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The Regulatory Mechanism of Cerebral Blood flow of Adenosine A_2 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_2 receptor agonist in the rats, and to define whether its mechanism is mediated by nitric oxide (NO), 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_2 receptor agonist [5'-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA; 4 umol/l)] increased cerebral blood flow. This effect of CPCA (4 umol/l) was blocked by pretreatment with NO synthase inhibitor [N^G-nitro-L-argine methylester (L-NAME; 40 umol/l)] and adenylate cyclase inhibitor [MDL-12,330 (20 umol/l)]. But the effect of CPCA (4 umol/l) was not blocked by pretreatment with guanylate cyclase inhibitor [LY-83,583 (10 umol/l)]. These results suggest that adenosine A_2 receptor increases cerebral blood flow. It seems that this action of adenosine A_2 receptor is mediated via the NO and the activation of adenylate cyclase in the cerebral cortex of the rats.

Keywords □ adenosine A_2 receptor, 5'-(*N*-cyclopropyl)-carboxamidoadenosine, cerebral blood flow, nitric oxide, adenylate cyclase, guanylate cyclase

INTRODUCTION

The action of adenosine as a neurotransmitter or neuromodulator responsible for cardiovascular regulation has been suggested (Barraco *et al.*, 1987; Bredt *et al.*, 1991). It is generally accepted that systemic hypoxia exerts strong dilator influences upon the vasculature of skeletal muscle and the brain due to the action of locally released vasodilator substances. 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 : A_1 receptor that mediates an inhibition of adenylate cyclase and A_2 receptor that mediates a stimulation of adenylate cyclase (Choca *et al.*, 1987; Gerber and Gähwiler, 1994; Jiang *et al.*, 1992; Van Calker *et al.*, 1979). The A_1 and A_2 receptors have distinct distribution in the central nervous system (Bruns *et al.*, 1987; Stone *et al.*, 1988). And adenosine may cause vasodilatation by increasing intracelluar cAMP, so adenosine A_2 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 A_2 receptor agonist produces a substantial increase in cerebral blood flow but adenosine A_1 receptor agonist has minimal effects (Coney and Marshall, 1998).

Nitric oxide (NO) is a potent vasodilator that regulates the vascular tone in several vascular beds, including the brain, therefore NO might be of importance for the increase of cerebral blood flow (Akgoren *et al.*, 1994). And NO is a key coupling compound that links changes in cerebral blood flow and metabolism (Goadsby *et al.*, 1992). Adenosine causes NO release from cultured cortical astrocytes and mobilization of calcium from intracellular stores rather than influx is involved in the adenosine-induced activation of NO synthase (Janigro *et al.*, 1996). NO production leads to activation of guanylate cyclase and may help couple cerebral blood flow and metabolism (Dirnagl *et al.*, 1993). In the cerebral and other vascular beds, activation of guanylate cyclase cause relaxation of vascular smooth muscle (Hyman *et al.*, 1989). However, little is known about the regulatory mechanism of cerebral blood flow

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of adenosine A_2 receptor agonist in the rats. This study was performed to examine the regulatory mechanism of cerebral blood flow of adenosine A_2 receptor agonist in the rats, and to define whether its mechanism is mediated by NO, adenylate cyclase and guanylate cyclase.

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*-cyclopropyl)-carboxamidoadenosine (CPCA; 4 umol/l), an adenosine A_2 receptor agonist, topically to the cerebral cortex. The second group was treated with N^G-nitro-L-arginine methylester (L-NAME; 40 umol/l), a NO synthase inhibitor, topically to the cerebral cortex 10 min before the injection of 4 umol/l of CPCA. The third group was treated with MDL-12,330 (20 umol/l), an adenylate cyclase inhibitor, topically to the cerebral cortex 10 min before the injection of 4 umol/l of CPCA. The fourth group was treated with LY-83,583 (10 umol/l), an guanylate cyclase inhibitor, topically to the cerebral cortex 10 min before the injection of 4 umol/l of CPCA. 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 $NaHCO_3$, 1.45 mM $CaCl_2$, 1.00 mM Na_2HPO_4, 0.876 mM $MgCl_2$) 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 ± 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). PO_2, PCO_2 and pH of arterial blood were measured with blood gas analyzer before and after stereotaxic experiment. All results are expressed as mean ± 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 CPCA on cerebral blood flow is illustrated in Table I and Fig. 1. Topical application of CPCA caused an increase in cerebral blood flow that reached a maximum in 30 min after application. The increase in cerebral blood flow evoked by CPCA was dose-dependent (2, 4 and 6 umol/l of CPCA increased the cerebral blood flow by 24± 3, 72± 7 and 98± 12%, respectively; n=10; Fig. 1). Pretreatment with 3,7-dimethyl-1-propargylxanthine (DMPX, 40 umol/l), an adenosine A_2 receptor antagonist, blocked the CPCA-induced cerebral blood flow responses. Topical application of CPCA (4 umol/l) caused an increase in cerebral blood flow that reached a maximum in 30 min after application. The responses evoked by CPCA were 146± 14, 150± 17, 165± 14, 172± 19, 170± 19,

