Comparison of the inhibitory response to tendon and cutaneous afferent stimulation in the human lower limb

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Rogasch NC, Burne JA, Türker KS. Comparison of the inhibitory response to tendon and cutaneous afferent stimulation in the human lower limb. J Neurophysiol 107: 564–572, 2012. First published October 26, 2011; doi:10.1152/jn.00751.2011.—A powerful early inhibition is seen in triceps surae after transcutaneous electrical stimulation of the Achilles tendon [tendon electrical stimulation (TES)]. The aim of the present study was to confirm results from surface electromyogram (SEMG) recordings that the inhibition is not wholly or partly due to stimulation of cutaneous afferents that may lie within range of the tendon electrodes. Because of methodological limitations, SEMG does not reliably identify the time course of inhibitory and excitatory reflex components. This issue was revisited here with an analysis of changes in single motor unit (SMU) firing rate [peristimulus frequencygram (PSF)] and probability [peristimulus time histogram (PSTH)] to reexamine the time course of inhibitory SMU events that follow purely cutaneous (superficial sural) nerve stimulation. Results were then compared with similar data from TES. When compared with the reflex response to TES, sural nerve stimulation resulted in a longer onset latency of the primary inhibition and a weaker effect on SMU firing probability and rate. PSF also revealed that decreased SMU firing rates persisted during the excitation phase in SEMG, suggesting that the initial inhibition was more prolonged than previously reported. In a further study, the transcutaneous SEMG Achilles tendon response was compared with that from direct intra-tendon stimulation with insulated needle electrodes. This method should attenuate the SEMG response if it is wholly or partly dependent on cutaneous afferents. However, subcutaneous stimulation of the tendon produced similar components in the SEMG, confirming that cutaneous afferents made little or no contribution to the initial inhibition following TES.

tendon electrical stimulation; sural nerve stimulation; peristimulus time histogram; peristimulus frequencygram

ELECTRICAL STIMULATION of the tendon results in inhibition of ongoing agonist muscle activity in humans. The inhibition following tendon electrical stimulation (TES) is present in both upper (Burne and Lippold 1996; Priori et al. 1998) and lower (Khan and Burne 2007) limbs, is of a short latency (Khan and Burne 2009; Rogasch et al. 2011), and requires low stimulation intensities to evoke (Khan and Burne 2009). This profile suggests that the inhibition following TES may reflect an autogenic inhibition mediated by group Ib tendon afferents (Khan and Burne 2009), although group III tendon afferents have also been suggested (Priori et al. 1998). Given the difficulties in assessing group Ib afferents with mixed nerve stimulation (Heckman et al. 1984; Pierrot-Deseilligny et al. 1981), transcutaneous stimulation of the tendon provides a promising technique to assess the reflex contribution of group Ib afferents in humans.

Despite the growing evidence for group Ib afferents mediating the reflex response to TES, stimulation of other afferents such as cutaneous afferents may also contribute (Floeter 2003). For instance, in the lower limb tactile stimulation of the plantar surface of the foot (Fallon et al. 2005) and electrical stimulation of the sural nerve, a nerve comprising primarily cutaneous afferent fibers, results in inhibition of ongoing muscle activity in flexor and extensor muscles of the ankle (Aniss et al. 1988, 1992; Burke et al. 1991). Currently, studies assessing inhibition following sural nerve stimulation in humans have used probabilistic methods such as rectified averaging of surface electromyogram (SEMG) and calculating single motor unit (SMU) firing probability with peristimulus time histograms (PSTHs). Although these probabilistic methods are excellent in determining the onset of inhibition, a major limitation of these methods is their tendency to produce count- and synchronization-related errors that obscure the true end of the inhibitory event. An alternative method that analyzes the SMU firing rate called the peristimulus frequencygram (PSF) provides a more accurate approximation of inhibitory postsynaptic potential (IPSP) duration without count and synchronization errors (Türker and Powers 1999, 2003). Recent studies in humans have utilized a combined PSTH/PSF approach to demonstrate prolongation of inhibitory responses following cutaneous stimulation of the upper limb (Kahya et al. 2010) and TES of the Achilles tendon in the lower limb (Rogasch et al. 2011) that were not detectable with SEMG or PSTH alone. However, the cutaneous reflex in the lower limb has yet to be reassessed with this method.

