Reduced motoneuron excitability in a rat model of sepsis

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Submitted 25 October 2012; accepted in final form 5 January 2013

Many critically ill patients in intensive care units suffer from an infection-induced whole body inflammatory state known as sepsis, which causes severe weakness in patients who survive. The mechanisms by which sepsis triggers intensive care unit-acquired weakness (ICUAW) remain unclear. Currently, research into ICUAW is focused on dysfunction of the peripheral nervous system. During electromyographic studies of patients with ICUAW, we noticed that recruitment was limited to few motor units, which fired at low rates. The reduction in motor unit rate modulation suggested that functional impairment within the central nervous system contributes to ICUAW. To understand better the mechanism underlying reduced firing motor unit firing rates, we moved to the rat cecal ligation and puncture model of sepsis. In isoflurane-anesthetized rats, we studied the response of spinal motoneurons to injected current to determine their capacity for initiating and firing action potentials repetitively. Properties of single action potentials and passive membrane properties of motoneurons from septic rats were normal, suggesting excitability was normal. However, motoneurons exhibited striking dysfunction during repetitive firing. The sustained firing that underlies normal motor unit activity and smooth force generation was slower, more erratic, and often intermittent in septic rats. Our data are the first to suggest that reduced excitability of neurons within the central nervous system may contribute to ICUAW.

WEAKNESS FOLLOWING CRITICAL illness is a common problem that greatly complicates weaning from ventilators and rehabilitation (for review, see Bolton 2005; Khan et al. 2008; Latronico and Bolton 2011; Stevens et al. 2009). The syndrome of profound weakness following critical illness is currently termed intensive care unit (ICU)-acquired weakness (ICUAW). It is widely thought that the primary cause of ICUAW is dysfunction of the peripheral nervous system. The primary mechanisms underlying dysfunction of the peripheral nervous system have been identified as critical illness neuropathy and critical illness myopathy (Bolton 2005; Khan et al. 2008; Latronico and Bolton 2011; Stevens et al. 2009). Despite the focus on the peripheral nervous system as the locus of ICUAW, there is also evidence of central nervous system (CNS) dysfunction in critical illness. Many patients with ICUAW have septic encephalopathy. The etiology of septic encephalopathy is poorly understood (Flierl et al. 2010; Pytel and Alexander 2009), but it provides clear evidence of CNS dysfunction. No studies have been performed to determine whether CNS dysfunction contributes to ICUAW.

During a prospective electromyographic (EMG) study of ICUAW (Khan et al. 2006), we identified myopathy and neuropathy, but in many patients these factors seemed insufficient to explain the virtual paralysis exhibited by conscious patients attempting to produce maximal voluntary contraction. With further study, we identified an additional factor in recordings of motor unit activity obtained using concentric needle EMG. In these recordings, recruitment was limited to very few motor units, which fired at low rates. Thus the two primary mechanisms by which the CNS regulates muscle force, recruitment of motor units and rate modulation of motor unit firing, were both reduced. This suggested that dysfunction of the CNS contributes to ICUAW. To explore potential mechanisms underlying poor recruitment and rate modulation of motor units, we moved to a rat model of sepsis to assess motoneuron excitability. Motoneurons from septic rats fired at slower rates and demonstrated abnormal pauses in firing during current injection. These data raise the possibility that deficits in motoneuron excitability occurring within the CNS contribute to ICUAW.

MATERIALS AND METHODS

Ethical approval. All procedures performed on patients were approved by the Institutional Review Board of Emory University, and each patient or legally authorized representative gave informed written consent. All procedures involving animals were approved by the Wright State University Laboratory Animal Care and Use Committee (LACUC).

Nerve conduction/EMG in patients. Subjects with weakness following a diagnosis of severe sepsis were enrolled from the medical intensive care units at two participating institutions, Emory University Hospital and Grady Health System, using criteria for the diagnosis of sepsis that we used previously (Khan et al. 2006). Subjects less than 18 years of age and those with known preexisting neuromuscular disorders were excluded.

