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RESEARCH ARTICLE

Hisayoshi Ogata
Yukiharu Higuchi
Toru Ogata
Shinya Hoshikawa
Masami Akai
Kimitaka Nakazawa

Pressor response to passive walking-like exercise in spinal cord-injured humans

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H. Ogata, PhD (✉) · T. Ogata, MD, PhD
S. Hoshikawa, MD, PhD
K. Nakazawa, PhD
Dept. of Rehabilitation for Movement Functions
Research Institute of National Rehabilitation Center for Persons with Disabilities
4-1 Namiki, Tokorozawa
Saitama 359-8555, Japan
Tel.: +81-4/2995-3100
Fax: +81-4/2995-3132
E-Mail: hisaoga@rehab.go.jp

Y. Higuchi, PhD · M. Akai, MD, PhD
Dept. of Physical Training for Remedial Gymnastics
Hospital, National Rehabilitation Center for Persons with Disabilities
Tokorozawa, Japan

Abstract *Objective* To examine blood pressure responses during passive walking-like exercise in the standing posture (PWE) in spinal cord-injured (SCI) humans. *Methods* Twelve motor-complete SCI individuals (cervical level 6 to thoracic level 12, ASIA grade: A or B) and twelve able-bodied controls (CON) participated in this study. SCI individuals were divided into a group with injury level at or above thoracic (T) 6 (HSCI, $n = 7$) and a group with injury level at or below T10 (LSCI, $n = 5$). Subjects carried out 6-minute quiet standing and then 12-minute PWE at 1 Hz using a gait training apparatus that enables subjects to stand and move their legs passively. *Results* Mean arterial blood pressures (MAPs) at standing in HSCI, LSCI and CON were 69 ± 5, 83 ± 4 and 93 ± 2 mmHg, respectively. MAP changed significantly during PWE only in HSCI and CON, increasing to 88 ± 4 ($P < 0.001$) and 98 ± 1 mmHg ($P < 0.01$), respectively. The former group showed a larger increase in MAP ($P < 0.001$). *Interpretation* Spinal sympathetic reflexes can be induced in a region isolated from the brainstem in response to a stimulus originating below the level of the spinal cord injury, and the magnitude of increase in blood pressure is greater in SCI individuals with lesion level at or above T6 due to loss of supraspinal control of the major sympathetic outflow. This central mechanism may be one of the reasons why greater pressor response to PWE was observed in HSCI.

Key words blood pressure · standing posture · passive exercise · rehabilitation · spinal cord injuries

Introduction

There have been several studies on the effects of passive leg movement on blood pressure responses in able-bodied individuals and in spinal cord-injured (SCI) individuals. In able-bodied persons, passive leg cycling (PLC) has been found to increase mean arterial blood pressure (MAP) via an increase in stroke volume [27] and/or heart rate [28]. Nobrega et al. [27] suggested that the increase in stroke volume was induced by an increase in venous return from the passively moved lower limbs or by a muscle mechanoreceptor-evoked increase in myocardial contractility. The increases in myocardial contractility and HR during PLC are thought to be mediated by a reflex neural mechanism that involves the brainstem cardiovascular center by afferent signals [27, 28]. However, an increase in blood pressure during PLC in able-bodied individuals was not found in some studies [24, 30].

In SCI individuals, it is assumed that there is little or no afferent input to the brainstem cardiovascular center during passive leg movement due to the spinal cord lesion. The effect of PLC in SCI individuals has been examined in several studies, but most studies failed to show a rise in any of the blood pressure parameters (i.e., systolic and diastolic arterial blood pressure and MAP) in individuals with cervical (C) [25], thoracic (T) or lumbar (L) spinal cord injury [24, 30]. Dela et al. [10] found that MAP increased significantly in cervical (C6) and thoracic (T3–5) injured individuals with PLC and that the magnitude of increase was larger in cervical injured individuals. In contrast, in studies in which no significant increase in blood pressure was found, lesion level of the subjects covered a wide range (T2–L1) [30] or was lower (T8–L1) [24]. These findings indicate the possibility that blood pressure can be increased even in SCI individuals depending on lesion level. However, Nash et al. [25] reported that MAP did not increase significantly during PLC even in individuals with cervical spinal cord injury. Recently, Ballaz et al. [4] found an increase in leg blood flow during PLC in SCI individuals with a lesion level between T3 and L1, although blood pressure responses were not reported. Ter Woerds et al. [30], on the other hand, showed no increase in leg blood flow or blood pressure during PLC in SCI individuals with lesion levels similar to that in the study by Ballaz et al. [4] (T2–L1). Ballaz et al. [4] used a slightly different exercise mode, i.e., higher revolution speed (40 vs. 35 rpm), that provided greatest ranges of motion of the knee and hip. These findings indicate the possibility that the occurrence of pressor response is also dependent on the mode of passive leg movement.

With regard to exercise mode, Nash et al. [26] reported that an individual with cervical spinal cord injury, who is at a high risk of orthostatic hypotension [6, 18], could complete prolonged (40 minutes) passive body weight-supported treadmill walking, suggesting an increase in blood pressure by performing this kind of exercise. However, there has been no study in which blood pressure response during passive walking-like exercise (PWE) in SCI individuals was examined. The purpose of the present study was to determine whether blood pressure increases during PWE in SCI individuals with motor-complete paralysis of their legs (C6–T12). We hypothesized that blood pressure increases during PWE even in SCI individuals but that the magnitude of increase in blood pressure is dependent on lesion level. To determine the effect of lesion level, we divided SCI individuals into two groups, i.e., individuals with a lesion level at or above T6 (higher SCI; HSCI, C6–T6) and individuals with lesion level at or below T10 (lower SCI; LSCI, T10–T12). The reason for this is as follows: it is known that spinal sympathetic reflex activity can be induced in the paralyzed region in response to a stimulus originating below the level of the spinal cord injury. Although spinal reflexes can be seen in upper and lower thoracic injured individuals, systemic pressure response is confined to upper level injured individuals who have lost supraspinal control of most of the greater splanchnic nerve (GSN) outflow [14]. The uppermost ganglion contributing to the GSN resides in T5–7 and the lowermost ganglion contributing to the GSN resides in T9–11 in most cases [33]. Therefore, a pressor response should be greater in HSCI than in LSCI if spinal sympathetic reflexes are induced. Although noxious stimuli are thought to be the primary (and sole) initiators of spinal reflexes, there are arguments for and evidence indicating that spinal reflexes have the potential to be provoked by "innocuous" stimulation [5, 20]. Therefore, considering the potential effect of spinal reflexes during PWE, it is reasonable to divide SCI individuals into HSCI and LSCI.

In the present study, simultaneous measurements of electromyographic activity of the paralyzed leg and pulmonary oxygen uptake were conducted to determine the influence of muscle activity on pressor response based on the assumption that muscle activity level determines in part the magnitude of muscle pumping effect. These parameters were also used to detect muscle spasm, because muscle spasms have been shown to lead to an increase in blood pressure, possibly due to the muscle pump effect or spinal reflexes acting on the heart or on peripheral vessels [9]. The exercise time of PWE was set at 12 minutes because the therapeutic potential of PWE on muscle activity has previously been found to decline between 5 and 10 minutes from initiation [11].

Materials and methods

Subjects

Twelve male SCI individuals and twelve male able-bodied controls (CON) participated in the present study. Characteristics of the SCI individuals are shown in Table 1. There were no significant differences in age, height and weight among the three groups. There was also no significant difference in post-injury period between the two groups of SCI individuals. All SCI individuals had a spinal cord lesion of traumatic origin and had complete motor paralysis of their lower limb muscles. None of the subjects had cardiopulmonary disease, and none were taking medications likely to affect the results of our study. We chose subjects with hip joint range of motion of more than 30° and without any continuous muscle spasm during the standing posture. One of the SCI individuals (subject No. 10) had been performing regular body weight-support treadmill training (40 minutes/twice a week) for 9 months prior to testing, while the other SCI individuals reported that they occa-

Table 1 Characteristics of individuals with spinal cord injury and able-bodied controls

Subject No.	Age (years)	Height (cm)	Weight (kg)	Time since injury (months)	Lesion level	ASIA grade
1	20	176	43	39	C6	A
2	27	169	53	26	C7	B
3	29	160	54	17	T1	A
4	21	168	51	38	T3	A
5	36	173	75	178	T5	A
6	50	169	57	48	T5	A
7	37	179	65	232	T6	A
8	22	171	57	53	T10	A
9	42	167	53	281	T11	A
10	20	180	100	14	T12	B
11	35	183	68	111	T12	A
12	35	178	61	7	T12	A
HSCI (n = 7) Mean ± SD	31 ± 11	171 ± 6	57 ± 10	83 ± 86		
LSCI (n = 5) Mean ± SD	31 ± 9	176 ± 7	68 ± 19	93 ± 113		
CON (n = 12) Mean ± SD	33 ± 8	171 ± 5	67 ± 8			

C and T: cervical and thoracic levels, respectively, *HSCI*: individuals with spinal cord injury with lesion at or above thoracic level 6, *LSCI*: individuals with spinal cord injury with lesion at or below thoracic level 10, *CON*: able-bodied controls

sionally experienced quiet standing and/or passive walking-like movement. On the other hand, none of the control subjects had been performing any regular physical exercises.

Voluntary consent for participation in this study was obtained from all subjects after they had been informed of the purpose of the experiment, the procedure and possible risks. The study was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee of the National Rehabilitation Center for Persons with Disabilities in Tokorozawa, Japan.

Protocol

The subjects were instructed to refrain from eating for at least 3 hours before the test, to refrain from smoking and taking caffeine for at least 5 hours before the test, to refrain from drinking alcohol and doing heavy exercise for 12 hours before the test, and to urinate just before they reported to the laboratory. During measurements, the temperature in the experimental room was set within the range of 23–25°C.

Quiet standing and PWE were carried out using a commercially available device (Easy Stand Glider 6000, Altimate Medical, Inc. USA) as shown in Figure 1. Briefly, this device enables subjects to change their posture from sitting to standing by pulling a built-in hydraulic lever. Standing posture is stabilized by fixing the trunk, pelvis and knees using front and back pads, lateral pelvic pads, and kneepads. Bilateral handles located in front of the trunk are linked to the footplates, thus allowing one leg to move forward while the other moves back by pushing and pulling the handles alternately. In the present study, an experimenter (H.O.) manually pulled the hydraulic lever and moved the handles.