Several studies have compared the inhibition of SEMG in lower limb muscles following sural nerve stimulation and TES of the Achilles tendon. TES results in a shorter-latency SEMG inhibition that is reduced to a greater extent after tibial nerve block compared with sural nerve stimulation, suggesting a greater contribution of group I afferents to TES-evoked inhibition (Khan and Burne 2009). The mechanisms underlying SEMG inhibition following TES and sural nerve stimulation may also differ. TES inhibition is most likely mediated by presynaptic inhibition of Ia afferent input to the motor neuronal pool, whereas inhibition following sural nerve stimulation is more consistent with a postsynaptic inhibitory mechanism (Khan and Burne 2010). Given that a combined PSTH/PSF

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method appears to give a more accurate account of reflex postsynaptic potentials (PSPs) than SEMG alone, a direct comparison of TES and sural nerve inhibition using this technique would better inform the contribution of cutaneous afferents to the inhibition following TES.

The aim of the present study was therefore twofold. First, we sought to reexamine the time course and nature of inhibitory events that follow cutaneous (sural) nerve stimulation in the lower limb, using a combination of PSTH and PSF methods. This method minimizes the count-related errors associated with probability-based analyses such as SEMG and PSTH and permits an estimate of the resultant PSPs in motor neurons after nerve stimulation. Second, we aimed to confirm earlier SEMG studies on the contribution of cutaneous afferents to the inhibitory response after TES by comparing the time course of IPSPs following both TES and cutaneous nerve stimulation in medial gastrocnemius (MG) SMUs. To further clarify the contribution of cutaneous afferent stimulation to the TES reflex response, transcutaneous SEMG tendon responses were compared with those from direct intratendon stimulation with insulated needle electrodes. This method should attenuate the SEMG response if it is wholly or partly dependent on cutaneous afferents.

**MATERIALS AND METHODS**

**Subjects**

Thirteen neurologically healthy volunteers (4 women, 9 men; age range 23–57 yr) participated in experiment 1, and two male volunteers participated in experiment 2. Protocols were approved by the Human Ethics Committee of Ege University in accordance with the Declaration of Helsinki. All subjects provided informed written consent prior to participation.

**Protocols**

**Experiment 1.** SEMG and SMU potentials were recorded from the medial head of left gastrocnemius (MG). Subjects were instructed to perform a weak to moderate isometric contraction until SMUs were recruited and firing regularly at 6–10 Hz as determined by auditory feedback (see Experimental Arrangements and Recordings for details). Electrical stimuli were delivered transcutaneously to either the tendon or the sural nerve for trains of 500–1,000 ms duration at 0.1–2 Hz. As the reflex response to sural nerve stimulation is altered under different experimental and functional circumstances (Aniss et al. 1992; Burke et al. 1991; Zehr et al. 1998), SEMG and SMU reflex responses that followed tendon and sural nerve stimulation were collected under similar conditions. In six units, subjects were able to maintain SMU firing long enough for both tendon and sural nerve stimulation to be performed on the same SMU. At the conclusion of the experiment, subjects performed three maximal voluntary contractions (MVCs) to allow quantification of the relative contraction strength maintained during stimulation trials.

**Experiment 2.** SEMG was recorded from the medial (MG) and lateral (LG) heads of left gastrocnemius and the left soleus (SOL). Subjects were instructed to perform a weak to moderate contraction while TES was given in one of three conditions: 1) transcutaneously (stimulus intensity 40–90 mA); 2) with the cathode positioned subcutaneously in the tendon and the anode positioned on the skin over the proximal tendon (stimulation intensities 40–50 mA); or 3) with both the cathode and anode positioned subcutaneously in the tendon (stimulation intensities 30–60 mA). For both subcutaneous conditions, the depth of the cathode was varied between 1 and 3 cm below the skin surface. Stimuli were delivered at 1-s intervals until >100 responses were collected and ensemble averaged.

