The Journal of Applied Pharmacology, 14, 119-124(2006)

Effects of K_{ATP} Channel Blocker, cAMP and cGMP on the Cardiovascular Response of Adenosine A_1 Agonist in the Spinal Cord of the Rats

In Chul SHIN*

Department of Pharmacology, College of Medicine, Hanyang University, 17 Haengdang-Dong, Seongdong-Gu, Seoul, 133-791, Korea

(Received May 29, 2006; Accepted June 19, 2006)

Abstract – This study was performed to investigate the influence of the spinal adenosine A_1 receptors on the central regulation of blood pressure (BP) and heart rate (HR), and to define whether its mechanism is mediated by cyclic AMP (cAMP), cyclic GMP (cGMP) or potassium channel. Intrathecal (i.t.) administration of drugs at the thoracic level were performed in anesthetized, artificially ventilated male Sprague-Dawley rats. I.t. injection of adenosine A_1 receptor agonist, N^6-cyclohexyladenosine (CHA; 1, 5 and 10 nmol) produced dose dependent decrease of BP and HR and it was attenuated by pretreatment of 50 nmol of 8-cyclopentyl-1,3-dimethylxanthine, a specific adenosine A_1 receptor antagonist. Pretreatment with a cAMP analogue, 8-bromo-cAMP, also attenuated the depressor and bradycardiac effects of CHA (10 nmol), but not with cGMP analogue, 8-bromo-cGMP. Pretreatment with a ATP-sensitive potassium channel blocker, glipizide (20 nmol) also attenuated the depressor and bradycardiac effects of CHA (10 nmol). These results suggest that adenosine A_1 receptor in the spinal cord plays an inhibitory role in the central cardiovascular regulation and that this depressor and bradycardiac actions are mediated by cAMP and potassium channel.

Keywords □ adenosine A_1 receptor, spinal cord, N^6-cyclohexyladenosine, blood pressure, heart rate, cAMP, cGMP, ATP-sensitive potassium channel

INTRODUCTION

The action of adenosine as a neurotransmitter or neuromodulator responsible for cardiovascular regulation has been suggested (Barraco *et al.*, 1987; Barraco *et al.*, 1988; Barraco *et al.*, 1990). Its two receptors mediate different cardiovascular effects in the peripheral and central nervous system. In the peripheral autonomic nervous system, A_1 receptor mediates negative inotropic effects on the heart, while A_2 receptor mediates depressive effects on most of blood vessels including the coronary arteries. As for the central nervous system, the administration of adenosine or its agonist into the 3rd ventricle (Stella *et al.*, 1993), 4th ventricle (Barraco *et al.*, 1987), the nucleus tractus solitarius (Barraco *et al.*, 1990; Mosqueda *et al.*, 1991) and spinal cord (Koh *et al.*, 1996) all resulted in a dose dependent decrease of the blood pressure (BP) and heart rate (HR). Adenosine is coupled to adenylate cyclase via two types of receptors: A_1 receptor that mediates an inhibition of adenylate cyclase and A_2 receptor that meditates a stimulation of the enzyme (Daly *et al.*, 1983; Gerber and Gahwiler, 1994; Londos *et al.*, 1987; Van Calker *et al.*, 1979). The A_1 and A_2 receptors have distinct distributions in the central nervous system (Bruns *et al.*, 1987; Stone *et al.*, 1988), and evidence for the existence of both receptors in the spinal cord of rats were also demonstrated by Choca *et al.* (Choca *et al.*, 1987).

The autonomic preganglionic neurons in the spinal cord play an important role in central regulation of the cardiovascular system, and this is supported by experiments which show the alterations of cardiovascular responses after intrathecal (i.t.) administration of drugs. Alterations in cardiovascular responses by i.t. administration of glutamate (Tao and Abdel-Rahman, 1983) were reported. However, little is known about the cardiovascular regulatory effects of adenosine A_1 receptors in the spinal cord. This study was performed to examine the cardiovascular effects of adenosine A_1 receptor stimulation in the spinal cord, and to define whether its mechanism is mediated by potassium channel, cAMP or cGMP.

