

Effects of Cyclic Nucleotides on the Cerebral Blood flow Response Induced by Adenosine A_{2B} Receptor Agonist in the Rats

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Abstract – This study was performed to investigate the regulatory mechanism of cerebral blood flow of adenosine A_{2B} receptor agonist in the rats, and to define whether its mechanism is mediated by adenylate cyclase and guanylate cyclase. In pentobarbital-anesthetized, pancuronium-paralyzed and artificially ventilated male Sprague-Dawley rats, all drugs were applied topically to the cerebral cortex. Blood flow from cerebral cortex was measured using laser-Doppler flowmetry. Topical application of an adenosine A_{2B} receptor agonist, 5'-N-ethylcarboxamidoadenosine (NECA; 4 μmol/l) increased cerebral blood flow. This effect of NECA (4 μmol/l) was not blocked by pretreatment with adenylate cyclase inhibitor, MDL-12,330 (20 μmol/l). But effect of NECA (4 μmol/l) was blocked by pretreatment with guanylate cyclase inhibitor, LY-83,583 (10 μmol/l). These results suggest that adenosine A_{2B} receptor increases cerebral blood flow. It seems that this action of adenosine A_{2B} receptor is mediated via the activation of guanylate cyclase in the cerebral cortex of the rats.

Keywords □ adenosine A_{2B} receptor, 5'-N-ethylcarboxamidoadenosine, cerebral blood flow, adenylate cyclase, guanylate cyclase

INTRODUCTION

Adenosine A₂ receptors are regarded as coupling to stimulation of adenylate cyclase activity and the production of cAMP (Fredholm *et al.*, 1994) and to mediate the dilator response of cerebral arterioles to adenosine (Edvinsson and Fredholm, 1983). Adenosine level increases in the brain with moderate hypotension, and its increment is related to the regulation of cerebral blood flow (Win *et al.*, 1981). Adenosine that mediates cerebral vasodilatation during systemic hypoxia is released from the endothelium (Coney and Marshall, 1998). Adenosine is coupled to adenylate cyclase via 2 types of receptor: A₁ receptor that mediates an inhibition of adenylate cyclase and A₂ receptor that mediates a stimulation of the enzyme (Choca *et al.*, 1987; Gerber and Gähwiler, 1994; Jiang *et al.*, 1992; Van Calker *et al.*, 1979). The A₁ and A₂ receptors have distinct distribution in the central nervous system (Bruns *et al.*, 1987; Stone *et al.*, 1988). The adenosine A₂ receptors are further classified as adenosine A_{2A} receptor and A_{2B} receptor subtypes

based on the potency of agonist and the receptor affinity (Fredholm *et al.*, 1994). Cloning techniques have confirmed the existence of distinct A_{2A} receptor and A_{2B} receptor subtypes of A₂ receptor (Stehle *et al.*, 1992). And adenosine may cause vasodilatation by increasing intracellular cAMP, so adenosine A₂ receptor may produce vasodilatation in cerebral cortex. The role for adenosine in the regulation of cerebral blood flow has been proposed by a number of investigators (Dirnagl *et al.*, 1994; Wysham *et al.*, 1986). Adenosine A₂ receptor agonist produces a substantial increase in cerebral blood flow but adenosine A₁ receptor agonist has minimal effects (Coney and Marshall, 1998). Although the precise physiological functions of adenosine A_{2B} receptors remain undefined, role for the adenosine A_{2B} receptors have been suggested in the regulation of neuroglia functions (Fiebich *et al.*, 1996), myocardial contractility (Liang and Haltiwanger, 1995) and vascular smooth muscle tone (Martin, 1992).

However, little is known about the regulatory mechanism of cerebral blood flow of adenosine A_{2B} receptor agonist in the rats. This study was performed to examine the regulatory mechanism of cerebral blood flow of adenosine A_{2B} receptor agonist in the rats, and to define whether its mechanism is mediated by adenylate cyclase and guanylate cyclase.