**Experimental Arrangements and Recordings**

Subjects lay prone on a modified physiotherapy table with their hip and knee joints at 180° to the torso and limb, respectively. The left foot was fastened to a force plate with the ankle joint angle fixed at 90° to the limb. In experiments 1 and 2, SEMG was recorded with bipolar Ag-AgCl electrodes (4-mm diameter) positioned ~2 cm apart over the belly of the MG (experiments 1 and 2) and LG and SOL (experiment 2) muscles. SEMG signals were amplified (1,000×), bandwidth filtered (20 Hz high pass, 500 Hz low pass), digitized at 1–5 kHz1 with a CED interface system (Cambridge Electronic Design), and recorded for off-line analysis. In experiment 1, SMU potentials were recorded with Teflon-insulated (except for their tips) silver bipolar wire electrodes (100-μm diameter with insulation; 70-μm core diameter). The wires were inserted into the MG muscle belly with a 25-gauge surgical needle that was then withdrawn, leaving the fish hooked wires in the belly of the muscle. SMU signals were amplified (1,000×), bandwidth filtered (high pass 200 Hz, low pass 10 kHz), digitized at 20 kHz with a CED interface system, and recorded for off-line analysis. SMU signals were displayed on a monitor for subject feedback. SMU potentials were also discriminated online with a microprocessor-based waveform analysis method, which matches the SMU potentials’ shape with preestablished templates (CED; Spike2 systems). Acceptance pulses from the discriminator were used to deliver auditory feedback to the subject on the SMU discharge rate. A circular self-adhesive electrode (5-cm diameter) positioned laterally from the SEMG electrodes served as a common ground for all signals. This site was chosen because it was found to be optimal in reducing the stimulus artifact observed in the SEMG recording after electrical stimulation. As a measure of system noise, the rectified baseline SEMG without muscle activity was calculated over three separate 1-s windows at the beginning of each trial and averaged. The mean baseline SEMG noise across trials was 1.12 μV (SD 0.2) for sural nerve stimulation trials and 1.85 μV (SD 1.3) for TES trials.

**Stimulation**

In experiments 1 and 2, the left Achilles tendon was stimulated transcutaneously with a 3 cm × 3 cm square cathode (self-adhesive electrode) positioned over the skin ∼1 cm distal to the musculotendinous junction with an anode positioned directly below (Khan and Burne 2007). The experimenter identified the musculotendinous junction by finding the distal border of the MG muscle while subjects performed a moderate plantarflexion. For the subcutaneous conditions, Teflon-insulated cathodal and anodal needle electrodes bared at the tip were inserted into the tendon transcutaneously. The sural nerve was stimulated with an Ag-AgCl cathode and an anode (4-mm diameter) positioned below the lateral malleolus over the sural nerve trunk. Single square pulse stimuli of 500-μs duration were delivered by a constant-current stimulator (Digitimer) triggered by Spike software (CED).

For both tendon and sural nerve stimulation in experiment 1, perceptual thresholds (PTs) were determined by increasing the stimulator intensity in 1-mA increments until the subject reported sensation. Stimulator intensity was then reduced until the subject reported no sensation, and the last intensity with sensation present was taken as threshold. Reflex stimulation intensity was determined by increasing the stimulator output in 2-mA increments above threshold until

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1The single motor unit data collection was all performed at 5 kHz. However, the tendon needle stimulation experiments were performed at 1 kHz since they were based on SEMG data only where higher sampling rates were not required.
subjects reported that the stimuli became painful or, for tendon stimulation, a visible muscle twitch occurred. In either case, the stimulator intensity was then reduced in 1-mA increments until neither a muscle twitch nor a painful sensation was present, and this was used as the reflex stimulation intensity. An accumulative online average of the rectified SEMG was continuously monitored by the experimenters to ensure that reflexes were present. In experiment 2, stimulation intensities were increased in 10-mA increments between 30 and 90 mA depending on the condition (see Protocols).

Data Analysis

For SEMG, the data were full wave rectified, extracted around the time of stimulation (±250 ms), and averaged. To account for system noise, baseline SEMG noise calculated for each trial was subtracted from the rectified average SEMG recording. To account for the stimulus artifact, data from −5 ms before the stimulus to +10 ms after the stimulus were removed and replaced by the mean prestimulus SEMG value. After artifact replacement a 0 phase ± 11 bin filter was applied. SEMG normalization was achieved by dividing the averaged trace by the average prestimulus value, effectively making the prestimulus value equal to 1. A cumulative sum (CUSUM) (Ellaway 1978) of the normalized SEMG was then constructed (Brinkworth and Türker 2003). From the prestimulus period of the CUSUM records, maximum and minimum deflections from the prestimulus average were obtained. The larger of the two CUSUM values was then used to make a symmetrical “error box” (Türker et al. 1997). Significant changes in the SEMG following the stimulus were determined by comparing deflections in the CUSUM with the “error box,” with deflections in the CUSUM greater in size than the “error box” considered a significant reflex response (Türker and Powers 2003). The turning points of each significant reflex response were marked (latency and end point) and the reflex strength calculated by dividing the size of each deviation by its duration (% maximum possible deflection) (Brinkworth and Türker 2003).

