Inactivation of sodium channels underlies reversible neuropathy during critical illness in rats

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Neuropathy and myopathy can cause weakness during critical illness. To determine whether reduced excitability of peripheral nerves, rather than degeneration, is the mechanism underlying acute neuropathy in critically ill patients, we prospectively followed patients during the acute phase of critical illness and early recovery and assessed nerve conduction. During the period of early recovery from critical illness, patients recovered from neuropathy within days. This rapidly reversible neuropathy has not to our knowledge been previously described in critically ill patients and may be a novel type of neuropathy. In vivo intracellular recordings from dorsal root axons in septic rats revealed reduced action potential amplitude, demonstrating that reduced excitability of nerve was the mechanism underlying neuropathy. When action potentials were triggered by hyperpolarizing pulses, their amplitudes largely recovered, indicating that inactivation of sodium channels was an important contributor to reduced excitability. There was no depolarization of axon resting potential in septic rats, which ruled out a contribution of resting potential to the increased inactivation of sodium channels. Our data suggest that a hyperpolarized shift in the voltage dependence of sodium channel inactivation causes increased sodium inactivation and reduced excitability. Acquired sodium channelopathy may be the mechanism underlying acute neuropathy in critically ill patients.

Introduction

Weakness is a frequent neurologic complication of critical illness (1–4). Disorders of both muscle (myopathy) and nerve (neuropathy) cause weakness during critical illness. Myopathy is due to both structural abnormalities, which include loss of myosin thick filament and atrophy, and physiologic abnormalities caused by reduced excitability (5–7). It has appeared that neuropathy is accounted for by degeneration of axons (8–10) such that physiologic problems do not contribute. However, 2 studies suggest that physiologic problems may contribute to neuropathy in critically ill patients. First, sural nerve biopsy samples from patients with reduced sensory nerve response amplitudes are often normal (11). Second, in a study of nerve threshold electrotonus and current-threshold relationships in critically ill patients, nerve excitability was reduced (12). These studies suggest that reduction in nerve excitability, rather than degeneration of axons, may underlie weakness in some patients with neuropathy during critical illness.

The possibility that reduced axon excitability contributes to neuropathy has implications for both treatment and prognosis of patients with neuropathy during critical illness. We predicted that if a defect of excitability underlies neuropathy, recovery from the neuropathy would be rapid, since recovery from the defect in muscle excitability occurs rapidly following resolution of critical illness. In contrast, neuropathy caused by axon degeneration recovers slowly, since regrowth of axons is slow (13). To determine whether some critically ill patients develop rapidly reversible neuropathy, we prospectively followed patients and performed serial nerve conduction studies. Our studies suggest that there is what we believe to be a previously unrecognized syndrome of rapidly reversible neuropathy in critically ill patients. To determine the mechanisms underlying this neuropathy, we moved to a rat model of sepsis. In this model, neuropathy was also rapidly reversible, and data suggested that the mechanism underlying neuropathy was a hyperpolarized shift in the voltage dependence of sodium channel inactivation. We suggest acquired sodium channelopathy as a novel mechanism underlying the neuropathy present early in the course of critical illness.

Results

Patients with rapidly reversible neuropathy. To determine whether neuropathy that develops during critical illness (14–16) can be rapidly reversible, we prospectively followed patients during the acute phase of critical illness and early recovery. This was an extension of a previous study in which we followed patients during the development of neuromuscular dysfunction early in the course of critical illness (14). We enrolled 48 patients, 20 of whom were in the intensive care unit long enough to have undergone serials studies. Of these 20 patients, 9 developed neuropathy. Of the 9 patients with neuropathy, 5 died; the 4 survivors were followed until they had recovered from sepsis. The clinical features and longitudinal nerve conductions and electromyography (EMG) findings for 3 of the 4 survivors are presented in Table 1. In these 3 patients, sensory and motor nerve response amplitudes were either decreased at the time of the initial study (within 3 days of the onset of sepsis) or rapidly decreased during worsening of critical illness (Table 1), demonstrating rapid onset of neuropathy. EMG demonstrated the coexistence of myopathy in all 3 patients.

Conflict of interest: The authors have declared that no conflict of interest exists.

Nonstandard abbreviations used: CIM, critical illness myopathy; CIP, critical illness polyneuropathy; EMG, electromyography.

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Table 1
Patient nerve conduction amplitudes and EMG

Medical conditions	Nerve	Initial study	1 wk	2 wk	Final study[A]	EMG at 2 wk
Patient 1						
Urosepsis, ARDS, HIV, asthma	Sural	0	0	7	9	Myopathic[B]
	Median sensory	5	6	22	19	
	Peroneal motor	1.8	0.1	2.1	4	
	Median motor	4.7	5.0	11.4	10.2	
Patient 2						
Legionella pneumonia, sepsis, ATN	Sural	10	0	3	7	Myopathic[B]
	Median sensory	25	10	15	15	
	Peroneal motor	6.1	0.7	0.5	0.8	
	Median motor	10.0	5.3	4.0	6.7	
Patient 3						
Paraesophageal hernia repair, perioperative abscess, sepsis	Sural	0	4	8		Myopathic[B]
	Median sensory	19	21	25		
	Peroneal motor	0.3	0	0.7		
	Median motor	2.4	1.6	4.8		

[A]Week 12 in patient 1; week 3 in patient 2. The final study time for patient 3 was week 2. [B]Early recruitment of small motor units with positive spontaneous activity and increased insertional activity. Sural and median sensory amplitudes are in microvolts; peroneal and median motor responses are in millivolts. Normal values for nerve response amplitudes: sural, ≥6 μV; median sensory, ≥15 μV; peroneal motor, ≥2 mV; median motor, ≥4 mV. ARDS, acute respiratory distress syndrome; ATN, acute tubular necrosis.

