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Effects of $GABA_B$ Receptor Antagonist on the Cardiovascular Response of Adenosine A_1 and Adenosine A_2 Receptor Agonist in the Spinal Cord of the Rats

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Abstract – Adenosine and GABA are known to be major inhitory neurotransmitters in the central nervous system and its receptors mediate various neurophamacological effects including cardiovascular modulatory effects. Inhibitory cardiovascular effects induced by intrathecal (i.t.) administration of adenosine A_1 receptor agonist and its modulation by cyclic AMP was suggested by our previous report. In this experiment, we examined the modulation of cardiovascular effects of adenosine A_1 receptor and adenosine A_2 receptor by $GABA_B$ receptors antagonist in the spinal cord. I.t. administration of 10 nmol of N^6-cyclohexyladenosine (CHA, an adenosine A_1 receptor agonist), I.t. administration of 2 nmol of 5'-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA, an adenosine A_2 receptor agonist), pretreatment with 5-aminovaleric acid (a $GABA_B$ receptor antagonist, 50 nmol, i.t.) prior to administration of CHA and pretreatment with 5-aminovaleric acid (a $GABA_B$ receptor antagonist, 50 nmol, i.t.) prior to administration of CPCA were performed in anesthetized, artificially ventilated Sprague-Dawley rats. I.t. administration of 50 nmol of 5-aminovaleric acid significantly attenuated the inhibitory cardiovascular effects of CHA but did not attenuated the inhibitory cardiovascular effects of CPCA. It is suggested that cardiovascular responses of adenosine A_1 receptor is modulated by $GABA_B$ receptor and adenosine A_2 receptor is not modulated by $GABA_B$ receptor in the spinal cord.

Keywords □ Adenosine A_1 receptor, adenosine A_2 receptor, $GABA_B$ receptor, spinal cord, blood pressure, heart rate

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).

GABA is one of the major inhibitory neurotransmitter in central nervous system and it is suggested to have a significant role in modulating the activity of sympathetic preganglionic neuons in the spinal cord (Bacon and Smith, 1988; Choca *et al.*, 1987). Both $GABA_A$ and $GABA_B$ receptors in these neurons mediate cardiovascular regulatory effects of GABA in the spinal cord (Hong and Henry, 1991). Colocalization and functional relations between adenosine receptors and GABA

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receptors, were suggested by some authors (Goodman and Snyder, 1982; Lee *et al.*, 1983). Gerber and Gahwiller (Gerber and Gahwiller, 1994) suggested that actions of adenosine and $GABA_B$ receptors in hippocampal pyramidal cells are mediated by a common signal transduction pathway. This study was performed to examine the effects of $GABA_B$ receptor antagonist on the cardiovascular depressive action induced by stimulation of adenosine A_1 receptor and adenosine A_2 receptor in lower thoracic cord level and also the relationship of $GABA_B$ receptor with adenosine A_1 receptor or adenosine A_2 receptor.

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 A1 receptor agonist. The second group was treated with only with 5'-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA; 2 nmol), an adenosine A_2 receptor agonist. The third group was treated with 5-aminovaleric acid (50 nmol), an $GABA_B$ receptor antagonist, 10 minutes before the administration of the CHA (10 nmol). The fourth group was treated with 5-aminovaleric acid (50 nmol), an $GABA_B$ receptor antagonist, 10 minutes before the administration of the CPCA (2 nmol). Another group was sham-operated animal group. All drugs were purchased from RBI Chemical Company (USA). CHA, CPCA and 5-aminovaleric acid were dissolved in 0.9% NaCl 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. 50 nmol of 5-aminovaleric acid were injected 10 minutes before administration of CHA and CPCA. 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 16.3±4.1 min after administration. The depressor response evoked by CHA was dose dependent (1, 5 and 10 nmol of CHA decreased the MAP by 2.6±1.5, 12.3±2.1, 25.5±4.2 mmHg, respectively; n=5; Fig. 1). Dose dependent decrease of HR (heart rate) was also induced by CHA administration (1, 5 and 10 nmol of CHA decreased the HR by 11±7.5, 56±10.9, 126±29.2 mmHg, respectively; n=5; Fig. 2), maximum 15.7±3.2 min after injection. Baseline MAP and HR for these rats were 88.2±5.7 mmHg and 326.5±8.2 bpm respectively. Pretreatment with 8-cyclopentyl-1,3-dimethylxanthine (50 nmol), an adenosine A_1 receptor antagonist, completely attenuated the CHA-induced cardiovascular responses. Administration (i.t.) of an equivalent volume of normal saline did not affect the basal MAP and HR.

