Korean J Physiol Pharmacol
Vol 10: 131~135, June, 2006

Effects of Phenylephrine on the Excitability of Medial Vestibular Nuclear Neurons in Rats

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Coeruleo-vestibular pathway which connects locus coeruleus and vestibular nuclei is noradrenergic. This study was designed to elucidate the effects of phenylephrine on the spontaneous activity of acutely isolated medial vestibular nuclear neurons of rat by whole-cell patch-clamp technique. Sprague-Dawley rats, aged 14 to 16 days, were used. After enzymatic digestion, dissociated medial vestibular neurons were transferred to a recording chamber mounted on an inverted microscope, and spontaneous action potentials were recorded by standard patch-clamp techniques. In current-clamp mode, the frequency of spontaneous action potential of medial vestibular nuclear neurons was decreased by phenylephrine (n=15). Phenylephrine increased the amplitude of afterhyperpolarization without changes in the resting membrane potential and spike width. In voltage-clamp mode, the whole potassium currents of the medial vestibular nuclear neurons were increased by phenylephrine (n=12). These experimental results suggest that α-receptor mediates the inhibitory effects on the neuronal activity of the medial vestibular nuclear neuron.

Key Words: Medial vestibular nucleus, Phenylephrine, Action potential, Potassium currents

INTRODUCTION

Locus coeruleus lies in the pons beneath the floor of the fourth ventricle. In the rat central nervous system, each locus coeruleus contains about 1500 cells, which accounts for approximately one-half of all norepinephrine-containing cells in the brain. Locus coeruleus is a major source of noradrenergic innervation to all levels of neuraxis, and involves attention, arousal, circadian rhythms, and changing sensorimotor responsiveness (Moore & Bloom, 1979).

The vestibular nuclear complex receives afferent fibers from the utricle, saccule, semicircular canals and cerebellum. And they send efferent fibers to the extraocular motor nuclei, the cerebellum, and all spinal levels. Medial vestibular nucleus is the largest among the vestibular nuclei, and it sends large numbers of nerve fibers into the medial longitudinal fasciculus to cause corrective movements of the eye and also signals through the medial vestibulospinal tract to cause appropriate movements of the neck and head (Smith & Darlington, 1991).

Earlier studies suggest that coeruleo-vestibular pathway is predominantly noradrenergic (Ross, 1976; Jonsson et al, 1981; Jonsson et al, 1982; Steinbusch & Tilders, 1987; Fritschy & Grzanna, 1989). mRNAs for both α and β-adrenergic receptors are expressed in vestibular nuclear neurons, and high turnover rate of norepinephrine in the vestibular nuclei has been reported (Cransac et al, 1996; Rosin et al, 1996).

Despite many reports on the effects of norepinephrine on the neuronal activity of the vestibular nuclei, the effect of α-receptor agonist in isolated medial vestibular nuclear neurons has not been studied (Yamamoto et al, 1967; Kirsten et al, 1976; Cransac et al, 1996; Podda et al, 2001). In the present study, we performed whole-cell patch-clamp experiments to investigate the effects of phenylephrine, a α-receptor agonist, on the potassium currents and spontaneous electrical activity of the acutely isolated rat medial vestibular nuclear neurons.

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METHODS

Preparation of medial vestibular nuclear neurons

Institutional Committee of Laboratory Animal Care and Use approved the experimental protocol. Coronal slices of the brain stem of Sprague-Dawley rats, aged 14 to 17 days, were prepared as described previously for rats (Kay & Wong, 1986). Briefly, animals were anesthetized with ether and decapitated. The brain stem was rapidly removed into ice-cold artificial cerebrospinal fluid. The coronal slices (400 μm thick) of the brain stem were prepared 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 hour. 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 this enzyme digestion, a portion of medial vestibular nucleus neuron was removed by micropunching and gently agitated. The dissociated neurons were transferred to a recording chamber mounted on an inverted microscope (IX 70, Olympus, Tokyo, Japan).

Whole-cell patch-clamp

Whole-cell membrane currents and spontaneous firing of medial vestibular nuclear neurons were recorded at room temperature by using standard patch-clamp techniques. Patch pipette had a resistance of 3~6 MΩ, when filled with a pipette solution. Membrane currents were measured with an Axopatch 200B voltage-clamp amplifier (Axon instrument, Foster City, CA, USA). Command signals were applied using IBM-compatible computer and pCLAMP 7 software (Axon instrument). 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).

