

Threshold of the soleus muscle H-reflex is less sensitive to the change in excitability of the motoneuron pool during plantarflexion or dorsiflexion in humans

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Abstract. In the present study, we investigated whether weak (10% of maximal voluntary contraction) tonic dorsiflexion (DF) and plantarflexion (PF) affects the two conventional parameters used for evaluating the excitability of the soleus motoneuron (MN) pool, i.e. the ratio of the threshold of H-reflex to that of M-response ($H_{th}:M_{th}$) and the ratio of the maximal amplitude of H-reflex to that of M-response ($H_{max}:M_{max}$) in human subjects. The results showed that the $H_{max}:M_{max}$ decreased during DF and increased during PF compared with that during rest, whereas no clear alteration was observed in $H_{th}:M_{th}$. These results are consistent with the scheme proposed by earlier workers, who have argued that neither inhibitory nor facilitatory effects of the conditioning stimulus apply to specific spinal reflex circuits occurring around the threshold of the test H-reflex. It is suggested, therefore, that the conventional use of the $H_{th}:M_{th}$ ratio as a parameter reflecting the excitability of the MN pool should be reconsidered.

Key words: H-reflex – Threshold – Maximal amplitude – Dorsiflexion – Plantarflexion

Introduction

A number of previous studies using the Hoffmann (H)-reflex technique have investigated the effects of a conditioning stimulus applied to a specific spinal reflex circuit on the amplitude of the test H-reflex, which reflects the excitability of the proprioceptive spinal motoneuron (MN) pool. It has been noted, however, that the quantitative effect of the conditioning stimulus on the amplitude of the H-reflex depends greatly on the size of the test H-reflex used in the examination itself (Meinck 1980; Crone et al. 1990). Crone et al. (1990) have proposed a scheme showing that the sensitivity of the test H-reflex to facilitatory or inhibitory (including

presynaptic inhibition) effects induced by some conditioning stimuli is a quadratic function of the size of the test H-reflex itself. As the size of the test H-reflex increases (by increasing the stimulus intensity), the effect of the conditioning stimulus on the relative size of the test H-reflex (which is expressed as the ratio to the size of the maximal M-response) initially increases, then reaches a peak (or plateau) and finally decreases. It is therefore, reasonable to infer from Crone's scheme that the conditioning stimulus may not clearly cause either facilitatory or inhibitory effects on the amplitude of the test H-reflex in the range of its smallest sizes, i.e. around the threshold of the test H-reflex. Nevertheless, the threshold of the H-reflex has often been used as a parameter for evaluating the excitability of the MN pool varying under different conditions within an individual subject (Davies 1985; Davies and Lader 1985; Tanaka 1986; Kayser et al. 1993).

In the present study, we investigated whether the ratio of the threshold of the H-reflex relative to that of the M-response ($H_{th}:M_{th}$) is actually affected by either the additional facilitatory or inhibitory synaptic inputs into the soleus MN pool. As a reference, the maximal amplitude of the H-reflex relative to that of the M-response ($H_{max}:M_{max}$) was also investigated to determine the changes in excitability of the MN pool under the same conditions, because the $H_{max}:M_{max}$ ratio has been widely accepted as a parameter reflecting the excitability of the MN pool (Casabona et al. 1990; Nielsen et al. 1993).

Methods

Subjects. Six neurologically normal volunteers, aged between 20 and 36 years, all of whom gave informed consent participated in the present study.

Experimental procedure. The subjects were comfortably seated in an armchair and instructed to maintain a relatively stable arousal level of consciousness. The subject's left foot was fixed on an immobile pedal, with angles of the knee and ankle of that leg being maintained at approximately 120° and 100°, respectively. This leg

posture enabled the subjects to maintain their efforts for the isometric contraction of ankle musculature at a given strength level during the experiment.

