Changes in Reciprocal Inhibition Across the Ankle Joint With Changes in External Load and Pedaling Rate During Bicycling

H. S. Pyndt, M. Laursen, and J. B. Nielsen
Division of Neurophysiology, Department of Medical Physiology, The Panum Institute, University of Copenhagen, DK-2200 N Copenhagen, Denmark

Submitted 8 May 2003; accepted in final form 17 July 2003


First published July 23, 2003; 10.1152/jn.00444.2003. The purpose of this study was to investigate the role of reciprocal inhibition in the regulation of antagonistic ankle muscles during bicycling. A total of 20 subjects participated in the study. Reciprocal inhibition was induced by stimulation of the peroneal nerve (PN) at 1.2 times threshold for the M-response in the tibialis anterior muscle (TA) and recorded as a depression of the rectified soleus (SOL) EMG. Recordings were made during tonic plantar flexion and during bicycling on an ergometer bicycle. During tonic contraction, the amount of inhibition in the SOL EMG was linearly correlated to the amount of background EMG. This linear relation was used to calculate the expected amount of reciprocal inhibition at corresponding EMG levels during bicycling. During the early phase of down-stroke of bicycling at 60 revolutions per minute (RPM) and an external load of 1.0 kg, the amount of recorded reciprocal inhibition was significantly smaller than that calculated from the linear relation during tonic contraction. In nine subjects, the SOL H-reflex was used to evaluate the amount of inhibition. At a short conditioning test interval (2–3 ms), the PN stimulation depressed the SOL H-reflex when the subjects were at rest. This short latency inhibition was absent during downstroke, but appeared during upstroke just prior to and during TA activation. A positive linear relation was found between the level of SOL background EMG in early downstroke and the external load (0.5–2.5 kg) as well as the rate of pedaling (30–90 RPM at 1.0 kg external load). The amount of inhibition in the SOL EMG when expressed as a percentage of the background EMG activity decreased significantly with increasing load. During increased pedaling rate, a similar decrease was seen, but it did not reach a statistically significant level. The data illustrate that reciprocal inhibition of the soleus muscle is modulated during bicycling being small in downstroke when the SOL muscle is active and large in upstroke where the muscle is inactive and its antagonist becomes active. The depression of the inhibition in relation to increased load and pedaling rate likely reflects the need for reducing inhibition of the SOL motoneurons to ensure a sufficient activation of the muscle.

INTRODUCTION

Bicycling is a recently developed movement form, which is almost exclusively seen in human subjects. Bicycling resembles other forms of rhythmic locomotion such as walking, running, and swimming, and it is reasonable to assume that the brain has used similar strategies to control bicycling as these more “natural” forms of locomotion. Although we have detailed knowledge of how the brain and spinal cord control locomotion in animals, it is only quite recently that it has become possible to address similar issues in human experiments (see reviews by Capaday 2002; Dietz 2001; Nielsen 2002), and only a few studies regarding the central control of bicycling have been published (Boorman et al. 1992; Brooke et al. 1992; Pyndt and Nielsen 2003). Consequently, we still have insufficient information about the central control of human locomotion in general and of bicycling in particular. In this study, we addressed how reciprocal inhibition between antagonistic muscles is regulated during bicycle movements.

Reciprocal inhibition is essential for normal extension-flexion movements to occur because it ensures that antagonistic muscles are relaxed when the prime mover is activated. Experiments in the cat have documented that so-called IA inhibitory interneurons are organized to ensure this inhibitory control (see Lundberg 1970 and Hultborn 1972 for reviews). The interneurons in the pathway are activated in parallel with the antagonist motoneurons, both by descending motor tracts and peripheral sensory afferents, such as the group IA afferents, which have given the interneurons their name (reviewed by Edgley 2001). Subsequent human experiments have documented that the activity of the interneurons is regulated accordingly: prior to and during activation of ankle dorsiflexors transmission in the IA inhibitory pathway to ankle plantarflexors is increased, thereby ensuring that these muscles remain relaxed during the movement and that unwanted stretch reflex activity is not evoked (Crone et al. 1987; Nielsen et al. 1992; Tanaka 1974).

It was an early idea that reciprocal inhibition via the IA inhibitory pathway could be an integrated part of the spinal network responsible for eliciting the alternating flexor-extensor activity, which characterizes locomotion. However, Pratt and Jordan (1987) demonstrated that, although IA inhibitory interneurons were modulated rhythmically during fictive locomotion in the cat (i.e., alternating rhythmic activity in motor nerves evoked in the decerebrated cat by stimulation in the mesencephalic locomotor region) and probably contributed to the inactivation of the antagonists in the appropriate phases of the movement, the rhythmic alternation continued after the
inhibitory effect of the interneurons was blocked by strychnine. The interneurons are therefore not an integrated part of the network generating the rhythmic alternation but seem to be controlled by this network as well as by sensory and descending pathways. In human subjects, transmission in the 1a inhibitory pathway seems to be regulated in a similar way as in the cat (Petersen et al. 1999). In the stance phase of walking reciprocal inhibition from ankle dorsi-flexors to the active ankle plantar-flexors is thus small, whereas it is large in the swing phase where the ankle dorsi-flexors become active.