Figure 1 shows the protocol of the experiment. First, subjects spent 6 minutes in a sitting position and this was followed by a 6-minute quiet standing period to determine the baseline levels in sitting and standing states. Thereafter, they performed 12-minute rhythmic PWE at 1 Hz. The hip joint range of motion (ROM_{hip}) was set at 30°. In some SCI individuals, muscle spasm occurred, especially during the first 20 seconds after the commencement of PWE (Figure 2), thus restricting the ROM_{hip}. In such cases, the experimenter narrowed the distance transiently. After the PWE, the subjects spent 6 minutes in a quiet standing position and this was followed by a 6-minute sitting period. The subjects were occasionally instructed to relax their upper limbs during the experiment.

During PWE, the experimenter always checked angle data displayed on an oscilloscope to maintain the predetermined pattern (i.e., ROM_{hip} and swing frequency). The angle was measured by an electrogoniometer (Goniometer system, Biometrics Ltd, USA) placed at the junction of the handle and footplate. The experimenter (H.O.) conducted a sufficient number of practice sessions before the main tests so that he could adjust the leg motion to the predetermined pattern by monitoring the angle data displayed on the oscilloscope (Figure 2). In addition, knee angle was measured by another electrogoniometer (Goniometer system, Biometrics Ltd, USA) to define the starting and end points of standing. As shown in Figure 2, it took about 30 seconds to change from the sitting position to standing position and it took about 15 seconds to change from the standing position to sitting position.

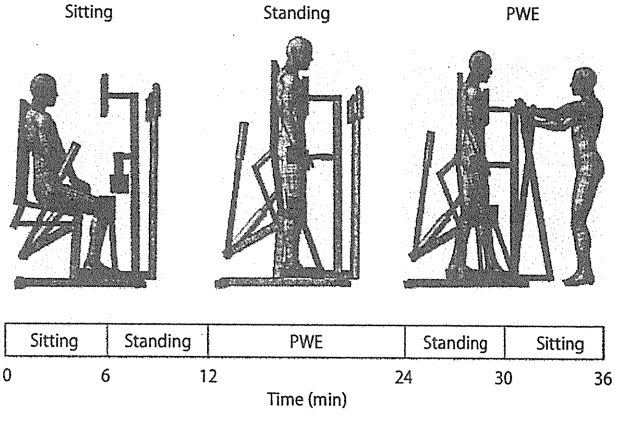

Fig. 1 Experimental protocol. The device enables subjects to change their posture from sitting to standing by pulling a built-in hydraulic lever. Standing posture is stabilized by fixing the trunk, pelvis and knees using front and back pads, lateral pelvic pads, and kneepads. Bilateral handles located in front of the trunk are linked to the footplates, thus allowing one leg to move forward while the other moves back by pushing and pulling the handles alternately. *PWE*: passive walking-like exercise

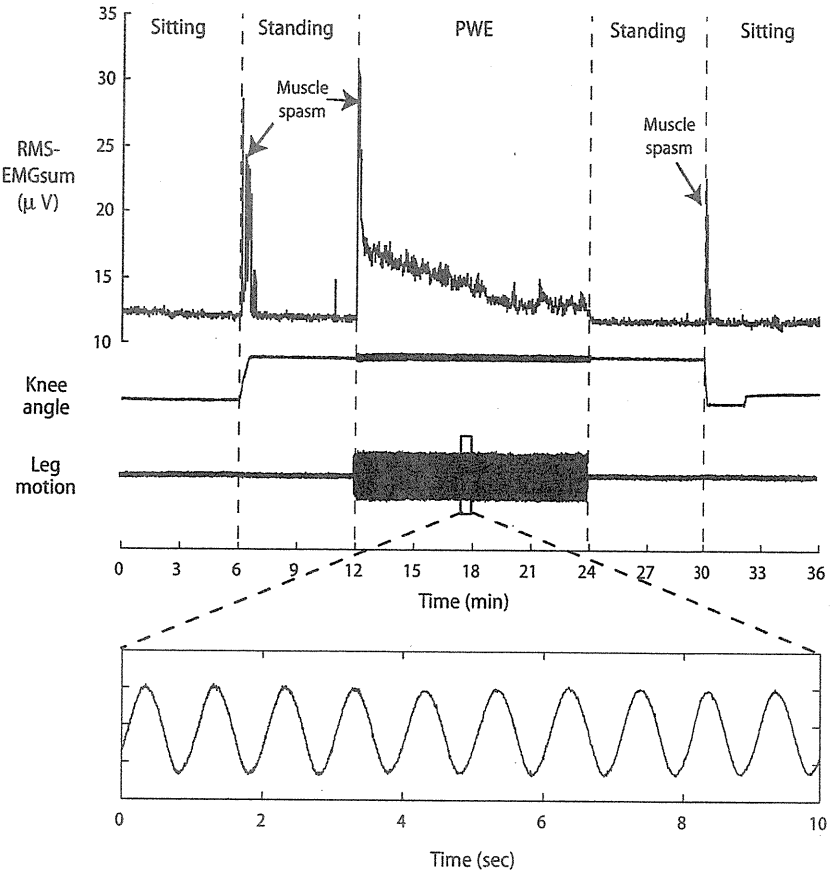

Fig. 2 Example of changes in RMS-EMG_{sum}, knee angle and leg motion throughout an experiment in an individual with spinal cord injury. RMS-EMG_{sum}: sum of root mean square of electromyographic activity of the left leg muscles (rectus femoris muscle, biceps femoris muscle, medial gastrocnemius muscle and tibialis anterior muscle). *PWE*: passive walking-like exercise

Measurements

Blood pressure and heart rate

Systolic and diastolic arterial blood pressures (SAP and DAP, respectively) were determined noninvasively using an electrosphygmomanometer (Tango, Sun Tech Medical Instrument Inc. USA). A pneumatic cuff was fixed to the left upper arm, and Korotkov sound was detected by a lavalier microphone fixed on the left brachial artery. The sampling interval was set at 1 minute because it took about 30 seconds to terminate one data sampling. An electrocardiograph was installed in the device and average heart rate for 5 heart beats just before termination of blood pressure measurement was recorded. MAP was calculated as diastolic pressure plus one third of pulse pressure.

Electromyographic activity

Continuous surface electromyograms (EMGs) of the rectus femoris muscle, biceps femoris muscle, medial gastrocnemius muscle and tibialis anterior muscle of the left leg were recorded using bipolar electrodes (DE-2.1, Delsys, Boston, USA). Prior to EMG electrode application, the measurement site was prepared by the removal of dead skin by gentle abrasion with abrasive paper. Then the EMG electrodes were attached to the skin in a direction parallel to the muscle fiber orientation with double-sided adhesive tape. The reference electrode was placed over the patella. The electrode contacts were made from two silver bars that were each 10 mm in length and 1 mm in diameter, and they were spaced 10 mm apart. The detected signals were amplified before being sent along a shielded cable to the rest of the EMG system. The EMG signals were then amplified using a main amplifier (Bagnoli-8, Delsys, Boston, USA) with a gain of 1000 fold. The analog signals of EMG were sampled at 1,000 Hz (Powerlab; AD Instruments, Castle Hill, Australia). In the present study, root mean square of the EMG signal (RMS-EMG, μV) for every 1-second interval for each muscle was calculated. The sum of RMS-EMG of the muscles (RMS-EMG_{sum}) was calculated.

Pulmonary oxygen uptake

Data on pulmonary oxygen uptake ($\dot{V}O_2$) were obtained breath-by-breath using a respiratory gas analyzer (AE-300S, Minato Medical Science, Osaka, Japan). O_2 was measured by a zirconium sensor. The gas analyzer was calibrated by known standard gas (O_2 15.18%, CO_2 5.06%). $\dot{V}O_2$ was determined for each 1-minute interval.

Data analysis

For each of the cardiorespiratory values and RMS-EMG_{sum}, the averages for each 3-minute interval were used for statistical analysis. In the present study, since two subjects complained of nausea during the standing position after PWE probably due to orthostatic hypotension, subsequent data collection in these two subjects was abandoned. Therefore, our analysis of responses includes only data obtained during pre-PWE and PWE periods. These data were analyzed through a two-way repeated measures analysis of variance (ANOVA) with the group (HSCI vs. LSCI vs. CON) and condition (sitting vs. standing vs. PWE in the first half vs. PWE in the second

Table 2 Cardiorespiratory parameter values and electromyographic activity of the leg ($RMS\text{-}EMG_{sum}$) during passive walking-like exercise (PWE) and during quiet sitting and quiet standing before PWE in individuals with spinal cord injury and able-bodied controls (CON)

		Sitting	Standing	PWE (4–6th minute)	PWE (10–12th minute)
HSCI ($n = 7$)	SAP (mmHg)	$97 \pm 4^{***}$	$86 \pm 7^{***, \#}$	108 ± 5	$105 \pm 5^{**}$
	MAP (mmHg)	$77 \pm 3^{***, \#}$	$69 \pm 5^{***, \#}$	88 ± 4	$85 \pm 4^{*}$
	DAP (mmHg)	$66 \pm 2^{***}$	$60 \pm 4^{***, \#}$	78 ± 4	75 ± 4
	HR (bpm)	$76 \pm 3^{**}$	$87 \pm 3^{**}$	$76 \pm 2^{*}$	$76 \pm 1^{*}$
	$\dot{V}O_2$ (ml min^{-1})	$190 \pm 8^{*, \#\#}$	$214 \pm 10^{\#}$	230 ± 16	$207 \pm 10^{\#\#}$
	$RMS\text{-}EMG_{sum}$ (μV)	11.8 ± 0.4	11.8 ± 0.3	13.7 ± 0.3	13.4 ± 0.4
LSCI ($n = 5$)	SAP (mmHg)	110 ± 4	106 ± 4	109 ± 7	110 ± 7
	MAP (mmHg)	87 ± 4	83 ± 4	88 ± 5	87 ± 5
	DAP (mmHg)	76 ± 4	72 ± 4	77 ± 5	76 ± 4
	HR (bpm)	$75 \pm 3^{*}$	80 ± 4	$78 \pm 5^{*}$	$81 \pm 6^{**}$
	$\dot{V}O_2$ (ml min^{-1})	268 ± 21	269 ± 20	275 ± 21	273 ± 18
	$RMS\text{-}EMG_{sum}$ (μV)	12.2 ± 0.5	12.0 ± 0.4	12.7 ± 0.7	12.4 ± 0.6
CON ($n = 12$)	SAP (mmHg)	116 ± 2	114 ± 2	122 ± 2	123 ± 2
	MAP (mmHg)	92 ± 2	93 ± 2	98 ± 1	97 ± 1
	DAP (mmHg)	81 ± 2	83 ± 1	86 ± 1	84 ± 2
	HR (bpm)	65 ± 2	72 ± 2	66 ± 2	66 ± 2
	$\dot{V}O_2$ (ml min^{-1})	241 ± 10	244 ± 8	247 ± 9	236 ± 8
	$RMS\text{-}EMG_{sum}$ (μV)	11.9 ± 0.4	12.7 ± 0.5	14.3 ± 0.8	14.4 ± 0.9

SAP, MAP and DAP: systolic, mean and diastolic arterial blood pressures, respectively. *HR*: heart rate. $\dot{V}O_2$: pulmonary oxygen uptake. $RMS\text{-}EMG_{sum}$: sum of root mean square of electromyographic activity of the left leg muscles (rectus femoris muscle, biceps femoris muscle, medial gastrocnemius muscle and tibialis anterior muscle). *HSCI*: individuals with spinal cord injury with lesion at or above thoracic level 6, *LSCI*: individuals with spinal cord injury with lesion at or below thoracic level 10
*, **, ***$P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared to the average in CON
#, ##, ###$P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared to the average in LSCI

half). If an interaction was found, one-way ANOVA with the group for each condition and one-way ANOVA for repeated measures with the condition for each group were performed. One-way ANOVA for repeated measures was performed with the standing level set to zero, because we focused on the amplitude of change from the level at quiet standing to the level at PWE. If a significant F value was observed following one-way ANOVA, comparison of average values was made using Tukey's HSD test. Differences between the two groups were assessed using Student's unpaired t test. A value of $P < 0.05$ was regarded as statistically significant. All data are presented as means ± S.E.M.

