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Short Communication

Nitric Oxide Modulation of the Spontaneous Firing of Rat Medial Vestibular Nuclear Neurons

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Abstract. Modulation of the spontaneous activity of rat medial vestibular nuclear neurons by nitric oxide was investigated using the whole-cell patch-clamp technique. The spike frequency was increased by sodium nitroprusside (SNP), a nitric oxide liberating agent, and it was also increased by another nitric oxide liberating agent, sodium-nitroso-*N*-acetylpenicillamine. L-Arginine, the substrate of nitric oxide synthase, increased the firing of the neurons. The increased SNP-induced firing was inhibited by 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinozalin-1-one (ODQ), a specific inhibitor of guanylate cyclase. These results suggest that nitric oxide increases the neuronal excitability of the neurons by a cGMP-dependent mechanism.

Keywords: nitric oxide, medial vestibular nucleus, action potential

Nitric oxide synthesized from L-arginine by nitric oxide synthase functions as a neurotransmitter in the central nervous system through the activation of guanylate cyclase, cycloxygenase, and protein kinase C (1). It mediates actions of glutamate and is involved in lasting changes of neuronal activity such as long-term suppression and long-term potentiation (2). Evidences suggest that nitric oxide is also involved in neuronal development, apoptosis, memory, control of cerebral blood flow, and hyperalgesia (3).

The medial vestibular nucleus (MVN) is the largest one among the vestibular nuclei and known to play important roles not only in normal vestibular information processing but also in vestibular compensation (4). Previous studies have shown that nitric oxide has effects on potassium currents that regulate cellular excitability, especially in neuronal cells. Park and Jeong reported that nitric oxide inhibits potassium currents of the rat MVN neuron by increasing intracellular cGMP (5). However, little is known about direct effects of nitric oxide on the neuronal excitability of the vestibular nuclear neurons. In the present study, we performed a whole-cell patch-clamp under the current clamp mode to investigate the direct action of nitric oxide on the spontaneous activity of acutely isolated rat MVN 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 (6). Briefly, the 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 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 this enzyme digestion, the portion of MVN neuron was removed by micropunching and gently agitated. The dissociated neurons were transferred into a recording chamber mounted on an inverted microscope (IX 70; Olympus, Tokyo).

Whole-cell membrane potentials were recorded at

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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 and 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 moniter, and a pen recorder (Polygraph; Grass, Quincy, MA, USA).

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

Drugs were made from stock solutions that were made up in distilled water and diluted to the desired concentration in external solution. Drugs were applied to the MVN cells by switching the perfusion inlet tube to the bath chamber. Drugs were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Data of the same drug tested in the study was obtained in the single neuron with sequential administration of different concentrations. 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 the *t*-test with Bonferroni correction and were considered to be significant when *P* values are less than 0.05.

Sodium nitroprusside (SNP) releases nitric oxide in physiological solutions and is used as a nitric oxide donor in studies to assess the actions of nitric oxide. Whole-cell patch-clamp recordings under the current clamp mode were performed on the MVN neurons to investigate direct effects of SNP on spontaneous activity of the MVN neuron. All MVN neurons responding to SNP showed excitatory responses (n = 18). The spike

