An evaluation of the muscle recruitment properties of intraspinal microstimulation

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Abstract

Functional electrical stimulation (FES) methods can be employed to restore function following spinal cord injury. However, many peripheral forms of FES activate motor units in reversed order. This can lead to inappropriately sharp force recruitment and fatigability. In the long term, chronic activation of fast-twitch motor units can result in dramatic fast-to-slow transformation of skeletal muscle and atrophy. Intraspinal microstimulation (ISMS) is a novel FES system that may be able to activate skeletal muscle in a more normal manner, producing smoother, more fatigue-resistant movements in the short term and rescuing the normal muscle phenotype in the long term while avoiding atrophy. Our ongoing work with acute and chronic spinal applications of ISMS in rat quadriceps has shown that ISMS recruits motor units in a more normal order and may be able to maintain a more healthy muscle in cases of chronic spinal injury.

1 Introduction

Spinal cord injury (SCI) represents a devastating neurological impairment with potentially life-threatening implications. The quality of life for a SCI sufferer often centers on the challenges to bladder and bowel function, respiration, skin and muscle health, freedom of movement, and general independence. Two of the associated difficulties following SCI are the dramatic muscle atrophy and slow-to-fast transformation of skeletal muscle downstream of the lesion (Burnham ET AL. 1997; Castro ET AL. 1999). These result in a muscle that is highly fatigable and incapable of performing at workloads required for standing or stepping. Previous rehabilitative interventions for restoring stepping have utilised functional electrical stimulation (FES) with some success (Prochazka ET AL. 2001; Stein ET AL. 2002). Unfortunately, the normal order of motor-unit recruitment is reversed by many peripheral stimulation systems so that the lowest currents recruit the fastest, most fatigable units leading to inappropriate force recruitment and high fatigability (Prochazka 1993).

Recently, intraspinal microstimulation (ISMS) has been suggested for therapeutic restoration of stable standing and stepping. This technique involves implanting microwires into the ventral horns of the lumbosacral spinal cord. Stimulation through implanted microwires has previously been effective in establishing coordinated single and multi-joint movements in both intact (Mushahwar ET AL. 2002) and spinalized (Saigal ET AL. 2004) cats including consistent bilateral stepping. Furthermore, previous reports show that ISMS produces graded force recruitment and activates muscles selectively (Mushahwar & Horch 1998; Mushahwar & Horch 2000). The production of fatigue-resistant, graded force suggests that ISMS is activating skeletal muscle in a more normal manner than peripheral FES systems. The purpose of this work was to examine the acute recruitment properties of ISMS in rat quadriceps muscle and to extend those findings with chronic experiments to determine if the recruitment properties of ISMS might rescue the normal muscle phenotype and prevent muscle atrophy.

2 Methods

2.1 Acute Experiments

In the first set of experiments, muscle fibres were activated by ISMS targeting the quadriceps motoneuron pool or by nerve-cuff stimulation (NCS) over the femoral nerve. Stimuli were delivered at 20 pulses per second (pps), at 1.2X threshold or at 20 pps and 3.0X threshold. Stimulation was delivered for 5 bouts of 5 minutes each, separated by 2 minute rest periods. Following stimulation, quadriceps muscles were frozen in isopentane cooled in liquid nitrogen. Rectus femoris and vastus...
lateralis muscles were subsequently sectioned on a cryostat to 10 µm thickness. The muscle fibres activated by each stimulation protocol were identified using the glycogen depletion method. Fibres that were depleted of glycogen were considered to have been active during the stimulation period. Depleted fibres were further classified according to their corresponding myosin heavy-chain (MHC) isoform content. A panel of monoclonal antibodies allowed direct identification of pure type-I, -IIA, and -IIB fibres, as well as associated hybrid fibre combinations. Pure type-IID/X fibres were identified as those fibres that remained unstained by this panel of antibodies.

2.2 Chronic Experiments
Using aseptic procedures 6 female Sprague-Dawley rats were spinalized at the T8 level. Animals were allowed to recover for 2 weeks following surgery while manual bladder expressions were performed twice daily. Animals were fed a high-calorie diet of 9% fat rat chow ad libitum for the duration of the experiment. After 14 days of recovery animals were implanted with bipolar nerve cuffs over the femoral nerve with leads running to a headpiece affixed to the skull. After 7 days of recovery from this second procedure stimulation was performed daily for 30 days. Stimulation through the nerve cuff consisted of 4 hours of 50 pulses per second (pps) at an amplitude of 5 times the stimulation threshold with a 50% duty cycle of 1 s on and 1 s off. Thresholds were checked regularly in order to ensure that all stimulation through the nerve cuffs was at a supramaximal level.

Following 30 days of stimulation animals were anaesthetized and terminal data collection methods were employed. Animals were fixed at the iliac crest and at the epicondyles of the femur with a stereotaxic array to prevent movement. The patellar tendon was dissected from its point of insertion and attached to a force transducer. Functional muscle data including peak twitch force data, tetanic force data, a sag test and a fatigue test were collected according to previously published methods (GALLO ET AL. 2004).

