Acute effects of high-frequency microfocal vibratory stimulation on the H reflex of the soleus muscle. A double-blind study in healthy subjects

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Summary

This study in healthy subjects examined the effects of a system delivering focal microvibrations at high frequency (Equistasi®) on tonic vibration stimulus (TVS)-induced inhibition of the soleus muscle H reflex. High-frequency microvibrations significantly increased the inhibitory effect of TVS on the H reflex for up to three minutes. Moreover, Equistasi® also significantly reduced alpha-motoneuron excitability, as indicated by the changes in the ratio between the maximum-amplitude H reflex (Hmax reflex) and the maximum-amplitude muscle response (Mmax response); this effect was due to reduction of the amplitude of the H reflex because the amplitude of muscle response remained unchanged. The present findings indicate that Equistasi® has a modulatory effect on proprioceptive reflex circuits. Therefore, Equistasi® might interfere with some mechanisms involved in both physiological and pathophysiological control of movement and of posture.

KEY WORDS: H reflex, high-frequency microvibrations, tonic vibratory stimulation.

Introduction

In neurophysiology it is an established concept that muscle tendon vibration determines a change in the discharge frequency of muscle spindle afferents. More specifically, it has been demonstrated that application of a high-frequency tonic vibratory stimulus (TVS) over a muscle tendon induces tonic activity of the muscle that is called the tonic vibration reflex (De Gail et al., 1966). A TVS over the soleus muscle tendon physiologically reduces the amplitude of the proprioceptive T and H reflexes (De Gail et al., 1966; Delwaide, 1973). The precise mechanisms responsible for this inhibitory effect are not completely understood, although it is likely that there are different contributory factors, such as postsynaptic mechanisms facilitating neurotransmitter depletion that leads to post-activation depression, and a supraspinal, presynaptic mechanism in which increased GABAergic transmission inhibits IA afferents (De Gail et al., 1966; Delwaide, 1973; Hultborn et al., 1987).

The recent development of smart devices has been paralleled by important advances in applied nanotechnologies, a field that has been a focus of growing interest in various branches of medical and physiological research (Lue, 2007). Nanogenerators have been developed that are able to transform minimal thermal variations into mechanical energy by self-producing a focal vibration. The Equistasi® system (Equistasi S.r.l., Milan, Italy) provides an example of the generation of microvibration energy. Equistasi® is a vibrotactile device, based on vibrational technology, which self-produces a mechanical focal vibration with a non-constant frequency of about 9000 Hz and with a very low pressure of about 3-4 E-6 Pa. In order to gain further insight into the mechanisms underlying the role of high-frequency microvibrations in motoneuron activation, we explored the effects, on motoneuron excitability and proprioceptive reflex pathways, of the application of Equistasi® in healthy subjects. Specifically, we used high-frequency microvibrations applied to the soleus muscle, investigating their effect on alpha motoneuron excitability and on the modulatory inhibition of the H reflex.

Materials and methods

Subjects

The study included 19 healthy volunteers (9 males and 10 females), aged 28.4 ± 7.9 years. Informed consent
was obtained from all the participants, none of whom had a history of neurological disease. The research protocol was approved by the Ethics Committee of the C. Mondino National Neurological Institute and the study complied with the Declaration of Helsinki.

**Electrophysiological investigations**

All the subjects were investigated at about the same time (between 2 p.m. and 5 p.m.) in order to minimize the possibility of circadian fluctuations of the electrophysiological measures. The electrophysiological investigations were performed using a Synergy SYN5-C electromyograph (© Viasys Healthcare, Old Woking, Surrey, UK) connected to a BMST6 constant current stimulation unit (Biomedical Mangoni, Pisa, Italy). All the procedures were carried out in a soundproof room kept at a constant temperature (25°C).

Motor nerve conduction studies of the fibular nerve and sensory nerve conduction studies of the sural nerve were performed bilaterally in each subject to rule out the presence of subclinical signs of sensorimotor neuropathy.

