

Mechanisms of Secondary Injury to Spinal Cord Axons *In Vitro*: Role of Na^+ , $\text{Na}^+-\text{K}^+-\text{ATPase}$, the Na^+-H^+ Exchanger, and the $\text{Na}^+-\text{Ca}^{2+}$ Exchanger

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There is evidence that intracellular Na^+ entry potentiates hypoxic–ischemic cell death by causing cytotoxic cell edema, intracellular acidosis, and gating of Ca^{2+} entry by reverse activation of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger. In this study, we examined the role of Na^+ in mediating traumatic injury to spinal cord axons. Dorsal column segments from adult rats ($n = 87$) were isolated and maintained in an *in vitro* recording chamber while being superfused with oxygenated Ringer's solution (95% $\text{O}_2/5\%$ CO_2 , 25°C). Selected experiments ($n = 10$) also were done at 33°C . Compound action potentials (CAP) were recorded from microelectrodes. Injury was performed by compression of the dorsal column segment for 15 sec with a modified aneurysm clip exerting a closing force of 2 gm. With injury, the CAP decreased to $72.1 \pm 9.6\%$ of baseline values. Removal of extracellular Na^+ and replacement with the impermeant cation *N*-methyl-D-glucamine enhanced recovery of the CAP to $98.3 \pm 18.3\%$ ($p < 0.05$) of baseline. The Na^+ channel blockers tetrodotoxin and procaine also improved recovery of the CAP to $96.3 \pm 23.7\%$ ($p < 0.05$) and $82.8 \pm 4.6\%$ ($p < 0.05$) of baseline values, respectively. In contrast, increasing Na^+ permeability with veratridine resulted in greater attenuation of CAP amplitude after 1 hr of trauma ($60.1 \pm 8.4\%$, $p < 0.05$). Similarly,

prevention of extrusion of Na^+ from the intracellular compartment by inhibiting the $\text{Na}^+-\text{K}^+-\text{ATPase}$ pump with ouabain resulted in greater attenuation of CAP amplitude at 1 hr after trauma ($56.7 \pm 3.6\%$, $p < 0.05$). The Na^+-H^+ exchange blockers amiloride ($100 \mu\text{M}$) and harmaline ($100 \mu\text{M}$) significantly improved recovery after injury to $89.6 \pm 17.0\%$ ($p < 0.05$) and $85.7 \pm 7.2\%$ ($p < 0.05$) of baseline, respectively. However, administration of the $\text{Na}^+-\text{Ca}^{2+}$ exchange blockers benzamil (100 or $500 \mu\text{M}$) and bepridil ($50 \mu\text{M}$) was ineffective. In summary, reduction of extracellular Na^+ confers neuroprotection after spinal cord injury *in vitro*. Intracellular sodium rises appear to be mediated by voltage-gated Na^+ channels. Blockade of the Na^+-H^+ exchanger also is neuroprotective, possibly by reducing intracellular acidosis. Furthermore, prevention of extrusion of intracellular Na^+ by the $\text{Na}^+-\text{K}^+-\text{ATPase}$ pump exacerbates the effects of compression trauma. However, reverse operation of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger does not explain the injurious effects of Na^+ in traumatically injured CNS white matter.

Key words: rat; spinal cord injury; sodium channel; tetrodotoxin; procaine; $\text{Na}^+-\text{K}^+-\text{ATPase}$; Na^+-H^+ exchanger; $\text{Na}^+-\text{Ca}^{2+}$ exchanger

The pathophysiology of spinal cord injury involves a primary mechanical injury and a delayed secondary injury attributable to a number of proposed mechanisms including ischemia, abnormal intracellular shifts of ions including Na^+ and Ca^{2+} , free radical-associated lipid peroxidation of cell membranes, and excitotoxic cell death (Fehlings et al., 1989; Regan and Choi, 1991; Tator and Fehlings, 1991). Importantly, the mechanisms of axonal injury may differ from those operating in gray matter (Bengtsson and Siesjo, 1990; Ransom et al., 1990). For example, axons in spinal cord white matter lack both receptor-coupled and voltage-sensitive calcium channels (Waxman, 1991; Stys et al., 1992a), in contrast to neuronal somata.

Recent work suggests that the rise in intracellular calcium level ($[\text{Ca}^{2+}]_i$) occurring during ischemia and hypoxia is driven in large part by an increase in $[\text{Na}^+]_i$ and is mediated by “reverse mode” of $\text{Na}^+-\text{Ca}^{2+}$ exchange (Haigney et al., 1992; Stys et al., 1992a;

Ziegelstein et al., 1992). In the present study, we sought to clarify the role of Na^+ in mediating secondary injury to axons after compression spinal cord injury. Potential avenues for the increase in $[\text{Na}^+]_i$ in traumatic spinal cord white matter and/or axons include a nonspecific leak of extracellular Na^+ through the disrupted myelin, failure to extrude Na^+ by the $\text{Na}^+-\text{K}^+-\text{ATPase}$ pump, and entry through Na^+-H^+ exchanger or through voltage-gated Na^+ channels. In pathological conditions, the Na^+-H^+ exchanger has been the subject of numerous investigations in different models. Several investigators have shown that amiloride, a Na^+-H^+ exchange blocker, and its derivatives reduce intracellular Na^+ and Ca^{2+} loading and improve postischemic or post-hypoxic recovery of myocardiocytes (Karmazyn, 1988; Weiss et al., 1990; Murphy et al., 1991). However, amiloride-type agents have many mechanisms of action including blockade of the Na^+-H^+ exchanger, voltage-dependent Na^+ channels (Tytgat et al., 1990; Imai et al., 1991), and the $\text{Na}^+-\text{K}^+-\text{ATPase}$ pump (Floresani and Luciani, 1984). Recently, Stys et al. (1992a) reported that the $\text{Na}^+-\text{Ca}^{2+}$ exchange blockers benzamil and bepridil attenuated hypoxic injury to optic nerve axons *in vitro*.