For SMUs, the recordings were discriminated off-line with the microprocessor-based waveform analysis method, which matches the SMU potentials’ shape with preestablished templates (CED; Spike2 systems). These discriminated units were then used to construct PSTHs and PSFs around the time of stimulation (250 ms). PSTH and PSF CUSUMs were then constructed from data normalized to the average prestimulus values (see description for SEMG). Considering the conservative nature of the “error box” technique and the possibility of type II errors, deviations that were greater than half the size of the “error box” but were not significant were considered a trend toward significance for SEMG, PSTH, and PSF data.

Statistical Analysis

To assess whether different populations of SMUs were more susceptible to sural nerve stimulation, a Kruskal-Wallis test was used to compare differences in contraction strength, stimulation strength, SEMG inhibition strength, and baseline SMU firing rate between sural nerve stimulation trials with significant, trend, and nonsignificant decreases in SMU firing probability and SMU firing rate. A Mann-Whitney U-test was used to compare differences in I1 latency, I2 latency, contraction level, SEMG inhibition strength, and stimulation strength between sural nerve stimulation and TES. To assess whether different populations of SMUs were significantly affected by TES and sural nerve stimulation, a Mann-Whitney U-test was used to compare contraction strength, SEMG inhibition strength, and SMU firing rate between sural nerve stimulation trials and TES trials with significant decreases in both SMU firing probability and firing rate. Values are reported as mean (standard deviation) in the text and tables unless otherwise stated.

RESULTS

Experiment 1: Sural Nerve Stimulation vs. TES

Response to sural nerve stimulation. Figure 1 illustrates the SEMG, PSTH, and PSF CUSUMs and raw data from an individual trial after sural nerve stimulation. Stimulation of the sural nerve resulted in a significant inhibition (I1) of the SEMG at 78 ms followed by a significant facilitation (E1) at 108 ms.

Fig. 1. Raw surface electromyogram (SEMG), peristimulus time histogram (PSTH), and peristimulus frequencygram (PSF) and their cumulative sums (CUSUMs) from an individual trial after sural nerve stimulation. The latencies and end points of significant reflex phases in SEMG CUSUM are marked with vertical lines (top; solid lines = inhibition, dashed lines = excitation). Note that the PSTH CUSUM (3rd panel) reflects the changes in SEMG, with decreases in firing probability during I1 and I2 and increases during E1; however, each failed to reach significance. In contrast, the PSF CUSUM (5th panel) revealed no change in single motor unit (SMU) firing rate throughout each reflex phase. Arrows indicate the time of stimulus, and horizontal dotted lines represent the error box.
A secondary inhibition (I2) of the SEMG was also recorded at 138 ms after stimulus. SMU firing probability as measured by PSTH also decreased during I1 and I2 and increased during E1, although in each case it failed to reach significance. Despite the trend toward increases in SMU firing probability during E1, SMU firing rate (PSF) remained unchanged throughout each reflex phase. Significant changes in the SEMG CUSUM are indicated with vertical lines in Fig. 1 to allow a comparison of the reflex responses evident with each technique.

Results of the group data are reported in Table 1. Significant inhibition of SEMG was reported in 24 separate sural nerve stimulation trials. In 20 trials the inhibition was followed by a significant facilitation (E1) of SEMG. A secondary inhibition (I2) and facilitation (E2) were also observed in 16 and 8 trials, respectively. Despite significant inhibition in the SEMG for all 24 trials after sural nerve stimulation, only 5 trials resulted in significant decreases in SMU firing probability as measured with PSTH. However, 11 SMUs trended toward significant decreases in firing probability (deviation > than half the error box), with 8 remaining unchanged. Similarly, only 5 trials resulted in significant decreases in SMU discharge rate as measured with PSF, with 9 trials trending toward significance and 4 remaining unchanged. Although discharge rates as measured by PSF tended to decrease or remain unchanged during the initial facilitation measured by SEMG (Fig. 1), 6 trials (5 from 1 subject) all resulted in an increase in SMU discharge rate corresponding with the facilitatory phase of SEMG. This occurred in the absence of significant decreases in firing probability despite inhibition in SEMG.

