Sympathetic outflow to muscle in humans during short periods of microgravity produced by parabolic flight

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Exposure to microgravity (µG) in space has consistently induced hemodynamic changes in crew members during spaceflight. One of the most serious problems among astronauts after spaceflight is cardiovascular deconditioning. A recent study disclosed that 64% of the crew members examined suffered from orthostatic intolerance after spaceflight, i.e., 9 of 14 astronauts could not complete the 10-min stand test that all of the astronauts could complete before the flight (2).

The pathophysiology of the development of cardiovascular deconditioning has not yet been well clarified. Several studies investigating the underlying mechanisms focused mainly on hemodynamic changes (3, 12, 14–15, 20, 22–25), but no studies have yielded a direct insight into changes in sympathetic nerve function. Therefore, the identification of the changes in sympathetic outflow to antigravity skeletal muscles in µG might clarify the mechanism of cardiovascular deconditioning.

For exposing human subjects to µG in ground-based experiments, parabolic flight is the only maneuver that produces the actual µG, although it is of short duration. Parabolic flight utilizing a jet aircraft yields ~20 s of relative weightlessness of the crew members and equipment inside the aircraft (4, 17, 20, 24). Because weight is defined as the force produced by the acceleration of an object by gravity, then that object can be considered “weightless” when no relative acceleration is present.

Under this type of unusual condition, the stabilization of blood pressure is achieved by changes in the autonomic nervous system, especially changes in sympathetic nerve activity. Sympathetic nerve activity innervating skeletal muscles, i.e., muscle sympathetic nerve activity (MSNA), plays an important role in stabilizing the systemic arterial pressure (18). Because responses of MSNA in arterial baroreflex and cardiopulmonary reflex are reported to be blunted after exposure to actual/simulated µG, the altered MSNA response to orthostasis might be associated with the development of cardiovascular deconditioning (3, 9–10).

The investigation of sympathetic function in humans would be most effectively achieved by microneurographically recorded MSNA, which has been reported to respond to various types of environmental stimuli. Among them, gravitational stimuli were reported to be the most potent factor altering the MSNA (12–14, 18, 28) from the tibial nerve leading to antigravity muscles (4, 11). Our previous studies under simulated µG, i.e., bed rest or head-out water immersion, indicated that MSNA might be suppressed under µG (19, 21, 26). The MSNA recording during parabolic flight would provide a preferable consideration on MSNA alteration in an actual µG environment, even in a brief duration.

The present study was conducted to determine the MSNA changes in sitting humans during the short period of hypergravity and µG produced by parabolic flight.
METHODS

Subjects

Ten healthy males and three healthy females, age $27 \pm 1.6$ yr (mean $\pm$ SE), weight $61.1 \pm 2.6$ kg, and height $169.9 \pm 1.9$ cm, participated in the study. They were all normotensive without any cardiovascular, pulmonary, or kidney diseases. Approximately 20% of the subjects selected were unsuitable for MSNA recording because MSNA recording was not possible during the control reading before lift off. All subjects were informed about the procedure and risks of the experiment, and consent was obtained from each subject in written form. The protocol was approved by The Human Research Committee, Research Institute of Environmental Medicine, Nagoya University, and the Ethical Committee on Human Research, National Space Development Agency of Japan.

Parabolic Flight in an MU-300 Aircraft

The present study employed an MU-300 jet aircraft, which is slightly different in size and power than the KC-135 plane used by the National Aeronautics and Space Administration (NASA) in related studies.

The plane took a straight and level flight for 2 min, then dived from the altitude of 30,000 feet to 15,000 feet, followed by a 30-s "pull-up" at a 2-G level. The engine power was then reduced to 60% to make a 20-s "pushover" into the 0-G condition. The plane was then accelerated to achieve a 1.8-G state for 30 s, followed by a return to a straight and level flight. The intervals between two consecutive parabolas were $\sim 6$ min on average, and the time except for the parabola was spared for a 1-G straight and level flight (Fig. 1).

The MU-300 parabolic flight was different from KC-135 flight in its pause for several minutes between one parabola and a subsequent parabola due to the small Japan Self Defense Force training area; the MU-300 jet aircraft must make a turn before each parabola in this area. The parabolic flight using the KC-135 at NASA employed a continuous flight consisting of 10 parabolas. Another difference from KC-135 flight was that the MU-300 must dive several thousand feet before pull-up due to its small-sized engine power. However, no significant differences occurred in the $+G_z$ (gravitational acceleration from head to foot) profiles when the two planes were used.

