Polish Journal of Pharmacology
Pol. J. Pharmacol., 2001, 53, 83–92
ISSN 1230-6002

EFFECT OF ADENOSINE A1 AND A2 RECEPTOR STIMULATION ON HYPOXIA-INDUCED CONVULSIONS IN ADULT MICE

Witold Zgodziński[1,2,#], Andrzej Rubaj[3], Zdzisław Kleinrok[4], Maria Sieklucka-Dziuba[1]

[1]Department of Hygiene, Medical University, Radziwiłłowska 11, PL 20-085 Lublin, Poland, [2]2nd Department of General Surgery, Medical University, Staszica 16, PL 20-081 Lublin, Poland, [3]Department of Cardiology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland, [4]Department of Pharmacology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland

Effect of adenosine A1 and A2 receptor stimulation on hypoxia-induced convulsions in adult mice. W. ZGODZIŃSKI, A. RUBAJ, Z. KLEINROK, M. SIEKLUCKA-DZIUBA. Pol. J. Pharmacol., 2001, 53, 83–92.

Clinical observations indicate that seizures induced by hypoxia are common kind of convulsive activity in both infants and elderly patients. The occurrence of seizure episode during hypoxia is important risk factor of epilepsy development in the future. Experimental hypoxia was obtained by exposure of adult (20–23 g) Albino Swiss mice to spontaneous breathing in gas mixture composed of 5% oxygen and 95% nitrogen. The latency time to convulsive activity was determined. Single sublethal episode of seizures induced by hypoxia (HS) resulted in higher susceptibility to pentetrazol (PTZ), bicuculline (BCC), N-methyl-D-aspartic acid (NMDA) but not in electrically induced convulsions. Adenosine A1 receptor agonist, R(–)N6-(2-phenyl-isopropyl)adenosine (R-PIA) (0.01; 0.05; 0.1 mg/kg, *ip*) prolonged the latency to HS-induced convulsions. A1 receptor antagonist, 8-cyclopentyltheophylline (CPT), reversed the protective action of R-PIA. A2 receptor agonist, N(6)-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)]ethyladenosine (DPMA), only at the highest dose (5 mg/kg *ip*) prolonged the latency time to convulsive activity. This effect was only partially reversed by A2 antagonist 3,7-dimethyl-1-propargylxanthine (DMPX). Administered immediately after episode of HS R-PIA diminished the higher susceptibility to PTZ, BCC, NMDA at 3rd day after HS, while DPMA appeared to be ineffective.

These results confirm the important role of adenosine A1 receptor agonist in protection against acute and chronic epileptogenic effect of hypoxia. The role of adenosine A2 receptors seems to be of minor importance.

Key words*: adenosine receptors, hypoxia, convulsions*

[#] *correspondence*; e-mail: wzgodz@wp.pl

Abbreviations: BCC – *bicuculline,* CPT – *8-cyclopentyltheophylline,* DMPX – *3,7-dimethyl-1-propargylxanthine,* DPMA – *N(6)-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)]ethyladenosine,* NMDA – *N-methyl-D-aspartic acid,* PTZ – *pentetrazol,* R-PIA – *R(–)N6-(2-phenyl-isopropyl)adenosine*

INTRODUCTION

Adenosine is considered as one of the most important endogenous anticonvulsive factors [16, 18, 33]. Experimental investigations and clinical observations proved that convulsions significantly increased the level of endogenous adenosine in the brain and cerebrospinal fluid [13, 19, 49]. Adenosine and its analogues (mainly A1 receptor agonists) showed anticonvulsive activity in many models of experimental epilepsy. Adenosine A1 agonists appeared to be protective in convulsions induced by leptazole [34], picrotoxin [18], NMDA [47, 48] kainic and quisqualinic acid [46], pilocarpine [45] and activator of Ca^{2+} channels Bay k-8644 [14]. Moreover, adenosine A1 agonists are effective in kindling model of experimental epilepsy [2, 9, 16] and maximal electroshock seizures (MES) [12].

Considerably less data refers to anticonvulsive activity of A2 receptor agonists. Adenosine A2a agonists in dose-dependent manner acted against convulsions induced by pentetrazol (PTZ) [2], sound in DB2 mice [15] and inverse agonist of benzodiazepine receptor [30]. Hypoxia itself can be a factor, which induces convulsive activity. This special kind of convulsions constitutes about 30–65% of all cases in neonatal period [7, 40] and their occurrence is important risk factor of future epilepsy [6, 31, 35]. Seizures induced by hypoxia develop also in adult people in the course of stroke [5, 24, 28] or during sudden cardiac/respiratory arrest [41]. Clinical observations allowed to conclude that occurrence of convulsive episode in the course of brain hypoxia worsened prognosis and significantly increased the chance of epilepsy development in the future.

In the present study we wished to characterize the influence of hypoxia-induced seizures on further long-term susceptibility to convulsions induced by PTZ, bicuculline (BCC), N-methyl-D-aspartic acid (NMDA) and electrical shock (ES). Furthermore, we wished to test the possibility of preventing acute and chronic epileptogenic role of hypoxia by adenosine A1 and A2 receptors agonists.

MATERIALS and METHODS

Animals

Female Albino Swiss mice weighing 20–25 g were used in the studies. The animals were allowed to settle under standard conditions: at the temperature of 20 ± 1°C, under a natural light-dark cycle with free access to food and water. The animals were used only once in an experiment.