During the early period of recovery from critical illness (between 1 and 2 weeks of illness for patients 1 and 3; and between 1 and 3 weeks for patient 2), both motor and sensory nerve response amplitudes increased (Table 1). Patient served as their own controls, and in none of the patients could the rapid recovery of nerve response amplitudes be ascribed to technical factors such as resolution of edema. Although recovery from myopathy likely contributed to the increases in motor amplitudes, the parallel increase in sensory amplitudes indicated that these patients had rapidly reversible neuropathy. The finding that neuropathy had largely resolved within days in 3 of 4 affected patients suggests that neuropathy may be rapidly reversible in a majority of affected patients. Rapid recovery from neuropathy in critically ill patients has not to our knowledge been previously described and implies a novel neuropathy that is distinct from classically described critical illness polyneuropathy (CIP), which is due to axon degeneration and causes long-term disability. To determine the mechanism underlying rapidly reversible neuropathy, we moved to the cecal ligation and puncture model of sepsis in rats.

Following induction of sepsis, rats develop a reversible neuropathy. It has previously been shown that induction of sepsis in rats by cecal ligation and puncture induces neuropathy (17, 18). We performed nerve conduction studies on 29 rats that survived for 3 days following induction of sepsis by cecal ligation and puncture. We previously diagnosed neuropathy in critically ill patients if nerve response amplitude dropped by 30% relative to baseline levels (14). On day 3 following induction of sepsis, tail nerve response amplitude in 8 of 29 rats was reduced by greater than 30% (Figure 1, A and B). The reduction in mean tail nerve amplitude for all 29 rats was statistically significant (from 220 ± 13 μV to 163 ± 13 μV, $P < 0.01$). In 5 untreated rats studied on days 0 and 3, there was no change in tail nerve response amplitude ($P = 0.6$). Distal latency was increased greater than 30% in 8 of 29 rats, and duration was increased by greater than 30% in 9 of 29 rats. Mean distal latency was increased by 15% ($P < 0.01$), and mean duration was increased by 20% ($P < 0.01$). Prolonged distal latencies and temporal dispersion of nerve responses have been reported in a subset of patients with CIP (19–21).

To determine whether the neuropathy induced by sepsis was rapidly reversible, sepsis was induced in a second set of 14 rats. Given the difficulty of achieving recovery in rats with severe sepsis, rats in which tail nerve response decreased by at least 20% following induction of sepsis were included in the study. Of the 14 rats made septic, 8 had a greater than 20% drop in tail nerve response and recovered from sepsis. By the time of recovery, 7 days following induction of sepsis, the mean tail nerve response amplitude had increased to the baseline level (Figure 1, C and D; $n = 8$, $P < 0.01$, day 7 vs. day 2). The rapid recovery of nerve response amplitude was similar in time and magnitude to that in the patients presented in Table 1 and suggested that a similar mechanism might underlie the neuropathy.

A potential cause of reversible reduction in nerve excitability in vivo is electrolyte abnormalities. To examine this possibility, we measured blood chemistry in 5 untreated rats and 5 septic rats with decreased tail nerve response amplitudes. There were no significant differences in any of the electrolytes measured (Table 2). This suggested that the reduction in nerve response amplitudes was due to an intrinsic problem with axon function.

To determine whether there was any contribution of structural problems such as demyelination or axon loss, both cross sections and longitudinal sections were made of the distal portions of rat sural sensory and tibial nerves 7 days after induction of sepsis. The 7-day time point was used because structural changes in axons after acute injury may take several days to develop (22). In rats with neuropathy as determined by electrophysiology on day 3, there was no evidence of demyelination or axon loss in nerve 7 days after induction of sepsis (Figure 2, A–E, $n = 3$ rats). At a shorter time point, 3 days after induction of sepsis, nerve was also morphologically normal (data not shown, $n = 2$ rats). While these data did not rule out axon loss or demyelination as contributors to neuropathy, it appeared likely that another mechanism predominated.

Reduction in the number of sodium channels available to open during action potentials contributes to reduced excitability of axons in septic rats. To study the mechanism underlying the reduction in tail nerve

