Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1038 (2005) 118–121

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Short communication

Effects of CGS-12066A on medial vestibular nuclear neurons

Han-Seong Jeong, Myung-Joo Jang, Jong-Seong Park*

Department of Physiology, Chonnam National University Medical School, Gwangju 501-190, South Korea

Accepted 9 January 2005

Abstract

This study aims to explore the effects of a selective 5-HT_{1B} receptor agonist, CGS-12066A, on the neuronal excitability of the rat medial vestibular nuclear neurons. The spontaneous firing rate was decreased, and the membrane potential was hyperpolarized by CGS-12066A. The whole potassium currents were inhibited by CGS-12066A. After the calcium-dependent potassium, currents were blocked, however, CGS-12066A did not inhibit the potassium currents, suggesting that the 5-HT action site is calcium-dependent potassium currents.

Theme: Motor systems and sensorimotor integration
Topic: Vestibular system

Keywords: 5-hydroxytryptamine; Medial vestibular nucleus; Action potential; Potassium current

The vestibular nuclei in the brainstem process the information from the peripheral vestibular organs such as semicircular canals and otolith organs, cerebellum, contralateral vestibular nuclei and other CNS structures [15]. Known as one of the major factors controlling the neuronal excitability of vestibular nuclei, the dorsal raphe nucleus in the medulla is the main source of 5-hydroxytryptamine (5-HT) in CNS [9].

Immunohistochemical studies have revealed the profound 5-hydroxytryptaminergic terminations and 5-HT receptors in the vestibular nuclear complex [13]. There are several lines of evidences demonstrating that 5-HT influences the excitability of the vestibular nuclei. Licata et al. reported that the microiontophoretic ejection of 5-HT in the superior and medial vestibular nuclei modifies the mean firing rate of the neurons in vivo [10,11]. The bath applied 5-HT also influences the background firing rate of medial and lateral vestibular nuclei in the brainstem slice preparation [4].

We had previously reported that the 5-HT_2 receptors of the rat medial vestibular nucleus mediate the excitatory effects by inhibiting calcium-dependent potassium currents [3]. However, there has been no report on the effects of the 5-HT_{1B} receptor and the cellular mechanisms following 5-HT_{1B} receptor activation in vestibular nuclei. This study is designed to clarify the effects of 5-HT_{1B} receptor activation on medial vestibular nuclear neurons by whole-cell configuration patch-clamp technique using CGS-12066A as a selective 5-HT_{1B} receptor agonist.

Institutional Committee of Laboratory Animal Care and Use approved the experimental protocol. Coronal slices of the brainstem of Sprague–Dawley rats aged 14 to 17 days were prepared as described previously for rats [6]. Briefly, the animals were anesthetized with ether and decapitated. The brainstem was rapidly removed into ice-cold artificial cerebrospinal fluid. The coronal slices (400-μm-thick) of the brainstem were made with a sliding microtome (Vibroslice; WPI, Sarasota FL, USA). These slices were incubated in artificial cerebrospinal fluid well saturated with 95% O_2/5% CO_2 at room temperature for 1 h. The slices were treated with pronase (0.2 mg/ml) for 40–60 min and subsequently exposed to thermolysin (0.2 mg/ml) for 10 min at 32 °C. After the enzyme digestion, a portion of medial vestibular nuclear neurons was removed by micropunching and gently agitated. The dissociated neurons were transferred into a

* Corresponding author. Fax: +82 62 232 1242.
E-mail address: parkjs@chonnam.ac.kr (J.-S. Park).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.01.025

recording chamber mounted on an inverted microscope (IX 70; Olympus, Tokyo, Japan).

The whole-cell membrane potentials were recorded at room temperature by using standard patch-clamp techniques. The patch pipette had a resistance of 3–6 MΩ when filled with a pipette solution. Membrane potentials were measured with an Axopatch 200B voltage-clamp amplifier (Axon instrument, Foster City, CA, USA). Command pulses were applied using an IBM-compatible computer and pCLAMP 7 software (Axon instrument, Foster City, CA, USA). The data were filtered at 5 kHz and displayed on an oscilloscope (Tektronik, Wilsonville, OR, USA), a computer monitor, and a pen recorder (Polygraph; Grass, Quincy, MA, USA).

The artificial cerebrospinal fluid had the following composition (mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$, 1.3, $CaCl_2$, 2.4, D-glucose 10, $NaHCO_3$ 24. The external solution for recordings had the following composition in mM: NaCl 124, KCl 5, $MgSO_4$ 1.3, $NaHCO_3$ 26, $CaCl_2$ 2, NaH_2PO_4 1, glucose 11 (pH 7.4 with KOH). The internal solution (the patch pipette solution) had the following composition in mM: K-gluconate 122.5, KCl 17.5, NaCl 8, HEPES 10, EGTA 0.5, Mg-ATP 4 (pH 7.3 with KOH).

