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Further evidence to support different mechanisms underlying intracortical inhibition of the motor cortex

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Abstract Paired-pulse magnetic stimulation has been widely used to study intracortical inhibition of the motor cortex. Inhibition at interstimulus intervals (ISIs) of 1–5 ms is ascribed to a GABAergic inhibitory system in the motor cortex. However, Fisher et al. have proposed that different mechanisms are operating at an ISI of 1 ms and 2.5 ms. In order to confirm their concept and clarify whether inhibition at all these intervals is produced by a single mechanism, we compared effects of paired-pulse stimulation at ISIs of 1 ms, 2 ms, and 3–5 ms. We evaluated how intracortical inhibition affected the I3-wave, I1-wave, magnetic D-wave, and anodal D-wave components of electromyographic (EMG) responses using previously reported methods. The data suggest that three separate effects occur within these ISIs. At ISIs of 3–5 ms, inhibition was evoked only in responses to I3-waves, whereas no inhibition was elicited in responses to I1-waves or magnetic D-waves. In contrast, at an ISI of 1 ms, responses to I3-waves and I1-waves were moderately suppressed. Moreover, even magnetic D-waves were slightly suppressed, whereas anodal D-waves were unaffected. At an ISI of 2 ms, none of the descending volleys were inhibited. We propose that we should use ISIs of 3–5 ms for estimating function of the GABAergic inhibitory system of the motor cortex by paired-pulse transcranial magnetic stimulation (TMS). Our results support the idea of Fisher et al. that the mechanism responsible for the inhibition at an ISI of 1 ms is not the same as that responsible for suppression at ISIs of 3–5 ms (GABAergic inhibitory circuits in the motor cortex). At an ISI of 2 ms, we suggest that the inhibitory influence evoked by the first stimulus (S1) should collide with or be occluded by

the second stimulus (S2), which leads to the lack of inhibition when the subjects make a voluntary contraction of the target muscle.

Keywords Magnetic stimulation · Motor cortex · Intracortical inhibition · I-wave · GABA

Introduction

Paired-pulse magnetic stimulation techniques have been used for studying the intracortical circuitry of the motor cortex in humans. When the first stimulus (conditioning stimulus) is subthreshold, electromyographic (EMG) responses to a suprathreshold second (test) stimulus are inhibited when the interstimulus interval (ISI) is 1–5 ms (intracortical inhibition; Kujirai et al. 1993). The interaction between the stimuli was considered to occur at the motor cortex, because I-waves (indirect-waves: descending volleys produced by indirect activation of pyramidal-tract neurons (PTNs) via presynaptic neurons) were suppressed, whereas D-waves produced by anodal electrical stimuli were not. D-waves elicited by anodal electrical stimulation (anodal D-waves) are considered to originate from direct activation of corticospinal tracts in the white matter. Therefore, they should be unaffected by changes in cortical excitability. The inhibition was proposed to reflect activity of intracortical GABAergic inhibitory systems in the motor cortex (Kujirai et al. 1993; Ziemann et al. 1996b, 1996c). The effect was most powerful in responses to later rather than earlier I-waves (Nakamura et al. 1997; Hanajima et al. 1998; Di Lazzaro et al. 1999).

In previous studies, investigators have measured the amount of inhibition at an ISI of 2 ms or calculated an average value of size ratios at ISIs of 1–5 ms as representing the level of GABAergic intracortical inhibition (Ridding et al. 1995a, 1995b, 1995c; Hanajima et al. 1996; Ziemann et al. 1996a, 1996b, 1996c, 1997; Chen et al. 1998; Muellbacher et al. 2000). However, one recent paper (Fisher et al. 2002) has shown different effects of

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voluntary contraction on the inhibition and differences in the threshold for eliciting suppression between the inhibition at ISIs of 1 and 2.5 ms. The authors concluded that different mechanisms should operate at 1-ms and 2.5-ms intervals. We also noticed that inhibition at an ISI of 1 ms was normal in some patients in whom inhibition at ISIs of 3–5 ms was reduced (Hanajima et al. 1996). When we closely examined figures in previous reports, such a phenomenon was seen in some patients with Parkinson disease (Ridding et al. 1995a), focal dystonia (Ridding et al. 1995b), and amyotrophic lateral sclerosis (Ziemann et al. 1997). Even in normal subjects, this occurred when they maintained a constant contraction of a target muscle (Ridding et al. 1995c) or when transcranial magnetic stimulation (TMS) elicited magnetic D-waves (Hanajima et al. 1996). These led us to suspect that inhibition at certain ISIs may be produced by different mechanisms from that at other intervals. The aim of the present paper is to give further evidence to support the conclusion by Fisher et al. (2002) that different mechanisms must be responsible for the intracortical inhibition at different ISIs.