Drugs

The following substances were used in the experiments: adenosine A1 receptor agonist, R(–)N6--(2-phenyl-isopropyl)adenosine, (RBI, Natick, USA); adenosine A1 receptor antagonists, 8-cyclopentyltheophylline, (RBI, Natick, USA); adenosine A2 receptor agonists, N(6)-[2-(3,5-dimethoxyphenyl)--2-(2-methylphenyl)]ethyladenosine, (RBI, Natick, USA); adenosine A2 receptor antagonist, 3,7-dimethyl-1-propargylxanthine, (RBI, Natick, USA). Moreover we used: pentetrazol, (Sigma, St. Louis, USA); bicuculline, (Sigma, St. Louis, USA); N-methyl-D-aspartic acid, (Sigma, St. Louis, USA). Substances were dissolved in water and administered intraperitoneally (*ip*) or subcutaneously (*sc*) in constant volume of 10 ml/kg and NMDA intracerebroventricularly (*icv*) in constant volume of 2 l. Animals in the control groups received adequate volumes of vehicle.

Seizures induced by hypoxia

Mice (n = 10) were placed in special observation, airtight chamber in which the gas mixture (5% oxygen and 95% nitrogen) was maintained at steady flow of 10 l/min, and observed up to the appearance of the generalized clonic seizures (alternate limbs flexion and extension connected to loss of posture). The latency time to onset of seizures was measured. Adenosine A1 receptor agonist R-PIA, (0.01; 0.05; 0.1 mg/kg), adenosine A1 receptor antagonist CPT, (5; 10 mg/kg), as well as adenosine A2 receptor agonist DPMA, (1; 2.5; 5 mg/kg) and adenosine A2 receptor antagonist DMPX, (2.5; 5 mg/kg) were administered to mice, alone or in combination, 30 min before subjecting them to hypoxia. The latency time from the moment of placing the animals in hypoxic conditions to the occur-

rence of first episode of clonic convulsions was measured. Experimental and control groups consisted of 10 animals.

Clonic seizures induced by PTZ, BCC, NMDA

Mice were injected with PTZ (*sc*), BCC (*sc*) or NMDA (*icv*) at the doses equal to their ED_{16}, i.e. 65 mg/kg, 2.8 mg/kg, 0.8 nmol/mice, respectively. The proportion of mice in which clonic convulsions occurred (alternate flexion and extension of the limbs lasting at least 3 s, connected with loss of posture) within 30 min after the injection of the convulsant, was noted. Seizures were always induced between 11:00 and 16:00 hour to minimize possible inconsistencies arising from circadian rhythms. Experimental groups consisted of 15 mice.

Electrically induced seizures

Electrical seizures were induced by alternating current (50 Hz, 0.2 s) applied by ear-clip electrodes, according to the method of Swinyard et al. [43]. The intensity of current used, i.e. 5mA, corresponded to CS_{16}, which is the current strength in mA necessary to produce convulsions in 16% of control animals. The criterion of the convulsions was the tonic extension of the hind limbs. Furthermore ES test, with a current of 25 mA, was performed. The proportion of mice exhibiting tonic convulsions was noted. In both tests seizures were induced between 11:00 and 16:00 hour to minimize circadian effects. Experimental groups consisted of 15 mice

Testing of further seizure susceptibility induced by PTZ, BCC, NMDA, and electrical current in animals after hypoxia-induced convulsion episodes

Animals were subjected to hypoxia in the manner described above. After the episodes of convulsions lasting at least 10 s followed by occurrence of apnea and symptoms of urine incontinence, animals were evacuated from the container and transferred to home cages. After 1, 3, 10, 21 days from episode of hypoxia-induced convulsion, the susceptibility to clonic seizures induced by PTZ, BCC, NMDA at the doses equal their ED_{16} were investigated. Furthermore, the animals' excitability in electric convulsions elicited by electric current with an intensity equal to CS_{16}, and usually used for maximal electroshock seizures (25 mA) was determined. In order to assess the influence of adenosine, ligands of A1 and A2 receptors: R-PIA (0.01; 0.05; 0.1 mg/kg), DPMA, (1; 2.5; 5 mg/kg), CPT (10 mg/kg), and DMPX (5 mg/kg) were given alone or in combination immediately after hypoxic seizures episodes. Seizure susceptibility in PTZ, BCC, NMDA and electrically induced convulsions were investigated 3 days later.

Histological analysis

Three experimental and three control animals were randomly selected. Animals were killed 3 days after the hypoxia-induced seizures. Their brains were removed and postfixed with Baker solution (1% $CaCl_2$ in 10% buffered formalin) for 2 weeks. Then, the brains were dehydrated, embedded in paraffin and sectioned at 6 m thickness for staining with hematoxylin and eosine or cresyl violet for light microscopic analysis.

Statistical analysis

Latency to hypoxia-induced seizures was expressed as arithmetic mean ± SD and ANOVA test was used for analysis. The results from chemically and electrically induced convulsions were compared statistically by using Fisher's exact probability test.

RESULTS

The influence of adenosine receptor agonists and antagonists on latency time to hypoxic seizures (HS)

Adenosine A1 receptor agonist, R-PIA, administered 30 min before the hypoxia episode at the doses of 0.01, 0.05 and 0.1 mg/kg, *ip*, significantly prolonged the latency time to convulsive activity (440.2 ± 120.3 s, 555.6 ± 205 s and 632.4 ± 258 s, respectively) comparing with the control group (370.2 ± 55 s). A1 receptor antagonist CPT, applied at the doses of 5 and 10 mg/kg, 30 min before hypoxia, did not influence the latency time to convulsive activity. Moreover, CPT at 10 mg/kg diminished the protective activity of R-PIA (Fig. 1). A2 receptor agonist DPMA, administered at the doses of 1 and 2.5 mg/kg, 30 min before hypoxic episode, did not influence the latency time to convulsive activity. At 5 mg/kg it significantly prolonged the la-

tency time (430 ± 98 s) comparing with the control group. A2 receptor antagonist, DMPX, applied at 2.5 and 5 mg/kg, 30 min before hypoxia, did not exert any effect on latency time to convulsions, and at the dose of 5 mg/kg it diminished the protective activity of DPMA at the dose of 5 mg/kg (Fig. 2).

