Autonomic Neuroscience: Basic and Clinical 134 (2007) 55–60

AUTONOMIC NEUROSCIENCE: Basic & Clinical

www.elsevier.com/locate/autneu

Involvement of guanylate cyclase in the cardiovascular response induced by adenosine A_{2B} receptor stimulation in the posterior hypothalamus of the anesthetized rats

Min Jeong Kang, Hyun Chul Koh*

Department of Pharmacology, College of Medicine, Hanyang University, 17 Haengdang-Dong, Sungdong-Ku, Seoul 133-791, Republic of Korea

Received 11 July 2006; received in revised form 31 January 2007; accepted 11 February 2007

Abstract

Cardiovascular inhibitory effects induced by the posterior hypothalamic adenosine A_2 receptors were suggested by our previous reports. In this experiment, we examined the influence of the posterior hypothalamic adenosine A_{2B} receptors on central cardiovascular regulation of blood pressure (BP) and heart rate (HR). Posterior hypothalamic injection of drugs was performed in anesthetized, artificially ventilated male Sprague–Dawley rats. Injection of 5′-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA; 2 nmol), an adenosine A_2 receptor agonist, showed the decrease of arterial blood pressure and heart rate, and the alloxazine, an adenosine A_{2B} receptor antagonist, partially blocked the depressor and bradycardiac effects of CPCA (2 nmol). To examine the role of adenosine A_{2B} receptors among the adenosine A_2 subtypes, we applied the 5′-*N*-Ethylcarboxamidoadenosine (NECA), an adenosine A_{2B} receptor agonist, to the posterior hypothalamus. Injection of NECA (1, 4 and 8 nmol) produced a dose-dependent decrease of arterial blood pressure and HR. Pretreatment with alloxazine (5 nmol) partially blocked the depressor and bradycardiac effects of NECA (4 nmol). Also, pretreatment with LY-83,583 (5 nmol), a soluble guanylate cyclase inhibitor, attenuated the depressor and bradycardiac effects of NECA (4 nmol). However, pretreatment with MDL-12,330 (10 nmol), an adenylate cyclase inhibitor, did not affect these effects of NECA (4 nmol). These results suggest that adenosine A_{2B} receptor in the posterior hypothalamus plays an inhibitory role in central cardiovascular regulation, and that guanylate cyclase mediates the depressor and bradycardiac actions of adenosine A_{2B} receptors.

Keywords: Adenosine A_{2B} receptor; Posterior hypothalamus; Blood pressure; Heart rate; Guanylate cyclase; Adenylate cyclase

1. Introduction

Adenosine is a potent modulator of cardiovascular function. Its effects are depressant and involve both central and peripheral mechanisms as well as different adenosine receptor subtypes, mostly adenosine A_1, A_{2A}, A_{2B} and A_3 receptors. (Scislo et al., 2001; Spyer and Thomas, 2000). It has been demonstrated that adenosine A_2 receptors are involved in the central regulation of the cardiovascular system. Indeed, since many years, evidence exist that activation of adenosine A_2 receptors causes depressor and bradycardiac response in the central nervous system. The administration of adenosine A_2 receptor agonists into the 3rd ventricle (Stella et al., 1995), nucleus tractus solitarius (Tanaka et al., 1995), intrathecal (Koh et al., 2000) and posterior hypothalamus (Kang et al., 2003; Song et al., 2002), all resulted in depression of blood pressure (BP) and a decrease in heart rate (HR). Most likely, the inhibitory response is mediated by central adenosine A_2 receptor stimulation through adenylate cyclase and guanylate cyclase, and nitric oxide (NO) is also involved in the cardiovascular response of the receptors (Kang et al., 2003).

There is accumulating evidence suggesting that the posterior hypothalamus plays an important role in the central cardiovascular regulation and that many substances can also

* Corresponding author. Tel.: +82 2 2220 0653; fax: +82 2 2292 6686.
E-mail address: hckoh@hanyang.ac.kr (H.C. Koh).

1566-0702/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.autneu.2007.02.004

affect the cardiovascular regulatory response in the posterior hypothalamus. These major substances included GABA (Singewald et al., 1993), histamine (Finch and Hicks, 1977; Iwase et al., 1995), acetylcholine (Buccafusco, 1996; Hagiwara and Kubo, 2005), glutamate (Singewald et al., 1995; Spencer et al., 1990) and NO (Gerova et al., 1995; Hashiguchi et al., 1997). Also, an adenosine is an important substance in the central nervous system. In a previous study, we suggest that adenosine modulates the central cardiovascular regulation in the posterior hypothalamus (Kang et al., 2003; Song et al., 2002).