For measuring H-reflexes and M-responses, paired surface electrodes were attached to the skin over the belly of the soleus muscle with less than 5 k Ω electrical resistance. For electrical stimulation, an anodal electrode was placed on the skin of the patella and a ball cathode was placed at a suitable spot to stimulate the tibial nerve at the popliteal fossa, with careful exclusion of undesirable current spread to other nerve branches. Rectangular electrical pulses, 1 ms in duration, were applied at a rate of once every 3 s. The intensity of these pulses was gradually increased from one lower than the threshold of H-reflex to one higher than the saturated state of the maximal M-response. Gradual increases in stimulus intensity were employed in an attempt to avoid causing any psychological stress by giving a high intensity pulse unexpectedly to the subject. Seven pulses were sequentially provided at each stimulus intensity, and the muscle responses (i.e. the H-reflexes and/or M-responses) evoked by these pulses were amplified via a band-pass filter with a range of 20 Hz–3 kHz. All amplification procedures were performed by software run on a signal processor (NEC San-ei, 7S12). A stimulator (Nihonkouden, SEN-7013) coupled with an isolator (Nihonkouden, SS-102J), of which the output ranged from 0 to 200 V, was used for electrical stimulation. Analog outputs from the signal processor were transformed into digital data at a sampling rate of 10 kHz by an A-D converter installed in a personal computer (NEC, PC9801VM), and stored on a hard disk unit as source data for subsequent analyses.

All data concerning the amplitude of both the H-reflex and M-response obtained within a trial (consisting of a series of stimulations with the lowest to the highest stimulus intensities) were adopted to form the respective recruitment curves (see Fig. 1). To standardize the recruitment curves among all trials, the raw amplitudes of both the H-reflex and M-response obtained within each trial were converted to relative amplitudes (ordinate) calculated by dividing the respective raw amplitudes by the mean maximal amplitude of the M-response (which was calculated on the basis of the seven M-responses evoked at the highest intensity of electric stimulation). Stimulus intensity (abscissa) was also calibrated with the ratio to the threshold of the M-response. Such standardization of the recruitment curves should have cancelled possible inter-trial differences in absolute amplitude of both the H-reflex and M-response among all trials. The $H_{\max}:M_{\max}$ was thus defined as the ratio of the highest mean amplitude of H-reflexes to the mean maximal amplitude of the M-responses, and the $H_{\text{th}}:M_{\text{th}}$ was defined as the relative threshold of the H-reflex (i.e. the stimulus intensity evoking the H-reflex first) to that of the M-response (i.e. stimulus intensity evoking the M-response first).

During either the isometric dorsiflexion (DF) or plantarflexion (PF), ankle force was measured using a strain gauge attached to the immobile pedal. The voltage output from the strain gauge (the linearity of voltage output against the ankle force having been previously adjusted) was amplified (NEC San-ei, 6M82) and visually presented as the level of a horizontal beam on the display of an oscilloscope, which was placed at a distance of approximately 1 m in front of the subjects. Another horizontal beam appeared as a target on the same display indicating a force of 10% of the maximal voluntary contraction (MVC). We (Funase et al. in press) have recently found that isometric contraction at a force of 10% of MVC clearly changed the excitability of the soleus MN pool, with peripheral neuromuscular conditions (observed as the changes of the slope in the recruitment curve of the M-response which was normalized in terms of both the maximal amplitude and the threshold of M-response in an attempt to cancel actual, inter-trial variations in the maximal amplitude and the threshold of M-response arising between different experiments) being stable, whereas isometric contraction at a force level of 20% of the MVC affected both the excitability of the soleus MN pool and the peripheral neuromuscular conditions, in particular, during

