Role of Adenosine Receptors in Neuroprotective Effect during Global Cerebral Ischemia

V. I. Kulinsky, L. N. Minakina, and L. A. Usov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 5, pp. 536-538, May, 2001
Original article submitted July 10, 2000

Selective A_1 adenosine receptor agonists produced a considerable neuroprotective effect during global cerebral ischemia. The neuroprotective effect decreased in the order: A_1 agonists—NECA—adenosine—A_{2A} agonist CGS 21680, while selective A_3 adenosine receptor agonist was ineffective. Inhibitory analysis showed that A_1 adenosine receptors mediate the neuroprotective effect of CPA, are involved in the effects of NECA and adenosine (but not CGS 21680), and participate in natural resistance to cerebral ischemia. The role of A_{2B} adenosine receptors in the realization of neuroprotective effects was also demonstrated.

Key Words: *adenosine receptors; neuroprotectors; cerebral ischemia*

Recent studies demonstrated a pronounced neuroprotective effect (NPE) of A_1 adenosine receptor agonists during cerebral ischemia [2,7,9,10,12-14]. The efficiency of selective A_1 adenosine receptor agonist N^6-cyclohexyladenosine (CHA) [4] and A_{2A} agonist CGS 21680 [3] was proved using global cerebral ischemia (GCI), the most rigorous model of cerebral ischemia. Here we compared the effects of various adenosine receptor agonists and evaluated the role of A_1 receptors in the realization of NPE.

MATERIALS AND METHODS

Experiments were performed on 425 outbred male and female mice aging 2-4 months and weighing 18-25 g. We used highly selective ligands [5,14]: A_1 adenosine receptor agonists CPA (Sigma), 2-Cl-CPA, CHA (ICN), and adenosine amine derivative ADAC (adenosine amine congener, RBI), A_1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, RBI), A_{2A} adenosine receptor agonist CGS 21680 (ICN), A_{2A} adenosine receptor antagonist ZM 241385, and A_3 adenosine receptor agonists N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide (IB-MECA, Tocris) and N^6-benzyl-NECA (ICN). Nonselective adenosine receptor agonists adenosine-5'-N-ethyluronamide (NECA, Sigma) and adenosine (Siberian Chemical and Pharmaceutical Plant) were also used. All drugs were applied in water solutions. ZM 241385 and N^6-benzyl-NECA were preliminary dissolved in 20% 2-hydroxypropyl-β-cyclodextrine (RBI), DPCPX was dissolved in 20% 2-hydroxypropyl-β-cyclodextrine (RBI) or 0.1 N NaOH (the effects were similar), IB-MECA was dissolved in dimethylsulfoxide (1 mg/0.125 ml) and then water was added. The agents were injected subcutaneously (10 ml/kg). The optimum doses and the time of injections were determined experimentally. Since adenosine receptor agonists possess pronounced antinociceptive properties, no analgesics were administered [8]. GCI was modeled by the method of Lowry (decapitation model). The duration of gasping (agonal breathing) was recorded [4]. As differentiated from standard occlusion models, this rigorous procedure reproduces true GCI (vasodilators are ineffective) [11]. In humans GCI develops after complete arrest of systemic and cerebral circulation [12]. Since gasping duration does no fit normal Gaussian distribution, the results were expressed as medians and analyzed by nonparametric Mann—Whitney *U* test [1].

Department of Biochemistry, Department of Pharmacology, Irkutsk Medical University. ***Address for correspondence:*** kulinsky@pp.irkutsk.ru. Kulinsky V. I.

0007-4888/01/1315-0454$25.00

RESULTS

All selective A_1 adenosine receptor agonists displayed pronounced NPE (Table 1). The resistance to GCI increased by 6.9 times after administration of CPA and by 5.2-5.8 times after treatment with 2-Cl-CPA, CHA, and ADAC. NPE of CPA (5 series, 5.6-fold increase in GCI resistance, n=51) and its blockade with DPCPX (2 series, by 95-100% compared to the control, n=34) were reproducible over 3 years of observations. NPE of nonselective $A_{1,2A,2B}$ adenosine receptor agonist NECA [6], adenosine and, in particular, selective A_{2A} adenosine receptor agonist CGS 21680 were much less pronounced compared to that of selective A_1 adenosine receptor agonists. These substances increased resistance to GCI by 4.3, 2.8, and 1.6 times, respectively. The selective A_3 adenosine receptor agonist IB-MECA had no effect on the resistance to GCI, but decreased it during moderate ischemia [14]. The less selective A_3 adenosine receptor agonist N^6-benzyl-NECA injected 1-3 h before ischemia produced an insignificant NPE (1.8-fold increase in resistance); administration of this substance 20 min and 6 h before ischemia had no effect on animal's resistance to GCI. These data indicate that although NPE can be realized not only via A_1 adenosine receptors, they play the major role in this effect.

