

# Control of Membrane Sealing in Injured Mammalian Spinal Cord Axons

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Control of membrane sealing in injured mammalian spinal cord axons. *J Neurophysiol* 84: 1763–1769, 2000. The process of sealing of damaged axons was examined in isolated strips of white matter from guinea pig spinal cord by recording the “compound membrane potential,” using a sucrose-gap technique, and by examining uptake of horseradish peroxidase (HRP). Following axonal transection, exponential recovery of membrane potential occurred with a time constant of  $20 \pm 5$  min, at  $37^\circ\text{C}$ , and extracellular calcium activity ( $[\text{Ca}^{2+}]_o$ ) of 2 mM. Most axons excluded HRP by 30 min following transection. The rate of sealing was reduced by lowering calcium and was effectively blocked at  $[\text{Ca}^{2+}]_o \leq 0.5$  mM, under which condition most axons continued to take up HRP for more than 1 h. Sealing at higher  $[\text{Ca}^{2+}]_o$  was blocked by calpain inhibitors (calpeptin and calpain inhibitor-1) indicating a requirement for type II (mM) calpain in the sealing process. Following compression injury, the amplitude of the maximal compound action potential conducted through the injury site was reduced. The extent of amplitude reduction was increased when the tract was superfused with calcium-free Krebs’ solution ( $\text{Ca}^{2+}$  replaced by  $\text{Mg}^{2+}$ ). These results suggest that the fall in  $[\text{Ca}^{2+}]_o$  seen following injury in vivo is sufficient to prevent membrane sealing and may paradoxically contribute to axonal dieback, retrograde cell death, and “secondary” axonal disruption.

## INTRODUCTION

Influx of calcium ions is recognized as an essential mediator of intracellular damage and cell death in a range of pathological conditions, including mechanical trauma (Schanne et al. 1979; Schlaepfer and Bunge 1973). Repair of damage to nerve membranes has also been shown to be a calcium-dependent process (Xie and Barrett 1991; Yawo and Kuno 1985), but this observation has received less attention, and the potential conflict between these two phenomena in injury has not been examined. The possibility of opposing effects is particularly significant in the context of CNS trauma, where extracellular calcium activity ( $[\text{Ca}^{2+}]_o$ ) at the injury site falls immediately by 1–2 orders of magnitude and can remain depressed for hours (Stokes et al. 1983; Young et al. 1982). This prolonged depression of calcium, which may be common to a number of other pathological conditions, has been viewed as potentially beneficial, by reducing the driving force for cellular calcium influx. However, the sealing of severed and partially damaged axons may be compromised by such conditions, which would affect the positive interpretation of these ionic changes.

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The present study was designed to examine the process of membrane sealing in adult mammalian spinal cord axons isolated in vitro, and the dependence of sealing on extracellular calcium activity. Sucrose gap recordings were used to monitor membrane potential changes and to record axonal conduction in strips of white matter isolated from guinea pig spinal cord, by techniques that have been described previously (Shi and Blight 1996). Uptake of horseradish peroxidase (HRP) by the injured axons correlated with electrophysiological findings and provided additional information on the distribution of axonal damage and the time course of resealing (to large molecules) of the cut membrane. The response of isolated spinal cord axons to blunt compression was also examined, using an electrically controlled manipulator (Shi and Blight 1996). These experiments aimed to determine the effects of altered sealing on the recovery of action potential conduction in nerve fiber tracts subjected to more clinically relevant injuries.

## METHODS

All experiments were performed in vitro on strips of spinal cord white matter, isolated from 64 adult, female, Hartley strain guinea pigs. Histological studies were performed on the same tissues that were used for physiological analysis.

### *Isolation of spinal cord*

The technique for isolation of the cord was described previously (Shi and Blight 1996). Briefly, guinea pigs were anesthetized deeply with ketamine (80 mg/kg), xylazine (12 mg/kg), and acepromazine (0.8 mg/kg) and were perfused through the heart with 500 ml oxygenated, cold Krebs’ solution to remove blood and lower core temperature. The vertebral column was excised, and the spinal cord was removed, immersed in cold Krebs’ solution, and immediately subdivided, first along the sagittal midline, and then each half of the cord was cut radially, to produce ventral, lateral, and dorsal strips of white matter. The composition of the Krebs’ solution was as follows (in mM): 124 NaCl, 2 KCl, 1.2  $\text{KH}_2\text{PO}_4$ , 1.3  $\text{MgSO}_4$ , 2  $\text{CaCl}_2$ , 20 dextrose, 10 sodium ascorbate, and 26  $\text{NaHCO}_3$ , equilibrated by bubbling with 95%  $\text{O}_2$ -5%  $\text{CO}_2$  to produce a pH of 7.2–7.4. In calcium-free Krebs’ solution, the calcium chloride was replaced by equimolar magnesium chloride.