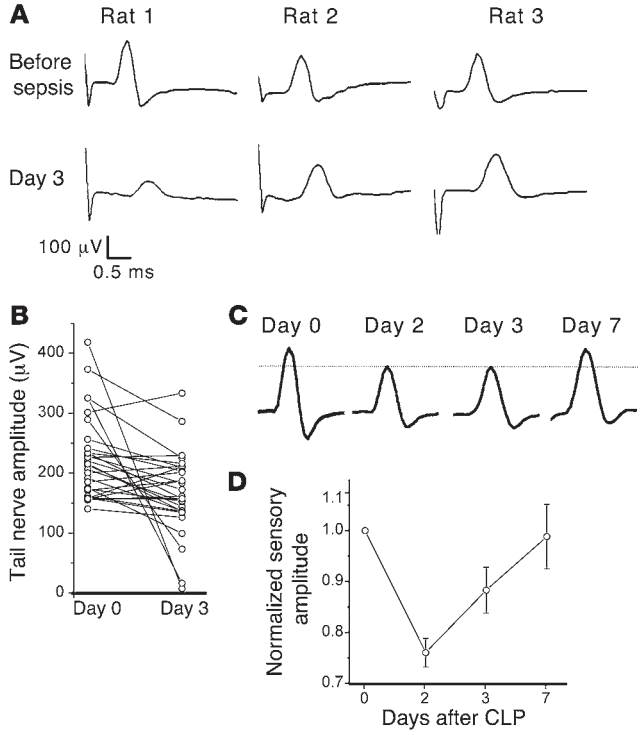

Figure 1
Rats develop reversible neuropathy following induction of sepsis. (**A**) Example of 3 tail nerve responses before and 3 days after cecal ligation and puncture to induce sepsis. There is a range of responses in the 3 rats. In rat 1, there was a 70% drop in amplitude and a substantial prolongation of distal latency. In rat 2, there was a 20% drop in amplitude and moderate prolongation of distal latency and duration. In rat 3, there was little effect on amplitude, but distal latency and duration were prolonged. (**B**) Paired scatter plot of tail nerve response amplitude before and after 3 days of sepsis for each of 29 rats ($P < 0.01$, paired Student's *t* test). (**C**) An example of the tail nerve response in a rat before, during, and after recovery from sepsis. The amplitude is reduced by 20% days 2 and 3 following cecal ligation and puncture but recovers by day 7. The dotted line is placed to aid in comparison of amplitudes. (**D**) A plot of the normalized average tail nerve response prior to cecal ligation and puncture (CLP), 2, 3, and 7 days later demonstrates the recovery of nerve amplitude as the rats ($n = 8$) recovered from sepsis. At day 2, the reduction in tail nerve amplitude is statistically significant relative to both the baseline and post-recovery amplitude ($P < 0.01$ for both). Data are presented as mean ± SEM.

response amplitudes, we obtained intracellular recordings from individual dorsal root sensory axons in untreated rats and in rats that had reduction of tail nerve amplitudes after 3 days of sepsis. In agreement with a previous study, action potentials did not consistently exceed 0 mV (23). The average axon action potential amplitude after 3 days of sepsis was reduced from 47.3 ± 1.7 mV in untreated rats to 32.9 ± 3.6 mV in septic rats ($P < 0.01$, $n = 60$ axons from untreated rats and 33 axons from septic rats; Figure 3 A and B). This established that reduced axon excitability contributed to neuropathy in septic rats.

Several mechanisms can reduce excitability of electrically active tissues (7, 24). One is decreased membrane resistance, which has been used experimentally to reduce excitability of neurons (25). We used input resistance as an indicator of membrane resistance and found that while input resistance was reduced in axons from septic rats, the difference was not statistically significant (7.2 ± 0.5 MΩ in untreated vs. 5.5 ± 0.3 MΩ in septic, $P = 0.06$). Since there was a trend toward lower input resistance in axons from septic rats, we further analyzed the contribution of input resistance to reduced action potential amplitude. As shown in Figure 3B, axons from untreated rats rarely had action potentials less than 30 mV in amplitude. Therefore, we classified axons from septic rats with action potential amplitudes of less than 30 mV as having small action potentials and axons with action potential amplitudes greater than 30 mV as having normal action potentials. There was no statistically significant difference in the input resistance of the subset of axons from septic rats with small action potential amplitudes and those with normal action potentials (5.8 ± 0.5 MΩ in axons with small action potentials vs. 5.4 ± 0.5 MΩ in axons with normal action potentials; $n = 15$ and 18 axons, respectively; $P = 0.53$). These data suggest that decreased membrane resistance is not an important contributor to reduced excitability of affected axons in septic rats.

A second mechanism that can reduce excitability is depolarization of the resting potential, which determines the degree of inactivation of sodium channels and thus controls the number of channels that are available to open during action potentials (26, 27). In muscle, depolarization of the resting potential underlies inexcitability in hyperkalemic periodic paralysis (28) and contributes to reduced excitability in the rat model of critical illness myopathy (CIM) (29, 30). We impaled dorsal root axons to determine the average resting potential and found values in both control and septic rats that were similar to values reported previously for dorsal root neurons (31, 32). The average resting potential in axons from septic rats was slightly more depolarized than in axons from untreated rats, but the difference was not statistically significant (–55.7 ± 1.0 mV in untreated vs. –52.6 ± 1.2 mV in septic, $P = 0.08$). Although this suggested that resting potential was not an important contributor to reduced excitability, we wished to determine whether excitability was decreased in axons from septic rats when resting potential was eliminated as a consideration. Action poten-

Table 2
Electrolytes in untreated and septic rats

	Untreated	Septic
Sodium	146.4 ± 2.1	150.6 ± 1.5
Potassium	6.3 ± 0.6	6.7 ± 0.5
Calcium	12.0 ± 0.4	11.3 ± 0.2
Glucose	198.4 ± 53.2	105.4 ± 7.0
Creatinine	0.28 ± 0.05	0.28 ± 0.05

Shown are the mean ± SEM of electrolyte values (in mM) for 5 untreated and 5 septic rats. None of the differences are statistically significant according to Student's *t* test. Blood glucose in 2 of 5 of the control rats was very high, and this resulted in a high mean value and a large standard error.

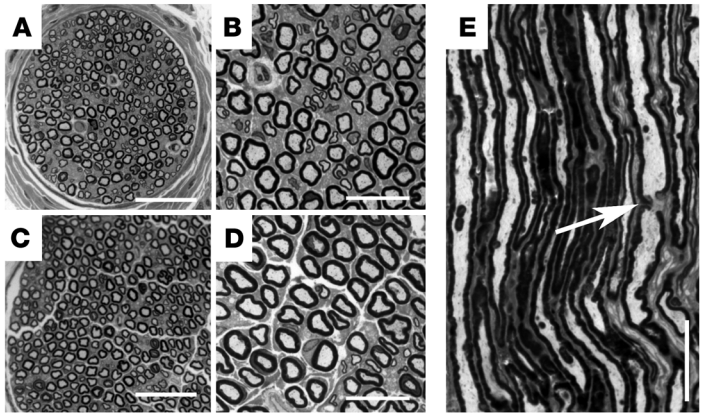

Figure 2
Nerve morphology is normal in septic rats with neuropathy. Toluidine blue–stained sural (**A** and **B**) and distal tibial (**C**–**E**) nerve sections from a rat 7 days after induction of sepsis. The nerves have normal axon morphology and myelination despite a 35% reduction in tail nerve amplitude on nerve conduction studies (on day 3 following sepsis) in the rat from which the nerves were harvested. **A**–**D** show 1-μm cross sections, and **E** shows a 1-μm longitudinal section. The arrow in **E** indicates a normal node of Ranvier. Scale bars: 50 μm (**A** and **C**); 20 μm (**B**, **D**, and **E**).

tial amplitudes were compared in selected axons from untreated and septic rats with resting potentials between –55 and –50 mV. When axons were matched by resting potentials (–52.9 ± 0.4 mV in untreated vs. –52.7 ± 0.4 mV in septic), the difference in action potential amplitude remained (46.7 ± 2.5 mV in untreated rats vs. 32.2 ± 3.0 mV in septic rats, $P < 0.01$; Figure 3B). This suggests that the slight depolarization of axonal resting potential in septic rats did not account for the reduction in action potential amplitude.

