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RESEARCH NOTE

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Saccular and utricular influences on sympathetic nerve activities in cats

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Abstract We studied the effects of stimulation of the utricular and saccular nerves on sympathetic nerve activity in decerebrated cats. Bipolar electrodes were fixed in place on the utricular and/or saccular nerve under visual observation; the other branches of the vestibular nerve were transected. Baroreceptors and vagus nerves were inactivated bilaterally so that inputs from baroreceptors and other visceral receptors did not influence the sympathetic nerve outflow. Postganglionic sympathetic nerve activity was recorded from the renal branch of the sympathetic nerve, which is known to be more sensitive to vestibular stimuli than other types of sympathetic fibers. With stimulation of either the saccular or utricular nerve at low stimulus intensity, a prominent inhibition followed by a rebound excitation was evoked on spontaneous renal nerve discharges. The latency of the inhibition ranged from 65 to 130 ms, and the duration of inhibitory responses was about 90–150 ms. An increase in stimulus intensity in both the saccular and utricular nerves induced inhibitory effects preceded by short-term excitation. The latency of this excitation, which was superimposed on the initial phase of the inhibitory responses, ranged from 55 to 90 ms.

Key words Vestibulosympathetic reflex · Utricular nerve · Saccular nerve · Renal nerve · Autonomic nervous system

Introduction

The vestibular labyrinth comprising the otolith organs and semicircular canals has long been recognized to play a role in motor control. In addition, it is well established that the vestibular system influences sympathetic nerve activity and blood pressure (Ishikawa and Miyazawa 1980; Tang and Gernandt 1969; Uchino et al. 1970; Yates 1992). It has been reported that stimulation of the whole vestibular nerve evoked a variable response (excitation, inhibition, or a combination of excitation and inhibition) on sympathetic nerve outflow (Kerman and Yates 1998; Uchino et al. 1970). Variable responses may be due to the activation of different populations of vestibular afferents, depending on the placement of silver ball electrodes in the labyrinth. Vestibular afferents that innervate the hair cells in the semicircular canal ampullae and in otolith organs signal head movements in many different directions, and stimulation might produce different patterns of responses on sympathetic outflow. The afferent fibers, even those innervating a single vestibular end organ, have been found to differ in size and branching patterns within the neuroepithelium. These afferent fibers vary in their responses to head movements, activation of efferent fibers, and in the central pathways to which they contribute (Goldberg 1991). Thus, vestibular afferents having different properties may produce different types of responses on sympathetic nerve activity.

It has been reported that natural vestibular stimulation in the vertical plane (near pitch) produces a maximal modulation of splanchnic nerve activity. The properties of the splanchnic nerve responses to head rotation were consistent with an origin from the otolith organs (Yates and Miller 1994). However, the particular types of otolith end organs (i.e., utricular or saccular) which influence the sympathetic nerve outflow are yet to be identified. In the present experiment, we studied the sympathetic nerve responses to selective stimulation of the saccular and utricular nerves. Sympathetic nerve activity was recorded from the renal branches of postganglionic efferents. Previous reports have suggested that these branches are more sensitive to vestibular stimuli than other types of sympathetic fibers (Kerman and Yates 1998).

Some of these results have previously been presented in abstract form (Zakir et al. 1999).

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Materials and methods

Experiments were conducted in 11 adult cats in conformity with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences* (The Physiological Society of Japan, 1988). The animals were initially anesthetized with ketamine hydrochloride (15–20 mg/kg, i.m.) and then with a halothane-nitrous oxide mixture after tracheotomy was performed. The carotid artery and vagus nerves were ligated bilaterally. All animals were decerebrated at the precollicullar level for recording without anesthesia. For recording vestibular field potentials, a small portion of the cerebellum dorsal to the vestibular nucleus was aspirated. All animals were paralyzed by intravenous administration of pancuronium bromide. Bilateral pneumothorax was done to reduce respiration pump-related movements. Body temperature was maintained at around 37°C with a heating pad. Arterial blood pressure was routinely monitored from the femoral artery and, when necessary, 5–10% glucose was administered intravenously to maintain the systolic pressure over 100 mmHg. At the end of each experiment the animal was put to death with an intravenous injection of pentobarbital sodium.

