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Effects of a δ-opioid Agonist on the Brainstem Vestibular Nuclear Neuronal Activity of Rats

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This study was undertaken to investigate the effects of [D-Ala^2, D-Leu^5]-enkephalin (DADLE) on the spontaneous activity of medial vestibular nuclear neurons of the rat. Sprague-Dawley rats, aged 14 to 16 days, were anesthetized with ether and decapitated. After enzymatic digestion, the brain stem portion of medial vestibular nuclear neuron was obtained by micropunching. The dissociated neurons were transferred to a recording chamber mounted on an inverted microscope, and spontaneous action potentials were recorded by standard patch-clamp techniques. The spontaneous action potentials were increased by DADLE in 12 cells and decreased in 3 cells. The spike frequency and resting membrane potential of these cells were increased by DADLE. The depth of afterhyperpolarization was not affected by DADLE. The potassium currents were decreased in 20 cells and increased in 5 cells. These results suggest that DADLE increases the neuronal activity of the medial vestibular nuclear neurons by altering resting membrane potential.

Key Words: Medial vestibular nuclear neurons, Action potential, [D-Ala^2, D-Leu^5]-enkephalin (DADLE)

INTRODUCTION

The vestibular nuclear complex in the brainstem consists of four major nuclei, including lateral, superior, medial, and inferior vestibular nuclei, which situated below the floor of the fourth ventricle. These nuclei receive 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 (MVN) is the largest among the vestibular nuclei and sends a large number of nerve fibers into the medial longitudinal fasciculus to induce corrective movements of the eye. In addition, MVN also sends signals through the medial vestibulospinal tract to cause appropriate movements of the neck and head (Smith et al, 1991).

It is well known that opioid has an analgesic effect. Besides analgesia, opioid has other CNS effects, including depression of respiratory center, constriction of pupils and emesis (Brody et al, 1994). It has been reported that the MVN contains not only a number of neurons expressing the mRNA and peptide of enkephalin, but also its opiate receptors, especially δ and μ receptors (Mansour et al, 1994; de Waele et al, 1995; Zanni et al, 1995).

Using extracellular recordings, Lin and Carpenter (1994) observed that the majority of MVN neurons were excited by ionophoretically applied μ and δ-opioid recepor agonists, such as morphine and [D-Ala^2]-leucine-enkephalin. This observation contrasts with the results of Sulaiman and Dutia (1998), who performed extracellular and whole-cell patch clamp intracellular recordings on MVN in vitro, and observed that δ-opioid receptor agonists, [D-Ala^2, Dleu^5]-enkephalin (DADLE) and [D-Pen^2, Pen^5]-enkephalin, inhibited MVN neurons, but μ, and κ-opioid receptor agonists did not affect the tonic discharge rate of neurons.

The present study was designed to clarify the difference between the experimental works of Lin and Carpenter (1994) and Sulaiman and Duita (1998), and to further-explore the effects and action mechanisms of δ-opioid receptor agonist, DADLE, on the spontaneous action potential of rat MVN neurons by whole cell patch-clamp recordings.

METHODS

Preparation of medial vestibular nuclear cells

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 (Kay & Wong, 1986). 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 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, the portion of MVN neuron was removed by micropunching and gently

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ABBREVIATIONS: MVN, medial vestibular nucleus.

agitated. The dissociated neurons were transferred into 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 MVN neurons were recorded at room temperature by using standard patch-clamp techniques (Hamill et al, 1981). 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_2PO4 1, and 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, and Mg-ATP 4 (pH 7.3 with KOH).

Drugs

Drugs from Sigma Chemical Co (St. Louis, MO, USA) were dissolved in distilled water as stock solutions and diluted to desired concentrations in external solution. They were applied to the MVN cells by switching the perfusion inlet tube to the bath chamber.