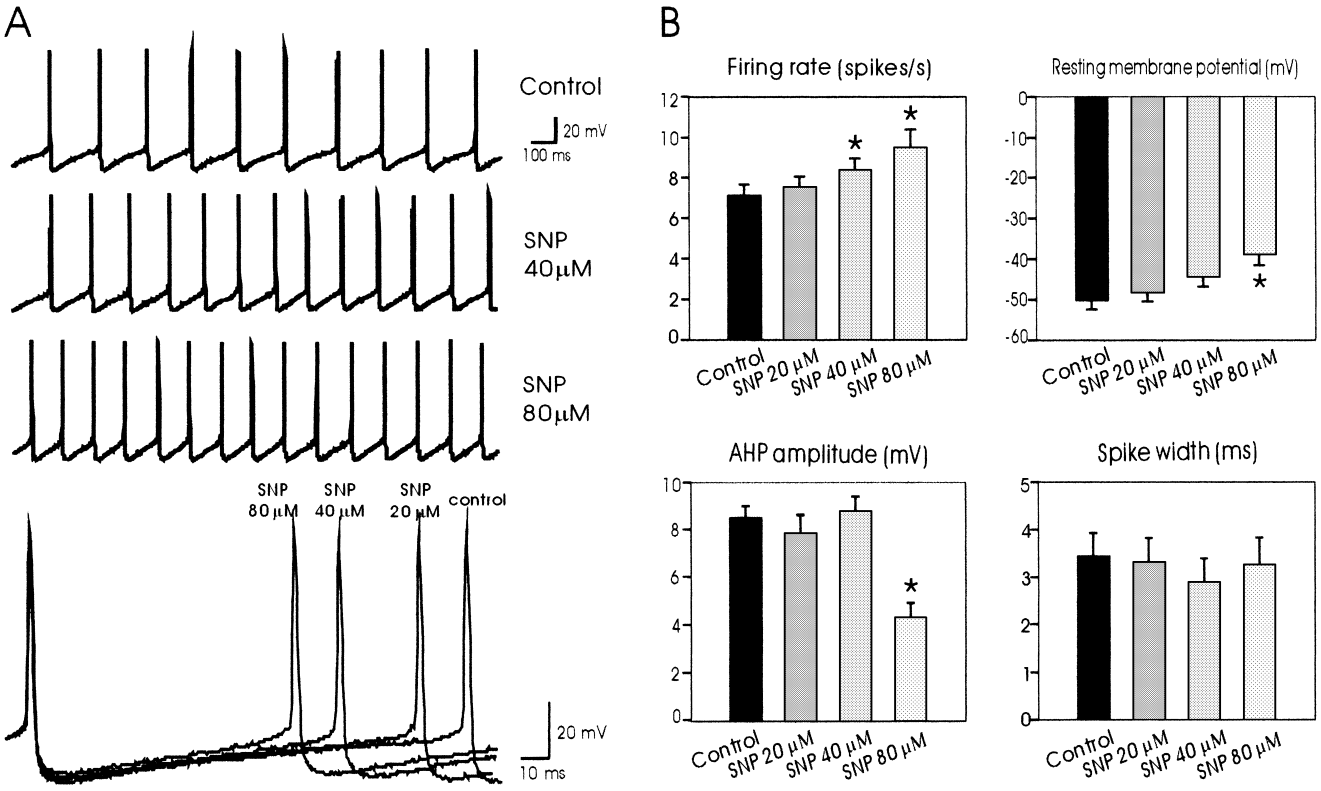

Fig. 1. Effects of SNP on spontaneous firing action potential of the medial vestibular nuclear neuron. A: Changes of the shape of the action potential by 40 and 80 μM SNP. B: SNP effects on the firing rate, resting membrane potential, afterhyperpolarization amplitude, and spike width (n = 18, *significantly different from the control with $P<0.05$).

frequency was increased to 7.54 ± 0.48, 8.38 ± 0.53 ($P<0.05$), and 9.54 ± 0.84 spikes/s ($P<0.05$) by 20, 40, and 80 μM SNP from the control level of 7.13 ± 0.49 spikes/s, respectively. The resting membrane potential of cells was increased to -48.36 ± 1.93, -44.53 ± 2.18, -38.86 ± 2.64 mV ($P<0.05$) from -50.36 ± 1.94 mV by 20, 40, and 80 μM SNP, respectively. The depth of afterhyperpolarization was decreased to 7.88 ± 0.75, 8.82 ± 0.56, and 4.35 ± 0.56 mV ($P<0.05$) from 8.55 ± 0.45 mV by 20, 40, and 80 μM SNP, respectively. The spike width of cells was not affected (Fig. 1). The effect of SNP on MVN neurons was abolished by perfusion with bath solution without SNP.

To confirm the effect of SNP on the spontaneous firing of the MVN neuron, effects of SNAP (sodium-nitroso-*N*-acetylpenicillamine), another nitric oxide liberating agent were tested. All MVN neurons responding to SNAP showed excitatory responses in 18 cells. The spike frequency was increased from 7.99 ± 0.60 spikes/s to 8.62 ± 0.72 and 10.00 ± 0.85 spikes/s ($P<0.05$) by 10 and 20 μM SNAP, respectively. The resting membrane potential was increased significantly from -52.27 ± 1.29 mV to -51.95 ± 1.28 and -49.40 ± 1.58 mV ($P<0.05$) by 10 and 20 μM SNAP, respectively. The depth of afterhyperpolarization was decreased from 5.42 ± 0.46 mV to 4.82 ± 0.34 and 4.41 ± 0.39 mV ($P<0.05$) by 10 and 20 μM SNAP, respectively. The spike width of cells was not affected (data not shown). The effect of SNAP on the MVN neurons was reversible.