In our previous paper (Hanajima et al. 1998), we compared intracortical inhibitory effects on I3- and I1-waves at ISIs of 3–20 ms using a method which elicits one group of I-waves preferentially (Sakai et al. 1997). Our main message was that I3-waves were inhibited at all these ISIs, but I1-waves were not. In the present communication, we compared suppressive effects of paired magnetic stimulation on different descending volleys at three groups of ISIs (1 ms, 2 ms, and 3–5 ms) using the same method. Our results support a previously reported conclusion (Fisher et al. 2002). We suggest that the excitability of GABAergic inhibitory interneuronal systems in motor cortex is best studied by measuring the effect of paired magnetic stimulation at ISIs of 3–5 ms. At ISIs of 1 ms and 2 ms, other mechanisms must overlap GABAergic inhibitory mechanisms.

Methods

Subjects

Ten healthy volunteers (eight men and two women, 32–47 years old) were studied. Written informed consent was obtained from all the subjects. The experimental procedures were performed according to the Declaration of Helsinki and approved by the Ethics Committee of the University of Tokyo. No side effects were noted in any of the individuals.

Electromyographic recordings

Surface electromyograms (EMGs) were recorded from the right first dorsal interosseous (FDI) with 9-mm-diameter, Ag–AgCl surface cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified (Biotop; GE Marquette Medical Systems Japan, Tokyo) through filters set at 100 Hz and 3 kHz, then recorded by a computer (Signal Processor DP-1200; GE Marquette Medical System Japan, Tokyo), on which

a randomized, conditional averaging was performed with a sampling rate of 10 kHz. During the experiments, subjects maintained a slight contraction of the right FDI (50–10% of the maximum voluntary contraction) with the aid of an oscilloscope monitor.

Stimulation

Transcranial electrical stimulation (TES) was performed with a high-voltage electrical stimulator D180A (Digitimer, UK) in order to determine the anodal D-wave latency for each muscle in all the subjects. Stimuli were given through two Ag–AgCl cup electrodes (9 mm in diameter) fixed to the scalp; the cathode was placed at the vertex, the anode over the hand motor area (about 5–6 cm lateral to the vertex). Electrical stimuli were given during a slight contraction of the target muscle. As a reference for the following experiments, anodal D-wave latencies were measured from a trace of several superimposed electrical responses.

TMS was done with a Magstim 200 magnetic stimulator (The Magstim Company, UK). A figure-eight-shaped coil (external diameter at each wing, 9 cm) was placed over the hand motor area. Before the main experiments, we determined the current directions at which I1- or I3-waves were preferentially elicited in every subject. In this experiment, a figure-eight-shaped coil was placed over the hand motor area and held at eight different orientations, each separated by 45°. We chose two current directions: the direction which was effective to elicit responses about 1.5 ms later (I1-waves) than anodal D-waves, and the direction effective to evoke responses about 4.5 ms later (I3-waves) than anodal D-waves. In most of the subjects, anteriorly directed induced currents in the brain preferentially elicited I1-waves and posteriorly directed induced currents, I3-waves. In three subjects, TMS with medially directed currents in the brain evoked responses whose latencies almost corresponded to the anodal D-waves determined by TES (Werhahn et al. 1994). In this paper, we use the term “anodal D-wave” to indicate the D-wave evoked by anodal electrical stimulation of the motor cortex, and “magnetic D-wave” as the D-wave elicited by TMS. In all of the three subjects, we performed paired-pulse stimulation experiments on magnetic D-waves. Since magnetic D-waves were suppressed at an ISI of 1 ms by the medially directed conditioning TMS (see Results) in paired-pulse magnetic stimulation, to investigate where this suppression occurs, we also studied effects of the same magnetic conditioning TMS (–5%) on anodal D-waves with the paired stimulation method (conditioning stimulus, medially directed MTS; test stimulus, TES of M1).

Paired-pulse magnetic stimulation

We studied intracortical inhibition (Kujirai et al. 1993) of I3-, I1-waves, and magnetic D-waves with the selected current directions (Hanajima et al. 1998). It has been reported that relatively pure descending volleys were evoked by different coil orientations in TMS using poststimulus time histograms (PSTH) of single motor units (Sakai et al. 1997; Hanajima et al. 1998). Conditioning and test stimuli were given through the same figure-eight-shaped coil by connecting two magnetic stimulators linked with a Bistim module.