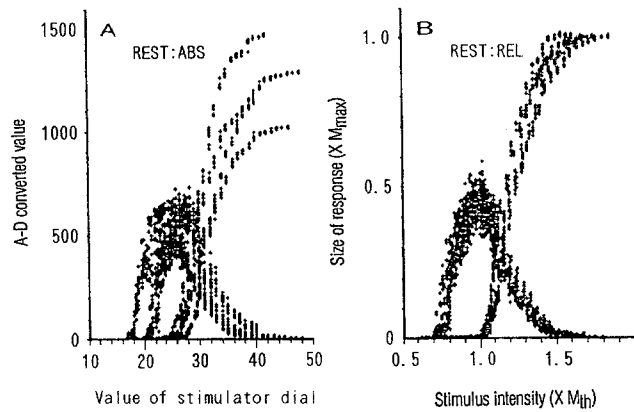


Fig. 1. Superimposed recruitment curves of the H-reflex (inverted U-shape) and M-response (S-shape) constructed from all plots in five trials on a single subject at rest. **A.** Both axes were calibrated by the absolute (ABS) scales, i.e. A-D converted value (1 mV equals 96 points) on the ordinate and the digits of the volume dial (0–100) of the stimulator on the abscissa. Maximal output range of the isolator was 200 V. **B.** Both axes were calibrated by the relative (REL) scales, i.e. the ratio of the amplitude of H-reflex relative to the mean maximal amplitude of M-response on the ordinate and the ratio of the threshold of H-reflex relative to that of the M-response on the abscissa. Subsequent recruitment curve diagrams of H-reflex and M-response in Figs. 2 and 3 were calibrated by the relative scales as in Fig. 1B

PF. These previous results suggested that both the background neuromuscular conditions and the excitability of the MN pool would change during voluntary contraction of 20% of MVC. In the present study, we therefore adopted a force of 10%, rather than 20%, of MVC in attempting to change only the excitability of the soleus MN pool but not the peripheral neuromuscular conditions. To maintain the force level of isometric contractions during either DF or PF, the subjects were asked to hold the beam of the output force level in line with the target beam. The subjects were allowed to take a short rest between the seven-stimulus sessions to minimize muscle fatigue.

Results

Figure 1A shows all the raw data plotted to form five sets of recruitment curves of both the H-reflexes (inverted U-shape) and M-responses (S-shape) obtained from five trials conducted in one typical subject while at rest. The abscissa is calibrated with the absolute stimulus intensity in terms of the digits of the volume dial (0–100) of the stimulator, which were adjusted by the experimenter to select actual intensity of the stimulus to be provided to evoke the H-reflexes through the isolator, of which the voltage output ranged from 0 to 200 V. The ordinate in this figure is calibrated with the digital points converted from the analog amplitude of evoked potentials (1 mV corresponds to 96 points). All these recruitment curves were obtained from trials performed with a single subject over a period of a few days with duplicated measurement procedures. Despite the identical procedures, wide variations were observed in these five recruitment curves. As shown in Fig. 1B, both axes are, in turn, calibrated with relative units such as the ratio of the threshold of H-reflex to

that of M-response (the $H_{th}:M_{th}$) on the abscissa, and the ratio of the amplitude of H-reflex to the mean maximal amplitude of M-response (the $H_{max}:M_{max}$) on the ordinate. With these new relative units of measurement being used in the two axes, five sets of recruitment curves appear to form clusters of H-reflexes and M-responses with much less variation than those in Fig. 1A. This would suggest that the recruitment curve of both the H-reflex and the M-response may indicate some property of each individual with respect to the monosynaptic reflex loop, as we have previously suggested (Funase et al. in press). The means ($n=5$) of the $H_{max}:M_{max}$ and $H_{th}:M_{th}$ at rest were 0.440 (SD 0.034) and 0.717 (SD 0.017), respectively. In the following sections, all the recruitment curves of H-reflexes and M-responses are regarded as those in the relative units of measurement as in Fig. 1B.

The effects of isometric DF or PF on the recruitment curve (Fig. 2A, B) were investigated using a set of data obtained from the same subject as in Fig. 1. The mean of the $H_{max}:M_{max}$ in fixe trials appeared to decrease [0.349 (SD 0.026)] during DF and to increase [0.562 (SD 0.038)] during PF, compared to that at rest, whereas the $H_{th}:M_{th}$ did not appear to change during either contraction.