A third mechanism that can reduce excitability is a reduction is the number of sodium channels available to open during action potentials. The relative number of sodium channels can be estimated by measuring the maximal rate of rise of action potentials (dV/dt max) (33). The mean maximal rate of rise of dorsal root axon action potentials was 62.2 ± 2.5 mV/ms in untreated rats ($n = 6$ rats). In septic rats, the mean peak rate of rise decreased to 45.4 ± 6.7 mV/ms ($P < 0.05$ vs. untreated, $n = 4$ rats). The decrease indicates a reduction in the number of sodium channels able to participate in the upstroke of the action potential.

Increased inactivation of sodium channels contributes to the reduction in the number of channels available to open. A reduction in the number of sodium channels available to open during action potentials can be attributed to either a reduction in the number of channels present or inactivation of channels. Both reduced channel number and increased fast inactivation of channels occur in muscle in CIM (29, 30, 34). If reduction in channel number is the cause of reduced excitability, relief of sodium channel inactivation should not greatly increase action potential amplitude. This would result in axons with the smallest action potentials having the smallest increases in action potential amplitude following relief of inactivation. On the other hand, if channel number is normal but inactivation increases, axons with the smallest action potentials should have the largest increases in action potential amplitude following relief of inactivation.

To distinguish between reduced channel number and increased fast inactivation, we used anode break excitation. In dorsal root axons, action potentials can be triggered after termination of a hyperpolarizing pulse (anode break excitation) due to activation

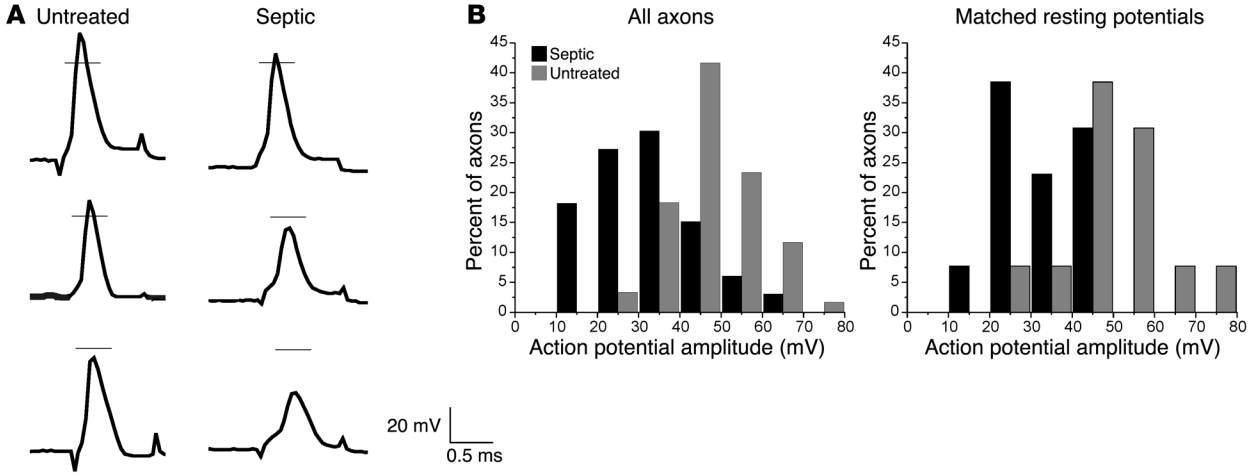

Figure 3
Amplitude of action potentials is reduced in dorsal root axons 3 days after induction of sepsis. (**A**) Representative dorsal root axon action potentials from untreated and septic rats, showing the range of amplitudes in each group. While the largest action potentials from axons in septic rats were normal in amplitude, half the axons had action potentials that were smaller than those normally found in untreated rats. The horizontal line represents 0 mV. In untreated rats, 53% of axons had action potentials that exceeded 0 mV. In septic rats only 27% of axons had action potentials that exceeded 0 mV. Scale bars: 20 mV (vertical); 0.5 ms (horizontal). (**B**) Left: Action potential amplitude for axons from all untreated and septic rats ($P < 0.01$ by the Kolmogorov-Smirnov test, $n = 60$ axons from untreated rats and 33 axons from septic rats). Right: Action potential amplitude when resting potentials were matched between –50 and –55 mV. The difference in action potential amplitudes is unchanged when resting potential is matched ($P < 0.01$, $n = 13$ axons from untreated rats and $n = 13$ axons from septic rats).

