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Modification of the cardiovascular response of posterior hypothalamic adenosine A_{2A} receptor stimulation by adenylate cyclase and KATP channel blockade in anesthetized rats

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ABSTRACT

In this experiment, we examined the influence of the posterior hypothalamic adenosine A_{2A} receptors on the 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 CGS-21680HCl (CGS; 20 nmol), an adenosine A_{2A} receptor agonist, elicited a decrease of arterial BP and HR, while injection of 8-(3-Chlorostyryl)caffeine (CSC; 10 nmol), an adenosine A_{2A} receptor antagonist, blocked the depressor and bradycardiac effects of CGS (20 nmol). To examine the mechanisms of cardiovascular regulation of adenosine A_{2A} receptors in the posterior hypothalamus, we applied the adenylate cyclase and guanylate cyclase inhibitors, to the posterior hypothalamus. Pretreatment with MDL-12,330 (MDL; 10 nmol), an adenylate cylase inhibitor, attenuated the depressor and bradycardiac effects of CGS. However, pretreatment with, LY-83,583 (LY; 5 nmol), a soluble guanylate cyclase inhibitor, did not alter the effects of CGS. Additionally, we examined the modification of the cardiovascular effects of adenosine A_{2A} receptors through the ATP-sensitive K^+ channel in the posterior hypothalamus. Posterior hypothalamic administration of glipizide (20 nmol) significantly attenuated the cardiovascular depressor actions elicited by CGS. These results suggest that adenosine A_{2A} receptors in the posterior hypothalamus play an inhibitory role in central cardiovascular regulation, and that adenylate cyclase, but not guanylate cyclase, mediates the depressor and bradycardiac actions of adenosine A_{2A} receptors. Furthermore, ATP-sensitive K^+ channels mediate the posterior hypothalamic cardiovascular regulation of adenosine A_{2A} receptors.

1. Introduction

Adenosine is a potent modulator of cardiovascular function. Its effects are depressant and involve both central and peripheral mechanisms as well as a variety of adenosine receptor subtypes, particularly 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 since the activation of adenosine A_2 receptors causes a depressor and bradycardiac response in the central nervous system. Additionally, the action of adenosine A_{2A} receptors at both central and peripheral levels may play a role in the cardiovascular effects of systemically administered adenosine agonist (Schindler et al., 2005). The administration of adenosine A_{2A} receptor agonists into nucleus tractus solitarius (NTS; Kitchen et al., 2000) results in the depression of blood pressure (BP) and a decrease in heart rate (HR).

There is accumulating evidence suggesting that the posterior hypothalamus plays an important role in the central cardiovascular regulation and that many substances can affect the cardiovascular regulatory response in the posterior hypothalamus. These major substances include 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). In addition to these, adenosine is an important substance in the central nervous system, and in a previous study we suggested that adenosine modulates the central cardiovascular regulation in the posterior hypothalamus (Kang et al., 2003; Song et al., 2002).

All adenosine receptors are G protein coupled. The A_1 and A_3 receptors are 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 in addition to changes in cyclic AMP (Fredholm et al., 2000). However, the mechanism of cardiovascular regulation through adenosine A_{2A} receptors is controversial. The main intracellular pathway allocated downstream of adenosine A_{2A} receptors is the cAMP/PKA cascade. Stimulation of adenosine A_{2A} receptors will lead to the 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 protein (Carroll et al., 2006; Duart-Araujo et al., 2004).