Table I. Percent changes in cerebral blood flow of 5'-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA; 4 umol/l) only, CPCA (4 umol/l) after pretreatment with N^G-nitro-L-argine methylester (L-NAME; 40 umol/l), CPCA (4 umol/l) after pretreatment with MDL-12,330 (MDL; 20 umol/l) and CPCA (4 umol/l) after pretreatment with LY-83,583 (LY; 10 umol/l). Data are the mean ± SEM. Pretreatment with L-NAME, MDL-12,330 or LY-83,583 have no effects on cerebral blood flow

Treatment	Cerebral Blood Flow (%)								
	Minute after Treatment								
	10	0	5	15	30	45	60	75	90
CPCA	100 ± 11.4	146 ± 14.2	150 ± 16.8	165 ± 14.2	172 ± 19.2	170 ± 18.6	168 ± 19.2	170 ± 17.6	172 ± 13.9
L-NAME+CPCA	100 ± 13.6	106 ± 14.8	103 ± 12.2	105 ± 14.1	98 ± 13.6	102 ± 11.6	102 ± 13.6	102 ± 15.1	97 ± 8.6
MDL+CPCA	100 ± 12.9	114 ± 14.9	108 ± 15.7	112 ± 13.6	119 ± 17.8	115 ± 13.6	117 ± 14.7	119 ± 18.2	116 ± 13.7
LY+CPCA	100 ± 11.7	139 ± 13.2	145 ± 15.7	157 ± 14.6	166 ± 17.2	168 ± 16.8	165 ± 17.4	166 ± 15.3	169 ± 12.6

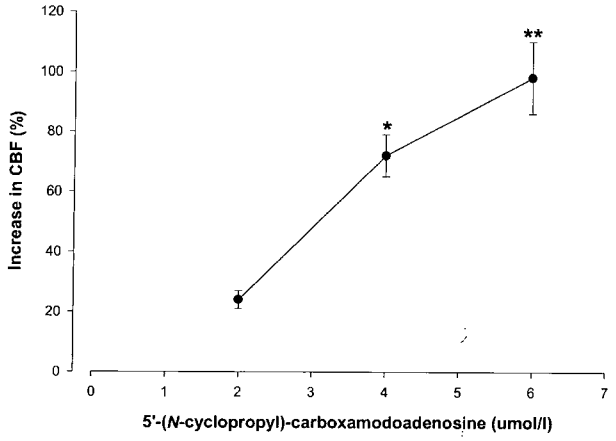

Fig. 1. Dose-dependent increase of cerebral blood flow (CBF) by topical application of 5'-(*N*-cyclopropyl)-carboxamidoadenosine (2, 4 and 6 umol/l). Data represent mean ± EM. Significant differences were determined by ANOVA with a multiple comparisons test; **P*<0.01 (2 umol/l vs. 4 umol/l), ***P*<0.01 (2 umol/l vs. 6 umol/l and 4 umol/l vs. 6 umol/l). All doses significantly differ from *P*<0.01 as compared to basal CBF.