The aim of this study was to investigate whether reciprocal inhibition is also regulated in this way during bicycling. This would further strengthen the idea that the brain uses similar strategies to control bicycling as other forms of locomotion. Bicycling furthermore provides an opportunity to study how the brain controls reciprocal inhibition in relation to changes in external load and the rate of movement. We report that reciprocal inhibition to ankle plantar-flexors measured in early downstroke during bicycling gradually decreases as the load is increased. These findings demonstrate that reciprocal inhibition is specifically controlled in relation to bicycling.

METHODS

Twenty subjects, ages 22–39 yr (10 females; 10 males), participated in the study. Not all subjects participated in all parts of the study. The subjects received written and oral information about the procedures of the experiments before giving their written consent. The experiments were conducted in accordance with the Helsinki declaration and approved by the local ethics committee.

The study consisted of two parts: part I, reciprocal inhibition during tonic plantar flexion and bicycling; and part 2, reciprocal inhibition during bicycling at different loads and cadences.

General setup

The subjects pedaled with their feet strapped to the pedals on a bicycle ergometer (Monarch 834E), modified to monitor the position of the crank continuously, at an external load from 0.5 to 2.5 kg (frictional torque ranging from 5 to 25 N) at 60 RPM (power output 30–150 W) and/or at an external load of 1.0 kg (frictional torque of 10 N) at 30, 60, and 90 RPM.

Recordings

EMG recordings were made from the right medial tibialis anterior (TA) and soleus (SOL) with bipolar (1-cm² recording area; 2 cm between poles) Ag-AgCl electrodes placed over the belly of the muscles. The EMG signals were amplified (2,000–5,000 times), band-pass-filtered at 25–1,000 Hz, and sampled at 2,000 Hz before being stored on a PC for later analysis. The signals were recorded in a window from 50 ms before until 200 ms after stimuli (see Fig. 1B).

Stimulations

To evoke reciprocal inhibition of SOL, stimulation of TA afferents was applied by bipolar stimulation of the peroneal nerve (PN). The anode was placed distal and lateral to the insertion of the patellar ligament and the cathode just below the neck of the fibula above PN. The stimulation electrodes were secured with adhesive tape to prevent them from moving during the experiment. Care was taken that the electrodes were placed so that the TA motor threshold was below the motor threshold of the peroneal muscle group. This was done by the investigator holding his fingers on the insertion tendons of TA and peroneus muscles, simultaneously perceiving the threshold of the muscle activation. The stimulation intensity of PN was adjusted to 1.2 times TA motor threshold throughout the experiments. At this stimulation, intensity activation of the part of the PN innervating the peroneus muscle group could not be avoided in most subjects, but

FIG. 1. Effect of peroneal nerve (PN) stimulation on soleus (SOL) EMG during tonic contraction. A: connection between the tibialis anterior muscle (TA) afferents and the SOL motoneurons, believed to be responsible for the observed effects. B: rectified averaged SOL EMG of 100 recordings with (black line) and 100 recordings without (gray line) stimulation of PN during a moderate level of tonic plantar flexion (approximately 10% of maximal voluntary contraction). C: inhibition of the SOL EMG during 6 different levels of tonic plantar flexion. To illustrate the inhibition, the abscissa of the plot is limited to the part where the inhibition is observed, from 35 to 65 ms after PN stimulation. Note that the ordinates are different in the right and left columns. Black line shows the rectified averaged SOL EMG after stimulation; gray line shows the SOL background EMG. Each line is an average of 100 recordings. D: linear regression between the level of SOL background EMG and recorded inhibition at 4 levels of plantar flexion. R² = 0.90, P < 0.05.
activation of the peroneus muscles were only minor compared with TA.

The SOL H-reflex was evoked by monopolar stimulation of the right tibial nerve (TN). The anode was placed above the patella, and the ball-shaped cathode was pressed into the popliteal fossa. The stimulation electrodes were secured with adhesive tape to prevent them from moving during the experiment. Seven to 10 crank-angles during the crank-cycle were investigated. At each crank-angle, the maximal muscle response (M_max) after TN stimulation was recorded, and the H-reflex was afterward adjusted to approximately 5% of M_max. The H-reflex was kept low, since it is not possible to elicit an H-reflex larger than 5–10% of M_max during late downstroke and upstroke in most subjects.

Part I: reciprocal inhibition during tonic plantar flexion and bicycling

This part of the study consisted of two different types of experiments. In the first experiment, the inhibition of the SOL EMG evoked by PN stimulation was measured at different levels of tonic plantar flexion and compared with the inhibition during bicycling. In the second experiment, the SOL H-reflex was conditioned by stimulation of PN at rest and during bicycling at 60 RPM and an external load of 1.0 kg.

