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Changes in Vestibular Nerve Activity Following Acute Hypotension in Rats

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The basic mechanism for the excitation of the peripheral vestibular receptors following acute hypotension induced by sodium nitroprusside (SNP) or hemorrhage was investigated in anesthetized rats. Electrical activity of the afferent vestibular nerve was measured after pretreatment with kynurenic acid, an NMDA receptor antagonist. The activity of the vestibular nerve at rest following acute hypotension induced by SNP or simulating hemorrhage was a greater increase than in control animals. The gain of the vestibular nerve with sinusoidal rotation following acute hypotension increased significantly compared to control animals. The acute hypotension induced by SNP or hemorrhage did not change the activity of the afferent vestibular nerve after kynurenic acid injection. These results suggest that acute hypotension produced excitation of the vestibular hair cells via glutamate excitotoxicity in response to ischemia.

Key Words: Peripheral vestibular receptor, Hypotension, Kynurenic acid, Electrical activity, Vestibular nerve

INTRODUCTION

The vestibular system is involved in the control of posture and movement via the vestibulo-ocular and vestibulospinal reflexes (Wilson & Melvill Jones, 1979). It also influences sympathetic outflow and blood pressure via the vestibulo-autonomic reflex (Yates, 1992). However, abnormal stimulation of the vestibular system may evoke nausea, vomiting, vertigo, and tachycardia. Electrical stimulation of the vestibular nerve increases sympathetic activity, but loss of vestibular function impairs the ability to compensate for orthostatic hypotension (Doba & Reis, 1974; Yates et al, 1995; Park et al, 1999) and does not produce motion sickness (Kennedy et al, 1968). The neural pathways involved in vestibulo-autonomic interactions include the nucleus tractus solitarius, the dorsal motor nucleus of the vagus nerve, the rostral ventrolateral medulla, and other brain-stem nuclei (Yates et al, 1995; Balaban & Porter, 1998; Biaggioni et al, 1998).

Although many studies have addressed the influence of the peripheral vestibular system on autonomic functions, the effects of changes in autonomic function on vestibular function are poorly understood. Excitation of peripheral vestibular receptors by postural changes produces functional changes in the cardiovascular system, including changes in blood pressure, pulse rate, the baroreceptor reflex, and blood flow to the extremities (Kolev & Tibbling, 1992; Normand et al, 1997; Convertino, 1998). Conversely, patients with idiopathic hypertension, myocardial infarction, arrhythmia, or congestive heart failure complain vertigo, which can be explained by changes in blood flow to the peripheral vestibular system. Recently, we reported the effects of changes in blood pressure on the peripheral vestibular receptors (Park et al, 2001), and described how the peripheral vestibular receptors after acute hypotension might contribute to controlling blood pressure by exciting type I neurons and inhibiting type II neurons in the medial vestibular nuclei. However, the basic mechanism for the excitation of the peripheral vestibular receptors following acute hypotension still remains unclear.

In this study, electrical activity of the afferent vestibular nerve was measured after pretreatment of rats with kynurenic acid, an NMDA receptor antagonist, to investigate the basic mechanism for the excitation of the peripheral vestibular receptors following acute hypotension induced by sodium nitroprusside (SNP) or hemorrhage in rats.

METHODS

Materials

Thirty-seven Sprague-Dawley rats, weighing 250~300 g, were used for examination of the vestibular function by rotatory test to select intact labyrinthine animals. Animals were anesthetized with 300 mg/kg chloral hydrate intra-

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ABBREVIATIONS: SNP, sodium nitroprusside; NMDA, *n-methyl-d-aspartate*.

peritoneally. The procedures used were approved by the Institutional Ethics Committee on the Experimental Use of Animals.

Measurement of blood pressure and blood flow

A polyethylene tube was cannulated into the femoral artery in prone position and the other end of the tube was connected to preamplifier (Gould Co, USA). Blood pressure was analyzed using the Spike 2 program (Cambridge Electronic Design, UK), and mean arterial pressure was calculated as "diastolic pressure+1/3 pulse pressure". Blood flow in the peripheral vestibular system was measured using a laser Doppler (Moor Instruments, UK). A laser probe (MP3 50 mm, Moore Instruments, UK) fixed into a micromanipulator was placed just above the ampullary portion of the posterior semicircular canal, and petroleum jelly was injected into the space between the tip of laser probe and the ampulla to prevent stagnation of fluid or blood. The laser Doppler has a time constant of 0.1 sec and a high cut filter of 15 kHz.