We first determined the threshold for each current direction using averaged rectified EMGs for active muscles (average of at least 10 responses). The intensity of stimulation was changed in steps at 2% of the maximum stimulator output. We defined the threshold as the lowest intensity that evoked a small response (about 50 μ V) as compared to the prestimulus background activities. To investigate the effect of the intensity of the conditioning stimulus, we used several intensities of the conditioning stimulus (–15% to –2 % below the motor threshold for active muscles). The test stimulus was adjusted to evoke a response with an amplitude of approximately 0.2–0.4 mV peak to peak in the active FDI, which was 10–15% above the threshold. The reasons

why we used control responses of this size are as follows: The intensity eliciting responses of this size usually produces one kind of descending volley judging from PSTH experiments (Sakai et al. 1997; Hanajima et al. 1998). When using a higher intensity and when responses are larger, a few groups of descending volleys contribute to generation of EMG responses. Therefore, responses of this size are appropriate for seeing effects on one kind of descending volley. The response latencies were measured from a trace of several superimposed, single responses. We confirmed that latencies of control responses elicited by stimulation with the two selected currents were compatible with the I1- and I3-waves. We used a randomized conditioning-test design similar to that reported previously (Hanajima et al. 1996). In short, various conditions (a test or conditioning stimulus given alone, and a test stimulus preceded by a conditioning stimulus at various ISIs) were intermixed randomly in one block. Several blocks of trials were performed to investigate the complete time-course of the studied effect.

ISIs between 1 and 5 ms (1 ms, 2 ms, 3 ms, 4 ms, and 5 ms) were used. Eight to ten responses were collected and averaged for each condition in which both stimuli were given, and 20 responses for a control condition in which the test stimulus was given alone. The peak-to-peak amplitude of each single response under each condition was measured to statistically compare the amplitudes of control and conditioned responses in the same block with a *t*-test corrected for multiple comparisons (Bonferroni correction) in each single subject. We calculated the ratio of the mean amplitude of the conditioned response to that of the control response for each ISI in every subject. Since we should see the effects on the first part of EMG responses (D-, I1-, or I3-waves in each experiment), we also measured the first deflection of every response in a few experiments and compared those with the peak-to-peak amplitude. These results showed that size changes were almost the same between the two kinds of response sizes. We then used size ratios of peak-to-peak amplitudes in the analysis of time courses of the effects. The graphs plot the mean (\pm SE) time course of the effect of the conditioning stimulus with these ratios averaged over all subjects. We compared these time courses using repeated-measures analysis of variance (ANOVA) and a Turkey's method for post-hoc analysis. To compare response sizes between control trials and conditioned trials at different ISIs in one time course, we used control condition and different ISIs as one factor and performed one-factor ANOVA for response sizes in all single trials.

In the three subjects who had magnetic D-waves, the same paired-pulse magnetic stimulation was performed on magnetic D-waves. In the same three subjects, we also studied effects of the conditioning, medially directed magnetic stimulus (-5%) on anodal D-waves at an ISI of 1 ms.

Results

Mean (\pm SD) onset latencies were 22.8 ± 0.7 ms for I3-waves, 20.1 ± 1.0 ms for I1-waves, 18.4 ± 0.2 ms for magnetic D-waves, and 18.4 ± 0.3 ms for anodal D-waves. These latencies support the hypothesis that each stimulation elicited an appropriate descending volley. Even though latencies of magnetic and anodal D-waves did not differ significantly, they must have originated from different sites within the corticospinal tract. Anodally elicited D-waves are produced by activation of axons of the corticospinal tract neurons at the site deep in the white matter, and therefore they should be unaffected by cortical excitability changes. However, Patton and Amassian (1954) reported that a portion of D-waves might originate from cell bodies or basal dendrites of the corticospinal neurons. Another report (Gorman 1966) suggested that D-waves evoked by anodal electrical

stimulation originate from direct activation of corticospinal tracts in the white matter, and activation occurs at a more superficial point of the corticospinal tract neurons (axon hillock) in the cathodal electrical stimulation. Such cathodal D-waves should be moderately affected by cortical excitability changes. Since TMS can activate superficial structures and cannot activate deep structures, magnetic D-waves must originate from a space near the axon hillock of corticospinal tract neurons. The magnetic D-waves, therefore, should be affected by the cortical excitability changes, whereas the anodal D-waves must be unaffected. The mean (\pm SD) threshold for I3-, I1-, and magnetic D-waves were $49\pm 10\%$, $36\pm 9\%$, and $35\pm 6\%$ of the maximum stimulator output, respectively.

