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Effect of spinal cord injury severity on alterations of the H-reflex

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Abstract

The monosynaptic motoneuron response to stimulation of Ia afferents is known to be altered by spinal cord injury (SCI). Although the Hoffman (H)-reflex is a tool that is often used to measure this reflex in patients, there has not been a systematic study investigating the effect of SCI severity and time on the H-reflex. We used a clinically relevant model of spinal cord contusion (Mild and Moderate) as well as complete surgical transection to measure the H-reflex at 1, 4 and 8 weeks after injury. The H-reflex was recorded from rat hindpaw plantar muscles in order to measure the baseline reflex amplitude and its response to increased stimulus frequency, i.e. rate depression. We correlated the reflex amplitude at each frequency to spared white matter at the injury epicenter, hindlimb function and serotonin immunoreactivity associated with retrogradely labeled plantar muscle motoneurons. The three injury groups displayed different behavioral deficits and amount of spared white matter at all three times tested. H-reflex rate depression was abnormal in all three injury groups at all three time points. At 8 weeks, transected animals displayed more H-reflex rate depression than those with a mild contusion. Baseline H-reflex amplitude was increased in both contusion groups at 4 weeks and showed a positive linear correlation with serotonin immunoreactivity. This baseline amplitude was not increased after transection. Furthermore, in the contusion group, there was a U-shaped relationship between behavioral scores and H-reflex rate depression, suggesting that an intermediate sensitivity of the motoneuronal pool to afferent input is associated with better recovery of hindlimb function.

Keywords: MASCIS; Contusion; Transection; Spasticity; Serotonin; Monosynaptic Ia afferent reflex; White matter; Hyperreflexia; Spontaneous recovery; Motoneuron recruitment

Introduction

The H-reflex is a commonly used clinical tool (Misiaszek, 2003; Yablon and Stokic, 2004) and has yielded information about altered properties of the monosynaptic reflex after spinal cord injury (SCI). The H-reflex is elicited by an electrical stimulation of a peripheral nerve that innervates the muscle from which the reflex is recorded. A single stimulus generates a short-latency M-wave, resulting from direct stimulation of the motor axons innervating the muscle, and a long-latency H-wave, which is a measure of the alpha motoneurons activated by Ia afferents (Gozariu et al., 1998). In normal subjects, the H-wave amplitude decreases with increasing stimulus frequency, while the M-wave amplitude remains constant (Fig. 3A; Hasegawa and Ono, 1996a,b). However, after SCI, this decrease in H-wave amplitude becomes less sensitive to stimulus frequency, resulting in a higher H/M ratio compared to corresponding frequencies in uninjured controls (Fig. 3B; Calancie et al., 1993; Thompson et al., 1992). Furthermore, the H/M ratio at the baseline stimulus frequency is elevated (Calancie et al., 1993; Thompson et al., 1998). These reflex changes are thought to contribute to spasticity in SCI patients (Schindler-Ivens and Shields, 2000; Yablon and Stokic, 2004) and have been studied in rat models of spasticity (Thompson et al., 2001).

To date, there has not been a systematic investigation of how the H-reflex is affected by SCI severity, which is important for several reasons. First, it provides insight into the role of spared pathways in H-reflex alterations after SCI. Second, it allows the establishment of possible correlations between the H-reflex and the behavioral and anatomical effects of SCI, which would give the H-reflex important predictive value. Third, the H-reflex provides an objective quantitative measure of physiological changes that are occurring in the lumbar spinal cord after SCI.

Serotonin (5-HT) has been shown to affect the monosynaptic Ia afferent reflex (Nagano et al., 1988a,b; Samathanam et al., 1989; Sinclair and Sastry, 1974), and cyproheptadine, a 5-

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HT_2 receptor antagonist, has been shown to alleviate spasticity (Barbeau et al., 1982; Nance, 1994; Wainberg et al., 1990). Serotonergic fibers are often partially spared after contusive SCI (Faden et al., 1988; Pikov and Wrathall, 2001), and 5-HT immunoreactivity can recover below the injury site with time after SCI (Holmes et al., 2005; Saruhashi et al., 1996). These studies suggest the involvement of serotonin in the alteration of the H-reflex after SCI.

The aim of this study was to investigate the alterations in the H-reflex at specific times after different SCI severities. We employed a standard clinically relevant weight-drop model of incomplete contusion SCI (Basso et al., 1996) as well as complete surgical transection to test the hypothesis that more severe injury will produce more abnormal H-reflex rate depression and baseline H/M ratio that correlate with loss of hindlimb function, white matter loss at the injury site and reduced serotonergic innervation of the plantar motoneurons.