Acute hypotension

SNP (0.5 ml with 5, 10, 15 μg/kg) was infused into the femoral vein. Also, acute hypotension was induced by depletion of 1~2 ml of blood for 30 sec from the femoral artery. The blood was then reperfused into the femoral vein to restore the pressure. To block NMDA receptors, 300 mg/kg kynurenic acid (Sigma Co, USA) was injected into the femoral vein 30 min before experiment.

Electrophysiological recordings

The animals were anesthetized with thiopental sodium (30 mg/kg, ip), secured in a head holder of stereotaxic device (Narishige Co, Japan), and mounted on a servo-controlled rotator, head centered over the axis of rotation with nose 30° down to bring the horizontal semicircular canals close to the horizontal plane of rotation. The body was supported in a horizontal position by a plastic plate hinged to the stereotaxic frame. The rotator was driven by DC servo-motor (400 W, LG Co). Rotatory range of the turntable was 180°, and maximum angular velocity was 220°/sec at 0.2 Hz. Artificial respiration was achieved using a ventilator (Phipps & Bird, U.S.A.) during rotation, and body temperature was maintained by heating pad. Action potentials from single neurons were recorded extracellulary using stainless steel microelectrodes (A & M, USA) with impedence of 5~10 MΩ. The recording site was confirmed by making a lesion with a current of 2~5 mA. Signals were amplified and filtered by signal processing system (SPS-8701, Australia) and displayed on an oscilloscope (Tektronix, 5113), which were analysed by data analysis program (Spike 2, Cambridge Electronic Design, UK). For the measurement of gain of response to horizontal sinusoidal acceleration, the averaged cycle histogram of the response and the stimulus in 5~10 cycles were separately subjected to a non-linear curve fitting routine to compute the least-squares fit of a sinusoid. Gain was computed as peak to peak firing divided by peak to peak velocity, and expressed as impulses/s per deg/s.

Statistical analysis

All data are presented as mean±SD. The statistical significance of differences was assessed using Statview 4.0 (Abacus Concepts, USA). Values of $p<0.05$ were considered significant.

RESULTS

Hypotension induced by SNP or hemorrhage

The mean arterial blood pressure was 97.3±12.6 mmHg. SNP decreased the blood pressure within 2 min of the beginning of the injection, and the hypotension was maintained for 2~3 min thereafter. With injection of 5, 10, 15 μg/kg SNP, the blood pressure decreased to 87.2±9.8, 68.4±8.5, 49.2±11.5 mmHg, respectively. After 1- and 2-ml blood loss for simulating hemorrhage, the blood pressure decreased by 30% and 50%, respectively. Reperfusion of the removed blood restored the blood pressure to control levels. The rate of blood flow in the peripheral vestibular system was proportional to the change in arterial pressure (r=0.99)(Fig. 1).

Effects of acute hypotension on the vestibular nerve activity

The resting activity of the vestibular and cochlear nerves was 75.4±25.3 (n=86) and 42.3±13.6 spikes/sec (n=128), respectively. Cochlear nerve activity was recorded more frequently than vestibular nerve activity during implantation of the microelectrode into the VIII nerve. In the VIII nerve, the activity of the vestibular nerve was increased by ipsilateral sinusoidal rotation to the recorded site and decreased by contralateral sinusoidal rotation. Moreover, the cochlear nerve was activated by auditory stimuli, such as sound of motor during bidirectional sinusoidal rotation

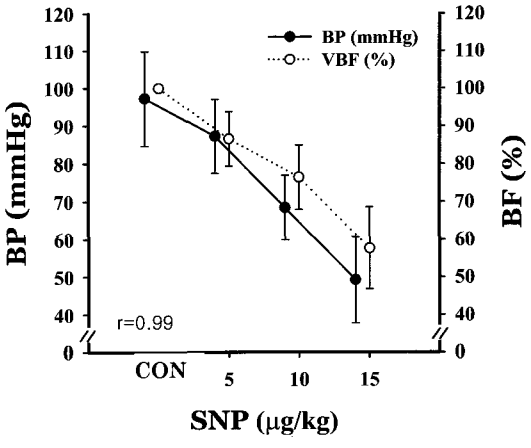

Fig. 1. Relationship between mean arterial blood pressure (BP) and blood flow in the vestibular system (VBF) following intravenous injection of different doses of sodium nitroprusside (SNP). Changes in blood flow of the vestibular system were measured by laser doppler. Sodium nitroprusside of 5, 10, 15 μg/kg doses were injected into the femoral vein. CON, control; r, coefficient of correlation.

