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H-reflex and M-wave studies in the fore- and hindlimbs of rabbit

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Abstract: The aim of this study was to investigate the H-reflex in the forelimb and to compare the forelimb and hindlimb H-reflex results and Hmax/Mmax ratios in rabbit. For this purpose, we used 15 healthy adult female New Zealand albino rabbits. Left limbs of rabbits were used for stimulation and recording procedures using general anesthesia. Electrophysiological tests were performed using Nikolet Viking Quest (USA) 2-channel EMG equipment. The median and the tibial nerves were used to evaluate H-reflex and M-waves. Latencies of the H-reflex were almost the same in both limbs. However, M-wave latencies and amplitudes were significantly different, P-values being $P < 0.001$ and $P = 0.001$, respectively. Hmax/Mmax ratios of the forelimb (0.15 ± 0.075) and the hindlimb (0.15 ± 0.068) were similar in rabbits. One possible reason for our Hmax/Mmax ratio results may be related to interspecies differences in descending pathways from red nucleus and reticular formation to cervical and lumbar spinal segments.

Key words: H-reflex, rabbit, Hmax/Mmax ratio, forelimb, hindlimb

1. Introduction

The Hoffmann reflex (H-reflex) is an electrically stimulated reflex and it is analogous to the mechanically induced spinal stretch reflex. This reflex was described by Paul Hoffman (1,2). The H-reflex pathway consist of the group Ia sensory fibers, which make a synapse with α-motoneurons and their axons. Although the neural pathway of the spinal stretch reflex and the H-reflex are similar, the H-reflex bypasses the muscle spindle of the muscle stretch reflex arch (1,3). Because of this, the H-reflex is a significant test method that assesses organization of monosynaptic reflex activity in the spinal cord. Stimulation of a mixed nerve with a low-intensity percutaneous electrical stimulus is required to obtain the H-reflex (1,2,4). During the recording of the H-reflex, characteristically 2 responses are observed in electromyograms (EMGs). Since the low-intensity electrical stimulus produces discharge with a large diameter of axon sensory Ia afferents, the H-reflex (H-wave) is visible in EMGs. After that, increasingly higher stimulus intensity of application to the same mixed nerve causes discharge also in thinner axons of the α-motoneurons travelling to the muscle as a direct M-response (M-wave) (5). In addition, the M-wave has a shorter latency period than the H-wave latency (1).

H-reflex measurements are useful in examining the response of the nervous system to a wide variety of neurologic conditions (e.g., radiculopathy, plexopathy, and polyneuropathy), musculoskeletal injuries, pain, exercise training, and performance of motor tasks (2,3). In addition, the ratio of the maximal H-reflex (Hmax) to the maximal M-wave (Mmax) is a proper index for revealing the level of excitability of the motor neuron pool (2,6,7). Because of widespread usage, the H-reflex is the most inclusively studied reflex in the literature on mammalian and human neurophysiology (3). As an animal model the rabbit is mostly used in neurophysiological and electrophysiological studies, for instance in nerve compression studies (8–10), entrapment neuropathy studies (10–12, and neuropathic pain and nerve crush studies (13,14). In addition to these studies, the rabbit is also used in H-reflex studies (15,16). According to the authors' knowledge, the technique and results of H-reflex studies in forelimbs in rabbit have not been clarified yet. The purpose of the present study was to show the H-reflex results of the forelimb in rabbit as well as to compare to H-reflex results and Hmax-to-Mmax ratios of the forelimb and hindlimb.

2. Materials and methods

H- and M-waves were recorded in both forelimbs and hindlimbs from 15 healthy adult female New Zealand albino rabbits weighing an average of 2562 ± 407 g (range: 1790–3283 g). The Animal Ethics Committee of