The influence of sublethal episode of HS on susceptibility to seizures induced by PTZ, BCC, NMDA and ES

Convulsion susceptibility was examined on 1, 3, 10, 21 day after HS. Increased susceptibility to

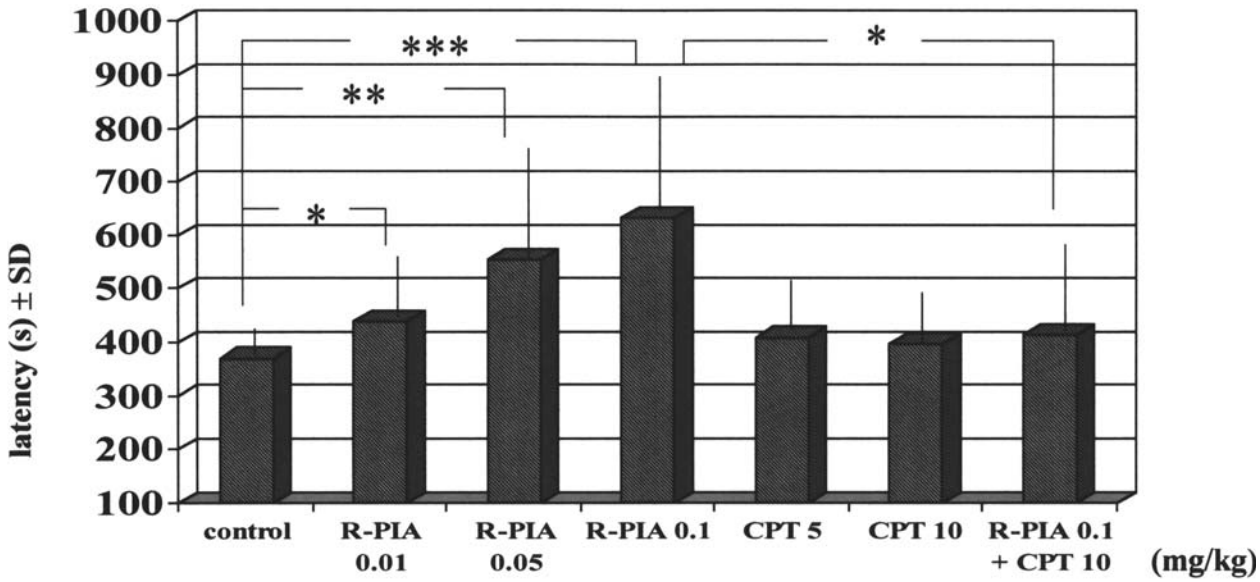

Fig. 1. The influence of A1 receptor agonist R-PIA, and antagonist CPT on latency to seizures induced by hypoxia. * $p < 0.05$. ANOVA test

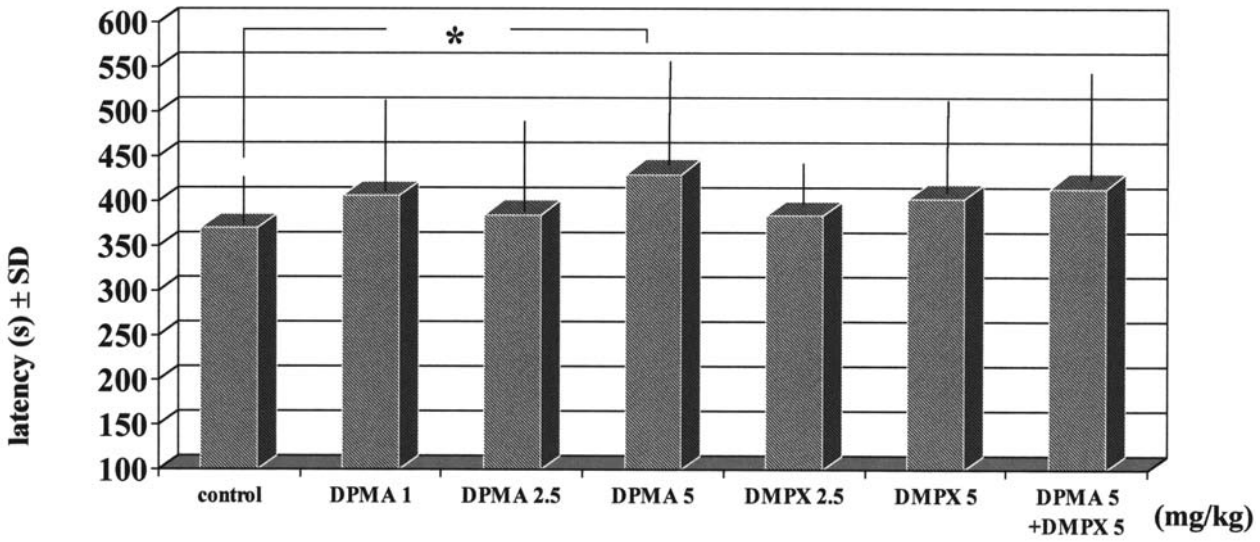

Fig. 2. The influence of A2 receptor agonist DPMA, and antagonist DMPX on latency to seizures induced by hypoxia. * $p < 0.05$. ANOVA test

convulsions induced by PTZ, BCC, NMDA was noted during whole observation period. The difference was most significant at lower doses of chemoconvulsants, and ED_{16} was selected for further investigations (Fig. 3). In case of ED_{50} and also ED_{97} for all abovementioned convulsants, the differences were not statistically significant. Differences in convulsive excitability, elicited by current strength equal to CS_{16}, or electric shock (25 mA) during whole period of observation were not significant.

