect (30% of mice impaired). However, the combined treatment of VPA (228 mg/kg) with CHA (1.0 mg/kg) led to a profound impairment of motor coordination, as reflected in all 100% of animals unable to perform the task (Table 4).

Effect of adenosine agonists on the free plasma and brain concentrations of AEDs

Only combinations showing an enhanced anticonvulsant effect of an AED were verified pharmacokinet-

ically. Out of these, no involvement of pharmacokinetic interactions in plasma was evident in the case of CBZ (7.4 mg/kg) + 2-CADO (1.0 mg/kg) and VPA (228 mg/kg) + CHA (1.0 mg/kg). The concentration of CBZ alone reached 0.64 ± 0.11 and in combination with CHA – 0.68 ± 0.11 µg/ml. Also, the free plasma level of VPA was 51.9 ± 4.2 µg/ml and following combination with CHA, it was insignificantly increased to 52.8 ± 2.5 µg/ml. However, N⁶-benzyl-NECA (1.0 mg/kg) significantly elevated the free plasma concentration of CBZ (10.3 mg/kg) from 1.22 ± 0.23 to 1.55 ± 0.24 µg/ml (P < 0.05). Only this combination was verified in terms of total brain concentration of CBZ, showing also an existence of the pharmacokinetic mechanism – the total brain level of CBZ of 1.20 ± 0.26 was elevated to 1.73 ± 0.53 µg/ml (P < 0.05) upon the combined treatment with N⁶-benzyl-NECA.

DISCUSSION

The effects of adenosine agonists, tested in this study, were already evaluated in a comparable experimental design, upon the anticonvulsant action of phenobarbital and phenytoin. Briefly, 2-CADO (1.0 mg/kg) enhanced the protective activity of phenytoin, CHA (1.0 mg/kg)

that of phenobarbital and phenytoin, whilst N⁶-benzyl-NECA (up to 1.0 mg/kg) and CGS 21 680 (4.0 mg/kg) remained ineffective upon the anticonvulsant action of both classical AEDs (Jasiński et al., 2011). Combinations of these AEDs with adenosine agonists potentiating their anticonvulsant efficacy also produced motor impairment in 40–60% of mice. In no case, pharmacokinetic interactions were present in terms of free plasma concentrations of phenobarbital and phenytoin.

Of the combinations evaluated in the present study, CBZ + 2-CADO (1.0 mg/kg) is the most promising one. First, the potentiation of the anticonvulsant activity of CBZ by 2-CADO was associated with no pharmacokinetic interaction and second, no motor impairment was noted. Very similar results were shown by Borowicz et al. (2002) who observed a potentiating effect of 2-CADO upon the anticonvulsant action of CBZ with no adverse effects or pharmacokinetic interactions. However, the dose effective range for 2-CADO was lower as it enhanced protection offered by CBZ against MES at 0.25 mg/kg. The remaining combinations evaluated in the present study were associated with disturbed motor coordination, which in case of CBZ + N⁶-benzyl-NECA (1.0 mg/kg) or VPA + CHA (1.0 mg/kg) reached 90 and 100% mice impaired, respectively. Also, the combined treatment of CBZ + N⁶-benzyl-NECA (1.0 mg/kg) resulted in the elevated total brain CBZ concentration, which suggests a pharmacokinetic interaction; it might, eventually, contribute to the enhanced anticonvulsant activity of CBZ. The interaction of CBZ with adenosine receptor agonists is particularly interesting when its mechanism of action are considered. Apart from the blockade of neuronal voltage-operated sodium channels and N-methyl-D-aspartate (NMDA)-induced currents in cultured neurons of the spinal cord (Lampe, Bigalke, 1990; Macdonald, 2002), this AED has been also shown to interact with adenosine receptors. CBZ actually displaced at therapeutic concentrations preferential adenosine A₁ receptor agonists from brain synaptosomal membranes (Marangos et al., 1983; Skerrett et al., 1983). After all, CBZ was demonstrated to reduce adenosine A₁ receptor-mediated responses in second messengers, the responses mediated by A_{2A} receptors being not affected (Van Calker et al., 1991). Quite recent data by Booker et al. (2015) indicate that CBZ may behave as an antagonists of A₁ adenosine receptors especially in the low range of therapeutic concentrations which was evident in terms of the increased excitatory postsynaptic currents in hip-

