MEP sizes were observed (Fig. 4B and C). The TA MEPs in response to TES during air stepping were not facilitated compared with those during ground stepping.

Figure 5 shows the mean TMS- and TES-evoked MEP amplitudes and BGAs in the Sol and TA muscles from four subjects. The stimulus intensity was set to evoke the MEP amplitude of ~0.1 mV in the TA during upright standing with 40% body weight unloading at both stimulus conditions. The mean TA MEP amplitudes during upright standing were 0.120 ± 0.024 mV for TMS and 0.128 ± 0.057 mV for TES in all subjects. The results from all subjects also showed that the MEPs evoked by TES had similar phasic modulation to those evoked by TMS in both stepping conditions.

Discussion

In the present study, TMS was applied to investigate the corticospinal excitability in the lower limb muscles during passive stepping on the ground (ground stepping) and in the air (air stepping) by the DGO. We hypothesized that the corticospinal excitability might be phasically modulated by afferent inputs induced by passive stepping, especially when load-related afferent inputs were included. For this study, the maintenance of the TMS coil position across the step cycle was a crucial point. During stepping, the subjects did not move backwards or forwards due to their fixation to the DGO and harness. Therefore, it appears that, across the step cycle, the coil position for TMS was well stabilized despite the rhythmic lower limb movements during passive stepping. Indeed, we confirmed from video images that the position of the coil relative to the subject's head remained unchanged during the step cycle. The present results showed that the MEPs of the TA muscle during passive stepping were significantly facilitated by load-related afferent inputs.

Motor evoked potential modulation in the tibialis anterior muscle

The DGO could generate stepping motions by motor drives for the hip and knee joint movements. Therefore, the effect of voluntary command was largely reduced during passive stepping by the DGO with the foot lifters. In the present study, no EMG activity in the RF, BF and TA muscles was observed in either stepping condition, whereas Sol EMG activity often occurred at the latter stance phase during ground stepping, which is similar to the findings in a previous study of passive stepping with the same DGO (Dietz *et al.*, 2002; Lünenburger *et al.*, 2006). In the present study, the stepping speed was 1.5 km/h, which was much less than that during normal walking. However, the recording of the EMG activity in the lower limb muscles during treadmill walking over a wide speed range (1–7 km/h) indicated that, although the mean EMG activity increased with speed, a similar pattern of EMG activity was obtained at the different speeds (Ivanenko *et al.*, 2006). In addition, in a previous study that used the

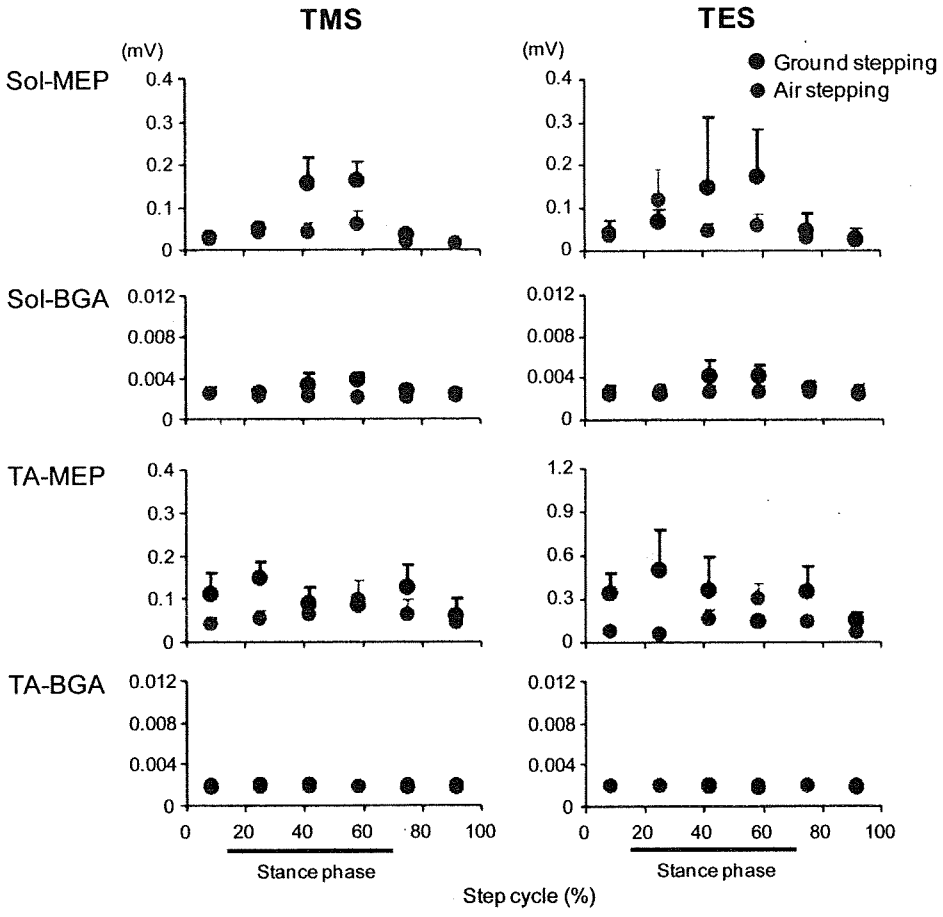

FIG. 5. Averages of TMS- and TES-evoked MEP peak amplitudes and EMG BGAs in the Sol and TA muscles. The black and grey circles depict the average data during ground and air stepping, respectively. Each plot represents mean (+SEM) value across four subjects. The vertical axis of the TA MEP responses induced by TES is three times larger than that of the TA MEP responses induced by TMS.

Lokomat®, locomotor-like EMG activations of the lower limb muscles were observed in complete spinal cord-injured patients during stepping at a speed of 1.5–2.5 km/h (Lünenburger *et al.*, 2006). The result suggests that the spinal neuronal circuits for locomotor activity can be driven by afferent inputs in this speed range in spinal cord-injured patients. In the present study, a noteworthy result was that the TA MEPs evoked by TMS increased at the early- and late-swing phases as well as at the early-stance phase only in the ground stepping condition, whereas no BGAs in the TA were observed during ground and air stepping (Figs 2 and 3). The facilitation pattern of the TA MEPs during ground stepping was similar to that observed during normal treadmill walking, as reported by Schubert *et al.* (1997). These authors found that the TA MEP induced by applying below-threshold TMS was largely modulated in parallel with the TA BGA during treadmill walking.