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MATERIALS AND METHODS

The experimental animals, male Sprague-Dawley rats (250-300 gm), were categorized into four groups. The first group of these groups was treated only with 5'-N-ethylcarboxamidoadenosine (NECA; 4 $\mu\text{mol/l}$), an adenosine A_{2B} receptor agonist, topically to the cerebral cortex. The second group was treated with MDL-12,330 (20 $\mu\text{mol/l}$), an adenylate cyclase inhibitor, topically to the cerebral cortex 10 min before the injection of 4 $\mu\text{mol/l}$ of NECA. The third group was treated with LY-83,583 (10 $\mu\text{mol/l}$), an guanylate cyclase inhibitor, topically to the cerebral cortex 10 min before the injection of 4 $\mu\text{mol/l}$ of NECA. Another group was sham-operated animal group. All drugs were purchased from RBI chemical company (USA) and SIGMA chemical company (USA). All drugs except LY-83,583 were dissolved in artificial cerebrospinal fluid (composition : 120.00 mM NaCl, 2.8 mM KCl, 22.00 mM NaHCO₃, 1.45 mM CaCl₂, 1.00 mM Na₂HPO₄, 0.876 mM MgCl₂) prior to administration and applied topically to the cerebral cortex. LY-83,583 was dissolved in 1% ethanol prior to administration and applied topically to the cerebral cortex. The drug administrations were performed in pentobarbital-anesthetized (50 mg/kg, i.p.), pancuronium-paralyzed (0.1 mg/kg/min, i.v.) and artificially ventilated (Harvard, USA), male Sprague-Dawley rats (250-300 gm). Rectal temperature was maintained at 37 \pm 0.5°C with a heating pad, and the rats were placed in a stereotaxic instrument (Kopf, USA) in the prone position and the parietal bone was removed by gradually thinning the bone bilaterally between the temporal and transverse suture lines using a dental burr: bone wax was used to achieve hemostasis. Cerebral blood flow levels were measured with a laser-Doppler flowmetry. 'Zero' blood flows were determined in each preparation after sacrifice at the conclusion of the experiment. Blood pressure and heart rate were continuously monitored via a femoral arterial catheter (PE-50) connected to a pressure transducer (Statham P23, USA) and a polygraph

(Grass, USA). PO₂, PCO₂ and pH of arterial blood were measured with blood gas analyzer before and after stereotaxic experiment. All results are expressed as mean \pm SEM with $P < 0.05$ and $P < 0.01$ considered as the level of significance. The statistical analysis of mean values was performed by analysis of variance (ANOVA). Students *t*-test for paired data was also used for statistical evaluation of the results.

RESULTS

A representative experiment showing the effects of topical application of NECA on cerebral blood flow is illustrated in Table I and Fig. 1. Topical application of NECA caused an increase in cerebral blood flow that reached a maximum in 30 min after application. Pretreatment with alloxazine (4 $\mu\text{mol/l}$), an adenosine A_{2B} receptor antagonist, blocked the NECA-induced cerebral blood flow responses. Topical application of NECA (4 $\mu\text{mol/l}$) caused an increase in cerebral blood flow that reached a maximum in 30 min after application. The responses evoked by NECA were 135 \pm 13.6, 137 \pm 12.5, 149 \pm 15.1, 164 \pm 18.1, 163 \pm 17.7, 164 \pm 17.9, 163 \pm 16.6 and 163 \pm 18.8% compared to the value of baseline cerebral blood flow, respectively in 5, 10, 15, 30, 45, 60, 75 and 90 min after topical application of NECA ($n=10$, Table I and Fig. 1). Baseline cerebral blood flow for these rats were 100 \pm 11.2%. Topical application of an equivalent volume of artificial cerebrospinal fluid did not affect the basal cerebral blood flow. Cerebral blood flow was not affected in sham-operated animal group.

Pretreatment with MDL-12,330 (20 $\mu\text{mol/l}$) did not attenuate the NECA-induced cerebral blood flow responses; 129 \pm 13.8, 133 \pm 13.1, 141 \pm 16.2, 157 \pm 16.8, 159 \pm 19.3, 156 \pm 18.8, 157 \pm 17.6 and 155 \pm 16.9% compared to the value of baseline cerebral blood flow, respectively in 5, 10, 15, 30, 45, 60, 75 and 90 min after topical application of NECA ($n=10$, Table I, Fig. 2 and Fig. 4). Baseline cerebral blood flow for these rats were 100 \pm 13.2%. Another different groups were treated with

Table I. Percent changes in cerebral blood flow of 5'-N-ethylcarboxamidoadenosine (NECA; 4 $\mu\text{mol/l}$) only, NECA (4 $\mu\text{mol/l}$) after pretreatment with MDL-12,330 (MDL; 20 $\mu\text{mol/l}$) and NECA (4 $\mu\text{mol/l}$) after pretreatment with LY-83,583 (LY; 10 $\mu\text{mol/l}$). Data are the mean \pm S.E. Pretreatment with MDL-12,330 or LY-83,583 have no effects on cerebral blood flow