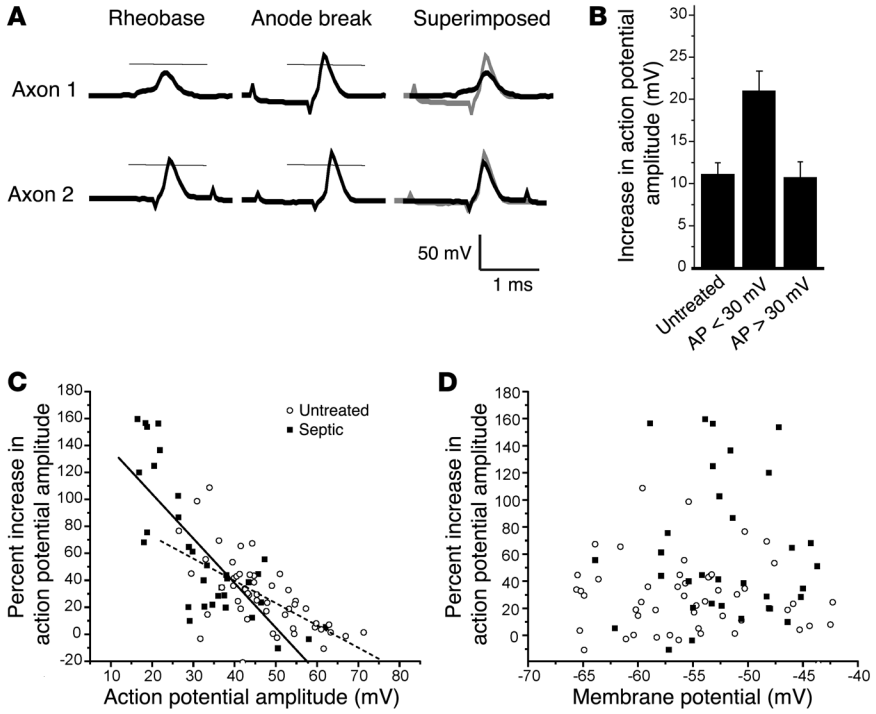

Figure 4

Increase in action potential amplitude following anode break excitation suggests that inactivation of sodium channels is an important contributor to reduced excitability. (**A**) Examples of depolarization-induced (rheobase) and anode break–induced action potentials from individual axons in septic rats 3 days following induction of sepsis. In axon 1, the small action potential following rheobase excitation gets much larger after anode break. In axon 2, the large action potential following rheobase excitation is only modestly larger following anode break. The horizontal line represents 0 mV. (**B**) Plot of the mean (±SEM) increase in action potential amplitude following anode break excitation for untreated and septic axons. The increase in action potential amplitude following anode break was greater in septic axons with small action potentials ($P < 0.01$ vs. both axons from untreated rats and axons from septic rats with normal action potentials; $n = 58$ normal action potentials [AP] from untreated rats, $n = 15$ small action potentials from septic rats, and $n = 18$ normal action potentials from septic rats). (**C**) Plot of the percent increase in action potential amplitude following anode break versus rheobase action potential amplitude. The increase in action potential amplitude following anode break is inversely correlated with rheobase action potential amplitude in septic rats ($r = –0.78$, $P < 0.01$, solid line). In control rats, there was also a relationship, but it was not as strong ($r = –0.63$, $P < 0.01$, dashed line). (**D**) The increase in action potential amplitude following anode break is not related to resting potential ($r = 0.04$, $P = 0.69$ for axons from septic rats).

of sodium channels during repolarization. During the hyperpolarized pulse, there is partial relief of sodium channel fast inactivation (35). We compared the amplitude of action potentials triggered by 1-ms depolarizing current pulses (rheobase excitation) with the amplitude of the action potentials triggered following 1-ms hyperpolarizing current pulses (anode break) in the same axon. Shown in Figure 4A are the action potentials triggered by rheobase and anode break excitation of 2 axons from septic rats. One axon had a small rheobase-triggered action potential, while that of the other axon was of normal amplitude. After anode break excitation, the difference in action potential amplitudes in the 2 axons was largely eliminated.

To quantitate the difference in response to anode break excitation, axons were categorized based on the amplitude of their action potentials, as was done for analysis of input resistance. In untreated axons and axons from septic rats with normal action potentials (>30 mV), the average increases in action potential amplitude following anode break excitation were 11.1 ± 3.3 and 10.8 ± 1.8 mV, respectively (Figure 4B). Since only 2 of 60 axons from untreated rats had small action potentials (<30 mV), meaningful analysis could not be performed on untreated axons with small action potentials. Fifteen of 33 axons from 4 septic rats had small action potentials, and in these axons, the average increase in action potential amplitude following anode break excitation was 21.0 ± 2.3 mV ($P < 0.01$ vs. both untreated axons and axons from septic rats with action potentials in the normal range; Figure 4B). To examine the contribution of sodium channel inactivation to reduced excitability in a different way, we plotted the percent increase in action potential amplitude following anode break versus rheobase action potential amplitude for both untreated and axons from septic rats. In septic rats, there was a significant negative correlation (Figure 4C, $r = –0.78$, $P < 0.01$). For axons from untreated rats, there was also a relationship, but it was not as steep ($r = –0.63$, $P < 0.01$). The finding that axons with small action potential amplitudes had greater increases in action potential amplitude after anode break suggests that inactivation of sodium channels, rather than reduced number, is the primary defect underlying reduced excitability.

Both resting potential and voltage dependence of sodium channel inactivation determine the percentage of sodium channels inactivated. The finding that sodium channel inactivation contributes to reduced excitability leads to the prediction that depolarization of the resting potential should decrease excitability. However, as shown in Figure 3, reduction in excitability appeared to be independent of resting potential. We reexamined the role of resting potential on excitability by determining whether it affected our indirect measure of sodium channel inactivation. The percent increase in action potential amplitude following anode break was plotted versus resting potential. We found no relationship between the increase in action potential amplitude following anode break and resting potential (Figure 4D, $r = 0.04$, $P = 0.69$). This is consistent with the data on excitability presented in Figure 3 and suggests that increased inactivation of sodium channels is not related to depolarization of the resting potential.