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Adnan Menderes University approved the study. During all examinations, laboratory ambient temperature was kept at 28–30 °C using an air-conditioner. Records were taken from all rabbits that were kept in the laboratory for 1 h. A digital thermometer was used for measuring the rectal temperature before the recordings. The general anesthesia was induced with a combination of 2 mg/kg xylazine (Rompun; Bayer) and 25 mg/kg ketamine (Ketanes; Alke) intramuscularly. Each rabbit was placed in right lateral recumbency and left limbs of rabbits were used for stimulation and recording procedures. Before the electrophysiological examination, hair on the stimulation and recording sites of the limbs were shaved. The middle-medial side of the cubital joint was used as the stimulation site for the median nerve and the recordings were taken from the flexor carpi radialis muscle. Tibial nerve stimulation was carried out from the popliteal fossa and the recordings were taken from the gastrocnemius muscle. The bipolar surface electrode (S 403, VIASYS Viking Quest) was used to stimulate both the median and the tibial nerves. For the recordings, bipolar surface disk electrodes were used in this study. An active electrode (cathode) and the reference (anode) electrodes were placed at the middle portion of the muscle belly and the tendon of the muscle, respectively. A grounding electrode was also placed on the limb in all animals. Electrophysiological tests were performed using VIASYS Nikolet Viking Quest (USA) 2-channel EMG equipment. The data were analyzed using the Nikolet Viking Quest software program. The settings of the equipment were as follows: filter setting: 2 Hz to 10 kHz, duration: 0.2 ms. Stimulus intensity was increased by increments of 1–2 V until the responses of the H-reflex and M-wave reached maximum amplitudes. The stimulating current intensity that evoked the largest H-reflex peak-to-peak amplitude was considered as maximum H-reflex intensity. The obtained H-reflex was accepted as Hmax. Stimulus intensity that would not lead to any further changes in the largest peak-to-peak amplitude of the M-wave was determined as the maximum M-wave stimulus intensity. Stimulus intensities of maximal M-wave responses and H-reflex responses in forelimbs and hindlimbs were 20–50 V and 31–59 V and 1–15 V and 7–26 V, respectively. Latencies of the H-reflex and M-wave were measured at the first negative deflection of traces.

For the calculation of the Hmax/Mmax ratios, peak-to-peak amplitudes of Hmax and Mmax waves were recorded for each rabbit. All data were given as mean values and standard errors of means. Statistical differences of latency and amplitude of H-reflex and M-wave in forelimb and hindlimb were investigated using SPSS 18.0 for Windows with the independent t-test.

3. Results

We chose healthy female rabbits and the rectal temperatures varied from 38 to 39 °C in all rabbits. Stimulus intensity, amplitudes and latencies of H-reflexes and M-waves, and Hmax/Mmax ratio values are shown in Figures 1a–1d. The shapes of the H-reflex and M-wave of both forelimb and hindlimb were diphasic with the initial negative deflection. Examples of the M- and H-waves in the forelimbs showing characteristic features are demonstrated in Figures 2a and 2b. The Hmax value was obtained at low stimulus intensities. However, it was usually observed also at supramaximal stimulation intensities. On the other hand, amplitudes of the H-reflexes that were obtained at supramaximal stimulation were not maximal (Figure 2c). H-reflex latencies and amplitudes of fore- and hindlimbs were not statistically different. However, M-wave latencies and amplitudes were significantly different between the forelimb ($P < 0.001$) and hindlimb ($P = 0.001$).

4. Discussion

Experimental H-reflex has been studied mostly in the rat. These studies have been focused on the hindlimb (17,18). Hosoido et al. (5) first showed that the H-reflex can be recorded not only from hindlimbs, but also from forelimbs in the rat. H-reflex in the hindlimb of rabbits was described in previous studies (15,19). The purpose of the present study was to characterize H-reflex data obtained from forelimbs of rabbit and to evaluate differences between the H- and M-waves that were recorded from forelimbs and hindlimbs.

We preferred the belly tendon method for the H-reflex recording for both forelimbs and hindlimbs. According to our experience, the rabbit could be considered as a proper animal for location of electrodes in the belly tendon method. The stimulus intensities required to induce H-reflex (maximal) was about 2 times higher in hindlimbs (13.86 ± 4.52 V) than forelimbs (6.36 ± 3.94 V). The same was true for the stimulus intensities for inducing M-waves between forelimbs and hindlimbs. We thought that these findings were related to the fact that the median nerve has a more superficial course at the stimulus site than the tibial nerve. The superficial course of the median nerve at the stimulus site might lead to alterations in the sensitivity to electrical stimulation. In the forelimb, stimulus intensity required to induce M-waves was around 6 times higher than H-reflex stimulus intensity. This ratio was around 3 in the hindlimb. Latencies of the H-reflex were almost the same in forelimbs (7.59 ± 1.03 ms) and hindlimbs (7.67 ± 1.2 ms). However, M-wave latencies and amplitudes were significantly different, P-values being $P < 0.001$ and $P = 0.001$, respectively. While the amplitude of the M-wave (39.01 ± 8.94 mV) was almost 7 times higher than Hmax amplitude (5.56 ± 2.63 mV) in forelimbs, in the hindlimb