In the present study, we report evidence suggesting that traumatic injury to spinal cord axons depends largely on a persistent membrane Na^+ conductance which, in turn, allows intracellular Na^+ to rise sufficiently to promote operation of the Na^+-H^+

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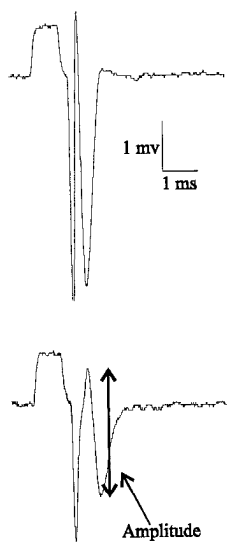
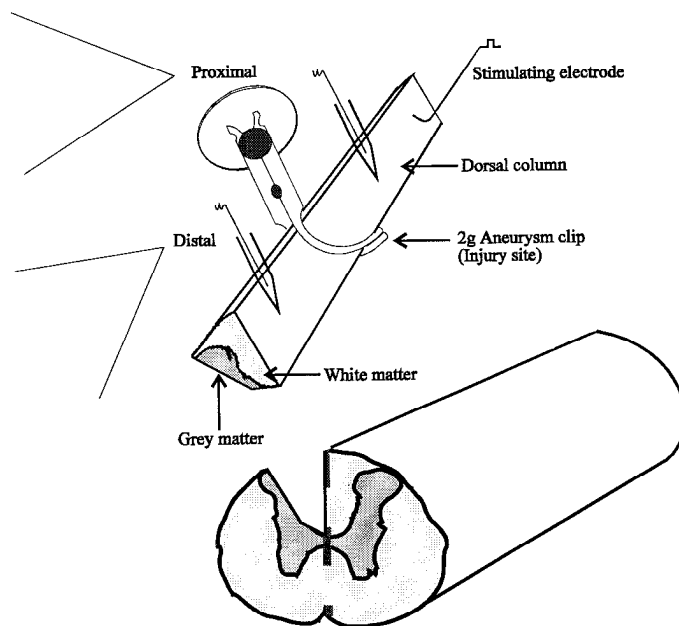


Figure 1. The experimental preparation, including recording setup, method of analysis of compound action potential waveforms, and schematic representation of dorsal column strip, is represented (see Materials and Methods for details) (calibration pulse, 1 msec at 1 mV). Injury was performed by compression of the dorsal column segment *in vitro* with a modified aneurysm clip exerting a closing force of 2 gm. CAPs were recorded at two points (proximal and distal) relative to the site of injury.



exchanger. However, this does not appear to be associated with “reverse operation” of the Na^+ – Ca^{2+} exchanger. Pharmacological blockade of voltage-gated Na^+ channels and inhibition of the Na^+ – H^+ exchanger are neuroprotective after spinal cord injury. Furthermore, increasing intracellular Na^+ with veratridine or preventing Na^+ extrusion by inhibiting Na^+ – K^+ –ATPase activity with ouabain exacerbates traumatic axonal injury.

A preliminary report of this work has been published previously (Agrawal et al., 1994).

MATERIALS AND METHODS

Experimental preparation. Experiments were performed on 87 adult male Wistar rats (250–350 gm) ranging in age from 4 to 11 months. After anesthetizing the rat with sodium pentobarbital (40 mg/kg, i.p.), a laminectomy was performed between T3 and T10 to expose the spinal cord. A 25 mm section of cord was removed rapidly and placed in cold (4–7°C) Ringer’s solution. The spinal cord segment was hemisected, and the dorsal column was sectioned longitudinally with microscissors as schematically shown in Figure 1. The dorsal column segment was pinned in an *in vitro* recording chamber and was perfused constantly (2–5 ml/min drip rate) with Ringer’s solution bubbled with 95% O_2 /5% CO_2 . The bath medium temperature was maintained at $25 \pm 0.5^\circ\text{C}$ with a microprocessor-controlled thermistor (Omega CN9000, Omega Engineering, Stamford, CT).

A bipolar platinum wire stimulating electrode was placed on one end of the dorsal column segment and delivered a 100 μsec constant current pulse at a supramaximal stimulus intensity, which was 50% greater than that required to elicit a maximal response. The responses were recorded extracellularly by two glass microelectrodes (2–4 μm tip, 5–10 M Ω resistance) filled with 150 mM KCl. The signals were amplified 100 \times (Axoprobe-1A, Axon Instruments, Foster City, CA), digitized (ISC-16 A/D converter, R.C. Electronics, San Diego, CA) at 12 bit resolution, and stored on a microcomputer and VCR. Each sweep of recording had a duration of 8 msec and was digitized to 512 points (i.e., sampling rate of 64 kHz).

Experimental protocol. The dorsal column segment was allowed to stabilize for 90 min after dissection before starting the experiment. A set of recordings was obtained consisting of 100 sweeps at 0.2 Hz. After the control set of recordings was obtained, the drug- or ion-substituted solutions were infused 15 min before injury. Then the dorsal column segment was injured (Fig. 1) between the proximal and distal recording sites for 15 sec with a 2 gm modified aneurysm clip (1 mm wide) (Dolan and Tator, 1979; Fehlings and Nashmi, 1995). The effects of drug infusion were studied for at least 20 min after injury and then substituted with

Ringer’s solution. Response recovery was monitored for at least 2 hr after injury. The same set of control experiments was used for all groups.

Solutions and drugs. The perfused solutions were bubbled with 95% O_2 /5% CO_2 , and the composition of solutions were (in mM): (1) Ringer’s solution: NaCl 124, KCl 3, Na_2HPO_4 1, NaHCO_3 26, MgSO_4 1.5, CaCl_2 1.5, and glucose 10; (2) zero sodium [substituted with the impermeant cation *N*-methyl-D-glucamine (NMDG $^+$): NMDG $^+$ 150, KCl 3, glucose 10, MgSO_4 1.5, KH_2PO_4 1.5, and CaCl_2 1.5; and (3) HEPES 5 bubbled with 100% O_2 , pH-adjusted to 7.4. Tetrodotoxin (TTX) (10 nM, Sigma, St Louis, MO), procaine hydrochloride (1 mM, Sterling-Winthrop, Collegeville, PA), amiloride hydrochloride (100 μM , Research Biochemicals, Natick, MA), harmaline hydrochloride (100 μM , Sigma), ouabain (25 μM , Sigma), benzamil hydrochloride (100 and 500 μM , Sigma), and bepridil (50 μM , Research Biochemicals) first were aliquoted in distilled water and then dissolved in Ringer’s solution. Amiloride, benzamil, and bepridil were dissolved in distilled water at 60°C and, after being allowed to cool, were added to Ringer’s solution.