EXPERIMENT 1: INHIBITION OF THE SOL EMG DURING TONIC PLANTAR FLEXION AND BICYCLING. The subjects (n = 12) sat in a reclining armchair performing four to six different levels of tonic isometric plantar flexion with the ankle plantar-flexed to approximately 110°. The levels of contraction ranged from approximately 2 to 30% of the size of the maximal rectified and integrated SOL EMG recorded during a maximal voluntary contraction. During tonic contraction, the rectified and integrated SOL EMG was monitored on an oscilloscope in front of the subjects for visual feedback. Inhibition of SOL EMG during tonic contraction and bicycling was calculated as the difference between the rectified average SOL EMG of 100 sweeps with and 100 sweeps without stimulation (see Fig. 1B). Trials with and without stimulation were alternated randomly with ≥2 s between stimulations. During tonic contraction, the amount of inhibition and the level of SOL background EMG data were fitted to a first order regression line, and the equation of the regression line was used to calculate the expected inhibition at the different levels of SOL background EMG during bicycling (see Fig. 1, C and D, as previously described by Petersen et al. 1999, comparing tonic contraction and walking). The calculated inhibition was later compared with that actually recorded (see below). Since the regression between background EMG and inhibition was essential for the comparison between inhibition during bicycling and tonic plantar flexion, only data from subjects where a significant regression between background EMG and inhibition was found during tonic contraction were used for further analysis (15 subjects volunteered for the experiments; in 12 subjects, a significant linear regression was found between background EMG and inhibition during tonic plantar flexion). The reason why we found no regression in the three subjects excluded was mainly because the post hoc analysis showed that the stimulation intensity used was not strong enough to evoke a clear detectable inhibition in the background EMG.

During bicycling, the subjects pedaled at 60 RPM with an external load of 1.0 kg. The effect of PN stimulation on SOL EMG was investigated at 0, 23, 45, 67, 90, and 180° after top dead center (TDC; where the pedal-arm of the investigated leg is at the top position, see Fig. 2A). In some subjects, SOL EMG activity was present from 23 to 155° after TDC, only. The inhibition was therefore not calculated for 0 and 180° after TDC. The inhibition of the SOL EMG during bicycling was calculated as described during tonic contraction, because the area between the integrated and averaged SOL EMGs with and without PN stimulation (see Figs. 1C and 2A).

EXPERIMENT 2: CONDITIONING OF THE SOLEUS H-REFLEX BY PN STIMULATION. At rest, a time-course of the effect of PN stimulation on the SOL H-reflex was constructed. Conditioning test intervals of 0, 1, 2, 3, 4, and 5 ms were used. Conditioning test interval, at which the largest inhibition of the SOL H-reflex was observed, was used in the rest of the experiment (see Fig. 3A). Crone and Nielsen (1989) showed that the amount of inhibition of the SOL H-reflex depends on the size of the control H-reflex and that the largest inhibition is usually recorded for reflexes around 20–50% of M_max. When comparing the amount of inhibition in different situations such as different crank-angles during bicycling, it is therefore important to ensure that the control H-reflex has a comparable size in the investigated situations. In most subjects, it is not possible during upstroke of bicycling to obtain a test-reflex larger than 5% of M_max. Caution was therefore taken to adjust the test-reflex to approximately 5% of M_max throughout the experiment. During the crank-cycle, at 0, 23, 45, 67, 90, 135, 180, 225, 270, and 315° after TDC, M_max was found, and the unconditioned SOL H-reflex was adjusted to approximately 5% of M_max before evoking a minimum of 10 conditioned and 10 unconditioned H-reflexes. Conditioned and unconditioned H-reflexes were altered randomly and evoked every 2–3 s. The very first H-reflex in each trial was discarded to avoid any influence of postactivation depression (Crone and Nielsen 1989).

Part II: reciprocal inhibition during bicycling at different loads and cadences

This part of the study consisted of three different set-ups. In the first set-up, the inhibition of the SOL EMG after PN stimulation was measured at different levels of tonic plantar flexion. In the second and third set-up, the subjects pedaled on the ergometer-bicycle with three to five different loads at 60 RPM and with different cadences at an external load of 1.0 kg. Whether the second or third set-up was performed first was randomly chosen. This part of the experiment was performed as described above in part I, experiment 1. In brief, 100 sweeps with and without stimulation of PN were recorded at different levels of tonic plantar flexion and during different bicycling set-ups. Inhibition was, similar to above, calculated as the difference between the rectified and averaged SOL EMG with and without stimulation within the time window for the analysis.

During bicycling at different loads, the subjects pedaled at 60 RPM at a workload of 0.5, 1.0, 1.5, 2.0, and 2.5 kg. First, the subjects worked at the lowest load, and a crank-angle for stimulation was chosen during downstroke. The subjects hereafter worked on each of the workloads in random order while the same crank-angle was used for stimulation. The crank-angle for stimulation was chosen to be during downstroke, when the SOL EMG activity was increasing, and at approximately 50% of the maximal SOL EMG during the crank-cycle. The crank-angle for stimulation usually corresponded to 45–67° after TDC in the individual subjects. The SOL EMG patterns during bicycling at different loads were very similar (Fig. 4A). At the crank-angle at which the stimulation was applied, there was no activity in the antagonist (Fig. 4A).

During bicycling at different cadences, the subjects pedaled with an external load of 1.0 kg at 30, 60, and 90 RPM. First, the subjects pedaled at 60 RPM, and the crank-angle at which the level of EMG activity corresponded to approximately 50% of the maximal SOL EMG during the crank-cycle was chosen for stimulation. The subjects hereafter pedaled on each of the cadences in random order while the same crank-angle was used for stimulation. The crank-angle for stimulation usually corresponded to 45–67° after TDC in the individual subjects.

Data analysis

Inhibition was calculated as the area between the rectified averaged unconditioned SOL EMG and the rectified averaged conditioned SOL
EMG. The start- and end-time for the inhibition was determined by visual inspection. During the different tasks (tonic contraction and bicycling at different load and cadences), the time where the inhibition started and especially the time where the inhibition ended varied. To be able to compare the levels of background EMG during the different tasks, the window, where the level of background EMG was measured, was set to be the same during all tasks for each individual subject. The size of the window was determined by the subject's very first start-time and latest end-time of inhibition of all the measurements recorded.