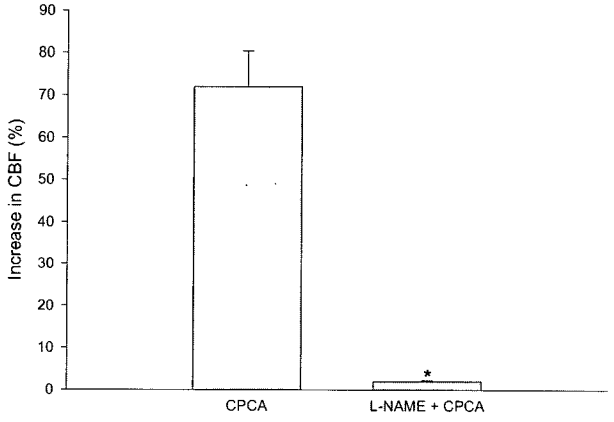

Fig. 2. Changes in cerebral blood flow (CBF) of 5'-(*N*-cyclopropyl)- carboxamidoadenosine (CPCA; 4 umol/l) only and CPCA (4 umol/l) after pretreatment with N^G-nitro-L-argine methylester (L-NAME; 40 umol/l) topically. Values are recorded at the end of 30 minutes after topical application of CPCA. Values are the mean ± EM in 10 rats. **P*<0.01, compared to CPCA only group.

168 ± 19, 170 ± 18 and 172 ± 14% 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 CPCA (n=10, Table I). Baseline cerebral blood flow for these rats were 100 ± 11%. 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 L-NAME (40 umol/l) significantly attenu-

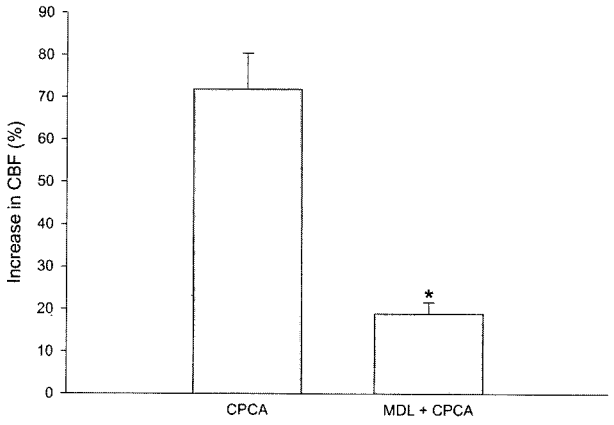

Fig. 3. Changes in cerebral blood flow (CBF) of 5'-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA; 4 umol/l) only and CPCA (4 umol/l) after pretreatment with MDL-12,330 (MDL; 20 umol/l) topically. Values are recorded at the end of 30 minutes after topical application of CPCA. Values are the mean ± EM in 10 rats. **P*<0.01, compared to CPCA only group.

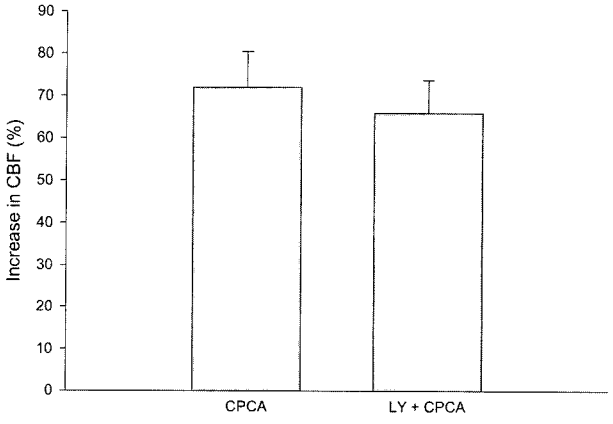

Fig. 4. Changes in cerebral blood flow (CBF) of 5'-(N-cyclopropyl)-carboxamidoadenosine (CPCA; 4 umol/l) only and CPCA (4 umol) after pretreatment with LY-83,583 (LY; 10 umol/l) topically. Values are recorded at the end of 30 minutes after topical application of CPCA. Values are the mean ± EM in 10 rats.

ated the CPCA-induced cerebral blood flow responses; 103 ± 12, 105 ± 14, 98 ± 14, 102 ± 12, 106 ± 15, 102 ± 14, 102 ± 15 and 97 ± 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 CPCA (*n*=10, Table I and Fig. 2). Baseline cerebral blood flow for these rats were 100 ± 14%. Pretreatment with MDL-12,330 (20 umol/l) significantly attenuated the CPCA-induced cerebral blood flow responses; 114±15, 108 ± 16, 112 ± 14, 119 ± 18, 115 ± 14, 117 ± 15, 119 ± 18 and 116 ± 14% compared to the value of baseline cerebral blood flow, respectively in 5, 10, 15, 30, 45, 60, 75 and 90 min after topical

Table II. Physiological variables: blood gas analysis; Data are the mean ± SEM.