### *Recording chamber*

The construction of the recording chamber is illustrated in Fig. 1. A strip of isolated spinal cord white matter, approximately 35 mm in

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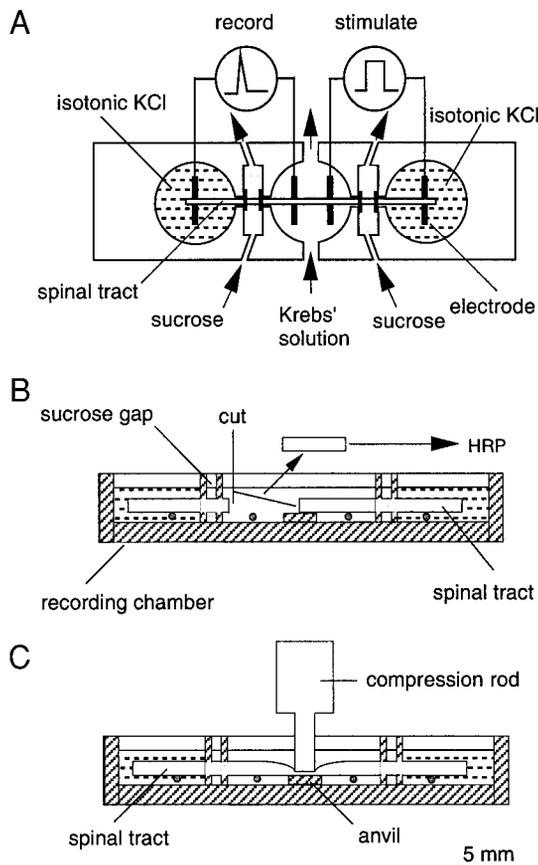


FIG. 1. Diagram to show the recording apparatus and injury technique. *A*: the recording arrangement, viewed from above. The isolated spinal cord tract is shown mounted in the apparatus, with the injury site placed in the middle of the central well, continuously perfused with oxygenated Krebs' solution. The two ends of the tract were placed in separate wells filled with isotonic KCl, divided from the central well by narrow channels filled with flowing, isotonic sucrose solution. Electrodes were formed of silver/silver chloride wires. Action potentials were generated at the right hand sucrose gap, conducted through the injured part of the spinal cord and were recorded at the left hand gap, using a bridge amplifier. *B*: to examine the response to transection, the tissue was cut near the face of the recording gap, seen here in diagrammatic view from the front of the chamber. The tissue was cut also in the middle of the chamber and was transferred to horseradish peroxidase (HRP) solution at particular times after injury, to examine the sealing of axons to HRP. *C*: compression injuries were produced with a vertically mounted Plexiglas rod, advanced downward with a micromanipulator. The movements of the rod were monitored with a displacement transducer and activated with a stepper motor control.

length, was supported in the central compartment and continuously superfused with oxygenated Krebs' solution ( $\sim 2$  ml/min). The ends of the tissue were carried through the sucrose gap channels to side compartments filled with isotonic (120 mM) potassium chloride (Fig. 1A). The white matter strip was sealed on either side of the sucrose gap channels, using fragments of plastic cover-slip and a small amount of silicone grease to attach the cover slip to the walls of the channel and seal around the tissue. Isotonic sucrose solution was continuously run through the gap channels at a rate of 1 ml/min. The temperature of the chamber was maintained with a thermostatically controlled Peltier unit in the base (Cambion Instruments). The axons were stimulated and compound action potentials recorded at opposite ends of the strip of white matter by silver/silver chloride wire electrodes positioned within the side chambers and the central bath. The central bath was similarly connected to instrument ground.

Stimuli, in the form of constant-current unipolar pulses of 0.1 ms duration, were controlled with a pulse generator connected through a WP Instruments isolation unit. Recordings were made using a bridge

amplifier and Neurocorder (both from Neurodata Instruments) for digital data storage on videotape. Subsequent analysis was performed using custom Labview software (National Instruments) on a Macintosh Power PC computer.

### Transection

To study the response of the nerve fibers to transection, the tissue strip was cut at the face of the recording sucrose gap, using microscissors. The scissors were also used to cut through the tissue at the center of the chamber, so that the isolated tract could be transferred to HRP solution at different times after injury for evaluation of sealing at the cut ends (Fig. 1B).