Data analysis was performed with SigmaStat statistical software (SigmaStat for Windows Version 2.03, SPSS). The difference between calculated and recorded inhibition was tested using a Wilcoxon's signed rank nonparametric test. The difference between conditioned and unconditioned SOL H-reflex was tested using the Student's t-test. To test for differences in the amount of inhibition during bicycling at different loads and at different cadences, a one-way repeated ANOVA was used. Throughout the study, the level of significance was set at 0.05. Where nothing else is stated, the values reported are mean ± SE.

RESULTS

Part I: reciprocal inhibition during tonic plantar flexion and bicycling

Figure 1, C and D, shows the amount of inhibition of the SOL EMG during tonic plantar flexion at different levels of

FIG. 2. Effect of PN stimulation on the SOL EMG during bicycling. A: SOL activation pattern during the crank-cycle, pedaling at 60 RPM with an external load of 1.0 kg. Insets: effect of PN stimulation at 7 crank-angles. Black line is an average of 100 recordings with PN stimulation; gray line is an average of 100 recording without stimulation. B: gray columns show the amount of recorded inhibition (area between the 2 lines representing the average EMG without and with stimulation; µV × ms) and black columns show the amount of inhibition calculated from the regression between background EMG and inhibition obtained at tonic contraction. Data in A and B are from the same subject. C: population average amount of inhibition in percent of calculated from the regressions obtained during tonic contraction. Columns represent an average of n = 8, 12, 11, 12, 10, 5, and 2 for the crank-angles 0, 23, 45, 67, 90, 135, 180° after top dead center (TDC), respectively. D: regressions between background SOL EMG and recorded inhibition during tonic contraction and during bicycling for the 8 subjects who showed a significant relation between background SOL EMG and recorded inhibition during bicycling. Correlations were $R^2 = 0.88 \pm 0.03$ and $0.82 \pm 0.04$ for tonic contraction and bicycling, respectively, $P < 0.05$ for all 8 subjects. The y intercepts of the regressions were not significantly different between tonic contraction and bicycling ($-0.10 \pm 0.08$ vs. $-0.05 \pm 0.04$). Slopes of the curves were $0.24 \pm 0.06$ and $0.10 \pm 0.01$ ($P < 0.05$) for tonic contraction and bicycling, respectively.
background EMG for one representative subject. The correlation coefficient \( R^2 \) for the relation between recorded inhibition and background EMG was 0.98 for this subject. For the population of subjects, the average \( R^2 \) was calculated to be 0.88 ± 0.03 (n = 12). Figure 2A shows the background EMG, and the insets show the effect of the PN stimulation on the SOL EMG at seven different crank-angles investigated during bicycling at 60 RPM and an external load of 1.0 kg. From the regression line between the inhibition and background EMG during tonic plantar flexion, it was possible to calculate the size of inhibition corresponding to each of the EMG levels recorded during bicycling. The result of this calculation is shown as black columns in Fig. 2B for each of the seven crank-angles. In the same graph, the amount of inhibition that was actually recorded at the seven crank angles is shown as gray columns. It is noticed that the amount of inhibition recorded during bicycling was smaller than the amount of inhibition calculated from the regression line between the inhibition and the background EMG activity during plantar flexion. This was also the case in the other subjects as evidenced from the population average shown in Fig. 2C. To pool data from different subjects, the size of the inhibition recorded at the different crank angles was expressed as a percentage of the amount of inhibition calculated from the regression line between the inhibition and background EMG activity during plantar flexion. A significant difference between the recorded inhibition and the calculated inhibition was found at crank angles of 0, 23, 45, 67, and 90° (Mann-Whitney rank sum test, \( P < 0.05 \)). Part of the reason why no difference was found at 135 and 180° may be that it was only possible to record SOL activity at these crank-angles in five and two subjects, respectively. This also partly explains the large variability in the results obtained at these crank-angles. However, calculating the population average for only the five subjects in whom we could record SOL activity at 135° after TDC did not alter the results. A two-way ANOVA revealed no statistical differences between the various recordings during bicycling and thus suggested that there was no modulation of the amount of inhibition during the crank cycle. Part of the reason for this may also be that EMG activity was only present in a few subjects at crank angles of 135 and 180°.

As was the case during tonic plantar flexion, there was a significant positive correlation between the background EMG and the inhibition data obtained during the crank-cycle of bicycling in 8 of the 12 subjects. The average \( R^2 \) value of the relation between recorded inhibition and background EMG activity during bicycling for the eight subjects was 0.82 ± 0.04. The average slope-parameter of the regression between background EMG and inhibition was significantly larger (0.24 ± 0.06) during tonic contraction compared with bicycling (0.10 ± 0.01, Student’s t-test, \( P < 0.05 \)). The x intercepts were not significantly different, being −0.001 and −0.050 during tonic contraction and bicycling, respectively. The slope of the regression line for the data recorded during bicycling was on average 63 ± 21% of the slope during tonic contraction.