Treatment	Cerebral Blood Flow (%)								
	Minute after Treatment								
	0	5	10	15	30	45	60	75	90
NECA	100 \pm 11.2	135 \pm 13.6	137 \pm 12.5	149 \pm 15.1	164 \pm 18.1	163 \pm 17.7	164 \pm 17.9	163 \pm 16.6	163 \pm 18.8
MDL+NECA	100 \pm 13.2	129 \pm 13.8	133 \pm 13.1	141 \pm 16.2	157 \pm 16.8	159 \pm 19.3	156 \pm 18.8	157 \pm 17.6	155 \pm 16.9
LY+NECA	100 \pm 14.2	105 \pm 14.2	107 \pm 12.8	108 \pm 13.7	107 \pm 12.7	109 \pm 15.1	107 \pm 14.1	110 \pm 15.8	108 \pm 16.3

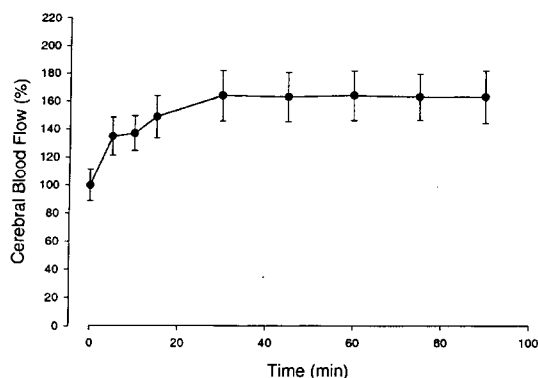


Fig. 1. Effects of topical application of 5'-N-ethylcarboxamidoadenosine (NECA; 4 $\mu\text{mol/l}$) on cerebral blood flow responses in rats. Values are mean \pm S.E. in 10 rats.

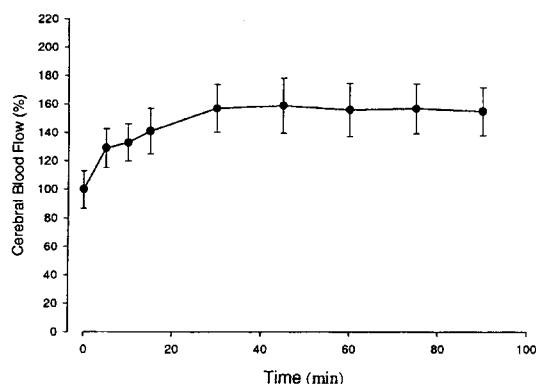


Fig. 2. Effects of topical application of 5'-N-ethylcarboxamidoadenosine (NECA; 4 $\mu\text{mol/l}$) after pretreatment with MDL-12,330 (20 $\mu\text{mol/l}$) on cerebral blood flow responses in rats. Values are mean \pm S.E. in 10 rats

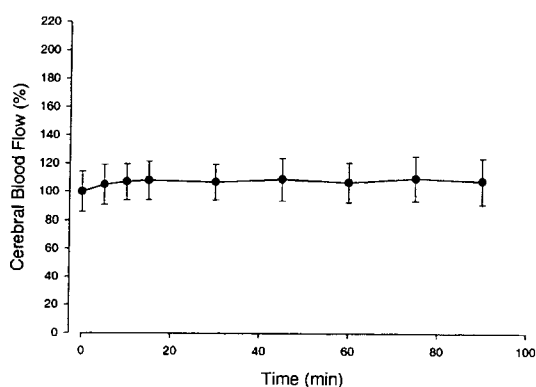


Fig. 3. Effects of topical application of 5'-N-ethylcarboxamidoadenosine (NECA; 4 $\mu\text{mol/l}$) after pretreatment with LY-83,583 (10 $\mu\text{mol/l}$) on cerebral blood flow responses in rats. Values are mean \pm S.E. in 10 rats.

MDL-12,330 (10 $\mu\text{mol/l}$, 30 $\mu\text{mol/l}$) did not affect NECA-induced cerebral blood flow responses. Therefore in this study authors chose the middle concentration.

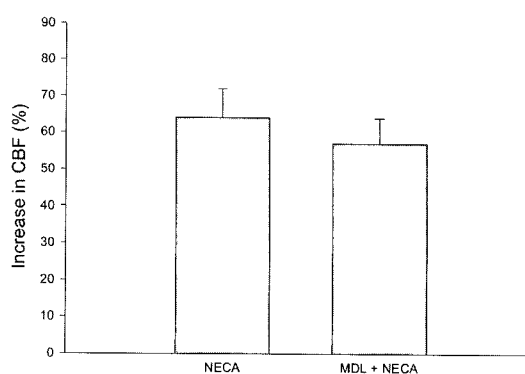


Fig. 4. Changes in cerebral blood flow (CBF) of 5'-N-ethylcarboxamidoadenosine (NECA; 4 $\mu\text{mol/l}$) only and NECA (4 $\mu\text{mol/l}$) after pretreatment with MDL-12,330 (MDL; 20 $\mu\text{mol/l}$) topically. Values are recorded at the end of 30 minutes after topical application of NECA. Values are the mean \pm S.E. in 10 rats.