*Corresponding author
Tel: 02-2220-0651, Fax: 02-2292-6686
E-mail: icshin@hanyang.ac.kr

MATERIALS AND METHODS

The experimental animals were categorized into five groups. The first group of the these groups was treated only with N^6-cyclohexyladenosine (CHA; 10 nmol), an adenosine A_1 receptor agonist. The second group was treated with cAMP analogue, 8-bromo-cAMP (10 nmol) 10 minutes before the administration of the CHA (10 nmol). The third group was treated with cGMP analogue, 8-bromo-cGMP (10 nmol) 10 minutes before the administration of the CHA (10 nmol). The fourth group was treated with glipizide (20 nmol), an ATP-sensitive potassium channel blocker, 10 minutes before the administration of the CHA (10 nmol). Another group was sham-operated animal group. All drugs were purchased from RBI Chemical Company (USA). CHA, 8-bromo-cAMP and 8-bromo-cGMP were dissolved in 0.9% NaCl solution. Glipizide was dissolved in 2.0% dimethylsulfoxide solution. Male Sprague-Dawley rats (300-350 gm) were anesthetized with urethane (1.15 gm/kg, i.p.), paralyzed with d-tubocurarine (0.5 mg/kg, i.m.) and artificially ventilated (Ugo Basile, Italy). BP and HR were continuously monitored via a femoral arterial catheter (PE-50) connected to a pressure transducer (Spectramed, USA) and a polygraph (Grass, USA). Mean arterial pressure (MAP) was calculated as diastolic pressure + 1/3 (systolic pressure - diastolic pressure). Rectal temperature was maintained at 37 ± 0.5°C with a heating pad. The rats were placed in stereotaxic instrument (Stoelting, USA) in prone postion. The posterior atlantooccipital membrane was exposed by an occipital incision. The atlantooccipital membrane was cut and a guide cannula (PE-10) was inserted intrathecally; its tip was positoned at the lower thoracic vertebral level (6.5 cm from the lower margin of the occipital bone). I.t. administration of drugs were made using the injector cannula (33-gauge stainless steel) through the guide cannula. 10 nmol of CHA were delivered in a volume of 5 ml in 1 min with Hamilton syringe mounted on a micrometer. 10 nmol of 8-bromo-cAMP was injected 10 minutes before administration of CHA. 10 nmol of 8-bromo-cGMP was injected 10 minutes before administration of CHA. 20 nmol of glipizide was injected 10 minutes before administration of CHA. Data were expressed as mean ± S.E. of the maximal response following drug administration. Student's *t*-test for paired or unpaired data was used for statistical evaluation of the results.

RESULTS

I.t. administration of CHA caused a decrease in MAP (mean arterial pressure) that reached maximum in 19.3 ± 2.1 min after administration. The depressor response evoked by CHA was dose dependent (1, 5 and 10 nmol of CHA decreased the MAP by 2.4 ± 2.0, 11.0 ± 1.9, 27.7 ± 3.8 mmHg, respectively; n=5; Fig. 1, Koh *et al.* 1996). Dose dependent decrease of HR (heart rate) was also induced by CHA administration (1, 5 and 10 nmol of CHA decreased the HR by 10 ± 7.1, 54.1 ± 11.4, 108 ± 25.0 mmHg, respectively; n=5; Fig. 2, Koh *et al.* 1996), maximum 15.7 ± 3.2 min after injection. Base-