Stimulation procedure

Procedures for selective stimulation of the utricular (UT) (Sasaki et al. 1991) and saccular (SAC) (Uchino et al. 1997) nerves have been described elsewhere. Briefly, a pair of bipolar silver electrodes, diameter 250 μm, insulated except for the tips (150–500 μm), interelectrode distance approximately 1.0 mm, were implanted into selected nerves. To prevent drying of the nerves and stimulus spread, the nerves and tips of the electrodes were covered with a semisolid paraffin-Vaseline mixture. The UT or SAC nerve was stimulated using a cathodal current pulse of width 0.2 ms, a train of five or ten pulses, and an interpulse interval of 4 ms. The stimulus was repeated every 6 or 10 s.

Recording of vestibular field potential

The validity of the selective stimulation was tested by recording the field potentials in the lateral or descending vestibular nuclei following electrical stimulation of the UT and/or SAC nerves with glass microelectrodes filled with 2 M sodium chloride and saturated fast green dye (impedance 1–3 MΩ). In some cases, recording locations were marked by dye ejection (Thomas and Wilson 1965). Dye marks were later recovered histologically.

Recording of sympathetic nerve responses

The kidney was approached through an incision in the right or left flank and the renal nerve branches were identified as fine nerve filaments running along the renal artery and vein and entering the kidney at the renal pelvis. The nerve was dissected and mounted on a bipolar silver hook electrode immersed in a mineral oil pool. Signals were amplified and filtered, and also full-wave rectified and integrated (Nihon Koden EI-601G). Typically, 100–300 response waves were averaged (Nihon Koden QC-111 J). Data were taken several times at various stimulus intensities.

Results

Field potentials in the vestibular nuclei

At the beginning of each recording session we checked the validity of selective stimulation by recording the field potentials in the vestibular nucleus. Stimulation of the UT and/or SAC nerve evoked a small positive (P) wave and a negative (N1) potential (Fig. 1A, inset), which resulted from the arrival of afferent impulses and monosynaptic activation of second-order vestibular neurons, respectively (Precht and Shimazu 1965). The amplitude of the N1 field potentials increased as the stimulus intensity increased, then reached a plateau at a stimulus intensity of about 4–6 × N1 threshold (Fig. 1A), implying that stimulus currents do not spread to other vestibular nerves, as observed previously (Bolton et al. 1992; Uchino et al. 1997). The threshold current for activation of the N1 field potential (N1T) ranged from 10 to 15 μA (13±3 μA, mean ± SD, *n*=6) and from 10 to 25 μA (15±6 μA, *n*=8) for the UT and SAC nerves, respectively.

Effect of sympathetic nerve activities following utricular nerve stimulation

In all experiments, the UT nerve was stimulated with a stimulus strength at or below the plateau level of the stimulus responses curve. Stimulation of the UT nerve evoked a stable predominant inhibition, which was followed by a rebound excitation in sympathetic nerve activity when the stimulus strength was low. Typical examples of inhibition (downward arrow), as shown in Fig. 1B, were evoked by a train of ten shocks. The threshold of elicited inhibitory responses measured several times in a single preparation was found to be low, 25–40 μA (33±10 μA, *n*=12) (2.5±0.6 × N1T). The latency of inhibitory responses, measured at a stimulus strength of around 3 × N1T, ranged from 70 to 130 ms (106±19 ms, *n*=27) (Table 1), and the total duration of the inhibitory period was about 100–150 ms. When stimulus intensity increased, a short-term excitation of sympathetic nerve activity was induced with a latency of

Table 1 Threshold and latencies for eliciting inhibition and excitation of sympathetic nerve activity. Values are means ± SD; n=number of observations. The onsets of latencies and the thresholds for eliciting inhibition and excitation were measured in traces when applying a train of five or ten shocks; the onsets of latencies were measured from the first shock of stimulus train (UT utricules, SAC sacculus)

