Noninvasive Stimulation of Human Corticospinal Axons Innervating Leg Muscles

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INTRODUCTION

In primates, the corticospinal tract is the major descending pathway for the control of voluntary movement (for review, see Lemon et al. 2004). This pathway includes a direct monosynaptic pathway between the motor areas of the cortex and alpha motoneurons innervating upper limb muscles (e.g., Maertens de Noordhout et al. 1999; Palmer and Ashby 1992). Similarly, most motoneurons innervating lower limb muscles receive a monosynaptic input from the motor cortex as demonstrated by recording from nerves (Bernhard et al. 1953) and motoneurones in primates (Jankowska et al. 1975) and by single motor-unit responses to corticospinal inputs in humans (e.g., Maertens de Noordhout et al. 1999). A common way of investigating the corticospinal pathway is by transcranial magnetic stimulation (TMS) over the motor cortex. The stimulus activates corticospinal neurons and produces electromyographic (EMG) responses (motor evoked potentials, MEPs) in many muscles of the human leg. TMS is an important technique for understanding corticospinal control of leg muscles, particularly during natural activities such as walking (e.g., Capaday et al. 1999; Petersen et al. 2001; Schubert et al. 1997). Changes in the size of MEPs under different conditions are used to infer changes in the nervous system. However, changes in the MEP can result from changes in the excitability of cortical neurons and spinal motoneurons. It is not possible to interpret changes in the MEP in terms of what might be happening in the motor cortex without an independent measure of any change at the motoneurons. In humans, it is difficult to test the responses of motoneurons in a controlled way. The tests that are commonly used for lower limbs are the H-reflex (the muscle response to activation of Ia afferents) and F-waves (the muscle response to antidromic activation of motoneurons). Although these approaches can help describe motoneuron behavior, each has characteristics that limit its effectiveness as a test of motoneuron excitability (for reviews, see Espiritu et al. 2003; Pierrot-Deseilligny and Burke 2005; Zehr 2002). Hence in studies of the lower limbs it is often difficult to isolate the precise location of changes in the motor pathway.

An alternative way of assessing motoneuronal excitability for upper limbs is by stimulation of descending corticospinal axons at a subcortical level (for reviews, see Taylor 2006; Taylor and Gandevia 2004). An electrical pulse passed between the mastoids elicits a single descending volley, which in turn evokes a motor response in biceps brachii (Butler et al. 2003; Gandevia et al. 1999), triceps brachii (Martin et al. 2006b, 2008), and first dorsal interosseus (Ugawa et al. 1991). A significant proportion of the motoneuronal response to this stimulus appears to be monosynaptic (Petersen et al. 2002), and there is evidence that descending tracts are not subject to presynaptic inhibition (e.g., Jackson et al. 2006; Nielsen and Petersen 1994). These characteristics make it the most direct assessment of the motoneurons’ response to synaptic input in awake human subjects. Moreover, the antidromic volley produced by magnetic stimulation between the mastoids can occlude the response produced by cortical stimulation, which suggests that the responses to both stimuli travel in the same axons (Taylor et al. 2002). Hence it is probably the most appropriate comparison to allow interpretation of changes in MEPs. However, stimulation between the mastoids rarely evokes responses in leg muscles at rest (Ugawa et al. 1991, 1995) although stimulation over the cervical or thoracic spine can evoke motor responses in tibialis anterior and extensor digitorum brevis (Claus et al. 1991; Maertens de Noordhout et al. 1992; Mills and Murray 1986; Ugawa et al. 1995). Limited data suggest that a cervical spine stimulus can occlude small responses to TMS during a weak contraction, which indicates that the spinally evoked responses may have a corticospinal component (Maertens de Noordhout et al. 1992).
primary aim of the current study was to determine whether stimuli over the thoracic spine activate descending corticospinal axons innervating various leg muscles and if so, whether a sufficient number of axons can be activated to produce motor responses in these muscles. We also investigated whether the technique is an effective means of testing motoneuron excitability in relaxed and contracting leg muscles.

METHODS

The effects of interaction of magnetic cortical stimulis and thoracic spine electrical stimulis on motor responses evoked in leg muscles were studied in neurologically intact subjects [28.6 ± 7.5 (SD) yr]. Eight subjects were tested in the first study and six subjects in the second study. Five of the subjects completed both studies. The procedures were approved by the local ethics committee and the study was conducted according to the Declaration of Helsinki. All subjects gave their informed written consent to participate.