Discussion

The present findings indicate that the neuropathy present during the acute period of critical illness is due to abnormality in nerve excitability in advance of, or possibly without, actual nerve degeneration. The first indication that this is the case derived from our observation of a return toward normal nerve response amplitudes within days of their decline during the onset of acute

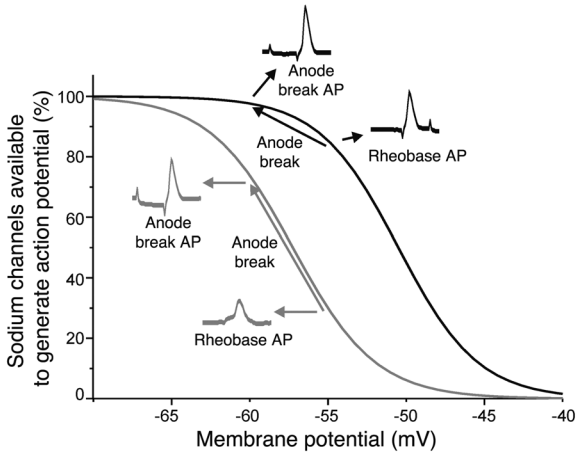

Figure 5

The effect of shifting the voltage dependence of sodium channel inactivation on action potentials triggered by both depolarizing and hyperpolarizing current injection. The black curve represents the hypothetical voltage dependence of sodium channel inactivation in an axon from an untreated rat. The sodium channel inactivation curve (gray) for an axon with reduced excitability from a septic rat is shifted to the left. In the septic axon, at a resting potential of –55 mV, only 30% of sodium channels are available to participate in generating an action potential. In the normal axon, at the same resting potential, 80% of sodium channels are available. The difference in percentage of sodium channels available leads to a small action potential in the axon from the septic rat (Rheobase AP, gray curve) and a large action potential in the normal axon (Rheobase AP, black curve). An anode break pulse hyperpolarizes each axon by 5 mV. In the axon from the septic rat, the resting potential is on the steep part of the inactivation curve, so a 5-mV hyperpolarization causes substantial relief of sodium channel inactivation and a large increase in action potential amplitude (Anode break AP, gray curve). In the normal axon, the resting potential is on the shallow part of the curve, so a 5 mV hyperpolarization does not greatly relieve inactivation and causes only a small increase in action potential amplitude (Anode break AP, black curve).

illness. Together with earlier data (12) (see Introduction), our finding that rapid reversal of decreases in nerve response amplitudes challenged assertions that nerve degeneration accounts for neuropathy in all critically ill patients. In order to test what other mechanism(s) might explain neuropathy, we studied nerve excitability in a rat model of sepsis. The model shared several features with critically ill patients, including abnormal evoked nerve responses in the absence of either widespread axon pathology or demyelination. This model enabled identification of reduced axonal excitability, probably occurring as a result of altered properties of sodium channel inactivation, i.e., the result of an acquired channelopathy. Acquired sodium channelopathy was similarly identified as the basis for the myopathy observed in critical illness and may, therefore, prove to be a common etiology in the nerve and muscle pathology observed in critical illness.

Reduced action potential amplitude in axons underlies reversible neuropathy in septic rats. It has previously been established in a rat model of sepsis that motor response amplitudes are rapidly reduced (17, 18). Here we report that the rat tail nerve (a mixed sensory and motor nerve) response amplitude was rapidly reduced after induction of sepsis. Similar to our finding in patients, the reduction in rat tail nerve response amplitude was rapidly reversible, and nerve morphology was normal. Intracellular recording from dorsal root axons in septic rats revealed reduced axon action potential amplitudes. Although all axons studied could generate action potentials at the site of current injection in the dorsal root, it is likely that some axons with small action potentials could not support conduction of action potentials along the length of the axon (36). Failure of conduction would result in reduction in nerve response amplitudes, since, in this type of recording, responses are measured from the nerve several centimeters away from the site of stimulation. The rapid recovery of nerve response amplitudes is consistent with recovery of ion channel function that appears to occur over days.

The mechanism underlying reduced action potential amplitude. Our data suggest that abnormalities of neither passive membrane properties nor resting potential account for the reduction in nerve excitability. Partial relief of sodium channel inactivation by hyperpolarized anode break stimulation largely corrected the reduction in action potential amplitude. Figure 5 explains how a hyperpolarized shift in the voltage dependence of sodium channel inactivation in axons from septic rats might account for data obtained after both rheobase (depolarizing) and anode break (hyperpolarizing) stimulation. Plotted in Figure 5 are the hypothetical voltage dependences of sodium channel inactivation in a normal axon and an axon from a septic rat. Both axons have a resting potential of –55 mV (the approximate average resting potential of axons in our study). In the axon from the septic rat, the majority of sodium channels are inactivated at the resting potential of –55 mV, so the rheobase action potential is small. In the axon from the untreated rat, the voltage dependence of sodium channel inactivation is positioned such that only a minority of sodium channels are inactivated and the rheobase action potential is of normal amplitude. During anode break excitation, each axon is hyperpolarized by 5 mV. In the axon from the septic rat, the resting potential is on the steep part of the inactivation curve, such that a 5-mV hyperpolarization relieves a substantial percentage of sodium channel inactivation, and the action potential amplitude following anode break is markedly increased. In the axon from the untreated rat, the resting potential is on the shallow part of the inactivation curve, such that there is little additional relief of sodium channel inactivation by a 5-mV hyperpolarization, and action potential amplitude is only slightly increased. Thus a hyperpolarized shift in sodium channel inactivation in axons from septic rats could result in small rheobase action potentials that get much larger with anode break excitation.