Equipment

Electrodes for bipolar chest cardiography were pasted, and tape electrodes for impedance plethysmography were taped circumferentially around the neck and the chest at the level of the xyphoid process. Electrocardiography (ECG) was monitored with a bioamplifier (AB-621; Nihon Kohden, Tokyo, Japan), and instantaneous heart rate (HR) was calculated from the R-R intervals of the ECG. Instrumentation for thoracic impedance measurements using an impedance plethysmograph (AI-601; Nihon Kohden) was put in place. Noninvasive impedance cardiography (16) was used to measure the thoracic fluid index. Thoracic fluid index values were normalized to values obtained during the steady-state period of 1 G for 1 min preceding the parabola (23, 24).

The subjects were prepared at Diamond Air Service (Toyoyama, Aichi, Japan). As illustrated in Fig. 2A, the subject was seated, and the bilateral knee joints were fixed at the thighs and calves by leg braces. The braces fixing the knees in the extended position were attached to both legs with Velcro tape at the upper and lower thigh, the calf, and the ankle. Blood pressure waves were monitored with a Finapres device (Finapres 2300; Ohmeda, Englewood, CO) at the subject's left middle finger, which was fixed with adhesive tape at the level of the right atrium, and the mean arterial pressure (MAP) was calculated from the averaged areas under the Finapres waveform trace. The respiration curve was recorded with a thermistor at the nose.

Microneurography

The discharge of postganglionic sympathetic nerve (MSNA) was recorded from the left tibial nerve by microneurography. A tungsten microelectrode with a shaft diameter of 120 µm, a tip diameter of 1 µm, and an impedance of $\sim 3$–5 MΩ (26–05–01; Frederick Haer, Brunswick, ME) was inserted manually and percutaneously without anesthesia in the muscle nerve fascicle of the tibial nerve at the popliteal fossa. The sympathetic nerve signals were fed into a high-impedance preamplifier (×2,000 in gain; Kohno II, Kohno Instruments, Nagoya, Japan) with band-pass filtration of $500$–$5,000$ Hz. The filtered signals were rectified, amplified, and integrated with a time constant of 0.1 s and were displayed on a digital oscilloscope (VC6023; Hitachi, Tokyo, Japan). MSNA was identified by the criteria described by Mano and colleagues (14, 18) as follows: 1) pulse-synchronous spontaneous and rhythmic efferent burst discharges recorded from muscle nerve fascicle, 2) modulation by respiration, 3) increase by a fall and decrease by a rise in systemic blood pressure, and 4) enhancement by maneuvers increasing intrathoracic pressure such as Valsalva's maneuver.

Experimental Protocol

The experiments were carried out on 13 separate days with each subject being investigated in a single day. The subjects were required to come to the laboratory after an overnight fast. After placement of the electrodes, the subject was seated in a passenger seat with his/her knees extended. After acceptable signal-to-noise ratio recordings were obtained, the MSNA, respiratory curve, ECG, blood pressure wave, thoracic impedance, and concurrent acceleration data with a head-to-foot direction of resultant inertial force ($+G_z$) were stored in a multichannel digital audio tape (DAT) recorder (PC-216A; Sony-Magnescale, Tokyo, Japan) for a 5-min control reading.

The jet aircraft departed from Nagoya International Airport for the training areas of the Self Defense Forces of Japan and performed at most 10 parabolas for 1 h. The cabin was
always pressurized to sea-level altitude. The changes in the G profile are illustrated in Fig. 1.

The subjects were instructed to breathe as naturally as possible and not to bend their knee joints. If the MSNA recording was lost because of movements by the subject, the electrode was adjusted to identify the MSNA again with the aid of a sound monitor.

Data Analysis

All data stored in the DAT recorder were digitized by Spike-2 software (Cambridge Electronic Design, Cambridge, UK) and a microcomputer (Macintosh 7500/100: Apple, Cupertino, CA) at the sampling rates of 16 kHz (MSNA), 100 Hz (respiration, thoracic impedance, +Gz), 500 Hz (ECG), and 200 Hz (blood pressure measured by the Finapres device).

The recorded MSNA potentials were discriminated to increase the signal-to-noise ratio and were full-wave rectified and fed through an analog circuit at the time constant of 0.1 s to obtain the integrated MSNA as a trace of the mean voltage neurogram.

The total MSNA was defined here as the burst area, which was measured every second by the area under the integrated MSNA trace, from the baseline. The total MSNA at 1 G just before entering 2-G conditions for 1 min (~90 s to ~30 s before the dive for parabola) was taken as 100%, and the changes from this value were calculated in 2-G and 0-G conditions (Fig. 2B). Changes in MSNA were also compared per second in the 2-G and 0-G conditions.