Figure 1 shows an example of EMG responses from a single subject produced by I3-waves, I1-waves, and magnetic D-waves in the paired-pulse magnetic stimulation experiments. In each figure part, the top trace is a control response and has a peak-to-peak amplitude of about 0.2 mV, and the others are conditioned responses at ISIs of 1–5 ms. The onset latency of the response elicited by posteriorly directed currents in the brain was 24.3 ms, which corresponded to an I3-wave (Fig. 1A). Anteriorly directed currents in the brain elicited a response whose onset latency was 21.5 ms (I1-wave; Fig. 1B). In this subject, medially directed currents in the brain elicited a response with an onset latency of 20.1 ms, which corresponded to the anodal D-wave latency (Fig. 1C). The intensity of the conditioning stimulus was fixed at 5% below the threshold for an active FDI (-5%). The sizes of responses to I3-waves were significantly reduced at ISIs of 1 ms, 3 ms, 4 ms, and 5 ms ($P<0.01$, *t*-test with Bonferroni correction; Fig. 1A). The first deflection after the onset, which should be produced by I3-waves, was markedly reduced or absent at these ISIs. On the other hand, the size of responses to I1-waves or magnetic D-waves was not significantly reduced at ISIs of 2–5 ms, even though significant suppression was elicited at an ISI of 1 ms ($P<0.02$, *t*-test with Bonferroni correction; Fig. 1B, C). In these waves, the first deflection after the onset was reduced at an ISI of 1 ms and was not affected at ISIs of 2–5 ms. These indicate that the descending volleys responsible for the early part of EMG responses were influenced by the conditioning stimulus in the similar manner to the peak-to-peak EMG response sizes.

A similar pattern of effects was observed in all subjects. Mean (\pm SE) time courses obtained from the results of all subjects are shown in Fig. 2. The time courses of inhibition evoked by conditioning stimuli at an intensity of 5% below the threshold (-5%) are shown (Fig. 2A: I3-waves, Fig. 2B: I1-waves, Fig. 2C: magnetic D-waves). For I3-waves (Fig. 2A), ANOVA revealed that the ISI had a significant effect on response sizes (one-factor ANOVA: $F=6.773$, $P<0.01$). Post-hoc analysis showed that responses at ISIs of 1 ms, 3 ms, 4 ms, 5 ms were significantly smaller than the control response, whereas the response was not significantly different from the control response at an ISI of 2 ms (ISI=1 ms, 3 ms, 4 ms, and 5 ms, $P<0.01$; ISI=2 ms, $P>0.05$). These

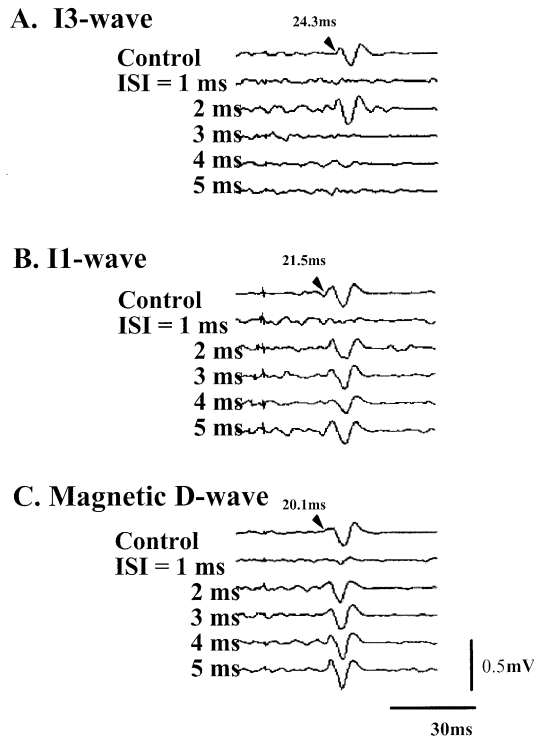


Fig. 1A-C Comparison between effects on responses elicited by different kinds of descending volleys. Responses to posteriorly directed induced currents (A), anteriorly directed induced currents (B), and medially directed induced currents (C) from a single subject are shown. A control response is shown in the first row, and responses to the test stimulus preceded by the conditioning stimulus by 1 ms, 2 ms, 3 ms, 4 ms, and 5 ms in subsequent five rows in every set of responses. The intensity of a conditioning stimulus was fixed at 5% below the active threshold. The sizes of control responses were about 0.2 mV. Their onset latencies were compatible with I3- (A), I1- (B), and D-waves (C). Responses to I3-waves were significantly suppressed by the conditioning stimulus at ISIs of 1 ms, 3 ms, 4 ms, and 5 ms (*t*-test with Bonferroni correction: $P < 0.01$), whereas no suppression was seen at an ISI of 2 ms. In contrast, amplitudes of responses to I1-waves or magnetic D-waves were unaffected by the conditioning stimulus at ISIs of 2 ms, 3 ms, 4 ms, and 5 ms. At an ISIs of 1 ms, responses to both I1-waves and magnetic D-waves, including the first deflection just after the onset, were significantly suppressed (*t*-test with Bonferroni correction: $P < 0.02$)