In the present study, there was phasic MEP modulation in the TA muscle during air stepping (Fig. 3). This result suggests that the corticospinal excitability of the TA muscle is modulated by peripheral afferent inputs associated with locomotor-like movements in the lower limb. However, the TA MEPs during air stepping were similar or smaller than those in the upright standing, whereas MEP facilitation in the TA was observed during ground stepping (Fig. 3). The movement trajectories of the hip and knee joints were not different between ground stepping and air stepping, as the angles were controlled by the computer of the DGO. In addition, the ankle angle was maintained in a constant position with the ankle foot orthosis. Thus, the trajectories of the lower limb joints in both stepping conditions were very similar (Figs 1–3). The factors facilitating corticospinal excitability of the TA muscle during ground stepping appear to be the load-related afferent inputs. The significance of load receptor input for regulating locomotion has been stressed not only for animals but also for humans (Stephens & Yang, 1999; Dietz & Duysens, 2000; Faist *et al.*, 2006; Nakajima *et al.*, 2008). The load-related effects may be mediated by afferent information from the Golgi tendon organs, joint receptors and cutaneous mechanoreceptors (Van de Crommert *et al.*, 1998; Dietz & Duysens, 2000; Faist *et al.*, 2006). By using the same DGO as in the present study, we recently observed facilitation of the cutaneous reflex in the TA muscle by tibial nerve stimulation during passive ground stepping but not during air stepping (Nakajima *et al.*, 2008). The facilitation of the cutaneous reflex was observed at the stance-swing transition during ground stepping (Nakajima *et al.*, 2008), whereas the facilitation of the MEP response in the present study occurred at the early-swing phase and swing-stance transition. Thus, the step phases at which the facilitation effects are mediated were different between the cutaneous reflex and corticospinal pathways. However, the modulation patterns of both the cutaneous reflex and corticospinal pathways during passive-loaded stepping are similar to those observed during normal walking (Yang & Stein, 1990; Schubert *et al.*, 1997). From animal studies, it is well known that spinal neuronal circuits can be activated passively by adequate afferent inputs that might be induced during normal locomotion (Grillner, 1981; Rossignol *et al.*, 2006). Such activities of the neuronal circuits induced during ground stepping may rhythmically facilitate corticospinal excitability of the TA muscle.

Although the main purpose of the present study was to investigate the modulation pattern in corticospinal excitability during passive stepping and not to clarify the level of neuroaxis at which the facilitation is mediated, TES was applied under the same stepping conditions as an additional experiment. As a method of obtaining information about the level (cortical or subcortical) at which a given change occurs, comparison of MEPs evoked by TMS and TES has been proposed (Petersen *et al.*, 2003). With the appropriate stimulation-electrode configuration, it is generally accepted that the current induced by TES at intensities just above the MEP threshold mainly activates the axons of corticospinal cells directly, whereas TMS activates the corticospinal cells either directly close to the cell soma or indirectly through the activation of fibers or neurons projecting onto corticospinal cell (Day *et al.*, 1989; Nielsen *et al.*, 1995; Rothwell, 1997; Di Lazzaro *et al.*, 2001). Therefore, it is considered that the MEPs induced by TES are not influenced by the change in excitability at the cortical levels. The latency of TES-evoked MEPs occurred 1–2 ms earlier than that evoked by TMS in the present study (Fig. 4A). This probably reflects the fact that TES activates the axons of corticospinal cells deep in the cortex (Nielsen *et al.*, 1995). If TES-evoked MEPs remained unchanged between ground and air stepping, whereas TMS-evoked MEPs during ground stepping were larger than those observed during air stepping, it can be concluded that the change of the corticospinal excitability probably occurred within the cortical level. The results of the TA MEPs by TES during ground stepping, however, also showed similar modulation to those by TMS (Figs 4B and C, and 5). Therefore, the level of neuroaxis at which the MEP facilitation in the TA muscle occurred during ground stepping could not be determined from the present study. As the MEP responses by TMS were measured without TA muscle activity, we could not determine the change in the subliminal fringe in the α-motoneurons during ground stepping. In addition, the change of the excitability in the segmental interneurons to the MEP facilitation was not eliminated. Further experiments are needed to specify the neuroaxis underlying facilitation of corticospinal excitability.

Motor evoked potential modulation in other lower limb muscles

The MEP modulation during ground stepping appeared not only in the TA but also in the Sol muscle. In the Sol muscle, weak EMG activity was observed at the late-stance phase during ground stepping and MEPs at this phase increased in a similar fashion (Figs 2 and 3). The EMG activity appeared to be reflexively generated because the stepping was performed passively (i.e. largely reduced the voluntary command). It has been indicated that the load receptors contribute to the afferent-mediated enhancement of ankle extensor muscle activity at the late stance of walking (Grey *et al.*, 2007). As simple rhythmic muscle stretching or loading alone does not lead to a locomotor EMG pattern (Dietz *et al.*, 2002), a combination of locomotor-like afferent inputs would be necessary to evoke locomotor EMG activities. In accordance with the previous study, the Sol EMG activity during air stepping was not observed in the present study. The phase-dependent Sol MEP modulation induced by TES during ground stepping was similar to that induced by TMS (Figs 4B and C, and 5). Therefore, the MEP facilitation would be mainly attributable to changes in the increased excitability of the α-motoneurons in this muscle. In the case of the locomotor activity, the flexors are considered to be predominantly under central control, whereas the extensors are mainly activated by afferent feedback (Dietz, 2002). Therefore, it is interesting that the TA MEP was facilitated by the load-related afferent feedback during passive stepping.