### Compression

A flat, raised surface was provided at the center of the recording chamber, against which the isolated white matter strip could be compressed, using a rod attached to a motorized micromanipulator (Fig. 1C). The end of the rod provided a compression surface of 2.5 mm along the length of the tissue, with a transverse width of 7 mm, such that it was wider than the tissue, even under compression. The compression rod was positioned perpendicularly to the tissue and was brought to a point of contact with its surface. After baseline measurements of conduction were obtained, the rod was advanced by means of the manipulator motor at a speed of  $24 \mu\text{m/s}$ . The compound action potential and the displacement of the rod were monitored during the compression, and the compression was stopped when the potential reached a set, target amplitude. The rod was then removed rapidly upward, to relieve pressure on the tissue, and the recovery of the compound potential was monitored.

### HRP histochemistry

To examine disruption of axons, segments of white matter strips were transferred at different times after injury (the times and the number of tissue samples depending on the physiological recordings required) to oxygenated Krebs' solution containing 0.015% HRP (Sigma type VI). After incubation for 1 h at room temperature, the tissue was fixed by immersion in 2.5% glutaraldehyde in phosphate buffer. Transverse sections of the tissue were cut at  $30 \mu\text{m}$  on a Vibratome and stained with diaminobenzidine reaction to reveal the extent of HRP uptake into damaged axons. Sections at approximately 1 mm from the plane of transection and at 5 mm distance were compared, to control for the presence of damaged fibers that might be unrelated to the deliberate injury. Sections were examined and photographed with a Nikon Optiphot microscope.

Initial experiments were performed to determine whether quantitative morphometry could be performed, using  $1\text{-}\mu\text{m}$  plastic sections. The density of staining with HRP was insufficient to provide a clear distinction between lightly stained and unstained fibers in the semi-thin sections. The presence or absence of stain was relatively unequivocal in the thicker Vibratome sections, but these sections did not allow accurate quantitative analysis of axon numbers, particularly for the smaller diameter nerve fibers. Observations were therefore restricted to major shifts in axonal uptake of HRP under different conditions.

## RESULTS

Spinal cord strips placed in the recording chamber showed a period of stabilization of the resting membrane potential, requiring 30–60 min. During this time, the "compound resting membrane potential" recorded across the sucrose gap became more negative, and the amplitude of the maximal evoked compound action-potential increased. The form and quantitative characteristics of the compound action potential and of the

“gap potential” or “compound resting membrane potential” have been described previously (Shi and Blight 1996). The action potential corresponds closely to the action potential recorded in single large mammalian spinal cord axons using intracellular microelectrodes (Blight and Someya 1985). The amplitude of the gap potential was determined to be  $16 \pm 3$  (SD) mV, or approximately three times the amplitude of the peak of the compound action potential. To provide a “normalized value” of gap depolarization for different white matter strips, the measured gap potential (in mV) at any given time following injury was related to the initial peak of gap depolarization seen immediately following transection (which was normalized to a value of 1). This served to overcome the variability in measured absolute potential. The amplitude of the potential recorded from a given tissue strip is arbitrary in itself, with a value based on the particular configuration of the tissue in the sucrose gap, and its passive electrical characteristics.

#### Response to transection

When the white matter strip was cut near the central face of the sucrose gap, the gap potential was reduced within seconds toward bath potential (0 mV) and then began to repolarize slowly (Fig. 2A). Full recovery of the initial resting potential required approximately 45–60 min at 37°C and 2 mM  $[Ca^{2+}]_o$ . The form of the recovery curve was approximately exponential, with a time constant of 20 min. At 25°C, the rate of recovery of resting potential was slower, with a time constant of approximately 40 min, but was still exponential to the same baseline (Fig. 2B).

#### Staining cut axons with HRP

HRP histochemistry showed a comparable rate of sealing of the axonal membrane to entry of the enzyme molecule. At 37°C and 2 mM  $[Ca^{2+}]_o$ , almost all axons were stained when transferred to HRP solution between 1 and 5 min after transection (Fig. 3A), but very few fibers were stained when transferred at 30 min or 1 h post transection (Fig. 3, B and C).

#### Effects of low calcium activity

The rate of recovery of the gap potential decreased with decreasing calcium concentration in the medium, from 2 to 0.5 mM (Fig. 4A). Further reduction in calcium concentration had little effect on the rate of recovery, and, at all concentrations  $\leq 0.5$  mM, there was no approach to the original baseline polarization (Fig. 4B). This change in the rate of recovery of compound membrane potential was reflected in the maintained uptake of HRP. Most axons in spinal cord strips maintained in Krebs' solutions with 0.5 mM  $[Ca^{2+}]_o$  continued to take up HRP for more than 1 h after transection (Fig. 3E). Spinal cord strips maintained in Krebs' solution with 1 mM  $[Ca^{2+}]_o$  showed an intermediate rate of sealing to HRP, the number of axons staining at 1 h (Fig. 3D) being greater than the number at 30 min in solutions with 2 mM  $[Ca^{2+}]_o$  (Fig. 3B).