The influence of adenosine receptor agonists and antagonists on susceptibility to convulsions induced by chemoconvulsants 3 days after HS episode

A1 receptor agonist, R-PIA, at the doses of 0.01, 0.05 and 0.1 mg/kg, administered immediately after HS, produced statistically significant lowering of seizure susceptibility in convulsions induced by PTZ and BCC, and at the doses of 0.05

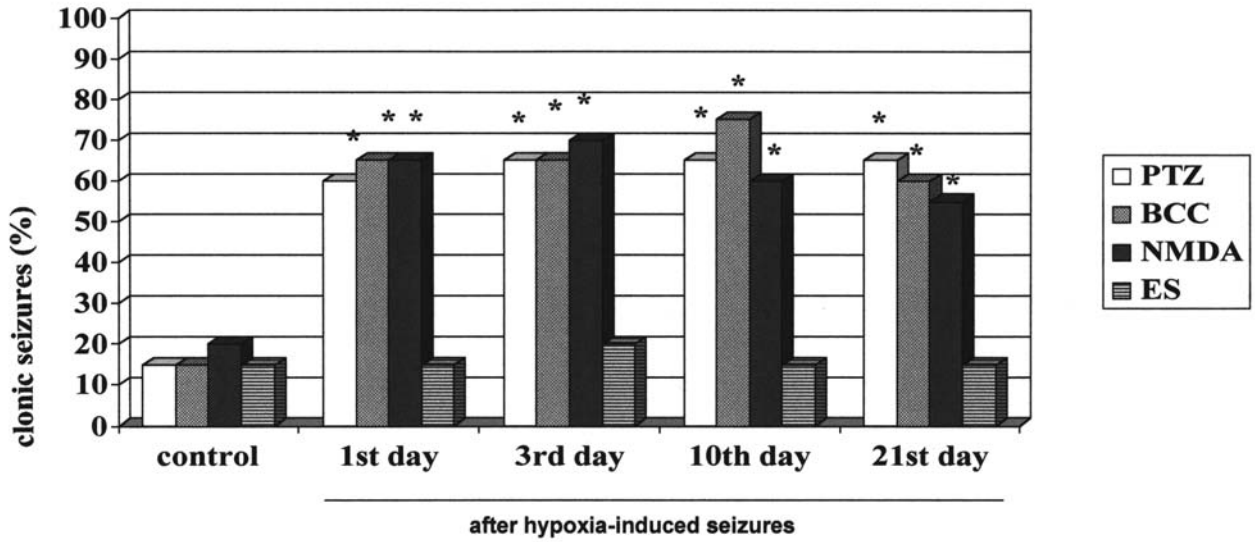

Fig. 3. The influence of single episode of hypoxia-induced seizures on susceptibility to seizures induced by PTZ, BCC, NMDA (at the doses equal ED_{16} and ES CS_{16}).* $p < 0.05$ vs. respective control group. Fisher test

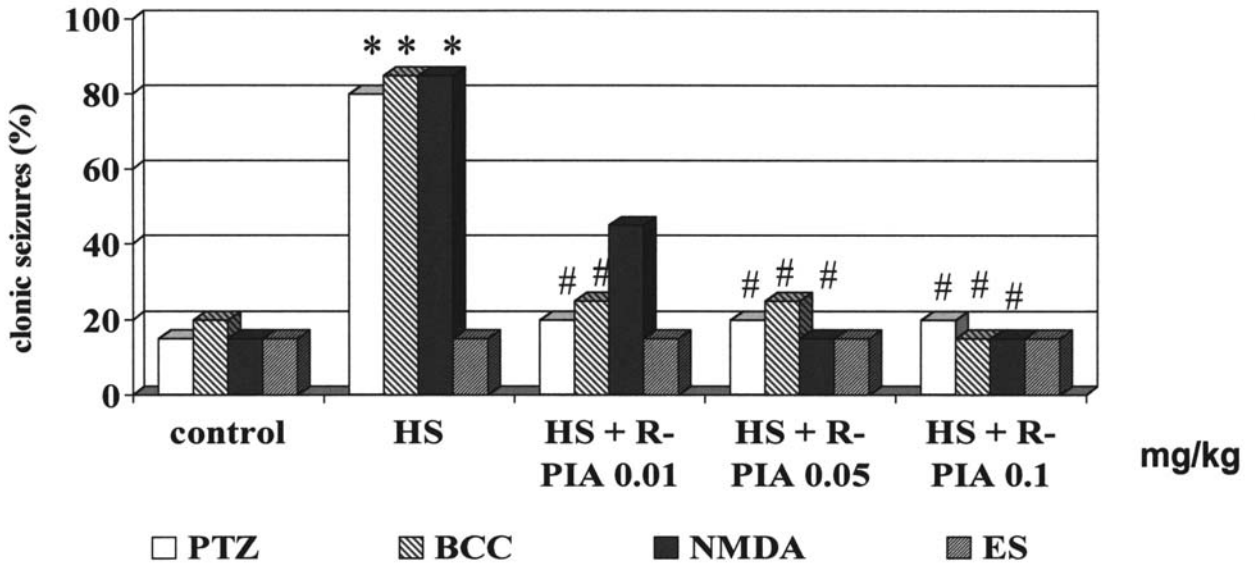

Fig. 4. The influence of adenosine A1 receptor agonist R-PIA on convulsive susceptibility in selected models of experimental epilepsy at 3rd day after hypoxia-induced seizures. * $p < 0.05$ vs. respective control group. # $p < 0.05$ vs. respective HS group. Fisher test