FIG. 3. Conditioning of the SOL H-reflex with PN stimulation. A: time course of the SOL H-reflex conditioned with stimulation of PN for 1 representative subject at rest. Arrow indicates conditioning test interval (CTI) where the largest inhibition of the reflex was observed (conditioned reflex was 28% of the unconditioned reflex). Unconditioned reflex was on average 6% of \( M_{\text{max}} \). This CTI was hereafter used throughout the experiment. B: size of the PN conditioned SOL H-reflex at 8 different crank-angles during pedaling at 60 RPM at an external load of 1.0 kg for the same subject as in A. During bicycling, the average test reflex was 7 ± 1% of \( M_{\text{max}} \). Bottom: EMG of SOL and TA during the crank-cycle. C: population average of PN conditioned SOL H-reflex during the crank-cycle. On average, the conditioned H-reflex was significantly smaller than the unconditioned H-reflex at 225 and 270° after TDC (\( P < 0.05 \), *Student’s t-test; **at 315° after TDC, the \( P \) value was 0.055). Bottom: average SOL and TA EMGs are superimposed.
Modulation of disynaptic reciprocal inhibition

It is not possible to evaluate the amount of reciprocal inhibition from averages of EMG activity when no EMG activity is present, and a full evaluation of the modulation of reciprocal inhibition during the crank cycle is not possible with that method. We therefore also investigated the modulation of reciprocal inhibition from averages of EMG activity when no EMG activity is present, and a full evaluation of the modulation of reciprocal inhibition was achieved by the H-reflex technique. Data from these experiments are presented in Fig. 3. Figure 3A shows a time course of the effect of PN stimulation on the SOL H-reflex for one representative subject at rest. The largest inhibition was found at a conditioning test interval of 2 ms (the size of the conditioned H-reflex being $28\%$ of the unconditioned H-reflex). Figure 3B shows the modulation of the conditioned H-reflex in percent of the unconditioned H-reflex through the crank-cycle (the unconditioned H-reflex was on average $4.27 \pm 0.96\%$ of $M_{\text{max}}$) for the same subject. In Fig. 3C, the same is presented for the population average ($n = 9$). As can be seen, the conditioned H-reflex was significantly smaller than the unconditioned H-reflex at 225 and 270° after TDC (Student’s t-test, $P < 0.05$). At 315° after TDC, the $P$ value was 0.055. Figure 3C reflects the trend observed in seven of nine subjects that reciprocal inhibition was most pronounced prior to or when TA was active.

Figure 3, B and C (bottom), shows the activity of SOL and TA. On average, SOL was active from 10 ± 5 to 149 ± 1° (28 ± 15 to 413 ± 6 ms) after TDC, whereas TA was active from 16 ± 7° before TDC to 19 ± 9° after (44 ± 20 ms before TDC to 53 ± 26 ms after).

Part II: reciprocal inhibition during bicycling at different loads and cadences

For the subjects participating in this part of the study, the regression between the average background EMG and the average recorded inhibition at different levels of tonic contraction showed an average $R^2 = 0.82 \pm 0.03$. During bicycling, with different loads, the level of SOL background EMG increased with increasing external load in 10 of the 11 subjects (Fig. 4C; thin lines). The population average (Fig. 4C; thick line) showed a statistically significant increase of the background EMG with the external load ($R^2 = 0.95, P < 0.01$). Contrary to the background EMG, the recorded inhibition decreased with increasing external load as shown in Fig. 4D.
When the size of the inhibition was expressed as a percentage of the background EMG for each subject, a significant decrease between the lowest and the highest load was found (Fig. 4E, Student’s $t$-test, $P = 0.05$). Thus, there was a positive relation between inhibition and background EMG during tonic plantar flexion (Fig. 4F; black line; $R^2 = 0.92$, $P < 0.01$), but a negative relation during bicycling (Fig. 4F; gray line; $R^2 = 0.72$ and $P = 0.07$).

Figure 5A shows the SOL and TA EMG during bicycling at 30, 60, and 90 RPM, and Fig. 5B shows examples of the effect of PN stimulation on the averaged SOL EMG activity at these three cadences of bicycling. The stimulation was applied at the ascending part of the SOL EMG activity at the same crank-angles. Figure 5C shows the soleus background EMG during bicycling at different cadences. As during bicycling with increased load, there was a linear increase in the SOL background EMG with increased pedaling rate ($R^2 = 0.96$, $P = 0.03$). The regression between recorded inhibition and speed (Fig. 5D), however, showed no correlation ($R^2 = 0.598$, $P = 0.437$), and no differences between the amounts of inhibition recorded at the different cadences could be detected using the one-way repeated ANOVA. In Fig. 5E, the amount of inhibition is presented as percentage of the background EMG at the cadences pedaled. No differences (one-way repeated ANOVA) were found between the averaged inhibition normalized to background EMG at the different cadences. No differences in the values were found. This is also illustrated in Fig. 5F, where the relation between background EMG and recorded inhibition is plotted for tonic contraction and for bicycling at different speeds. The regression during tonic contraction showed a linear relation between increased background EMG and recorded inhibition (black line; $R^2 = 0.95$, $P < 0.01$) and during bicycling with increased speed, we found no relation (gray line; $R^2 = 0.39$, not significant: $P = 0.57$).