#### Calpain inhibition

The dependence of sealing on relatively normal concentrations of calcium raised the possibility that the process of repair was dependent on millimolar or type II calpain. This possibility

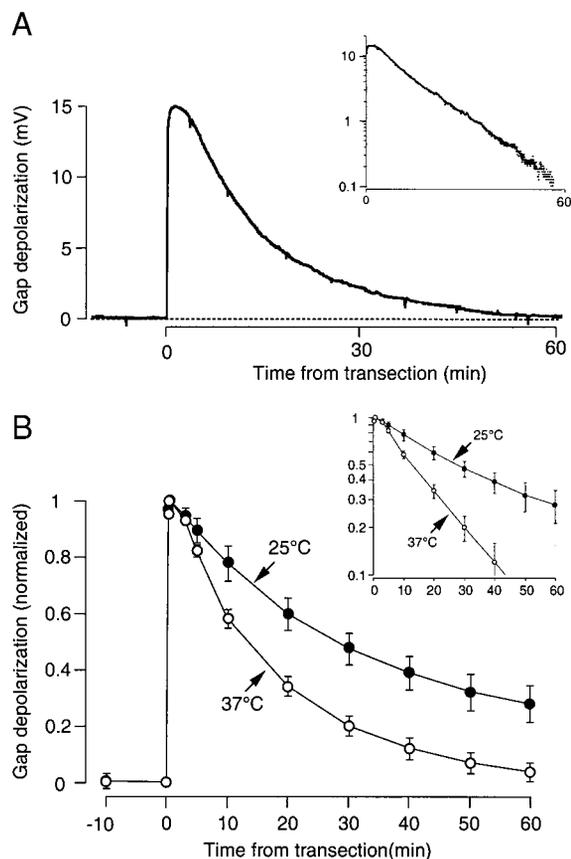


FIG. 2. Exponential rate of recovery of the gap potential following transection of a spinal cord white matter strip in normal Krebs' solution, containing 2 mM  $Ca^{2+}$ . A: an example gap potential recording displayed on a linear scale and (inset) on a log scale. Immediately after cutting the tissue at the outer edge of the gap, the gap potential depolarized by 15 mV and then began to repolarize toward the initial level. B: averaged responses to transection in 2 groups of white matter strips maintained at 37°C ( $n = 8$ ) and 25°C ( $n = 8$ ), respectively. The gap potential in each case was normalized to the peak of the depolarization produced by transection. The rate of recovery of the gap potential was reduced with temperature.

was examined by incubating spinal cord strips in Krebs' solution containing 2 mM  $Ca^{2+}$  and either calpeptin or calpain inhibitor-I. Both blockers of calpain activity produced a similar suppression of gap potential recovery (Fig. 5) and maintenance of HRP uptake (Fig. 3F) to those seen with calcium concentration below 0.5 mM (Figs. 3E and 4A).

#### Response to compression

The loss and recovery of action potential conduction in the isolated strips exposed to focal compression injury was similar to that reported previously (Shi and Blight 1996). Briefly, the normal compound action potential recorded at one end of the strip in response to electrical stimulation at the other was reduced in amplitude by focal compression in the middle of the strip. On removal of compression, there was a partial recovery of amplitude within 5–6 min, which then became stable for the succeeding hour of incubation.

The amplitude of this partial recovery was compared in two groups of spinal cord strips, one incubated throughout in 2 mM  $Ca^{2+}$  Krebs' solution, and the other in which the superfusion medium was changed to 0 mM  $Ca^{2+}$  Krebs' at the time of