The MEP amplitudes in the upper leg muscles were also changed depending on the step phase (Figs 2 and 3). In the RF, MEPs increased from the initial swing to the swing-stance transition phase during stepping. With regard to the BF, MEPs showed larger amplitudes around the early-stance phase. These phases of MEP facilitation corresponded to the phases at which EMG activities in the respective muscles were seen during normal walking (Winter & Yack, 1987) and

active stepping with the DGO (Lünenburger *et al.*, 2006). Bonnard *et al.* (2002) observed stimulation-induced hip movements by above-threshold TMS to the motor cortex of the leg area during treadmill walking. When TMS was applied at the initial swing phase, the stimulation increased the movement of the hip flexion, whereas TMS applied at the mid-stance phase increased hip extension. These results seem to suggest that the excitability of the corticospinal tract in the RF was relatively increased at the initial swing and that the excitability to the BF was relatively increased at the mid-stance. In the present study, similar MEP modulation patterns were induced during passive stepping. As the stimulus intensity was weak for the RF and BF MEPs, the effects of the task and/or step phase on the MEPs may be shown more clearly by investigating the input (stimulus intensity)–output (MEP amplitude) curve of the corticospinal tract at pre-determined step phases (Devanne *et al.*, 1997).

Clinical implications for rehabilitation

In a study using TMS, Thomas & Gorassini (2005) found that intensive locomotor training for patients with incomplete spinal cord injury increased the corticospinal excitability in the lower limb muscles in association with the recovery of locomotor ability. The present data show that the corticospinal excitability to the TA muscle is facilitated during passive ground stepping but not during passive air stepping. This indicates that load-related afferent inputs have a role in amplifying the corticospinal excitability in the TA muscle during stepping. In the locomotor training of patients with spinal cord injury, it is already known that body reloading is crucial to the generation of locomotor EMG activity and recovery of locomotion (Harkema *et al.*, 1997; Dietz & Harkema, 2004; Nakazawa *et al.*, 2004). On the basis of the present results, we cannot determine the effect of passive DGO stepping on the functional recovery of locomotion ability in patients with incomplete spinal cord injuries. However, the supply of appropriate load-related afferent inputs during locomotor training by passive stepping may partly be useful for activating the neuronal circuitry for the locomotion because the MEP responses during passive stepping were facilitated – a similar facilitation pattern to that seen in normal walking. Knowledge about the neuronal control of human locomotion, including passive stepping, should be accumulated to identify a more effective approach for locomotor recovery.

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Abbreviations

BF, biceps femoris; BGA, background activity; DGO, driven-gait orthosis; EMG, electromyographic; MEP, motor evoked potential; RF, rectus femoris; Sol, soleus; TA, tibialis anterior; TES, transcranial electrical stimulation; TMS, transcranial magnetic stimulation.

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RESEARCH ARTICLE

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

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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

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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.

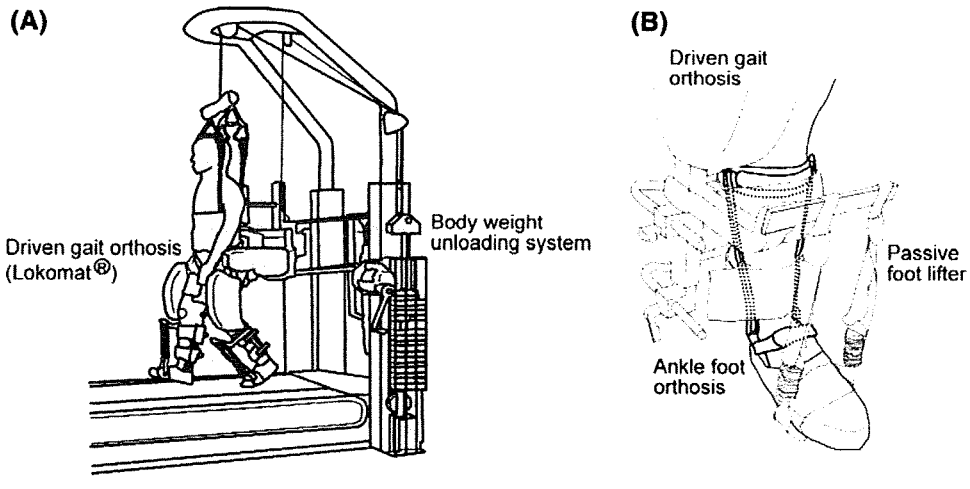

Fig. 1 **a** Schematic illustration of the experimental set-up for this study. Passive stepping was controlled by a driven gait orthosis (DGO; Lokomat®). Body weight of a subject was unloaded by a parachute harness. **b** Schematic illustration of the ankle joint with a passive foot lifter (spring-assisted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