pocampal neurons. Interestingly, when the concentration of endogenous adenosine was significantly diminished by adenosine deaminase, CBZ was no longer effective as an excitatory drug in hippocampal neurons. At higher concentrations, CBZ via blockade of voltage-operated sodium channels evoked clear cut depressive effects on hippocampal neurons (Booker et al., 2015). Basing upon the above *in vitro* data, it is very likely that CBZ may be regarded as an A₁ receptor antagonists *in vivo* because its anticonvulsant activity was not modified by the adenosine A₁ agonist – CHA. The interaction of CBZ with 2-CADO could partly involve A₂ adenosine receptor-mediated events as the enhancement of the anticonvulsant activity of CBZ was not fully reversed by aminophylline. As regards N⁶-benzyl-NECA, an involvement of A₃ adenosine receptors may be postulated in the potentiation of the CBZ protective activity against MES, as the ED₅₀ value of CBZ in this combination was not affected by aminophylline at all. However, the interaction of CBZ with adenosine A₁ agonists may be more complex – the existing data indicate that 2-chloro-N⁶-cyclopentyladenosine potentiated the protective activity of this AED against MES in mice and this particular effect was reversed by a selective A₁ adenosine receptor antagonist, 8-cyclopentyl-1,3-dimethylxanthine (Łuszczki et al., 2005). In the light of the cited above papers (Van Calker et al., 1991; Booker et al., 2015) the result obtained by Łuszczki et al. (2005) are difficult to interpret.

Interestingly, Sun et al. (2015) reported on the augmentation by NECA (a preferential A₂ receptor agonist) of the anticonvulsant activity of phenytoin against amygdala-kindled seizures in mice. Also, they found that this adenosine receptor agonist, apart from the enhancement of the anticonvulsant activity of this AED, elevated the brain concentration of phenytoin, in terms of general influence of adenosine agonists on the permeability of the blood-brain barrier (Bynoe et al., 2015). In this context, adenosine agonists could interact with AEDs both pharmacodynamically and pharmacokinetically, both effects leading to the potentiation of the anticonvulsant efficacy of AEDs. However, Sun et al. (2015) did not evaluate the adverse potential of the combined treatment and as indicated above, only a limited number of such combinations (AEDs + adenosine receptor agonists) are free from profound impairment of motor coordination (Jasiński et al., 2011; this study).

Some additional data are available as regards the combined treatment of AEDs with adenosine receptor

agonists. CHA (in a dose of 2.0 mg/kg) was also shown to enhance the protective efficacy of CBZ, VPA, phenobarbital and phenytoin against MES in mice (Assi, 2001). Somewhat broader interaction of this adenosine receptor agonist with classical AEDs may be explained in terms of its 2-fold higher dose than this used in the present study. Also, the combined treatment of VPA + CHA (2.0 mg/kg) resulted in a motor impairment (Assi, 2001). A non-selective agonist of A_3 receptors, N^6 -2-(4-aminophenyl) ethyl adenosine (APNEA) at a higher dose of 1.0 mg/kg significantly increased the protective potential of CBZ, phenobarbital, phenytoin and VPA against MES in mice without altering their plasma concentrations (Borowicz et al., 1997). Interestingly, APNEA (at an extremely low dose of 0.0039 mg/kg) also positively modulated the protection offered by CBZ, being inactive when combined with the remaining classical AEDs. The combined treatment (including the low dose of APNEA) produced also adverse effects – an impairment of long-term memory and decrease in body temperature. The authors concluded that the interaction between APNEA (1 mg/kg) with AEDs resulted from the stimulation of A_1 receptors whilst that of APNEA (at 0.0039 mg/kg) with CBZ involved A_3 receptors (Borowicz et al., 1997). APNEA was also tried in combinations with CBZ, VPA, phenobarbital, phenytoin or clonazepam against amygdala-kindled seizures in rats (Borowicz et al., 2000). The adenosine agonist and AEDs were applied in subprotective doses. The results indicate that the combined treatment of APNEA (2.0 mg/kg) with all AEDs except for phenytoin, resulted in a significant anticonvulsant protection (without any pharmacokinetic interactions) which was sensitive to reversal induced by an adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dimethylxanthine (at 5.0 mg/kg). Only, in the case of the combination of APNEA with CBZ, there was a partial reversal by the adenosine receptor antagonists of the observed anticonvulsant action. Strikingly, the combined treatment of APNEA (at a lower dose of 0.5 mg/kg) with CBZ was totally resistant to the adenosine receptor antagonist. Again, this particular interaction of APNEA with CBZ may be ascribed to only A_3 receptors (when a lower dose of APNEA was used) and to both, A_1 and A_3 adenosine receptors when APNEA was administered at the higher dose (Borowicz et al., 2000). The results obtained in the present study as regards combinations of CBZ with N^6 -benzyl-NECA point to the ineffectiveness of aminophylline (a non-selective adenosine A_1 and A_2

receptor antagonist; 5.0 mg/kg) to inhibit the enhancing effect of N^6 -benzyl-NECA. This may be interpreted in terms of the involvement of A_3 receptor-mediated events in this interaction. Because, aminophylline (5.0 mg/kg) partially reversed the anticonvulsant action of the combined treatment of CBZ with 2-CADO and VPA + CHA, an involvement of both A_1 - and A_2 -receptor-mediated events is likely in these particular cases.