indicate that there was significant suppression of I3-waves at ISIs of 1 ms, 3 ms, 4 ms, and 5 ms; whereas, at an ISI of 2 ms, the conditioning stimulus failed to evoke significant inhibition. For I1-waves (Fig. 2B) and magnetic D-waves (Fig. 2C), the ISI had a significant effect on response sizes (one-factor ANOVA, I1-wave: $F = 5.202$, $P < 0.01$; D-wave: $F = 3.775$, $P < 0.05$). EMG responses produced by I1-waves or magnetic D-waves were not significantly suppressed at ISIs of 2–5 ms (post hoc: I1-wave, magnetic D-wave; ISI=2 ms, 3 ms, 4 ms, and 5 ms, $P > 0.05$). At an ISI of 1 ms, responses elicited by I1-waves were significantly suppressed by the conditioning stimulus (post hoc: $P < 0.01$). Responses to magnetic D-waves were also suppressed by the conditioning stimulus (post hoc: $P < 0.05$; C). The time courses of

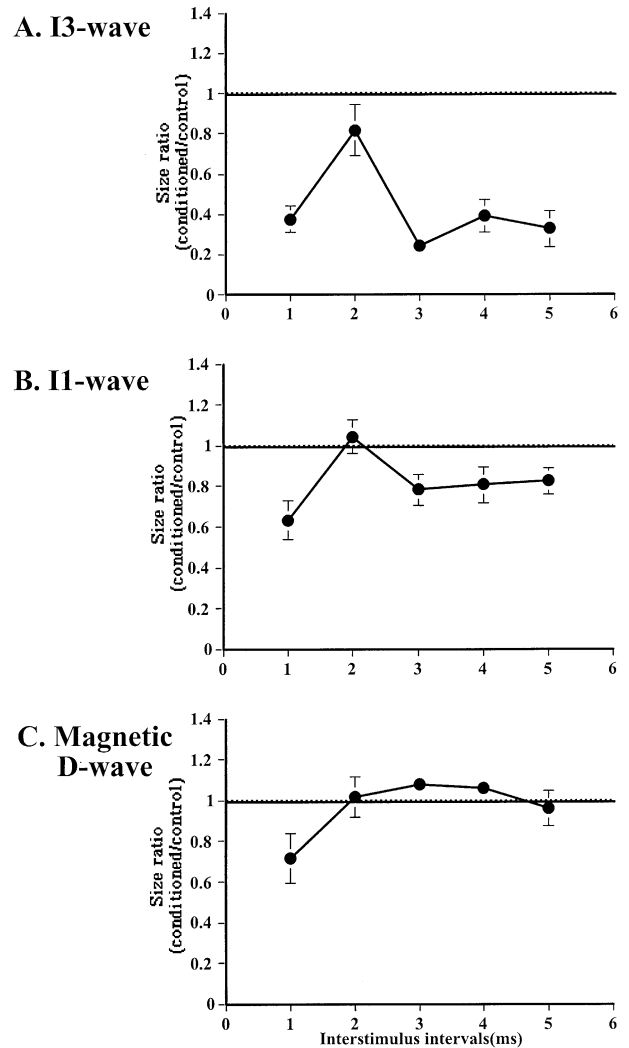


Fig. 2A-C Mean (\pm SE) time courses of effects on three different control responses. Time courses of effects evoked by the conditioning stimulus at an intensity of 5% below the active threshold are shown. **A** Effects on responses to I3-waves. Inhibition occurred at ISIs of 1 ms, 3 ms, 4 ms, and 5 ms; whereas, at an ISI of 2 ms, no significant inhibition was evoked. **B** Effects on responses to I1-waves. The size of responses to anteriorly directed currents was not suppressed at ISIs of 2–5 ms. On the other hand, responses were significantly suppressed at an ISI of 1 ms. **C** Effects on responses to magnetic D-waves. Mean time courses obtained from three subjects in whom D-waves were elicited by transcranial magnetic stimulation. Inhibition was not observed at ISIs of 2–5 ms. At an ISI of 1 ms, suppression was evoked by conditioning stimuli

inhibitory effects were significantly different between I3-waves, I1-waves, and magnetic D-waves (repeated-measures ANOVA: effect of descending volleys, $F = 8.19$, $P < 0.01$; post-hoc analysis between I3-wave and I1-wave, I3-wave and magnetic D-wave, $P < 0.01$). In the three subjects who had magnetic D-waves, there were no significant suppression of anodal D-waves at an ISI of 1 ms ($P > 0.05$, *t*-test with Bonferroni correction; size ratios were 0.96, 0.88, 1.02).