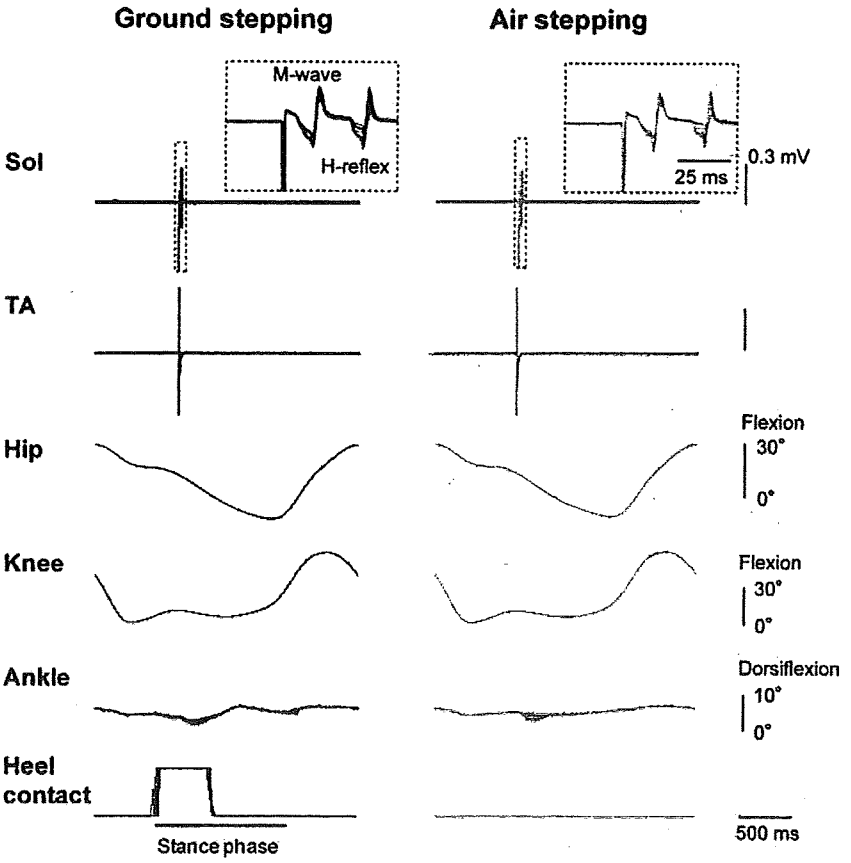

Fig. 2 Typical example of superimposed waveforms (10 sweeps) from the electromyographs (EMGs) of the soleus (Sol) and tibialis anterior (TA) muscles, angles of hip, knee, and ankle joints, and heel contact during ground and air stepping in a healthy subject. The electrical stimuli were applied at the early stance phase. The evoked responses are enlarged at each stepping condition

similar pattern in both loading conditions because of foot orthosis, but the angular variation in the stance phase increased slightly during ground stepping. No EMG activity was observed in the Sol and TA muscles at both loading conditions during passive stepping in this subject. Stimuli to evoke the H-reflex were applied at the early stance phase, and the evoked H-reflex waveforms are shown in the enlarged display. Due to the minimal variability in joint trajectories and muscle activities during stepping, the H-reflexes with a constant M-wave size could be easily evoked.

Figure 3 shows raw waveforms of the Mmax and H-reflex responses at two standing conditions and at six phases of two stepping conditions from a healthy subject. The stimulus intensity for the H-reflex was adjusted to evoke an M-wave size of 10% Mmax. Five sweeps for the Mmax and ten sweeps for the H-reflex are superimposed at each condition in this figure. There was phase-dependent modulation of the H-reflex excitability in both passive stepping conditions, showing that the Sol H-reflex at the early swing phase was markedly suppressed. In the present study, the H-reflex at the swing phase was completely suppressed in half of the subjects, while a small H-reflex response at the swing phase was observed in the remaining subjects.

Figure 4 represents the mean values of M-wave, H-reflex, and background EMG levels during passive stepping for all healthy subjects. The mean background EMG level of the Sol muscle, normalized to Mmax, was 0.09% Mmax for air standing and 0.08–0.09% Mmax through the six phases of air stepping. During ground standing, the background EMG level in the Sol was 0.0046 ± 0.0018 mV, which corresponding to 0.13% Mmax. Small muscle activation in the Sol was observed at the late-stance phase of ground stepping in four of ten subjects. The mean Sol background levels were 0.08–0.11% Mmax through the six phases of ground stepping. Although Sol background EMG levels tended to be larger during ground stepping than during air stepping, two-way ANOVA tests (2 loading conditions × 6 phases) showed that the main effect of loading was not significant ($F_{1,9} = 5.08$, $P = 0.051$). No significant effects of the phase and loading × phase interaction were observed in the Sol background EMG. Similarly, background EMG levels in the TA were not significantly different in loading conditions and among the step phases. In the present study, the Mmax size was not measured in the TA muscle. Instead, the EMG level during maximum voluntary contraction was recorded. The TA background levels, normalized by the EMG level during maximum voluntary

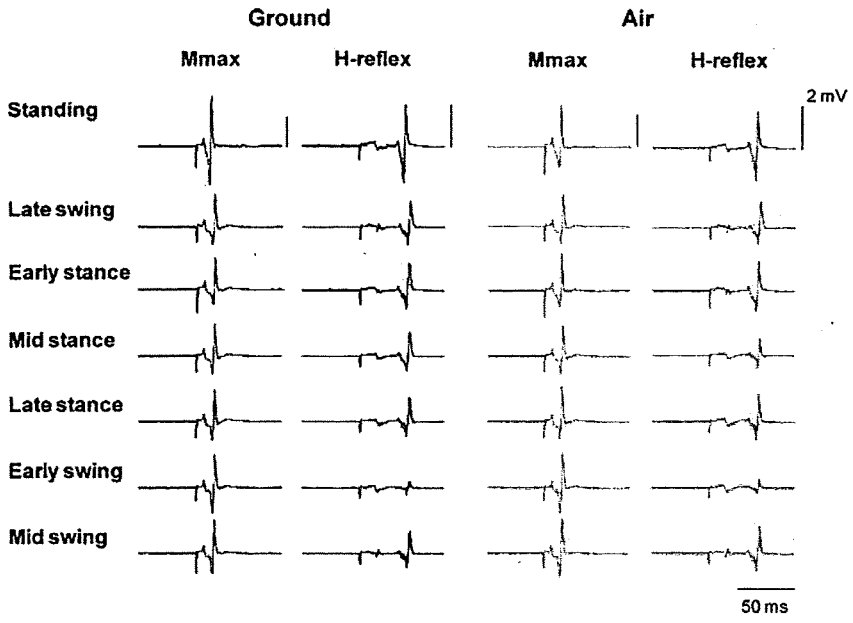

Fig. 3 Typical superimposed maximum M-wave (Mmax, 5 sweeps) and H-reflex (10 sweeps) waveforms with an M-wave size of 10% Mmax at standing and at six phases of passive stepping in a healthy subject