3 Results
3.1 Acute Experiments
Glycogen depleted fibres were typed according to their MHC content and the fatigue-resistant (FR) fibres (MHC type-I, -IIA and -I/IIA) were grouped together for further analysis. At a stimulation amplitude corresponding to 1.2X threshold, the proportion of FR fibres recruited by ISMS was 2.8-fold that of NCS, however this difference did not reach statistical significance (p < 0.059). In contrast, the 3.0X threshold stimulation condition produced consistent and highly significant increases (p < 0.0001) in the proportion of FR fibres recruited by ISMS over NCS. Only 0.4% of fibres recruited by 20 pps 3.0X threshold NCS were FR fibres, whereas 44.4% of the total fibres depleted by ISMS at 20 pps 3.0X threshold were FR. In summary the 1.2X threshold stimulation did not evoke significant differences in the number of FR fibres depleted by ISMS vs. NCS. In contrast the 3.0X threshold stimulation activated significantly more FR fibres with ISMS than with NCS.

3.2 Chronic Experiments
Functional quadriceps muscle data were collected from spinalized rats following 30 days of NCS. Mean wet muscle mass of unstimulated control quadriceps was significantly larger than that of stimulated muscle (unstim 2.07 ± 0.06 g, stim 1.57 ± 0.09 g). Despite this, mean force from single twitches was significantly greater from stimulated than from control muscle (unstim 1.92 ± 0.26 N, stim 2.99 ± 0.39 N). Measurements of mean twitch width such as ½ rise-time (unstim 8.58 ± 0.28 s, stim 9.42 ± 0.26 s), ½ fall time (unstim 14.83 ± 1.63 s, stim 17.92 ± 1.22 s) and time-to-peak-tension (unstim 19.50 ± 1.10 s, stim 21.92 ± 1.10 s) were significantly longer in stimulated muscle than unstimulated control. Stimulated muscle was also significantly more fatigue-resistant according to a modified Burke fatigue test where the ratio of maximum force to force at 2 minutes showed significantly less attenuation of force from stimulated muscle (unstim 0.32 ± 0.05, stim 0.49 ± 0.04). Twitch force profiles produced from sag tests showed gradual potentiation in stimulated muscle while unstimulated control muscle exhibited a sag followed by potentiation indicating slow and fast twitch profiles in stimulated and unstimulated muscle, respectively. In summary, stimulated muscle underwent a fast-to-slow transformation relative to the unstimulated control while also undergoing significantly more atrophy.

4 Discussion and Conclusions
Peripheral methods of FES can be employed to reanimate skeletal muscle following SCI. However, these methods often result in sharp recruitment of force and high fatigability owing to their propensity to recruit muscle in a reversed order. Our recent work has shown that ISMS can recruit skeletal muscle in a more normal manner at high amplitudes of stimulation. In the acute 3.0X threshold stimulation group FR fibres were preferentially recruited by ISMS while NCS preferentially recruited fatigable IID and IIB fibres. Ultimately, this pattern of ISMS activation is similar to the normal physiological activation of motor units in which the smallest, most fatigue-resistant motor units are activated first, holding the larger units in reserve for activities requiring large force or power. Recent work has shown that ISMS activates muscle fibres before directly activating afferents. In the acute 3.0X threshold stimulation group FR fibres were preferentially recruited by ISMS while NCS preferentially recruited fatigable IID and IIB fibres. Finally, our recent work has confirmed that ISMS can recruit muscle fibres in a reversed order. Our recent work has shown that ISMS activates muscle fibres in a reversed order. Our recent work has shown that ISMS activates muscle fibres in a more normal manner at high amplitudes of stimulation. In the acute 3.0X threshold stimulation group FR fibres were preferentially recruited by ISMS while NCS preferentially recruited fatigable IID and IIB fibres. Ultimately, this pattern of ISMS activation is similar to the normal physiological activation of motor units in which the smallest, most fatigue-resistant motor units are activated first, holding the larger units in reserve for activities requiring large force or power. Recent work has shown that ISMS activates muscle fibres through afferents before directly activating motoneurons (Gaunt et al. 2006). The centrally mediated activation of skeletal muscle by afferents may explain our findings of near normal muscle recruitment.

Surprisingly, our results with ISMS are not maintained at stimulation amplitudes near the twitch threshold. It is possible that 1.2X threshold stimulation through the nerve cuff is activating Ia afferents primarily, leading to a centrally mediated H-reflex response. This would explain the activation of FR fibres by NCS at low amplitudes and the near absence of FR fibres activated by NCS at higher amplitudes where direct activation of motoneurons would predominate.

Given the ability of ISMS to recruit muscle in a more normal manner we are now attempting to apply NCS and ISMS paradigms in a chronically spinalized animal to determine which method will best rescue the normal muscle phenotype and prevent atrophy. Towards this end we have completed chronic NCS experiments which entailed 30 days of stimulation for 4 hours per day. Following this, the NCS muscles were significantly slower than contralateral controls according to measurements of single twitch width as well as a sag test and a modified Burke fatigue test. In addition chronic NCS muscles were smaller yet they produced greater force from a single twitch. The atrophy in stimulated muscle may be due to the smaller cross-sectional area of type I fibers which are presumably more prevalent following chronic stimulation. Chronic ISMS experiments are currently underway in our laboratory to determine whether ISMS can prevent atrophy and rescue a normal mixed muscle phenotype following spinal cord injury.

References


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