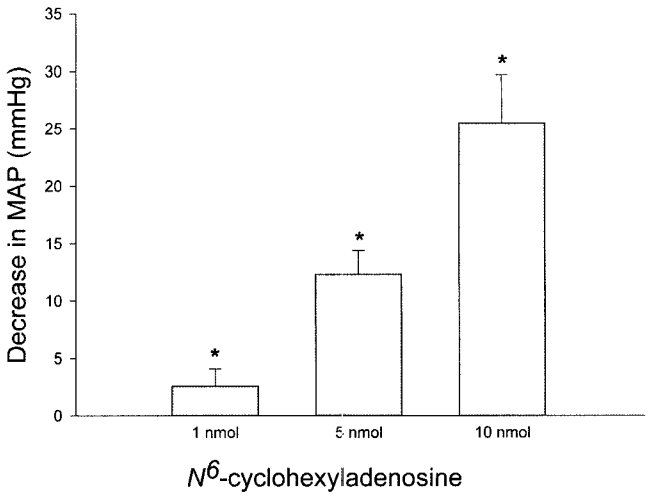

Fig. 1. Dose dependent decrease of mean arterial pressure (MAP) by I.t. administration of N^6-cyclohexyladenosine (CHA; 1, 5 and 10 nmol). Results are expressed as mean±SE. *$P<$ 0.05, compared to basal MAP (Koh *et al.* 1996).

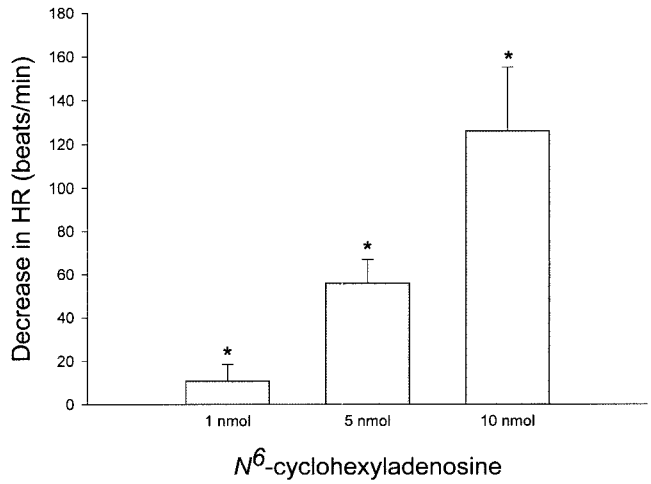

Fig. 2. Dose dependent decrease of heart rate (HR) by I.t. administration of N^6-cyclohexyladenosine (CHA; 1, 5 and 10 nmol). Results are expressed as mean±SE. *$P<0.05$, compared to basal HR (Koh *et al.* 1996).

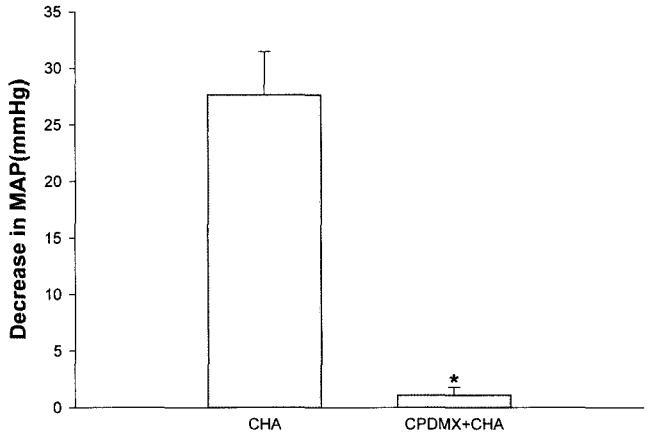

Fig. 3. Changes in mean arterial pressure (MAP) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 8-cyclopentyl-1,3-dimethylxanthine (CPDMX; 50 nmol, i.t.). Data are the mean± SE. *P<0.05, compared to CHA only group.