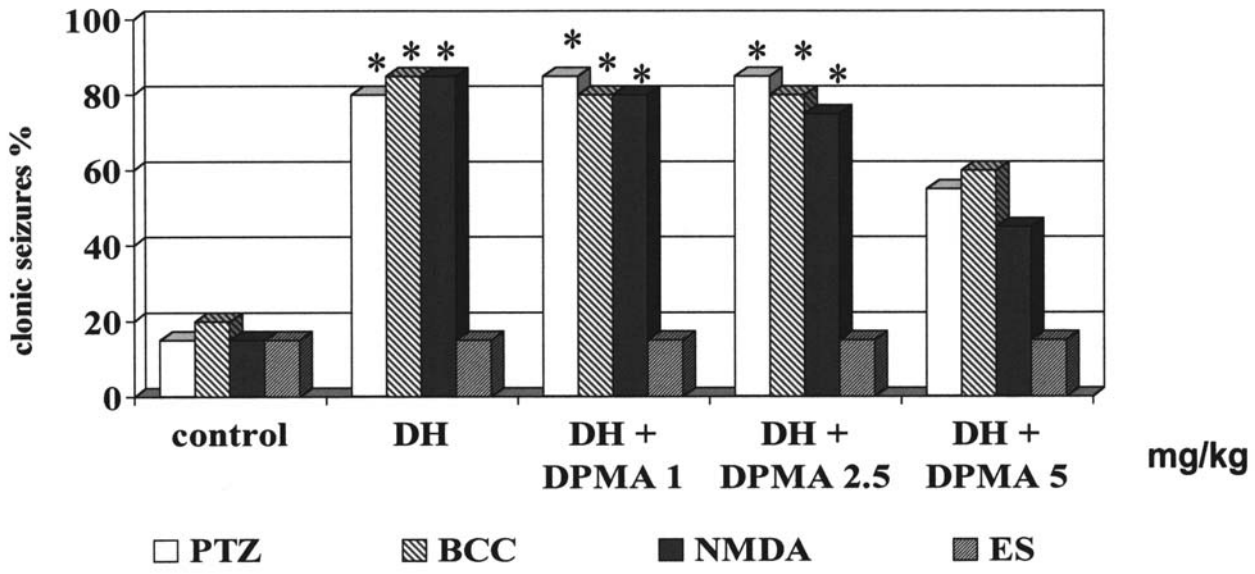

Fig. 5. The influence of adenosine A2 receptor agonist DPMA on convulsive susceptibility in selected models of experimental epilepsy at 3rd day after hypoxia-induced seizures. * $p < 0.05$ vs. respective control group. Fisher test

and 0.1 mg/kg in NMDA-induced convulsions, comparing with the control group (Fig. 4). A1 receptor antagonist CPT, administered at doses 5 and 10 mg/kg, did not influence the higher seizure susceptibility after HS, examined in similar experiment. At the dose of 10 mg/kg, CPT co-administered with R-PIA, reduced the protective activity of an agonist but the difference was not statistically significant (data not shown). A2 receptor agonist DPMA (1, 2.5 and 5 mg/kg), administered immediately after HS did not influence the higher seizure susceptibility in convulsions induced by PTZ, BCC and NMDA comparing with the control group (Fig. 5). Similarly, A2 receptor antagonist, DMPX (5 and 10 mg/kg), was not active in this experiment (data not shown).

Histology

In histological preparations of the brains assessed 1 and 3 days after sublethal episode of HS in light microscope at 500× no necrotic changes of neurons were detected.

DISCUSSION

According to experimental and clinical observations, convulsions can be one of complications of CNS hypoxia/ischemia [7, 26, 40, 44]. However, the existing evidences shows that hypoxia is not always the injuring or lethal factor. Hypoxic episodes of moderate intensity can induce the protective action by eliciting adaptive effects both, in young and adult animals. It was observed that moderate hypoxia can reduce later convulsion susceptibility [38, 39]. The effect (protection or injury) probably depends on intensity as well as the duration of hypoxic insult but precise conditions are difficult to establish.

In experimental model presented in this paper, hypoxia (5% oxygen in atmosphere) appeared to be a factor eliciting the convulsions in about 90% of animals. According to these results and data from other studies [21, 26, 42], it can be concluded that occurrence of convulsions in the course of hypoxia is a factor which determines and potentiates the possible complications and generally worsens the prognosis. Animals subjected to hypoxia-induced convulsions were characterized by increased seizure susceptibility in later period as determined in several different models of experimental epilepsy. It seems to be interesting that this phenomenon is observed only for lower doses (ED_{16}) of applied convulsants. In electrically induced seizures the differences between the control and group subjected to HS were not observed. Thus, the functional disturbances in neurons after hypoxia-induced convulsions in this experimental model are rather limi-

ted. The current results confirm previous data that hypoxia does not exert a general influence on seizure susceptibility, but rather a model-specific effect [3].