All of the adenosine receptors are G protein coupled. The A_1 and A_3 receptors coupled to $G_{i/o}$ proteins that inhibit adenylate cyclase, whereas the A_{2A} and A_{2B} receptors stimulate adenylyl cyclase via G_s proteins (Londos et al., 1980; van Calker et al., 1979). The adenosine receptors mediate many other effects than changes in cyclic AMP (Fredholm et al., 2000). However, the signal transduction mechanism of adenosine A_{2B} receptors is controversial. The main intracellular pathway allocated downstream of adenosine A_{2B} receptors is the cAMP/PKA cascade. Stimulation of adenosine A_{2B} receptors will lead to activation of adenylyl cyclase and the production of cAMP, causing activation of the cAMP-dependent protein kinase which in turn may phosphorylate an array of different proteins (Mayr and Montminy, 2001; Sitaraman et al., 2001). On the other hand, the adenosine A_{2B} receptors have been shown to be involved in the vasodilation via NO and cGMP in the peripheral cardiovascular system (Olanrewaju and Mustafa, 2000).

Many experimental evidence suggested that adenosine A_2 receptors are involved in the central cardiovascular regulation. However, the effects of adenosine A_{2B} receptors on central cardiovascular regulation were not reported. This study was performed to investigate the influence of adenosine A_{2B} receptor stimulation on the posterior hypothalamic cardiovascular regulation, and to define whether its mechanism is mediated by adenylate cyclase or guanylate cyclase.

2. Methods

2.1. Subjects

Experimental procedures were carried out following protocols approved by the ethical review committee of the School of Medicine, University of Hanyang. Male Sprague–Dawley rats weighing 250–300 g were used in the present experiment. Animals were housed individually in plastic cages in a temperature-controlled room (25 °C) in the animal care unit of the Department of Pharmacology of the School of Medicine under a 12:12 h light–dark cycle. Animals had free access to water and standard laboratory chow, except during the experimental period. The experimental animals were categorized into eight groups. The three groups consisted of the dose response groups of 5′-*N*-Ethylcarboxamidoadenosine (NECA; 1, 4 and 8 nmol), an adenosine A_{2B} receptor agonist. The other groups were individually treated with alloxazine (5 nmol), an adenosine A_{2B} receptor antagonist, MDL-12,330 (10 nmol), an adenylate cyclase inhibitor and LY-83,583 (5 nmol), a soluble guanylate cylcase inhibitor respectively, 10 min before injection of 4 nmol of NECA. The last of these groups was injected with alloxazine (5 nmol) 10 min before injection of 2 nmol of 5′-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA), an adenosine A_2 receptor agonist. The volumes used for microinjection of each compound were based on the solubility of each substance and were limited to the smallest possible amount and were injected over a time period of 60 s. Also, the doses of the drugs were followed according to previous reports (Kang et al., 2003; Song et al., 2002).

2.2. Drug microinjections in the posterior hypothalamus

The administration of drugs was performed in urethane-anesthetized (1.15 g/kg, i.p.), D-tubocurarine-paralyzed (0.5 mg/kg, i.m.) and artificially ventilated (Ugo Basile, Italy) male Sprague–Dawley rats. Rectal temperature was maintained at 37±0.5 °C with a heating pad, and the rats were placed in a stereotaxic instrument (Stoelting, USA) in the prone position. Microinjection into the right posterior hypothalamus was performed at the following coordinates: 4.3 mm posteriorly, 0.4 mm laterally and 8.3 mm ventrally from the bregma point (Paxinos and Watson, 1986). Administration of drugs was made using the injection cannula (30G) through a guide cannula. All drugs were delivered into the posterior hypothalamus in a volume of 5 μl (at a rate of 5 μl/min) with a Hamilton syringe mounted on a micrometer.

2.3. Measurement of arterial blood pressure and heart rate

Catheters (PE-50) were filled with heparinized saline (200 U/ml) and inserted into the left femoral artery to record blood pressure (BP) and heart rate (HR). For the measurement of cardiovascular responses, BP and HR were continuously monitored via femoral arterial catheter connected to a pressure transducer (Spectramed, Quincy, MA, USA) and a polygraph (Grass, Quincy, MA, USA). The amplifier for measurement of BP and HR was the polygraph DC driver (Grass). For BP a low-level DC preamplifier (Grass) and for HR a EKG tachograph preamplifier (Grass) were used. Mean arterial pressure (MAP) was calculated as diastolic pressure + 1/3 (systolic pressure − diastolic pressure).

2.4. Drugs

NECA, alloxazine, MDL-12,330 LY-83,583 and CPCA were purchased from Sigma (USA). MDL-12,330 and CPCA were dissolved in 0.9% NaCl solution, and NECA, alloxazine and LY-83,583 were dissolved in 0.5% ethanol