Results

In most cases, muscle spasms occurred in the transition from sitting to standing and immediately after the onset of PWE, probably due to sudden muscle stretching, and they tended to disappear within 30 seconds (Figure 2). Therefore, we used the averages for the last 3 minutes at sitting and standing and the averages for 4–6 and 10–12 minutes of PWE for statistical analysis, with the assumption that these values are minimally influenced by muscle spasms.

Significant main effect and interaction

A significant main effect for conditions, main effect for groups and their interaction were observed for all of the parameters except $RMS\text{-}EMG_{sum}$. Since only a significant main effect for conditions was observed for $RMS\text{-}EMG_{sum}$, data for $RMS\text{-}EMG_{sum}$ were analyzed to identify the simple main effect for conditions when data obtained in the three groups at each time point were combined.

At sitting

Table 2 shows the absolute values for cardiovascular parameters at sitting. All of the blood pressure values in HSCI were significantly lower than those in CON (SAP: $P < 0.001$, MAP: $P < 0.001$, DAP: $P < 0.001$). In addition, MAP in HSCI was significantly lower than that in LSCI ($P < 0.05$). HR in HSCI and that in LSCI were significantly higher than that in CON ($P < 0.01$ in HSCI, $P < 0.05$ in LSCI).

At sitting vs. at standing

In all of the three groups, there was no significant difference in any of the values of the three blood pressure parameters in the two conditions (Figure 3). In HSCI, however, P values for SAP and MAP were nearly significant (SAP: $P = 0.088$, MAP: $P = 0.088$). HR at standing was significantly larger than that at sitting in HSCI and in CON, but not in LSCI (Figure 4). $RMS\text{-}EMG_{sum}$ and $\dot{V}O_2$ did not differ significantly in the two conditions, indicating that blood pressure is minimally affected by muscle spasms.

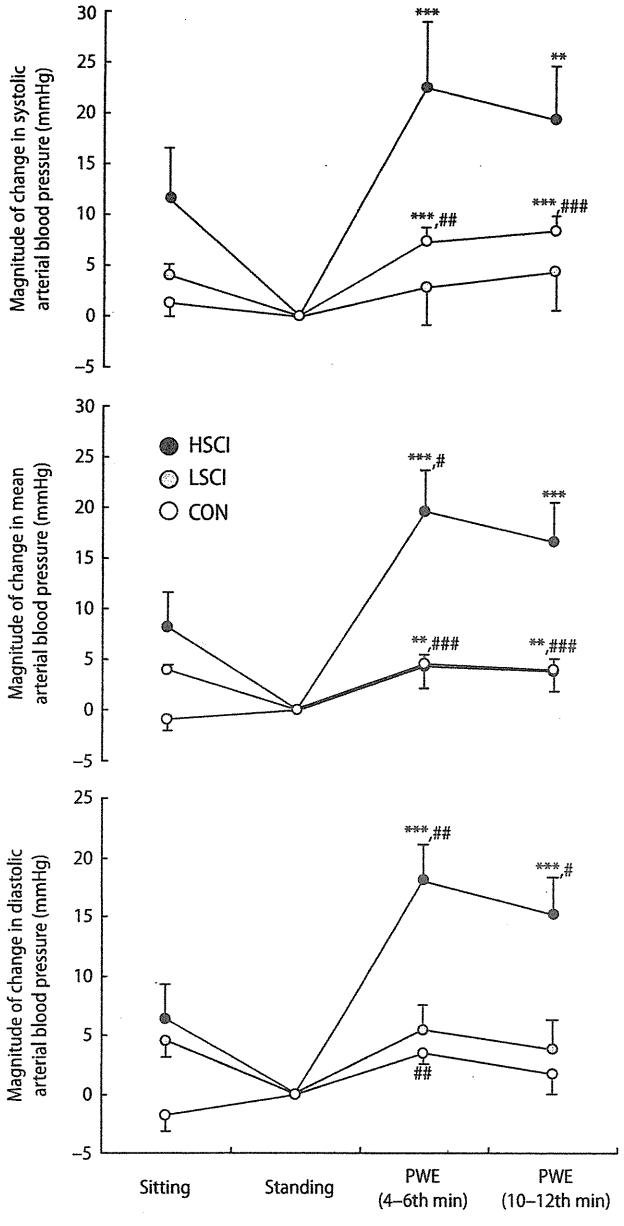

Fig. 3 Magnitudes of changes in systolic, mean and diastolic arterial blood pressures from the quiet standing level. *HSCI*: individuals with spinal cord injury at or above thoracic level 6. *LSCI*: individuals with spinal cord injury at or below thoracic level 10. *CON*: able-bodied controls. *PWE*: passive walking-like exercise. *, **, ***$P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared to the quiet standing level. #, ##, ###$P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared to the quiet sitting level

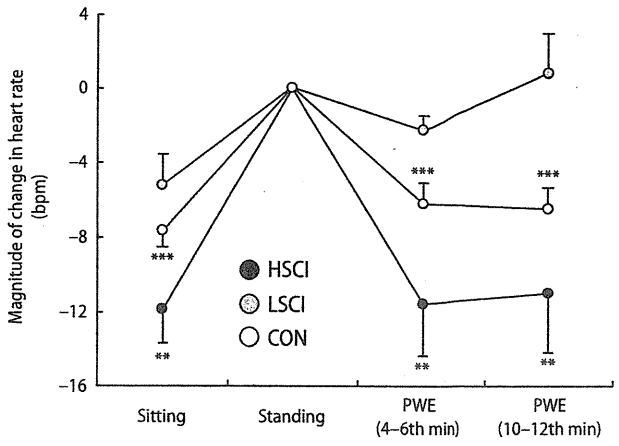

Fig. 4 Magnitudes of changes in heart rate from the quiet standing level. See legend to Figure 3 for definitions of symbols and abbreviations

At standing vs. at PWE

In both HSCI and CON, all of the blood pressure values at PWE were significantly higher than those at standing except for DAP in CON (Figure 3). In LSCI, on the other hand, there was no significant difference in either of the blood pressure values in the two conditions (Figure 3). $RMS\text{-}EMG_{sum}$ at PWE was significantly higher than that at standing ($P < 0.001$), whereas $\dot{V}O_2$ did not differ significantly in the two conditions in any of the groups.

For all of the blood pressure parameters and HR, there was no significant difference between the value in the first half of PWE and that in the second half of PWE in all three groups (Figures 3 and 4). Therefore, we selected the values in the first half of PWE and compared the magnitude of changes in SAP, MAP and HR from the standing level in HSCI and CON (See Table 2). The magnitudes of increase in SAP and MAP in HSCI (22 ± 7 and 20 ± 4 mmHg, respectively) were significantly larger than those in CON (7 ± 1 and 5 ± 1 mmHg, respectively) (SAP: $P < 0.05$, MAP: $P < 0.001$). When blood pressure increases were expressed in relative terms (%) and compared, the magnitudes of increase in SAP and MAP in HSCI (30 ± 11 and 31 ± 8%, respectively) were also larger than those in CON (7 ± 1 and 5 ± 1%, respectively) (SAP: $P < 0.01$, MAP: $P < 0.001$). On the other hand, the magnitude of decrease in HR was not significantly different (12 ± 3 vs. 6 ± 1 bpm or 13 ± 3 vs. 9 ± 2% in HSCI and CON, respectively).

At sitting vs. at PWE

In HSCI, MAP and DAP during PWE were significantly higher than those during sitting, whereas there was no significant difference in the values of SAP. In CON, on the other hand, all of the blood pressure parameters showed significantly higher values during PWE than during sitting.

In HSCI and CON, HR decreased significantly during PWE compared with that during standing (Figure 4), resulting in no significant difference in HR during PWE and sitting.

Discussion

The new findings in this study were that PWE raises blood pressure above that present during quiet standing in able-bodied individuals and in SCI individuals with a lesion level at or above T6. This increase was significantly larger in the latter group. In individuals with a lesion level at or below T10, on the other hand, there was no significant change in blood pressure. We discuss below the possible physiological mechanisms underlying increases in blood pressure during PWE and the clinical implications of our results.

Circulatory responses at quiet sitting

In the present study, all of the blood pressure values in HSCI were significantly lower than those in CON, and MAP in HSCI was significantly lower than that in LSCI. On the other hand, none of the blood pressure parameters in LSCI were significantly different from those in CON. Several studies have shown that blood pressure level in a supine [6, 19, 21, 23] or seated [6, 10] resting state is lower in individuals with quadriplegia than in normal controls. However, the resting supine blood pressure level in individuals with lower thoracic and lumbar SCI is almost normal [21]. These findings are in accordance with the findings in the present study. Previous studies have shown that the lower blood pressure in individuals with quadriplegia is accompanied by a lower plasma catecholamine level [6, 10, 19, 21, 23], and the lower blood pressure has been attributed to reduction in basal sympathetic activity and subsequent low plasma catecholamine levels [6, 23].

Circulatory responses to postural change from sitting to standing

Orthostatic hypotension is characterized by a decrease in SAP by more than 20 mmHg and a decrease in DAP by more than 10 mmHg within three minutes of standing according to The Consensus Committee of the American Autonomic Society and the American Academy of Neurology [31]. In the present study, although there was no significant decrease in blood pressure by postural change from sitting to standing in any of the three groups, two individuals with cervical SCI (subject No. 1 and 2) in HSCI showed decreases in SAP and DAP to levels in the definition of orthostatic hypotension. Several studies have shown that individuals with cervical SCI are prone to orthostatic hypotension by postural change from supine to upright position, but this is not the case in individuals with thoracic SCI or able-bodied individuals [6, 18]. The development of orthostatic hypotension in chronic SCI individuals is thought be mediated by multiple factors such as sympathetic nervous system dysfunction, impaired baroreflex function, lack of skeletal muscle pumping activity and impaired water and sodium retention [7].