I.t. administration of CPCA caused a decrease in MAP that reached maximum in 18.9±2.7 min after administration. The depressor response evoked by CPCA was dose dependent (1, 1.5 and 2 nmol of CPCA decreased the MAP by 2.6±1.4, 14..3±2.5, 26.3±2.7 mmHg, respectively; n=5; Fig. 3). Dose dependent decrease of HR was also induced by CPCA administration (1, 1.5 and 2 nmol of CPCA decreased the HR by

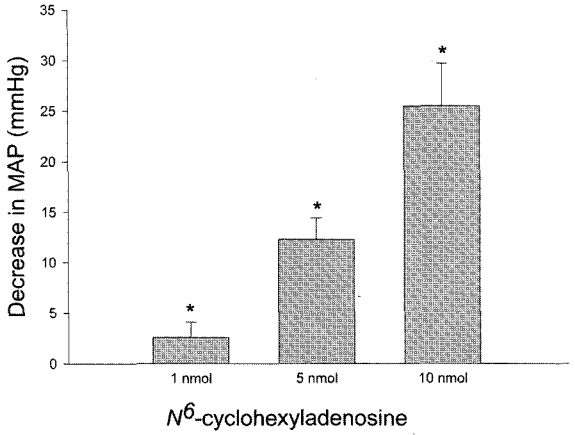

Fig. 1. Dose dependent decrease of mean arterial pressure (MAP) by I.t. administration of N^6-cyclohexyladenosine (CHA; 1, 5 and 10 nmol). Results are expressed as mean±SE.
*P< 0.05, compared to basal MAP.

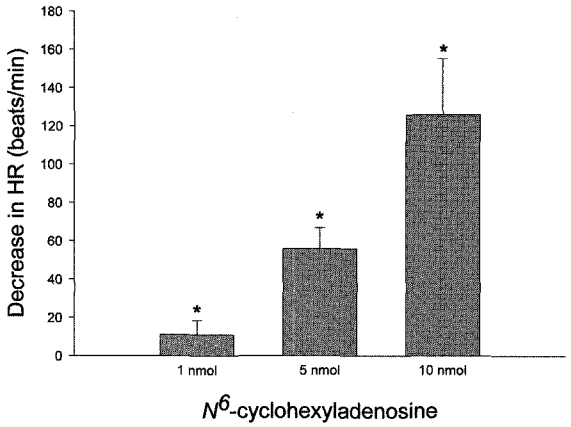

Fig. 2. Dose dependent decrease of heart rate (HR) by I.t. administration of N^6-cyclohexyladenosine (CHA; 1, 5 and 10 nmol). Results are expressed as mean±SE.
*P<0.05, compared to basal HR.

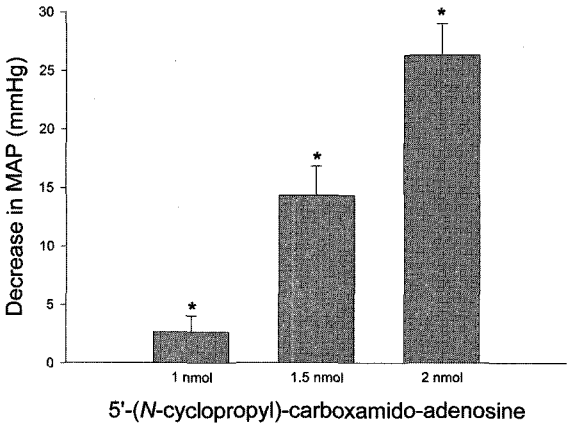

Fig. 3. Dose dependent decrease of mean arterial pressure (MAP) by I.t. administration of 5'-(*N*-cyclopropyl)-carboxamido-adenosine (CPCA; 1, 1.5 and 2 nmol). Results are expressed as mean±SE.
*P<0.05, compared to basal MAP.

14±5.5, 36±6.2, 58±8.4 mmHg, respectively; n=5; Fig. 4), maximum 17.7±3.6 min after administration. Baseline MAP and HR for these rats were 87.7±8.1 mmHg and 376.8±10.7 beats per min (bpm). Pretreatment with 3,7-dimethyl-1-propargylxanthine (10 nmol), an adenosine A_2 receptor antagonist, completely attenuated the CPCA-induced cardiovascular responses. Administration (i.t.) of an equivalent volume of normal saline did not affect the basal MAP and HR.

