Original Article

Effects of measurement posture and stimulation intensity on the nociceptive flexion reflex (RIII reflex)

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Abstract. [Purpose] The purpose of this study was to examine the effects of the measurement posture and stimulation intensity on the nociceptive flexion reflex (RIII reflex). [Subjects and Methods] Thirty normal female adult subjects were selected for this study. Their RIII reflexes were measured in three positions and with three degrees of stimulation intensity. The measurement posture was randomly selected. The analysis items were the stimulation intensity of the induced RIII reflex, the amplitude of the RIII reflex, and the numeric rating scale (NRS). [Results] The study results showed statistically significant differences in the interaction effects between the measurement posture and the stimulation intensity. The NRS showed no statistically significant differences in the interaction effects but showed statistically significant differences in the main effect. The amplitude of the RIII reflex showed no statistically significant differences in the interaction effects and showed statistically significant differences only in the stimulation intensity. [Conclusion] The study results suggest that the RIII reflex may be influenced by the measurement posture and stimulation intensity.

Key words: Nociceptive flexion reflex, RIII reflex, Numeric rating scale

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INTRODUCTION

The nociceptive flexion reflex (NFR), also known as the flexion reflex (FR) or withdrawal reflex, is a physiological, polysynaptic reflex allowing for painful stimuli to activate an appropriate withdrawal response1). The NFR is a withdrawal reflex of the leg, typically recorded using a biceps femoris muscle electromyogram (EMG) following stimulation of the sural nerve at the foot2). Whereas this reflex has been poorly investigated in the upper limb, the lower-limb flexion reflex (LLFR) in humans and the hind-paw FR in animals have been widely investigated and represent a useful window into the function of the central nervous system (CNS)3). To assess the NFR, the biceps femoris muscle activity is monitored using EMG during the application of varying intensities of electrocutaneous stimulation to the ipsilateral sural nerve4). Most of the previous relevant studies used rectification and averaging of traces to improve the quantitative assessment of the EMG reflex activity compared to baseline3).

The NFR is made up of the RII and RIII reflexes. The RII reflex is the threshold of the tactile component of the LLFR3), and the RIII reflex is elicited by the activation of the A delta afferent fibers5). As the threshold of the RIII reflex has been shown to correspond to the pain threshold, and as the size of the reflex has been shown to be related to the level of pain perception, it has been suggested that the RIII reflex may be a useful tool for investigating the pain processing at the spinal and supraspinal levels, the pharmacological modulation, and the pathological pain conditions3). The RIII reflex is highly correlated with the visual analogue scale (VAS)6) and is used as an objective index of the nociceptive threshold in studies related to pain7). The measurement postures of the RIII reflex are the prone position8) and the sitting position9). The RIII reflex
threshold is used as the degree of intensity of the stimulation required to elicit a stable response at 1.2 times\(^3\) and 1.4 times\(^5\).

Despite the fact that the RIII reflex is the most widely used among the nociceptive reflexes, the most effective measurement posture and stimulation intensity in the RIII reflex test are not yet known. Therefore, this study aimed to examine the effects of the measurement posture and stimulation intensity on the nociceptive flexion reflex (RIII reflex).

**SUBJECTS AND METHODS**

The subjects of this study were 30 normal female in their 20s who attended the women’s university. The general characteristics listed in Table 1. This study was conducted in accordance with the Declaration of Helsinki. All the participants signed the informed consent form before participating in the study. The research subjects were those who had no nerve muscular or musculoskeletal diseases, who did not do regular exercises in the last six months, and who did not take any medicine that might have affected the experiment results.

This study measured the numeric rating scale (NRS) and RIII reflex. NRS was used to quantify the subjective pain intensity evoked by the stimuli, and the volunteers were instructed to rate each perceived stimulus on the NRS. The NRS ranged from 0 (without pain) to 10 (with the severest pain), and the subjects were asked to inform the measurer of the degree of their pain.