Table 2 summarizes the characteristics of SMU populations displaying significant, trend, and nonsignificant decreases in SMU firing probability (PSTH) and firing rate (PSF). There was no difference in contraction strength, stimulation strength, SEMG inhibition strength, or baseline SMU firing rate between SMU populations displaying significant, trend, and nonsignificant decreases in SMU firing probability or in SMU firing rate after sural nerve stimulation.

Table 2. Comparison between single motor unit populations displaying significant, trend, and nonsignificant decreases in firing probability and firing rate after sural nerve stimulation

<table>
<thead>
<tr>
<th>SEMG</th>
<th>PSTH</th>
<th>PSF</th>
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<tbody>
<tr>
<td>Significant Trials</td>
<td>Onset, ms</td>
<td>Duration, ms</td>
</tr>
<tr>
<td>I1</td>
<td>24 [0]</td>
<td>80.9 (10)</td>
</tr>
<tr>
<td>E1</td>
<td>20 [2]</td>
<td>115.3 (27)</td>
</tr>
<tr>
<td>I2</td>
<td>16 [0]</td>
<td>136.8 (24)</td>
</tr>
<tr>
<td>E2</td>
<td>8 [0]</td>
<td>176.9 (29)</td>
</tr>
</tbody>
</table>

Values are means (SD); total number of trials = 24. The number of trials that are not significant but trending toward significance are given in square brackets. SEMG, surface electromyogram; PSTH, peristimulus time histogram; PSF, peristimulus frequencygram; I1, initial inhibitory period; E1, initial excitatory period; etc.

Sural nerve stimulation vs. TES. The effect of TES on SEMG and on SMU firing probability and discharge rate has been described in detail elsewhere (Rogasch et al. 2011). Significant inhibition in SEMG after TES was reported in 22 separate trials. When compared with TES, sural nerve stimulation resulted in a significantly more latent first inhibition in SEMG [sural = 80.9 ms (SD 10), TES = 43.7 ms (SD 3); P = 0.0001]. However, the inhibition latency of I2 was not significantly different between the two types of stimulation [sural = 136.8 ms (SD 24), TES = 129.0 ms (SD 35); P = 0.76]. Sural nerve stimulation resulted in fewer trials with significant decreases in SMU firing probability as measured by PSTH (20.8% of trials after sural vs. 72.7% after TES) and fewer trials with significant decreases in SMU discharge rate as measured by PSF (20.8% of trials after sural vs. 59.1% after TES) compared with TES. SEMG inhibition strength, contraction level, and stimulation intensities during both sural nerve stimulation and TES are presented in Fig. 2. There was no significant difference in SEMG inhibition strength or contraction levels between types of stimulation; however, sural nerve stimulation required higher stimulation intensities than TES.

Table 3 summarizes the characteristics of SMU populations displaying significant decreases in firing probability and firing rate after sural nerve stimulation and TES. There were no differences in contraction strength, SEMG inhibition strength, or SMU firing rate between SMUs with significantly decreased firing probability (PSTH) after sural nerve stimulation and TES. Similarly, there was no difference in contraction strength or SEMG inhibition strength between SMUs with significantly decreased firing rate (PSF) after sural nerve stimulation and TES.

Two individuals were able to maintain SMU firing long enough for both TES and sural nerve stimulation to be applied during firing of the same SMU. Between these individuals six different SMUs were tested with both protocols, and the results are summarized in Table 4. In the SEMG, sural nerve stimulation resulted in a significantly more latent I1 than TES. In
addition, there was a differential effect of stimulation type on SMU response. Whereas TES resulted in significant reductions in SMU firing probability and firing rate in five of the six SMUs, there were only nonsignificant trends toward decreases in five of the six SMUs after sural nerve stimulation. Figure 3 compares the raw SEMG and SEMG, PSTH, and PSF CUSUMs from one SMU. The onset, duration, and strength of I1 are clearly different in both the SEMG and the SMU after sural nerve stimulation and TES. However, the onset, duration, and strength of I2 in both the SEMG and SMU firing probability and firing rate are similar.

Experiment 2: Transcutaneous TES vs. Subcutaneous TES

Transcutaneous stimulation resulted in a significant inhibition (I1) followed by a facilitation (E1) in MG at stimulus intensities of 50, 70, 80, and 90 mA but not 40 mA. The mean latency of the inhibition was 39.0 ms (SD 3), with duration of 69.6 ms (SD 34). Inhibition and facilitation were also observed in other synergistic muscles at similar inhibitory latencies [LG 40.2 ms (SD 4); SOL 36.8 ms (SD 2)]. A secondary inhibition (I2) was also observed, though at longer latencies than previously reported (>250 ms), falling outside the analysis window.