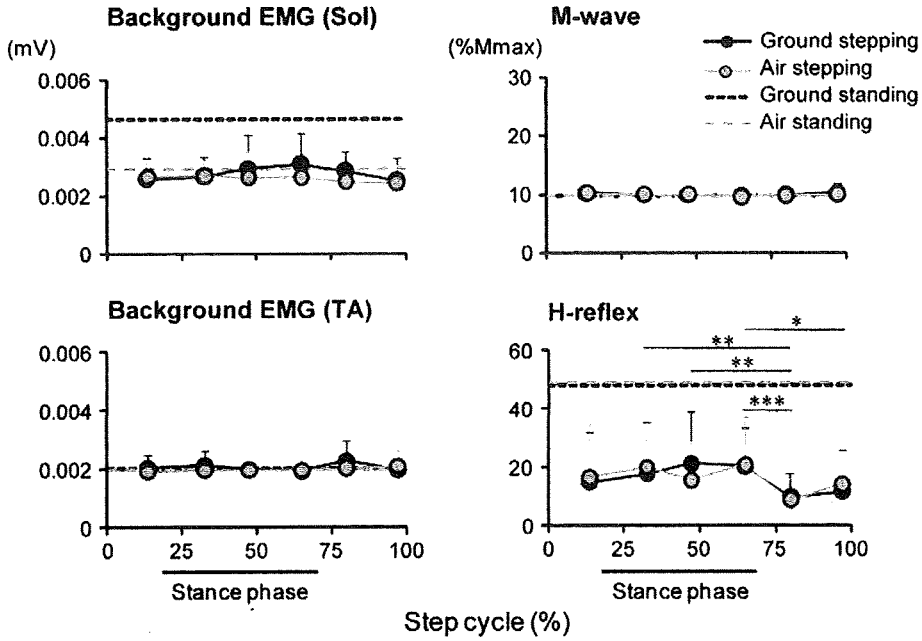

Fig. 4 Mean M-wave, H-reflex, and background EMG activity in the soleus (Sol) and tibialis anterior (TA) muscles at each phase during passive ground and air stepping in all healthy subjects. Significant difference between step phases during passive stepping, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The values for two unloading standing conditions are shown by *black* (ground standing) and *gray* (air standing) *dashed lines*

contraction, were less than 1% for both the standing conditions and at each phase for both stepping conditions.

In standing condition, the mean sizes of the H-reflex with M-wave corresponding to 10% of Mmax were not significantly different between ground and air standing (ground standing 48.3 ± 22.4% Mmax, air standing 49.1 ± 25.8% Mmax). Although the M-wave size was constant throughout the recording, the H-reflexes evoked at each phase during passive stepping were significantly inhibited compared to those at standing. Two-way ANOVA for the H-reflex during stepping revealed a significant main effect for phase ($F_{5,45} = 3.61$, $P < 0.05$), but not for loading ($F_{1,9} = 0.07$, $P > 0.05$), and no loading × phase interaction was observed ($F_{5,45} = 1.63$, $P > 0.05$). These results suggest that the H-reflex excitability was modulated in a phase-dependent manner during passive stepping, but no difference between ground and air stepping conditions. Post hoc test demonstrated that the Sol H-reflex was significantly inhibited at the early swing phase compared to the three stance phases ($P < 0.01$), and at the mid-swing phase compared to the late-stance phase ($P < 0.05$).

Figure 5 shows the M-wave and H-reflex (H-M) recruitment curves at air standing and at the stance and

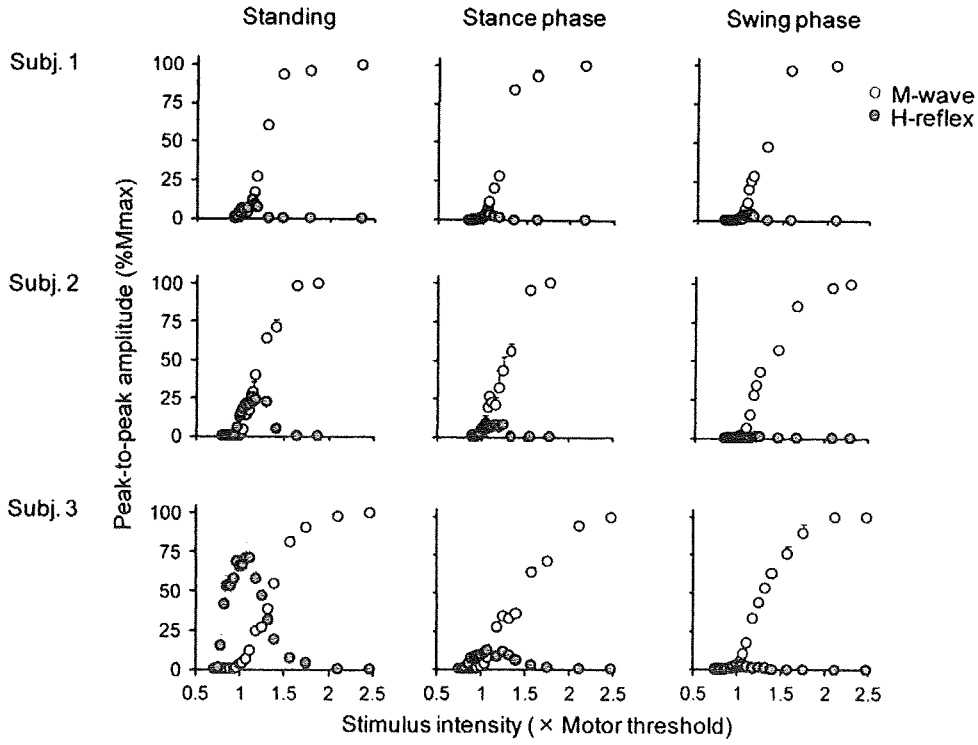

Fig. 5 Examples of H–M recruitment curves at standing and at the swing and stance phases of passive air stepping from three healthy subjects. Each plot shows the mean value of three responses at each stimulus intensity