The instantaneous HR was calculated from the ECG R wave and is expressed in beats per min (min⁻¹). The respiratory curve was monitored throughout the parabolic flight, and apnea >2 s was regarded as “breath holding.”

Standard descriptive statistics were calculated for the baseline levels of thoracic impedance, HR, MAP, and total MSNA, with results presented as means ± SE. Paired t-test was employed to examine the effects of gravity, i.e., the
differences between 1, 2, and 0 G. The effects of +Gz on thoracic impedance, HR, mean arterial blood pressure, and total MSNA in the course of <10 parabolas were evaluated by using a repeated-measures ANOVA with Statview version 4.5 (Abacus Concepts). For all determinations, statistical significance was accepted at $P < 0.05$.

**RESULTS**

In 13 flights on separate days, 91 parabolas were performed, and analyzable MSNA data were obtained from 6 subjects who completed 19 parabolas. Impedance data were obtained in 6 subjects with 46 parabolas, and blood pressure and HR could be obtained in 13 subjects with 91 parabolas.

**Changes in MSNA Under $\mu$G Induced by Parabolic Flight**

Figure 2B shows typical changes in a head-to-foot direction of inertial force (+Gz), thoracic impedance, integrated MSNA, blood pressure wave, ECG, and instantaneous HR in a 24-yr-old male subject. During 2-G state produced by a pull-up of the aircraft, thoracic impedance was slightly increased (i.e., the thoracic fluid volume was decreased) with a transient MSNA enhancement and a HR increase.

In the transition from 2G to $\mu$G produced by the pushover of the aircraft, the thoracic impedance was decreased, with accompanying sympathetic silence and HR reduction, followed by a gradual blood pressure drop, a moderate MSNA enhancement, and a slight HR increase.

The changes in MSNA during 2 G and $\mu$G produced by parabolic flight were confirmed by the array display of integrated MSNA traces (Fig. 3). The array traces were drawn from a full-wave rectified and integrated neurogram of MSNA in a subject at the time constant of 0.1 s. They were adjusted at the starting point of $\mu$G. MSNA was enhanced at the beginning of the 2-G condition and then returned to the control level. At the $\mu$G entry, MSNA appeared to be slightly enhanced, was suppressed during the early stage of $\mu$G, and then was enhanced in the latter part of $\mu$G. This slight enhancement continued until several seconds after the 1.8-G entry after $\mu$G.

The MSNA changes were as follows: control state → enhanced → suppressed → enhanced → slightly suppressed, according to the gravity changes of 1 G → 2 G → $\mu$G → 1.5 G → 1 G, respectively. Taking the total MSNA at 1 G as 100%, the MSNA under 2 G was significantly enhanced, to 191.4 ± 14.2% ($P < 0.0001$ vs. 1 G), and the MSNA under $\mu$G was significantly suppressed to 82.8 ± 2.5% ($P < 0.0001$ vs. 1 G) in six subjects with 19 parabolas (Fig. 4, top right). Total MSNA were significantly different among various G conditions by repeated-measures ANOVA ($F = 17.8$, $P = 0.0072$).

**Changes in Hemodynamic Variables Under $\mu$G Induced by Parabolic Flight**

Thoracic blood volume. In average of the six subjects with 46 parabolas, the thoracic fluid index increased to 103.2 ± 3.0% during 2 G, and it decreased to 96.6 ± 1.0% under $\mu$G (Fig. 4, top left). This percentage change means that the thoracic fluid volume decreased by 3.2 ± 3.0% under the 2-G condition and increased by 3.4 ± 1.0% under $\mu$G (Fig. 4, top left), and thoracic fluid indexes were significantly different among various G conditions by repeated-measures ANOVA ($F = 21.3$, $P = 0.019$).
Arterial blood pressure. The arterial blood pressure wave measured with the Finapres device exhibited phasic changes throughout 2 G and µG. During 2 G, a gradual increase in arterial blood pressure was observed. On µG entry, arterial blood pressure was relatively high, fell down in the former part of µG, reached the trough midway in µG, and maintained the same level or rather increased in the latter part of µG until the transition to hypergravity (1.5 G). During the pull-out phase at the hypergravity of 1.5 G, the arterial blood pressure increased gradually (Figs. 2B and 5).

When the MAPs under 2 G and µG conditions were averaged in 13 subjects with 91 parabolas, there was a significant increase from 89.5 ± 1.7 to 100.2 ± 1.7 mmHg in the 2-G condition and a significant decrease (vs. under 1 G) to 77.9 ± 2.3 mmHg under the µG (0 G) condition (Fig. 4, bottom left). It has been clarified that MAPs were significantly different among various G conditions (F = 28.3, P = 0.0022).