Based on these physiological characteristics, we classified effects evoked by the paired-pulse magnetic

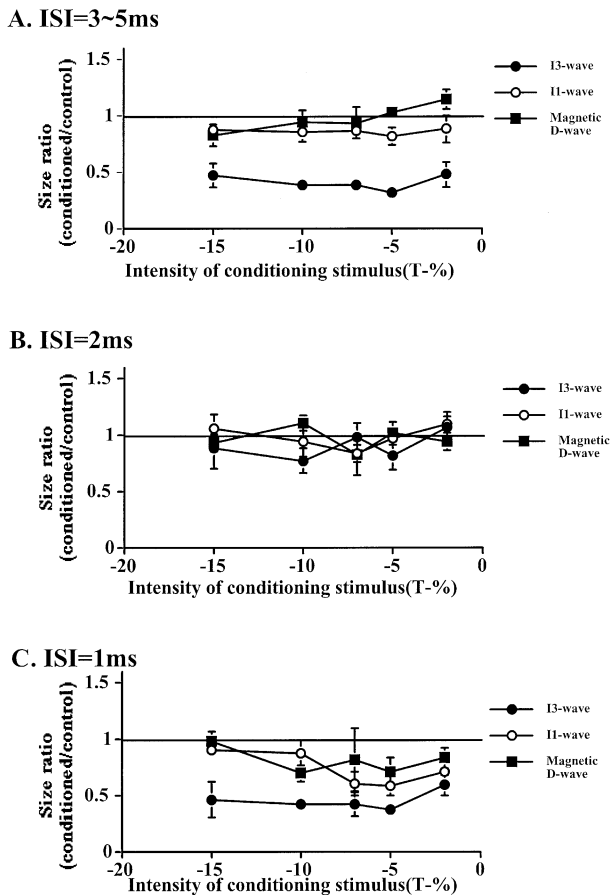


Fig. 3A-C Effects of the intensity of the conditioning stimulus in three groups of ISIs. Mean (\pm SE) average-size ratios at each group of ISIs are plotted against intensities of the conditioning stimulus. *Dots* show the ratios for responses to I3-waves, *circles* for I1-waves, and *squares* for magnetic D-waves. **A** At ISIs of 3–5 ms, average-size ratios were significantly less than 1.0 at any intensities of the conditioning stimulus in responses to I3-waves. In contrast, no inhibition was evoked in responses to I1- or magnetic D-waves. **B** At an ISI of 2 ms, no suppression was evoked in any kinds of responses. **C** Responses to I3-waves were suppressed by any conditioning stimuli at an ISI of 1 ms. Responses to I1-waves were significantly suppressed by conditioning stimuli at an intensity of -7 , -5 , or -2% , and those to magnetic D-waves by conditioning stimuli at an intensity of -5% or -2%

stimulation into three groups at different ISIs (1 ms, 2 ms, and 3–5 ms). For a representative value of the effect at ISIs of 3–5 ms, we calculated the average of size ratios at ISIs 3 ms, 4 ms, and 5 ms in each subject. In order to clarify effects of the intensity of the conditioning stimulus on the suppression, we plotted the mean (\pm SE) of these average size ratios against the intensity of the conditioning stimulus (Fig. 3) for all three groups of ISIs. At ISIs of 3–5 ms (Fig. 3A), there was significant inhibition in responses to I3-waves, but no inhibition in responses to I1 or magnetic D-waves. For the average size ratio (3–5 ms), ANOVA with two factors, descending volleys (magnetic D-, I1-, and I3-waves) and the intensity of conditioning stimulus, showed a significant effect of descending volleys ($F=11.6$, $P<0.05$), but no effect of the condition-

ing stimulus intensity, nor any interaction between them (conditioning stimulus intensity: $F=0.54$, $P>0.05$, descending volleys \times conditioning stimulus intensities: $F=0.03$, $P>0.05$). Significant suppression was evoked by the conditioning stimuli at any intensity in I3-waves (post hoc, $P<0.05$), but no inhibition in I1-waves or magnetic D-waves (post hoc, $P>0.05$). This is consistent with our previous report (Hanajima et al. 1998). At an ISI of 2 ms (Fig. 3B), there were no significant differences among responses to magnetic D-, I1-, and I3-waves (ANOVA: $F=0.02$, $P>0.05$). The intensity of conditioning stimulus had also no significant effect (ANOVA: $F=0.12$, $P>0.05$). No inhibition was elicited in responses to any waves. At an ISI of 1 ms, ANOVA showed a significant effect of the descending volley ($F=5.0$, $P<0.05$), but no effect of the conditioning stimulus intensity ($F=0.07$, $P>0.05$), nor any interaction between them ($F=0.52$, $P>0.05$). Deeper suppression was elicited in I3-waves than in I1-waves or magnetic D-waves (post hoc, $P<0.05$). To see the effect of conditioning stimulus intensity on each descending volley at an ISI of 1 ms, we performed one-factor (control trials and conditioned trials using several intensities of the conditioning stimulus) ANOVA for each single curve. Post-hoc analysis was performed with a Tukey's method. In all curves for magnetic D-, I1-, and I3-waves, the intensity of the conditioning stimulus had a significant effect on response sizes (ANOVA, $P<0.05$). Post-hoc analysis revealed the following: Significant inhibition was evoked by all conditioning stimuli in I3-waves, whereas it was evoked by conditioning stimuli at an intensity of -2% , -5% , and -7% in I1-waves and at an intensity of -2% and -5% in magnetic D-waves.