Demographic and clinical information regarding patient’s sex, age, race, medical comorbidities, and source of infection if known were recorded. None of the patients received neuromuscular blocking agents or glucocorticoids. All aspects of patient care, including nutritional support, ventilator management, and general ICU supportive care were left to the discretion of the primary physician caring for the patient.

Nerve conduction studies were performed in the ICU using standard techniques with surface stimulation and recording. Responses were categorized as normal or abnormal based on standard normal values used at the EMG laboratories at Grady Memorial Hospital and Emory University Hospital. When possible, sural sensory, peroneal motor, tibial motor, median sensory, radial sensory, and median motor studies were obtained. Amplitudes were measured from baseline to negative peak for motor and sensory responses. Concentric needle
EMG was performed on both proximal and distal arm and leg muscles in each patient. Muscles studied included the deltoid, biceps, and triceps, first dorsal interosseous, tibialis anterior, gastrocnemius, and vastus lateralis.

Studies of motoneuron excitability in the septic rat. We used the cecal ligation and puncture procedure to induce sepsis in rats, which we used previously (Novak et al. 2009). Briefly, rats were anesthetized with inhaled isoflurane (1–3% mixed in 100% O_2), and the anterior abdomen was shaved, cleaned, and incised. The cecum was ligated halfway 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 \mu g·kg^{-1}·h^{-1} of oxymorphone was inserted into the abdomen before 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 oxymorphone took effect.

Following 1 day of sepsis, data were collected in vivo from motoneurons in rats anesthetized by inhalation of isoflurane (1.2–1.5% mixed in 100% O_2) and fixed in a rigid recording frame. Properties of medial gastrocnemius motoneurons were measured by intracellular recording with glass micropipettes (K-acetate, 5–10 MΩ) advanced through the dorsal spinal cord, exposed by laminectomy as detailed in earlier reports from this laboratory (Bullinger et al. 2011). All recordings were performed using an Axoprobe 1A (Sunnyvale, CA) amplifier in bridge mode. Action potentials were initiated in motoneurons by injecting suprathreshold depolarizing current (square pulses lasting \( \geq 100 \) ms) directly into a motoneuron soma within the spinal cord, bypassing synaptic activation of the motor unit. In normal rats, motoneurons fire one action potential at rheobase current and fire repetitively throughout longer pulses as current is increased. Repetitive firing during 5-s pulses was assessed at several different levels of current. Both rheobase current and repetitive firing behaviors reflect the translation of current into firing rates by motoneurons and are standard indices of the intrinsic excitability of motoneurons (Powers and Binder 2001).

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 were pooled and tested for group differences using nested ANOVA, which identified the effect of sepsis as well as its dependence on individual rats.

RESULTS

Reduced recruitment of motor units contributes to ICUAW in patients. We performed nerve conduction/EMG studies in 4 patients in the early stage (2–3 wk after onset) of recovery from sepsis. Clinical and neurophysiological characteristics of the patients are included in Table 1. EMG was performed in both proximal and distal muscles in an arm and leg of each patient. At the time of EMG study, all patients were awake and able to cooperate but had severe weakness with an average Medical Research Council (MRC) scale of \( \leq 3 \) or less in both proximal and distal muscles. Despite apparent cooperation, EMG in all 4 patients revealed an inability to recruit more than a few motor units during maximal voluntary contraction of both distal and proximal limb muscles (bottom traces in Fig. 1).