Data analysis and statistics. Peak-to-peak amplitude of the individual CAP was analyzed by computer (Fehlings and Nashmi, 1995) after completion of the experiment (Fig. 1). All data were expressed as mean \pm SD. Significant differences in amplitude ($p < 0.05$) between control (Ringer’s solution) and treatment CAPs at a particular time point of the experiment were determined by two-way analysis of variance (ANOVA) with post hoc analysis by the Student–Neuman–Keuls test.

RESULTS

Removal of extracellular Na^+ protects against compression injury

Experiments were performed to determine whether reduction of influx of Na^+ ions protects against compression injury to dorsal column white matter. The dorsal column was injured between the proximal and distal recording sites for 15 sec with a modified aneurysm clip. The effects of drug infusion were studied for at least 20 min after injury and then substituted with Ringer’s solution. The Na^+ in the perfusing solution was replaced in an equimolar manner with NMDG $^+$, a cation that does not permeate the Na^+ channel (Friedman and Haddad, 1994). The zero sodium solution was infused 15 min before the 2 gm clip compression injury and continued until 20 min after injury. Recovery was observed for 2 hr after trauma. During infusion of NMDG $^+$, action potential conduction ceased (Fig. 2e,g) but returned promptly during washout with Ringer’s (Fig. 2f,g). As shown in

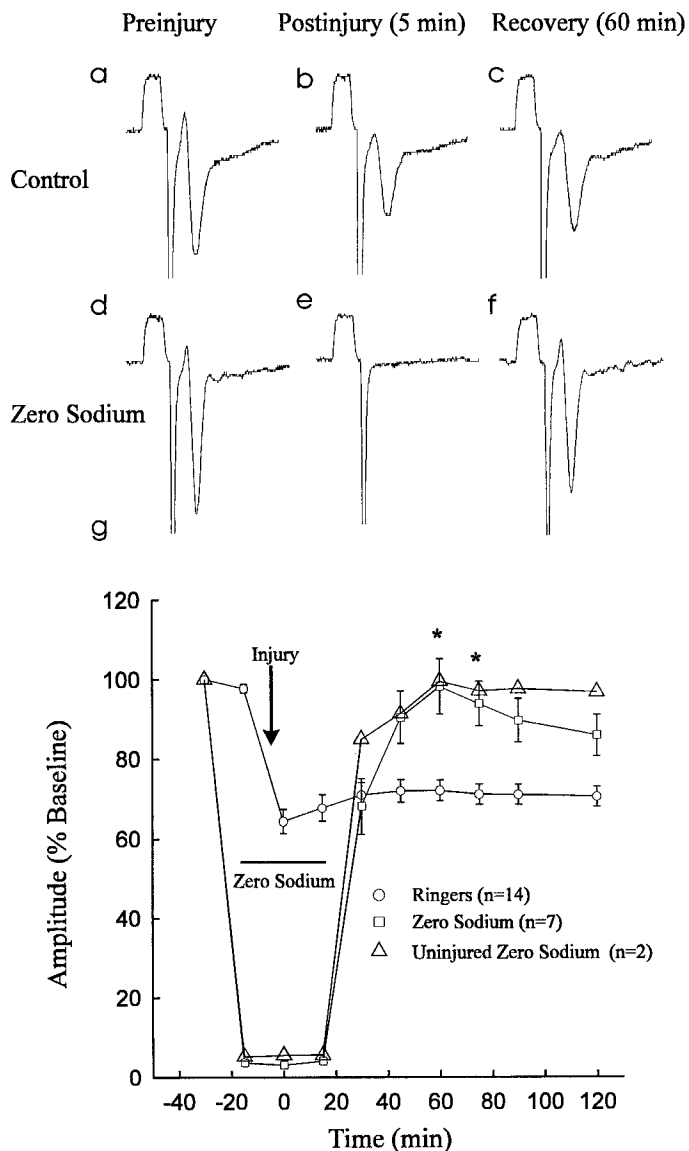


Figure 2. Effect of removing Na^+ from the extracellular perfusing solution (equimolar substitution with NMDG^+) on CAP recovery after 2 gm clip compression injury. Perfusion of test solution was started 15 min before injury and continued for 20 min after injury. Typical CAP waveform before injury, after injury, and at 60 min after injury in the control (Ringer's solution) group (*a–c*). A typical CAP waveform before injury, after injury while being perfused with zero sodium solution, and at 60 min after injury in normal Ringer's solution (*d–f*). Graph of normalized CAP amplitude versus time for the control and zero sodium groups (*g*) (significant differences at $p < 0.05$ depicted by asterisks). As an additional control, the effect of zero sodium solution on an uninjured dorsal column preparation ($n = 2$) is shown.

Figure 2, the CAP amplitude was significantly greater ($p < 0.05$) in the NMDG^+ -treated group ($98.3 \pm 18.3\%$ of baseline; $n = 7$) compared with the Ringer's solution group ($72.1 \pm 9.6\%$ of baseline; $n = 14$) at 1 hr after injury. After 60 min, there was a gradual decay of CAP amplitude in the NMDG^+ group, possibly because of other cytotoxic events. As an additional control, the effect of zero sodium solution on the uninjured dorsal column segment ($n = 2$; Fig. 2*g*) was observed. During infusion of NMDG^+ -substituted Ringer's solution, action potential conduction ceased. During washout, CAP amplitude returned to baseline levels and remained stable. Representative waveforms are de-

picted in Figure 2*a–f*; summary data with statistical analyses are shown in Figure 2*g*.