Subjects sat comfortably in a chair with the hip, knee, and ankle flexed at ~90°. EMG activity was recorded from rectus femoris, tibialis anterior, and soleus muscles via self-adhesive electrodes (Ag-AgCl, 10 mm diam). For rectus femoris, electrodes were placed 5 and 15 cm proximal to the superior edge of the patella on a line from the anterior superior iliac spine to the patella. For tibialis anterior, one electrode was placed over the motor point with the second electrode on the tibial tuberosity. For soleus, electrodes were placed 10 and 15 cm proximal to the superior aspect of the calcaneus. EMG activity was filtered (16–1,000 Hz), amplified and sampled at 2 kHz for off-line analysis using customized software (CED 1401 with Signal software; Cambridge Electronic Design, Cambridge, UK).

Prior to each experiment the maximal M-wave (Mmax) was evoked for each muscle with stimulation of the peripheral nerves innervating each muscle. The tibial nerve was stimulated in the popliteal fossa to evoke responses in soleus, and the common peroneal nerve was stimulated below the head of the fibula to elicit responses in tibialis anterior (100 µs, Digitimer DS7, Welwyn Garden City, Hertfordshire, UK). For soleus, the mean amplitudes of Mmax were 21.0 ± 9.2 and 17.2 ± 8.0 mV in the two studies, and for tibialis anterior, they were 9.3 ± 3.9 and 10.5 ± 6.6 mV. Responses in rectus femoris were evoked by magnetic (figure-of-8 coil; Magstim 200, Magstim) or electrical stimulation over the femoral nerve. For magnetic stimulation, the coil was placed tangentially to the leg over the femoral triangle. Electrical stimuli were delivered to the femoral nerve with the cathode and anode 30 mm apart, pressed into the femoral triangle with a Velcro strap (e.g., Martin and Rattey 2007). For this muscle, Mmax was 11.2 ± 7.1 and 9.5 ± 6.3 mV in the two studies.

Transcranial magnetic stimuli to the motor cortex were delivered via a double cone coil positioned just lateral to the vertex (Magstim 200). The coil was orientated to induce a posterior to anterior current in the underlying cortex. The stimulus intensity (35–70% of stimulator output) was adjusted to produce a MEP of 5–10% of Mmax in the relaxed muscles.

Thoracic spine stimulation was carried out by passing a high-voltage electrical current (100 µs, Digitimer D180) between surface electrodes with the cathode and anode 30 mm apart, pressed into the femoral triangle with a Velcro strap (e.g., Martin and Rattey 2007). For this muscle, Mmax was 11.2 ± 7.1 and 9.5 ± 6.3 mV in the two studies.

Data analysis

The area of each response to thoracic spine or transcranial stimulation was measured for each muscle and the mean area of the responses in each condition in each block was calculated for each subject. To determine whether responses were facilitated or reduced when the two stimuli were given together, the averaged response to the thoracic stimulus was graphically subtracted from the averaged response to both stimuli together for each ISI for each subject. The
area of this subtraction was then calculated and compared with the area of the response to TMS (see Fig. 1 in Taylor et al. 2002). The subtracted responses [(cortex + thoracic) – thoracic] were compared with the responses to cortex alone with Student’s paired t-test. No statistics were performed for the interaction between stimuli for responses evoked in soleus due to the small number of subjects (n = 3; see RESULTS). For the studies of contraction strength, the mean area of TMEPs evoked at each contraction strength was calculated and normalized to Mmax. One-way repeated-measures ANOVA with Student’s-Newman-Keuls post hoc procedure were used to compare the changes in TMEPs with contraction. Group data are presented as means ± SD in the text and as SE in the figures (with n in the legends). Statistical significance was set at P < 0.05.

RESULTS

Electrical stimulation over the thoracic spine evoked small TMEPs in relaxed muscles of the lower limb in some subjects. Apart from one subject in whom responses were obtained at 40% stimulator output, the stimulus intensity needed to be set at 99% of stimulator output (750 V) to evoke responses in relaxation. Of the eight subjects tested, TMEPs were evoked in rectus femoris and tibialis anterior in six subjects, whereas only three subjects had responses in relaxed soleus. The interaction between cortical and thoracic stimuli was performed only for muscles in which a resting TMEP was evoked.