Effects of L-arginine, the substrate of nitric oxide synthase on the firing of the MVN neuron were tested. The spontaneous firing of the MVN neurons increased in 12 cells and was not changed in 3 cells by L-arginine. The spike frequency was increased from 7.22 ± 0.62 spikes/s to 7.51 ± 0.41, 11.86 ± 0.84 ($P<0.05$), and 12.75 ± 1.16 spikes/s ($P<0.05$) by 1, 10, and 100 μM L-arginine, respectively. The resting membrane potential was changed from -52.20 ± 0.55 mV to -51.75 ± 0.58, -52.70 ± 0.97, and -52.96 ± 1.09 mV by 1, 10, and 100 μM L-arginine, respectively. The depth of afterhyperpolarization was decreased from 12.94 ± 1.25 mV to 12.40 ± 1.25, 9.35 ± 0.86 ($P<0.05$), and 7.77 ± 0.94 mV ($P<0.05$) by 1, 10, and 100 μM L-arginine, respectively. The spike width of cells was not affected by L-arginine (Fig. 2).

In order to determine whether SNP increases the spontaneous firing directly or through other second

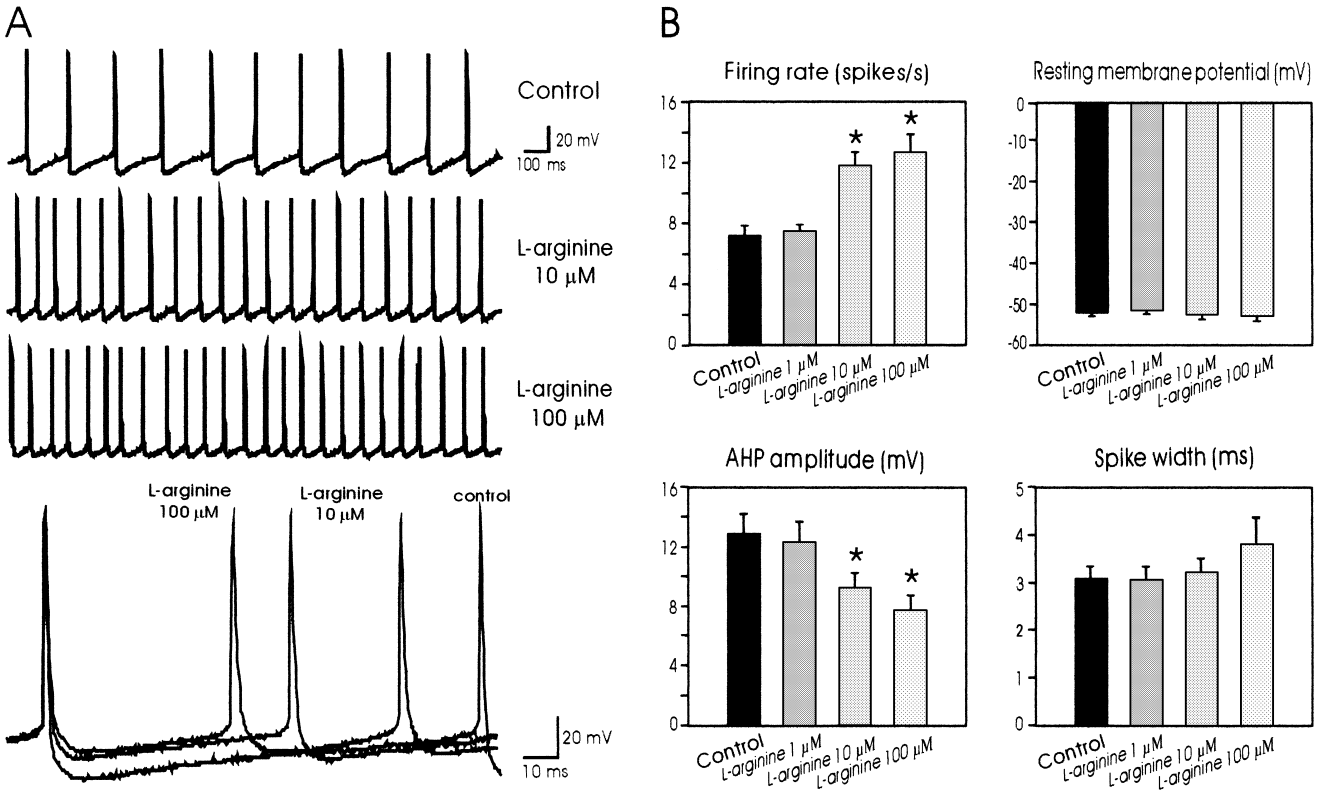

Fig. 2. Effects of L-arginine on spontaneous firing action potential of the medial vestibular nuclear neuron. A: Changes of the shape of the action potential by 10 and 100 μM L-arginine. B: L-Arginine effects on the firing rate, resting membrane potential, afterhyperpolarization amplitude, and spike width (n = 12, *significantly different from the control with $P<0.05$).

messengers, we studied the effects of ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinozalin-1-one), a specific inhibitor of soluble guanylate cyclase, on the SNP-induced firings. The change of spontaneous firing was recorded with cotreatment of 20 μM SNP and 4 μM ODQ. SNP increased the spontaneous firing rate, but combined application of ODQ and SNP abolished the effects of SNP on the action potential firing (Fig. 3). The spike frequency of the medial vestibular nuclear neurons (n = 6) increased from 8.22 ± 0.65 spikes/s to 10.25 ± 0.58 spikes/s ($P<0.05$) by 20 μM SNP, and it was decreased to 8.23 ± 0.66 spikes/s ($P<0.05$ *vs* SNP alone) by the combined application.