Internal and external solutions

The external solution for recordings had the following composition in mM: NaCl 124, KCl 5, $MgSO_4$ 1.3, $NaHCO_3$ 26, $CaCl_2$ 2.5, 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 0.2, Mg-ATP 4 (pH 7.3 with KOH).

Drugs

Drugs were prepared from stock solutions that were made up in distilled water and diluted to desired concentration in external solution. Drugs were applied to the medial vestibular nuclear cells by switching the perfusion inlet tube to the bath chamber. Phenylephrine used in the experiment was purchased from Sigma Chemical Co (St. Louis, MO, USA).

Statistics

The resting membrane potential was measured at the lowest point of rising phase of the spike. The afterhyperpolarization amplitude of the action potential was measured as the difference of membrane potential between spike threshold and the minimum after falling phase of the spike.

All values are expressed as mean±S.E.M. Differences between two groups were determined by Student's t test and were considered to be significant when p values were less than 0.05.

RESULTS

Effects of phenylephrine, α-receptor agonist, on th spontaneous firing of medial vestibular nuclear neurons

Medial vestibular nuclear neurons isolated from rat brainstem have round or pyramidal shaped cell bodies. We tested the effects of phenylephrine on 15 neurons with whole cell patch-clamp recordings under current-clamp mode. When command current was fixed at 0 nA, the neurons revealed spontaneous firing action potentials. Inhibitory responses to phenylephrine were seen in all of MVN neurons (Fig. 1).

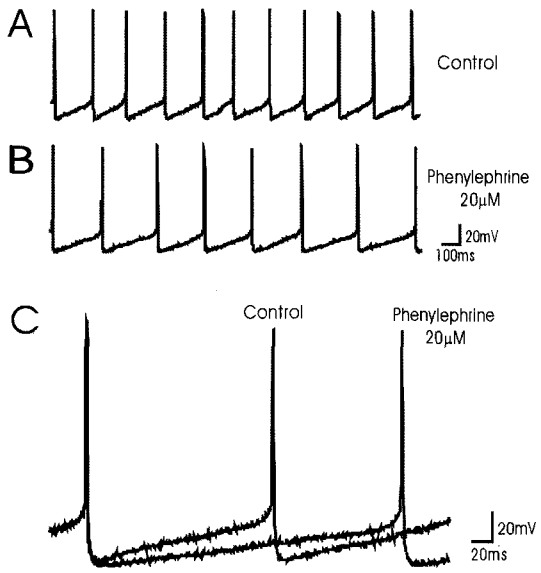

Fig. 1. Inhibitory effects of phenylephrine on the spontaneous activity of medial vestibular nucleus neurons. (A) Control, (B) effects of 20 μM phenylephrine, (C) comparison of effects of phenylephrine on the shape of action potentials.

Effects of phenylephrine on the spike frequency of medial vestibular nuclear neurons: The spike frequency was decreased from 8.00±0.73 spikes/sec to 5.86±0.56 spikes/sec ($p<0.05$) by 20 μM phenylephrine (Fig. 2A).

Effects of phenylephrine on the resting membrane potential of medial vestibular nuclear neurons: The resting membrane potential was changed from −51.05±1.48 mV to −52.29±1.57 mV by 20 μM phenylephrine, which was not significant (Fig. 2B).

Effects of phenylephrine on the afterhyperpolarizaion of medial vestibular nuclear neurons: The depth of afterhyperpolarization was increased from 9.15±0.79 mV to 10.68±0.97 mV ($p<0.05$) by 20 μM phenylephrine (Fig. 2C).

Effects of phenylephrine on the spike width of medial vestibular nuclear neurons: The spike width was changed from 3.32±0.13 msec to 3.38±0.17 msec by 20 μM phenylephrine, which has not significant (Fig. 2D).

Effects of phenylephrine on the whole potassium currents of medial vestibular nuclear neurons

To investigate whether phenylephrine affects the potassium currents in medial vestibular nuclear neurons, the effects of phenylephrine were tested over a broad range of membrane potentials. Whole-cell patch clamp recordings under voltage-clamp mode were performed on 12 medial vestibular nuclear neurons. The potassium currents were activated by 400 ms test pulses from −60 to +40 mV in 10 mV increments from a holding potential of −70 mV. Phenylephrine at 20 μM concentration applied to the bath increased the potassium currents. The mean peak current of the medial vestibular nuclear neurons was 2,787±152 pA in control cells, and it was increased to 3,345±148 pA by treatment with 20 μM phenylephrine (n=12) (Fig. 3).