**H reflex**

The H reflex was studied with the subject lying prone on a bed with the head in a neutral position. It was recorded from the soleus muscle with surface electrodes; the active electrode (cathode) was placed over the soleus muscle, two fingerbreadths proximal to the anode (inter-electrode distance of 2.5 cm). The ground electrode was applied over the calf, between the stimulating and the recording electrodes. The EMG signals of the H reflex were band-pass filtered between 10 Hz and 2 kHz. The sweep time for the EMG recordings was 10 ms/div. The tibial nerve was stimulated at the popliteal fossa using percutaneous bipolar electrodes, with the electrode placed proximal to the anode (inter-electrode distance of 2.5 cm). The duration of the stimulus was 1 ms and the frequency of stimulation was 0.1 Hz. In order to elicit a maximum-amplitude H reflex (Hmax reflex) from the soleus muscle, tibial nerve stimulation was performed by increasing the stimulation intensity in steps of 1 mA until the Hmax reflex was reached. To elicit a compound muscle action potential (CMAP) of maximum amplitude from the soleus muscle, i.e. maximum-amplitude muscle response (Mmax response), the tibial nerve was stimulated by increasing the stimulation intensity beyond that needed to elicit the Hmax reflex, again in steps of 1 mA, until the Mmax response was obtained (Preston and Shapiro, 2005). The TVS was delivered by applying a vibrator (Vibration Exciter Type 4809, Brüel and Kjaer, Skodsborgvej, Denmark), driven by monophasic rectangular pulses of 2-ms duration, 100 Hz frequency, over the Achilles tendon for 100 seconds. The strength of the vibration was monitored by means of a power amplifier and the amplitude of the TVS was constantly kept below 1.0 mm (De Gail, 1966; Preston and Shapiro, 2005). To evaluate motoneuron pool excitability of the soleus muscle at baseline, before application of the TVS, we examined the ratio between the Hmax reflex and Mmax response (Delwaide, 1984; Hagbärth et al., 1973). This ratio, denoted H1/Mmax, was obtained by calculating the average values of 10 consecutive Hmax reflexes (H1) and of 10 consecutive Mmax responses (Mmax). This intensity of stimulation was kept constant for the evaluation of the average of 10 Hmax reflexes, both at baseline (H1) and subsequently during application of the TVS (H2). The frequency of stimulation was always 0.1 Hz (1 stimulus every 10 seconds).

To evaluate the effects of TVS on the Hmax reflex a “vibratory index” (VI) was calculated using the following formula: (H2/H1)x100. This parameter is used to quantify the inhibitory effect of the TVS on the H reflex (Delwaide, 1973). Three minutes after delivery of the TVS, the Hmax reflex was again recorded as the average of 10 responses (H3) obtained with a 0.1 Hz stimulation frequency and constant intensity of stimulation. This was done in order to investigate the late effects of TVS. Therefore, a “vibratory index late effect” (VI late) was calculated as: (H2/H3)x100. This parameter was used to quantify long-lasting effects of TVS on the H reflex. At this time, we also reassessed motoneuron pool excitability by again evaluating the Mmax response and by calculating the H3/Mmax ratio.

The parameters investigated in this study are summarized in table I.

### Table I - The neurophysiological parameters considered in the study.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Electrophysiological parameters</th>
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<tbody>
<tr>
<td>Hmax reflex</td>
<td>Maximum-amplitude H reflex</td>
</tr>
<tr>
<td>Mmax response</td>
<td>Maximum-amplitude muscle response (maximum-amplitude CMAP)</td>
</tr>
<tr>
<td>Mmax</td>
<td>Mean of 10 consecutive Mmax responses</td>
</tr>
<tr>
<td>H1</td>
<td>Mean of 10 maximum H-reflex amplitudes at baseline</td>
</tr>
<tr>
<td>H2</td>
<td>Mean of 10 maximum H-reflex amplitudes during TVS</td>
</tr>
<tr>
<td>H3</td>
<td>Mean of 10 maximum H-reflex amplitudes 3 minutes after TVS</td>
</tr>
<tr>
<td>H1/Mmax</td>
<td>Ratio between 10 consecutive Hmax reflexes and 10 consecutive Mmax responses at baseline</td>
</tr>
<tr>
<td>H3/Mmax</td>
<td>Ratio between 10 consecutive Hmax reflexes and 10 consecutive Mmax responses 3 minutes after TVS</td>
</tr>
<tr>
<td>Vibratory index (VI)</td>
<td>(H2/H1)x100</td>
</tr>
<tr>
<td>VI late - vibratory index late effect</td>
<td>(H2/H3)x100</td>
</tr>
</tbody>
</table>
Experimental paradigm and study design

The study was conducted according to a randomized, double-blind, placebo-controlled design (Fig. 1). In particular, electrophysiological investigations were performed twice in all the subjects. First, all the subjects underwent baseline electrophysiological investigations without Equistasi® or placebo devices (T₀) and then they repeated these investigations while wearing Equistasi® or placebo devices (T₁). The subjects were randomly divided into two groups: the Equistasi® group (n=10 subjects) and the Placebo group (n=9 subjects). The Equistasi® and placebo devices were of the same shape and size and they were applied to the skin of the tendon of the triceps surae muscle 100 mm proximal to the lower edge of the heel three minutes before the electrophysiological investigations. Both the volunteers and the neurophysiologist were blind to the procedure.

Statistical analyses

The statistical tests were carried out using XSTAT-2014 software (Addinsoft SARL, New York). We compared electrophysiological variables H₁, H₂, H₃, H₁/Max, H₃/Max, VI, and VI late in the two groups under investigation (Equistasi® vs Placebo). For these variables we applied both inferential parametric (Student’s t for paired samples or two-way ANOVA) and non-parametric (Wilkinson’s) tests. Then we tested the null hypothesis H₀, in which the two means (before and after treatment) are the same, against an alternative hypothesis (H₁), in which the pre-treatment mean is significantly larger than the post-treatment mean: H₀: μ₁ = μ₀ vs H₁: μ₀ > μ₁. For all analyses the level of statistical significance was set at p<0.05.