**DISCUSSION**

In this study, we have demonstrated that the inhibition in the SOL background EMG induced by stimulation of PN is smaller during downstroke of bicycling compared with a matched level of background EMG during tonic plantar flexion and that the inhibition of the SOL H-reflex after PN stimulation is modu-
lated during the crank-cycle of bicycling, being largest during upstroke when the antagonist muscle (TA) is active. The inhibition in the SOL background EMG, when expressed as a percentage of the background EMG, decreased as the background EMG increased with increasing load during bicycling. The inhibition in contrast remained constant when the background EMG was increased with increasing tonic plantar flexion.

**Disynaptic Ia inhibition**

Previous studies have demonstrated that the short latency depression of the SOL H-reflex evoked by PN stimulation is mediated by the disynaptic reciprocal Ia inhibitory pathway (reviewed by Crone and Nielsen 1994). In all likelihood, the depression of the SOL background EMG after PN stimulation is also mediated, at least partly, by this pathway. Petersen et al. (1998) demonstrated that the two inhibitions have the same threshold, and Petersen et al. (1999) showed that the inhibition of the SOL background EMG disappeared when transmission in large-diameter afferents were blocked by ischemia, suggesting that group Ia afferents are also responsible for this inhibition. Finally, Crone et al. (2001) found that the inhibition in both the SOL background EMG and the SOL H-reflex is absent in patients with a known defect in the glycine receptor, which is known to be involved in mediating reciprocal inhibition (Bradley et al. 1953). Although other mechanisms may contribute to the inhibition in the EMG, it is thus our opinion that disynaptic reciprocal inhibition via Ia inhibitory interneurons is the main responsible mechanism.

**Decreased reciprocal inhibition in downstroke during bicycling**

The inhibition in the SOL background EMG during bicycling was reduced compared with a similar level of tonic SOL background EMG. This resembles what has previously been reported during walking by Petersen et al. (1999), but it is contrary to what was reported by Capaday et al. (1990). As discussed by Petersen et al. (1998, 1999), there may be several reasons for this discrepancy. In this study and in the studies by Petersen et al. (1998, 1999) special care was taken to ensure that the PN stimulation was as selective as possible for the branch supplying the TA muscle and rather weak stimuli compared with the study by Capaday et al. (1990) were used. Furthermore, in the study by Capaday et al. (1990), the regression line calculated for data recorded in different parts of the stance phase was used for comparison to the regression line calculated for data recorded during tonic plantar flexion. In this study and in the studies by Petersen et al. (1998, 1999), on the other hand, a direct comparison was made for each individual recording during walking to that obtained during tonic plantar flexion at a corresponding level of EMG activity. This was done since changes in the amount of inhibition in specific parts of the gait cycle may be overlooked when all the data are pooled together.

It was ensured that the PN stimulation evoked an M-response of a constant size in the TA muscle in all situations and the smaller amount of inhibition in downstroke during bicycling as compared with tonic plantar flexion therefore is not likely to be caused by changes in the stimulation conditions. As we ensured that the background SOL EMG activity was comparable in the two situations, simple differences in the excitability of the SOL motoneurons are also not likely to be involved. We cannot entirely exclude the possibility that different motoneurons were active in the two situations, but as the soleus muscle is rather homogeneous and consists mainly of type S motor units (Gollnick et al. 1974), which receive an almost equal amount of inhibition (Binder and Powers 1999), we find that this is unlikely to explain our observations. We also cannot fully exclude that it may not be valid to compare the very stable background EMG during tonic contraction to the rapidly changing EMG during bicycling. The motoneurons may be very differently sensitive to inhibition when the background EMG is rapidly changing than when it is stable, and it may in any case be difficult to compare the levels of EMG exactly. However, Petersen et al. (1999) found that reciprocal inhibition was still depressed during walking compared with the inhibition measured in sitting subjects, who were asked to perform a voluntary dynamic plantar flexion where both the level and rate of change of the background EMG was matched to that recorded in the stance phase of walking. We therefore think that the decreased inhibition during bicycling that we have observed in this study is most likely explained by a reduced transmission in the inhibitory pathway at a premotorneuronal stage.

Another concern could be that SOL contract eccentrically during early downstroke but concentrically during late downstroke. This could change the background afferent activity and interfere with measurements of reciprocal inhibition. However, similar amount of inhibition was observed during early and late downstroke (cf. Fig. 3C).

**Mechanisms involved in controlling the transmission in the disynaptic reciprocal inhibitory pathway**

Several different mechanisms may be involved in setting the transmission in the disynaptic reciprocal inhibitory pathway (Enriquez-Denton et al. 2000; Hultborn 1972), and we can only speculate on which of these mechanisms is responsible for the observed decrease of inhibition. Ia inhibitory interneurons receive inhibitory influences from the Ia inhibitory interneurons projecting in the opposite direction (i.e., interneurons that inhibit extensor motoneurons are inhibited by interneurons that inhibit flexor motoneurons), and it would not be unlikely if increased activity of such interneurons were responsible for the decreased inhibition. The interneurons are also inhibited by Renshaw neurons, which have been shown to be modulated during various voluntary movements in human subjects (Katz and Pierrot-Deseilligny 1999) and during fictive locomotion in the cat (Pratt and Jordan 1987). Increased recurrent inhibition during bicycling may therefore also contribute to decreased reciprocal inhibition. Iles et al. (2000) showed that recurrent inhibition of quadriceps motoneurons from SOL was stronger during downstroke of bicycling compared with matched contraction in sitting subjects. The authors argued that their findings may also apply to recurrent inhibition of SOL motoneurons, but whether they also apply to recurrent inhibition of the inhibitory interneurons projecting to these motoneurons is unknown. The transmission in the disynaptic reciprocal pathway is also controlled at the level of the synapses of the Ia afferents on the interneurons (Enriquez-Denton et al. 2000).
Nielsen (1989) demonstrated that the long-lasting (more than 10 s) change in transmitter release from Ia afferents following their previous activation (postactivation depression) may interfere with the determination of the amount of reciprocal inhibition. If postactivation depression is more pronounced during downstroke of bicycling, possibly secondary to larger dorsiflexor Ia afferent activity, than during tonic plantar flexion, this would explain the smaller amount of reciprocal inhibition.