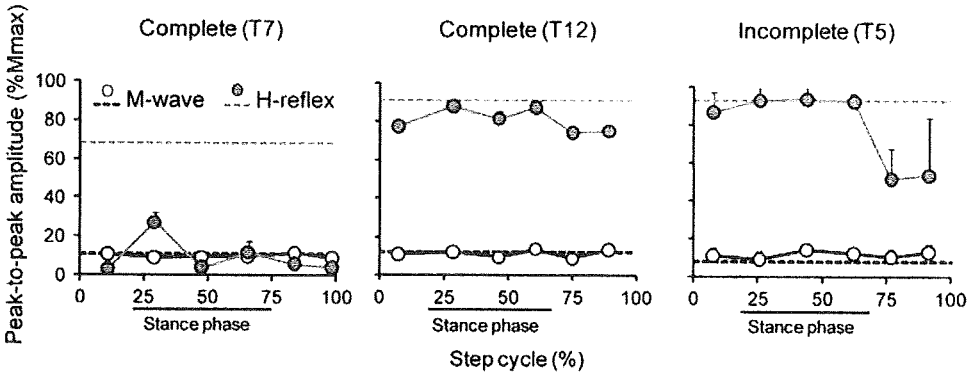

Fig. 6 Mean M-wave and H-reflex amplitudes during passive ground stepping in three spinal cord-injured (SCI) patients. The black and gray dashed lines represent the mean amplitudes of the M-wave and H-reflex at ground standing, respectively

swing phases of air stepping from three healthy subjects. The angle of the hip joint was similar at the instant when these H-M recruitment curves were measured. Although there were marked differences in the maximal H-reflex amplitude at the standing posture among the three subjects, the maximal H-reflex amplitude decreased from the standing to the stance and to the swing phase in all subjects. In addition, it was evident that the H-reflex recruitment curves at the swing phase of passive stepping were reduced across a wide range of stimulus intensities.

Furthermore, in the present study, we recorded H-reflex during ground stepping for two complete and one incomplete SCI patients. These patients showed elevated H-reflex amplitudes at ground standing (mean value in three SCI patients: 84.3 ± 12.0% Mmax, Fig. 6). During ground stepping, spastic muscle activities were rarely observed for a short time at the beginning of stepping and after the electrical stimulation in two SCI patients. Therefore, we applied the simulation when no spastic EMG activation was observed. Although the extent of H-reflex inhibition during ground stepping differed among the three patients (Fig. 6), the mean H-reflex amplitude at three (early-, mid-, and late-) swing phases was smaller than that at three stance phases (mean values at the stance and swing phases for all SCI patients: 64.2 ± 43.5 and 47.9 ± 38.3% Mmax, respectively).

Discussion

The aim of this study was to clarify the effect of peripheral sensory inputs on the Sol H-reflex excitability during

walking. In the present study, passive stepping was conducted using a DGO in healthy subjects and SCI patients who were asked to keep their lower-limb muscles relaxed during stepping. During passive stepping, the H-reflexes were inhibited at the swing phase compared to the stance phase, which showed phase-dependent modulation. No significant difference was observed in the H-reflex excitability between different body weight unloading conditions for passive stepping.

Mechanism for H-reflex modulation during passive stepping

A constant M-wave size normalized to Mmax is typically used as an index of stimulus intensity to excite the group Ia afferents for H-reflex experiments to dynamic movement in humans. However, there is a possibility that the Mmax amplitude itself differs among the step phases due to changes in muscle geometry with respect to the surface electrodes and stimulus efficacy to nerves (Simonsen and Dyhre-Poulsen 1999). Therefore, in the present study, the Mmax size was recorded at each phase of stepping, and the H-reflex was evoked at a stimulus intensity in which the M-wave amplitude was 10% Mmax (Fig. 3). Compared to the stance phase, the Sol H-reflex with a constant M-wave was markedly reduced in the swing phase of the passive stepping (Fig. 4). Moreover, the H-reflex amplitudes across a wide range of stimulus intensities were inhibited from the standing posture to the stance phase to the swing phase (Fig. 5), indicating that any change in the H-reflex amplitude during passive stepping was not due to a change in the stimulus efficacy to the posterior tibial nerve.

The present study attempted to reduce the effect of voluntary command on the Sol H-reflex during walking by applying DGO to the healthy subjects. Indeed, no TA or Sol EMG activity was observed during ground and air stepping, except the Sol EMG activity at the latter-stance phase of ground stepping (Figs. 2, 4). Thus, with substantially reduced descending drive to the stepping task in healthy subjects, the results obtained during passive stepping would provide the effect of peripheral sensory inputs on the H-reflex excitability. Moreover, we investigated the H-reflex modulation during passive stepping in the SCI patients whose commands from the supraspinal center were completely or incompletely interrupted. Three SCI subjects who showed exaggerated H-reflex excitability at standing posture revealed that the Sol H-reflex was inhibited at the swing phase of passive stepping (Fig. 6). Recently, Phadke et al. (2007) investigated the effects of different walking environments (treadmill with body weight support and manual assistance by trainers as well as overground walking with an assistive device and brace) on the Sol H-reflex in two phases of mid-stance and mid-swing in incomplete SCI patients. They found that the H-reflexes during mid-swing phase were smaller than those during mid-stance phase for both walking environments in incomplete SCI patients. While they investigated the Sol H-reflex during active voluntary stepping for incomplete SCI, we also found a similar tendency for H-reflex modulation in six phases during passive stepping in SCI patients.

From previous studies, it is known that passive movements around the hip or knee joint significantly inhibit the Sol H-reflex. For example, reflex inhibition appears at the flexion phase of hip and knee joints, and peaks close to full flexion during passive pedaling (Cheng et al. 1995). Knikou et al. (2007) reported that the passive flexion of the hip significantly depressed the Sol H-reflex excitability when SCI patients were in the supine position. Moreover, afferent signals from the quadriceps muscles in decerebrate cats inhibit the Sol H-reflex during locomotion (Misiaszek and Pearson 1997). These results suggest that the inhibition of the H-reflex arises from movement-elicited sensory receptors discharging at the hip and knee joints. The findings of the present study also demonstrated that the H-reflex inhibition increased at the early- and mid-swing phases when the hip and/or knee joints were flexed. Therefore, the source underlying the phase-modulation of the H-reflex during passive stepping can be attributed to the sensory inputs, which likely arise from muscle spindle primary endings when the joints are flexed. The modulation pattern of the Sol H-reflex during passive stepping was similar to that observed during normal walking (Capaday and Stein 1987; Simonsen and Dyhre-Poulsen 1999; Ethier et al. 2003). Therefore, it is considered that the modulation pattern in the Sol H-reflex excitability during normal walking might be largely formed by peripheral sensory inputs associated with the lower-limb movements.