The drugs were made from stock solutions that were made up in distilled water and diluted to the desired concentration in external solution. The drugs were applied to the medial vestibular nuclear cells by switching the perfusion inlet tube to the bath chamber. They were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Data of the same drug tested in this study were obtained in the single neuron with sequential administration of different concentrations. The average firing rate and membrane potential were calculated in recordings over 10 min. The resting membrane potential was measured at the lowest point of the rising phase of the spike. The afterhyperpolarization amplitude of the action potential was measured as the difference of membrane potential between the spike threshold and the minimum post-falling phase of the spike. All values are expressed as mean ± SEM. The one-way ANOVA test (Bonferroni post hoc comparison) was used to analyze the differences between groups, with $P < 0.05$ being considered significant.

The whole-cell patch-clamp recordings under the current-clamp mode were performed on the medial vestibular nuclear neurons to investigate the direct effects of the 5-HT_{1B} receptor agonist on the spontaneous activity of the medial vestibular nuclear neuron. When the command current was fixed to 0 nA, the neurons revealed spontaneous firing action potentials with a frequency of 7.07 ± 0.20 spikes/s. All of the 18 cells responding to CGS-12066A showed inhibitory responses under current-clamp mode. The spontaneous action potential of the medial vestibular nuclear neurons was not affected by 10 μM CGS-12066A. The spike frequency was decreased to 5.90 ± 0.21 ($P < 0.05$) and 5.58 ± 0.20 ($P < 0.05$) by 20 and 40 μM CGS-12066A from the control level of 7.07 ± 0.20 spikes/s, respectively. The resting membrane potential of the cells showing inhibitory response was decreased to −53.50 ± 0.62 ($P < 0.05$) and −54.45 ± 0.64 ($P < 0.05$) from −51.23 ± 0.71 mV by 20 and 40 μM CGS-12066A, respectively. The depth of afterhyperpolarization was increased to 11.90 ± 0.54 and 11.01 ± 0.49 from 10.12 ±

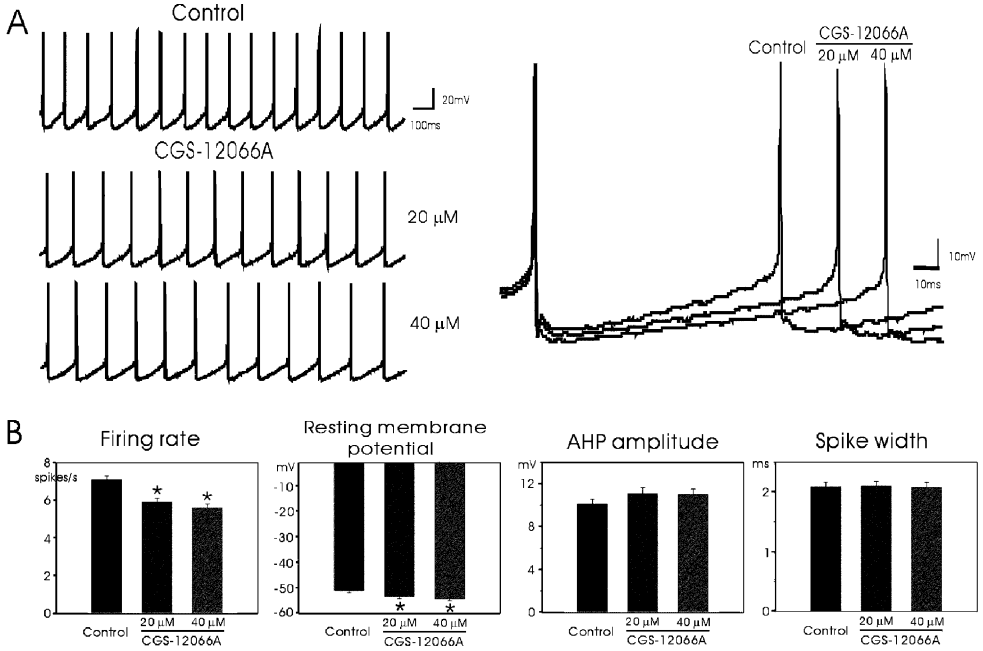

Fig. 1. Effects of CGS-12066A, a specific 5-HT_{1B} receptor agonist, on the spontaneous activity of medial vestibular nucleus neurons. (A) Changes in the shape of the action potential by 20 and 40 μM CGS-12066A; (B) CGS-12066A effects on the firing rate, resting membrane potential, after hyperpolarization amplitude, and spike width ($n = 18$, *significantly different from control with $P < 0.05$).