Variables	Before Experiments	After Experiments
PaO_2, mmHg	86.9 ± 2.4%	91.4 ± 3.8%
$PaCO_2$, mmHg	41.6 ± 2.1%	40.7 ± 1.7%
pH	7.30 ± 0.31%	7.41 ± 0.22%

application of CPCA (n=10, Table I and Fig. 3). But pretreatment with LY-83,583 (10 umol/l) did not attenuate the CPCA-induced cerebral blood flow responses (n=10, Table 1 and Fig. 4). Baseline cerebral blood flow for these rats were 100± 13%. Topical application of L-NAME (40 umol/l), MDL-12,330 (20 umol/l) and LY-83,583 (10 umol/l) had no effects on cerebral blood flow. No significant changes in PO_2, PCO_2 and pH of arterial blood were seen following stereotaxic experiment (Table II).

DISCUSSION

In this present study, topical application of CPCA 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 is a potent dilator of cerebral vessels (Ibayashi *et al.*, 1991; Ngai and Winn, 1993) and has been implicated in the regulation of cerebral blood flow (Phillis, 1989; Winn *et al.*, 1981). Ngai (Ngai and Winn, 1993) investigated the receptors involved in adenosine-induced dilation in cerebral resistance arterioles and adenosine acts primarily via A_2 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 A_2 receptor agonist produces a substantial increase in cerebral blood flow (Coney and Marshall, 1998; Van Wylen *et al.*, 1989). Our result is consistent with the opinion of above author. But little is known about the regulatory mechanism of cerebral blood flow of adenosine A_2 receptor agonist. Systemic hypoxia exerts strong dilator influences upon the vasculature of skeletal muscle and the brain due to the action of locally released vasodilator substances. Adenosine plays a major part in this vasodilatation (Mian and Marshall, 1991; Skinner and Marshall, 1996; Thomas and Marshall, 1994). Adenosine makes a major contribution to the vasodilatation induced in the cerebral cortex of the rat by systemic hypoxia. Adenosine is involved in coupling of cerebral blood flow to neuronal activation. Adenosine A_2 receptor agonist produces a substantial increase in cerebral blood flow but adenosine A_1 receptor agonist has minimal effects (Coney and Marshall, 1998).

This effect of CPCA (4 umol/l) was blocked by pretreatment with NO synthase inhibitor, N^G-nitro-L-argine methylester (L-NAME; 40 umol/l). NO is a potent vasodilator and a key coupling compound that links changes in cerebral blood flow and metabolism (Goadsby *et al.*, 1992). Adenosine is involved in coupling of cerebral blood flow to neuronal activation and NO is involved in this response as well, so there is an interaction between the vasodilator pathways of adenosine and NO (Dirnagl *et al.*, 1994). Vials and Burnstock (Vials and Burnstock, 1993) documented that in the guinea pig coronary artery, a major part of vasodilator action of adenosine is directly mediated via adenosine A_2 receptors on the smooth muscle and activation by adenosine of A_2 purinoceptors on endothelial cells induces relaxation via production of NO. Therefore our results suggest that the effects of CPCA on the cerebral blood flow is mediated by NO synthase.

This effect of CPCA (4 umol/l) was blocked by pretreatment with adenylate cyclase inhibitor, MDL-12,330 (20 umol/l). Adenosine A_2 receptor is mediated a stimulation of adenylate cyclase (Choca *et al.*, 1987; Van Calker *et al.* 1979). Forskolin, a drug which stimulates adenylate cyclase, is a direct cerebral vasodilator (Wysham *et al.*, 1986). 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 cAMP-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). In fetal chicken ventricular myocardium cells express adenosine A_2 receptor and positively coupled to stimulation of adenylate cyclase and myocyte contractility and adenosine A_2 receptor can also couple to other intracellular pathways, including calcium channels and phospholipase C (Liang and Haltiwanger, 1995). And adenosine may cause vasodilatation by increasing intracelluar cAMP, so adenosine A_2 receptor may produce vasodilatation in cerebral cortex by stimulation of adenylate cyclase. Our result is consistent with the opinion of above authors. But this effect of CPCA (4 umol/l) was not 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 increases 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). But adenosine A_2 receptor is not mediated a stimulation of guanylate cyclase in this present study. Therefore adenosine A_2 receptor may increase cerebral blood flow by increasing intracelluar cAMP. So adenosine A_2 receptor may produce vasodilatation in cerebral cortex by stimulation of adenylate cyclase. In this present study we demonstrated that the regulation of cerebral blood flow in adenosine A_2 receptor is mediated by adenylate cyclase through a NO.