Na^+ channel blockade attenuates traumatic spinal cord axonal injury

To test whether voltage-gated Na^+ channels are involved in the mechanism of compression spinal cord white matter injury, the effects of TTX and procaine were examined. TTX (10 nM), a selective Na^+ channel antagonist, was infused for 15 min before the 2 gm clip compression injury and continued until 20 min after injury. Recovery of CAP was observed for 2 hr after injury. With 10 nM TTX ($n = 6$), a dose sufficiently small to maintain action potential propagation in the dorsal column segment, the recovery of the CAP was significantly improved ($p < 0.05$; $96.3 \pm 23.7\%$ of baseline) compared with Ringer's solution ($72.1 \pm 9.6\%$ of baseline; $n = 14$) at 60 min after injury. Figure 3*a* illustrates the

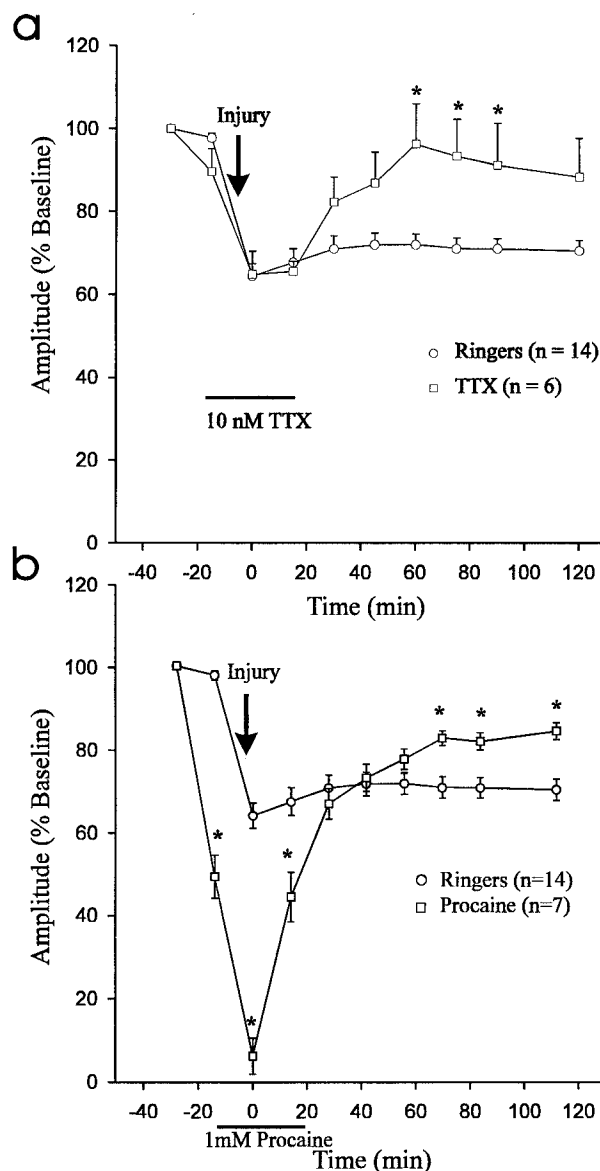


Figure 3. Effect of TTX (10 nM) and procaine (1 mM) on CAP recovery after 2 gm clip compression injury. Perfusion of test solutions was started 15 min before injury and continued for 20 min after injury. *a*, Graph of normalized CAP amplitude versus time for the control and TTX groups. *b*, Graph of normalized CAP amplitude versus time for the control and procaine groups (significant differences at $p < 0.05$ depicted by asterisks).

recovery curves of CAP amplitude in the Ringer's solution- and TTX-treated groups.

Infusion of procaine (1 mM; $n = 7$), a "local anesthetic" with Na^+ channel-blocking properties (Stys et al., 1992b), was started 15 min before 2 gm clip compression injury and continued until 20 min after injury. Recovery of CAPs was monitored for 2 hr after injury. During infusion of procaine, axonal conduction was blocked and returned during the washout phase. Procaine, as with TTX, conferred significant neuroprotection from traumatic spinal cord injury (Fig. 3b). With procaine, recovery of CAP was significantly enhanced ($p < 0.05$; $82.8 \pm 4.6\%$ of baseline) compared with Ringer's solution ($71.1 \pm 9.5\%$ of baseline; $n = 14$) at 75 min after injury.

Because strategies to reduce intracellular Na^+ influx by ion substitution with NMDG $^+$ or blockade of voltage-gated Na^+ channels with TTX or procaine appear to be neuroprotective after spinal cord injury, we hypothesized that increasing Na^+ permeability would result in more severe injury to axons after compression trauma. This hypothesis was tested by pretreating the dorsal column segment with veratridine, an alkaloid that increases Na^+ permeability (Catterall, 1980; Stys et al., 1992, 1993). The infusion of veratridine began 15 min before the 2 gm clip compression injury and continued until 20 min after injury. Recovery of CAPs was observed for 2 hr after injury. With veratridine, we observed significantly ($p < 0.05$) poorer recovery of the CAP ($60.1 \pm 8.4\%$ of baseline; $n = 5$) than with control Ringer's solution ($72.1 \pm 9.6\%$; $n = 14$) at 60 min after injury. The graph of normalized CAP amplitude as a function of time for the veratridine and control groups is depicted in Figure 4a.

Effect of blockade of the Na^+ - K^+ -ATPase pump

The Na^+ - K^+ -ATPase pump plays a key role in regulating $[\text{Na}^+]_i$. Under conditions of $[\text{Na}^+]_i$ loading, this pump extrudes Na^+ . We tested whether blockade of this mechanism of Na^+ extrusion would exacerbate traumatic axonal injury. Accordingly, we examined the effect of ouabain (25 μM ; $n = 5$), a potent Na^+ - K^+ -ATPase pump inhibitor, on recovery after compression injury to the isolated dorsal column segment. Ouabain was infused 15 min before the 2 gm clip compression injury and continued until 20 min after injury. Recovery of CAP was observed for 2 hr after injury. With ouabain, we observed that CAP recovery was significantly poorer ($p < 0.05$; $56.7 \pm 3.6\%$ of baseline) than under control (Ringer's solution) conditions ($72.1 \pm 9.6\%$; $n = 14$) at 60 min after injury (Fig. 4b).

