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Research Paper

Reduced motor neuron excitability is an important contributor to weakness in a rat model of sepsis

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ABSTRACT

The mechanisms by which sepsis triggers intensive care unit acquired weakness (ICUAW) remain unclear. We previously identified difficulty with motor unit recruitment in patients as a novel contributor to ICUAW. To study the mechanism underlying poor recruitment of motor units we used the rat cecal ligation and puncture model of sepsis. We identified striking dysfunction of alpha motor neurons during repetitive firing. Firing was more erratic, and often intermittent. Our data raised the possibility that reduced excitability of motor neurons was a significant contributor to weakness induced by sepsis. In this study we quantified the contribution of reduced motor neuron excitability and compared its magnitude to the contributions of myopathy, neuropathy and failure of neuromuscular transmission. We injected constant depolarizing current pulses (5 s) into the soma of alpha motor neurons in the lumbosacral spinal cord of anesthetized rats to trigger repetitive firing. In response to constant depolarization, motor neurons in untreated control rats fired at steady and continuous firing rates and generated smooth and sustained tetanic motor unit force as expected. In contrast, following induction of sepsis, motor neurons were often unable to sustain firing throughout the 5 s current injection such that force production was reduced. Even when firing, motor neurons from septic rats fired erratically and discontinuously, leading to irregular production of motor unit force. Both fast and slow type motor neurons had similar disruption of excitability. We followed rats after recovery from sepsis to determine the time course of resolution of the defect in motor neuron excitability. By one week, rats appeared to have recovered from sepsis as they had no piloerection and appeared to be in no distress. The defects in motor neuron repetitive firing were still striking at 2 weeks and, although improved, were present at one month. We infer that rats suffered from weakness due to reduced motor neuron excitability for weeks after resolution of sepsis. To assess whether additional contributions from myopathy, neuropathy and defects in neuromuscular transmission contributed to the reduction in force generation, we measured whole-muscle force production in response to electrical stimulation of the muscle nerve. We found no abnormality in force generation that would suggest the presence of myopathy, neuropathy or defective neuromuscular transmission. These data suggest disruption of repetitive firing of motor neurons is an important contributor to weakness induced by sepsis in rats and raise the possibility that reduced motor neuron excitability contributes to disability that persists after resolution of sepsis.

1. Introduction

The syndrome of profound weakness following critical illness is termed ICU acquired weakness (ICUAW) and greatly complicates patient recovery. The current view is that the primary causes of ICUAW are neuropathy and myopathy (for review see Friedrich et al., 2015; Khan et al., 2008; Latronico and Bolton, 2011; Stevens et al., 2009). However, during a prospective EMG study of ICUAW we identified a number of patients in which myopathy and neuropathy appeared insufficient to explain their severe weakness (Khan et al., 2006). EMG records taken during voluntary contractions demonstrated that these patients had trouble recruiting motor units (Nardelli et al., 2013). The findings raised the possibility that there might be a defect in the central nervous system that contributes to weakness.

Reduction in excitability due to sepsis has been reported in skeletal muscle, peripheral nerve and the heart (Koesters et al., 2014; Novak et al., 2009; Rich et al., 1997; Rich et al., 2002; Rossignol et al., 2007). We wished to determine whether there might also be reduction of excitability in the central nervous system that contributes to the difficulty in recruiting motor units. In the rat cecal ligation and puncture model of sepsis, we examined motor neuron excitability by microelectrode injection of depolarizing current into spinal motor neurons in vivo. We

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identified a novel form of reduced motor neuron excitability that is only expressed during repetitive firing (Nardelli et al., 2013). Our findings identified a defect in mechanisms specific to central portions of motor neurons that encode repetitive firing, thereby suggesting that reduced motor neuron excitability may contribute to weakness in patients.

In the current study we used the same rat model of sepsis to assess the relative contributions to weakness of reduced excitability of motor neurons, myopathy, neuropathy, and failure of neuromuscular transmission. Results of the present study suggest that in the rat model of sepsis, prolonged reduction of motor neuron excitability is a more important contributor to weakness than myopathy or neuropathy. We propose that difficulty with normal recruitment of motor units arising from reduced motor neuronal excitability within the central nervous system may be a major contributor to motor difficulties in patients with ICUAW.

2. Materials and methods

2.1. Ethical approval

All procedures involving rats were approved by the Wright State IACUC committee and followed the NIH guide for care and use of laboratory animals.

2.2. Induction of sepsis

We used the cecal ligation and puncture procedure to induce sepsis in rats, which we used previously (Nardelli et al., 2013; Novak et al., 2009). Briefly, rats were anesthetized for survival surgery with inhaled isoflurane (1–3% mixed in 100% O_2) and the anterior abdomen was shaved, cleaned and incised. The cecum was ligated half way between its tip and the ileum, and punctured with an 18 gauge needle. For continuous relief of pain an Alzet 2 mL osmotic pump (Durect, Cupertino CA) that delivered 30 μg/kg/h of oxymorphine was inserted into the abdomen prior to closing the incision. At the end of surgery rats were given a single dose of buprenorphine (0.12 mg/kg) subcutaneously for pain relief until the oxymorphine took effect. All rats were given 5 ml of subcutaneous 0.9% sodium chloride 24 h after surgery. If rats appeared lethargic on postoperative days 2, 3 and 4, they were given daily additional injections of 5 ml of saline. If rats were so lethargic that they had little response to handling they were also given 10 mg/kg subcutaneous Baytril every 12 h until they began to improve behaviorally (usually by 24 h).