In conclusion, our results show that adenosine A_2 receptor increases cerebral blood flow and this action of adenosine A_2 receptor is mediated via the NO and the activation of adenylate cyclase in the cerebral cortex of the rats.

REFERENCES

Akgoren, N., Fabricius, M. and Lauritzen, M. (1994). Importance of nitric oxide for local increases of blood flow in rat cerebellar cortex during electrical stimulation. *Proc. Natl. Acad. Sci.* **91**, 5903-5907.

Barraco, R. A., Campbell, W. R., Parizon, M., Shoener, E. P. and Shein, S. E. (1987). Cardiovascular effects of microinjections of adenosine analogs into the fourth ventricle of rats. *Brain Res.* **424**, 17-25.

Bredt, D. S., Glatt, C. E., Hwang, P. M., Fotuhl, M., Dawson, T. M. and Snyder, S. H. (1991). Nitric oxide synthetase protein and m-RNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. *Neuron* **7**, 615-624.

Bruns, R. F., Fergus, J. H., Badger, E. W., Bristol, J. A., Santay, L. A., Hartman, J. D., Hays, S. J. and Huang, C. C. (1987). Binding of the A_1-selective adenosine antagonist 8-cyclopentyl-1,3-diproxylxanthine to rat brain membranes. *Naunyn-Schmiedebergs Arch. Pharmacol.* **335**, 59-63.

Choca, J. I., Proudfit, H. K. and Green, R. D. (1987). Identification of A_1 and A_2 adenosine receptors in the rat spinal cord. *J. Pharmacol. Exp. Ther.* **242**, 905-910.

Coney, A. M. and Marshall, J. M. (1998). Role of adenosine and its receptors in the vasodilatation induced in the cerebral cortex of the rat by systemic hypoxia. *J. Physiol.* **509**, 507-518.

Dirnagl, U., Lindauer, U. and Villringer, A. (1993). Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. *Neurosci. Lett.* **149**, 43-46.

Dirnagl, U., Niwa, K., Lindauer, U. and Villringer, A. (1994). Coupling of cerebral blood flow to neuronal activation: role of adenosine and nitric oxide. *Am. J. Physiol.* **267**, H296-H301.

Fredholm, B. B., Abbrachio, M. P., Burnstock, G., Daly, J. W., Harden, T. K., Jacobson, K. A., Leff, P. and Williams, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.* **46**, 143-156.

Garthwaite, J., Charles S. L. and Chess-Williams R. (1988). Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* **336**, 385-388.

Gerber, U. and Gähwiler, B. H. (1994). $GABA_B$ and adenosine receptors mediate enhancement of the K^+ current, I_{AHP}, by reducing adenylate cyclase activity in rat CA3 hippocampal neurons. *J. Neurophysiol.* **72**, 2360-2367.

Goadsby, P. J., Kaube, H. and Hoskin, K. L. (1992). Nitric oxide synthesis couples cerebral blood flow and Metabolism. *Brain Res.* **595**, 167-170.

Hong, K. W., Pyo, K. M., Lee, W. S., Yu, S. S and Rhim, B. Y. (1994). Pharmacological evidence that calcitonin-related peptide is implicated in cerebral autoregulation. *Am. J. Heart. Circ. Physiol.* **266**, H11-H16.

Hong, K. W., Shin, H. K., Kim, H. H., Choi, J. M., Rhim, B. Y. and Lee W. S. (1999). Metabolism of exogenous cAMP to adenosine and its role for vasodilation during cerebral autoregulation in rat pial artery. *Am. J. Heart. Circ. Physiol.* **276**, H376-H382.