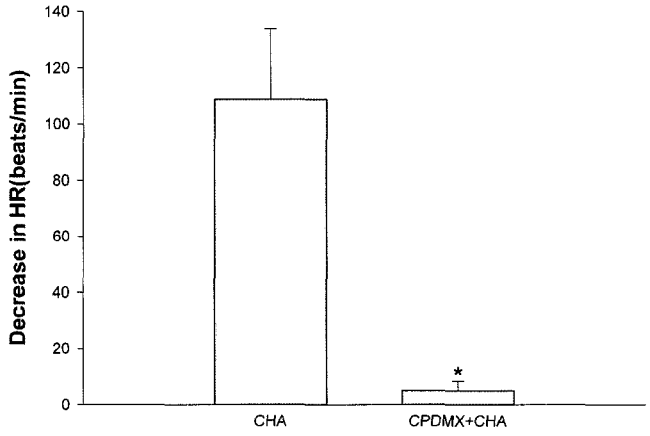

Fig. 4. Changes in heart rate (HR) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 8-cyclopentyl-1,3-dimethylxanthine (CPDMX; 50 nmol, i.t.). Data are the mean±SE. *P<0.05, compared to CHA only group.

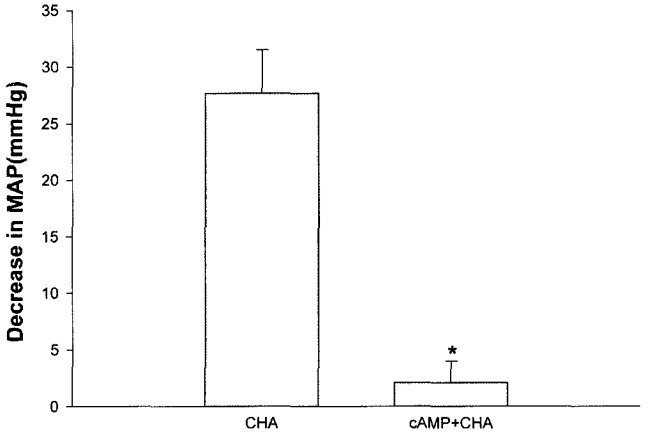

Fig. 5. Changes in mean arterial pressure (MAP) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 8-bromo-cAMP (cAMP; 10 nmol, i.t.). Data are the mean±SE. *P<0.05, compared to CHA only group (Koh *et al.* 1996).

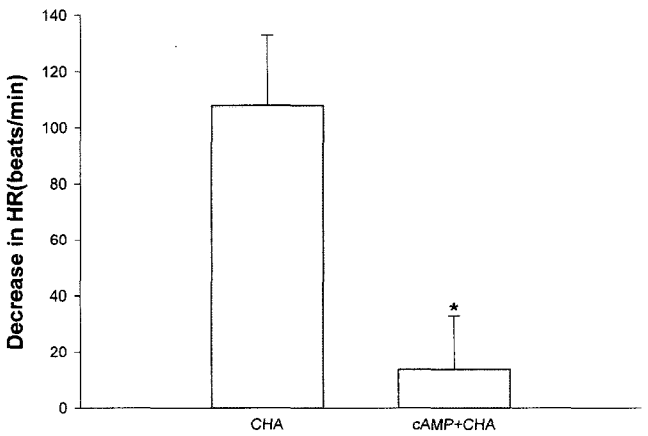

Fig. 6. Changes in heart rate (HR) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 8-bromo-cAMP (cAMP; 10 nmol, i.t.). Data are the mean±SE. *P<0.05, compared to CHA only group (Koh *et al.* 1996).

line MAP and HR for these rats were 85.7 ± 6.5 mmHg and 334.5 ± 9.3 bpm respectively. Pretreatment with 8-cyclopentyl-1,3-dimethylxanthine (50 nmol), an adenosine A_1 receptor antagonist, completely attenuated the CHA-induced cardiovascular responses; decrease in MAP and HR were 1.1 ± 0.7 mmHg and 5.0 ± 3.5 bpm (n=5, Fig. 3, Fig. 4), respectively. Administration (i.t.) of an equivalent volume of normal saline did not affect the basal MAP and HR.