In the present study, we monitored the effects of nitric oxide on the spontaneous action potential of the MVN neurons and studied whether its effects are direct or second messenger-mediated. SNP, a nitric oxide liberating agent increased the firing frequency of the cell and another nitric oxide liberating agent, SNAP also increased the frequency. Because the effect of nitric oxide on the spontaneous firing of the MVN cells was reversed by ODQ, it is likely that the effects of nitric oxide on the neuron is associated with a cGMP-dependent mechanism (7). Some studies addressed nitric oxide modulation of neuronal excitability and synaptic plasticity. SNP increases the firing frequency and decreases the action potential latency of ganglial neurons of the snail and nitric oxide increases excitability of the rat supraoptic nuclear neurons via cGMP-dependent mechanisms (8, 9).

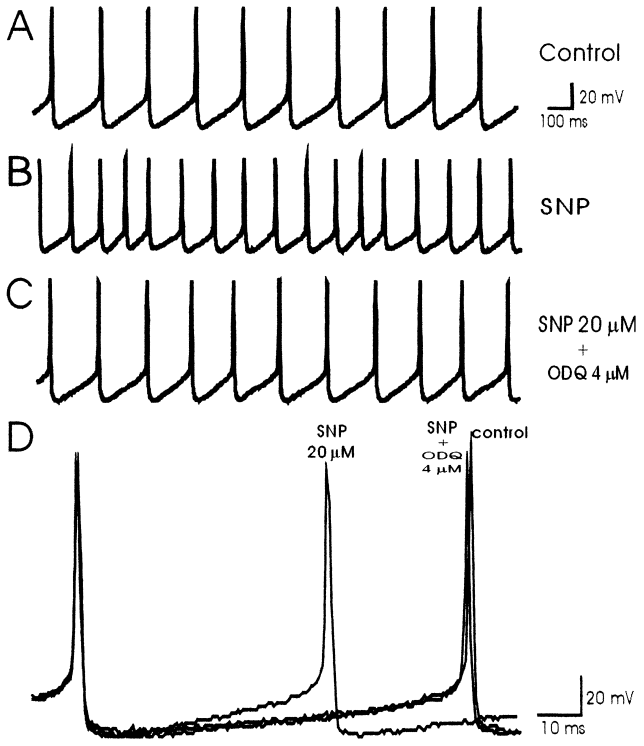

Fig. 3. Effects of ODQ on SNP-induced firing. A: Normal firing; B: 20 μM SNP; C: Cotreatment of 20 μM SNP and 4 μM ODQ, a soluble guanylyl cyclase inhibitor.

The presence of nitric oxide synthase in the vestibular system was reported by other authors (10, 11). It is known to be present in peripheral vestibular neurons surrounding type I hair cells, vestibular ganglionic neurons, and MVN neurons in the brainstem. Park and Jeong (5) reported that potassium currents in the isolated medial vestibular neurons were inhibited by nitric oxide liberating agents and after blockade of calcium-dependent potassium currents by high EGTA solution, SNP did not inhibit the outward potassium currents, suggesting that the target of nitric oxide is calcium-dependent potassium currents. Generally, calcium-dependent potassium currents contribute to the repolarizing phase of the action potential and to control the repetitive discharge of spikes. The calcium-dependent potassium currents of the vestibular nuclear neurons are known to underlie the afterhyperpolarization of action potential (12, 13). In the present study, the depth of afterhyperpolarization was decreased by nitric oxide liberating agents and L-arginine, which coincides with the results of Park and Jeong (5).

Vestibular compensation is a process of partial behavioral recovery that occurs following lesions to the vestibular inner ear (14, 15). During the process of vestibular compensation, rebalancing of the neuronal activity of vestibular nuclei in the brainstem is an essential factor for the functional recovery. Decreased activity of the ipsilateral vestibular nuclei after lesion of vestibular inner ear gradually increases, whereas increased activity of the contralateral vestibular nuclei gradually decreases (14). Nitric oxide, which increases spontaneous firing of the MVN neuron in the present study could be an important factor for increasing the neuronal activity of the ipsilateral vestibular nucleus after unilateral vestibular lesion.

Acknowledgments

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