Pretreatment with 5-aminovaleric acid (50 nmol, i.t.) significantly attenuated the CHA-induced cardiovascular responses; decrease in MAP and HR were 11.3±3.2 mmHg and 60.0± 10.0 bpm (n=5, Fig. 5, Fig. 6), respectively. However, i.t. administration of 5-aminovaleric acid (50 nmol, i.t.) prior to

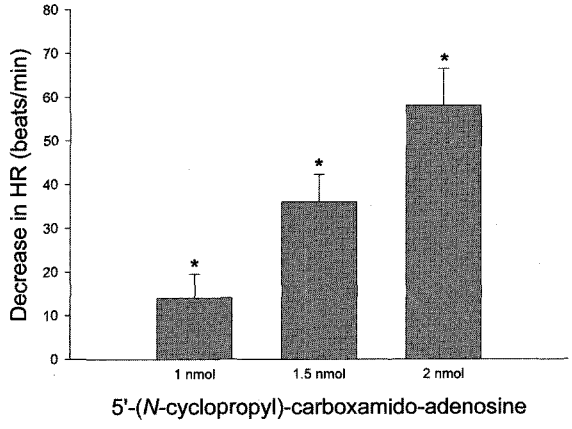

Fig. 4. Dose dependent decrease of heart rate (HR) by I.t. administration of 5'-(*N*-cyclopropyl)-carboxamido-adenosine (CPCA; 1, 1.5 and 2 nmol). Results are expressed as mean±SE.
*P<0.05, compared to basal HR.

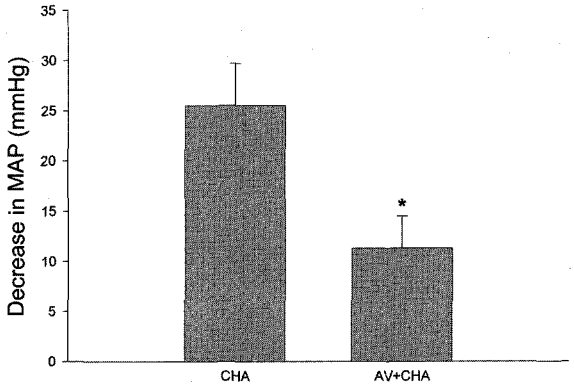

Fig. 5. Changes in mean arterial pressure (MAP) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 5-aminovaleric acid (AV; 50 nmol, i.t.). Data are the mean±SE.
*P<0.05, compared to CHA only group.

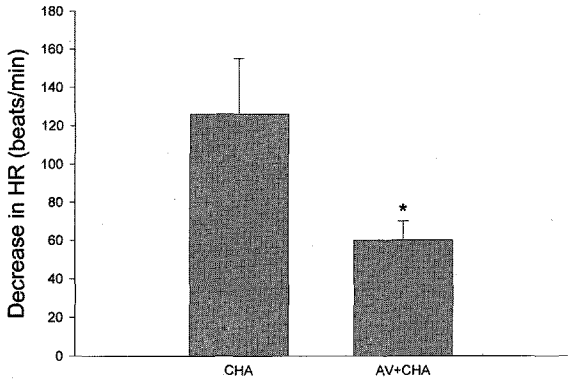

Fig. 6. Changes in heart rate (HR) following treatment with N^6-cyclohexyladenosine (CHA; 10 nmol, i.t.) only and CHA after pretreatment with 5-aminovaleric acid (AV; 50 nmol, i.t.). Data are the mean±SE.
*P<0.05, compared to CHA only group.

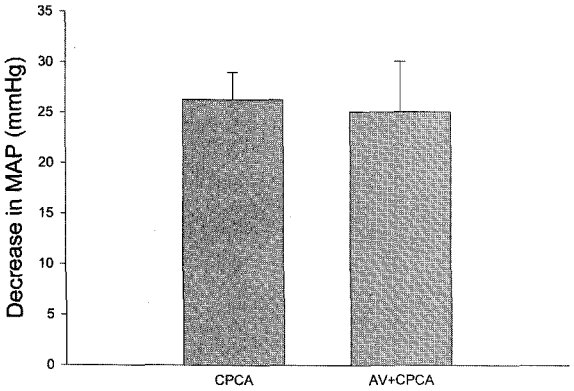

Fig. 7. Changes in mean arterial pressure (MAP) following treatment with 5'-(*N*-cyclopropyl)-carboxamido-adenosine (CPCA; 2 nmol, i.t.) only and CPCA after pretreatment with 5-aminovaleric acid (AV; 50 nmol, i.t.). Data are the mean±SE.