Nerve	Response	Threshold, μA and N1T	n	Latency (ms)	n
UT	Inhibition	33±10 μA (2.5±0.6)×N1T	12	106±19	27
SAC	Inhibition	33±16 μA (2.5±0.6)×N1T	14	100±18	30
UT	Excitation	39±10 μA (3.2±0.8)×N1T	6	74±13	10
SAC	Excitation	48±14 μA (4.2±1.2)×N1T	7	75±22	12

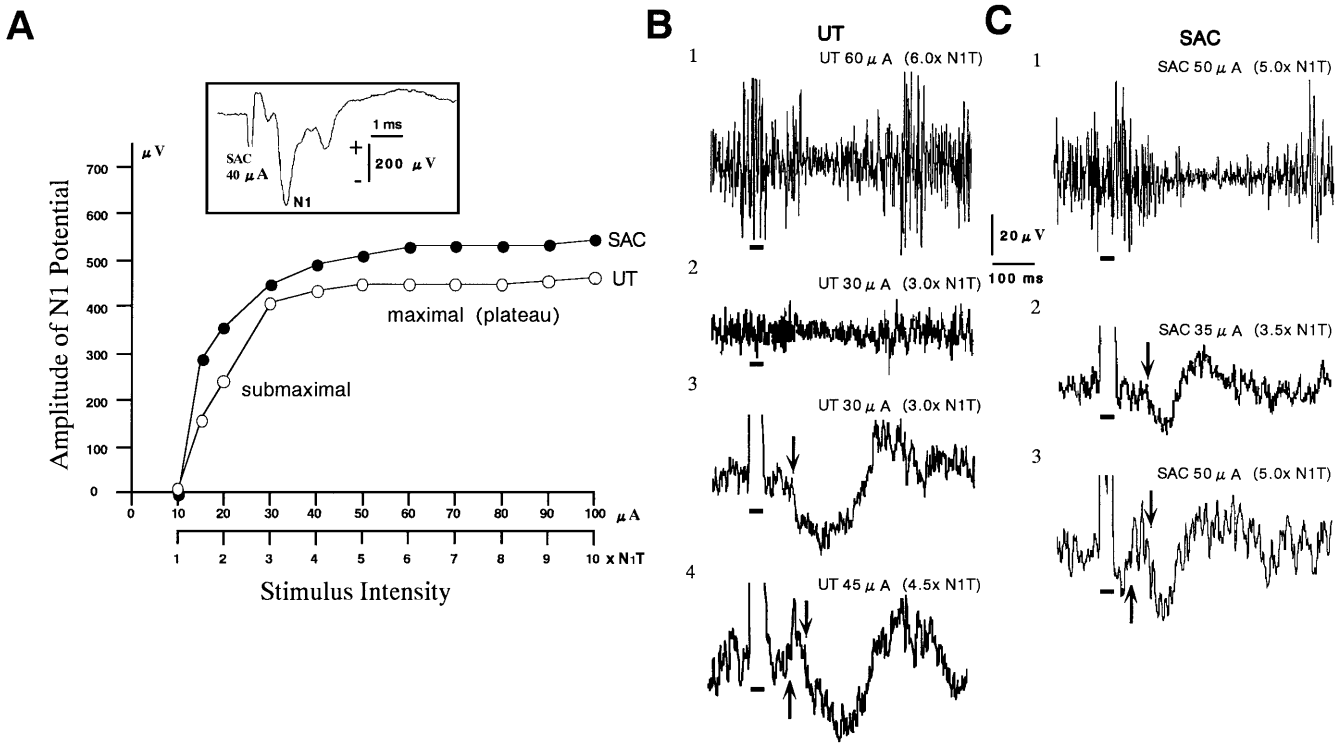

Fig. 1 Effect of utricular (UT) and saccular (SAC) nerve stimulation. **A** Plot of growth of N1 field as stimulus intensity increased for UT and SAC nerves. Inset Typical field potential (averaged) recorded in the vestibular nuclei in response to saccular nerve stimuli. **B** Responses of sympathetic nerves after stimulation of UT nerves. 1 A single sweep of typical response of sympathetic nerve discharge to UT nerve stimulation with a train of ten shocks. Bars indicate the duration of the stimulus train. Response was a short-term excitation followed by prominent decreases in spontaneous discharge of sympathetic nerves. 2 Average of more than 100 sweeps of raw wave sympathetic nerve responses to stimulation of the UT nerve by a train of ten shocks. The response was a decrease in sympathetic nerve discharge. 3 Rectified, integrated wave of sympathetic nerve responses. Downward deflections indicate a decrease in nerve activity (inhibition) and upward deflections indicate an increase (excitation) in nerve activity. The response consisted of a prominent inhibition (downward arrow) followed by a rebound excitation elicited at a low stimulus intensity. 4 When stimulus intensity increased, an early excitation (upward arrow) was elicited followed by a prominent inhibition. **C** Responses of sympathetic nerves after stimulation of the SAC nerve. 1 Single sweep of responses of renal nerve discharge to SAC nerve stimulation evoked by a train of ten shocks. The response was a prolonged decrease in spontaneous sympathetic nerve discharges. 2 Rectified, integrated wave form averaged from more than 100 sweeps. The response consisted of a prominent inhibition followed by a rebound excitation. 3 When stimulus intensity increased, an early excitation was elicited which was followed by a prominent inhibition

about 55–90 ms (74±13 ms, *n*=10) (Table 1). The excitation was superimposed on the initial phase of the inhibitory responses shown in Fig. 1B4 (upward arrow). Latency of facilitation was measured at a stimulus strength of around 5 × N1T. The threshold of elicited excitatory responses was higher than that needed to evoke inhibitory responses: 35–55 μA (39±10 μA, *n*=6) (3.2±0.8 × N1T) (Table 1).

Effect of sympathetic nerve activities following saccular nerve stimulation