Materials and methods

Spinal cord injury and animal care

All animal protocols were in accordance with NIH *Guide for the Care and Use of Laboratory Animals* and approved by the Georgetown University Animal Care and Use Committee. Adult female Sprague–Dawley rats (200–250 g, Taconic, Germantown, NY and Zivic Miller, Pittsburgh, PA) were housed 2 or 3 per cage, with food and water provided ad libitum, and kept on a 12 h light–dark cycle. Rats were anesthetized with chloral hydrate (360 mg/kg, i.p., Sigma, St. Louis, MO), and a laminectomy was performed at T8 to remove the vertebral bone overlying the spinal cord, exposing a circle of dura. The spinal column was stabilized by clamping the T7 and T9 vertebra, and the MASCIS weight-drop device (Basso et al., 1996) was used to produce a contusive SCI with a 10 g impounder dropped from either 12.5 mm (Mild) or 25 mm (Moderate). For transections, irridectomy scissors were used to completely sever the spinal cord at T8, and gel foam was placed into the lesion site. Age-, sex-, and weight-matched controls were only laminectomized. Rats were assigned using a randomized block design with the aim of having 5 per control group and 7–8 per injury group as shown in Table 1. After injury, bladders were manually expressed twice daily until no longer needed, and oral antibiotics (sulfamethoxazole and trimethoprim oral suspension, 4 mg/1 mg, Hi-Tech Pharmacal Co., Inc, Amityville, NY) were given in cases of suspected urinary tract or bladder infections.

Table 1
Number of animals in each experimental group[a]

	Cont	Mild	Mod	Trans
1 week	5	8	7	7
4 weeks	5	7	8	7
8 weeks	5	7	8	7

[a] Rats received a T8 laminectomy alone (Cont) or were subjected to Mild (10 g × 12.5 mm) or Moderate (10 g × 25 mm) contusion or complete surgical transection (Trans).

Behavioral analysis

All rats were tested for behavioral deficits on day 1 after injury and weekly thereafter, using the Combined Behavioral Score (CBS, Gale et al., 1985) and the Basso, Beattie and Bresnahan (BBB, Basso et al., 1995) scale. The CBS provides an overall measure of hindlimb functional deficits, ranging from 100 (most deficits) to 0 (normal hindlimb function). The BBB is a neurological rating scale of use of hindlimbs in open field locomotion after SCI, ranging from 0 (complete hindlimb paralysis) to 21 (normal hindlimb locomotion). All animals in the study displayed a BBB score of ≤ 1 for both hindlimbs at 1 day after SCI, confirming the expected behavioral effect of SCI.

Retrograde labeling and H-reflex recording

One week before recording the H-reflex, FluoroGold (5 μl of 5% solution in water, Biotium, Hayward, CA) was pressure-injected with a Hamilton syringe near the recording site to retrogradely label the motoneurons innervating the hindpaw plantar muscle. At 1, 4 or 8 weeks after injury or laminectomy, animals were lightly anesthetized with chloral hydrate (220 mg/kg, i.p.), and additional anesthesia (approximately 50 mg/kg every 20 min) was given to suppress voluntary movement and whisker tremors but maintain corneal reflex. This was done to ensure a similar plane of anesthesia among animals. Chloral hydrate has been shown to produce similar negligible effects on the H-reflex as ketamine, which is commonly used for H-reflex recordings (Cliffer et al., 1998). Saline (2 cm^3 s.c.) was injected approximately 30 min before recording to ensure proper hydration of each animal. The animal's forelimbs and hindlimbs were secured with tape onto a grounded heated (approximately 37°C) metal platform. Hindlimbs were only slightly extended away from the trunk (kept consistent between animals), and care was taken not to apply any unnecessary pressure or stretch to any part of the limbs. H-reflex was recorded from the plantar muscles of the hindpaw, with the active needle electrode (30-gauge) inserted between the fourth and fifth metatarsals, and the reference electrode inserted in the skin of the fifth digit. To elicit the H-reflex and study its rate sensitivity, the tibial nerve at the ankle was stimulated for 0.1 ms at 0.1, 0.3, 1, 2, 3 and 5 Hz using a Grass S88 stimulator (Astro-Med, Inc., West Warwick, RI). The cathode needle was inserted subcutaneously at the ankle, just above the heel, and the anode needle was inserted subcutaneously at the plantar surface of the heel. The intensity of the stimulus was adjusted to elicit the maximal consistent H-wave amplitude. The recorded signal was passed to a differential amplifier (Tektronix AM 502, Richardson, TX) and bandpass filtered at 0.1 Hz and 10 kHz. The analog signal was then sent to an A/D converter (model PCI-MIO-16E-4; National Instruments, Austin, TX), and the digital waveform (recorded at 30 kHz) was stored and displayed online using LabView 7 data acquisition software (National Instruments, Austin, TX). The H-reflex was recorded from the right or left side chosen at random. Twelve consecutive waveforms were collected at each frequency, but