Discussion

We have shown that inhibitory effects studied with paired-pulse magnetic stimulation at short ISIs have different physiological characteristics at different ISIs: (1) at ISIs of 3–5 ms, inhibition was evoked only in I3-waves, which is consistent with our previous report (Hanajima et al. 1998); (2) at an ISI of 1 ms, inhibition was observed in I1-waves and magnetic D-waves, as well as in I3-waves, whereas anodal D-waves were unaffected; (3) at an ISI of 2 ms, no significant inhibition was evoked by any conditioning stimuli. These results are consistent with the recently reported finding that the inhibition at an ISI of 1 ms had physiological differences from that at an ISI of 2.5 ms (Fisher et al. 2002). We will discuss inhibition in these three groups of ISIs separately.

Inhibition at ISIs of 3–5 ms

The characteristics of inhibition at these intervals are consistent with our previous report (Hanajima et al. 1998). This showed that the duration of I3-wave inhibition was 20 ms or longer, which is compatible with the GABAergic inhibitory mechanisms reported in the animal

motor cortex (Krnjevic et al. 1964, 1965; Matsumura et al. 1992; Bekenstein et al. 1993). We conclude that the inhibition at ISIs of 3–5 ms in responses evoked by I3-waves is produced by a GABAergic inhibitory system in the motor cortex (Alger and Nicoll 1982; Dingledine and Korn 1985; Chen et al. 1995), which has been proposed by Ziemann et al. (1996b, 1996c) based on the results of pharmacological studies on the motor cortical inhibition.

Inhibition at an ISI of 1 ms

At an ISI of 1 ms, there was inhibition of EMG responses evoked by both I1-waves and magnetic D-waves, as well as those evoked by I3-waves, whereas anodal D-waves were unaffected. This suggests that the mechanism for inhibition at an ISI of 1 ms is different from that at ISIs of 3–5 ms, even though it occurs at the cortex. This supports the conclusion by Fisher et al. (2002), who proposed a possible mechanism for the inhibition at an ISI of 1 ms as follows: The first conditioning stimulus activates axons of interneurons normally involved in response to the second test stimulus. The test stimulus activates those that are not refractory at the 1-ms interval. Although the amount of net inputs to PTNs produced by the two stimuli were the same as those produced by the test stimulus alone, they are less synchronized than those elicited by a single test stimulus. This loss of synchrony of inputs to PTNs may cause a reduction of I-wave generation. This explanation must be applied to the inhibition of I3- or I1-waves.

What mechanisms underlay inhibition of magnetic D-waves at an ISI of 1 ms? Inhibition of PTN axons at an ISI of 1 ms has also been noted to have special characteristics in animal experiments. Bindman et al. (1979) have used paired electrical stimulation of the motor cortex and show that inhibition at ISIs of 2–8 ms but not at an ISI of 1 ms is reduced by blocking synaptic transmission with magnesium. The conclusion is that inhibition at an ISI of 1 ms does not require activation of inhibitory synapses. Patton and Amassian (1954) have reported that the D-wave is attenuated by paired cortical stimuli at ISIs shorter than 1.2 ms in cats and monkeys. In humans, recordings of descending volleys with subdural electrodes show that D-waves elicited by the second stimulus are smaller than those elicited by the first stimulus in paired-pulse transcranial electrical stimulation at ISIs shorter than 1 ms (Katayama et al. 1988; Inghilleri et al. 1989). The possible explanation of these effects is the relative refractory period of PTNs. Refractoriness of PTNs must lead to the desynchronization of descending volleys by the similar mechanism mentioned above for the motor cortical interneurons. However, one problem in applying this explanation to magnetic D-waves is the intensity of the conditioning stimulus. Inhibition of magnetic D-waves was elicited when the conditioning stimulus had an intensity of -2% or -5% . The threshold for active muscles must be almost equal to the threshold for axon hillocks or axons of PTNs, which is almost the same as the threshold for D-wave production (activation of PTNs)

when eliciting D-waves. In contrast, in the case of I-wave generation, the threshold for I-waves may not be strong enough for activation of PTNs. Some activation occurs in the motor cortical interneurons, but it may not be enough for activation of the target cells (PTNs). It is, therefore, not obvious that relative refractoriness of the corticospinal tract neurons evoked by the first conditioning stimulus causes inhibition of magnetic D-waves.