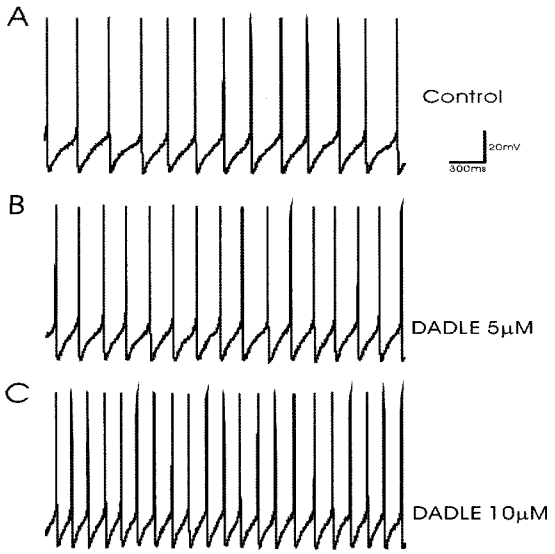

Fig. 1. Spontaneous action potentials of the medial vestibular nuclear neuron. Control (A), effects of 5 μM (B), and 10 μM [D-Ala^2, D-Leu^5]-enkephalin (DADLE), a δ-opioid receptor agonist (C).

Statistics

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

RESULTS

Effects of δ-opioid receptor agonist DADLE on spontaneous firing rate of medial vestibular nuclear neurons

MVN neurons isolated from rat brainstem have round or pyramidal shaped cell bodies. We tested the effects of DADLE on 15 neurons with whole cell patch-clamp recordings under current-clamp mode. When the command current was fixed to 0 nA, the neurons revealed spontaneous firing action potentials. Excitatory responses to DADLE were seen in majority of MVN neurons (n=12) (Figs. 1 and 2).

Effects of DADLE on resting membrane potential of MVN neurons

The resting membrane potential was increased from −40.02±0.68 mV to −39.01±0.73 mV ($p<0.01$) and −37.98±0.78 mV ($p<0.01$) by 5 μM and 10 μM DADLE, respectively (Fig. 3A).

Effects of DADLE on the spike width of MVN neurons

The spike width was decreased from 2.75±0.24 msec to 2.66±0.23 msec and 2.59±0.23 msec ($p<0.05$) by 5 μM and 10 μM DADLE (Fig. 3B).

Effects of DADLE on the afterhyperpolarization of MVN neurons

The depth of afterhyperpolarization was changed from 10.37±1.51 mV to 10.28±1.54 mV, and 9.09±1.45 mV by 5 μM and 10 μM DADLE, respectively (Fig. 3C).

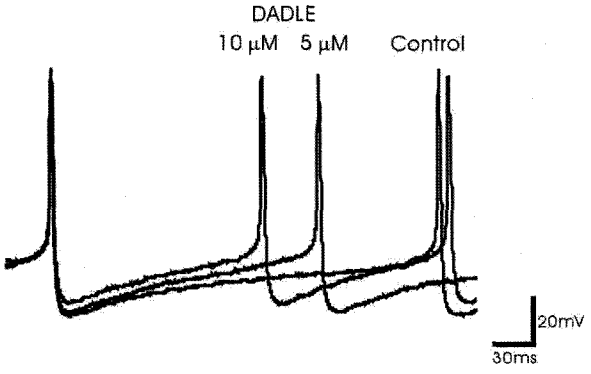

Fig. 2. Effects of different concentrations of [D-Ala^2, D-Leu^5]-enkephalin (DADLE) on the shape of medial vestibular nuclear neuron action potential.