Stimulation with a single subcutaneous cathode electrode resulted in significant inhibition followed by facilitation in all muscles at 40 and 50 mA and a depth of 1–2 cm below the skin. Inhibition latencies were similar in all muscles to transcutaneous stimulation (ranging from 34 ms in MG to 44 ms in LG). Stimulation with both cathode and anode needle electrodes positioned subcutaneously also resulted in significant I1 followed by E1 in MG and LG at depths of 1–3 cm below the skin and at intensities 30–60 mA. Inhibition latencies ranged from 36 ms to 48 ms in LG. Figure 4 describes individual raw SEMG and CUSUMs after transcutaneous, cathode subcutaneous, and anode subcutaneous tendon stimulation in MG. Figure 5 describes individual raw SEMG and CUSUMs after subcutaneous tendon stimulation in LG and SOL.

DISCUSSION

The aim of the present study was to investigate the PSPs resulting from cutaneous afferent stimulation and compare the time course of these potentials with those after TES. There are several novel findings from this study. First, probabilistic methods such as SEMG and PSTH described a phasic inhibitory/facilitatory reflex in ongoing voluntary muscle activity following stimulation of the superficial sural nerve, a nerve composed primarily of cutaneous afferents. However, in SMU firing rate-based analysis (PSF) revealed a tendency for decreased firing rates during the facilitatory phase of the reflex in low-threshold SMUs, suggesting a prolonged inhibition. Compared with the reflex response to TES, sural nerve stimulation resulted in longer latencies of the primary inhibition and a weaker effect on SMU firing probability and rate. Subcutaneous stimulation of the tendon produced similar components in the SEMG, confirming that cutaneous afferents made little or no contribution to the initial inhibition following TES, although a contribution to the later inhibitory events was not excluded.

Effect of Sural Nerve Stimulation on SEMG and SMU Firing

Several studies have reported the reflex response to cutaneous afferent stimulation in SEMG recordings of the human lower limb. In the triceps surae, studies have generally reported a phasic reflex pattern in SEMG and SMU divided into early (<70 ms), middle (70–120 ms), and late (>120 ms) components (Aniss et al. 1992; Burke et al. 1991; Fallon et al. 2005; Table 3. Comparison between single motor unit populations displaying significant decreases in firing probability and firing rate after tendon electrical stimulation and sural nerve stimulation

<table>
<thead>
<tr>
<th></th>
<th>Decreased Firing Probability</th>
<th>Decreased Firing Rate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TES</td>
<td>Sural</td>
</tr>
<tr>
<td>Contraction strength (% MVC)</td>
<td>8.8 (6)</td>
<td>4.9 (2)</td>
</tr>
<tr>
<td>SEMG inhibition strength (% max)</td>
<td>29.1 (9)</td>
<td>32.8 (21)</td>
</tr>
<tr>
<td>SMU firing rate, pps</td>
<td>6.4 (1.3)</td>
<td>7.1 (0.4)</td>
</tr>
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Values are means (SD). TES, tendon electrical stimulation.

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We observed a phasic response in SEMG and PSTH consisting of both inhibitory and facilitatory peaks. However, PSF indicated a trend toward significance. Kukulka (1994; Uncini et al. 1991). In the present study, we observed a predominant inhibition of ongoing voluntary muscle activity in MG measured with SEMG and SMU firing after transcutaneous sural nerve stimulation with a mean initial latency of ~80 ms. This was followed by a facilitation of SEMG and a secondary late inhibition (~137 ms). Interestingly, we did not observe any significant early inhibitory components in either SEMG or SMU firing such as those reported after sural nerve stimulation (Aniss et al. 1992; Kukulka 1994) and tactile cutaneous stimulation of the foot (Fallon et al. 2005). In several trials a small decrease in SEMG at ~40 ms was observed; however, in each case this did not meet either our criteria for a significant event or our relaxed criteria for a trend event and was therefore not further analyzed. The inhibitory response following sural nerve stimulation is highly dependent on various factors such as stimulation strength, muscle contraction strength, and functional state of the lower limb (Aniss et al. 1992; Burke et al. 1991; Zehr et al. 1998). To facilitate a comparison between sural nerve stimulation and TES, we endeavored to keep these conditions as constant as possible between trials. The low contraction strength and 90° limb position may not be optimal for eliciting the early inhibitory component, as other studies on nonnoxious or mildly noxious sural nerve stimulation have also failed to report this early component (Khan and Burne 2009, 2010; Uncini et al. 1991).

