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Better functional outcome of compression spinal cord injury in mice is associated with enhanced H-reflex responses

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ABSTRACT

Alterations in spinal reflexes and functional improvements occur after incomplete spinal cord injury but the relationship between these phenomena is not understood. Here we show that spontaneous functional recovery after compression injury of the spinal cord at low-thoracic level (Th10–12) in C57BL/6J mice is associated with a progressively increasing, over 3 months, excitability of the plantar H-reflex. The stimulation rate-sensitive H-reflex depression, already strongly reduced at 1 week after injury, when compared with non-injured mice, decreased further during the observation time period. Twelve weeks after injury, the degree of motor recovery estimated by single-frame motion analysis in individual animals correlated positively with their H-reflex responses at 2-Hz stimulation. Functional recovery and reflex alterations were accompanied by an increase in glycine/GABAergic and glutamatergic terminals around motoneuron cell bodies between 6 and 12 weeks after injury. Enhanced H-reflex responses at frequencies between 0.1 and 5 Hz were also observed in mice deficient in the extracellular matrix glycoprotein tenascin-R and the adhesion molecule close homolog of L1, mice previously shown to have better motor recovery after spinal cord injury than wild-type littermates. These results indicate that better functional outcome of compression spinal cord injury in mice is associated with alterations of the monosynaptic reflex pathway which facilitate motoneuron recruitment. Our observations support the view that plasticity of spinal circuitries underlies specific aspects of motor recovery and demonstrate the usefulness of H-reflex analyses in studies on spinal cord injury in mice.

Introduction

Acute spinal cord injury in humans and experimental animals like monkeys, cats and rats causes spinal shock, a syndrome consisting of loss of sensory and motor functions, loss of tendon (stretch) reflexes and flaccid muscle tone (Dietz and Colombo, 2004; Frigon and Rossignol, 2006). Over weeks and months after injury, a spasticity syndrome progressively develops. This syndrome is characterized by hyperreflexia (enhanced tendon reflexes), increased muscle tone, clonus (repetitive, rhythmic contractions of muscles upon stretching them) and muscle spasms (Little et al., 1989). Initially, development of spasticity is paralleled by recovery of some motor and sensory functions whereby the degree of spontaneous restoration is inversely related to the lesion severity and is generally limited. In the chronic phase of spinal cord injury, spasticity and functional recovery occur at lower rates and are no longer simultaneous. Spasticity is debilitating in that it causes pain, sleep disturbances and affects preserved motor functions, and it is believed that it limits functional restoration (Little et al., 1989; 1999). However, the latter view is contradicted by the fact that muscle hypertonia aids walking in many patients with CNS lesions and observations showing that antispastic drugs can worsen pareses (Dietz and Sinkjaer, 2007). The symptoms of spasticity have been considered to be consequences of exaggerated monosynaptic (tendon) and polysynaptic (cutaneous/flexor) reflexes (Abbruzzese, 2002; Sheean, 2002). It is now obvious that the role of hyperreflexia has been overestimated and a current view is that spastic movement disorders arise from loss of supraspinal control, defective use of afferent information in the context of altered spinal reflexes, and alterations in the paralyzed muscles (Dietz and Sinkjaer, 2007). It is thus evident that the relationships between functional recovery, spinal reflex properties and spasticity after spinal cord injury are not understood.

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In this study, we were interested whether the functional outcome of spinal cord injury in mice is influenced by alterations in motoneuron excitability. We analyzed mice subjected to compression spinal cord injury at low thoracic level using simultaneously two approaches: assessment of motor functions by means of a classical open-field locomotion rating and numerical parameters (single-frame motion analysis, Apostolova et al., 2006), on the one hand, and recording of H-reflexes, on the other. The H- (Hoffmann) reflex is an electrically elicited analog of the spinal stretch reflex providing information on the functional properties of Ia afferents and homonymous alpha-motoneurons under physiological and pathological conditions (Gozariu et al., 1998; Pierrot-Deseilligny and Mazevet, 2000). In the chronic phase of spinal cord injury, the H-reflex is facilitated upon single and repetitive low-frequency stimulation (Thompson et al., 1992; Little et al., 1999; Valero-Cabre et al., 2004; Lee et al., 2005). This is an objective measure of reflex hyperexcitability which, however, has barely been exploited to correlate reflex pathway properties with functional outcome in controlled animal experiments (Lee et al., 2005). Here we show that better functional recovery in mice is associated with enhanced H-reflex responses, an unexpected finding regarding the general view that hyperexcitability is a negative pathophysiological factor. Furthermore, this study has a methodological aspect as it is, to our best knowledge, the first to evaluate the potential of H-reflex analyses in a spinal cord injury paradigm in mice.

Materials and methods

Animals

The animals used in this study were adult female C57BL/6J mice, CHL1-deficient (CHL1−/−) mice and wild-type (CHL1+/+) littermates (mixed C57BL/6J-129Ola genetic background, Montag-Sallaz et al., 2002, subsequent 6 backcrosses into C57BL/6J), and TNR-deficient (TNR−/−) mice and wild-type (TNR+/+) littermates (mixed C57BL/6J-129Ola genetic background, Weber et al., 1999, 5 backcrosses into C57BL/6J genetic background). All mice were obtained from the animal facility of the Universitätsklinikum Hamburg-Eppendorf (Hamburg, Germany) at the age of 3 months. Genotyping of the mice from the CHL1 and TNR stocks was performed by PCR assays. The animals were kept under standard laboratory conditions. All experiments were conducted in accordance with the German and European Community laws on protection of experimental animals. Numbers of animals studied in different experimental groups are given in the text and figures. Data acquisition and analyses were performed in a blinded manner, i.e. without knowledge of genotype or performance of individual animals in different tests.