A potential explanation for poor recruitment of motor units in patients with ICUAW is neuropathy. In ICU-acquired neuropathy, limb weakness is worse distally (Zochodne et al. 1987). This pattern of weakness is due to more severe loss of motor units in distal muscles with relative sparing of proximal muscles. All patients studied had neuropathy as evidenced by reduction of nerve-evoked sensory and motor responses (Table 1). However, there was a disproportionate lack of motor unit recruitment. On average, motor amplitudes were reduced in distal muscles by \( \sim 50\% \) (Table 1). This should have been reflected in a 50% reduction in motor unit recruitment in distal muscles with milder reduction in motor unit recruitment in proximal muscles. Instead, proximal muscles also showed severe reduction in motor unit recruitment instead of the milder reduction expected. Our findings suggest that neuropathy is

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Medical Conditions</th>
<th>Time of EMG Study Relative to Onset of Sepsis</th>
<th>Mean Motor Amplitude as a % of the Lower Limit of Normal at the Time of EMG</th>
<th>Mean Sensory Amplitude as a % of the Lower Limit of Normal at the Time of EMG</th>
<th>Summary of EMG Findings</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Pneumonia Sepsis ARDS Hypertension CHF CRD</td>
<td>20 Days</td>
<td>58% ( n = 6 )</td>
<td>0% ( n = 4 )</td>
<td>+ Fibrillations, normal motor-unit amplitudes, reduced motor-unit activation, and firing rate in proximal and distal muscles</td>
</tr>
<tr>
<td>2</td>
<td>Pneumonia Sepsis ARDS ATN Sinusitis Hypertension</td>
<td>21 Days</td>
<td>76% ( n = 5 )</td>
<td>65% ( n = 4 )</td>
<td>+ Fibrillations, reduced motor-unit amplitudes, reduced motor-unit activation, and firing rate in proximal and distal muscles</td>
</tr>
<tr>
<td>3</td>
<td>Sepsis Leukemia Bone marrow transplant GI bleed</td>
<td>17 Days</td>
<td>42% ( n = 5 )</td>
<td>10% ( n = 4 )</td>
<td>No spontaneous activity, reduced motor-unit amplitudes, reduced motor-unit activation, and firing rate in proximal and distal muscles</td>
</tr>
<tr>
<td>4</td>
<td>Pneumonia Sepsis Alcohol Abuse Gout</td>
<td>14 Days</td>
<td>32% ( n = 1 )</td>
<td>38% ( n = 4 )</td>
<td>+ Fibrillations, no motor-unit activation in proximal and distal muscles</td>
</tr>
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</table>

For motor and sensory studies, \( n \) = the number of nerves studied. ARDS, adult respiratory distress syndrome; ATN, acute tubular necrosis; CHF, congestive heart failure; CRD, chronic renal disease; GI, gastrointestinal; EMG, electromyography.
Reduced motoneuron excitability.

Insufficient to account for the reduction in recruitment of motor units in patients with severe ICUAW.

In addition to the reduction in number of motor units recruited, we noticed a defect in the rate of motor unit firing. Rates of motor unit firing in these patients were uncommonly low (5–10 Hz) during maximum voluntary contraction and did not achieve the normal maximum values of 15 Hz and greater (De Luca and Contessa 2012). These subnormal values contrast starkly with those typically observed in patients with neuropathy wherein the firing rates of surviving motor units are high.

Reductions in maximal firing rate of motor units cannot be accounted for by either neuropathy or myopathy and strongly suggest that functional impairment within the CNS contributes to ICUAW. There are two possible explanations for reduced motor unit recruitment and firing rates in patients with ICUAW. One is a reduction in excitatory drive of motoneurons due to either poor effort secondary to encephalopathy or weakening of synaptic inputs onto motoneurons. A second explanation is reduction in motoneuron excitability so that motoneurons are less responsive to depolarizing current. The possibility that motoneurons have reduced excitability was particularly interesting to us in light of our earlier demonstrations of reduced excitability of muscle and sensory nerve axons early in the course of critical illness (Novak et al. 2009; Rich et al. 1996, 1997). To go beyond our qualitative studies in patients and quantify motoneuron excitability, it was necessary to move to an animal model of sepsis to perform intracellular recordings from motoneurons in vivo.