Hong, K. W., Yoo, S. E., Yu, S. S., Lee, J. Y. and Rhim, B. Y. (1996). Pharmacological coupling and functional role for CGRP receptors in the vasodilation of rat pial arterioles. *Am. J. Heart. Circ. Physiol.* **270**, H317-H323.

Hyman, A. L., Kadowitz, P. J. and Lippton, H. L. (1989). Methylene blue selectively inhibits pulmonary vasodilator responses in cats. *J. Appl. Physiol.* **66**, 1513-1517.

Ibayashi, S., Ngai, A. C., Meno, J. R. and Winn, H. R. (1991). Effects of topical adenosine analogs and forskolin on rat pial arterioles in vivo. *J. Cereb. Blood. Flow. Metab.* **11**, 72-76.

Janigro, D., Wender, R., Ransom, G., Tinklepaugh, D. L. and Winn, H. R. (1996). Adenosine-induced release of nitric oxide from cortical astrocytes. *NeuroReport* **7**, 1640-1644.

Jiang, H., Colbran, J. L., Francis, S. H. and Corbin, J. D. (1992). Direct evidence for cross-activation of cGMP-dependent protein kinase by cAMP in pig coronary arteries. *J. Biol. Chem.* **267**, 1015-1019.

Liang, B. T. and Haltiwanger B. (1995). Adenosine A_2 receptors in cultured fetal chick heart cells. *Circ. Res.* **76**, 243-251.

Mian, R. and Marshall, J. M. (1991). The role of adenosine in dilator responses induced in arterioles and venules of rat skeletal muscle by systemic hypoxia. *J. Physiol.* **443**, 499-511.

Ngai, A. C. and Winn, H. R. (1993). Effects of adenosine and its analogues on isolated arterioles: extraluminal and intraluminal application. *Circ. Res.* **73**, 448-457.

Olah, M. E. and Stiles, G. L. (1996). Adenosine receptor

subtypes: characterization and therapeutic regulation. *Annu. Rev. Pharmacol. Toxicol.* **35**, 581-606.

Phillis, J. W. (1989). Adenosine in the control of the cerebral circulation. *Cerebrovasc. Brain Metab. Rev.* **1**, 26-54.

Skinner, M. R. and Marshall, J. M. (1996). Studies on the roles of ATP, adenosine and nitric oxide in mediating muscle vasodilation induced in the rat by acute systemic hypoxia. *J. Physiol.* **495**, 553-560.

Stone, G. A., Jarvis, M. F., Sills, M. S., Weeks, B., Snowhill, E. W. and Williams, M. (1988). Species differences in high affinity adenosine A_2 binding sites in striatal membranes from mammalian brain. *Drug Develop. Res.* **15**, 31-46.

Thomas, T. and Marshall, J. M. (1994). Interdependence of respiratory and cardiovascular changes induced by systemic hypoxia in the rat: the role of adenosine. *J. Physiol.* **480**, 627-636.

Van Calker, D., Muller, M. and Hamprecht, B. (1979). Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem.* **33**, 999-1005.

Van Wylen, D. G. L., Park, T. S., Rubio, R. and Berne, R. M. (1989). The effect of local infusion of adenosine and adenosine analogues on local cerebral blood flow. *J. Cereb. Blood. Flow. Metab.* **9**, 556-562.

Vials, A. and Burnstock, G. (1993). A_2-purinoceptor-mediated relaxtion in the guinea-pig coronary vasculature: a role for nitric oxide. *Br. J. Pharmacol.* **109**, 424-429.

Winn, H. R., Rubio, R. and Berne, R. M. (1981). The role of adenosine in the regulation of cerebral blood flow. *J. Cereb. Blood. Flow. Metab.* **1**, 239-244.

Wysham, D. G., Brotherton, A. F. and Heistad, D. D. (1986). Effects of forskolin on cerebral blood flow : Implications for a role of adenylate cyclase. *Stroke* **17**, 1299-1303.

Zhou, H. L. and Torphy, T. J. (1991). Relationship between cyclic guanosine monophosphate accumulation and relaxation of canine trachealis induced by nitrovasodilators. *J. Pharmacol. Exp. Ther.* **258**, 972-978.