Pyndt and Nielsen (2003) demonstrated that presynaptic inhibition of Ia afferents on soleus motoneurons is increased in the late part of downstroke compared with early downstroke. If these observations are also relevant for presynaptic inhibition of Ia afferents on the antagonistic TA motoneurons and their corresponding interneurons, it would be expected that a similar difference was seen in the modulation of reciprocal inhibition in early and late downstroke. However, this was not the case (cf. Figs. 2 and 3).

It could also be argued that stretch of the TA muscle would increase Ia afferent activity and lead to refractoriness. This could explain the smaller amount of inhibition. However, TA is only stretched during late downstroke (e.g., Chen et al. 2001), and this is therefore not likely to explain our observations during downstroke.

It is very likely that the regulatory mechanisms in the spinal cord may also be influenced by a vast variety of afferent inputs arising from muscles from the ipsilateral as well as the contralateral leg (Brooke et al. 1997).

Modulation of reciprocal inhibition during the crank-cycle

Using the H-reflex technique, we demonstrated that reciprocal inhibition was much larger during upstroke than during downstroke (Fig. 3, B and C). Petersen et al. (1999) similarly reported that reciprocal inhibition was increased in the swing phase of walking compared with the stance phase. In this study, the increase of reciprocal inhibition was observed just prior to and during the time when the TA muscle was active. The inhibition disappeared again when the soleus muscle became active in the early part of downstroke. This underscores the parallel control of Ia inhibitory interneurons and their corresponding motoneurons during extension-flexion movements (Crone et al. 1987; Hultborn 1972; Lundberg 1970). It should also be noted that the pattern resembles that observed for the activity of Ia inhibitory interneurons during fictive locomotion in the cat (Pratt and Jordan 1987).

Pyndt and Nielsen (2003) observed that the soleus H-reflex was depressed in the late part of downstroke and especially during upstroke (see also Brooke et al. 1992). The observation in this study that reciprocal inhibition to soleus motoneurons is not increased until well into upstroke shows that increased disynaptic reciprocal inhibition cannot be responsible for the H-reflex depression. A more likely mechanism for that depression is, as also suggested by Pyndt and Nielsen (2003), increased presynaptic inhibition of the soleus Ia afferents.

Changes in reciprocal inhibition as a function of load and cadence

During tonic contraction the amount of depression of the SOL background EMG was linearly related to level of background SOL EMG. When expressed as a percentage of the background EMG the inhibition in other words remained nearly constant. This phenomenon has been described as “automatic gain compensation” (Marsden et al. 1972; Matthews 1986) and has previously been demonstrated for the PN induced inhibition of the SOL EMG (Capaday et al. 1990; Petersen et al. 1999). Automatic gain compensation is thought to ensure that reflexes remain appropriate to the level of contraction. In our study “automatic gain compensation” was observed during bicycling in 8 of 12 subjects (Fig. 2D). It is, however, meaningless to compare the measurements during bicycling, which include measurements from both early downstroke, where the background EMG is increasing and late downstroke where the EMG is decreasing. As we have already pointed out, reciprocal inhibition is likely to be controlled very differently in these two phases of the movement, and it is therefore not reasonable to calculate regression lines for such data or make any conclusions regarding the existence of automatic gain compensation. However, we also investigated the amount of inhibition as a function of the external load and the rate of pedaling. In these two cases, the amount of inhibition was measured at the same crank-angle during downstroke, but with increasing background EMG as the external load or the rate of pedaling was increased. In striking contrast to what was observed during tonic plantar flexion, the amount of inhibition when expressed as a percentage of the background EMG decreased with increasing background EMG produced by increased external load. The principle of “automatic gain compensation” thus does not apply to bicycling against different external loads. The data again rather suggest that reciprocal inhibition is differently controlled during bicycling than during tonic voluntary plantar flexion. It seems that reduction of the amount of inhibition of the soleus motoneurons is of importance during bicycling to increase the activation of the soleus muscle and overcome the increased external load. A similarly strong reduction of reciprocal inhibition is apparently not necessary during voluntary plantar flexion.

The SOL and TA EMG patterns during pedaling at increased cadences were very similar to the EMG patterns during pedaling with increased load. Although it was not significant, we also observed a small decrease in the amount of reciprocal inhibition with increased pedaling rate. The lack of significance may be caused by the large variability of the measurements (cf. Fig. 5D), but it may also reflect a difference in modulation of afferent inflow (Staines et al. 1997) or central command (Christensen et al. 2000) with increased pedaling rate compared with increased load.