Selective defects in central excitability of motoneurons in septic rats. Reduced excitability of the peripheral nervous system in rats was well-explained by sodium channelopathy (Filatov and Rich 2004; Novak et al. 2009; Rich et al. 1998; Rich and Pinter 2001, 2003), and it was intriguing that such problems might extend to the CNS where they would alter how neurons integrate and respond to synaptic current. To this end, we studied the capacity of motoneurons to respond to current injected directly into their soma within the spinal cord. Data from untreated rats were compared against those from rats made septic by cecal ligation and puncture 24 h earlier.

We examined central excitability of motoneurons using injection of current to elicit single action potentials. In rat models of critical illness neuropathy and critical illness myopathy, reduced peripheral excitability manifested as lower action potential amplitude and reduced rate of action potential rise (dV/dt; Novak et al. 2009; Rich and Pinter 2001). By contrast, there was no change in single action potentials generated by brief current pulses injected centrally into the soma of motoneurons in septic rats: both action potential amplitude and rate of rise of were normal (Table 2). Other measures of factors that affect baseline excitability were also normal. There was no difference between control and septic motoneurons in amplitude and decay time of the afterhyperpolarization of the action potential (Table 2). Resting potential was normal, consistent with our earlier finding that electrolyte concentrations are normal at early time points in sepsis in rats (Novak et al. 2009). Neither input resistance nor rheobase were significantly different between control and septic motoneurons (Table 2). Thus, with regard to generation of a single action potential, excitability was normal.

Normal operation of motoneurons is based on repetitive firing, and that is where we found evidence of reduced excitability. When repetitive firing of motoneurons was triggered using prolonged stimulation, there were striking abnormalities. Motoneurons from control rats respond to increasing current by increasing their rate of firing (Fig. 2). This is the mechanism underlying modulation of firing rate, which is essential for maximal generation of force. In motoneurons from septic rats, the increase in firing rate triggered by increased current injection was blunted such that firing rates were reduced at all levels of current injection (Fig. 2). The slope of the frequency-current (F–I) relation was reduced from 4.6 ± 1.3 pulses per second per nanoampere in the control to 3.6 ± 0.8 pulses per second per nanoampere in septic rats. In part because of the reduced firing rate, the number of spikes generated by septic motoneurons during 5 s of stimulation averaged only 1/5 that of control motoneurons injected with the same current (Fig. 2; P < 0.01). Reduction in firing rate was not the only difference between septic and control motoneurons. In control motoneurons, at each level of current injection, the rate of firing was relatively stable throughout the duration of that current injection. In motoneurons from septic rats, the firing rate was often normal.

Table 2. Action potential and passive properties of control and septic rat motoneurons

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<thead>
<tr>
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<th>Control</th>
<th>Septic</th>
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<tr>
<td>Resting potential, mV</td>
<td>−68.9 ± 3.0</td>
<td>−68.6 ± 2.0</td>
</tr>
<tr>
<td>Input resistance, MO</td>
<td>1.9 ± 0.3</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Rheobase current, nA</td>
<td>12.6 ± 2.3</td>
<td>12.9 ± 1.4</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>78.2 ± 4.7</td>
<td>76.7 ± 2.0</td>
</tr>
<tr>
<td>Rate of AP rise, mV/ms</td>
<td>405 ± 9</td>
<td>396 ± 6</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>1.3 ± 0.4</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>AHP half-decay time, ms</td>
<td>11.3 ± 1.5</td>
<td>11.6 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. Control, n = 6 motoneurons from 5 rats. Septic, n = 8 motoneurons from 4 rats. No differences were statistically significant.

AP, action potential; AHP, afterhyperpolarization.
initially but then fell, producing highly variable firing rates (Fig. 3). Further evidence of an activity-dependent reduction in excitability came from the pattern of firing of septic motoneurons. In ¾ of septic motoneurons, firing intermittently stopped completely for 1-2 s (Fig. 4). This “stuttering” pattern of firing has not been observed in any of ~50 motoneurons examined by us in control rats for other studies. These data strongly suggested that although the threshold properties of septic motoneurons are normal, their ability to sustain prolonged repetitive firing was severely impaired.