Pretreatment with 8-bromo-cAMP (10 nmol, i.t.) significantly attenuated the CHA-induced cardiovascular responses; decrease in MAP and HR were 2.1 ± 1.9 mmHg and 14.0 ± 19.0 bpm (n=5, Fig. 5, Fig. 6, Koh *et al.* 1996), respectively. However, i.t. administration of 8-bromo-cGMP (10 nmol, i.t.) prior to administration of CHA did not alter the depressor and bradycardiac response elicited by CHA; decrease in MAP and HR were 24.5 ± 5.2 mmHg and 113 ± 36.9 bpm (n=5, Fig. 7, Fig. 8, Koh *et al.* 1996), respectively. Injection of 8-bromo-cAMP (10 nmol, i.t.) and 8-bromo-cGMP (10 nmol, i.t.) had no effect on basal MAP and HR.

Pretreatment with glipizide (20 nmol, i.t.) significantly attenuated the CHA-induced cardiovascular responses; decrease in MAP and HR were 4.6 ± 2.9 mmHg and 34.0 ± 11.4 bpm (n=5, Fig. 9, Fig. 10), respectively. Injection of glipizide (20 nmol, i.t.) had no effect on basal MAP and HR.

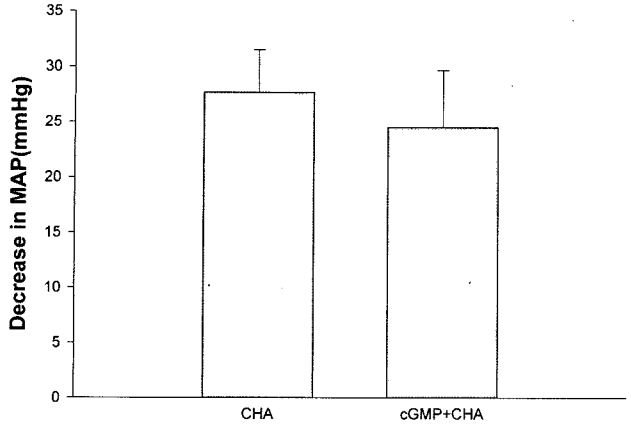

Fig. 7. Changes in mean arterial pressure (MAP) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 8-bromo-cGMP (cGMP; 10 nmol, i.t.). Data are the mean±SE (Koh *et al.* 1996).

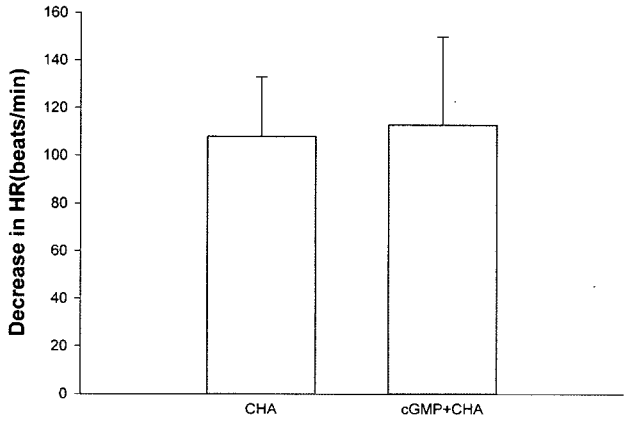

Fig. 8. Changes in heart rate (HR) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 8-bromo-cGMP (cGMP; 10 nmol, i.t.). Data are the mean±SE (Koh *et al.* 1996).