The RIII reflex was measured at the lower limb using EMG (neuro-EMG-micro, Ivanovo, Russia). The measurement posture was randomly selected. In the case of the sitting position, the subjects were asked to sit on a slightly higher stool without a backrest, with 90° flexion in the hip and knee and a neutral ankle position. In the case of the Semi-Fowler’s position, the subjects were asked to take a supine position, with the head of the bed at approximately 45°. Also, the subjects were positioned with 130° flexion in the hip and 120° flexion in the knee, using a pillow on the knee, with a neutral ankle position. In the case of the prone position, the subjects were asked to assume a prone position on the bed, and to bend their hip joint about 130° using a pillow on the abdomen, and their knee joint about 120°, with a neutral ankle position.

As the conditions for recording the action potential, the band-pass filter was set to 5–2,000 Hz, and the sampling rate to 20,000 Hz, and the notch filter was on. The test stimulation was a square wave made of six pulsations within one pulse train made of 1 ms.

Before attaching the ground and electrode, part of the attached electrical stimulator was cleaned with alcohol, and band-type-ground-applying gel was attached to the fibula. A 2.3 × 2.6 cm rectangular Ag-Cl electrode (disposable EMG electrode F3001, Fiab, Italy) was used. The active electrode was attached to the 1/2 position of the biceps femoris long head, and the record electrode was attached to the biceps femoris tendon at 2 cm from the knee. After applying the gel, electrical stimulation was applied to the passage of the sural nerve from the lateral malleolus. The negative pole was positioned proximally, and the positive pole was located 2 cm below the negative pole. The stimulation was increased by 1 mA at 10 sec. intervals, and then the first stimulation intensity showing the RIII reflex was set as the reflect threshold. The current values when the stimulation intensity was increased 1.2, 1.4, and 1.5 times from the minimum threshold were recorded. Stimulation was given 3 times at 10 sec. intervals, and the amplitude values above 15 µV of the RIII reflex in the 90–180 ms time window after the stimulation onset were analyzed.

Statistical analysis was performed using the Windows SPSS 12.0 program. Repeated-measures ANOVA was used to analyze the changes that occurred in the two groups according to the treatment duration. The significance level was set to α=0.05.

**RESULTS**

The NRS showed no significant differences in the interaction effects between the measurement posture and the stimulation intensity. The main effects showed significant differences both in the measurement posture (\(F_{2,58}=8.547; p=0.001\) and in the stimulation intensity (\(F_{2,58}=214.357; p=0.000\) (Table 2). In the post hoc test, the measurement posture showed significant differences between the sitting position and the Semi-Fowler’s position, and between the Semi-Fowler’s position and the prone position. In the post hoc test, the stimulation intensity showed significant differences in all the three degrees. The current values showed significant differences in the interaction effects between the measurement posture and the stimulation intensity, showing significant differences only in the

| Table 1. General characteristics of the subjects |
|-----------------------------------------------|
| Subjects (n=20)                               |
| Age (yrs)  | 21.2 ± 0.9 |
| Height (cm)| 162.3 ± 6.2|
| Weight (kg)| 58.2 ± 8.3 |
| Body fat (%)| 22.3 ± 2.9 |
stimulation intensity ($F_{2.58}=6.163; p=0.014$) (Table 4). In the post hoc test, significant differences were shown between the 1.2- and 1.4-time stimulation intensities and between the 1.2- and 1.5-time stimulation intensities.

**DISCUSSION**

As mentioned earlier, the aim of the present study was to investigate the effects of the measurement posture and the stimulation intensity on the RIII reflex. The threshold of the RIII reflex was needed as the lowest current value in the sitting position and 1.2-time stimulation intensity. The amplitude of the RIII reflex was influenced only by the stimulation intensity, and there was no significant difference between the 1.4- and 1.5-time stimulation intensities. The NRS showed significant differences only in the measurement posture ($F_{2.58}=8.547; p=0.001$) and stimulation intensity ($F_{2.58}=214.357; p=0.000$). In the post hoc test, the measurement posture showed significant differences between the sitting and Semi-Fowler’s positions and between the Semi-Fowler’s and prone positions. In the post hoc test, significant differences were shown in all the three degrees of stimulation intensity.