Possible mechanisms affecting circulatory responses during PWE

SAP and MAP increased significantly during PWE compared with those in the standing state in HSCI and CON, although DAP increased only in HSCI. In contrast to the response in blood pressure, HR decreased significantly during PWE compared to the level during standing, indicating that HR did not affect the increase in blood pressure. With regard to the HR response, HR at standing was below 100 bpm in the present study (Table 2). HR below 100 bpm is controlled mainly by cardiac vagal activity [29]. Therefore, the decrease in HR during PWE must have been due to baroreceptor-mediated parasympathetic vagal activation induced by increase in blood pressure.

Nobrega et al. [27] demonstrated that MAP increased during passive leg cycling via an increase in stroke volume in able-bodied individuals. They attributed the increase in stroke volume to an increase in venous return from the passively moved lower limbs (muscle pump effect) or to a muscle mechanoreceptor-evoked increase in myocardial contractility, the latter being thought to be mediated by a reflex neural mechanism that involves the brainstem cardiovascular center by afferent signals from the legs. Therefore, the pressor responses during PWE in CON might have been caused by a brainstem-mediated neural reflex mechanism and/or muscle pump effect. In SCI individuals, on the other hand, the possibility of contribution of a brainstem-mediated neural reflex mechanism to pressor response can be excluded because it is assumed that there is little or no afferent input to the brainstem cardiovascular center due to the spinal cord lesion.

The magnitudes of increase in SAP and MAP from the quiet standing level to the PWE level were larger in HSCI than in CON despite the fact that the magnitudes of decrease in HR in the two groups were not significantly different. It is possible that the larger

increase in blood pressure in HSCI is due to the greater muscle pump effect that enables venous return to increase by squeezing the blood out of the veins and venules of the dependent leg. The presence of this effect is suggested by the presence of electromyographic activity during PWE. However, the muscle activity level in HSCI is assumed to be very small and to be almost the same as that in CON because of no significant increase in $\dot{V}O_2$ in either HSCI or CON. Not only muscle activity level but also blood volume in the legs determines the muscle pump effect. However, Hopman et al. [16] found a decrease in venous capacity, venous distensibility and enhanced venous outflow resistance in the upright seated position in paraplegic individuals compared to those in able-bodied individuals and they attributed these changes to inactivity and muscle atrophy of the legs. From these findings, Hopman et al. [16] suggested that venous blood pooling in paralyzed legs is very unlikely. The lower blood volume capacity and the unlikelihood of blood volume pooling in the upright position suggest that the blood volume in paralyzed legs during quiet standing is also smaller in HSCI than in CON, resulting in a smaller muscle pump effect on venous return during PWE in the former group. Therefore, it is unlikely that the larger increase in blood pressure during PWE in HSCI is solely dependent on the magnitude of the muscle pump effect.

As far as we know, spinal reflexes are the only factor other than the muscle pump effect that can cause pressor responses in SCI individuals during PWE. This effect might have enhanced the magnitude of increase in blood pressure in HSCI. Mizushima et al. [23] found that MAP increased during ice-water immersion of the foot in individuals with cervical spinal cord injury with concomitant increase in plasma norepinephrine and they concluded that the rise in blood pressure by ice-water immersion was caused by a reflex sympathetic discharge through the isolated spinal cord. Mizushima et al. [23] also found that the rise in MAP during ice-water immersion, expressed as percent rise from pre-immersion baseline, was significantly higher in patients than in normal subjects. This finding is in accordance with the finding in the present study. Mizushima et al. [23] attributed the enhanced pressor response in SCI individuals to increased noradrenergic receptor sensitivity, postjunctional changes in the effector organs resulting from prolonged inactivity, and a lack of baroreceptor reflexes restraining a rise in blood pressure.

In LSCI, blood pressure was not changed significantly during PWE. In LSCI, there is little or no afferent input to the brainstem cardiovascular center from the legs as is the case in HSCI. However, the pool of blood vessels affected by spinal reflexes is smaller than that in HSCI because the affected area is limited mostly to the splanchnic vasculature innervated by the lesser (LSN) and/or least splanchnic nerves (lSN) [33] as well as to the leg vasculature. Garrison et al. [14] recently demonstrated that systemic pressure response due to spinal reflexes elicited by electrocutaneous stimulation to the arch of the foot is confined to upper level injured individuals (above T6) who have a larger pool of blood vessels affected, including the leg vasculature and splanchnic vasculature innervated not only by the LSN and lSN but also by the GSN. Therefore, the absence of pressor response in LSCI is logically explained by the loss of afferent input to the brainstem and the smaller effect of spinal reflexes. In other words, the results suggest that the enhanced pressor response in HSCI is due to exaggerated spinal reflexes resulting from a larger pool of blood vessels affected by stimuli imposed on the paralyzed area. Another possible explanation for enhanced pressure response in HSCI is as follows. Central and peripheral changes that may lead to exaggerated spinal reflexes, such as aberrant sprouting of sensory fibers in the dorsal horn [1, 32], inappropriate innervations of preganglionic axons to postganglionic neurons [22], and enhanced and prolonged contractile responses to nerve activity in arterial vessels [22], occur after SCI. These changes might have contributed to enhanced blood pressure response in HSCI.

In previous studies [24, 25, 30], SCI individuals performed passive leg cycling (PLC) in the sitting position, but this failed to increase blood pressure. The present study clearly demonstrated that whether blood pressure increases or not is dependent on lesion level. In addition, the difference in response might be dependent on the difference in stimuli imposed on the paralyzed area between PLC and PWE. It is possible that PWE is more noxious than PLC, but PWE itself is assumed to be "innocuous." Despite this, innocuous stimuli have recently been shown to have the potential to provoke spinal reflexes [20]. Since PWE is characterized by rhythmic hip joint flexion and extension with large body load on the foot, whereas PLC may produce lesser hip joint movement and body load on the foot, differences in mechanical stimuli associated with exercise modes such as joint movements and/or body load may lead to the difference in pressor response between PWE and PLC.

Clinical implications of our results

Disuse syndrome is one of the many health issues that arise following spinal cord injury and is characterized by muscle atrophy, loss of bone mineral density, joint

contracture, and circulation disorders (which can lead to the occurrence of deep vein thrombosis and pressure sores) in the paralyzed area. Passive standing therapy is thought to be useful for preventing bone mass loss [3, 13] (which is caused by mechanical stress due to weight bearing) [15], preventing joint contracture by providing an adequate range of motion [3, 13] and allowing sustained periods of relief to the sacral and ischial high-pressure areas of the buttocks, thus reducing the incidence of pressure sores [3, 13]. In addition to these positive effects, it has been shown that the imposition of passive walking-like movement in the standing posture induces locomotor-like muscle activity and an increase in oxygen supply in the paralyzed lower limb muscles in individuals with motor-complete spinal cord injury [17]. Furthermore, 4 months of thrice-weekly body weight-supported treadmill training has been shown to improve muscle morphology in a chronic motor-complete individual with spinal cord injury [2]. These physiological and morphological responses indicate that PWE has the potential to attenuate deterioration of disuse syndrome that arises in paralyzed lower limbs.

On the other hand, with the upright position, there are risks of orthostatic hypotension and its secondary symptoms such as dizziness, nausea, lightheadedness, and even loss of consciousness. In the present study, although two individuals with cervical SCI showed decreases in SAP and DAP at standing to levels in the definition of orthostatic hypotension, they also showed increases in SAP and DAP during PWE above the level at sitting. Therefore, we believe that PWE itself has an inhibitory effect on orthostatic hypotension.

In the present study, one of the SCI individuals (subject No. 2) showed an increase in SAP during PWE by more than 20 mmHg from the sitting level (usual level). This value matches that of autonomic dysreflexia defined by the Consortium for Spinal Cord Medicine [8]. If other hypertensive stimuli such as bladder distension and bowel impaction are added during PWE, extreme hypertension may be induced. Therefore, appropriate treatment or preparation, such as urination, is needed for SCI individuals before the start of PWE.

Limitations

Since the population of SCI individuals is a very specific and complex population, it was difficult to find a large number of subjects who fulfill the inclusion criteria. However, even with the relatively small number of subjects, it was shown that the main outcome parameter of the present study, i.e., blood pressure, was increased significantly during PWE even in SCI individuals, especially in SCI individuals with lesion level at or above T6. Therefore, we do not believe that a larger number of subjects would have dramatically influenced the main outcome of the study. On the other hand, blood pressure responses in LSCI might have been different with a large number of subjects and/or depending on the choice of subjects. For example, in the present study, one subject in LSCI (subject No. 10) had been performing regular body weight-supported treadmill training. Ditor et al. [12] found that thrice-weekly 4-month treadmill training in motor-complete SCI individuals affected resting heart rate variability. Such an adaptation to training may also have occurred in response to PWE in subject No. 10 and thus have altered our results, although the statistical results for cardiovascular responses in LSCI were the same even when subject No. 10 was removed.

Conclusion

Blood pressure increases during passive walking-like exercise in SCI individuals with lesion level at or above thoracic 6. The increase in blood pressure elicited by the application of passive walking-like exercise could represent an integrated response involving both an effect of spinal reflex in the region below the spinal cord injury and a muscle pump effect. Our findings aid evaluation of the safety of passive walking-like exercise, which is becoming a common rehabilitation technique, in SCI individuals of various lesion levels, including high level SCI individuals who are susceptible to orthostatic hypotension and autonomic dysreflexia.

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Age-related changes of the stretch reflex excitability in human ankle muscles

Hiroki Obata [a,b,*], Noritaka Kawashima [b,c], Masami Akai [b], Kimitaka Nakazawa [b], Tatsuyuki Ohtsuki [a]

[a] Department of Life Sciences, Graduate School of Arts and Sciences, Sports Science Laboratory, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan
[b] Department of Rehabilitation for Movement Functions, Research Institute, National Rehabilitation Center for Persons with Disabilities, 4-1 Namiki, Tokorozawa, Saitama 359-8555, Japan
[c] Japanese Society for the Promotion of Science, Japan

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ABSTRACT

The purpose of this study was to characterize the effects of aging on the stretch reflex in the ankle muscles, and in particular to compare the effects on the ankle dorsi-flexor (tibialis anterior: TA) and the plantar-flexor (soleus: SOL). Stretch reflex responses were elicited in the TA and SOL at rest and during weak voluntary contractions in 20 elderly and 23 young volunteers. The results indicated that, in the TA muscle, the elderly group had a remarkably larger long-latency reflex (LLR), whereas no aging effect was found in the short latency reflex (SLR). These results were very different from those in the SOL muscle, which showed significant aging effects in the SLR and medium latency reflex (MLR), but not in the LLR. Given the fact that the LLR of the TA stretch reflex includes the cortical pathway, it is probable that the effects of aging on the TA stretch reflex involve alterations not only at the spinal level but also at the cortical level. The present results indicate that the stretch reflexes of each of the ankle antagonistic muscles are affected differently by aging, which might have relevance to the neural properties of each muscle.