However, the stimulus subthreshold for an EMG response in active FDI may be suprathreshold for some PTNs. Epidural recording experiments (Di Lazzaro et al. 1999) have shown that descending volleys are often elicited even by transcranial magnetic stimuli subthreshold for some EMG responses. This suggests that some corticospinal axons can be activated by the subthreshold stimulus for an active muscle. In this case, some PTNs are activated, but not enough for activation of spinal motoneurons. This should cause a relative refractory period in a small number of PTNs. This refractoriness should affect magnetic D-waves even though it should not affect anodal D-waves because of the difference in origin between the magnetic and anodal D-waves. Actually, anodal D-waves were unaffected by the same conditioning stimuli.

Based on these all arguments, we propose the mechanisms underlying the inhibition at an ISI of 1 ms as follows. Even in responses to magnetic D-waves, as well as I1- and I3-waves, the inhibition must be produced by desynchronization of the descending volleys due to the relative refractory period of the corticospinal neurons.

Effects at an ISI of 2 ms

At an ISI of 2 ms, there was no inhibition in any responses. The ISI of 2 ms is a unique interval. We have sometimes noticed that suppression was shallower at an ISI of 2 ms than at the other short ISIs in normal subjects (Ridding et al. 1995c; Ashby et al. 1999; Civardi et al. 2000) and patients (Ridding et al. 1995a). This suggests that there should be something which precludes the cortical inhibition at an ISI of 2 ms. Inhibitory effects elicited by S1 must act on their target cells at about 2 ms later. If so, effects elicited by suprathreshold S2 given 2 ms later (ISI of 2 ms) may mask that inhibitory effect. In the intracortical inhibition experiment, S1 should activate inhibitory interneurons and S2 should activate the target cells of those inhibitory interneurons (motor cortical interneurons for generation of I1- or I3-waves, PTNs for D-waves). Activation of the target cells by S2 may occlude almost simultaneously reaching inhibitory effects elicited by S1 at the target cells, or antidromic volleys in the inhibitory interneurons elicited by S2 may collide with the orthodromic impulses elicited by S1 somewhere in the inhibitory interneurons. This may explain the lack of inhibition at an ISI of 2 ms. A similar peculiar finding at the 2-ms interval happened in the intracortical I-wave facilitation (Di Lazzaro et al. 1999). At this interval, the EMG responses were not facilitated

even though descending volleys were facilitated. This phenomenon was explained by an associated spinal disinhibitory effect. Facilitatory effects were masked by an inhibitory effect which needs one more synapse than the facilitatory effect.

If these mechanisms are responsible for the lack of inhibition, why was inhibition seen in most previous experiments at an ISI of 2 ms? This phenomenon, causing the lack of inhibition, may occur synchronously in most cells when the subject voluntarily contracts the target muscle, whereas it may not occur so synchronously when the subject does not contract the muscle for some reason. Because most of the previous experiments were done when the subject made no contraction, this phenomenon cannot be as accurate as our experiments using active muscles.

Because voluntary contraction alters the pattern of intracortical inhibition (Ridding et al. 1995c), we are not sure whether results during contraction are useful to speculate about mechanisms for inhibition observed at rest. However, the conclusion deduced from our previous experiments during voluntary contraction (Hanajima et al. 1998) is consistent with those of the experiments using epidural recordings in the relaxed condition (Di Lazzaro et al. 1998). We are sure, therefore, that the present results during voluntary contraction should mostly explain the mechanisms for inhibition at rest.

In conclusion, the intracortical inhibition of the motor cortex studied with paired-pulse TMS is produced by GABAergic interneurons of the motor cortex at ISIs of 3–5 ms. At an ISI of 1 ms, the relative refractory period of the target cells for S2 and resulting desynchronization of descending volleys must contribute to the inhibition. At an ISI of 2 ms, the inhibition is not often elicited, because S2 must occlude or collide with the inhibitory effect evoked by S1. Based on these, we recommend using ISIs of 3–5 ms when we evaluate GABAergic function of the motor cortex by the paired-pulse TMS, because mechanisms other than GABAergic systems should not contribute to inhibition at these ISIs.

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