The role of adenosine in ischemic preconditioning phenomenon in the brain is worth noting [25, 38], however, its molecular mechanism is not fully clarified. Administration of R-PIA, A1 receptor agonist, before period of hypoxia significantly prolonged the latency time to seizure onset, in dose--dependent manner. The effect was abolished by A1 receptor antagonist CPT. Administration of A1 antagonist, CPT alone, did not influence the time of latency to convulsions. Moreover, R-PIA administered immediately after episode of hypoxia-induced convulsions prevented the increase in susceptibility in seizures induced by PTZ, BCC and NMDA. Neuroprotective and anticonvulsive activity of adenosine and its analogues mediated *via* A1 receptor is widely supported by several evidences. One of the results of A1 receptor stimulation is inhibition of presynaptic glutamate release [11]. As the effect of potassium current induction [22] adenosine maintains blockade of voltage-dependent NMDA receptor, and reduces Ca^{2+} influx. In consequence of A1 receptor activation NMDA--dependent production of nitric oxide is also reduced, that can confirm the neuroprotective profile of activity [8]. Furthermore, *in vitro* experiments revealed that adenosine neuroprotection may result from inhibition of free radical production in neutrophils [10], as well as from diminution of endogenous, antioxidant enzyme activity [32]. Above-described neuroprotective activity of adenosine acting *via* A1 receptor is also fundamental for its anticonvulsive activity. This mechanism of activity is dependent mostly on restrictive influence on excitatory amino acid system in CNS. Immediate protective influence of A1 agonist CPA (N6-cyclopentyladenosine) in convulsions induced by NMDA administration in mice was proved. This protective effect was not dependent on hypothermia, which can occur after adenosine A1 stimulation [47]. Similar protection (prolonged latency and reduced mortality) was noted after dizocilpine administration as well as some adenosinergic compounds (N6-cyclohexyladenosine, 2-chloroadenosine and dipyridamol) in hypoxia-induced convulsions in mice. This effect was reversed in both cases by teophylline, a non-selective adenosine receptor antagonist [44]. Another evidences for the involvement of adenosinergic system in pathomechanism of convulsions induced by hypoxia are provided by the observations of seizures elicited by administration of caffeine in rat hippocampal slices exposed to short period of anoxia. The effect was prevented by A1 receptor agonist N6-cyclopentyladenosine. Moreover, results similar as in the case of caffeine treatment were obtained for selective A1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine [20]. These results decidedly support the hypothesis about important role of adenosine A1 receptor in pathophysiology of hypoxia-induced convulsions.

Adenosine A2 receptor agonist DPMA, used in the present experiments, did not influence the latency to convulsions, and only at higher dose (5 mg/kg) it prolonged the latency time. Adenosine A2 receptor antagonist DMPX did not change the time to convulsion onset. Furthermore, DMPX reversed the protective activity of DPMA at 5 mg/kg. Action of DPMA and/or DMPX given after hypoxia-induced convulsions consisting in the increased susceptibility to PTZ-, BCC- and NMDA-induced seizures was not observed. To date there is no enough information about the role of adenosine A2 receptor agonists in protective action of adenosine during ischemia or hypoxia. Stimulation of A2a receptor by specific agonists may considerably enhance the extracellular glutamate [37] and acetylcholine level [29] in the rat striatum, that perhaps is unfavorable in hypoxia. Other data indicate that A2a receptor activation causes diminution of electric activity of neurons [36]. A2a agonist HE-NECA, in dose-dependent manner prolonged the latency to convulsions elicited by PTZ in rats, and reduced mortality of animals in an experiment [2]. Mechanism of the protective action can depend on the increased cerebral blood flow and reduced free radical formation [22]. Moreover, it was observed that chronic A2 receptor stimulation was not related with down-regulation which cannot be excluded in case of A1 receptor [1]. Protective effect of A2a stimulation was observed in audiogenic convulsions in DBA/2 mice [15]. DPMA blocked the onset of seizures induced by inverse agonist of benzodiazepine receptor [30]. It was proved that in some experimental models, tolerance to anticonvulsive activity develops more quickly for A1 receptor rather than for A2a [13].

Results of the present work seem to confirm the fact that A2 receptor agonists play minor role in an-

ticonvulsive effect of adenosine. Moreover, accessible data do not permit for identification of cellular mechanisms, by which adenosine analogues acting *via* A2 receptor exert their anticonvulsive effects in individual experiments mentioned above. As it was noted in the experiments, single, sublethal episode of hypoxia-induced convulsions resulted in prolonged, increased susceptibility in some models of experimental epilepsy.

Adenosine A1 receptor agonist R-PIA, administered immediately after episode of hypoxia-induced convulsions-significantly prevented the occurrence of higher susceptibility in PTZ-, BCC- and NMDA-induced seizures 3 days later. Adenosine A1 antagonist CPT in combination with R-PIA reversed the protective effect of the agonist. A2 receptor agonist A2 DPMA did not influence the increased susceptibility to seizures in this experiment.

In previous studies concerning the relation between hypoxic conditions and epileptogenesis, it was observed that total hypoxia in rats on postnatal days 5–17 resulted in seizures, which induced higher susceptibility to convulsions in future. Administration of NBQX, AMPA/KA receptor antagonist, protected against the epileptogenic effect of hypoxia, examined in convulsions induced by flurothyl. The authors postulated significant role of AMPA/KA receptor activation in prolonged epileptogenic effect of hypoxia in this period of development [26]. Increased seizure susceptibility after HS in convulsions induced by PTZ and NMDA suggests disturbances related to glutamergic transmission. These disturbances are connected mostly with higher release of excitatory amino acids, which occurs even after period of hypoxia. It is possible that protective effect of A1 receptor agonist (R-PIA) observed in the present experiments may be explained with limitation of this phenomenon in post-hypoxic period.

In the present work, behavioral experiments were complemented with histologic assessment of the brain. In animals subjected to hypoxia-induced convulsions histologic examination was performed with light microscope. Assessment was focused mostly on the hippocampus, the most sensitive structure for injury under hypoxic conditions. In *in vitro* studies on hippocampal slices, Kawasaki et al. revealed that convulsions induced by hypoxia are generated mostly in CA1 region and only at 2.5% in CA3 [27]. In cited above Jensen's experimental model, no morphological indications of neuronal injury or necrosis of neurons were found in young rats subjected to hypoxia-induced convulsions [26]. In our experiments, existence of any morphologic changes, which would confirm neuronal injury in the course of hypoxia-induced convulsions at adult mice, was not observed too. This refers only to necrosis of neurons. Investigation in light microscope does not permit to estimate possible lesions connected with apoptosis. Lack of morphological signs of brain injury suggests that the increased susceptibility to convulsions in the described model has functional character.