In our previous studies of animal models of ICUAW, we found that increased inactivation of the sodium channels that generate the action potential was the mechanism of reduced excitability of peripheral nerve and skeletal muscle (Filatov and Rich 2004; Novak et al. 2009; Rich and Pinter 2001, 2003). We wished to determine whether a similar mechanism could account for failure to sustain firing of motoneurons in septic rats. If sodium channel inactivation is the sole mechanism underlying stuttering in firing, the degree of inactivation should be greatest before pauses in firing, and relief of inactivation should occur before resumption of firing. The rate of action potential rise in motoneurons is proportional to the number of sodium channels available to open (Brownstone et al. 2010; Miles et al. 2005). To estimate the relative degree of sodium channel inactivation, we measured the maximal rate of rise of action potentials (dV/dt) during repetitive firing in response to a 5 s suprathreshold current step (Fig. 4C). We compared the maximal dV/dt of the last action potential before each pause (Fig. 4B, left) with that of the first action potential following resumption of firing (Fig. 4B, right). The first action potential after a pause had a significant reduction in the rate of rise relative to the final action potential before the pause (210 ± 14 vs. 243 ± 14 mV/ms; P < 0.01, paired t-test) as well as reduction in amplitude (48.9 ± 2.9 vs. 55.1 ± 2.5 mV; P < 0.01, paired t-test). These data demonstrate resumption of firing following a pause occurs when sodium channel availability is relatively low. This suggests that resumption of firing is not solely due to relief of sodium channel inactivation.

**DISCUSSION**

During EMG studies of patients recovering from sepsis, we found that neither neuropathy nor myopathy appeared sufficient to account for profound weakness. In looking for an explanation for weakness, we identified reduced firing rates of motor units as a potential contributor. Using a rat model of sepsis, we identified a novel form of reduced motoneuron excitability that is only expressed during repetitive firing. Our findings suggest there is a defect in mechanisms specific to central portions of motoneurons that encode repetitive firing.
Our findings suggest that reduced motoneuron excitability may contribute to ICUAW.

**Comparison between studies of septic patients and rats.** We used an in vivo cecal ligation and puncture model of sepsis in rats to determine whether reduced motoneuron excitability was a potential contributor to reduced motor unit recruitment in sepsis. The rat model of sepsis we used closely mimics the clinical setting in patients who are septic; however, EMG studies of patients were performed several weeks after the onset of sepsis, when patients were recovering, whereas rats were studied within 24 h of the onset of sepsis. In patients, we could not examine motor unit recruitment during the acute phase of critical illness because patients were too encephalopathic to cooperate with voluntary motor unit examination. This might not be coincidental if septic encephalopathy and reduced motoneuron excitability are due to the same underlying mechanism. Although the time point at which they were studied differed, the parallels in motoneuron behavior raise the possibility that reduced motoneuron excitability identified in rats also underlies reduced motor unit firing rates identified in patients. It also suggests the possibility that a mechanism in the CNS, which can be observed within 24 h of the onset of sepsis, might contribute to the early phase of ICUAW.

**Mechanisms underlying reduced excitability of rat motoneurons.** In the central portion of motoneurons in septic rats, properties of single action potentials as well as resting potential and membrane conductance were all normal. Defects in the central excitability of motoneurons only emerged during repetitive firing. In previous studies where we identified reduced excitability of peripheral nerve and muscle, we found abnormalities of single action potentials fired from rest (Novak et al. 2009; Rich et al. 1998). This difference suggests the mechanism underlying reduced motoneuron excitability is distinct from the hyperpolarized shift in sodium channel inactivation that we previously identified as the mechanism underlying reduced excitability of skeletal muscle and peripheral nerve (Filatov and Rich 2004; Novak et al. 2009; Rich and Pinter 2001, 2003). However, two recent computational studies suggest a hyperpolarized (left) shift in activation and inactivation of sodium channels, when coupled with reduced efficacy of the Na-K-ATPase, can lead to stuttering in firing of neurons with pauses that last for seconds (Boucher et al. 2012; Yu et al. 2012). These studies raise the possibility that reduced excitability in motoneurons involves multiple mechanisms but shares the shift in sodium channel inaction seen in peripheral nerve and muscle.