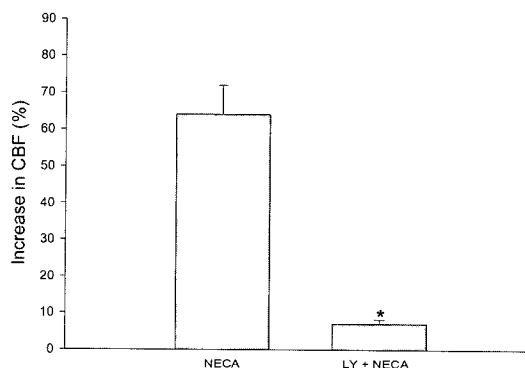


Fig. 5. Changes in cerebral blood flow (CBF) of 5'-N-ethylcarboxamidoadenosine (NECA; 4 $\mu\text{mol/l}$) only and NECA (4 $\mu\text{mol/l}$) after pretreatment with LY-83,583 (LY; 10 $\mu\text{mol/l}$) topically. Values are recorded at the end of 30 minutes after topical application of CPCA. Values are the mean \pm S.E. in 10 rats. * $P < 0.01$, compared to NECA only group

But pretreatment with LY-3,583 (10 $\mu\text{mol/l}$) significantly attenuated the NECA-induced cerebral blood flow responses; 105 ± 14.2 , 107 ± 12.8 , 108 ± 13.7 , 107 ± 12.7 , 109 ± 15.1 , 107 ± 14.1 , 110 ± 15.8 and $108 \pm 16.3\%$ compared to the value of baseline cerebral blood flow, respectively in 5, 10, 15, 30, 45, 60, 75 and 90 min after topical application of NECA ($n=10$, Table I, Fig. 3 and Fig. 5). Baseline cerebral blood flow for these rats were $100 \pm 14.2\%$. Another different groups were treated with LY-83,583 (5 $\mu\text{mol/l}$, 15 $\mu\text{mol/l}$) affected NECA-induced cerebral blood flow responses concentration-dependently. Therefore in this study authors chose the middle concentration. Topical application of MDL-12,330 (20 $\mu\text{mol/l}$) and LY-83,583 (10 $\mu\text{mol/l}$) had no effects on cerebral blood flow. No significant changes in PO_2 , PCO_2 and pH of arterial blood

Table II. Summary of blood gas analysis

Variables	Before Experiment	After Experiment
PaCO ₂ , mmHg	83.8 ± 2.7%	89.1 ± 4.1%
PaO ₂ , mmHg	39.9 ± 2.4%	40.1 ± 1.8%
pH	7.20 ± 0.36	7.29 ± 0.41

were seen following stereotaxic experiment (Table II).

DISCUSSION

Adenosine is an endogenous nucleotide that modulates many physiological processes. Its actions are mediated by interaction with specific cell membrane receptors. Four subtypes of adenosine receptors have been cloned: A₁, A_{2A}, A_{2B} and A₃. Significant advancement has been made in the understanding of the molecular pharmacology and physiological relevance of adenosine receptors (Feoktistov and Biaggioni, 1997). It is also recognized that adenosine A_{2B} receptors are coupled to intracellular pathways different from those of adenosine A_{2A} receptors, a finding that may provide the basis for their distinct physiological role. Adenosine A_{2B} receptors have been implicated in mast cell activation and asthma, vasodilation, regulation of cell growth, intestinal function and modulation of neurosecretion (Feoktistov and Biaggioni, 1997). Pharmacological identification of A_{2B} receptors, based on their affinity and characteristic order of potency for receptors. Adenosine A_{2B} receptors participate in the regulation of vascular tone (Webb *et al.*, 1992). Therefore adenosine-induced vasodilation is mediated via adenosine A_{2B} receptors. In this present study, topical application of NECA, an adenosine A_{2B} receptor agonist, in anesthetized and artificially ventilated rats elicited an increase in cerebral blood flow. Adenosine plays an important role in many physiological processes. Its actions are mediated by specific cell surface receptors coupled to G proteins (Fredholm *et al.*, 1994; Olah and Stiles, 1996). Adenosine vasodilates the vasculature of skeletal muscle and the brain (Mian and Marshall, 1991; Skinner and Marshall, 1996; Thomas and Marshall, 1994). Ngai (Ngai and Winn, 1993) investigated the receptors involved in adenosine-induced dilation in cerebral resistance arterioles and adenosine acts primarily via A₂ receptors to elicit cerebral vasodilation. Some experimental evidences suggest that adenosine receptor plays a critical role in the mediation of cerebral blood flow responses (Van Calker *et al.*, 1979; Hong *et al.*, 1999), and some experimental evidences suggest that adenosine A₂ receptor agonist produces a substantial increase in cerebral blood flow (Coney and Marshall, 1998;