To show clearly the distribution of the $H_{th}:M_{th}$ under DF, rest, and PF for this subject, part of the data within the range from 0.6 to 1.0 are plotted on the abscissa in Fig. 3. The means of $H_{th}:M_{th}$ for DF, rest, and PF were 0.709 (SD 0.019), 0.717 (SD 0.017), and 0.693 (SD 0.019), respectively. In contrast to the distinct changes in the $H_{max}:M_{max}$ showing a decrease during DF and an increase during PF compared to that at rest, the mean $H_{th}:M_{th}$, indicated by the solid triangles in Fig. 3, appeared less variable between these conditions. These results are inconsistent with the conventional use of $H_{th}:M_{th}$ to evaluate the excitability of the MN pool.

Figure 4 shows a summary of the results with respect to the changes in $H_{max}:M_{max}$ and $H_{th}:M_{th}$ during

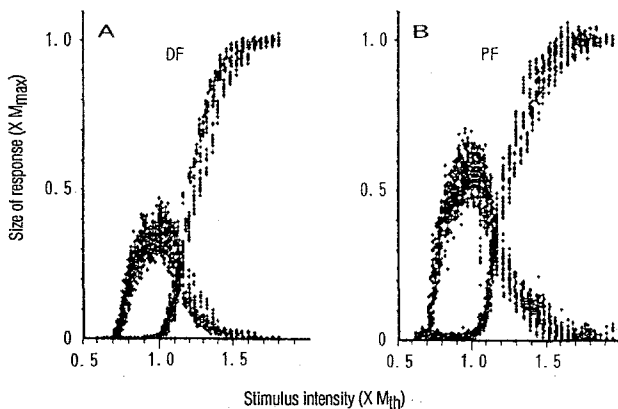


Fig. 2. Effects of weak (10% of the maximal voluntary contraction) isometric dorsiflexion (DF, A) or plantarflexion (PF, B) on the H-reflex (inverted U-shape) and M-response (S-shape) recruitment curves. Five trials were performed in each condition with the same subject as in Fig. 1

DF, rest, and PF. Figure 4A and B show the results obtained from analyses of the data collected from a single subject over five trials (these data have already been described in the previous sections explaining Figs. 1, 2, 3). The results shown in Fig. 4C and D were obtained from data collected from six different subjects in one trial; the means of $H_{th}:M_{th}$ (Fig. 4C) during DF, rest, and PF were 0.668 (SD 0.126), 0.677 (SD 0.122), and 0.648 (SD 0.106) ($n=6$), respectively, while those

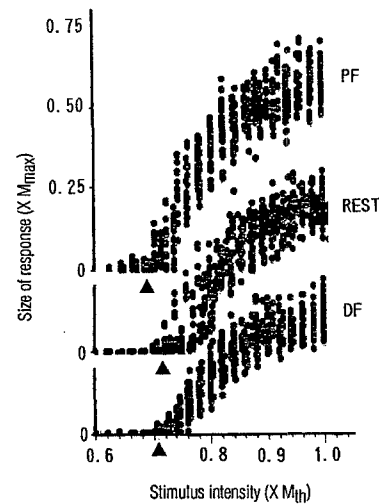


Fig. 3. Summary of Fig. 1B (REST), Fig. 2A (DF), and Fig. 2B (PF) within the range from 0.6 to 1.0 on the abscissa and from 0 to 0.75 on the ordinate. Each solid triangle shows the mean threshold of H-reflex calculated from five trials under each condition. All data were obtained from the same subject as in Figs. 1 and 2