CONCLUSIONS

It seems evident that adenosine-mediated inhibition may potentiate the protective effect of both CBZ and VPA, CBZ being more susceptible for the interaction with adenosine agonists. However, only a few combinations of adenosine agonists with AEDs are free from serious adverse effects (Borowicz et al., 1997; Assi, 2001; Łuszczki et al., 2005; Jasinski et al. 2011; this study) so the clinical significance of this strategy is low. However, the experiments regarding the enhancement of brain adenosinergic inhibition and the anticonvulsant activity of various AEDs must not be abandoned. A possibility exists that brain adenosine may be elevated via adenosine uptake inhibitors or inhibitors of brain adenosine kinase (Świader et al., 2014; Cieślak et al., 2017). Possibly, such an approach could still enhance the protection offered by AEDs, without being accompanied by significant adverse effects. Also, direct application of adenosine into the brain, with the help of a silk biopolymer drug delivery system, seems possible and this approach could overcome the serious problem of peripheral adenosine receptor-mediated untoward actions (for review, Younus, Reddy, 2017).

ACKNOWLEDGEMENT

This study was supported by a statutory grant from Medical University of Lublin (DS 475).

REFERENCES

- Abbracchio M.P., Brambilla R., Ceruti S., Kim H.O., von Lubitz D.K., Jacobson K.A. et al.: *G protein-dependent activation of phospholipase C by adenosine A_3 receptors in rat brain*. Mol. Pharmacol., 1995; 48: 1038–1045.
- Assi A.A.: *N^6 -cyclohexyladenosine and 3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid enhance the effect of antiepileptic drugs against induced seizures in mice*. J. Pharm. Sci., 2001, 4: 42–51.
- Booker S.A., Pires N., Cobb S., Soares-da-Silva P., Vida I.: *Carbamazepine and oxcarbazepine, but not eslicarbazepine, enhance excitatory synaptic transmission onto hippocampal CA1*