In conclusion we emphasize that adenosine A1 receptor agonist R-PIA and A2 receptor agonist DPMA produce protection against convulsions induced by hypoxic conditions by prolongation of latency time but the effect of DPMA seems to be not specific for A2 receptor. Furthermore, A1 receptor agonist R-PIA prevented the increased seizure susceptibility in the selected experimental models of epilepsy, which was observed in control animals after short episode of hypoxia-induced convulsions. These results confirm the important role of adenosine A1 receptors in counteracting an acute and chronic epileptogenic effect of hypoxia. The role of adenosine A2 receptors seems to be of minor importance.

REFERENCES

1. Abbracchio M.P., Brambilla R., Rovati G.E., Cattabeni F.: Prolonged agonist exposure induces imbalance of A1 and A2 receptor-mediated functions in rat brain slices. Drug. Develop. Res., 1993, 28, 364–368.
2. Adami M., Bertorelli R., Ferri N., Foddi M.C., 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.
3. Applegate C.D., Jensen F., Burchfiel J.L., Lombroso C.: The effects of neonatal hypoxia on kindled seizure development and electroconvulsive shock profiles. Epilepsia, 1996, 37, 723–727.
4. Barraco R.A., Swanson T.H., Phillis J.W., Berman R.F.: Anticonvulsant effects of adenosine analogues on amygdaloid-kindled seizures in rats. Neurosci. Lett., 1984, 46, 317–322.
5. Barry E., Hauser W.A.: Status epilepticus: the interaction of epilepsy and acute brain disease. Neurology, 1993, 43, 1473–1478.
6. Bergamasco B., Penna P., Ferrero P.: Neonatal hypoxia and epileptic risk: clinical prospective study. Science, 1982, 216, 899–901.

7. Bergman I., Painter M.J., Hirsch R.P., Crumrine P.K., David R.: Outcome in neonates with convulsions treated in an intensive care unit. Pediatrics, 1993, 91, 128–134.
8. Bhardwaj A., Northington F.J., Koehler R.C., Hanley D.F., Traystman R.J.: Adenosine A1 receptor stimulation attenuates the N-methyl-D-aspartate-induced nitric oxide production in the hippocampus: An in vivo microdialysis study. Stroke, 1995, 26**,** 168–170.
9. Bortolotto Z.A., Mello L.E., Turski L., Cavalheiro E.A.: Effects of 2-chloroadenosine on amygdaloid and hippocampal kindled seizures. Arch. Int. Pharmacodyn. Thér., 1985, 277**,** 313–320.
10. Burkey T.H., Webster R.O.: Adenosine inhibits fMLP--stimulated adherence and superoxide anion generation by human neutrophils at an early step in signal transduction. Biochim. Biophys. Acta, 1993, 1175**,** 312–318.
11. Choi D.W., Hartley D.M.: Calcium and glutamate--induced cortical neuronal death. Res. Publ. Assoc. Res. Nerv. Ment. Dis., 1993, 71, 23–34.
12. Czuczwar S.J., 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.
13. Delaney S.M., Shepel P.N., Geiger J.D.: Levels of endogenous adenosine in rat striatum. Regulation by ionotropic glutamate receptors, nitric oxide and free radicals. J. Pharmacol. Exp. Ther., 1998, 285, 561–567.
14. De Sarro G., Ascioti C., di Paola E.D., Vidal M.J., De Sarro A.: Effects of antiepileptic drugs, calcium channel blockers and other compounds on seizures induced by activation of voltage-dependent L calcium channel in DBA/2 mice. Gen. Pharmacol., 1992, 23, 1205––1216.
15. De Sarro G., De Sarro A., Di Paola E.D., Bertorelli R.: Effects of adenosine receptor agonists and antagonists on audiogenic seizure sensible DBA/2 mice. Eur. J. Pharmacol., 1999, 371, 137–145.
16. Dragunow M., Goddard G.V., Laverty R.: Is adenosine an endogenous anticonvulsant? Epilepsia, 1985, 26, 480–487.
17. Dunwiddie T.V., Diao L., Kim H.O., Jiang J.L., Jacobson K.A.: Activation of hippocampal adenosine A3 receptors produces a desensitization of A1 receptor-mediated responses in rat hippocampus. J. Neurosci., 1997, 17, 607–614.
18. Dunwiddie T.V., Worth T.: Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J. Pharmacol. Exp. Ther., 1982, 220, 70–76.
19. During M.J., Spencer D.D.: Adenosine: a potential mediator of seizure arrest and post-ictal refractoriness. Ann. Neurol., 1992, 32, 618–624.
20. Dzhala V., Desfreres L., Melyan Z., Ben-Ari Y., Khazipov R.: Epileptogenic action of caffeine during anoxia in the neonatal rat hippocampus. Ann. Neurol., 1999, 46, 95–102.
21. Ekert P., Perlman M., Steinlin M., Hao Y.: Predicting the outcome of postasphyxial hypoxic-ischemic encephalopathy within 4 hours of birth. J. Pediatr., 1997, 131, 613–617.
22. Fredholm B.B., Johansson B., van der Ploeg I., Hu P.S., Jin S.: Neuromodulatory role of purines. Drug Develop. Res., 1993, 28, 349–358.
23. Greene R.W., Haas H.L.: The electrophysiology of adenosine in the mammalian central nervous system. Prog. Neurobiol., 1991, 367, 329–341.
24. Gupta S.R.: Postinfarction seizures: a clinical study. Stroke, 1988, 19, 1477–1481.
25. Heurteaux C., Lauritzen I., Widmann C., Lazdunski M.: Essential role of adenosine, adenosine A1 receptors, and ATP-sensitive K 1 channels in cerebral ischemic preconditioning. Proc. Nat. Acad. Sci. USA, 1995, 92, 4666–4670.
26. Jensen F.E.: An animal model of hypoxia-induced perinatal seizures. Ital. J. Neurol. Sci., 1995, 16, 59–68.
27. Kawasaki K., Traynelis S.F., Dingledine R.: Different responses of CA1 and CA3 regions to hypoxia in rat hippocampal slice. J. Neurophysiol., 1990, 63, 385––394.
28. Kilpatrick C.J., Davis S.M., Hopper J.L., Rossiter S.C.: Early seizures after acute stroke. Risk of late seizures. Arch. Neurol., 1992, 49, 509–511.
29. Kirk I.P., Richardson P.J.: Adenosine A2a receptor--mediated modulation of striatal [3H]GABA and [3H]acetylcholine release. J. Neurochem., 1994, 3, 960–966.
30. Klitgaard H., Knutsen L.J., Thomsen C.: Contrasting effects of adenosine A1 and A2 receptor ligands in different chemoconvulsive rodent models. Eur. J. Pharmacol., 1993, 242, 221–228.
31. Lambroso C.T.: Neonatal seizures. In: Epilepsy: Diagnosis and Management. Ed. Browne T.R., Little Brown, Boston, 1983, 297–313.
32. Maggirwar S.B., Dhanraj D.N., Somani S.M., Ramkumar V.: Adenosine acts as an endogenous activator of the cellular antioxidant defense system. Biochem. Biophys. Res. Commun., 1994, 201, 508–515.
33. Maître M., Ciesielski L., Lehmann A., Kempf E., Mandel P.: Protective effect of adenosine and nicotinamide against audiogenic seizures. Biochem. Pharmacol., 1974, 23, 2807–2816.
34. Murray T.F., Sylvester D., Schultz C.S., Szot P.: Purinergic modulation of the seizure threshold for pentylenetetrazole in the rat. Neuropharmacology, 1985, 24, 761–767.
35. Nelson K.B., Ellenberg J.H.: Antecedents of cerebral palsy. Multivariate analysis of risk. N. Engl. J. Med., 1986, 315, 81–86.
36. Phillis J.W.: The selective A2 agonist CGS 21680, is potent depressant of cerebral cortical activity. Brain Res., 1990, 509, 328–330.
37. Popoli P., Betto P., Reggio R., Ricciarello G.: Adenosine A2a receptor stimulation enhances striatal glutamate levels in rats. Eur. J. Pharmacol., 1995, 287**,** 215–217.