| Table 2. Changes in NRS (unit: grade) |
|---------------------------------------|
| Position                             | 1.2 times | 1.4 times | 1.5 times |
| Sitting                               | 3.0 ± 1.7 | 3.9 ± 1.8 | 4.7 ± 2.2 |
| Semi-Fowler’s                        | 2.8 ± 1.2 | 3.8 ± 1.5 | 4.7 ± 1.8 |
| Prone                                | 4.1 ± 1.7 | 5.2 ± 1.9 | 6.2 ± 1.9 |
| Mean ± SD                            |           |           |           |

The NRS showed significant differences only in the measurement posture ($F_{2.58}=8.547; p=0.001$) and stimulation intensity ($F_{2.58}=214.357; p=0.000$). In the post hoc test, the measurement posture showed significant differences between the sitting and Semi-Fowler’s positions and between the Semi-Fowler’s and prone positions. In the post hoc test, significant differences were shown in all the three degrees of stimulation intensity.

| Table 3. Changes in the current values (unit: mA) |
|-----------------------------------------------|
| Position                                      | 1.2 times | 1.4 times | 1.5 times |
| Sitting                                       | 3.9 ± 2.7 | 4.5 ± 3.2 | 4.9 ± 3.4 |
| Semi-Fowler’s                                | 4.1 ± 3.4 | 4.8 ± 4.0 | 5.1 ± 4.3 |
| Prone                                        | 6.0 ± 3.7 | 7.0 ± 4.3 | 7.5 ± 4.5 |
| Mean ± SD                                     |           |           |           |

The current values showed significant differences in the interaction effects between the measurement posture and the stimulation intensity ($F_{4,116}=4.190; p=0.000$). The main effects showed significant differences both in the measurement posture ($F_{2.58}=5.395; p=0.013$) and in the stimulation intensity ($F_{2.58}=92.022; p=0.000$).

| Table 4. Changes in amplitude of RIII reflex (unit: µV) |
|--------------------------------------------------------|
| Position                                               | 1.2 times | 1.4 times | 1.5 times |
| Sitting                                                | 26.1 ± 10.2| 27.1 ± 12.1| 30.1 ± 25.7|
| Semi-Fowler’s                                         | 24.0 ± 5.5 | 26.4 ± 8.0 | 27.7 ± 9.7 |
| Prone                                                 | 22.5 ± 5.7 | 25.7 ± 8.5 | 30.4 ± 18.0|
| Mean ± SD                                             |           |           |           |

The amplitude of the RIII reflex showed significant differences only in the stimulation intensity ($F_{2.58}=6.163; p=0.014$). In the post hoc test, there were significant differences only between the 1.2- and 1.4-time stimulation intensities and between the 1.2- and 1.5-time stimulation intensities.
The stimulation of the sural nerve, with the subject in a sitting or supine position, provokes a response characterized by flexion of the hip and knee joint (frequently combined with adduction and rotation of the limb). In the study results, the electrical stimulation needed to induce the RIII reflex showed lower values in the sitting position than in the Semi-Fowler’s and prone positions. Also, the current values of the electrical stimulation needed to induce the RIII reflex were almost the same between the 1.2- and 1.4-time stimulation intensities in all the positions. The threshold of the RIII reflex was affected by a variety of physiological factors, including the circadian rhythm, age, and gender. Therefore, the results of this study suggest that the measurement posture and stimulation intensity as well as a variety of physiological factors may affect the threshold of the RIII reflex. Wang et al. investigated influence of upper extremity position on EMG signal measures calculated in time, frequency and time-frequency domains. They suggested that parameters analyzed in the time-frequency domain were more sensitive to changes in position than parameters analyzed in the frequency domain. We suggest that the measurement posture, especially lower extremity position, might be influence on EMG signal (root mean square amplitude) of time-frequency domain in RIII reflex test. The limitation of this study is a study that was only conducted by women.

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