2.3. Terminal recordings

Rats were anesthetized in terminal experiments by inhalation of isoflurane (1.2–1.5% mixed in 100% O_2) and fixed in a rigid recording frame. In all experiments, the medial gastrocnemius (MG) muscle in the left hindlimb was partially dissected from surrounding tissue and attached through its cut tendon of insertion to a transducer for measuring force in isometric contractions (Aurora Scientific Inc, 205B-LR, Spike2 Cambridge Electronic Design (CED)). Fine-wire electrodes were inserted into the belly of the MG muscle for recording EMG. With all muscle nerves crushed except the MG nerve, the tibial nerve was placed on a bipolar silver wire stimulating electrode. In experiments in which intracellular recording and stimulation of motor neurons was performed, dorsal laminectomy of L3-L6 was also performed.

Peripheral motor function: The efficacy of peripheral motor axons, neuromuscular junctions, and extrafusal muscle fibers in supporting muscle force generation was examined by direct electrical stimulation of the MG nerve or muscle. The MG muscle was fixed at optimal length for maximal isometric twitch force evoked by brief current pulses (0.04 ms) delivered to the tibial nerve. Whole-muscle isometric force was evoked by nerve stimulation to determine the force-frequency relation. Whole muscle force was also studied in response to electrical stimulation applied directly to the muscle through silver ball electrodes.

Central excitability of motor neurons: Defects in central excitability were tested by examining the action potentials fired by alpha motor neurons in response to suprathreshold current injected intracellularly through a micropipette (2 M K-acetate, 5–10 MΩ). In a subset of experiments, motor unit force was recorded simultaneously with recording of intracellular action potentials.

2.4. Statistical analysis of data

The effect of sepsis in rats was identified by statistical comparison of treated vs. control groups. Data sampled from rats in each group was pooled and tested for group differences using nested ANOVA, which identified the effect of sepsis as well as its dependence on individual rats. For analysis of standard deviation of firing rate, a repeated measure ANOVA was used to compare data from motor neurons from septic rats to controls at each level of current injection.

3. Results

We previously identified reduced excitability of motor neurons within the spinal cord of rats during sepsis. To study the contribution of this defect to weakness, we used the rat cecal ligation and puncture model of sepsis (Nardelli et al., 2013; Novak et al., 2009). Within 20 h of surgery, rats developed piloerection and sat hunched without moving. Of 71 rats made septic, 11 died: 6 within 1 day, 1 at 2 days and 4 at 3 days. No rats died after day 3, and by day 5 most rats appeared in no distress as judged by absence of piloerection and normal grooming. By behavioral criteria we estimate that rats were most severely septic days 1–3 following cecal ligation and puncture and had largely recovered by day 5. As we did not measure muscle force production during voluntary activation of motor units, we do not know the time course of development, or resolution, of weakness following sepsis.

We impaled motor neurons in the lumbosacral spinal cord of living, anesthetized rats, identified ones that innervated the medial gastrocnemius muscle and stimulated them to fire with a 5 s depolarizing current pulse delivered to the cell soma. At the same time we measured force production by the motor unit innervated by the impaled motor neuron. One to three days after induction of sepsis, the mean force produced during the 5 s stimulation was reduced by close to 50% in motor neurons from septic rats ($p < 0.01$, Fig. 1). Reduced excitability of motor neurons was a major contributor to the reduction in force. Motor neurons in control rats fired action potentials at a steady rate throughout the 5 s period of stimulation such that muscle force was sustained. In motor neurons from septic rats, firing was usually irregular such that muscle force often dropped during the 5 s stimulation. These data confirmed our previous finding of reduced motor neuron excitability and demonstrate that the defect in motor neuron excitability contributes significantly to weakness 1–3 days after induction of sepsis (Nardelli et al., 2013).

The defect in motor neuron excitability disrupted motor function in several ways. The first was reduction in accumulated (or total) force production. The primary cause of reduction in force was inability of motor neurons to sustain firing throughout the 5 s stimulation. In motor neurons from control rats, once current injection was 3 nA or more above rheobase current, motor neurons sustained firing throughout the 5 s period of stimulation. In motor neurons from septic rats, there were pauses of up to several seconds in firing, during which force production dropped to zero (Fig. 2). Increasing current injection did not eliminate pausing. The pauses were variable from trial to trial in the same motor neuron such that force production was relatively normal during one trial, only to be followed by a trial in which there was a prolonged pause and force production was greatly reduced (Fig. 2). We were unable to identify any history dependence of the pauses in firing and thus were unable to predict its occurrence.