- pyramidal cells through an antagonist action at adenosine A1 receptors. *Neuropharmacology*, 2015, 93: 103–115.
- Borowicz K.K., Kamiński R., Gasior M., Kleinrok Z., Czuczwar S.J.: Influence of melatonin upon the protective action of conventional anti-epileptic drugs against maximal electroshock in mice. *Eur. Neuropsychopharmacol.*, 1999, 9: 185–190.
- Borowicz K.K., Kleinrok Z., Czuczwar S.J.: N^6 -2-(4-aminophenyl) ethyl-adenosine enhances the anticonvulsive activity of antiepileptic drugs. *Eur. J. Pharmacol.*, 1997, 327: 125–133.
- Borowicz K.K., Kleinrok Z., Czuczwar S.J.: $N(6)$ -2-(4-aminophenyl) ethyl-adenosine enhances the anticonvulsive action of conventional antiepileptic drugs in the kindling model of epilepsy in rats. *Eur. Neuropsychopharmacol.*, 2000, 10: 237–243.
- Borowicz K.K., Łuszczki J., Czuczwar S.J.: 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.
- Bynoe M.S., Viret C., Yan A., Kim D.G.: Adenosine receptor signaling: a key to opening the blood-brain door. *Fluids Barriers CNS*, 2015, 12: 20. doi: 10.1186/s12987-015-0017-7.
- Cieślak M., Wojtczak A., Komoszyński M.: Role of the purinergic signaling in epilepsy. *Pharmacol Rep.*, 2017, 69: 130–138.
- Czapiński P., Błaszczuk B., Czuczwar S.J.: Mechanisms of action of antiepileptic drugs. *Curr. Top. Med. Chem.*, 2005, 5: 3–14.
- Daly J.W., Butts-Lamb P., Padgett W.: Subclasses of adenosine receptors in the central nervous system: Interactions with caffeine and related methylxanthines. *Cell. Mol. Neurobiol.*, 1983, 3: 69–80.
- Dragunow M.: Endogenous anticonvulsant substances. *Neurosci. Biobehav. Rev.*, 1986, 10: 229–244.
- Jasiński M., Chrościńska-Krawczyk M., Kaczmarczyk K., Czuczwar S.J.: Various adenosine receptor agonists differentially affect the anticonvulsant action of phenobarbital and phenytoin against maximal electroshock-induced seizures in mice. *Epileptologia*, 2011, 19: 5–15.
- Lampe H., Bigalke H.: Carbamazepine blocks NMDA-activated currents in cultured spinal cord neurons. *Neuroreport*, 1990, 1: 26–28.
- Litchfield, J.T., Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.*, 1949, 96: 99–113.
- Löscher W., Schmidt D.: Which animal models should be used in the search for new antiepileptic drugs? A proposal based on the experimental and clinical considerations. *Epilepsy Res.*, 1988, 2: 145–181.
- Löscher W.: New visions in the pharmacology of anticonvulsion. *Eur. J. Pharmacol.*, 1998, 342: 1–13.
- Łuszczki J.J., Czuczwar S.J.: How significant is the difference between drug doses influencing the threshold for electroconvulsions? *Pharmacol. Rep.*, 2005, 57: 782–786.
- Łuszczki J.J., Kozicka M., Świąder M.J., Czuczwar S.J.: 2-Chloro- N^6 -cyclopentyladenosine enhances the anticonvulsant action of carbamazepine in the mouse maximal electroshock-induced seizure model. *Pharmacol. Rep.*, 2005, 57: 787–894.
- Macdonald R.L.: Carbamazepine. Mechanisms of action. In: R.H. Levy, R.H. Mattson, B.S. Meldrum, E. Perucca (eds.), *Antiepileptic Drugs*, 5th edn. Lippincott Williams & Wilkins, Philadelphia, 2002, 227–235.
- Malhotra J., Gupta Y.K.: Effect of adenosine receptor modulation on pentylenetetrazole-induced seizures in rats. *Br. J. Pharmacol.*, 1997, 120: 282–288.
- Marangos P.J., Post R.M., 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.
- Miziak B., Chrościńska-Krawczyk M., Błaszczuk B., Radzik I., Czuczwar S.J.: Novel approaches to anticonvulsant drug discovery. *Expert Opin. Drug Discov.*, 2013, 8: 1415–1427.
- Ralevic V., Burnstock G.: Receptors for purines and pyrimidines. *Pharmacol. Rev.*, 1998, 50: 415–492.
- Schwartzkroin P.A.: Basic Mechanism of epileptogenesis. In: E. Wyllie (ed.), *The Treatment of epilepsy: Principles and practice*. Lea & Febiger, Philadelphia, 1993, 83–99.
- Sebastião A.M., Ribeiro J.A.: Adenosine A₂ receptor-mediated excitatory actions on the nervous system. *Prog. Neurobiol.*, 1996, 48: 167–189.
- Shorvon S.D.: The epidemiology and treatment of chronic and refractory epilepsy. *Epilepsia*, 1996, 37 (Suppl. 2): S1–S3.
- Skerritt J.H., Davies L.P., Johnston G.A.: Interactions of the anticonvulsant carbamazepine with adenosine receptors. 1. Neurochemical studies. *Epilepsia*, 1983, 24: 634–642.
- Sun Z., Zhong X.L., Zong Y., Wu Z.C., Zhang Q., Yu J.T. et al.: Activation of adenosine receptor potentiates the anticonvulsant effect of phenytoin against amygdala kindled seizures. *CNS Neurol. Disord. Drug Targets*, 2015, 14: 378–385.
- Świąder M.J., Kotowski J., Łuszczki J.J.: Modulation of adenosinergic system and its application for the treatment of epilepsy. *Pharmacol. Rep.*, 2014, 66: 335–342.
- Van Calker D., Muller M., Hamprecht B.: Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. *Nature*, 1978, 276: 839–841.
- Van Calker D., Steber R., Klotz K.N., Greil W.: Carbamazepine distinguishes between adenosine receptors that mediate different second messenger responses. *Eur. J. Pharmacol.*, 1991, 206: 285–290.
- Young D., Dragunow M.: Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. *Neuroscience*, 1994, 58: 245–261.
- Younus I., Reddy D.S.: A resurging boom in new drugs for ep-

ilepsy and brain disorders. Expert Rev. Clin. Pharmacol., 2017 – in press. doi: 10.1080/17512433.2018.1386553.

Zhang G., Franklin P.H., Murray T.F.: *Manipulation of endogenous adenosine in the rat prepiriform cortex modulates seizure susceptibility*. J. Pharmacol. Exp. Ther., 1993, 264: 1415–1424.

Zhou Q.Y., Li C., Olah M.E., Johnson R.A., Stiles G.L., Civelli O.: *Molecular cloning and characterization of an adenosine receptor: the A₃ adenosine receptor*. Proc. Natl. Acad. Sci. USA, 1992, 89: 7432–7436.