Time course

Figure 1 shows responses evoked in rectus femoris to magnetic stimulation of the motor cortex (A; MEPs), responses to electrical stimulation over the thoracic spine (B; TMEPs), and responses to combined stimulation (C) at different ISIs. For this subject, MEPs were 10% Mmax, whereas TMEPs were 4% Mmax. The response to combined stimuli was large at ISIs of −3, −2, and −1 ms and was small at ISIs of 0, 1, 2, and 3 ms. The bottom row of traces in Fig. 1 shows a subtraction of the mean TMEP from the mean response to the combined stimuli. If the responses to cortical and thoracic stimulation were independent, the subtraction would be identical to the response to cortical stimulation (Fig. 1, top row). However, instead there was facilitation above the sum of the individual responses when thoracic stimulation was delivered after the initial volley from magnetic stimulation is likely to have passed the site of activation for thoracic stimulation (see METHODS). Conversely, a reduction in the subtracted responses occurred at intervals when thoracic stimuli were delivered prior to the arrival of the initial volleys from cortical stimulation.

For rectus femoris, in the group of subjects (n = 6), magnetic cortical stimuli that evoked mean responses of 5.1 ± 3.7% Mmax (range, 1.6–10.6% Mmax; latency, 19.6 ± 1.5 ms) were given with thoracic stimuli that evoked responses of 1.7 ± 1.6% Mmax (range, 0.6–4.0% Mmax; latency, 12.2 ± 1.9 ms). Figure 2, A and B (●), shows the area of what remains of the response evoked in rectus femoris by both stimuli at different ISIs after the response to the thoracic stimulus alone was subtracted. The area of this subtracted response is expressed as a percentage of the response to magnetic cortical stimulation. The subtracted response was larger at −3, −2, and −1 ms and smaller at ISIs of 1, 2, and 3 ms (P < 0.05).

A similar pattern of facilitatory and inhibitory interactions between cortical and thoracic stimulation was also observed for responses evoked in tibialis anterior (Fig. 2, A and B, ▲) and soleus (■). For tibialis anterior (n = 6), magnetic cortical stimuli which evoked mean responses of 12.2 ± 5.6% Mmax (range, 5.3–19.4% Mmax; latency, 26.6 ± 1.9 ms) were given with thoracic stimuli which evoked responses of 5.6 ± 3.4% Mmax (range, 0.4–10.7% Mmax; latency, 19.1 ms). The response to both stimuli after subtraction of the response to the thoracic stimulus alone was facilitated at ISIs of −3, −2 and −1 ms but was reduced at 2 and 3 ms (P < 0.05; Fig. 2, A and B, ▲). For the three subjects in whom soleus responses could be

FIG. 1. Compound muscle action potentials recorded from rectus femoris in 1 subject. Each group of traces shows 5 responses superimposed. Responses were evoked by magnetic cortical stimulation (top row), thoracic electrical stimulation (2nd row) and both stimuli (3rd row) given at different interstimulus intervals (ISIs). ISIs −3, −1, 1, and 3 ms were recorded in 1 set of trials and −2, 0, and 2 ms in another. ISIs were calculated by comparing the latency of responses to cortical and thoracic stimulation and adjusted so that at negative ISIs the initial volley from the cortex preceded the thoracic spine volley to the motoneurons (see METHODS). The thoracic stimuli are offset for the corresponding interstimulus intervals. Times of stimulation can be judged from the stimulus artifacts. The interaction of the 2 stimuli can be judged from the bottom row of traces. These show a subtraction of the average of the responses to thoracic stimulation from the average of the responses to both stimuli.
evoked by magnetic (mean response, 2.8 ± 1.5% Mmax; range, 1.4–4.8% Mmax; latency, 29.5 ± 3.2 ms) and thoracic stimuli (mean response, 1.5 ± 2.4% Mmax; range, 0.4–5.1% Mmax; latency, 21.7 ± 2.3 ms), the responses to both stimuli were larger at 3, 2, and 1 ms and smaller at 1, 2, and 3 ms.