Effect of blockade of the Na^+ - H^+ and Na^+ - Ca^{2+} exchangers

The Na^+ - H^+ and Na^+ - Ca^{2+} exchangers have been implicated in the mechanism of hypoxic-ischemic cell injury. Accordingly, we sought to investigate the potential role of these exchangers in the pathophysiology of traumatic spinal cord axonal injury. Given that pharmacological blockers of the Na^+ - Ca^{2+} exchanger have some Na^+ - H^+ exchange-blocking properties, we first sought to examine the effect of amiloride and harmaline (a highly specific Na^+ - H^+ exchange blocker) (Hartley and Dubinsky, 1993).

Infusion of either amiloride (100 μM ; $n = 6$), a potent inhibitor of the NHE $_1$ isoform of the Na^+ - H^+ exchanger, or harmaline (100 μM ; $n = 5$), a more specific inhibitor of the Na^+ - H^+ exchange inhibitor, was commenced 15 min before compression injury and continued until 20 min after injury. Recovery was observed for 2 hr after injury. Both amiloride and harmaline caused a slight decrease in CAP amplitude, possibly because of

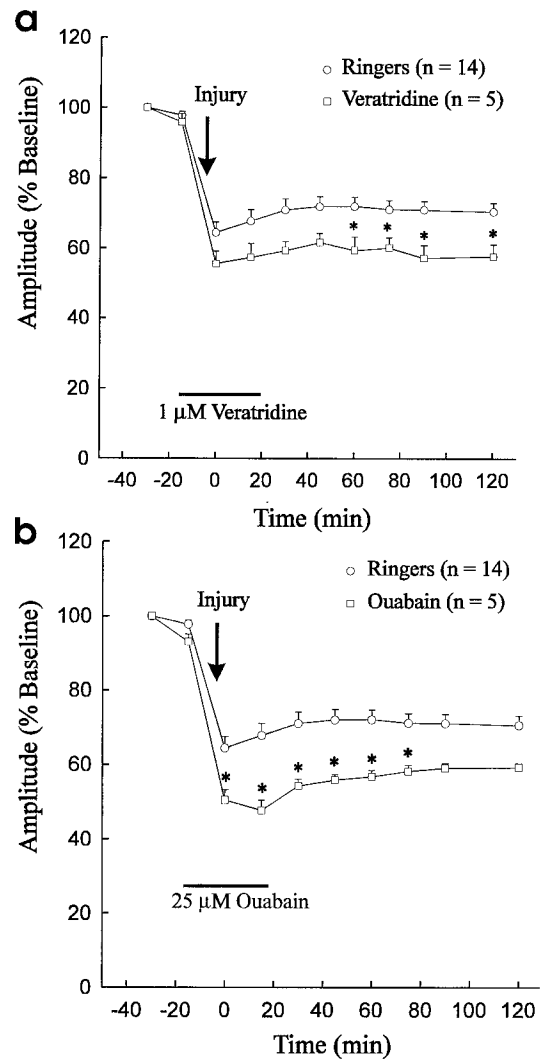


Figure 4. Effect of veratridine (1 μM), an alkaloid that increases Na^+ permeability, and ouabain (25 μM), a Na^+ - K^+ -ATPase inhibitor, on CAP after 2 gm clip compression injury. Perfusion of test solutions was started 15 min before injury and continued for 20 min after injury. *a*, Graph of normalized CAP amplitude versus time for the control and veratridine groups. *b*, Graph of normalized CAP amplitude versus time for the control and ouabain groups (significant differences at $p < 0.05$ depicted by asterisks).

partial blockade of Na^+ channels (Velly et al., 1988; Decher et al., 1992).

With amiloride, recovery of the CAP was significantly ($p < 0.05$; $89.6 \pm 17.0\%$ of baseline) improved compared with Ringer's solution ($72.1 \pm 9.6\%$ of baseline; $n = 14$) at 60 min after injury. Representative waveforms are depicted in Figure 5a-d. A graph of normalized CAP versus time is shown in Figure 5A (e).

With harmaline, recovery of the CAP was significantly ($p < 0.05$; $85.7 \pm 7.2\%$ of baseline) improved compared with Ringer's solution ($71.1 \pm 9.5\%$ of baseline; $n = 14$) at 75 min after injury. Representative waveforms are depicted in Figure 5B (a-d). A graph of normalized CAP versus time is shown in Figure 5B (e).

In hypoxic-ischemic CNS injury, rises in intracellular Na^+ have been postulated to gate reverse activation of the Na^+ - Ca^{2+} exchanger, leading to intracellular calcium influx. Therefore, we tested the applicability of this hypothesis to traumatic spinal cord injury by infusing benzamil and bepridil, potent Na^+ - Ca^{2+} ex-