Saccular nerve stimulation with a low stimulus strength evoked predominantly stable inhibition followed by excitation in the renal branch of the sympathetic nerve outflow. Figure 1C shows typical examples of inhibition (downward arrow), evoked by a train of ten shocks (1, single sweep, and 2, integrated wave form). The thresholds of elicited inhibitory responses were low, 30–50 μA (33±16 μA, n=14) (2.5±0.6× N1T). The latencies of inhibitory responses ranged from 65 to 130 ms (100±18 ms, n=30) (Table 1), and the total duration of the inhibitory period was about 90–130 ms. These latencies were measured at a stimulus strength of around 3.5 × N1T. As stimulus intensity increased, excitation of sympathetic nerve activity was induced with a latency of about 55–90 ms (75±22 ms, n=12) (Table 1). The facilitation was superimposed on the initial phase of the inhibitory responses, as shown in Fig. 1C3 (upward arrow). The thresholds of elicited excitatory responses were 40–70 μA (48±14 μA, n=7) (4.2±1.2× N1T) (Table 1).

Discussion

The present study revealed that a predominant inhibition in sympathetic nerve activity, followed by a late rebound excitation, was elicited by stimulation of the utricular and saccular nerves at low stimulus strength. A short-term excitation, superimposed on the initial phase of the inhibitory responses, was also evoked with increasing stimulus intensity. The latencies of the inhibitions ranged from 65 to 130 ms and the duration of the inhibitory pe-

riod was about 90–150 ms. The latencies of the short-term excitatory responses were 55–90 ms.

The stimulation technique employed in these experiments was identical to that used previously to study the responses of neurons in the vestibular nuclei (Kushiro et al. 2000; Sato et al. 2000; Zakir et al. 2000), neck motoneurons (Bolton et al. 1992; Kushiro et al. 1999; Uchino et al. 1997) and extraocular motoneurons (Isu et al. 2000; Uchino et al. 1996) to otolith inputs. In the present experiment, the absence of current spread was assessed on the basis of the stimulus intensity-response amplitude relationship (Uchino et al. 1997). In successful preparations, the amplitude of N1 potentials grew rapidly and then leveled to a plateau as stimulus intensity increased to about 4–6 × N1T (Fig. 1A). The growth of the N1 potential amplitude indicates an increase in the number of recruited fibers in the particular nerve stimulated, and the plateau indicates that the stimulus current recruited all available fibers without spreading to other vestibular nerves. In some preparations, additional increases in N1 amplitude were observed as stimuli increased further (not shown), indicating current spread to the central cut end of one or more of the other vestibular nerves. Consequently, the stimuli applied to a particular nerve were always smaller than the intensity that exceeded the plateau (maximal activation of the vestibular nerve branch) in the stimulus response curve of N1 field potentials. Thus, there was no possibility of stimulus-spread to non-target afferents.