Growth of response to thoracic spine stimulation with voluntary contraction

In the second set of experiments (n = 6), the mean sizes of TMEPs in relaxed muscles were 2.6 ± 3.2, 5.7 ± 4.6, and 3.0 ± 2.9% Mmax for rectus femoris, tibialis anterior, and soleus, respectively. These responses grew considerably with voluntary contraction (Fig. 3). For all three muscles, responses grew linearly with contraction strength (R² = 0.566, rectus femoris; R² = 0.375, tibialis anterior; R² = 0.416, soleus; P < 0.005). During contractions of 50% MVC, responses were 40.7 ± 17.9, 27.4 ± 15.9, and 30.2 ± 20.1% Mmax for rectus femoris, tibialis anterior and soleus, respectively. The latencies of TMEPs did not change with voluntary contraction (P > 0.05).

Blood pressure and heart rate

There were no changes for any subject (n = 3) in blood pressure or heart rate immediately before (i.e., in anticipation of the stimulus) or after thoracic stimuli delivered at 99% of stimulator output. Mean blood pressure and heart rate before or after each stimulus differed from baseline by <5% regardless of the number of beats used for calculation.

DISCUSSION

These experiments demonstrate that stimulation over the thoracic spine can evoke motor responses in tibialis anterior, soleus, and rectus femoris. These responses have features that suggest they are evoked transsynaptically and involve activation of descending corticospinal axons and that they have a monosynaptic component.

Background voluntary contraction markedly increased the responses to stimulation over the thoracic spine. This indicates that responses are evoked transsynaptically rather than through stimulation of the motor axons distal to the cell bodies. During contractions, ongoing input excites the motoneuron pool, causing repeated firing of some motoneurons and brings others close to their threshold for firing. Thus during contraction, more motoneurons in the pool are close to threshold and should fire with additional synaptic input. For the three leg muscles, the sizes of TMEPs increased linearly with contractions up to 50% MVC. Comparison of the facilitation of responses to stimulation of the descending tracts innervating arm muscles by different levels of voluntary contraction suggests that motoneuron “excitability” increases linearly up to 50–75% MVC.
Muscles, Data are shown as means responses were small in relaxation but grew considerably with contraction. Spine stimulation is complex and the ultimate size of potentials facilitation or suppression of responses depending on the in-recruitment of motoneuron pools innervating leg muscles. Especially for very strong contractions, may help elucidate the size of response. To simplify discussion, we will initially consider the pathway activated by each stimulus to be purely monosynaptic but the possible contribution of non-monosynaptic pathways is considered later in DISCUSSION.

The conduction time from the cortex to the site of thoracic stimulation determines the nature and magnitude of the interaction between stimuli. When thoracic stimuli are delivered after some or all of the volleys evoked by cortical stimulation have passed the site of thoracic stimulation (negative ISIs), the descending excitatory volley from the motor cortex precedes the thoracic volley down the spinal cord and hence the thoracic volley arrives at an excited motoneuron pool. This produces facilitation. Conversely, when thoracic stimuli are delivered just before descending volleys have reached the site of thoracic stimulation (positive ISIs) the descending volleys are susceptible to collision and this should reduce responses to combined stimulation. The three muscles investigated showed a similar pattern of interaction between stimuli. Responses were facilitated at negative ISIs. The intervals were set relative to the arrival at the site of thoracic stimulation of the first volley evoked by the cortical stimulus (see METHODS). Therefore later volleys may be partially occluded by the antidromic thoracic volley. However, the facilitatory interaction in the motoneuron pool is more than sufficient to mask such an occlusion. Conversely, responses were occluded (or possibly partly inhibited, see following text) at positive interstimulus intervals. At these intervals, all cortically evoked volleys would be susceptible to collision by the antidromic volley, although only one potential in each axon can be eliminated by collision within this period, so that if the same corticospinal neuron fired more than once, in each axon can be eliminated by collision within this period, the combined responses to responses evoked by combined stimulation (positive ISIs) the descending volleys are susceptible to collision and this should reduce responses to combined stimulation. 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with monosynaptic connections to motoneurons. In non-human primates, monosynaptic connections between corticospinal fibers and motoneurons of hind-limb muscles have been demonstrated by recording from muscle nerves (Bernhard et al. 1953) and by intracellular recordings from motoneurons (Jankowska et al. 1975). Similarly, single motor-unit recordings from tibialis anterior, soleus, and quadriceps indicate that monosynaptic corticospinal connections to these muscles exist in humans, although these may be relatively weak for quadriceps muscles and soleus (Bawa et al. 2002; Brouwer and Ashby 1992; Maertens de Noordhout et al. 1999; Taube et al. 2006; Valls-Sole et al. 1994). Our results indicate that responses evoked by thoracic stimulation probably have a large monosynaptic component. These responses grew significantly during voluntary contraction but their latency did not change. If there were multiple synapses in the pathway, increased excitability of each postsynaptic pool of cells should shorten each activation time and reduce the latency. Nevertheless, non-monosynaptic pathways may contribute to responses evoked by cortical or thoracic stimulation. Indirect disynaptic and polysynaptic pathways from the motor cortex to motoneurons have also been demonstrated for each of these muscles (Cowans et al. 1986; Iles and Pisini 1992; Marchand-Pauvert et al. 1999; Nielsen et al. 1993; Simonetta-Moreau et al. 1999). Moreover, some of the interneurons activated by cortical stimulation may produce inhibitory rather than excitatory effects at the motoneuron pool, and there is evidence that the inhibitory pathways have a lower threshold than the direct excitatory ones for some muscles (Nielsen et al. 1993). Therefore these indirect pathways may contribute to changes in the sizes of responses to combined stimulation. For example, an initial descending volley may act via inhibitory interneurons to reduce the excitability of the motoneuron pool so that subsequent volleys produce less facilitation via temporal summation. Even though a pattern of facilitation at negative ISIs occurred for the three muscles, the magnitude of this effect was far less pronounced for soleus and tibialis anterior than for quadriceps. This could occur if the inputs to tibialis anterior and soleus were more influenced by spinal inhibitory pathways, such as the Ia reciprocal inhibitory path.