A significant disadvantage of using probabilistic methods such as SEMG and PSTH to estimate spinal reflexes is the count- and synchronization-related errors associated with these techniques. This was highlighted in a series of experiments by Türker and Powers (for review see Türker and Powers 2005). Known potentials were injected into discharging rat motor neurons in slice preparations, and the firing probability and discharge rate of the motor neuron were recorded. Both excitatory and inhibitory potentials were better represented by measuring motor neuron discharge rate with PSF than by measuring firing probability with PSTH (Türker and Powers 1999, 2003). Recently, Kahya and colleagues (2010) used both PSTH and PSF to assess the cutaneous silent period in human upper limb motor units. Despite observation of both inhibitory and excitatory events in SEMG and PSTH, PSF revealed increases in MG SMU firing rate cated a tendency for low-threshold SMU firing rates to remain decreased or not change during these facilitatory peaks, suggesting a prolonged IPSP following sural nerve stimulation in the absence of excitation. This finding further highlights the importance of using an approach combining both probabilistic and rate-based methods when interpreting human reflexes.

It is of interest to compare the results of the present study with those obtained in animals in which PSPs were recorded directly from the motor neuron. In the cat and the nonhuman primate, stimulation of the sural nerve results in predominantly IPSPs recorded directly from triceps surae motor neurons (Hori et al. 1986; LaBella et al. 1989) These IPSPs generally occur in the absence of excitatory PSPs (EPSPs), as supported by our results; however, under certain circumstances such as at higher stimulation intensities EPSPs have also been recorded. In the present study, PSF revealed increases in MG SMU firing rate in six trials despite significant decreases in the global SEMG. The stimulation intensity used in these trials was higher than in other trials (10–12 × PT compared with 3–9 × PT). This result is comparable to that of Aniss and colleagues (1992), who also observed differential effects on MG motor neurons after sural nerve stimulation at different intensities in humans. Thus our results also support a potential differential effect between low- and high-threshold cutaneous afferents, with low-threshold afferent activation resulting in prolonged IPSPs in MG motor neurons and high-threshold afferent stimulation resulting in EPSPs in certain MG motor neurons.

### Comparing Sural Nerve Stimulation with TES

The second aim of this study was to assess the contribution of cutaneous afferents to the inhibition observed after TES. Several lines of evidence from the present data strongly suggest that cutaneous afferents do not contribute to the early inhibitory response observed after TES, although a contribution to the latter inhibitory component is possible. First, in accordance with previous studies (Khan and Burne 2009, 2010) a clear difference in inhibition latency was observed after sural nerve stimulation and TES in SEMG and SMU recordings under similar experimental conditions. Second, the effect of sural nerve stimulation on SMU firing probability and firing rate was weaker than that of TES. This difference was particularly evident when the effects of both TES and sural nerve stimulation were observed on the same SMU (Table 4, Fig. 3). The seemingly weaker inhibitory effects on individual SMUs of cutaneous afferent stimulation may reflect different inhibitory mechanisms of tendon and cutaneous afferents.
cutaneous afferents such as those proposed by Khan and Burne (2010). In that study, the authors concluded that the inhibition following TES may result from both presynaptic inhibition of group Ia and corticospinal input to the motor neuron pool in addition to postsynaptic inhibition via inhibitory interneurons. Conversely, the cutaneous afferent-evoked inhibition appeared predominantly due to a postsynaptic mechanism (Khan and Burne 2010). The stronger effect of TES on SMU firing than stimulation of cutaneous afferents may result from the convergent inhibitory mechanisms of the stimulated group Ib fibers.

Finally, the stimulation intensities required to evoke SEMG inhibition of comparable relative strength after sural nerve stimulation were higher than those used for TES. Higher stimulation intensities suggest that the afferents mediating cutaneous inhibition after sural nerve stimulation are of a higher threshold than those stimulated by TES under the present experimental conditions. This observation is in concurrence with previous studies in both the upper (Burne and Lippold 1996; Priori et al. 1998) and lower (Khan and Burne 2009) limbs, in which TES-mediated inhibition decreased or disappeared when the stimulating electrodes were moved away from the tendon. In addition, the cutaneous inhibition observed in the present experiment disappeared if the stimulating electrodes were not positioned directly over the sural nerve trunk. The implication of these findings is that the nonnoxious stimulation intensities used in the present experiment were not sufficient to stimulate cutaneous afferent across the skin. Therefore, surface electrical stimulation of low-threshold cutaneous afferents appears unlikely to contribute to the early reflex component observed after TES. A common source mediating the latter inhibitory components after both types of stimulation cannot be excluded, especially considering the similarities in timing and shape of the secondary inhibition. Given the latency of this latter component, a transcortical influence is also possible (Burke et al. 1991).