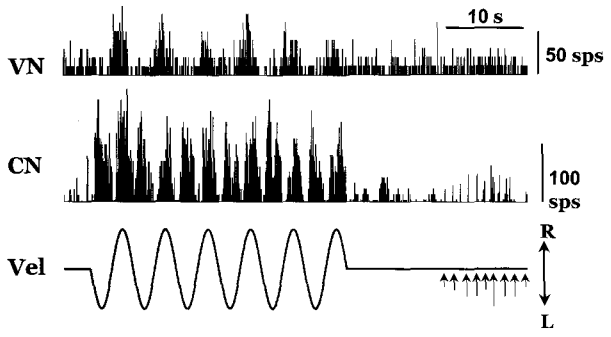

Fig. 2. Activities of the right vestibular and cochlear nerves with sinusoidal rotation of the whole body and auditory stimulation. Activity of the right vestibular nerve (VN) was increased by rotation to the right and decreased by rotation to the left. The right cochlear nerve (CN) was activated by rotation to the right or left, and was also activated by tapping on the stereotaxic frame. Vel, velocity curve of sinusoidal rotation; sps, spike/sec; R, rightward rotation; L, leftward rotation. The small arrows (↑) indicate tapping on the stereotaxic frame, and their size is proportional to the intensity of tapping.

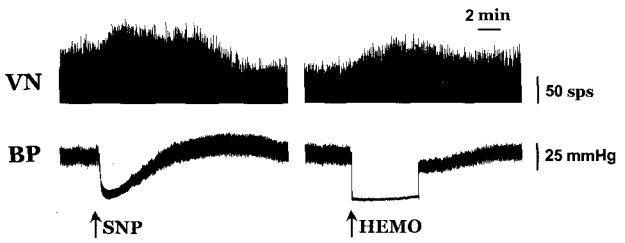

Fig. 3. Activity of the right vestibular nerve following acute hypotension induced by sodium nitroprusside or hemorrhage. VN, right vestibular nerve; BP, blood pressure; SNP, sodium nitroprusside (15 μg/kg); HEMO, hemorrhage of 2 ml.

or tapping on the stereotaxic frame. The cochlear nerve activity was proportional to the strength of auditory stimuli (Fig. 2). The gain in vestibular nerve activity with ipsilateral sinusoidal rotation was 1.40±0.83, and that with contralateral sinusoidal rotation was 0.52±0.50. The activity of the vestibular nerve following acute hypotension induced by SNP increased to 99.7±32.8 spikes/sec (n=56) (Fig. 3)($p<0.01$). In addition, the acute hypotension induced by hemorrhage also increased the activity of the vestibular nerve. The gain of the vestibular nerve with sinusoidal rotation following acute hypotension increased significantly compared to control animals: 1.72±0.83 with rotation ipsilateral to the recorded side and 0.65±0.44 with contralateral rotation.

Effects of kynurenic acid on activity of the vestibular nerve

As shown in Fig. 4, systemic injection of kynurenic acid did not change blood pressure or the vestibular nerve activity. Pretreatment of the animals with kynurenic acid did not affect the magnitude of the SNP-induced hypotension, but prevented the increase in the vestibular nerve activity during the hypotension. As shown in Fig. 5, the blood pressure was significantly decreased from the control