Practical implications

As a technique for studying motor control of human leg muscles, stimulation over the thoracic spine has several advantages and disadvantages. The most obvious disadvantage is that the technique is transiently painful because of activation of local skin afferents. Subjects described the stimulus as “sharp”, and some subjects indicated that it was more painful than when applied over the mastoids, which is commonly used to activate the corticospinal output to arm muscles. However, the stimulus was tolerated by all subjects, produced no transient changes in heart rate or blood pressure, and did not cause any lasting effects. Due to the anticipation of the impending stimulus, it is occasionally difficult for subjects to remain completely relaxed. Therefore it is important to monitor ongoing background EMG from the targeted muscle as well as remote muscles. Larger stimulating electrodes (Ugawa et al. 1995) or the application of a topical anesthetic under the electrodes may decrease the discomfort, although our preliminary observations suggest that any benefit from these approaches is minimal. The technique also stimulates nearby peripheral nerves and causes contraction of muscles in the back, neck, shoulders, and arms, although positioning the cathode over T3 rather than more rostrally minimizes the contraction of the arms. In addition, studying motoneuron behavior in relaxed muscles is difficult because responses are small (<10% Mmax) regardless of the muscle tested. In fact, in some subjects, even very high stimulus intensities fail to evoke responses, particularly for soleus. To improve the efficacy of the technique, we trialed a variety of electrode locations but were unable to identify a superior location. Hence it may be difficult to further improve the efficacy of the technique, and therefore it is probably not possible to test all subjects in relaxation using this approach. However, responses grow considerably with a weak voluntary contraction and to 30–50% Mmax with a moderate contraction. Under these conditions, the technique would be relatively simple to use. The technique may be an important measure of excitability of motoneurons innervating leg muscles. Unlike afferent pathways, descending tracts are not subject to classical presynaptic inhibition (Jackson et al. 2006; Nielsen and Petersen 1994; Rudomin and Schmidt 1999), and therefore techniques that activate corticospinal axons provide a relatively direct measure of motoneuron behavior. On the other hand, there is evidence that the efficacy of synapses from descending tracts to motoneurons may be altered by repetitive activity (e.g., Gandevia et al. 1999; Jackson et al. 2006; Petersen et al. 2003), and stimulation of the descending pathways makes it possible to test for these changes in humans. Finally, stimulation over the thoracic spine could be combined with other techniques to identify specific changes in the motor pathways during various tasks. For example, comparison with H-reflexes may provide information on changes in presynaptic inhibition of the Ia afferent volley. Most importantly, used in combination with TMS, it can help define changes at the motor cortex by providing an appropriate control for changes occurring at the segmental level.

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References