1. Introduction

It is well recognized that aging affects the performance of motor tasks, such as the maintenance of posture and balance control (Bohannon et al., 1984; Horak et al., 1989). It is presumed that both the central and peripheral nervous systems contribute to these phenomena, but the precise mechanisms are still unknown (Stelmach et al., 1989). Stretch reflex, a simple neural circuit that responds to the sudden stretch of a muscle, might be also affected by aging.

Some investigations have paid attention to the effect of aging on the spinal reflex modulation of the soleus (SOL) muscle, which is a key muscle for postural control and bipedal walking (Koceja et al., 1995; Angulo-Kinzler et al., 1998; Chalmers and Knutzen, 2002; Kawashima et al., 2004). We previously demonstrated that elderly subjects show augmented short stretch reflex response in the SOL muscle at rest as compared to those in young subjects (Kawashima et al., 2004). It has also been demonstrated that elderly subjects lack the ability to modulate the SOL motoneuronal excitability for instance, Koceja et al. (1995) showed abnormal modulation of the motoneuronal excitability by postural changes.

On the other hand, recent studies have suggested that the stretch reflex of the tibialis anterior (TA), the antagonistic muscle of the SOL muscle, also has an important role in stabilizing the ankle joint during upright standing (Nakazawa et al., 2003), and the early stance phase of walking (Christensen et al., 2001; Nakazawa et al., 2004). However, to the best of our knowledge, only one study has examined the changes of the TA muscle due to aging. Although Nardone et al. (1995) reported that the amplitude of the reflex action obtained in the TA muscle during upright standing did not depend on age; it remains unknown how the stretch reflex function of the TA muscle changes with age, and whether or not the aging process of this muscle is identical to those in other muscles.

It is well known that each of the SOL and TA muscle have different neuronal characteristics, such as different degrees of connection to the motor cortex (Bawa et al., 2002), and different modulation effects on the spinal reflex excitability (Katz et al., 1988). Interestingly, in contrast to the SOL muscle, the TA stretch reflex shows a larger long-latency reflex (LLR) component, which presumably involves the transcortical pathway (Petersen et al., 1998). Given these facts, it is very likely that the aging process of the stretch reflex in the TA muscle is different from that in the SOL muscle.

The purpose of this study was therefore to characterize the effect of aging on the stretch reflex in the ankle muscles, and in particular how they differ between the ankle dorsi-flexor TA and the plantar-flexor SOL.

2. Methods

2.1. Subjects

Twenty healthy elderly volunteers (mean age 68.0 ± 5.9 years, male = 11, female = 9) and twenty-three young healthy volunteers

* Corresponding author. Address: Department of Life Sciences, Graduate School of Arts and Sciences, Sports Science Laboratory, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan. Tel.: +81 3 5454 6887; fax: +81 3 5454 4317.
E-mail addresses: hirokiobata@gmail.com, obata@rehab.go.jp (H. Obata).

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(22.4 ± 1.8 years, male = 11, female = 12) with no history of neurological or muscle disorders participated in the present study. The subjects gave their informed consent to participate in this study, which was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the National Rehabilitation Center for Persons with Disabilities, Tokorozawa, Japan.

2.2. Procedures

Each subject remained seated comfortably in a chair with the right leg fixed to a footplate connected to a servo-controlled torque motor with a rotary encoder (Senoh Inc., Tokyo, Japan). The axis of rotation of the footplate was aligned with the axis of rotation of the ankle joint. The hip and knee angles were fixed at 40° and 50° flexion positions, respectively (anatomical position was 0°). The electromyographic (EMG) activities in the SOL and TA muscles were recorded using bipolar surface electrodes (Ag/AgCl, diameter 7 mm) placed on the muscle belly at an inter-electrode distance (center to center) of 15 mm. The EMG signals were amplified and band-pass-filtered by a bipolar differential amplifier (AB-621B Nihon Kohden, Tokyo, Japan) with low and high cut-off frequencies between 15 and 1000 Hz, respectively. The EMG, torque and angle signals were digitalized at a sampling rate of 1 kHz (WE7251; Yokogawa Electric Co., Tokyo, Japan) and stored in a computer.

Prior to the stretch reflex tests, the subjects were asked to exert the maximal isometric voluntary contraction for the SOL and the TA muscle in turn in order to determine the maximal EMG level. In this manuscript, the term "MVC" indicates the EMG level during maximal isometric voluntary contraction. The subjects were asked to maintain two contraction levels, i.e., rest (condition REST) and weak voluntary contraction (approximately 10% MVC: condition ACT) in the TA for the TA stretch test and in the SOL for the SOL stretch test. To control the contraction level, the smoothed full-wave rectified EMG and the reference line corresponding to 0% and 10% MVC were displayed on an oscilloscope, and the subjects were asked to keep a rectified EMG at a target level. A quick stretching of the TA or the SOL was given by imposing a quick rotation of the ankle joint at a range from 10° plantar-flexion to 5° dorsi-flexion. The direction of the rotation was plantar-flexion for the TA stretch test and dorsi-flexion for the SOL stretch test. Three different angular velocities, approximately 150 (slow: SL), 250 (moderate: MD), and 350°/sec (fast: FS) were applied five times in random order and at random stimulus intervals (10–15 s). The orders of stretching and contraction levels were randomized.

2.3. Data analysis

The digitized EMG signals were full-wave rectified after subtraction of the DC bias. The mean background EMG activity (BGA) level was then subtracted from the evoked EMG responses. The BGA was calculated during the 100 ms prior to the onset of stretches. In the present study, the incidence of reflex elicitation was calculated to show how often the stretch reflex response appeared in each group and for each contraction level and angular velocity. The number of observed responses was expressed as the ratio to the total number of stretches. The criterion of reflex appearance used for the probability was whether an EMG response reached a level higher than the BGA plus three times its standard deviation (BGA + 3SD). Stretch reflex responses were divided into their short- (SLR), middle- (MLR), and LLR components. The onset and the endpoint of the stretch reflex response were defined in the same manner as the probability of reflex elicitation. In accordance with previous studies (Schieppati and Nardone, 1997; Christensen et al., 2001), the onset of MLR was defined as 20 ms after the onset of SLR and that of LLR was defined in as 20 ms after the onset of MLR. Thus, the duration of SLR and MLR was defined as 20 ms, respectively. The duration of LLR was determined from the above mentioned onset to the endpoint of the stretch reflex response. In the present study, the mean amplitude of the rectified EMG with the BGA value subtracted was used to evaluate the size of each stretch reflex component.

The statistical differences of incidences were tested by the χ^2-test at each muscle contraction level and angular velocity. Statistical differences in each reflex component were tested by two-way analysis of variance (ANOVA, 2×2, muscle contraction level × group). Scheffé's post-hoc comparisons were used to determine the statistical differences between the REST and ACT conditions and between elderly and young groups. The significance level was set at $P < 0.05$.

3. Results

3.1. Background EMG activity

We confirmed that the level of BGA of the SOL and TA muscles were similar between the elderly and young groups. In the SOL stretch test, the SOL BGA at REST were 0.4 ± 0.07 and 0.4 ± 0.08% MVC for young and elderly, respectively and increased to 9.6 ± 0.59 and 9.9 ± 0.74% MVC at ACT. In the TA stretch test, the TA BGA at REST were 0.4 ± 0.19 and 0.5 ± 0.14% MVC for young and elderly, respectively and increased to 8.6 ± 0.33 and 9.6 ± 0.60% MVC at ACT.

3.2. Probability of reflex elicitation

In the present study, the stretch reflex responses of the TA appeared more frequently in the elderly subjects than in the young subjects. The probability of reflex elicitation in each condition, stretch speed, and muscle is summarized in Table 1. As shown in this table, a stretch reflex response could not be obtained in all subjects. Moreover, the number of responses increased with angular velocity and contraction level. The probability of the TA stretch reflex response in REST was statistically higher in the elderly subjects than in the young subjects at each angular velocity (χ^2-test, $P < 0.05$). On the other hand, the probability of the SOL stretch reflex response in REST tended to be lower in the elderly subjects (χ^2-test, $P < 0.05$). Therefore, we calculated the latency and amplitude of the reflex responses only at the fastest velocity and for subjects who responded to both the REST and ACT conditions in each stretch test.

3.3. Stretch reflex EMG responses

Fig. 1 shows typical waveforms of stretch reflex responses in the TA and the SOL under both postural conditions, REST and ACT, obtained from one subject in each group. As clearly shown in this figure, there was a remarkable difference in the reflex amplitude between the TA and SOL stretch reflex responses. The elderly subjects showed a relatively larger (Fig. 2) and longer response (Table 2) in both REST and ACT in the TA.

3.4. Latency and duration of the stretch reflex EMG responses

The onset of the TA stretch reflex response was earlier in the ACT than in the REST ($F_{(1, 32)} = 15.8$, $P < 0.01$), while no difference was found in latency between the two subject groups (Table 2). In contrast, there was no effect of the contraction level in the SOL stretch reflex response, whereas the effect of group was significant ($F_{(1, 32)} = 23.1$, $P < 0.01$) in this muscle.

An interaction between groups and contraction levels was found in the durations of the TA stretch reflex ($F_{(1, 32)} = 16.0$,

Table 1
The probability of reflex elicitation at each angular velocity for the (a) TA and (b) SOL stretch tests. *Significant difference ($P < 0.05$).

(a) TA

	Slow	Moderate	Fast
Elderly			
REST	39.0% (10/20)	57.0% (13/20)	71.0% (19/20)
ACT	58.0% (16/20)	75.0% (20/20)	81.0% (20/20)
Young			
REST	19.1% (5/23)	31.3% (12/23)	36.5% (14/23)
ACT	53.0% (15/23)	77.4% (22/23)	80.9% (22/23)

(b) SOL

	Slow	Moderate	Fast
Elderly			
REST	84.0% (17/20)	85.0% (18/20)	88.0% (18/20)
ACT	92.0% (20/20)	98.0% (20/20)	100.0% (20/20)
Young			
REST	99.1% (23/23)	98.3% (23/23)	99.1% (23/23)
ACT	98.3% (23/23)	100.0% (23/23)	100.0% (23/23)

Percentage of number of responses to number of stretches (number of responding subjects/total number of subjects)

$P < 0.01$). The duration of the TA stretch reflex was longer in ACT than in REST only in the young subjects ($P < 0.05$). On the other hand, there was no effect of group or contraction level in the SOL.