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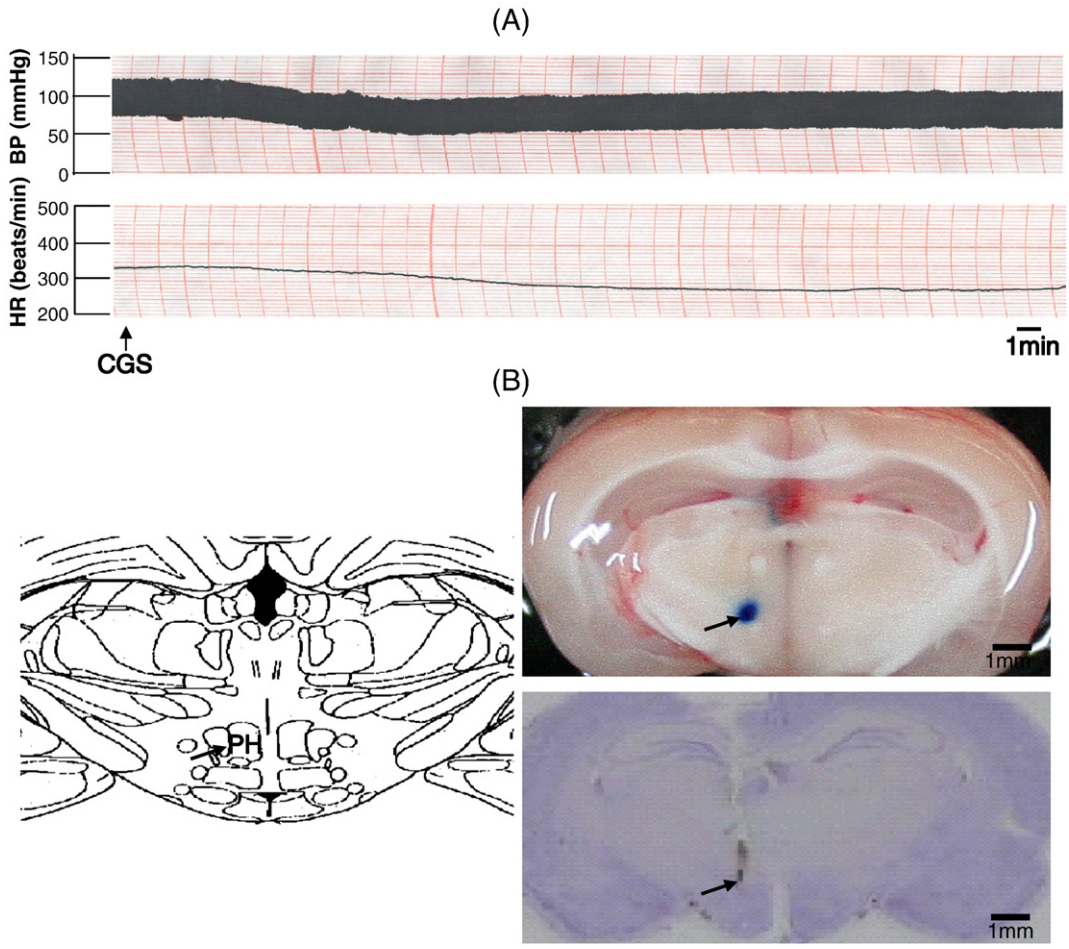

Fig. 1. Decreases of blood pressure (BP) and heart rate (HR) induced by posterior hypothalamic injection of CGS-21680HCl (CGS). (A) Representative tracings showing the changes of BP and HR following microinjection of CGS (20 nmol). (B) Diagram illustrating the location of injection sites of drugs for PH. Injection site corresponds with coordinate from Paxinos and Watson (1986). PH, posterior hypothalamus.

Some authors reported that cardiovascular actions of adenosine A_2 receptors have a relation with ATP-sensitive K^+ channels (Tang et al., 1999; Yoneyama et al., 1992). In addition, we previously reported the relationship between central cardiovascular regulation of adenosine A_2 receptors and the opening of the ATP-sensitive K^+ channels (Kang et al., 2003).

Many lines of experimental evidence suggest that adenosine A_2 receptors are involved in central cardiovascular regulation. Additionally, we reported that adenosine A_{2B} receptors play an inhibitory role in posterior hypothalamic cardiovascular regulation (Kang and Koh, 2007). However, the effects of adenosine A_{2A} receptors on hypothalamic cardiovascular regulation have not been reported. The purpose of this study was to investigate the influence of adenosine A_{2A} receptor stimulation on posterior hypothalamic cardiovascular regulation, and to define whether the mechanism is mediated by adenylate cyclase or guanylate cyclase. In addition, we examined the relationship between the central cardiovascular regulation of adenosine A_{2A} receptors and the opening of the ATP-sensitive K^+ channel.

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 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. Three groups consisted of dose response groups of CGS-21680HCl (CGS; 5, 10 and 20 nmol), an adenosine A_{2A} receptor agonist. Four other groups were individually treated with 8-(3-Chlorostyryl) caffeine (CSC; 10 nmol), MDL-12,330 (MDL; 10 nmol), LY-83,583 (LY; 5 nmol), or glipizide (20 nmol), an adenosine A_{2A} receptor antagonist, adenylate cyclase inhibitor, soluble guanylate cylcase inhibitor, and an ATP-sensitive K^+ channel blocker, respectively, 10 min before injection of 20 nmol of CGS. The last group was injected with 500 nl CGS (20 nmol) for the exclusion of volume effect. The last group was subdivided into four subgroups according to injection site. The volumes used for the microinjection of each compound were based on the solubility of each substance, were limited to the smallest possible amount, and were injected over a time period of 60 s.

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. We checked the respiratory or heart rate, and after animals were stable for the rate, skull exposed. A small hole was drilled for the stereotaxic implantation of a cannula.