It is widely thought that phase-modulation of the Sol H-reflex during normal walking is associated with presynaptic inhibition of the Ia terminal (Capaday and Stein 1987; Crenna and Frigo 1987; Zehr 2002). Because no EMG activity in the TA muscle was observed during stepping in the present study, it appears that postsynaptic effect from reciprocal inhibition might play a minor role in the Sol H-reflex inhibition. In addition, it has been reported that the Sol H-reflex was still inhibited during passive movement with voluntary tonic contraction of the Sol muscle (Brooke et al. 1995; Misiaszek et al. 1995). When excitability in the motoneuron pool is stabilized by tonic contraction during passive movement, the postsynaptic effects on the H-reflex inhibition are likely to be minimized. Thus, it appears that the major source of the H-reflex modulation during passive stepping can be attributed to the presynaptic inhibition. However, the postsynaptic effect on the H-reflex modulation cannot be excluded in the present study. Because

passive stepping was performed without the background EMG, any change in the subliminal fringe of the motoneuron during passive stepping was unknown. The change in the resting membrane potential of the motoneuron dependent on a step cycle might be partly involved in the H-reflex modulation during passive stepping.

Methodological limitations

In the present study, passive stepping was used to investigate the role of sensory inputs on the H-reflex modulation during walking. Although it appears that the modulation observed in the present study was generated by processes within the spinal cord, a few methodological limitations should be noted for the interpretation of these results. By instructing the subjects to relax during passive stepping, EMG activities in the lower limb muscles were not observed. However, the nearly complete disappearance of the EMG activity may not be a sufficient criterion to make the claim that there was no influence of descending drive on the H-reflex excitability. Also, because the sensory information that the subjects received during passive stepping with body weight unloading was different from that during normal walking, even a minor effect from the cortex due to the novel sensory perceptions during passive stepping may be related to modulations of the H-reflex. Furthermore, a possible implication of descending drive on the H-reflex excitability cannot be completely excluded, even in complete SCI patients, as it has been reported that a small percentage of individuals designated as complete SCI converted to incomplete SCI within 1 year after injury (Marino et al. 1999). Thus, the effect of the supraspinal input on the H-reflex modulation might not have been entirely eliminated in the present study.

Comparisons of reflex modulation during passive stepping and normal walking

In the present study, half of the subjects showed almost complete suppression at the swing phases of both stepping conditions, while the remaining subjects showed small H-reflex responses. Such inter-individual differences in the H-reflex inhibition have also been observed during normal walking (Simonsen et al. 2002). However, the amount of change in the H-reflex amplitude through the step cycle during passive stepping appeared to be less than that observed during normal walking. During the stance phase of normal walking, the Sol H-reflex increases progressively, nearly in parallel with the Sol background EMG (Capaday and Stein 1986). Ethier et al. (2003) demonstrated that, in most subjects, the H-reflex amplitude at late-stance during normal walking was larger than that during standing. However, the present study found that the H-reflex excitability at the stance phase in both loading conditions during passive stepping was significantly lower than that during standing. The lower H-reflex excitability in the stance phase during passive stepping when compared with normal walking might be attributed to little or no Sol EMG activity. As for the swing phase of normal walking, the H-reflex is almost completely suppressed due to the relevance of reciprocal inhibition, while TA is active (Ethier et al. 2003). Schneider et al. (2000) also observed that during the swing phase in a knee-locked walking task, the H-reflex inhibition was correlated with the TA EMG activity, and suggested that the modulation pattern during human walking follows the centrally produced reciprocal inhibition. In the present study, the H-reflexes were not completely suppressed at the swing phase of passive stepping in half of the subjects, presumably due to the absence of TA EMG activity. Thus, the EMG activity of the Sol and TA muscles that occurs normally during walking might quantitatively contribute to phase-modulation of the H-reflex. In addition, the difference in stepping speed between normal walking and this passive stepping condition might also be involved in the amount of the H-reflex inhibition during the swing phase because attenuation in the Sol H-reflex would depend on the velocity of the joint movement (McIlroy et al. 1992; Cheng et al. 1995).

Effect of load-related sensory inputs on reflex modulation during passive stepping

Inputs from load-related receptors during normal locomotion are well recognized to be significant for neural control (Harkema et al. 1997; Van de Crommert et al. 1998; Dietz and Duysens 2000; Dietz et al. 2002). Dietz et al. (2002) showed that afferent inputs from the hip joint in combination with those from the load receptors play crucial roles in generation of locomotor activity in SCI patients. However, the extent to which the Sol H-reflex is modulated by the load-related sensory inputs during walking is unclear. In the present study, there was no foot contact with the treadmill during air stepping (Fig. 2). Knikou and Conway (2001) observed that during sitting, the Sol H-reflex is inhibited by pressure (15–80 N) applied to the foot sole in healthy subjects and SCI patients. Therefore, we hypothesized that the Sol H-reflex during passive stepping would be inhibited by body weight loading. However, no significant difference was observed in the H-reflex between air and ground stepping in the present study (Fig. 4), i.e., no effect of the load-related sensory information was observed on the Sol H-reflex excitability during passive stepping. It is well known that the Sol H-reflex excitability is inhibited in a task-dependent manner from lying to sitting, to standing, and to walking (Capaday and Stein 1987; Crenna

and Frigo 1987; Brooke et al. 1991; Zehr 2002; Stein and Thompson 2006). Similarly, the difference of the load-related effect on the H-reflex excitability between sitting and passive stepping could be explained by task dependency. Alternatively, the different loading effects on the H-reflex between sitting and stepping may be attributed to the differences in pressure to the foot sole. While tonic pressure was applied to the metatarsal region of the foot sole in the sitting condition (Knikou and Conway 2001), during walking, the focus of the pressure on the foot sole in stance phase moved from the heel toward the toe, and the amount of foot pressure was also changed through the stance phase (Nakajima et al. 2008). Thus, the difference in the sensory feedback provided by the pressure on the foot sole might possibly account for the inconsistent results for the loading effects on the H-reflex excitability between two different tasks.