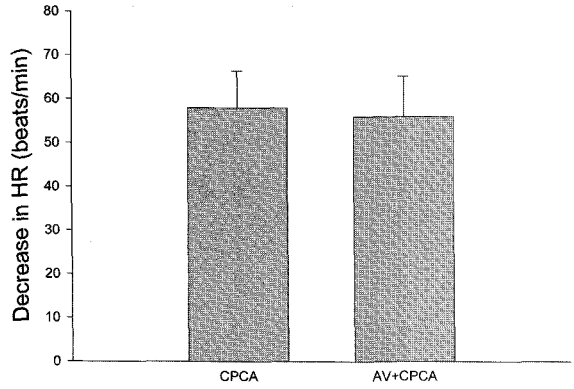

Fig. 8. Changes in heart rate (HR) following treatment with 5'-(*N*-cyclopropyl)-carboxamido-adenosine (CPCA; 2 nmol, i.t.) only and CPCA after pretreatment with 5-aminovaleric acid (AV; 50 nmol, i.t.). Data are the mean±SE.

administration of CPCA did not alter the depressor and bradycardiac response elicited by CPCA; decrease in MAP and HR were 25.1±5.0 mmHg and 56±9.2 bpm (n=5, Fig. 7, Fig. 8), respectively. Another different groups were administered with 5-aminovaleric acid (10 nmol, 50 nmol and 100 nmol, i.t.) had no effect on basal MAP and HR. Therefore in this study authors chose the middle concentration.

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 A1 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 $GABA_B$ receptor antagonist in the spinal cord.

Administration (i.t.) of 5-aminovaleric acid significantly attenuated the cardiovascular depressor actions elicited by CHA. Both adenosine A_1 and $GABA_B$ receptors mediate mainly inhibitory effects in central nervous system, and their actions are suggested to be closely related each other in terms of mechanism of cardiovascular regulation. Although the exact mechanism involved in this action requires further elucidation, this results were consistent with the observations made in previous reports. Accumulating evidences clearly demonstrate cardiovascular response of adenosine A_1 receptors were mediated by cAMP in the spinal cord (Koh *et al.*, 1996). Actions of $GABA_B$ receptors are also mediated by cAMP in cardiovascular modulatory action in the spinal cord (Karbon and Enna, 1985). We suggest that these mechanism, in the spinal cord, may contribute to modulation of cardiovascular regulation of CHA by 5-aminovaleric acid, and that adenosine A_1 receptor might directly interact with $GABA_B$ receptor in the spinal cardiovascular regulation because 50 nmol of 5-aminovaleric acid alone did not altered basal BP and HR. This can be supported by the following other studies. Kamatchi and Ticku (Kamatchi and Ticku, 1990; Kamatchi and Ticku, 1991) suggested that the action of $GABA_B$ receptors involved the G proteins and adenylate cyclase, and that this is under modulatory control by protein kinase A and C. Gerber and Gahwiler (Gerber and Gahwiler, 1994) also suggested that adenosine receptors and GABA receptors are seemed to be present in the cell membrane of same hippocampal neuron, and contact with same Gi protein

pool or separate G proteins which are converged to same adenylate cyclase. Administration (i.t.) of 5-aminovaleric acid did not attenuated the cardiovascular depressor actions elicited by CPCA. Both adenosine A_2 and $GABA_B$ receptors mediate mainly inhibitory effects in central nervous system, and their actions are suggested to be not closely related each other in terms of mechanism of cardiovascular regulation. We suggest that these mechanism, in the spinal cord, may contribute to modulation of cardiovascular regulation of CPCA by 5-aminovaleric acid, and that adenosine A_2 receptor might not directly interact with $GABA_B$ receptor in the spinal cardiovascular regulation because 50 nmol of 5-aminovaleric acid alone did not altered basal BP and HR.

In conclusion, we suggest that cardiovascular response of adenosine A_1 receptor is modulated by $GABA_B$ receptor and adenosine A_2 receptor is not modulated by $GABA_B$ receptor in the spinal cord.

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