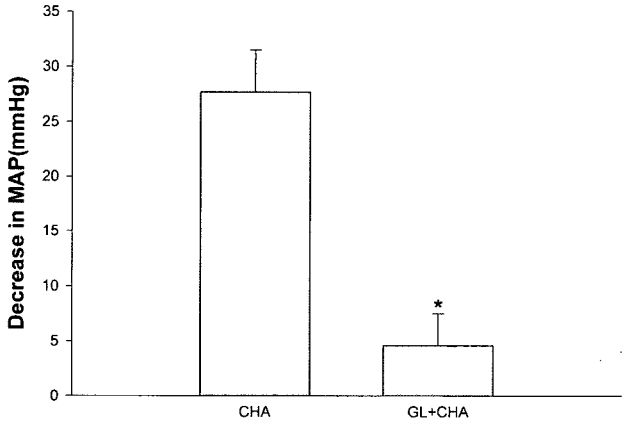

Fig. 9. Changes in mean arterial pressure (MAP) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with glipizide (GL; 20 nmol, i.t.). Data are the mean±SE. *P<0.05, compared to CHA only group.

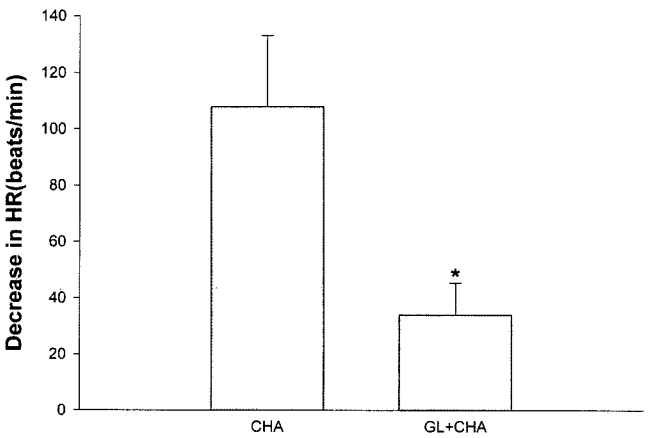

Fig. 10. Changes in heart rate (HR) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with glipizide (GL; 20 nmol, i.t.). Data are the mean±SE. *P<0.05, compared to CHA only group.

DISCUSSION

Adenosine A_1 receptor, which is found in both central and peripheral nervous system (Choca *et al.*, 1987), mediates various neuromodulatory action of adenosine, including cardiovascular regulatory effects. Adenosine A_1 receptors in the central nervous system mediates mainly inhibitory effects on synaptic transmission of glutamate, acetylcholine in hippocampus, striatum, hypothalamus and cerebral cortex (Brown *et al.*, 1990; Coardetti *et al.*, 1984; Phillis *et al.*, 1979). Adenosine A_1 receptor is responsible for cardiovascular inhibitory effects in central and peripheral nervous system (Koh *et al.*, 1996). The administration of adenosine or its agonist into the 4th ventricle (Barraco *et al.*, 1987) and nucleus tractus solitarius (Mosqueda *et al.*, 1991) resulted in depression of blood pressure (BP) and decrease of heart rate (HR). Adenosine A_2 receptor in the spinal cord plays an inhibitory role in the central cardiovascular regulation (Koh *et al.*, 2000).

Previously, we have reported that intrathecal (i.t.) administration of cyclohexyladenosine (CHA), an adenosine A_1 receptor agonist, leads the cardiovascular inhibitory effects which is mediated by cyclic AMP but, not by cyclic GMP (Koh *et al.*, 1996) In the previous report, we demonstrated that adenosine A_1 receptor-mediated cardiovascular response was modulated by cAMP in the spinal cord of rats. In present experiment, we examined the modulation of cardiovasuclar effects of CHA by cAMP, cGMP or potassium channel in the spinal cord.

In present study, i.t. administration of CHA in anesthetized and artificially ventilated rats elicited dose-dependent decrease of

MAP and HR and it was attenuated by pretreatment with adenosine A_1 receptor antagonist. Many experimental evidences suggested that adenosine A_1 receptors in the central nervous system plays a critical role in the mediation of cardiovascular responses (Barraco *et al.,* 1987; Barraco *et al.,* 1988; Mosqueda *et al.,* 1991). Cardiovascular inhibitory action induced by adenosine or adenosine A_1 receptor agonists in the caudal and rostral nucleustractus solitarius (Barraco *et al.,* 1988; Tao and Abdel-Rahman, 1983) were also reported. Our result is consistent with the opinion of above authors, but this is the first evidence of cardiovascular inhibitory action of adenosine A_1 receptor in spinal cord.