Caloric stimulation of the vestibular labyrinth lowers blood pressure, and this effect is abolished by vestibular nuclei lesions (Spiegel 1946). Direct electrical stimulation of the vestibular nerves lowers blood pressure through inhibition of sympathetic nerve activity (Ishikawa and Miyazawa 1980; Tang and Gernandt 1969; Uchino et al. 1970). In the present study, we found that stimulation of the UT or SAC nerves at low stimulus intensity evoked a prominent inhibition, followed by a rebound excitation, of spontaneous renal nerve discharge. Previously, it has been described that rotation around either the vertical or the longitudinal axis produced transient hypotension in cats, dogs, guinea pigs and rabbits (Spiegel 1946; Lindsay et al. 1945). Hypotension responses were not observed in animals with vestibular nerve transection. In contrast, recent studies in cats and humans have shown that only head pitch movements elicit changes in blood pressure and sympathetic nerve activity. Doba and Reis (1974) found that bilateral transection of the vestibular nerves in paralyzed chloralose-anesthetized cats impaired the reflex that compensates for orthostatic hypotension produced by 30° or 60° head upright tilt. This strongly suggests that the vestibular system contributes to sympathetic responses to upright posture. Woodering et al. (1997) used selective stimulation of vestibular receptors during 50° nose-up head pitch in cats. This study confirmed that nose-up movements result in large increases in blood pressure and that transection of the vestibular nerves immediately abolished this response. Yates and Miller (1994) reported a more complex interaction. This was maximal modulation of sympathetic nerve outflow with head rotations in a plane near pitch: nose-up rotation produced increased outflow while nose-down rotation reduced nerve discharges in cats. The properties of the sympathetic nerve responses to head rotation were consistent with an origin from otolith organs. In humans, a static head-down neck flexion, which would mainly activate the otolith organs, elicits a rapid and marked increase in muscle sympathetic nerve activity and in blood pressure (Hume and Ray 1999; Shortt and Ray 1997).

In the present study, we found an excitation, which preceded the inhibition of sympathetic nerve activity from the UT or SAC nerves at a strength of stimulus current somewhat higher than that needed to evoke inhibitory responses from these organs. Actually, inside the UT and SAC macula are numerous hair cells with polarization vectors in all directions. These hair cells bend in accordance with the earth's constant gravitational pull (Wilson and Melvill Jones 1979). At any given head position some hair cells are depolarized; at the same time others are hyperpolarized (Goldberg et al. 1990). In the mammalian vestibular macula, different sizes of afferents (thick, thin and medium) can be recognized (Fernandez et al. 1990; Goldberg 1991). Thick afferent fibers only innervate the hair cells in central (striolar) zones and thin fibers supply the peripheral (extrastriolar) zones. Medium-sized fibers supply the hair cells in all parts of the neuroepithelium (Goldberg 1991). The afferent fibers in the vestibular maculae also differ in branching patterns within the neuroepithelium, which vary in their response to head movements (Goldberg 1991). In our study, as the stimulus intensity increased, hair cells and primary afferents distal to the stimulating electrode were likely activated, resulting in complex effects on sympathetic nerve outflow.

In summary, the present study used selective UT and SAC nerve stimulation at several different intensities. We found that both UT and SAC afferent stimulation elicited changes in sympathetic nerve activity in cats. Weak stimuli to organs evoked a prominent inhibition followed by a rebound excitation of spontaneous renal nerve discharges. Increased stimulus intensities (smaller than the intensity that exceeded the plateau in the stimulus response curve of N1 field potentials) evoked complex sympathetic nerve responses, due to the activation of additional otolith afferents, which possess a wide range of properties.

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