HR. On 2 G entry, the HR increased slightly but not significantly from that under 1 G. Just after the µG entry, the HR was gradually decreased in association with the MAP decrease, in the former part of µG, and then turned to increase gradually during the latter part of µG. After pull-up, the HR gradually increased (Fig. 2B).

The HR increase from 1 to 2 G was 76.4 ± 1.6 to 82.1 ± 2.4 min⁻¹ (P < 0.001), and there was a significant decrease in HR from 2 G to µG of 78.1 ± 2.7 min⁻¹ (P = 0.0019), although the HR difference between 1 G and µG was not significant (P = 0.23) in 13 subjects with 91 parabolas (Fig. 4, bottom right). HRs were revealed to be not significantly different among various G conditions by the repeated-measures ANOVA (F = 2.046, P = 0.275).

Relationship Between MAP Change and MSNA Enhancement During µG

The arterial pressure waves during µG are array displayed in Fig. 5, which shows the gradual arterial pressure drop after the sudden blood pressure increase just after the µG entry in some parabolas. The blood pressure drop was 27.0 ± 8.3 mmHg in MAP, 38.9 ± 10.1 mmHg in systolic pressure, and 21.0 ± 8.3 mmHg in diastolic pressure in 13 subjects with 91 parabolas. The beat-to-beat changes in systolic and diastolic blood pressure were shown in Fig. 6, which indicates the blood pressure fall and MSNA suppression just after the µG entry and subsequent blood pressure stabilization or rather increase concomitant with MSNA enhancement.

In contrast, the MSNA enhancement in the latter stage of µG was analyzed in relation to this blood pressure drop in two subjects with eight parabolas each. Apparent blood pressure drops were observed in
both subjects (123.0 ± 3.8 to 111.9 ± 4.6 mmHg in subject 1 and 112.4 ± 6.2 to 75.4 ± 4.8 in subject 2), with sympathetic silence during the transition of 2 G to µG and to the midway of µG. In the latter stage of µG to the pull-out stage, MSNA was activated, which in turn raised the blood pressure to the control level.

During µG, the effect of breath holding on the MAP drop and MSNA enhancement was examined in these 19 parabolas. The MAP drop was significantly greater in the parabolas with breath holding (21.5 ± 1.3 mmHg, n = 12) than without it (13.2 ± 1.7 mmHg, n = 7, P = 0.0004), and the MSNA was significantly more enhanced in the parabolas with breath holding (817 ± 40%, n = 12) compared with those without it (575 ± 51%, n = 7, P = 0.018).

**DISCUSSION**

The present study examined the changes in sympathetic nerve activity leading to antigravity skeletal muscles in sitting humans with their knees extended, using a microneurographic technique. The results documented the activation of MSNA during 2 G and the suppression of MSNA during µG created by parabolic flight. These changes were phasic and dynamic, i.e., MSNA was enhanced at the initial phase of 2 G and was suppressed during the early stage of µG produced by parabolic flight, followed by a transient and slight MSNA enhancement during the later stage of µG. On average, the total MSNA was significantly increased under 2 G and decreased under 0 G.

Our findings suggest that these MSNA changes are modified by two factors: thoracic fluid volume and arterial blood pressure. Static MSNA changes may be related to cardiopulmonary reflex, since thoracic impedance shows changes analogous to the + Gz profile. This suggested that the MSNA changes during gravitational change depend on the loading of cardiopulmonary volume receptors represented by the thoracic fluid.
abrupt cephalad fluid shift elevates the arterial blood pressure by 26% on average and 46% on μG entry; see Ref. 15). This by an increased right ventricular filling velocity (by increases the venous return to the heart, as indicated the early phase of spaceflight. During this phase, it abruptly increases the status of the fluid redistribution under μG in the upper part of the body, which might approximate there is a sudden cephalad fluid shift from the lower to the head to the leg (Gz). During 2 G, circulatory fluid shifts caudadly from the upper to the lower part of the body to pool the blood volume. It reduces the blood volume shifted from the lower body to the intrathoracic cavity. However, the arterial baroreflex may also play a role in suppressing the MSNA in response to the arterial blood pressure increase at the end of 2 G, transition from 2 G to μG, and just after μG entry. Contrarily, MSNA enhancement in the latter part of μG to the beginning of 1.8 G of pull-out in response to the fall of arterial blood pressure may be associated with unloading of the arterial baroreceptor.