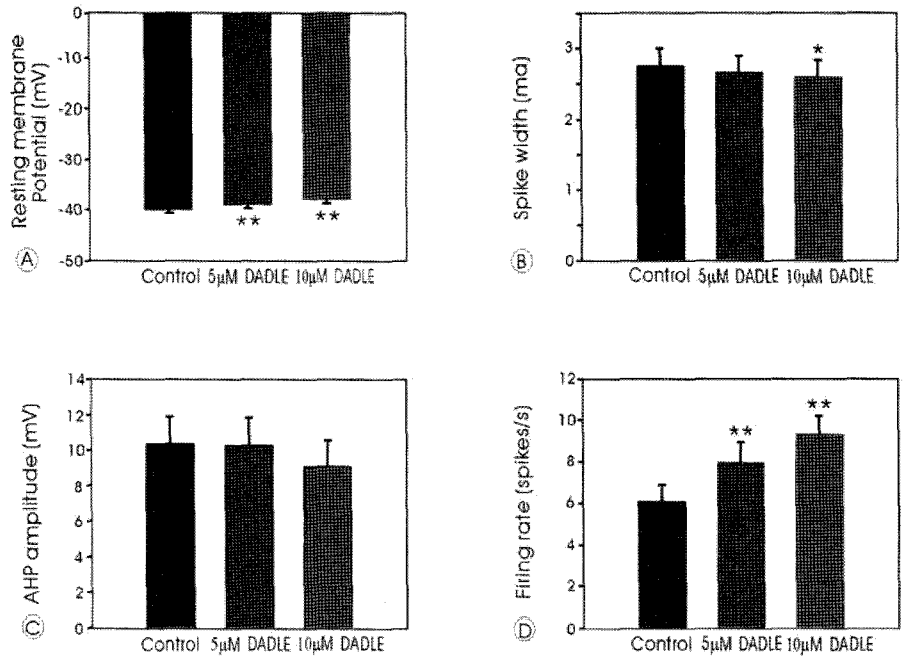

Fig. 3. Effects of δ-opioid receptor agonist, [D-Ala^2, D-Leu^5]- enkephalin (DADLE), on the resting membrane potential (A), spike width (B), depth of afterhyperpolarization (C), and firing rate (D). *Significantly different from control with $p<0.05$. **Significantly different from control with $p<0.01$.