Our conclusion that a hyperpolarized shift in the voltage dependence of sodium channel inactivation is the cause of reduced excitability differs from that of a study reporting reduced nerve excitability in patients with CIP (12). Z'Graggen et al. used nerve threshold electrotonus and current-threshold relationships to study excitability. Although resting potential cannot be directly determined using nerve threshold measurements, the authors speculated that raised extracellular potassium and depolarization of the resting potential were likely contributors to increased sodium channel inactivation and reduced excitability of axons in patients. Our data ruled out depolarization of resting potential as a significant contributor to reduced excitability of axons in septic rats. An alternate interpretation of the findings of Z'Graggen et al. is that a hyperpolarized shift in the voltage dependence of inactivation increases sodium channel inactivation. The increase in inactivation could induce changes in threshold electrotonus and current-threshold relationships independent of depolarization of resting potential.

Mechanisms that might underlie reduction of sodium current. As outlined in Figure 5, a hyperpolarized shift in the voltage dependence of sodium channel inactivation may underlie the reduction in sodium current. One way that voltage dependence of sodium current might be shifted is a change in sodium channel isoform expression (37, 38). Alternatively, there might be modification of gating of an individual sodium channel isoform. Potential mechanisms that can modify gating of individual sodium channel isoforms include secondary modifications of sodium channels through known molecular mechanisms such as glycosylation (39) or phosphorylation (40–42). There are also processes that strongly regulate the voltage dependence of sodium channel inactivation in living cells, where the molecular mechanism remains unknown (43, 44).

Although a change in the voltage dependence of sodium channel gating could account for our findings, we cannot rule out a contribution of other factors that might alter the density of functional sodium channels present in axons of septic rats. For example, phosphorylation of sodium channels in axons by kinases activated by injury may reduce sodium current amplitude (45). It is also possible that block of sodium channels by a soluble factor released during sepsis underlies the reduction in excitability. Some factors that block sodium channels (e.g., phenytoin) are use-dependent, and block by these factors could be relieved by hyperpolarization during anode break stimulation (46, 47). To definitively determine the relative importance of reduction in the number of functional channels versus a hyperpolarized shift in the voltage dependence of inactivation, it would be necessary to voltage clamp axons. This cannot currently be done on axons in vivo.

The relationship between reduced nerve excitability and CIP. CIP causes long-term disability in a subset of patients due to degeneration of axons (2, 10, 48). Our finding that reduced excitability of axons may underlie the majority of acute neuropathy in critically ill patients suggests that prognosis for recovery may be good in most patients. It also redirects research toward mechanisms regulating axon excitability and raises the possibility that therapy to enhance axon excitability may prevent acute neuropathy in critically ill patients.

It is not known whether reduced excitability of nerve puts patients at risk for development of axon degeneration and long-term disability. It is clear from our current study that some axons in both patients and rats recover excitability without degenerating. It could be that there are 2 unrelated processes occurring in patients, one that causes axon degeneration and one that causes reduced axon excitability. Alternatively, reduction of excitability and death of axons may be due to a single process and are differentiated only by severity. Patients who develop axon degeneration and long-term CIP generally require treatment that includes intubation, antibiotics, and blood pressure support. The septic rats in our study survived with administration of only subcutaneous fluids. The difference in severity of sepsis between the rats in our study and patients with CIP may account for the lack of axon degeneration in the rats.

Acquired sodium channelopathy may underlie both neuropathy and myopathy in critically ill patients. We have previously reported that reduced excitability of muscle fibers underlies CIM (49–51). As in our present study in nerves, in CIM neither changes in passive membrane properties nor depolarization of resting potential could account for the reduction in excitability (29, 30). In the CIM studies, we used voltage clamp techniques to directly study sodium currents in isolated muscle fibers and found that reduced numbers of channels did not fully account for the differences in excitability between severely affected and mildly affected muscle fibers (29). Instead, a hyperpolarized shift in the voltage dependence of sodium channel inactivation was the principal cause of reduced excitability (29, 30, 34). The hyperpolarized shift increased inactivation of sodium channels and reduced the number of channels available to open during action potentials. The hyperpolarized shift in inactivation of sodium channels in CIM is primarily due to altered gating of the $Na_v1.4$ sodium channel isoform (34). However, the $Na_v1.5$ sodium channel isoform is also expressed in muscle in CIM and appears to be similarly affected (34, 52). Thus, hyperpolarized inactivation of both $Na_v1.4$ and $Na_v1.5$ contributes to reduced excitability in CIM.

Our current study suggests that gating of sodium channels expressed in dorsal root axons is affected similarly to gating of $Na_v1.4$ and $Na_v1.5$ in skeletal muscle in CIM. In dorsal root axons, $Na_v1.6$ as well as other sodium channel isoforms are expressed (53, 54). Combining the current study of neuropathy with our previous work on CIM, we hypothesize that acquired sodium channelopathy in critical illness affects multiple sodium channel isoforms in both nerve and muscle.

If CIM and acute neuropathy have a common mechanism (acquired sodium channelopathy), one might expect them to frequently coexist. This appears to be the case. Three of 4 surviving patients in this study as well as critically ill patients from other studies appear to have both neuropathy and CIM (14, 55–57). The frequent coexistence of neuropathy and CIM led one investigator to term the syndrome neuromyopathy (56, 57). We proposed that neuromyopathy is due to an acquired sodium channelopathy occurring in both peripheral nerve and skeletal muscle.

In summary, data from both critically ill patients and a rat model of sepsis suggest that reversible reduction of nerve excitability underlies the neuropathy present in acutely ill patients. Data from the rat model of sepsis suggest that reduced excitability of nerve is due to a hyperpolarized shift in the voltage dependence of sodium channel inactivation. These data suggest that the neuropathy present during the acute phase of critical illness is a novel type of neuropathy in which the underlying mechanism is an acquired sodium channelopathy.

Methods

Nerve conductions in critically ill patients with sepsis. The Institutional Review Board of Emory University gave approval for this study, and each patient or legally authorized representative provided informed written consent. Subjects with a diagnosis of severe sepsis were prospectively enrolled from the medical intensive care units at 2 participating institutions: Emory University Hospital and Grady Healthcare System (Atlanta, Georgia, USA). The criteria for severe sepsis from the recombinant human activated protein C trial were used to identify patients (58), who were assessed clinically and underwent weekly nerve conductions and EMG as described previously (14).