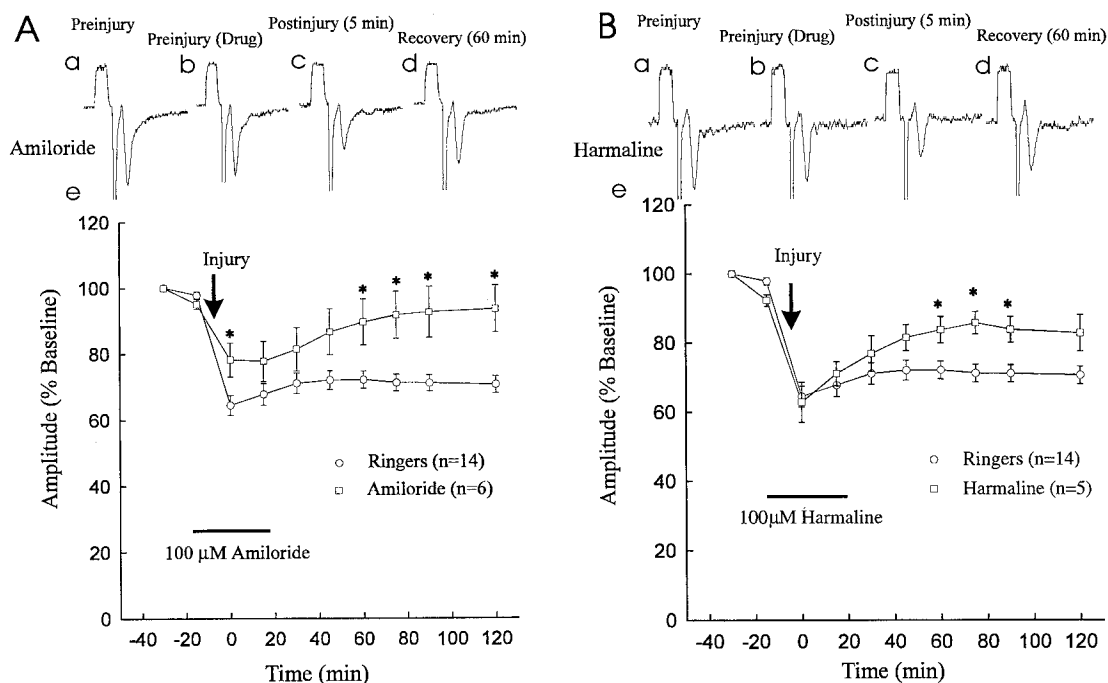


Figure 5. Effect of amiloride (100 μ M) and harmaline (100 μ M) on CAP recovery after 2 gm clip compression injury. Perfusion of test solutions was started 15 min before injury and continued for 20 min after injury. *A* (*a–d*), A typical CAP waveform before injury before amiloride perfusion, before injury after 15 min amiloride perfusion, after injury while being perfused with amiloride, and at 60 min after injury in normal Ringer's solution. (*e*) Graph of normalized CAP amplitude versus time for the control and amiloride groups. *B* (*a–d*), A typical CAP waveform before injury before harmaline infusion, before injury after 15 min harmaline perfusion, after injury while being perfused with harmaline, and at 60 min after injury in normal Ringer's solution. (*e*) Graph of normalized CAP amplitude versus time for the control and harmaline groups (significant differences at $p < 0.05$ depicted by asterisks).

change blockers. Benzamil (100 μ M, $n = 5$; 500 μ M, $n = 10$) or bepridil (50 μ M; $n = 5$) infusion was begun 15 min before the 2 gm clip compression injury and continued until 20 min after injury. CAP recovery was observed for 2 hr after injury. As shown in Figure 6, neither benzamil nor bepridil resulted in improved recovery after traumatic spinal cord injury *in vitro*.

The previously mentioned experiments were conducted at 25°C because at this temperature the dorsal column preparation remains extremely stable physiologically. To confirm that the results described above could be replicated at more physiological temperatures, a number of experiments ($n = 10$) were conducted at 33°C. The recovery of CAP amplitude with compression injury was similar at 33 and 25°C. Furthermore, zero sodium solution (equimolar substitution with NMDG⁺) conferred a highly significant ($p < 0.05$) degree of neuroprotection at 33°C, thus paralleling the results obtained at 25°C.

DISCUSSION

The principal findings of this study can be summarized as follows. (1) Reduction of extracellular Na⁺ by equimolar substitution with NMDG⁺ is neuroprotective after spinal cord compression trauma. (2) Potentiating intracellular Na⁺ entry with veratridine and preventing [Na⁺]_i extrusion by partial blockade of Na⁺–K⁺–ATPase activity with ouabain exacerbate traumatic axonal injury. (3) Blockade of Na⁺ entry via voltage-gated Na⁺ channels improves recovery of axonal function after injury. (4) Inhibition of the Na⁺–H⁺ exchanger with amiloride or harmaline also is neuroprotective after traumatic spinal cord injury, whereas blockade of the Na⁺–Ca²⁺ exchanger does not improve functional outcome.

Several studies have confirmed the neuroprotective effects of reducing extracellular Na⁺. For example, Regan and Choi (1991) reported that replacement of extracellular Na⁺ on an equimolar

basis with choline prevented glutamate-induced neuronal swelling in a spinal cord cell culture system. Friedman and Haddad (1994) found that replacement of Na⁺ with NMDG⁺ in the tissue culture medium completely protected freshly dissociated hippocampal CA1 neurons from anoxic injury. Similar results also have been reported in models of anoxic injury to hippocampal slice (Boening et al., 1989) and *in vitro* optic nerve (Stys et al., 1991, 1992a, 1993). Accordingly, the results of the present study are congruent with the above reports and point to a key role of Na⁺ in the pathogenesis of axonal injury after spinal cord trauma. It is noteworthy that the neuroprotective effects of the zero sodium solution were not sustained. After 60 min, the CAP amplitude gradually declined (Fig. 2g), suggesting additive effects of other cytotoxic events such as calcium influx (Goldberg and Choi, 1993).

The present study provides strong evidence that voltage-gated Na⁺ channels play a key role in trauma-associated intracellular Na⁺ entry. We studied TTX and procaine to block selectively voltage-gated Na⁺ channels, which are known to be in high concentration at the nodes of Ranvier of mammalian axons (Lee-Son et al., 1992; Ragsdale et al., 1994). We found that infusion of TTX (Fig. 3a) and procaine (Fig. 3b) resulted in greater recovery of CAP amplitude compared with control Ringer's solution. These results are similar to those of Haigney et al. (1994), who studied the effect of lidocaine on anoxic injury to cardiac myocytes. TTX and other Na⁺ channel modulators may reduce cellular swelling caused by active sodium influx through voltage-gated channels and passive chloride and water influx. For example, in hypoxic brain slices, pretreatment with TTX preserves cytosolic ATP stores and prevents Na⁺ loading (Kass et al., 1992). Moreover, Choi's group has reported that administration of TTX or low Na⁺ to cell cultures deprived of oxygen and glucose