Another possibility is that the mechanism underlying reduced motoneuron excitability is distinct from the one underlying reduced excitability of peripheral nerve and muscle. Striatal neurons normally fire in a stuttering pattern that is very similar to the pattern of firing of motoneurons from septic rats (Sciamanna and Wilson 2011). Stuttering of striatal neurons is...
dependent on the balance between persistent inward currents (PICs) and outward currents mediated by subthreshold voltage-activated K channels (Sciamma and Wilson 2011). Increasing the ratio of PICs to subthreshold K currents eliminated stuttering of striatal neurons. PICs are present in motoneurons from rats (Button et al. 2006; Dai and Jordan 2011; Hamm et al. 2010) and are under neuromodulatory control by monoamines, which play an important role in regulation of motoneuron excitability (Heckman et al. 2003, 2008; Powers and Binder 2001; Rekling et al. 2000). Changes in neuromodulatory drive could reduce PICs and diminish the ability of motoneurons to sustain firing. Further experiments will be necessary to distinguish between these and other mechanisms that could underlie reduced excitability of motoneurons in septic rats.

Implications of reduced motoneuron excitability. Motoneurons are the final common pathway through which the CNS encodes muscle force and its gradation. Motoneurons execute their role in modulating force output by converting the synaptic current delivered to the soma into a firing rate that determines muscle force output (Kernell 2006; Powers and Binder 2001). Our data suggest that this process is selectively disrupted by the reduction in motoneuron excitability triggered by sepsis. The fact that this change is restricted to a specific motoneuron function encourages the possibility of a selective treatment that normalizes firing. It is encouraging that the motoneuron is able to fire at least one normal action potential.

Motoneuron central excitability was significantly reduced within 24 h of induction of sepsis. If there is a similar rapid reduction in motoneuron excitability in patients, it might have implications for efforts to prevent ICUAW. The rapid loss of motoneuron excitability could trigger weaknesses before interventions have time to work. Reduced excitability of motoneurons might affect efforts to prevent later complications of critical illness such as muscle atrophy.

We have not determined whether reduced excitability of motoneurons is fully reversed on recovery from sepsis. If reduced excitability persists, it might be a contributing factor to the complaints of fatigue in patients who have recovered from sepsis (Cheung et al. 2006; Fletcher et al. 2003; Herridge et al. 2003). In rats, motoneurons were able to fire at normal frequencies for brief periods but unable to sustain firing rates. If this sort of defect in firing occurs in patients, it would manifest as the inability to sustain force and might be perceived as fatigue.

Septic encephalopathy is one of the most common and troubling complications of critical illness (Pytel and Alexander 2009). Despite a number of studies, the mechanism underlying septic encephalopathy remains a mystery (Pytel and Alexander 2009). Our findings raise the possibility that reduced excitability of neurons within the CNS might underlie septic encephalopathy. If this is the case, a single therapy to improve neuronal excitability might treat both ICUAW and septic encephalopathy.

ACKNOWLEDGMENTS

We thank Lori Goss for technical assistance.

REFERENCES


GRANTS

This work was supported by National Institute of Neurological Disorders and Stroke Grant P01-NS-057228 (M. M. Rich and T. C. Cope).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

P.N. and J.K. performed experiments; P.N., J.K., R.K.P., T.C.C., and M.M.R. analyzed data; P.N., J.K., R.K.P., T.C.C., and M.M.R. interpreted results of experiments; P.N., J.K., R.K.P., T.C.C., and M.M.R. prepared figures; P.N., J.K., R.K.P., T.C.C., and M.M.R. approved final version of manuscript; R.K.P., T.C.C., and M.M.R. conception and design of research; R.K.P., T.C.C., and M.M.R. drafted manuscript; R.K.P., T.C.C., and M.M.R. edited and revised manuscript.