3.5. Stretch reflex components

Summarized data for each of three reflex components (SLR, MLR, and LLR) are shown in Fig. 2. Differences in the effects of aging between the TA and SOL muscles were mainly found in LLR. The group effect was significant only in LLR of the TA stretch reflex ($F_{(1, 32)} = 4.7$, $P < 0.05$): the elderly subjects had larger LLR responses than the young subjects in both contraction levels ($P < 0.05$).

Another difference was found in the SOL, that is, the elderly subjects lacked a significant augmentation in the reflex response size according to the muscle contraction level in SLR and MLR. The augmentation was found only in the young subjects in SLR and MLR ($P < 0.05$). On the other hand, the effect of muscle contraction was significant in all three reflex components in the TA (SLR: $F_{(1, 32)} = 42.5$, MLR: $F_{(1, 32)} = 20.6$, LLR: $F_{(1, 32)} = 7.7$, $P < 0.05$): each component in both groups was larger in ACT than in REST.

The most remarkable age-related differences were found in the LLR component of the TA muscle, and there were distinct differences in the relative EMG magnitudes of the SLR and LLR components between the SOL and TA muscle. Fig. 3 shows the size of the LLR relative to the SLR component. It is clear that the LLR component is larger in the TA muscle, especially in the elderly group. Two-way ANOVA (muscle contraction level × group) revealed a significant main effect of "group" in the TA LLR ($F_{(1, 57)} = 13.12$, $P < 0.01$). A post-hoc test showed statistically significant differences between the elderly and young groups under the REST and ACT conditions in the TA LLR.

4. Discussion

The purpose of the present study was to examine the effect of aging on the stretch reflex in the ankle muscles. Our interest was whether the aging process of the ankle dorsi-flexor TA is identical to that in the ankle plantar-flexor SOL. We characterized the differences in the effects of aging on these muscles by observing not only the amplitude of each reflex component and the reflex latency, but also the incidence of the stretch reflex responses when perturbations were applied. The present results clearly demonstrated the following four differences in the elderly subjects in comparison to the young subjects, namely, (1) higher probability of reflex elicitation, (2) longer duration of the reflex response in the TA at REST, (3) larger LLR in the TA at both contraction levels, and (4) lack of contraction level-related augmentation of SLR and MLR in the SOL.

These results suggest that the effect of aging on the stretch reflex is different between the TA and SOL muscles. In the following section, the possible neurological mechanisms underlying these results are discussed.

4.1. Effects of aging on the short and medium stretch reflex component

As clearly shown in the data obtained from the young subjects, the SLR and MLR in both the TA and SOL muscles showed marked increases from the REST to ACT conditions. This facilitated stretch reflex response, called automatic gain compensation, is regarded as an important function to adjust the reflex excitability in accordance with the pre-activation level of each muscle (Matthews, 1986). It can be simply explained by the prior recruitment of more motor units or enhanced sensitivity of the muscle spindle due to α–γ linkage. However, in the elderly subjects, the SLR and MLR of the SOL muscle showed no significant changes between the REST and ACT conditions, although the level of BGA was very different. This result is consistent with the previous results (Kawashima et al., 2004; Chung et al., 2005). In our previous study, we suggested that histochemical alterations in muscle fibers accompanying the

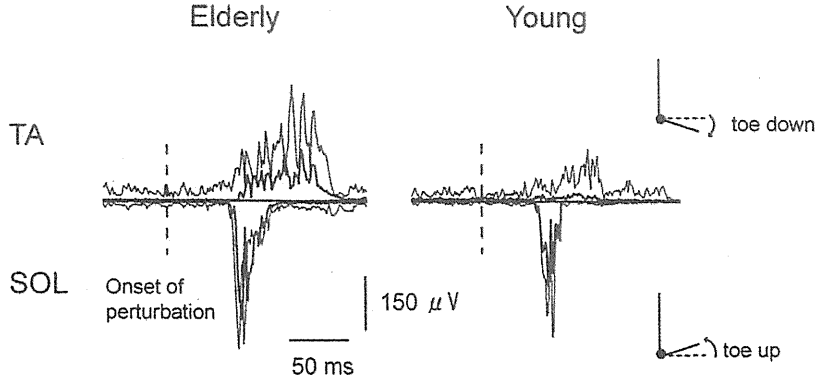

Fig. 1. Typical waveforms of the stretch reflex responses in the TA and the SOL at moderate velocity. The thick line indicates the EMG responses under the REST condition, and the thin line, under the ACT condition. The vertical dotted line indicates the onset of stretch.

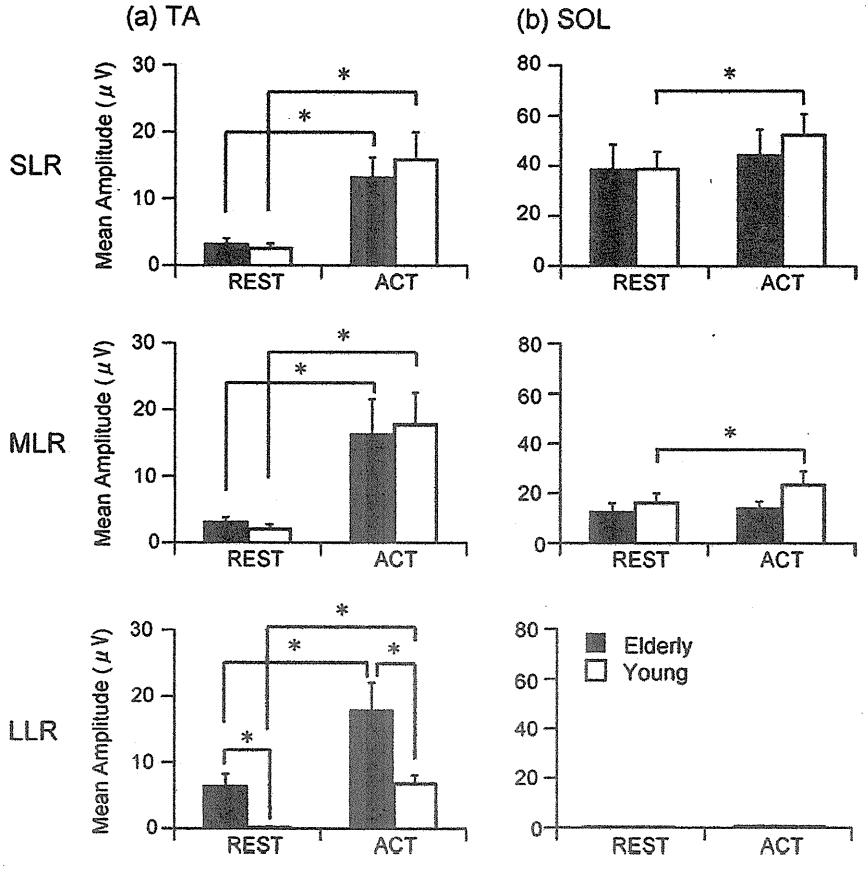

Fig. 2. Comparisons of the mean amplitude of each component between the elderly and young groups and REST and ACT conditions in the (a) TA and (b) SOL stretch reflex responses. The error bars indicate the SE of the mean value. *Significant difference ($P < 0.05$).

Table 2
Means ± SE for latency and duration of the stretch reflex responses in the (a) TA and (b) SOL stretch reflex responses. *Significant difference ($P < 0.05$).

(a) TA

	Latency (ms)	Duration (ms)
Older		
REST	76.8 ± 6.6	63.2 ± 7.8
ACT	65.6 ± 6.5 *	65.7 ± 4.2 *
Younger		
REST	69.5 ± 6.6	42.9 ± 5.1
ACT	60.2 ± 5.2 *	58.0 ± 3.8 *

(b) SOL

	Latency (ms)	Duration (ms)
Older		
REST	47.2 ± 0.9	33.9 ± 2.2
ACT	47.1 ± 0.7 *	35.7 ± 1.3 *
Younger		
REST	43.6 ± 0.9	34.9 ± 1.9
ACT	42.8 ± 0.7 *	37.0 ± 1.3 *

aging process, such as stiff intrafusal fibers, might affect the SLR in the SOL muscle because the excitability of the motor-neuron pool evaluated with the H-reflex was not different in elderly and young subjects (Kawashima et al., 2004). This explanation may also be applicable to the present result.

4.2. Effect of aging on the long-latency reflex component

There has been some dispute regarding the physiological pathways of the LLR. For the distal muscles in the upper extremities, which are more predominantly under cortical control, the LLR is commonly assumed to involve supraspinal mechanisms (Matthews, 1991). In the lower limb, the neural pathway underlying LLR is likely to depend on the muscle. In the SOL muscle, some authors have reported that the spinal pathway is responsible (Tracey et al., 1980; Koceja and Kamen, 1992), whereas others have indicated that it is the transcortical pathway (Taube et al., 2006). In the TA muscle, it is now assumed that the transcortical pathway is largely involved in the LLR response (Petersen et al., 1998; van Doornik et al., 2004). Therefore, the larger LLR response in the TA muscle in the elderly subjects may indicate that the effect of aging on the TA stretch reflex includes the change in the excitability of the motor cortex. Similarly, Lin and Sabbahi (1998) observed larger stretch reflex magnitude of the long-latency responses in the wrist muscles of the elderly group, and they suggested that the age-related changes of the stretch reflex system involve supraspinal mechanisms.