Comparison with walking

The modulation of reciprocal inhibition observed in this study during bicycling is very similar to the modulation reported during walking by Petersen et al. (1999). This strengthens the idea that the brain uses similar strategies to control bicycling and walking. In both cases, less inhibition is observed than during voluntary tonic plantar flexion, and in this study, we found a gradual decrease of inhibition with increasing activation of the soleus muscle. This may be related to differences in sensory inflow in the two situations as already pointed out by Brooke et al. (1997) or it may be related to a different central control of the segmental circuitry. During walking and bicycling it is reasonable to assume that a spinal
Synaptic integration in spinal motoneurons.
Binder MD and Powers RK.

REFERENCES

Ministry of Culture (The Sports Science Research Council), the Danish Society

rhythmicity, whereas the muscle activity to a larger extent is

1981) is responsible for generation of the basic locomotor
circuitry similar to that described in animals (Grillner et al.
1981) is responsible for generation of the basic locomotor
rhythmicity, whereas the muscle activity to a larger extent is

Modulation of the

The special nature of human walking and its neural control.
Capaday C.

Movement features and H-reflex modulation. I. Pedalling versus matched controls. J


Bradley K, Easton DM, and Eccles JC. An investigation of primary or direct

Brooke JD, Cheng J, Collins DF, McIlroy WE, Misiaszek JE, and Staines
WR. Sensori-sensory afferent conditioning with leg movement: gain control

Brooke JD, McIlroy WE, and Collins DF. Movement features and H-reflex
modulation. I. Pedalling versus matched controls. Brain Res 582: 78–84,

Capaday C. The special nature of human walking and its neural control. Trends

Capaday C, Cody FW, and Stein RB. Reciprocal inhibition of soleus motor
output in humans during walking and voluntary tonic activity. J

Chen G, Kautz SA, and Zajac FE. Simulation analysis of muscle activity changes
with altered body orientations during pedalling. J Biomech 34: 749–756,

Christensen LO, Johannsen P, Sinkjaer T, Petersen N, Pyndt HS, and Nielsen
JB. Cerebral activation during bicycle movements in man. Exp

Crone C, Hultborn H, Jespersen B, and Nielsen J. Reciprocal Ia inhibition

Crone C, Hultborn H, Mazieres L, Morin C, Nielsen J, and Pierrot-
Desilettigny E. Sensitivity of monosynaptic test reflexes to facilitation and
inhibition as a function of the test reflex size: a study in man and the cat. Exp

Crone C and Nielsen J. Methodological implications of the post activation

Crone C and Nielsen J. Central control of disynaptic reciprocal inhibition in

Crone C, Nielsen J, Petersen N, Tjølsen MA, and van Dijk JG. Patients with
the major and minor form of hyperekplexia differ with regards to disynaptic
reciprocal inhibition between ankle flexor and extensor muscles. Exp

Dietz V. Physiology of human gait. Neural processes. Adv

Edgley SA. Organisation of inputs to spinal interneuron populations.

Enriquez-Denton M, Nielsen J, Perreault M, Morita H, Petersen N, and
Hultborn H. Presynaptic control of transmission along the pathway medi-
ating disynaptic reciprocal inhibition in the cat. J Physiol 526: 623–637,
2000.

Gollnick PD, Sjödin B, Karlsson J, Jansson E, and Saltin B. Human soleus
muscle: a comparison of fiber composition and enzyme activities with other

Grillner S. Control of locomotion in bipeds, tetrapods and fish. In: Handbook
of Physiology. The Nervous System. Motor Control, edited by Brooks VB.

Hultborn H. Convergence on interneurones in the reciprocal Ia inhibitory

Iles JF, Ali A, and Pardoe J. Task-related changes of transmission in the pathway of heteronymous spinal recurrent inhibition from soleus to quad-

Katz R and Pierrot-Deseilligny E. Recurrent inhibition in humans. Prog

Lundberg A. The excitatory control of the Ia inhibitory pathway. In: Excita-
tory Synaptic Mechanisms, edited by Andersen P and Jansen JKS. Oslo,

Marsden CD, Merton PA, and Morton HB. Servo action in human voluntary

Matthews PB. Observations on the automatic compensation of reflex gain on
varying the pre-existing level of motor discharge in man. J Physiol 374:
73–90, 1986.

Nielsen JB. Motoneuronal drive during human walking. Brain Res Rev 40:

Nielsen J, Kagamihara Y, Crane C, and Hultborn H. Central facilitation of
Ia inhibition during tonic ankle dorsiflexion revealed after blockade of

Petersen N, Morita H, and Nielsen J. Evaluation of reciprocal inhibition of
the soleus H-reflex during tonic plantar flexion in man. J Neurosci Methods

Petersen N, Morita H, and Nielsen J. Modulation of reciprocal inhibition
between ankle extensors and flexors during walking in man. J Physiol 520:

Pratt CA and Jordan LM. Ia inhibitory interneurons and Renshaw cells as
contributors to the spinal mechanisms of fictive locomotion. J

Pyndt HS and Nielsen JB. Modulation of transmission in the corticospinal
and group Ia afferent pathways to soleus motoneurons during bicycling.

Staines WR, Brooke JD, Misiaszek JE, and McIlroy WE. Movement-
induced gain modulation of somatosensory potentials and soleus H-reflexes
evoked from the leg. II. Correlation with rate of stretch of extensor muscles

Tanaka R. Reciprocal Ia inhibition during voluntary movements in man. Exp