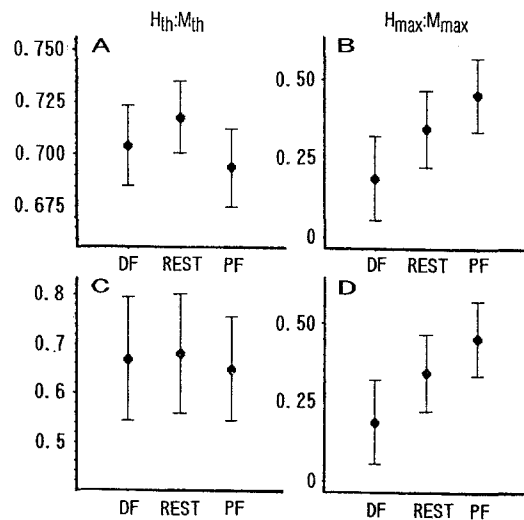


Fig. 4. Mean values and standard deviations of the ratio of the threshold of H-reflex relative to that of M-response ($H_{th}:M_{th}$, A and C), and the ratio of the maximal H-reflex relative to the maximal M-response ($H_{max}:M_{max}$, B and D) during dorsiflexion (DF), rest (REST), and plantarflexion (PF), obtained from a single subject in five trials (A and B) or six different subjects in single trials (C and D). Results of statistical analyses by separate one-way analyses of variance (ANOVAs) are described in detail in the text

of the $H_{\max}:M_{\max}$ (Fig. 4D) were 0.188 (SD 0.133), 0.340 (SD 0.121), and 0.451 (SD 0.118), respectively. Statistical analyses for comparison of the means of $H_{\text{th}}:M_{\text{th}}$ and $H_{\max}:M_{\max}$ among the three conditions were performed by separate one-way analyses of variance (ANOVA) for both the single subject and the six subjects. Results showed that the $H_{\max}:M_{\max}$ differed significantly between DF, rest, and PF for both the single subject (Fig. 4B; $F_{2,12}=51.979$, $P<0.001$) and the six subjects (Fig. 4D; $F_{2,15}=6.799$, $P<0.01$). In contrast, $H_{\text{th}}:M_{\text{th}}$ did not differ significantly among these conditions for either the single subject (Fig. 4A; $F_{2,12}=2.195$, $P>0.1$) or the six subjects (Fig. 4C; $F_{2,15}<1.0$).

Discussion

The main finding of the present study was that $H_{\text{th}}:M_{\text{th}}$ was not significantly influenced by changes in the excitability of the soleus MN pool, as manipulated by the isometric DF and PF, in contrast to the apparent changes in $H_{\max}:M_{\max}$ under the same conditions.

It has been found that during isometric contraction of the ankle flexor or extensor muscle, the excitability of the soleus MNs (as the final common path) is regulated by the summation of facilitatory and/or inhibitory synaptic inputs onto MNs in the soleus MN pool. Several types of neural mechanisms can conceivably modify the excitability of the soleus MN pool during weak contraction of the ankle flexor or extensor muscle. During DF (contraction of the pretibial muscle, i.e. the antagonist of the soleus muscle), for example, it has been shown that reinforcement of inhibitory synaptic inputs is mediated by both Ia inhibitory interneurons, which are facilitated by the direct motor commands descending from the upper brain, and the peripheral Ia feedback inputs induced by activation of the γ -loop, which accompanies contraction of the pretibial muscle as the antagonist of the soleus muscle (Tanaka 1976; Rothwell et al. 1984; Shindo et al. 1984; Nielsen et al. 1992). This reinforced inhibition is a main cause of the reduction of excitability of the soleus MN pool. During PF (contraction of the agonist) on the other hand, it has been found that the soleus MNs are facilitated by both direct motor commands descending from the upper brain to the soleus MN pool and increasing homonymous peripheral Ia feedback inputs caused by the activation of the γ -loop accompanying the contraction of the soleus muscle as the agonist of PF (Vallbo 1970; Kagamihara et al. 1992). It has been suggested that other possible neural mechanisms, such as presynaptic inhibition, recurrent inhibition etc., may play a role acting on the soleus MNs during the weak isometric contraction of either the agonist or antagonist (Schieppati 1987; Schomburg 1990). Accordingly, the present results with respect to the $H_{\max}:M_{\max}$ ratio would imply that a decrease in the excitability of the soleus MN pool during DF and an increase during PF relative to that under rest conditions are satisfactorily evaluated by the changes in $H_{\max}:M_{\max}$.