It is noteworthy that the larger magnitude and longer duration of the LLR component in the elderly subjects were found even at REST. This result suggests that elderly persons have a deficit of adequate suppression of the LLR under resting condition. The enhanced LLR in the TA muscle is known in patients with Parkinson's disease (Ridding et al., 1995). Given the fact that intracortical inhibition is reduced with aging (Peinemann et al., 2001) and in patients with Parkinson's disease (Ridding et al., 1995), the inhibitory mechanisms of the motor cortex might have contrib-

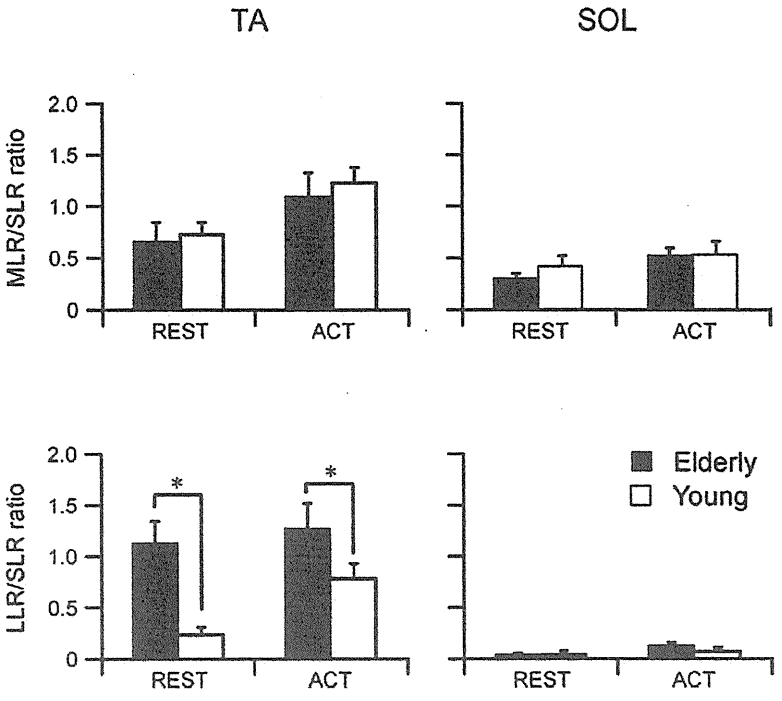

Fig. 3. Comparisons of the amplitude ratios between stretch reflex components between elderly and young groups and REST and ACT conditions in both the TA and the SOL muscles. The error bars indicate the SE of the mean value. *Significant difference ($P < 0.05$).

uted to the present result. Although it is not clear whether or not the same neural mechanism contributes, age-related alteration in the supraspinal mechanism, such as the transcortical feedback loop from the basal ganglia to the motor cortex, may at least in part contribute to the present result.

Some previous studies have investigated the age-related change in the cortico-spinal pathway by using transcranial magnetic stimulation. These studies have shown that greater stimulus intensities are required to reach the same maximal motor output in elderly subjects (Pitcher et al., 2003). Their result suggests that the enhanced LLR component observed in the present study cannot be solely explained by the age-related change in the cortico-spinal pathway. Nevertheless, since the previous investigations have been targeted at the FDI muscle, the involvement of the cortico-spinal pathway cannot be completely ruled out. Alternatively, the enlarged LLR response could be explained by age-related alterations in the peripheral muscle and/or tendon properties, such as the increased half-relaxation time of tendon reflexes previously reported in the elderly (Carel et al., 1979; Koceja et al., 1993). Because detailed information about the mechanism of the larger LLR in the elderly is not available at present, future studies using the TMS technique, such as a short-interval intracortical inhibition or intracortical facilitation will need to be conducted to examine this possibility.

4.3. Different aging process of the stretch reflex between TA and SOL muscles

The present results indicated the occurrence of remarkable age-related changes in the LLR in the TA and SLR and MLR in the SOL. Such differences between the ankle plantar- and dorsi-flexor muscles might be explained by the different neurological and biomechanical features of those muscles. It is well recognized that the connection to the motor cortex is stronger in the TA than in the SOL muscle (Bawa et al., 2002), and that the activity of the ankle dorsi-flexor is more under the control of a supraspinal mechanism (Armstrong, 1988). Dietz indicated in his review article that "the CNS determines the TA response, whereas the SOL response is dominantly modulated by peripheral information" (Dietz, 1992). Moreover, the present result of the TA stretch reflex is similar to that observed in the wrist muscles, in which a larger LLR has been reported with aging (Lin and Sabbahi, 1998). Biomechanical factors are also likely to contribute to the difference. It is well known that the ankle plantar-flexor muscle, but not the ankle dorsi-flexor muscle, acts as an antigravity muscle during standing. Since the ankle plantar-flexor is selectively exposed to body weight for a long time, some peripheral changes are more progressive within the ankle plantar-flexor than they are within the ankle dorsi-flexor.

In conclusion, the present results demonstrated that the effects of aging on the stretch reflex are different in the TA and the SOL. Such differences seem to be related to the different neurological and biomechanical properties of those muscles.

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RESEARCH ARTICLE

Effect of sensory inputs on the soleus H-reflex amplitude during robotic passive stepping in humans

Kiyotaka Kamibayashi · Tsuyoshi Nakajima · Masako Fujita · Makoto Takahashi · Tetsuya Ogawa · Masami Akai · Kimitaka Nakazawa

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Abstract We investigated the modulation of the soleus (Sol) Hoffmann (H-) reflex excitability by peripheral sensory inputs during passive stepping using a robotic-driven gait orthosis in healthy subjects and spinal cord-injured patients. The Sol H-reflex was evoked at standing and at six phases during passive stepping in 40 and 100% body weight unloaded conditions. The Sol H-reflex excitability was significantly inhibited during passive stepping when compared with standing posture at each unloaded condition. During passive stepping, the H-reflex amplitude was significantly smaller in the early- and mid-swing phases than in the stance phase, which was similar to the modulation pattern previously reported for normal walking. No significant differences were observed in the H-reflex amplitude between the two unloaded conditions during passive stepping. The reflex depression observed at the early part of the swing phase during passive stepping might be attributed to the sensory inputs elicited by flexion of the hip and knee joints. The present study provides evidence that peripheral sensory inputs have a significant role in phase-dependent modulation of the Sol H-reflex during walking, and that the Sol H-reflex excitability might be less affected by load-related afferents during walking.

Keywords Hoffmann reflex · Reflex modulation · Load-related afferent input · Locomotion · Spinal cord injury

K. Kamibayashi (✉)
Graduate School of Systems and Information Engineering, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan
e-mail: kamibayashi@iit.tsukuba.ac.jp

K. Kamibayashi · T. Nakajima · M. Takahashi · M. Akai · K. Nakazawa
Department of Rehabilitation for Movement Functions, Research Institute of National Rehabilitation Center for Persons with Disabilities, 4-1 Namiki, Tokorozawa, Saitama 359-8555, Japan

M. Fujita
Graduate School of Engineering, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama, Saitama 337-8570, Japan

M. Takahashi
Graduate School of Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, Hiroshima 734-8551, Japan

T. Ogawa
Graduate School of Human Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359-1192, Japan

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Introduction

It is now generally recognized that the Hoffmann (H-) reflex, which is evoked by an electrical stimulation of group Ia afferents, is strongly modulated in a task-dependent manner between different motor tasks, and in a phase-dependent manner during rhythmic movements, such as walking, running, and pedaling in humans (Capaday and Stein 1986, 1987; Crenna and Frigo 1987; Brooke et al. 1991; Simonsen and Dyhre-Poulsen 1999; Schneider et al. 2000; Zehr 2002; Ethier et al. 2003; Stein and Thompson 2006). For example, the H-reflex excitability in the soleus (Sol) muscle is significantly lower during walking than during standing (Capaday and Stein 1986; Zehr 2002; Ethier et al. 2003). Furthermore, during walking, the Sol H-reflex amplitude increases progressively in the stance phase, while it is very small or totally absent in the swing

phase (Capaday and Stein 1987; Simonsen and Dyhre-Poulsen 1999; Ethier et al. 2003).

Although modulation of reflex excitability is commonly considered to be functionally important for locomotion, neural mechanisms of reflex modulation are still not fully understood. A passive movement paradigm has often been used to investigate the mechanisms of reflex modulation during human movements (McIlroy et al. 1992; Brooke et al. 1993; Cheng et al. 1995; Misiaszek et al. 1995). Passive movement substantially reduces the influence of descending commands onto spinal motoneurons and presumably onto segmental interneurons. Therefore, changes in the H-reflex amplitude during passive movement are considered to be due to peripherally mediated sensory signals. Due to experimental difficulty in investigating the reflex modulation during walking, passive lower-limb pedaling has been substituted for passive limb movement in a walking manner (McIlroy et al. 1992; Brooke et al. 1993; Cheng et al. 1995; Misiaszek et al. 1995). The results obtained from the passive pedaling studies have revealed that the reflex amplitude in the Sol muscle substantially decreases with limb rotation, and that the degree of inhibition is dependent on the cycle phase, which is pronounced when the hip and knee joints are flexed (McIlroy et al. 1992; Cheng et al. 1995). In addition, increasing the speed of passive pedaling enhances the H-reflex depression (McIlroy et al. 1992; Cheng et al. 1995). Therefore, it has been concluded that sensory inputs from the muscle spindle have a powerful influence on the H-reflex excitability.

However, how observations from these passive "pedaling" studies can be generalized to the "walking" is still unclear. Although cyclic pedaling consists of multisegmental movements in the lower limb, the posture of pedaling differs from the upright posture of walking. Furthermore, load-related sensory inputs during walking, which occur rhythmically through foot contact with ground, are different from those that occur during pedaling. This load-related sensory information is considered to be one of the important sensory inputs that control the locomotor activity (Harkema et al. 1997; Van de Crommert et al. 1998; Dietz and Duysens 2000; Dietz et al. 2002). In cats, it has been suggested that the afferent inputs from load receptors act on the spinal central pattern generator (Duysens and Pearson 1980; Pearson and Collins 1993; Duysens et al. 2000). In humans, the essential role of the load-related inputs to locomotor activity has also been reported in patients with spinal cord injury (SCI) (Harkema et al. 1997). Bastiaanse et al. (2000) suggested that the load receptors are involved in phasic modulation of the medium latency response of the cutaneous reflex during walking. The main receptors for detecting load information in mammals are considered to be the Golgi tendon organs and cutaneous receptors on the soles of feet (Duysens et al. 2000). Additionally, muscle spindle and joint receptors, like Ruffini endings and Pacinian corpuscles, are thought to be accessory receptors for load information. Grey et al. (2007) found that the feedback from the load receptors, especially Golgi tendon organs, contributed to the enhancement of the ankle extensor muscle activity during the late-stance phase of human walking.

Brooke et al. (1995) reported that the Sol H-reflex was attenuated over an entire passive stepping cycle manipulated by an experimenter. The study by Brooke et al. (1995), however, was performed while subjects were lying supine or tilted from the vertical position. Knikou and Conway (2001) observed that applying mechanical pressure to the foot sole inhibits the H-reflex during sitting. Therefore, it is hypothesized that load-related inputs elicited during passive stepping may also affect the Sol H-reflex excitability. On the other hand, with regard to the Ib pathway from the gastrocnemius muscle to the Sol muscle, it has been shown that, although an electrical stimulation of Ib afferents inhibited the Sol H-reflex during lying supine and sitting, the Ib inhibition was mostly absent in conditions that involved a form of loading (Faist et al. 2006).