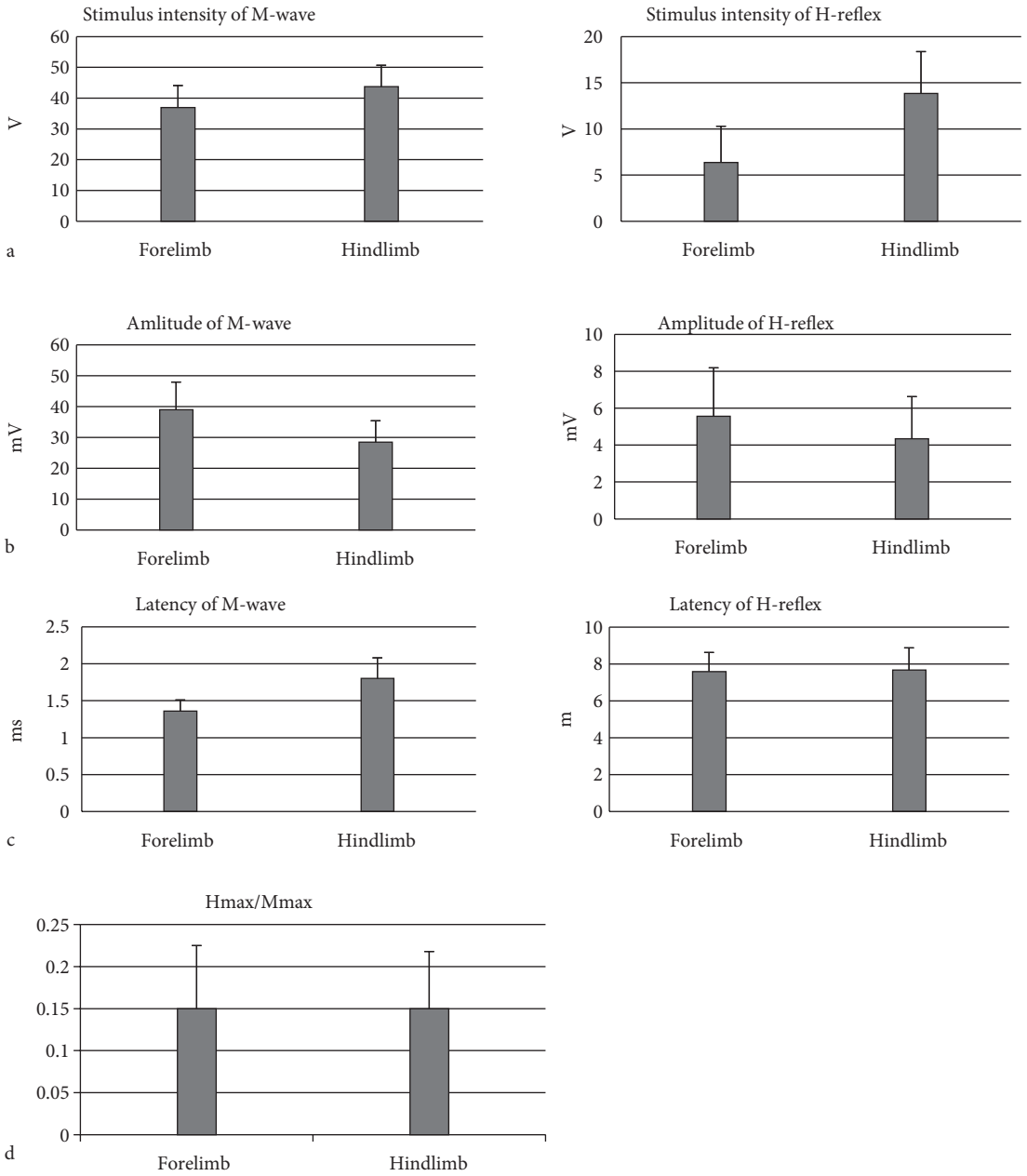

Figure 1. Stimulus intensity (a), amplitudes (b), and latencies (c) of H-reflexes and M-waves in the fore- and hindlimbs and the Hmax/Mmax ratio values (d).

Mmax amplitude (28.46 ± 6.94 mV) was 6.5 times higher than Hmax amplitude (4.34 ± 2.29 mV).

In human beings, the pattern of the H-wave is usually triphasic and it has initial positive deflection and a large negative deflection when recorded from the triceps surae muscle (1). In contrast, the first deflection of the H-reflex was negative and its shape was diphasic in both limbs in our study. The shape, amplitude, and latency of the canine H-reflex can be variable from trial to trial, unlike in human H-reflexes studies (20). Besides, the canine H-reflex can be abolished in deep anesthesia (21). It was reported that a probable reason for the difficulty in obtaining the H-reflex was the depressive effect of anesthesia on the motor neuron excitability (22). However, Leis et al. (23) stated that the H-reflex is quite robust in the range of anesthetic concentrations used to produce surgical immobility. Amplitudes of H-reflexes varied from 3.43 to 11.97 mV in their study. Similarly, in our study, amplitudes of the