Little is known about the autonomic preganglionic neurons which has adenosine receptors responsible for the cardiovascular regulation in the spinal cord. However, anatomical localization of nitric oxide synthetase in preganglionic autonomic neurons in intermediolateral cell column of thoracicand lumbar spinal cord was reported (Bredt *et al.,* 1991) and cardiovascular excitatory response elicited by i.t. administration of sodium nitroprusside, a nitric oxide donor, was demonstrated (Lee *et al.,* 1996). Presence of adenosine A_1 and A_2 receptors in spinal cords were also demonstrated through the autoradiographic binding method (Choca *et al.,* 1988; Choca *et al.,* 1987). However, those studies were primarily concerned with its localizations in substantia gelatinosa which has close association with pain modulation. Further experiments are required to identify morphologically the adenosine receptors responsible for cardiovascular regulation in the spinal cord.

I.t. administration of 8-bromo-cAMP significantly attenuated the cardiovascular depressor actions elicited by CHA, but 8-bromo-cGMP did not altered it. Adenosine receptors are linked to G-proteins known to be coupled negatively to the enzyme adenylate cyclase (Gerber and Gahwiler, 1994). The actions of cAMP and cGMP are mediated by cAMP and cGMP dependent protein kinase, respectively. In recent study, Jiang et al (Jiang *et al.,* 1992) reported that high concentration of cAMP could also activate cGMP dependent protein kinase as well as cAMP-dependent protein kinase, suggesting the close relationship between actions of cAMP and cGMP. But our result suggested that the cardiovascular inhibitory action of adenosine A_1 receptor in spinal cord is mediated by cAMP dependent adenylate cyclase, independent from cGMP.

I.t. administration of with ATP-sensitive potassium channel inhibitor, glipizide (20 nmol). significantly attenuated the cardiovascular depressor actions elicited by CHA. Some authors report that the action of adenosine is mediated via the activation of potassium channel (Trussel and Jackson, 1985; Nicoll, 1988; Gerber and Gahwiler, 1994). Adenosine receptor and GABA (Gamma-amino butyric acid) receptor act via the activation of potassium channel in substantia nigra (Watt *et al.,* 1995). Adenosine activates the potassium channel via adenosine A_1 receptor (Nicoll, 1988). It is known that ATP-sensitive potassium channel inhibitor is involved in the peripheral response of adenosine A_1 receptor. Therefore the regulation of cerebral blood flow in adenosine A_1 receptor is mediated by potassium channel.

In conclusion, our results show that adenosine A_1 receptor in the spinal cord plays an inhibitory role in central cardiovascular regulation and that this depressor and bradycardiac actions are mediated by cAMP and potassium channel.

REFERENCES

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.

Barraco, R. A., El-Ridi, M. R., Ergene, E. and Phillis, J. W. (1991). Adenosine receptor subtypes in the brain stem mediate distinct cardiovascular response. *Brain Res.* **26**, 59-84.

Barraco, R. A, Janusz, C. A., Parizon, M., Posalek, P. M and Roberts, P. A. (1988). Cardiovascular effects of microinjection of adenosine into the nucleus tractus solitarius. *Brain Res. Bull.* **20**, 129-132.

Barraco, R. A., Janusz, C. A, Shoener, E. P. and Simpson. L. L. (1990). Cardiorespiratory function is altered by picomol injections of 5'-*N*-ethylcarboxamidoadenosine into the nucleus tractus solitarius of rats. *Brain Res.* **507**, 234-246.

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.

Brown, S. J., James, S., Reddington, M. and Richardson, P. J. (1990). Both A_1 and A_{2a} purine receptors regulate striatal acetylcholine release. *J. Neurochem.* **55**, 31-38.