Results

Table II lists the electrophysiological parameters examined at T₀, while the larger set of electrophysiological parameters examined at T₀ and T₁ in the two groups (Equistasi® and Placebo) are shown in tables III and IV, respectively.

Intragroup comparisons

In the Equistasi® group we observed that both VI and VI late at T₁ were significantly lower as compared to the values obtained at T₀ (Fig.s 2 and 3). Furthermore, the decrease in the Hm reflex observed at the end of TVS (H₂) was statistically significant with respect to T₀. In addition, the H₁/Max and the H₃/Max values at T₁ were lower than the corresponding parameters obtained at T₀ (Table III and Fig. 4). In the Placebo group we did not detect any significant difference in any parameter (H₂, H₃ and H₃/Max) between T₀ and T₁ (Table IV, Fig.s 2, 3, and 4).

Table II - Demographic characteristics and baseline electrophysiological parameters of the subjects examined.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Equistasi® group</th>
<th>Placebo group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>28.4±7.9</td>
<td>28.1±8.2</td>
<td>ns</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/10</td>
<td>4/6</td>
<td>ns</td>
</tr>
<tr>
<td>H₁ (mV)</td>
<td>9.03±4.53</td>
<td>9.29±4.01</td>
<td>ns</td>
</tr>
<tr>
<td>Mmax (mV)</td>
<td>18.19±5.81</td>
<td>18.26±5.98</td>
<td>ns</td>
</tr>
<tr>
<td>H₁/Mmax</td>
<td>0.49±0.21</td>
<td>0.49±0.16</td>
<td>ns</td>
</tr>
</tbody>
</table>
| Abbreviations: H₁=mean of 10 maximum H-reflex amplitudes at baseline; Mmax=mean of 10 consecutive Mmax (maximum-amplitude CMAP) responses; H₁/Mmax=ratio between H₁ and Mmax.

Table III - Electrophysiological parameters without the device (T₀) and while wearing device (T₁) in the Equistasi® group.

<table>
<thead>
<tr>
<th></th>
<th>T₀</th>
<th>T₁</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₁ (mV)</td>
<td>9.29±4.01</td>
<td>8.7±4.7</td>
<td>ns</td>
</tr>
<tr>
<td>H₂ (mV)</td>
<td>1.3±1.52</td>
<td>0.83±2.34</td>
<td>0.025</td>
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<tr>
<td>H₃ (mV)</td>
<td>8.7±4.04</td>
<td>7.6±4.27</td>
<td>ns</td>
</tr>
<tr>
<td>Mmax (mV)</td>
<td>18.26±5.98</td>
<td>18.8±6.04</td>
<td>ns</td>
</tr>
<tr>
<td>H₁/Mmax</td>
<td>0.49±0.16</td>
<td>0.44±0.19</td>
<td>0.036</td>
</tr>
<tr>
<td>H₃/Mmax</td>
<td>0.47±0.19</td>
<td>0.40±0.19</td>
<td>0.042</td>
</tr>
<tr>
<td>VI</td>
<td>9.5±15.85</td>
<td>4.79±12.80</td>
<td>0.006</td>
</tr>
<tr>
<td>VI late</td>
<td>10.33±17.40</td>
<td>5.65±14.81</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abbreviations: H₁=mean of 10 maximum H-reflex amplitudes at baseline; H₂=mean of 10 maximum H-reflex amplitudes during TVS; H₃=mean of 10 maximum H-reflex amplitudes 3 minutes after TVS; Mmax=mean of 10 consecutive Mmax (maximum-amplitude CMAP) responses; H₁/Mmax=ratio between H₁ and Mmax; H₃/Mmax=ratio between H₃ and Mmax; VI (Vibratory Index)=(H₂/H₁)x100; VI late (Vibratory Index late effect)=(H₂/H₃)x100.
The Mmax response did not differ significantly between T₀ and T₁ in either the Equistasi® group or the Placebo group (Tables III and IV).

Equistasi® group vs Placebo group

At T₁, the inhibitory effect induced by TVS on the Hmax reflex was more marked in the Equistasi® group than in the Placebo group with a significant reduction of both VI and VI late recorded in the Equistasi® group (Fig.s 2 and 3).

Similarly, the decrease in H1/Mmax between T₀ and T₁ was significant in the Equistasi® group but not in the Placebo group (Fig. 4).

The Mmax response did not show significant differences at T₀ or at T₁ between the Equistasi® and Placebo groups.

Discussion

The results of the present study indicate that application of a microfocal vibratory stimulation at high...
Vibration exercise is also known to increase muscle force and power. In particular, the effects of vibration exercise are similar to those of resistance training. In conclusion, our study indicates that focal microvibratory stimulation is able to modulate phasic proprioceptive reflexes in healthy subjects. More targeted studies are needed to assess whether microvibrations induced by Equistasi® can improve the physiological mechanisms involved in motor strength and fatigue, and to confirm the potential effect of this kind of stimulation as a means of improving, also, balance abnormalities and motor disorders.

References