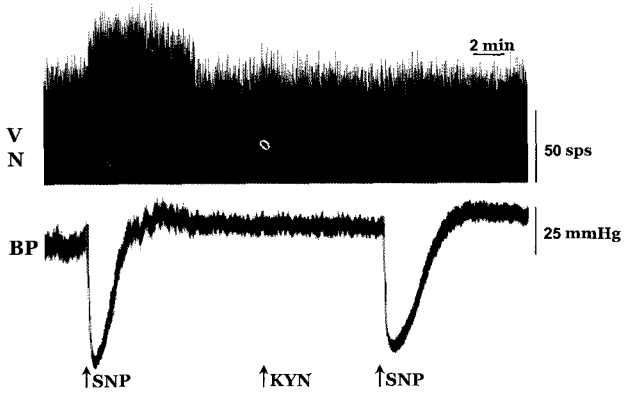

Fig. 4. Effect of kynurenic acid (KYN) on the activity of the right vestibular nerve following acute hypotension induced by sodium nitroprusside (SNP). VN, right vestibular nerve; BP, blood pressure; SNP, injection of sodium nitroprusside (15 μg/kg); KYN, kynurenic acid (300 mg/kg).

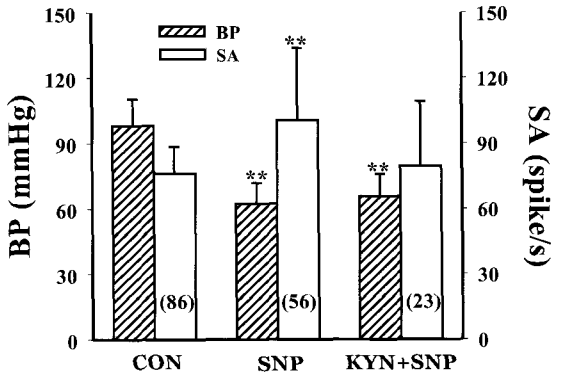

Fig. 5. Effects of pretreatment of animals with kynurenic acid on the activity of the afferent vestibular nerve evoked by acute hypotension. BP, blood pressure; SA, spontaneous activity of the afferent vestibular nerve; CON, control animals; SNP, intravenous injection of sodium nitroprusside; KYN+SNP, injection of sodium nitroprusside after pretreatment of the animals with kynurenic acid. Parentheses represent number of recorded neurons. Values are means±SD. $^{**}p<0.01$, significant difference from the CON.

level by injection of SNP with or without kynurenic acid. In contrast, the increase in vestibular nerve activity during the hypotension was prevented by kynurenic acid. Also the acute hypotension induced by hemorrhage did not change the activity of the vestibular nerve 30 min after kynurenic acid injection (Data not shown).

DISCUSSION

Vertigo is produced not only by abnormal stimulation of the vestibular system, but also by changes in blood pressure (Andrews et al, 1988; Kikuchi et al, 1993). However, the mechanism of vertigo during changes in blood pressure is not clear. Blood flow in the brain is well controlled, even when the flow decreases to less than 50% of control levels (Bunemann et al, 1991; Hamaguchi et al, 1992). SNP

decreases blood pressure by causing the release of nitric oxide (Hamaguchi et al, 1992). Decrease in blood pressure is proportional to the doses of SNP. The rate of blood flow measured by laser Doppler in the peripheral vestibular system was found to be proportional to the change in arterial blood pressure, which corresponded to the rate of blood flow in the cochlear system with changing arterial blood pressure (Preckel et al, 1995; Ueda & Matsunaga, 1995). These results suggest that blood flow in the vestibulo-cochlear system changes when blood pressure decreases by 10~30% of control levels, while the blood flow in the brain stem remains at control levels due to the autonomic regulation.

In recording electrical activity, the vestibular nerve was distinguished from the cochlear nerve in VIII nerve by sinusoidal rotation of the whole body. The vestibular nerve activity at rest in the present study was slightly less than that in cats, and the higher gain by sinusoidal rotation, compared to other studies, might have been caused by low impedance of the microelectrode used (Goldberg & Fernandez, 1971). And the cochlear nerve activity at rest presently observed was similar to that in birds (Manley et al, 1985). The cochlear nerve activity during sinusoidal rotation increased at the peak velocity of rotation due to increased noise made by the rotator, and the activity was proportional to the strength of the sound stimuli. The vestibular nerve activity with sinusoidal rotation was excited by rotation toward the recorded side and inhibited by rotation away from the recorded side, which was the response to stimulation of peripheral vestibular receptors alone. However, the activity of the vestibular nuclei showed both excitation and inhibition, since the vestibular nuclei receive inputs from both peripheral vestibular receptors and the contralateral vestibular nuclei via commissural connections (Wilson & Melvill Jones, 1979). Therefore, recording from the vestibular nerve appears to be the best electrophysiological method for evaluating the function of peripheral vestibular receptors.