These findings, in turn, necessarily lead us to question why the $H_{\text{th}}:M_{\text{th}}$ did not significantly shift during either isometric DF or PF relative to that at rest, despite the apparent changes in $H_{\max}:M_{\max}$. The change in the excitability of the MNs within an MN pool can theoretically be responsible for modifying $H_{\text{th}}:M_{\text{th}}$. However, given that the MNs in the soleus MN pool have been shown to be recruited according to the 'size principle' (Henneman et al. 1965; Henneman 1981), that is, in the order of small to large sized MNs as the stimulus intensity for evoking the test H-reflex increases, the $H_{\text{th}}:M_{\text{th}}$ should reflect the firing threshold of the smallest type of MNs alone. This may result in little change in the amplitude of H-reflex around its threshold even under DF or PF, and as a result this subtle change of H-reflex may not have been detected as a significant variation in the $H_{\text{th}}:M_{\text{th}}$. Crone et al. (1990) have also proposed as a tentative conclusion that the difference in the additional facilitatory or inhibitory effect which is dependent on the size of the test H-reflex is attributed to the distribution of the intrinsic excitability of the MNs within the MN pool. This hypothesis supports the present results showing reduced sensitivity of $H_{\text{th}}:M_{\text{th}}$ to changes in excitability of the soleus MN pool. In contrast, the $H_{\max}:M_{\max}$ should reflect the firing of the majority of MNs in the MN pool. Therefore, the changes in the excitability of these MNs caused by either DF or PF may have significantly altered the $H_{\max}:M_{\max}$. Such conditions suggest that $H_{\text{th}}:M_{\text{th}}$ is not a sensitive parameter for evaluating the changes in excitability of the smallest type of MNs, and more importantly, that $H_{\text{th}}:M_{\text{th}}$ is an inadequate parameter for evaluating the 'net' excitability of a given MN pool (as it is conventionally used). Rather, $H_{\max}:M_{\max}$ is a much more appropriate parameter for evaluating the 'net' excitability, although even this parameter cannot literally reflect the 'net' excitability of all the MNs in the MN pool.

The $H_{\text{th}}:M_{\text{th}}$ has also often been used to determine differences in the excitability of the MN pool between individuals. Both Shindo (1990) and Boorman et al. (1991) have reported a large dispersion of $H_{\text{th}}:M_{\text{th}}$ at rest in both normal subjects and patients showing spasticity, with no distinguishable differences appearing between the normal and patient groups. Because the MN pool of patients showing spasticity should exhibit much higher excitability than that of normal subjects, the absence of a distinguishable group difference in $H_{\text{th}}:M_{\text{th}}$ cannot be explained in view of such a likely difference in the excitability of the MN pool between both groups. Rather, the individual differences in $H_{\text{th}}:M_{\text{th}}$ may result from at least two factors other than the excitability of the MN pool. Firstly, the distribution of nerve diameters within both the Ia-afferent and α -efferent nerves may primarily be related to $H_{\text{th}}:M_{\text{th}}$, as the excitability threshold of nerves is directly proportional to their diameter. Secondly, the electrical impedance at the site on the skin at which the electrical stimulation is applied may affect $H_{\text{th}}:M_{\text{th}}$. Tanaka (1986) has suggested that the threshold of H-reflex may approach the threshold of M-response (i.e.

$H_{th}:M_{th}$ approaches 1.0) as the electrical impedance becomes very high. These two factors, in addition to the excitability of the MN pool, may differ greatly among different subjects. At present, it is unclear which factor is the most important in determining individual differences in $H_{th}:M_{th}$. This implies that the $H_{th}:M_{th}$ is neither a powerful nor appropriate parameter for evaluating alterations in the excitability of the MN pool, either within a subject or between subjects.

In conclusion, the present study clearly showed that, despite obvious changes in $H_{max}:M_{max}$ during both DF and PF relative to that at rest, $H_{th}:M_{th}$ was not significantly altered under these conditions. This suggests that the 'reflex gain' (i.e. the developmental slope of the recruitment curve of the H-reflex, which is strongly correlated with $H_{max}:M_{max}$), rather than the 'reflex threshold', is principally a function of the excitability of the MN pool. This is consistent with the findings of our previous study (Funase et al. in press). The conventional notion that $H_{th}:M_{th}$ can be regarded as a parameter for estimating the excitability of the MN pool should therefore be reconsidered.

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