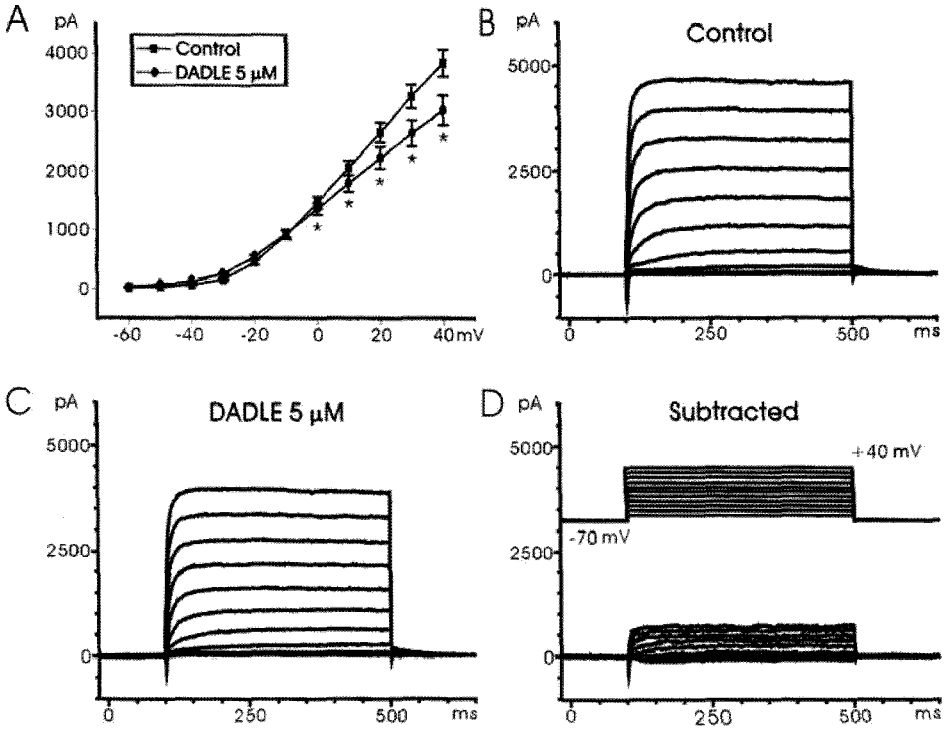

Fig. 4. Decreased effects of δ-opioid receptor agonist, [D-Ala^2, D-Leu^5]-enkephalin (DADLE) on potassium currents of medial vestibular nuclear neurons. (A) Current-voltage relationship, (B) control currents, (C) effects of 5 M DADLE, (D) 5 M DADLE- induced currents subtracted from control currents. *Significantly different from control with $p<0.05$.

Effects of DADLE on the spike frequency of MVN neurons

The spike frequency was increased from 6.12±0.74 spikes/sec to 7.96±0.98 spikes/sec ($p<0.01$) and 9.33±0.86 spikes/sec ($p<0.01$) by 5 μM and 10 μM DADLE (Fig. 3D).

Effects of δ-opioid receptor agonist DADLE on the whole potassium currents of medial vestibular nuclear neurons

We also performed voltage-clamp mode patch-clamp recordings to investigate the change of outward potassium currents. 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. The threshold for activation of the whole potassium currents was −30 mV. The potassium currents were decreased in 20 (80%) of 25 cells and increased in 5 (20%) of 25 cells.

The mean peak currents of the MVN neurons was 3825± 231 pA in control cells (n=20), whereas the peak current of the potassium currents of the vestibular nuclear neurons treated with 5 μM DADLE was decreased to 3015±257 pA ($p<0.05$) (Fig. 4).

DISCUSSION

The MVN in the brainstem processes various informations from many different sources, and glutamate is the major neurotransmitter controling the neuronal activity of the MVN. Besides glutamate, norepinephrine from locus coreuleus, GABA from cerebellum and opposite vestibular nuclei and 5-hydroxytryptamine from dorsal raphe nucleus are involved in sensory processing of the MVN (Zennou-Azogui et al, 1993; Yamanaka et al, 1995).

In the present study, we observed that selective δ-opioid receptor agonist, DADLE increased spontaneous action potential of majority of MVN neurons. These results are in agreement with the findings of Lin and Carpenter (1994), who demonstrated excitation of MVN neurons by ionophoretic application of δ-opioid receptor agonist [D-Ala2]-leucine-enkephalin using extracellular recordings.

In the present study, potassium currents of majority of MVN neurons were inhibited, and the resting membrane potential was increased by DADLE, implying that potassium currents of MVN underlying the resting membrane potential were affected by DADLE. Three types of potassium channel have been identified in MVN; type A potassium channel, delayed rectifier potassium channel and calcium-activated potassium channel (Serafin et al, 1991; Johnston et al, 1994). Although not tested, DADLE seems to depolarize MVN neurons by closing type A or delayed rectifier potassium currents rather than calcium-activated potassium currents, because calcium-activated potassium currents were shown to underly the afterhyperpolarization (Peusner et al, 1998).

Potassium currents of MVN neurons are modulated by many different neurotransmitters involved in the neuronal processing of MVN. Jeong et al (2003) reported that 5-α-hydroxytryptamine, a 5-HT_2 receptor agonist, increases spontaneous firing rate of MVN neurons by inhibiting calcium-dependent potassium currents, Park and Jeong (2000) reported that nitric oxide inhibits potassium currents of rat MVN neurons by increasing intracellular cGMP, and Kim et al (2004) demonstrated that nitric oxide liberating agents and L-arginine decrease the depth of afterhyperpolarization and increase the neuronal excitability of neurons.

Saika et al (1993) reported that preproenkephalin mRNA was increased in the MVN on the lesion side after unilateral labyrinthectomy, suggesting involvement of opioids in vestibular compensation. Based on the experimental results presented herein, DADLE appears to be a factor which facilitates the recovery of MVN neuron's activity after unilateral vestibular lesion by modulation of potassium currents.

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