As an alternative source for H-reflex modulation during normal walking, a central origin has been proposed. Some studies have shown that the Sol H-reflex inhibition is closely associated with the activation of the antagonistic muscle, and have suggested that the centrally produced reciprocal inhibition serves as the mechanism for H-reflex inhibition during the swing phase (Lavoie et al. 1999; Schneider et al. 2000). By investigating the H-reflex modulation during passive stepping without the tibialis anterior (TA) muscle activity, the contribution of reciprocal inhibition to the Sol H-reflex modulation might be excluded. In addition, Garrett et al. (1999) have suggested that the H-reflex modulation during walking is associated with the descending motor command that produces the stepping movement. Therefore, investigation of the H-reflex modulation in SCI patients during passive stepping might have considerable significance for clarifying the effect of the supraspinal input on the H-reflex modulation.

As driven gait orthosis (DGO) has recently been developed as a rehabilitation device for locomotor training of patients with gait disorders (Colombo et al. 2000), applying the DGO to healthy humans makes it possible to impose passive stepping. Therefore, the aim of this study was to investigate whether the Sol H-reflex is modulated in a phase-dependent manner by substantially reduced descending motor command during passive stepping using DGO in healthy subjects and SCI patients. Furthermore, this study also aimed to investigate the effects of the load-related inputs by comparing the Sol H-reflex amplitudes at different body weight unloading conditions during passive stepping.

Methods

Subjects

Ten healthy subjects (6 male and 4 female) with no history of neuromuscular disorders (22–32 years), two clinically motor-complete SCI subjects (32-year-old woman, lesion level T7, duration of injury 83 months; 19-year-old man, lesion level T12, duration of injury 6 months), and one motor-incomplete SCI subject (21-year-old male, lesion level T5, duration of injury 7 months) participated in this study. This study was conducted with an ethical approval from the local ethics committee. Each subject provided informed consent for the experimental procedures as required by the Declaration of Helsinki.

Stepping condition

Passive stepping was conducted using a DGO (Lokomat®, Hocoma AG, Switzerland, Fig. 1), a detailed description of which can be found elsewhere (Colombo et al. 2000). Briefly, DGO provides electromechanical drives for physiological hip and knee joint movements like normal walking, and imposes stepping in SCI patients and healthy subjects with substantially reduced descending command. The DGO was secured to the subject with straps across the pelvis and chest. The lower-limb parts of the orthosis were fixed to the subject with straps around the thigh and shank.

Two passive stepping conditions were performed at 1.5 km/h under different body weight unloading conditions. One was passive stepping on a treadmill with 40% unloading of body weight (ground stepping). The other stepping condition was full (100%) body weight unloading, which means that the subject was suspended with the DGO in air so that his/her feet did not touch the treadmill during passive stepping (air stepping). Body weight unloading was achieved by a parachute harness connected to counter weights (Fig. 1). Although dorsiflexion of the ankle joint during the swing phase was achieved by passive foot lifters (spring-assisted elastic straps), an ankle foot orthosis (fixed at 5° dorsiflexion) was attached to minimize movement of the ankle joint in both stepping conditions (Fig. 1). During passive stepping, the subject was instructed to relax and allow the lower-limb movements imposed by the DGO. Because body weight-supported treadmill training for SCI generally starts at a stepping speed of 1.5 km/h with 40% unloading, this stepping condition was selected from a safety standpoint for the SCI patients. In addition, the slow stepping speed allowed the healthy subjects and SCI patients to relax easily during passive stepping.

Measurements

Electromyographic (EMG) activities from the rectus femoris, biceps femoris, medial gastrocnemius, Sol, and TA muscles in the right leg were recorded with surface bar-electrodes (inter-electrode distance 10 mm) placed over the muscle bellies. The EMG signals were amplified (1,000×) and band-pass filtered (15–1,000 Hz) using a bioamplifier (MEG-108, Nihon Kohden, Japan). For measurements of the maximal voluntary EMG activities in the Sol and TA muscles, the healthy subjects performed maximum voluntary contractions of plantar flexion and dorsiflexion under the standing posture. During passive stepping, the joint angles at the hip and knee of orthosis were provided by potentiometers of the DGO. The ankle joint angle was recorded by an electrogoniometer (SG110, Biometric Ltd, UK) attached to the anterior aspect of the lower leg and foot. Ground contact of the heel during ground stepping was detected by a pressure-sensitive sensor (PH-463, DKH, Japan) placed under the heel. All signals from the right leg were sampled at 2 kHz using an A/D converter (WE 7000, Yokogawa Co. Ltd, Japan) and stored for later analyses.

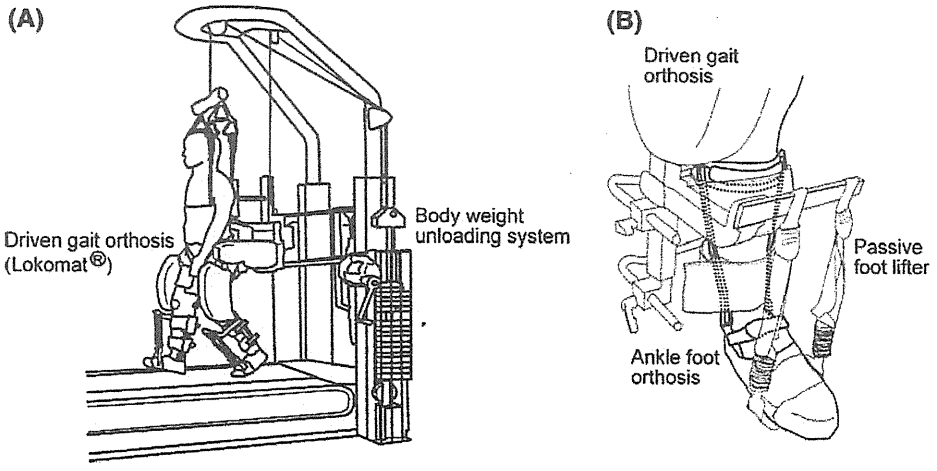

Fig. 1 **a** Schematic illustration of the experimental set-up for this study. Passive stepping was controlled by a driven gait orthosis (DGO; Lokomat®). Body weight of a subject was unloaded by a parachute harness. **b** Schematic illustration of the ankle joint with a passive foot lifter (spring-assisted elastic strap) and ankle foot orthosis

H-reflex recording

Before recording the Sol H-reflex, subjects had sufficient practice for passive stepping, while EMG signals of the lower limb muscles (rectus femoris, biceps femoris, medial gastrocnemius, and TA muscles) were continuously displayed on an oscilloscope (TDS 3014B, Tektronix, USA). During the H-reflex recording, an experimenter monitored the EMG activities in these muscles on a computer screen. When EMG activity during passive stepping was observed by the visual inspection, stimulation for the H-reflex was interrupted and the subject was instructed to relax. After disappearance of the EMG activity, stimulation was applied again.

The Sol H-reflex was elicited by stimulating the posterior tibial nerve (1-ms square pulse) using a cathode in the popliteal fossa and an anode placed over the patella with an electrical stimulator (SEN-7203, Nihon Kohden, Japan). During passive stepping, an output signal from the Lokomat® system was used as a trigger signal for electrical stimulation. The output signal was generated at a constant angle of right hip joint during stepping. Before the H-reflex recording, subjects performed passive ground stepping to determine six trigger delays for the application of stimulation after the output signal. The six trigger delays corresponded to six different step phases that were equivalent to the early-, mid-, and late-phases of the stance and swing. Because the hip and knee trajectories during passive stepping were under direct computer control for both stepping conditions, no differences were observed in the hip and knee joint angles at the determined stimulation timing between ground stepping and air stepping. By a stimulation method using a hip joint signal from the DGO, Querry et al. (2008) reported that stimulation accuracy was within 0.5° for a defined hip joint position. During passive stepping, the stimulation was randomly delivered at six predetermined phases of a step cycle with greater than 5-s intervals. The constancy of the M-wave size normalized to the maximum M-wave (Mmax) was needed for recording the H-reflexes evoked by the same stimulus strength during stepping. Since there is possibility that the amplitude of the Mmax itself vary considerably during walking (Simonsen and Dyhre-Poulsen 1999), measurements of the Mmax amplitudes by supramaximal stimulation in each phase for both loading conditions were performed. The H-reflexes were recorded at the stimulus intensity with the M-wave amplitude of ~10% Mmax in each phase. In addition to passive stepping, the amplitudes of the Mmax and H-reflex were measured at standing with 40 and 100% body weight unloading (ground standing and air standing, respectively). The stimuli were applied randomly with greater than 5-s intervals during the standing conditions. More than four sweeps for Mmax and more than ten sweeps for acceptable H-reflex in each measurement were recorded. For the SCI patients, the H-reflex was recorded only during ground stepping and standing.

As an additional experiment, recruitment curves of the M-wave and H-reflex were recorded in three of ten healthy subjects. Recording of the recruitment curve was performed at air standing and at the stance and swing phases of air stepping. The stimulus intensity was gradually increased from below the threshold of the H-reflex to supra-maximum stimulation of the M-wave. Three responses were recorded at each stimulus intensity.

Data analysis and statistics

The sizes of M-wave and H-reflex were assessed by peak-to-peak amplitudes, which were normalized to the respective Mmax amplitude recorded at each standing condition and at each phase of the stepping condition. The background EMG activities in the Sol and TA muscles were determined as the root mean square values of the EMG signals for 50 ms just prior to stimulation. If TA background activity was observed at the timing of H-reflex stimulation, the reflex response was removed from the data analysis. The data are shown as mean ± SD. The M-wave, H-reflex, and background EMG levels during passive stepping were analyzed by a two-way repeated measure ANOVA with factors of loading (ground and air stepping) and phase (6 phases in the step cycle). When the assumption of sphericity by Mauchly's test was violated, Greenhouse-Geisser adjustments were applied to adjust the degrees of freedom. When statistical significance was detected by ANOVA, post hoc multiple comparisons (Bonferroni) were used to identify the significant differences. The paired Student's t test was used for comparing between the two standing conditions. Statistical analyses were not performed for the data measured for the SCI patients. A statistical significant level was set at $P < 0.05$ in all cases.

Results

Figure 2 shows the EMG waveforms in the Sol and TA muscles, the angles at the hip, knee, and ankle joints, as well as the pressure-sensitive sensor signal at both loading conditions during passive stepping in a healthy subject. In this figure, 10 waveforms were superimposed based on the initiation of hip extension in the right limb. Duration of the one-step cycle was 2,750 ms at 1.5 km/h. Since the hip- and knee-joint trajectories of the DGO were computer-controlled, the joint movements were highly repeatable, and no difference was observed between the two loading conditions. The trajectory of the ankle joint showed a