Direct Tendon Stimulation

In a different approach to confirm that the stimulation of cutaneous afferents does not contribute to the short-latency inhibition observed after TES, we stimulated the tendon subcutaneously and recorded SEMG from the three muscles of the triceps surae. Burne and Lippold (1996) utilized a similar approach in the upper limb and reported an identical reflex profile after both transcutaneous and subcutaneous stimulation of the tendon. Here we also observed a similar inhibitory profile in the lower limb SEMG after both transcutaneous and subcutaneous stimulation of the tendon. The inhibitory profile remained similar regardless of whether one or both stimulating electrodes were positioned subcutaneously. As expected, lower intensities were required to achieve inhibition with subcutaneous stimulation compared with transcutaneous stimulation. The removal of current flow from across the skin largely excludes the stimulation of cutaneous afferents and further confirms that the inhibitory response to TES results from stimulation of Achilles tendon afferents.

A similar pattern of inhibition was observed in all three synergistic muscles of the triceps surae after TES, a result consistent with earlier studies on the reflex responses of Ib afferents in humans by Pierrot-Deseilligny and colleagues (1981). These authors also reported an inverse reflex pattern in antagonist muscles in line with animal studies (see Jami 1992). Further experiments investigating the pattern response in antagonistic muscles after TES of the Achilles tendon would further clarify the contribution of Ib afferents to this reflex response.

Limitations

There are several limitations to this study. First, the error box method utilized to identify significant reflex responses in the CUSUM is conservative and may be prone to type II errors.
However, this method was originally developed to prevent erroneous detection of reflex events (type I errors), meaning that the error box method is meant as a rigorous test of significance (Brinkworth and Türker 2003). Acknowledging the conservative nature of this technique, we have included a trend category (events greater than half the error box but less than the full error box) to minimize false rejection of true reflex events.

Second, MVCs may have been influenced by fatigue since they were performed at the end of each experiment after very small but sustained contractions. MVCs could have been performed both at the start and at the end of the experiment, but this might have led to increased effects of fatigue on the SMU reflex data.

Finally, experiment 2 was only performed in two individuals. Although SEMG inhibition was clearly evident in both subjects after subcutaneous TES, this result requires further exploration with a larger sample size.

Conclusions

When the effects of TES and cutaneous sural stimulation on SMU PSTH and PSF were compared in similar experiments and even on the same SMUs, TES clearly produced a primary inhibition of SMU firing of much shorter latency and greater strength than that due to sural stimulation. Similarly, direct tendon stimulation with subcutaneous insulated electrodes reproduced similar reflex response components in SEMG. Thus stimuli designed to optimize cutaneous afferent stimulation of the triceps surae group in the lower limb failed to reproduce that due to transcutaneous Achilles tendon stimulation in the same muscle group. Transcutaneous Achilles tendon stimulation therefore serves as a selective method to study the reflex effects of group I tendon afferents in the lower limb. In addition, this study further highlights the importance of using combined probabilistic and rate-based analyses when assessing reflexes in humans.

Fig. 4. SEMG measured from left head of gastrocnemius and CUSUMs after stimulation of the tendon transcutaneously at 80 mA (A), with cathode stimulating needle positioned subcutaneously at 50 mA (B), and with both stimulating needles positioned subcutaneously at 60 mA (C). Significant reflex phases measured in CUSUM are indicated with vertical lines (solid lines = inhibition, dashed lines = excitation). Arrows indicate the time of stimulus.

Fig. 5. SEMG and CUSUMs from triceps surae muscles after subcutaneous stimulation of the tendon. A: SEMG in soleus after tendon stimulation with cathode stimulating needle positioned subcutaneously at 50 mA. B: SEMG in right head of gastrocnemius after tendon stimulation with both cathode and anode stimulating needles positioned subcutaneously at 30 mA. Arrows indicate the time of stimulus.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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