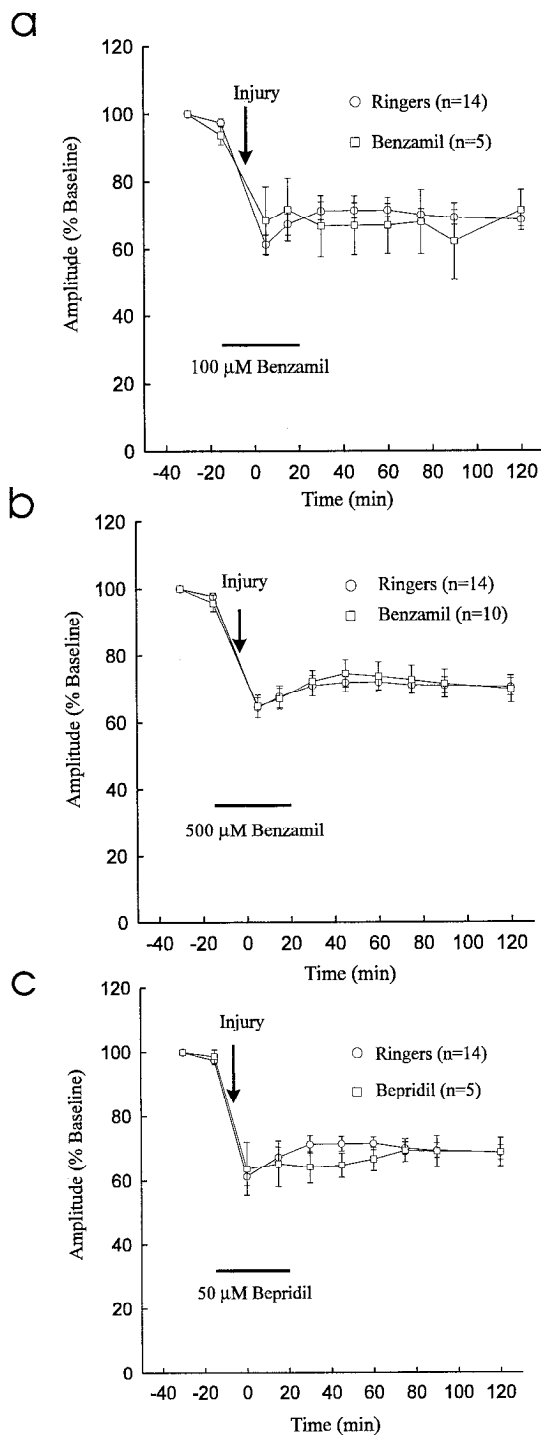


Figure 6. Effect of the Na^+ - Ca^{2+} exchange blockers benzamil (100 and 500 μM) and bepridil (50 μM) on CAP recovery after 2 gm clip compression injury. No evidence of neuroprotection was found with these drugs.

prevented cytotoxic cell edema but not eventual cell death (Goldberg and Choi, 1993). It also is possible that the effect of harmaline and amiloride is a partial blockade of Na^+ channels, because these drugs do have some degree of activity at voltage-gated Na^+ channels (Velly et al., 1988; Deecher et al., 1992).

Voltage-gated Na^+ channels are not perfectly selective, and they possess a finite permeability to other ions including Ca^{2+} .

However, because perfusion with zero sodium solution was highly neuroprotective, it is unlikely that influx of other ions such as Ca^{2+} occurs to a significant degree through these channels after trauma. The major source of Na^+ influx through these voltage-gated channels may be independent of action potential generation, because axonal conduction is impaired after injury. Furthermore, TTX exerted a neuroprotective effect at doses sufficiently small to allow action potential propagation (Fig. 3a). Accordingly, it is possible that Na^+ channel-modulating drugs such as procaine could reduce Na^+ influx through Na^+ channels that fail to inactivate completely during sustained depolarization (Taylor, 1993).

The potential mechanisms of Na^+ -induced cell injury include the following: (1) induction of cytotoxic edema (Regan and Choi, 1991); (2) stimulation of intracellular phospholipase activity (Gusovsky et al., 1986); (3) promotion of intracellular acidosis via gating of the Na^+ - H^+ exchanger (Haigney et al., 1994; Reithmeier, 1994); and (4) increasing intracellular Ca^{2+} by reverse operation of the Na^+ - Ca^{2+} exchanger (Stys et al., 1991, 1992a; Haigney et al., 1994). The latter two mechanisms were examined by using pharmacological blockers to inhibit these exchangers. Amiloride, a potent blocker of the NHE_1 isoform of the Na^+ - H^+ exchanger (Reithmeier, 1994), and harmaline, a specific inhibitor of the Na^+ - H^+ exchanger (Hartley and Dubinsky, 1993), were found to confer significant neuroprotection to traumatically injured spinal cord axons in the present study. This could be explained either by a reduction in intracellular Na^+ entry, because both amiloride and harmaline have some Na^+ channel-blocking properties, or by a reduction in intracellular acidosis caused by blockade of the Na^+ - H^+ exchanger. Indeed, harmaline and amiloride administered 15 min before injury produced a slight decrease in amplitude, suggesting partial blockade of Na^+ channels. It is noteworthy that amiloride and, to a lesser extent, harmaline affect a variety of receptors including Na^+ channels (Velly et al., 1988; Yu et al., 1993) and α and β adrenoreceptors, and that they also interact with adenylate cyclase and guanine nucleotide-binding proteins and G-proteins (Mahe et al., 1985; Howard et al., 1987; Friedrich and Burckhardt, 1988; Anand-Srivastava, 1989; Garristen et al., 1991, 1992). Of note, the GABA_B receptor is coupled directly through G-proteins to its effector channels in a manner similar to the adenosine receptor. It is possible that amiloride exerts an action through the GABA_B receptor/G-protein complex or through inactivation of adenosine receptors. Indeed, Fern et al. (1995) recently have shown that GABA improves recovery after anoxic white matter injury via recruitment of a G-protein/protein kinase C pathway. Fern et al. (1994) also have suggested that GABA and adenosine act synergistically to confer neuroprotection. On the other hand, having a primary role in regulating intracellular pH, Na^+ - H^+ exchange also acts as a major Na^+ pathway in many cells via Na^+ influx and influences intracellular volume (Stys et al., 1995). Amiloride and harmaline, which have been found to be effective in protecting traumatic spinal cord injury, also can inhibit Na^+ entry through Na^+ channels and transporters. It is possible then that both Na^+ - H^+ exchange and Na^+ currents contribute significantly in traumatic spinal cord injury.