Van Wylen *et al.*, 1989). The adenosine A₂ receptor is further classified as adenosine A_{2A} receptor and A_{2B} receptor subtypes based on the potency of agonist and the receptor affinity (Fredholm *et al.*, 1994). Cloning techniques have confirmed the existence of distinct A_{2A} receptor and A_{2B} receptor subtypes of A₂ receptor (Stehle *et al.*, 1992). Although the precise physiological functions of the A_{2B} receptors remain undefined, roles for the A_{2B} receptor has been suggested in the regulation of intestinal chloride secretion (Strohmeier *et al.*, 1995). Hong *et al.* (Hong *et al.*, 1999) demonstrated that when the cortical surface is suffused with cAMP, the release of adenosine is increased in the artificial cerebrospinal fluid and they suggested that the cAMP-adenosine pathway as a metabolic mechanism is implicated in the production of adenosine in the rat pial artery and contributes to the regulation of vasodilation in response to hypotension. But little is known about the regulatory mechanism of cerebral blood flow of adenosine A_{2B} receptor agonist. And adenosine A_{2B} receptor agonist produces vasodilation of rat pial artery and cerebral blood flow autoregulation (Shin *et al.*, 2000). In the present study NECA, adenosine A_{2B} receptor agonist, produces a substantial increase in cerebral blood flow. Martin and Potts (Martin and Potts, 1994) demonstrated a similar result in that renal artery of rat contains adenosine A_{2B} receptors that are located on the endothelium.

This effect of NECA (4 umol/l) was not blocked by pretreatment with adenylate cyclase inhibitor, MDL-12,330 (20 umol/l). Hong *et al.* (Hong *et al.*, 1994; Hong *et al.*, 1996) demonstrated that the vasodilation of the pial artery is closely related with accumulation of intracellular cAMP. An involvement of the metabolic AMP-adenosine pathway was demonstrated as a likely mechanism in the production of adenosine that acted as a regulator of vasodilation in response to hypotension (Hong *et al.*, 1999). And adenosine may cause vasodilation by increasing intracellular cAMP, so adenosine A₂ receptor may produce vasodilation in cerebral cortex by stimulation of adenylate cyclase. But the role of adenosine A_{2B} receptor agonist in the cerebral blood flow response is not related to cAMP.

This effect of NECA (4 umol/l) was blocked by pretreatment with guanylate cyclase inhibitor, LY-83,583 (10 umol/l). The level of cyclic GMP in vascular smooth muscle is an important regulator of blood flow. Many vasodilators work through increases in cyclic GMP (Hyman *et al.*, 1989; Zhou and Torphy, 1991). Generally NO production leads to increase in the level of cyclic GMP and may help couple cerebral blood flow and metabolism (Dirnagl *et al.*, 1993). It is also believed that NO is involved in the coupling of neurotransmitter receptor

stimulation with cellular cyclic GMP responses (Garthwaite, 1988). In this present study adenosine A_{2B} receptor is mediated by stimulation of guanylate cyclase. Therefore adenosine A_{2B} receptor may increase cerebral blood flow by increasing intracellular cGMP. So adenosine A_{2B} receptor may produce vasodilatation in cerebral cortex by stimulation of guanylate cyclase. We demonstrated that NECA-induced vasodilatation in cerebral cortex was significantly inhibited by guanylate cyclase inhibitor, LY-83,583, but not by adenylate cyclase inhibitor, MDL-12,330. Therefore the regulation of cerebral blood flow in adenosine A_{2B} receptor is mediated by guanylate cyclase. In further study we may determine the level of cGMP level to confirm this evidence.

In conclusion, our results show that adenosine A_{2B} receptor increases cerebral blood flow and this action of adenosine A_{2B} receptor is mediated by the activation of guanylate cyclase in the cerebral cortex of the rats.

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