Spinal cord injury

The mice were anesthetized by intraperitoneal injection of a ketamin–xylazin mixture (100 mg/kg ketamin, Ketanest, Parke-Davis/Pfizer, Karlsruhe, Germany; 10 mg/kg xylazin, Rompun, Bayer, Leverkusen, Germany). After shaving, the skin was opened and laminectomy was performed at T7–T9 level of the thoracic vertebral segments. The spinal cord was compressed by a mouse spinal cord compression device (Curtis et al., 1993). The force and duration of compression were regulated by an electromagnetic device operated through an electrical current flow controller. Compression was performed at the highest force for 1 s (100% compression according to the operational definition of Curtis et al., 1993; Apostolova et al., 2006). The skin was closed by surgical suture with 6-0 nylon stitches (Ethicon, Norderstedt, Germany). The operated mice were housed individually at 37 °C for 12 h to prevent hypothermia. Afterwards, three mice were housed in one cage and kept in a conditioned room (22 °C) with standard water and food provided *ad libitum*.

H-reflex recording

For analysis we selected the plantar reflex which, as indicated by pilot experiments, was reproducibly recorded in the mouse. Previous work (Valero-Cabre et al., 2004) has shown that after spinal cord injury in rats the plantar reflex is changed similar to reflexes elicited in other hindlimb muscles such as the gastrocnemius and the anterior tibial muscles. Therefore, we assume that the changes in the plantar H-reflex after spinal cord injury in mice are characteristic for the hindlimb musculature as a whole.

The experiments were performed in a Faraday cage made of copper metal mesh. All conductible devices used in the cage were grounded. An insulated stimulator (Model 2100 Isolated Pulse Stimulator, A-M systems, Calsborg, WA, USA) was located outside the cage and connected to the stimulating electrode. The signals from the recording electrode were transmitted to a differential amplifier (P55 General Purpose AC Preamplifier, Grass Technologies, West Warwick, RI, USA), magnified (x 1000) and filtered (300 Hz high pass and 1 kHz low pass). The amplified analog signal was delivered to an A/D converter (ADC42, Pico Technology, Cambridgeshire, U.K.) and the digitalized data were displayed and saved on a computer using the Picoscope data acquisition software (PicoScope 5, Pico Technology, Cambridgeshire, U.K.).

Mice were anesthetized using ketamin–xylazin mixture as described above. Ketamin is widely used for H-reflex recordings, because it has been shown to have negligible effects on the electrophysiological recording (Ho and Waite, 2002). The initial dose was strictly controlled according to the body weight for every recording to synchronize the state of anesthesia among animals and prevent voluntary movement during H-reflex recording. Additional anesthesia was given if necessary, with 25% of the initial dose every 30 min, to suppress whisker tremor or voluntary movements. The forelimbs and hindlimbs of the mouse were fixed with tape on a plastic plate. The positions of the hindlimbs were controlled to avoid unnecessary pressure and stretch which could affect the electrophysiological responses of muscles and nerves. To maintain body temperature, the plastic plate was placed on a warm water pad (37 °C) driven by a temperature regulating pump system (TP472T/pump, Gaymar Industries, Orchard Park, NY, USA). The eyes of animals were protected from drying with an eye cream (Bepanthen, Bayer, Leverkusen, Germany).

Sciatic nerve stimulation was performed using two needle electrodes (stainless steel, diameter: 0.4 mm). Small plastic discs were fixed at a distances of 1 cm from the stimulation electrode tips to ensure equal depth of subcutaneous penetration. The stimulating electrodes were inserted between the two heads of the biceps femoris muscle in the thigh so that the cathode was located rostrally to the anode. A ground needle electrode (stainless steel, diameter: 0.4 mm) was applied at the base of the animal's tail. For recording, a reference stainless steel electrode was fixed to the skin between the first and second digit and an active recording electrode (Tungsten, diameter: 0.25 mm) was inserted between the second (intermediate) and third (lateral) cuneiform bones. Electrode positioning was performed under a stereo microscope.

The H-reflex was elicited by delivering bipolar electrical pulses of 0.2-ms duration to the sciatic nerve. Stimulus intensity was gradually increased until both M- and H-waves with latencies of approximately 2 and 5 ms, respectively, were visible. After the threshold measurement, stimulus intensity was further increased until maximal and stable H-responses were elicited. Thereafter, stimulation continued at the defined suprathreshold level at frequencies of 0.1, 0.3, 0.5, 1, 2, 3, 5 Hz. Six consecutive responses were recorded at each frequency. The amplitudes of M- and H-waves were measured as peak-to-peak values, averaged (excluding the first response at each frequency) and used to calculate H/M ratios. The latencies of the responses were measured as time elapsed between trigger and peak of each waveform.