Another observation of our study is that the respiratory movement has a great influence on the rebound MSNA enhancement in the later stage of μG induced by a gradual MAP drop during μG. The significant differences in the MAP drop and MSNA enhancement between the parabolic flights with and without breath holding indicated that breath holding on μG entry accelerated the MAP drop and magnified the MSNA enhancement during μG.

The hemodynamic changes during parabolic flight can be considered as follows in two stages, 2 G and μG. When a subject is seated, body fluid shifts according to the changes in gravitational force in the direction from the head to the leg (+Gz). During 2 G, circulatory blood shifts caudally from the upper to the lower part of the body to pool the blood volume. It reduces the venous return to the heart (15) and cerebral blood flow (1) in the sitting position. This causes the unloading of intrathoracic low-pressure (or volume) receptors, which may be responsible in part for the elevation of the HR and enhancement of the sympathetic nerve activity that increase the peripheral vascular resistance.

Regarding the changes initiated just after μG entry, there is a sudden cephalad fluid shift from the lower to the upper part of the body, which might approximate the status of the fluid redistribution under μG in the early phase of spaceflight. During this phase, it abruptly increases the venous return to the heart, as indicated by an increased right ventricular filling velocity (by 26% on average and 46% on μG entry; see Ref. 15). This abrupt cephalad fluid shift elevates the arterial blood pressure and pulse pressure, suggesting that not only the cardiopulmonary low pressure (or volume) receptors but also the baroreceptors in the carotid sinus and aortic arch are activated to suppress the sympathetic nerve activity in the medulla oblongata via the glossopharyngeal and vagal nerves (7). Sympathetic suppression during the early stage of μG and slight activation in the later stage of μG were observed, although these changes varied among the individual subjects. On μG entry, MSNA appeared to be slightly enhanced in part due to artifacts produced by body movements upon μG entry and due in part to vestibular stimulation (6).

According to the present polygraphic recordings, it is reasonable to divide the 20-s μG period into two phases: early μG and late μG phases. In the early μG phase, the thrusting fluid shift in the thoracic cavity suppressed the MSNA and reduced the HR, which decreased the arterial blood pressure. Consequently, the MSNA was activated through the arterial baroreflex, the HR recovered, and the arterial pressure was stabilized with a slight increase in the late μG phase. This division was also pointed out by John et al. (15), who evaluated the cardiac filling and ejection properties by Doppler echocardiography. They reported a 20% increase in the mean left ventricular flow velocity in sitting subjects in the early phase, whereas a suppression was recorded in the late phase.

Previous studies have reported the cardiovascular variables during parabolic flight in sitting subjects, although there have been no comments on respiratory movements, including a HR reduction by ~10 min⁻¹, a reduction in intrathoracic impedance by 1.25 Ω, an increase in cardiac output by 0.5 l/min, and an increase in stroke volume from 60 to 80 ml (17, 22, 23, 24). These results are compatible to ours; however, the present study clarified that these changes are dynamic and phasic and are strongly influenced by respiratory movement during parabolic flight. A recent study by Schlegel et al. (27) also documented the significance of breathing maneuvers during parabolic flight.

The present study confirmed the dynamic suppression of MSNA in the early phase of μG. A 20-s period of μG is too short to observe static changes; however, sympathetic nerve traffic to muscle has been shown to be suppressed in response to the fluid shift in the early phase of μG, with a transient enhancement observed in the late phase of the μG in response to the blood pressure drop, which might be the consequence of the sympathetic silence in the early phase.

In conclusion, MSNA was suppressed during the early stage of μG induced by parabolic flight, but only transiently. Rebound activation in MSNA to a moderate degree depending on respiratory movement was induced through the baroreflex mechanism in the latter stage of μG, whereas a dynamic response in MSNA was observed during the entry to 2 G just before the parabolic flight. The suppression and activation of MSNA were dependent not only on the loading and unloading of cardiopulmonary low-pressure (volume) receptors, respectively, as observed during head-out water immersion studies (19, 21), but also on the
loading and unloading of arterial baroreceptors, which are dynamic and transient events.

Exposure to μG has been confirmed to induce sympathetic suppression in the dynamic phase; however, the question of what happens in the sympathetic nerve activity via the baroreflex or cardiopulmonary reflex under prolonged μG remains to be answered. Studies conducted on the long-term μG during spaceflight may resolve this issue.

Other issues are respiratory control and postural effect. The same kinds of studies should be carried out in the future by altering respiratory maneuvers (controlled and uncontrolled) and/or changing postures (supine, upright, sitting).

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