We recently reported that the resting activity of type I neurons in the medial vestibular nuclei increased and that of type II neurons decreased, following SNP-induced acute hypotension (Park et al, 2001). In this study, the increased vestibular nerve activity seen after SNP-induced acute hypotension was closely related to our recently published results. However, the activity of the vestibular nuclei following acute hypotension might have been affected by higher centers, since our previous study could not exclude central effects of the cerebellum, cerebrum, or other brain stem nuclei on vestibular nuclei activity following acute hypotension. The increased resting activity and increased gain in the vestibular nerve caused by sinusoidal rotation following acute hypotension induced by SNP or hemorrhage represent the response of peripheral vestibular receptors to acute hypotension.

It is not clear how a decrease in blood flow activates the peripheral vestibular receptors. One possible explanation is that decreased blood flow to the inner ear produces an ischemic environment for the hair cells. It has been shown that an excess of excitatory amino acids leads to the prolonged depolarization of ionic channel-gated postsynaptic receptors, thereby inducing large cation influxes and passive entry of Cl^-. Then, the resulting osmotic imbalance causes massive water entry into the postsynaptic element, leading to acute swelling (Choi & Rothman, 1990). The neurotransmitter glutamate has been implicated in acute excitotoxicity of the synapses between inner hair cells and radial auditory dendrites following cochlear ischemia (Pujol et al, 1993). It is highly likely that excitation of the vestibular nerve results from ischemic activation of hair cells via reduction in blood flow to the inner ear following acute hypotension. Glutamate is a major neurotransmitter in the peripheral vestibular receptors. In this study, pretreatment of animals with kynurenic acid, an NMDA receptor antagonist, blocked the excitation of hair cells following SNP-induced acute hypotension. This implies that excitation of the vestibular nerve following acute hypotension could result from the excitotoxic effect of glutamate on hair cells. Similarly, it has been reported that excitatory amino acid antagonists prevented the noise-trauma induced injury of cochlear auditory neurons (Puel et al, 1994, 1998).

Acute hypotension following SNP injection induces functional changes in hair cells temporarily; however, persisting swelling leads to cell death, possibly due to Ca^{2+} homeostasis defect (Choi & Rothman, 1990). Transient ischemia in the cochlea may cause hearing weakness or tinnitus, and transient ischemia in the vestibular system may also cause vertigo (Pujol et al, 1993; Hakuba et al, 2000). Excitation of peripheral vestibular receptors following acute hypotension may contribute to regulating blood pressure in addition to baroreceptors in the carotid artery and aortic arch. The baroreceptors keep blood pressure within a physiological range, while the peripheral vestibular receptors may control blood pressure via reversible ischemic response to decreased blood flow in the receptors. This suggests that a reversible pathological response caused by ischemia induces physiological effects that regulate blood pressure. This premise is based on the observations that blood flow in the peripheral vestibular receptors corresponds to changes in systemic blood pressure (Preckel et al, 1995; Ueda & Matsunaga, 1995), neural connections between vestibular nuclei and rostral ventrolateral medulla exist (Balaban & Porter, 1998; Biaggioni et al, 1998), and that afferent signals from the peripheral vestibular receptors during postural changes result in compensation in orthostatic hypotension (Doba & Reis, 1974). Moreover, c-Fos protein expression in the vestibular nuclei increases following acute hypotension in animals with intact labyrinths, and the expression increases in the medial vestibular nuclei contralateral to the injured side and decreases in the ipsilateral medial vestibular nuclei following acute hypotension in unilaterally labyrinthectomized animals (Park et al, 2002; Kim et al, 2003). This study indicates that excitation of the vestibular nerve following acute hypotension is caused by the excitotoxic effect of glutamate on hair cells. Moreover, prevention of the excitotoxicity triggered by the ischemic response might inhibit the induction of vertigo following acute hypotension.

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