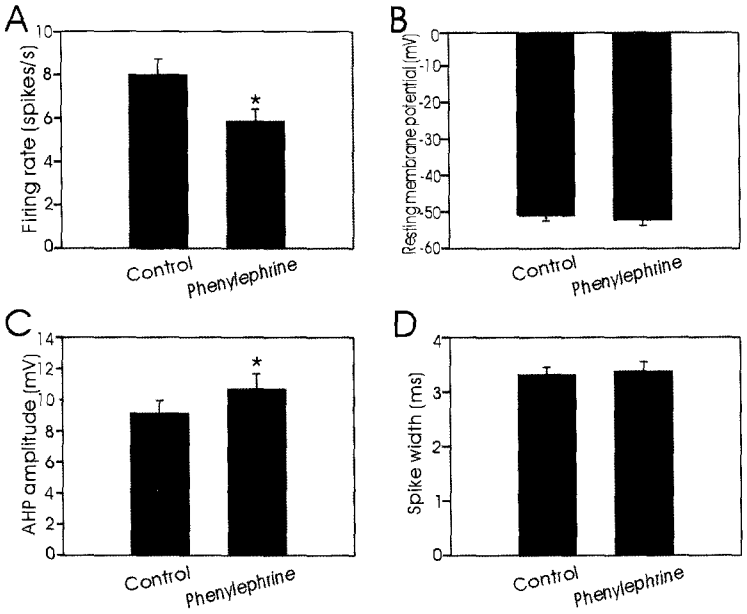

Fig. 2. Effects of phenylephrine (20 μM) on the firing rate (A), resting membrane potential (B), amplitude of afterhyperpolarization (C), and spike width (D) (*significantly different from control with $p<0.05$, n=15).

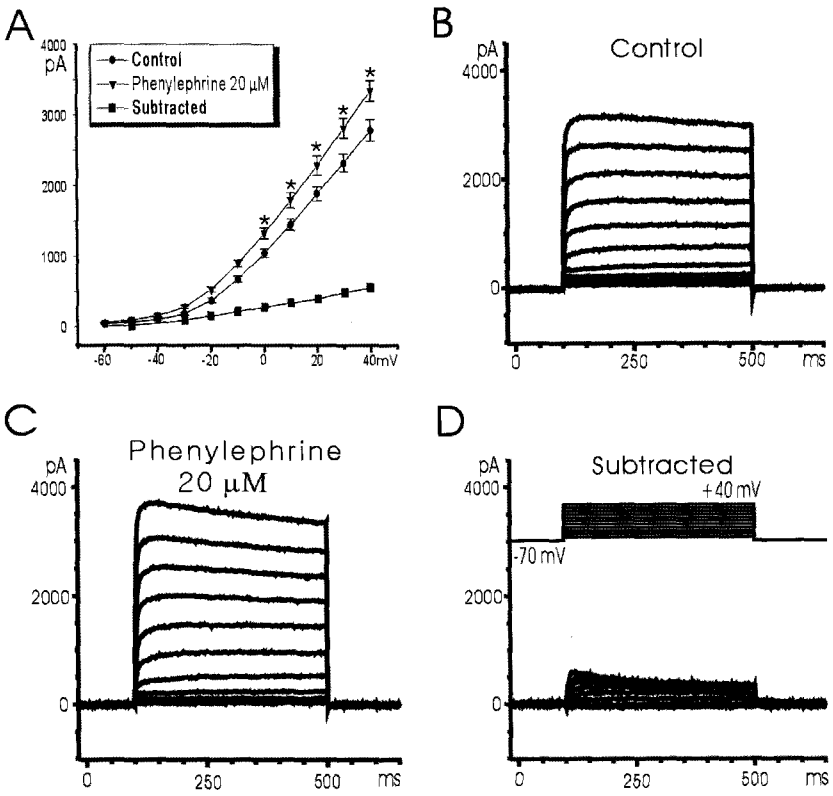

Fig. 3. Effects of phenylephrine on the whole potassium currents of medial vestibular nuclear neurons. (A) Current-voltage relationships for the potassium currents show that outward current was increased by 20 μM phenylephrine (*significantly different from control with $p<0.05$, n=12). (B) Control currents, (C) increasing effects of 20 μM phenylephrine, (D) Digitally subtracted currents represent the components sensitive to phenylephrine.