Regarding the load effect on the transmission in other afferent pathways, Faist et al. (2006) reported that Ib inhibition from the stimulation of the gastrocnemius nerve to the Sol H-reflex was reduced by loading of the leg, regardless of motor tasks. In the present study, although the load-related afferent inputs were involved during ground stepping, there were no significant differences in modulation of the Sol H-reflex between ground and air stepping. However, it was observed that slight difference of the H-reflex amplitude between two stepping conditions was observed only in the mid-stance phase (Fig. 4). During passive stepping on a treadmill, foot sole pressure was the greatest in this phase (Nakajima et al. 2008). Regarding the effect of loading on Ib inhibition during stepping, additional investigations may be required.

As for the effect of load on the cutaneous reflex pathway, Bastiaanse et al. (2000) previously suggested that load-related afferent inputs were involved in the regulation of the cutaneous reflex in the lower limb muscles evoked by sural nerve stimulation during normal walking. In addition, using the same DGO as in the present study, we have recently observed strong facilitation of the cutaneous reflex in the TA muscle during the late-stance to early swing phase of passive ground stepping, but not during passive air stepping (Nakajima et al. 2008). These results suggest that the load-related sensory inputs play a key role in modulation of cutaneous reflexes during walking. In contrast, no significant difference was observed in the H-reflex between ground and air stepping in the present study. Also in a recent study by Knikou et al. (2009), it was reported that Sol H-reflex modulation remained constant across 0, 25, and 50% body weight support levels during treadmill walking for healthy subjects. Thus, it appears that the effects of load-related sensory inputs to the reflex responses during stepping are different between the cutaneous and the H-reflex pathways.

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RESEARCH ARTICLE

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

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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.

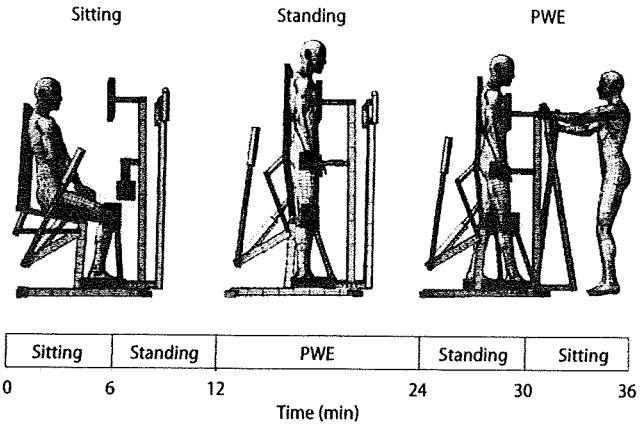

Fig. 1 Experimental protocol. The device enables subjects to change their posture from sitting to standing by pulling a built-in hydraulic lever. Standing posture is stabilized by fixing the trunk, pelvis and knees using front and back pads, lateral pelvic pads, and kneepads. Bilateral handles located 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

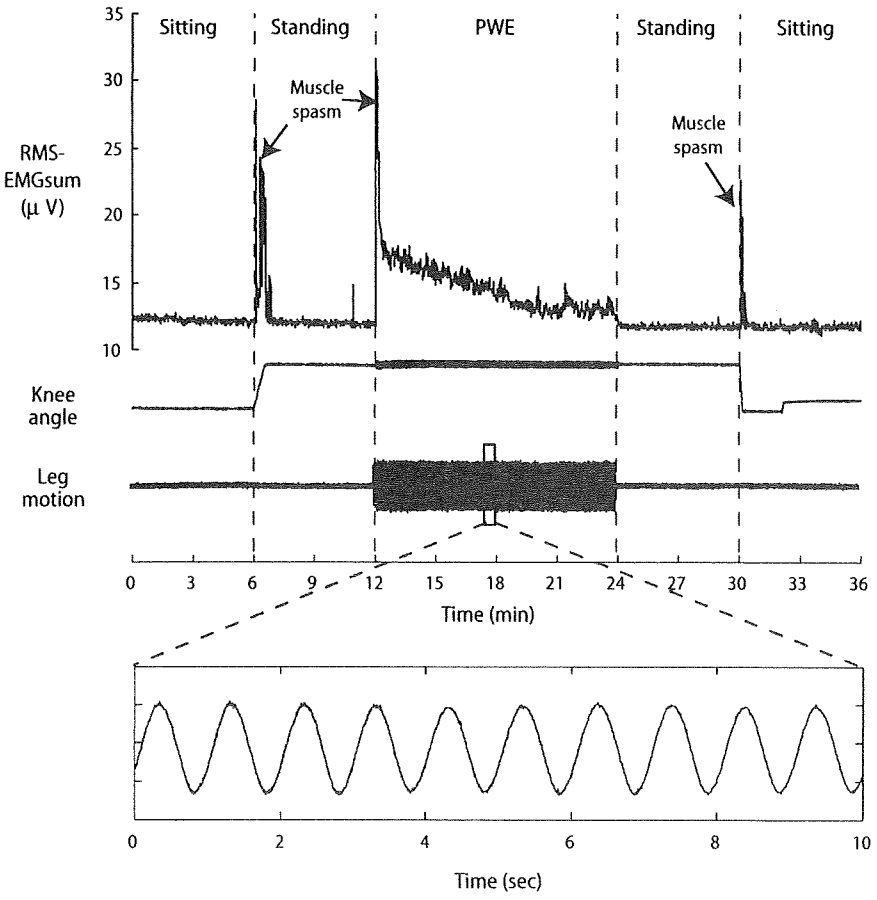

Fig. 2 Example of changes in RMS-EMG_{sum}, knee angle and leg motion throughout an experiment in an individual with spinal cord injury. RMS-EMG_{sum}: sum of root mean square of electromyographic activity of the left leg muscles (rectus femoris muscle, biceps femoris muscle, medial gastrocnemius muscle 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 ± 4***	86 ± 7***, #	108 ± 5	105 ± 5**
	MAP (mmHg)	77 ± 3***, #	69 ± 5***, #	88 ± 4	85 ± 4*
	DAP (mmHg)	66 ± 2***	60 ± 4***, #	78 ± 4	75 ± 4
	HR (bpm)	76 ± 3**	87 ± 3**	76 ± 2*	76 ± 1*
	$\dot{V}O_2$(ml min^{-1})	190 ± 8*, ##	214 ± 10#	230 ± 16	207 ± 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 ± 3*	80 ± 4	78 ± 5*	81 ± 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.