Cecal ligation and nerve conductions in rats. All animal procedures were approved by the Laboratory Animal Care and Use Committee of Wright State University. Prior to cecal ligation, female Wistar rats weighing 250–300 g were anesthetized with isoflurane and were subjected to nerve conduction experiments using a Nicolet Viking nerve conduction machine. The mixed nerve response of the tail nerve was recorded using subdermal recording electrodes inserted at the base of the tail, 1 cm apart along the length of the tail. A subdermal ground electrode was placed 2 cm distal to the active recording electrode. Two subdermal electrodes were used to stimulate the tail nerve 2 cm distal to the ground electrode. Before removal of the electrodes, a permanent black marker was used to mark the location

of the electrodes. This allowed for identical placement of the electrodes during nerve conductions, which were repeated 2–4 times. Temperature was monitored using a rectal thermometer and was maintained within 1 degree of 37°C using a heating blanket over the body and tail. Tail temperature was not directly measured.

After completion of the initial nerve conduction studies, 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. This caused sepsis that resulted in mortality of approximately 30% of rats. Most rats appeared ill within 24 hours of cecal ligation and puncture. Signs of illness included lethargy, lack of grooming, piloerection of fur, and pica. We found that unless rats were made very ill by sepsis, there were no changes on nerve conduction studies. For continuous relief of pain, an Alzet 2-ml osmotic pump (DURECT) that delivered 30 μg/kg/h of oxymorphone 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 oxymorphone took effect. Starting on day 1, rats were given a daily injection of 4 ml sterile saline subcutaneously in the back. For the initial study, 29 rats were studied on days 0 and 3. For the study of recovery from neuropathy, 8 rats were studied on days 2, 3, and 7 after the surgery. For repeat nerve conduction studies, rats were reanesthetized with isoflurane, and the nerve conductions were repeated.

Recording of action potentials from dorsal root axons in vivo. In terminal experiments, anesthesia was induced in a closed chamber and maintained via a tracheal cannula throughout the initial dissection with a gaseous mixture of isoflurane (2%–3%) in O_2. Artificial respiration was adjusted to hold end-tidal CO_2 between 3% and 4%. Temperature was monitored rectally and maintained between 37°C and 38°C via radiant heat, and animals were positioned in a stereotaxic recording frame. The lumbosacral enlargement was exposed via a laminectomy from L3 to S1 to provide access to dorsal roots associated with the sciatic nerve.

Records from sensory axons were obtained by advancing the tips of glass micropipettes (2 M K-acetate, 30–35 MΩ) into L5 dorsal roots, which were supported in continuity on bipolar silver hooks (59). When membrane potential exceeded 40 mV, 1-ms current pulses were injected at the rate of 1 pulse per second. We varied depolarizing current in 0.1-nA increments to determine axonal rheobase current (the current that triggered action potentials 100% of the time); we injected hyperpolarizing current (1–3 nA) to determine input resistance and to evoke action potentials at anode break. Records of intra-axonal membrane potential and current pulses were amplified (×100), filtered (0–10 kHz), digitized (20 kHz), stored on computer, and later analyzed using Spike2 software (version 5.14; CED). Recordings of 10 sensory axons were taken for each rat. The recording was always complete within 1 hour of the initial impalement. In 2 of the 4 septic rats studied, 5 and 8 axons were recorded before the rat died of complications dues to sepsis. At the end of the experiment, animals were euthanized by using a lethal dose of supersaturated KCl.

To determine whether intra-axonal measures of excitability were affected by oxymorphone treatment or a nonspecific effect of the surgery used to induce sepsis, we recorded action potential amplitude from 2 rats in which only mild sepsis was induced following cecal ligation and puncture. Both rats were eating and moving normally, and neither showed any signs of acute distress. In both rats, axon excitability was normal (mean action potential amplitude was 45.3 ± 2.9 mV, n = 20 axons). This was very similar to the action potential amplitude of 47.3 ± 1.7 mV in untreated rats and suggested that neither oxymorphone nor nonspecific effects of surgery account for the reduction in excitability of axons in severely septic rats.

Measurements of action potential threshold and peak were made by manually placing cursors at the point of inflection on the rising phase of the action potential and the peak of the action potential. Action potential amplitude was calculated by subtracting the threshold voltage from the peak voltage. To determine the maximal rate of rise of the action potential (dV/dt max), the first derivative of the action potential was taken using Spike2 software. We measured the maximal amplitude of the first derivative by manually placing cursors at the baseline and peak of the first derivative trace for the action potential.

Pathology and electrolytes. Rats were euthanized with CO_2, and the entire hind limb was separated from the animal and immersion fixed for at least 7 days in phosphate buffered 4% glutaraldehyde, pH 7.4. Sural (sensory) and tibial (motor and sensory) nerves were dissected and embedded in Epon resin. One-micrometer cross and longitudinal sections were stained with toluidine blue for light microscopic analysis, as previously described (60). Each nerve was examined at 2 separate levels for evidence of pathology.

For measurement of electrolytes, 1 ml of blood was drawn by cardiac ventricular puncture at the time of the terminal experiment. Electrolytes were measured using a VetScan Chemistry Analyzer (Abaxis).

Statistics. For comparisons of rat tail nerve responses and compound muscle action potentials before and after sepsis, paired 2-tailed Student's t tests were used, with Bonferroni correction for multiple comparisons. For comparisons of electrolyte values between untreated and septic rats, unpaired 2-tailed Student's t tests with Bonferroni correction were used. For comparisons of characteristics of axon properties between septic and untreated rats, the Kolmogorov-Smirnov test was used. A P value less than 0.05 was considered significant.

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