38. Rauca C., Ruthrich H.L.: Moderate hypoxia reduces pentylenetetrazole-induced seizures. Naunyn-Schmied. Arch. Pharmacol., 1995, 351, 261–267.
39. Rubaj A., Gustaw K., Zgodziński W., Kleinrok Z., Sieklucka-Dziuba M.: The role of opioid receptors in hypoxic preconditioning against seizures in brain. Pharmacol. Biochem. Behav., 2000, 67, 65–70.
40. Scher M.S., Aso K., Beggarly M.E., Hamid M.Y., Steppe D.A., Painter M.J.: Electrographic seizures in preterm and full-term neonates, clinical correlates, associated brain lesions, and risk for neurologic sequelae. Epilepsia, 1993, 34, 284–288.
41. Snyder B.D.: Neurologic prognosis after cardiopulmonary arrest: III. Seizure activity. Neurology, 1980, 30, 1292–1297.
42. So E.L., Annegers J.F., Hauser W.A., O'Brien P.C., Whisnant J.P.: Population based study of seizure disorder after cerebral infarction. Neurology, 1996, 46, 350–355.
43. Swinyard E.A., Brown W.C., Goodman L.S.: Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exp. Ther., 1952, 106, 319–330.
44. Thorat S.N., Kulkarni S.K.: Effect of MK-801 and its interaction with adenosinergic agents and carbamazepine against hypoxic stress-induced convulsions and death in mice. Meth. Find. Exp. Clin. Pharmacol., 1990, 12, 595–600.
45. Turski W.A., Cavalheiro E.A., Ikonomidou C., Mello L.E., Bortolotto Z.A., Turski L.: Effects of aminophylline and 2-chloroadenosine on seizures produced by pilocarpine in rats: morphological and electroencephalographic correlates. Brain Res., 1985, 361, 309–323.
46. Turski L., Niemann W., Stephens D.N.: Differential effects of antiepileptic drugs and beta-carbolines on seizures induced by excitatory amino acids. Neuroscience, 1990, 39, 799–807.
47. Von Lubitz D.K.J.E., Paul I.A., Carter M.F., Jacobson K.A.: Effects of N^6-cyclopentyl adenosine and 8-cyclopentyl-1,3-dipropylxanthine on N-methyl-D-aspartate-induced seizures in mice. Eur. J. Pharmacol., 1993, 249, 265–270.
48. Von Lubitz D.K.J.E., Paul I.A., Ji X-D., Carter M.F., Jacobson K.A.: Chronic adenosine A1 receptor agonist and antagonist stimulation: effect on receptor density and N-methyl-D-aspartate-induced seizures in mice. Eur. J. Pharmacol., 1994, 253, 95–99.
49. Winn H.R., Welsh J.E., Rubio R., Berne R.M.: Changes in brain adenosine during bicuculline-induced seizures in rats. Circ. Res., 1980, 47, 568–577.

Received: January 23, 2001.