DISCUSSION

Vestibular nuclei of the brainstem process diverse informations coming from the peripheral vestibular organs, cerebellum, proprioceptors, brainstem reticular nuclei, contralateral vestibular nuclei and locus coeruleus. They elicits vestibular reflexes such as vestibulo-ocular, vestibulocollic, and vestibulo-limb reflexes by sending secondary axons to the extraocular nuclei and skeletal muscle motor nuclei of spinal cord (Lin & Carpenter, 1993).

There are several lines of evidence that locus coeruleus affects vestibulo-spinal reflexes. Locus coeruleus facilitates vestibulo-spinal reflexes through coeruleo-spinal inputs by increasing the sensitivity of alpha motoneurons to descending lateral vestibulo-spinal tract inputs (Tanabe et al, 1990; Ono & Fukuda, 1995). Projections from locus coeruleus to the cerebellum alter the sensitivity of vestibulospinal reflexes (Pompeiano, 1994; Pompeiano et al, 1994), and projections from locus coeruleus to reticular formation alter the limb muscle responses to vestibular stimulation via action at β-receptors on pontine reticulospinal tract cells (Pompeiano, 1989; Pompeiano et al, 1990). Locus coeruleus also increases the sensitivity of vestibulo-spinal reflexes through direct action on the vestibular nuclei (Schuerger & Balaban, 1993).

The coeruleo-vestibular pathway, connecting directly the locus coeruleus and vestibular nuclei, seems to be involved in modulation of the excitability of vestibular nuclear neurons and, hence, affects central processing of vestibular information. The lateral bundle of coeruleo-vestibular pathway projects from locus coeruleus to superior vestibular and rostral lateral vestibular nuclei. And the medial bundle of the pathway projects from locus coeruleus to medial vestibular nucleus, lateral vestibular nucleus, group y and rostral nucleus prepositus hypoglossi (Ross, 1976; Steinbusch & Tilders, 1987).

Neuropharmacologic data indicate that exogenously applied norepinephrine and its agonists influence the firing rate of the vestibular nuclear neurons via both α and β-adrenergic receptors. Norepinephrine increases the firing rate (86%) of vestibular nuclear complex of anesthetized rats and it increases firing rate of lateral vestibular nucleus of midcollicular rats (Yamamoto et al, 1967; Kirsten et al, 1976; Cransac et al, 1996). Besides *in vivo* studies, bath applied norepinephrine alters the firing rate of medial vestibular nuclear neurons in brainstem slice studies. Podda et al (2001) reported that norepinephrine excites the majority (87% of norepinephrine-responsive cells) of medial vestibular nuclear neurons and inhibits the remaining cells. In the present study, we performed a whole-cell patch clamp to explore α-receptor-mediated effects and action mechanism on acutely isolated rat medial vestibular nuclear neurons without neuronal connections to the other structures in the CNS. Phenylephrine decreased the firing rate and increased the afterhyperpolarization amplitude without changes in the resting membrane potential and spike width. Under voltage-clamp mode, it increases the whole potassium currents of the medial vestibular nuclear neurons (Peusner et al, 1998).

Peusner et al. (1998) reported that medial vestibular nuclear neurons posess 3 types of potassium current; A type, delayed rectifier potassium and calcium-dependent potassium currents. It is well known that the afterhyperpolarizaion is caused by calcium-dependent potassium currents. The effect of phenylephrine on the firing rate in the present study may be due to increase of calcium- dependent potassium currents. Recently, Jeong et al. (2003) reported that α-methyl-5-hydroxytryptamine, 5-HT_{1A} receptor agonist, decreased the amplitude of afterhyperpolarization and increased the neuronal excitability of medial vestibular nuclear neurons by inhibition of calcium- dependent potassium currents, which is contrary to the effects of phenylephrine observed in the present study.

The coeruleo-vestibular pathway has been reported to be involved in vestibular compensation. The concentration of norepinephrine and its metabolite, MHPG in bilateral medial vestibular nuclei was increased after unilateral destruction of peripheral vestibular organ (Pompeiano et al. 1994). The experimental results in the study suggest that α-receptor of medial vestibular nuclear neurons could participate in the process of vestibular compensation by modulating the neuronal excitability.

ACKNOWLEDGEMENT

This study was financially supported by research fund of Chonnam National University in 2005.

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