For quantitative evaluation of the contribution of A_1 adenosine receptors into the resistance to GCI, we constructed dose-inhibition curves for DPCPX (Fig. 1). The natural resistance to GCI dose-dependently decreased by 25%, which was probably related to blockade of NPE produced by endogenous adenosine. DPCPX dose-dependently inhibited NPE of CPA, adenosine, and NECA, but not the effect of CGS 21680. DPCPX in optimum doses (0.3-1.0 μmol/kg) completely abolished NPE of CPA, while NPE of adenosine and NECA decreased to a minimum and remained at this level after increasing the dose of DPCPX to 1.6-2.6 μmol/kg. We assume that NPE of CPA during GCI are realized via A_1 adenosine receptors, while the effects of adenosine, and NECA are partially mediated through these receptors (67 and 50%, respectively). In contrast, A_{2A} adenosine receptors are not involved in NPE of CPA, while their contribution into NPE of NECA and adenosine is about 30% [3]. Since NECA produced NPE in the presence of DPCPX and highly selective A_{2A} adenosine receptor antagonist ZM 241385 (p<0.002, n=25), it can be hypothesized that this effect is also realized via A_{2B} adenosine receptors. This is consistent with the fact that NECA stimulates not only A_1 and A_{2A}, but also A_{2B} adenosine receptors [6]. Minor NPE of N^6-benzyl-NECA is probably related to its effects on A_{2B}, but not to A_1 adenosine receptors [6].

As differentiated from CPA, NECA, and adenosine, the effect of CGS 21680 was inhibited sharply and only by the maximum dose of DPCPX, which 10 times surpassed the dose of DPCPX blocking NPE of CPA (3.3 μmol/kg). These data attested to a nonspecific effect of high doses of DPCPX on NPE produced by CGS 21680. These findings together with the fact that various selective A_{2A} adenosine receptor antagonists ZM 241385 and chlorostyrylcaffeine [3] completely blocked the effect of CGS 21680 suggest that NPE is realized only via A_{2A} adenosine receptors. ZM 241385 also decreases natural resistance to GCI [3]. A_3 adenosine receptors do not play a role in the realization of the effects of adenosine, NECA, and selective A_1 and A_2 adenosine receptor agonists, since their NPE are completely blocked by theophylline [2] not interacting with A_3 receptors [7].

TABLE 1. Neuroprotective Effects of Adenosine Receptor Agonists

Series	Receptors	Dose, μmol/kg (mg/kg)	Time before ischemia, h	Gasping, sec	
				median	deciles (D_1-D_9)
Control (22)	—	—	0.25-6	16.0	15-17
CPA (14)	A_1	7.2 (2.4)	3-6	110*	93-115
CHA (16)	A_1	7.2 (2.5)	3-6	88.5*++	80-110
Cl-CPA (8)	A_1	7.2 (2.7)	3	83.0*	75-141
ADAC (12)	A_1	7.2 (4.2)	3-6	93.5*	83-127
NECA (11)	A_1, A_{2A}, A_{2B}	0.32 (0.1)	3	71.0*+	60-85
Adenosine (8)	A_1, A_2	749 (200)	1	42.5*+	36-48
CGS 21680 (30)	A_{2A}	1.8 (1.0)	1	25.5*+	23-28
IB-MECA (19)	A_3	0.2-2.0 (0.1-1.0)	0.25-6	17.0+	15-19
N^6-Benzyl-NECA (8)	A_3, A_{2B}	2.5 (1.0)	1-3	29.5*+	22-40

Note. Number of series is shown in brackets. *p<0.001 compared to the control; +p<0.001 and ++p<0.01 compared to the previous series.