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., Green, R. D. and Proudfit, H. K. (1988). Adenosine A_1 and A_2 receptors of the substantia gelatinosa are located predominantly on intrinsic neurons: an autoradiographic study. *J. Phamacol. Exp. Ther.* **247**, 757-764.

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.

Coardetti, R., Lo Conte, G., Moroni, F., Passani, M. B. and Peteu, G. (1984). Adenosine decrease aspartate and glutamate release from rat hippocampal slices. *Eur. J. Pharmacol.* **104**, 19-26.

Daly, J. W., Butts-Lamb, P. and Padgett, W. (1983). Subclass of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. *Cell Mol. Biol.* **3**, 69-80.

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

Gordon, F. J. (1985). Spinal GABA receptors and central cardiovascular control, *Brain Res.* **328**, 165-169.

Hassessian, H., Prat, A., Champlain, J. D. and Couture, R (1991). Regulation of cardiovascular sympathetic neuons by substance P and gamma aminobutyric acid in rat spinal cord. *Eur. J. Pharmacol.* **202**, 51-60.

Koh, H. C., Shin, I. C., Hwang, S. J. and Paik, D. J. (1996). Modification of cardiovascular response of adenosine A_1 receptor agonist by cyclic AMP in the spinal cord of the rats. *Neurosci. Lett.* **219**, 195-198.

Koh, H. C., Lee, T. K., Kang, J. S., Lee, C. H., Lee, H., Paik, D. J. and Shin, I. C. (2000). Modification of cardiovascular response of adenosine A_2 receptor agonist by adenylate cyclase in the spinal cord of the rats. *Neurosci. Lett.* **293**, 45-48.

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.

Lee, S. B., Koh, H. C., Kim, O. N., Sung, K. W. and Kim, S. Y. (1996). Intrathecal administration of sodium nitroprusside, a nitric oxide donor, increases blood pressure in anesthetized rats. *Neurosci. Lett.* **203**, 53-56.

Londos, S., Cooper, D. M. F. and Wolff, J. (1987). Subclasses of external adenosine receptors. *Proc. Natl. Acad. Sci.* USA **77**, 2551-2554.

Mosqueda, G. R., Tseng, C. J., Appalsamy, M., Beck, C. and Robertson, D. (1991). Cardiovascular excitatory effects of adenosine in the nucleus of the solitary tract. *Hypertension* **18**, 494-502.

Nicoll, R. A. (1988). The coupling of neurotransmitter receptor to ion channels in the brain. *Science* **241**, 545-551.

Phillis J. W., Edstrom, J. P. Kostopoulos, G. K. and Kirkpatrick, J. R. (1979). Effects of adenosine and adenine nucleotides on synaptic trasnmission in the cerebral cortex, *Can. J. Pharmacol.* **57**, 1289-1312.

Sighart, W. (1992). GABAA receptors: ligand-gated Cl^- ion channels modulated by multiple drug binding sites. *Trends Pharmacol. Sci.* **13**, 446-450.

Stella, L. Berrino, L., Maione, S., de Novellis, F. and Rossi, F. (1993). Cardiovascular effects of adenosine and its analogue in anesthetized rats. *Life Sci.* **53**, 755-763.

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 Dev. Res.* **15**, 31-46.

Tao, S. and Abdel-Rahman, A. A. (1983). Neuronal and cardiovascular response to adenosine microinjection into the nucleus tractus solitarius. *Brain Res. Bull.* **32**, 407-417.

Trussel, L. O. and Jackson, M. B. (1985). Adenosine-activated potassium conductance in cultured striatal neurons. *Proc. Natl. Acad. Sci. USA.* **82**, 4857-4861.

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.

Watts, A. E., Hicks, G. A. and Henderson, G. (1995). Putative pre- and postsynaptic ATP-sensitive potassium channels in the rat substantia nigra in vitro. *J. Neurosci.* **15**(4), 3065-3074.