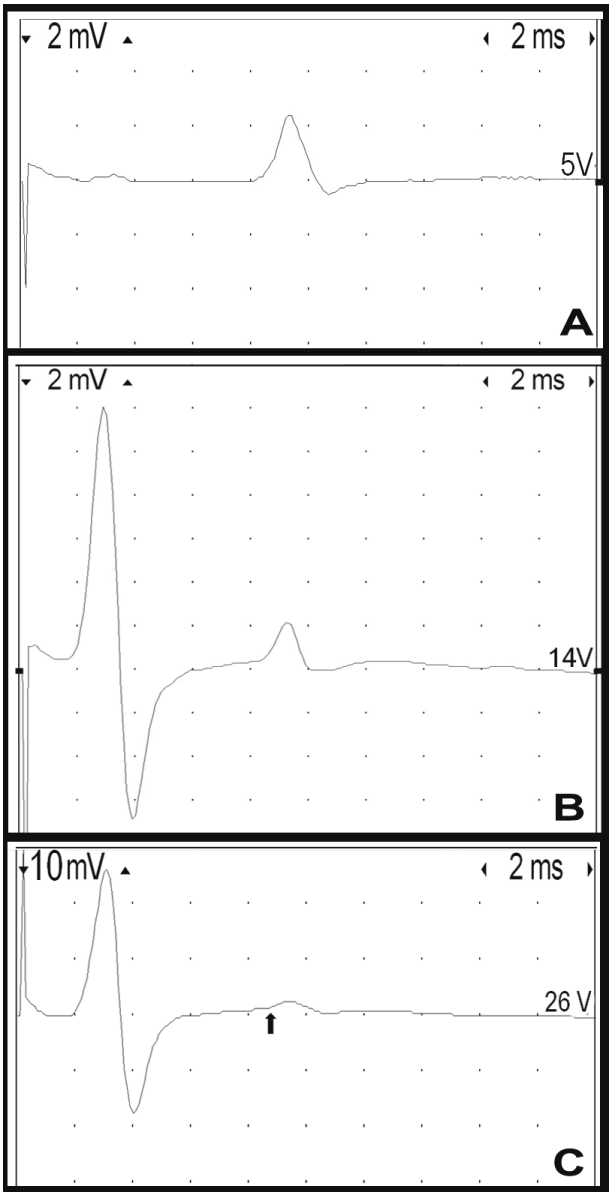

Figure 2. H-reflex and M-wave responses in the forelimbs of rabbits. (A) H-max wave at the lowest stimulus intensity. (B) M-wave and H-reflex responses at the moderate stimulus intensity. (C) M-max at the supramaximal stimulus intensity. Black arrow indicates that H-reflex response does not have maximal amplitude.

H-reflex were variable in both forelimbs and hindlimbs. The latencies of the H-reflex were almost stable in both limbs and there were small variations among individuals. Moreover, we could record H-reflex response in all rabbits. We think that the dose of the anesthetic agent has an effect on the recording of robust H-reflex response. The given anesthetic agent doses were low for rabbits in our study.

Cliffer et al. (17) in Sprague Dawley rats and Lagarce et al. (16) in rabbits observed persistent H-reflex at supramaximal stimulus intensity. Similarly, we usually observed H-reflex at supramaximal stimulation intensities. Classical nerve conduction studies indicate that the delayed signal after the M-wave may be an H-reflex or F-response (1). In spite of that, the delayed signal latencies that were recorded from both fore- and hindlimbs were not multivariate in our study. The shape and latency of F-responses has classically been variable, although the H-reflex has more regularity for the same neural circuitry (1). In our study, the shape of the H-reflex was stable and its latencies were 7.59 ± 1.03 ms and 7.67 ± 1.2 ms in the forelimb and hindlimb, respectively.

The Hmax/Mmax ratio shows the proportion of inducted motor neurons in the whole motor neuron pool (2,6,7). A study evaluating the Hmax/Mmax ratio between forelimbs and hindlimbs in the rat found that the Hmax/Mmax ratio in the forelimb was significantly smaller than that in the hindlimb. The authors speculated that the cause of their results could be related to the descending and/or propriospinal pathway of the forelimb having stronger control than that of the hindlimb (5). Furthermore, Huisman et al. (24) suggested that descending pathways from red nucleus and reticular formation were stronger in cervical spinal segments than in lumbar spinal segments in rat.

The results of our study have shown that Hmax/Mmax ratios of the forelimb (0.15 ± 0.075) and the hindlimb (0.15 ± 0.068) were almost similar in rabbit. We think that our Hmax/Mmax ratio results might be related to interspecies differences in descending pathways from red nucleus and reticular formation to cervical and lumbar spinal segments.

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