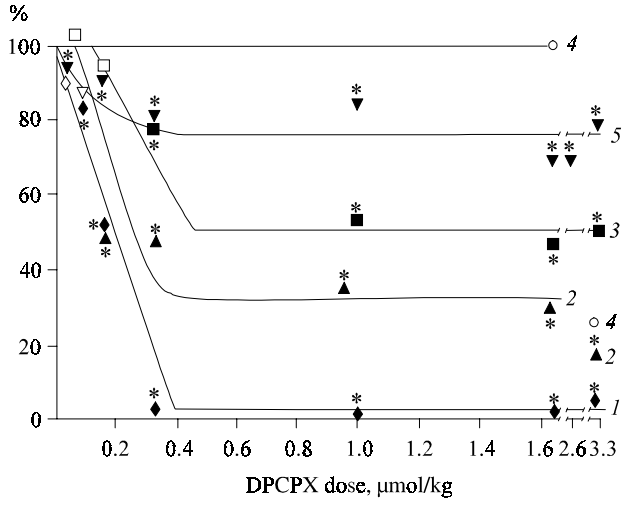

Fig. 1. Effect of A_1 adenosine receptor antagonist DPCPX on neuroprotective activity of adenosine receptor agonists: 7.2 μmol/kg cyclopentyladenosine 3 h before ischemia (*1*), 749 μmol/kg adenosine 1 h before ischemia (*2*), 0.32 μmol/kg NECA 3 h before ischemia (*3*), 1.8 μmol/kg CGS 21680 1 h before ischemia (*4*), and natural resistance to ischemia (*5*). DPCPX was injected 1 h before agonist treatment. *p<0.05 compared to the effect of agonist.

Thus, NPE of CPA and, probably, Cl-CPA, CHA, and ADAC is realized via A_1 adenosine receptors; A_{2A} adenosine receptors mediate the effect of CGS 21680. Both A_{2A} and, to a greater extent, A_1 adenosine receptors contribute to the natural resistance to GCI and mediate the effects of nonselective adenosine receptor agonists adenosine and NECA. A_{2B} adenosine receptors play a role in NPE of NECA and N^6-benzyl-NECA. A_3 adenosine receptors are not involved into realization of the effects of adenosine receptor agonists.

The efficiency of A_1 adenosine receptor agonists during GCI 3-7-fold higher than that of other neuroprotectors and 6-13 fold higher than that of other test substances [2]. Since GCI is much more severe condition compared to other types of ischemia, NPE of structurally different A_1 adenosine receptor agonists are of considerable importance.

This work was supported by the Russian Foundation for Basic Research (grant No. 97-04-49139).

REFERENCES

1. L. Zaks, *Statistical Analysis* [in Russian], Moscow (1976), pp. 91-98, 270-281.
2. V. I. Kulinsky, *Vestn. Ros. Akad. Med. Nauk*, No. 9, 39-43 (2000).
3. V. I. Kulinsky, L. N. Minakina, and L. A. Usov, *Eksp. Klin. Farmakol*., **63**, No. 6, 9-11 (2000).
4. V. I. Kulinsky, L. A. Usov, G. Z. Sufianova, *et al*., *Ibid*., **56**, No. 6, 13-16 (1993).
5. S. Alexander, J. Peters, A. Mead, and S. Lewis, *Trends Pharmacol. Sci*., **33**, Suppl. P. 9-10 (1999).
6. I. Feoktistov and L. Biaggoni, *Biochem. Pharmacol*., **55**, 627-633 (1998).
7. B. B. Fredholm, *Int. Rev. Neurobiol*., **40**, 259-280 (1997).
8. A. Fucunaga, G. Alexander, and H. Mamiya, *Drug. Dev. Res*., **43**, No. 1, 75 (1998).
9. I. J. Knutsen, J. Lau, H. Petersen, *et al*., *J. Med. Chem*., **42**, No. 18, 3463-3477 (1999).
10. V. I. Kulinsky, G. Z. Sufianova, L. N. Minakina, and L. A. Usov, *Drug. Dev. Res*., **43**, No. 1, 46 (1998).
11. M. Nonaka, T. Yoshimine, E. Kohmura, *et al*., *J. Neurol. Sci*., **157**, No. 1, 25-30 (1998).
12. A. Shuaib and M. M. Breker-Klassen, *Neurosci. Biobehav. Rev*., **21**, No. 2, 219-226 (1997).
13. M. I. Sweeney, *Ibid*., **21**, No. 2, 207-217 (1997).
14. D. K. J. E. Von Lubitz, *Eur. J. Pharmacol*., **371**, No. 1, 85-102 (1999).