Many other cellular functions depend on maintenance of the transmembrane gradient of Na^+ ions, including glutamate and GABA uptake from the extracellular space, and transport of hydrogen ions, calcium ions, and metabolic amino acids (Koch and Barish, 1994). Therefore, reducing sodium influx from traumatic spinal cord axonal injury may preserve these vital processes and may delay the loss of ion homeostasis caused by injury.

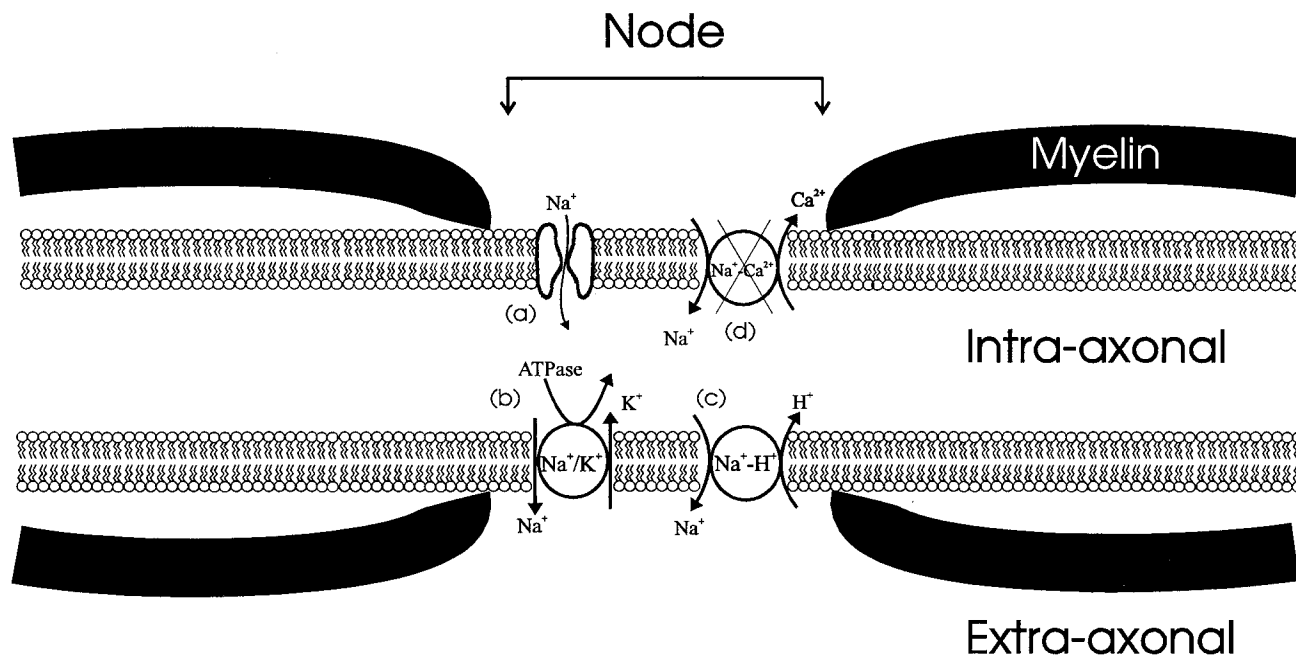


Figure 7. Hypothetical events in post-traumatic injury leading to Na^+ accumulation in spinal cord myelinated axons. Traumatic axonal injury is associated with a rise in $[\text{Na}^+]_i$ caused by influx of Na^+ through voltage-gated channels (a) and dysfunction of membrane-bound $\text{Na}^+-\text{K}^+-\text{ATPase}$ with a reduction in Na^+ efflux (b). Rises in $[\text{Na}^+]_i$ may lead to intracellular acidosis via operation of the Na^+-H^+ exchanger (c). Reverse operation of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger (d) does not appear to play a major role in traumatic spinal cord axonal injury, in contrast to some models of hypoxic-ischemic cell injury.

In contrast to our results with amiloride, pharmacological inhibition of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger with benzamil or bepridil (Fig. 6) did not improve outcome after compression injury to the dorsal column segment. These results suggest that reverse operation of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger does not explain the mechanisms of Na^+ -induced neurotoxicity in traumatic spinal cord injury. Thus, these results differ significantly from those with anoxic cell injury (Stys et al., 1991, 1992a). It is possible, for example, that spinal cord axons differ in their expression of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger from optic nerve or other cerebral white matter tracts. It also is important to note that these agents are not entirely selective for the $\text{Na}^+-\text{Ca}^{2+}$ exchanger and have action at voltage-gated Na^+ and Ca^{2+} channels (Galizzi et al., 1986; Kleyman and Cragoe, 1988; Garcia et al., 1990).

A majority of experiments conducted in this study were performed at 25°C , because the dorsal column preparations remain extremely physiologically stable under these conditions. Although hypothermia could provide some degree of neuroprotection, both control and test preparations were subjected to the same bath conditions. Accordingly, any neuroprotective effects of a hypothermic extracellular milieu can be controlled. However, to exclude this as a potentially confounding factor, selected experiments ($n = 10$) were conducted at 33°C . At this temperature, stable recordings could be obtained. The results at 33°C were very similar to data obtained at 25°C .

The results of the present study are summarized schematically in Figure 7. Traumatic axonal injury is associated with a rise in $[\text{Na}^+]_i$ caused by influx of Na^+ through voltage-gated channels and dysfunction of membrane-bound $\text{Na}^+-\text{K}^+-\text{ATPase}$ with a reduction in Na^+ efflux. Rises in $[\text{Na}^+]_i$ may lead to intracellular acidosis through operation of the Na^+-H^+ exchanger. However, the lack of effect of bepridil and benzamil suggests that the reverse operation of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger does not explain the inju-

rious effects of Na^+ in traumatically injured CNS white matter, in contrast to some models of hypoxic-ischemic cell injury. The high density of Na^+ channels at the nodes of Ranvier may render this region extremely vulnerable to compression injury.

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