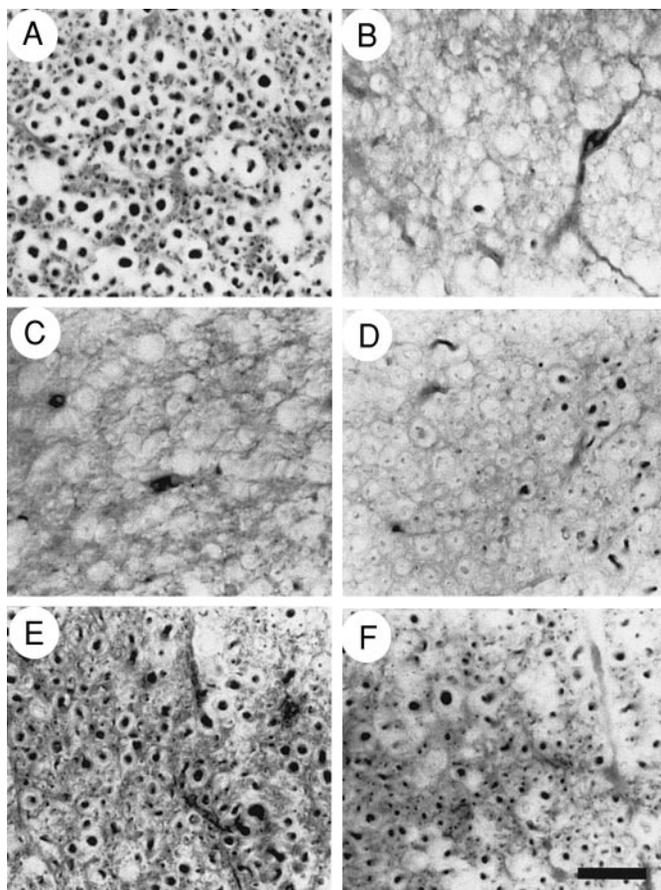


FIG. 3. Photomicrographs of Vibratome sections cut at 1 mm from the transected end of spinal cord tracts and stained for horseradish peroxidase (HRP) using the diaminobenzidine technique. The tracts were transferred to HRP containing Krebs' solution at different times following transection and then incubated for 1 h before fixation in glutaraldehyde. *A*: in tracts transferred at 2 min, following transection in Krebs' solution containing 2 mM  $\text{Ca}^{2+}$ , all the axons were stained darkly with diaminobenzidine reaction product, showing they had rapidly taken up and transported HRP. *B*: after 30 min of incubation in Krebs' solution containing 2 mM  $\text{Ca}^{2+}$ , the majority of axons were effectively sealed to uptake of HRP. *C*: after 1 h, all axons were sealed to HRP uptake, with reaction product appearing only in endothelial cells and some glial processes. *D*: after 1 h in 1 mM  $\text{Ca}^{2+}$  Krebs' solution, a significant proportion of axons, at varying density across the tissue, remained accessible to HRP. *E*: after 1 h incubation in Krebs' solution containing 0.5 mM  $\text{Ca}^{2+}$ , practically all axons were stained with HRP reaction product. *F*: after 1 h in 1 mM  $\text{Ca}^{2+}$  Krebs' solution containing 30  $\mu\text{M}$  calpain inhibitor I, practically all axons continue to take up HRP. Scale bar, 50  $\mu\text{m}$ . All experiments performed with tissue at 37°C.

compression. The amplitude of the recovered compound action potential was significantly greater in the group of spinal cord tracts maintained in Krebs' solution with 2 mM  $[\text{Ca}^{2+}]_o$  (Fig. 6).

In addition, experiments were performed, changing the perfusion of the injured spinal cord strip from calcium-free to 2 mM calcium Krebs' solution at 30 min following compression injury (Fig. 7A). Even after this delay, the amplitude of the compound potential was increased significantly within a few minutes of restoring extracellular calcium (Fig. 7B).

#### DISCUSSION

Neurons are able to recover from mechanical injury, including loss of dendritic or axonal processes, because they have the

ability to seal breaks in the plasmalemma, and do so surprisingly rapidly. This sealing of cut processes and the consequences for cell survival have been examined previously in large, isolated, invertebrate axons (Yawo and Kuno 1985), in cultured vertebrate cells (Lucas et al. 1985; Shi et al. 1989; Xie and Barrett 1991), and, more recently, in reptilian peripheral axons (David et al. 1997). While influx of sodium is clearly implicated in the cytotoxic effects of mechanical damage to cells (Rosenberg and Lucas 1996), the process of sealing itself is also clearly calcium dependent (Gallant 1988; Xie and Barrett 1991) and appears to involve an essential role of calcium-activated phospholipase-A2 (Yawo and Kuno 1985). The precise dependence of resealing on calcium-mediated mechanisms has not been established, nor has the phenomenon been examined in adult mammalian axons of the CNS, where repair is of key clinical relevance. The potential significance of these mechanisms is highlighted by the finding that extracellular calcium activity in the CNS is profoundly depressed by mechanical trauma (Stokes et al. 1983; Young et al. 1982). The present experiments were designed to use a dynamic measure

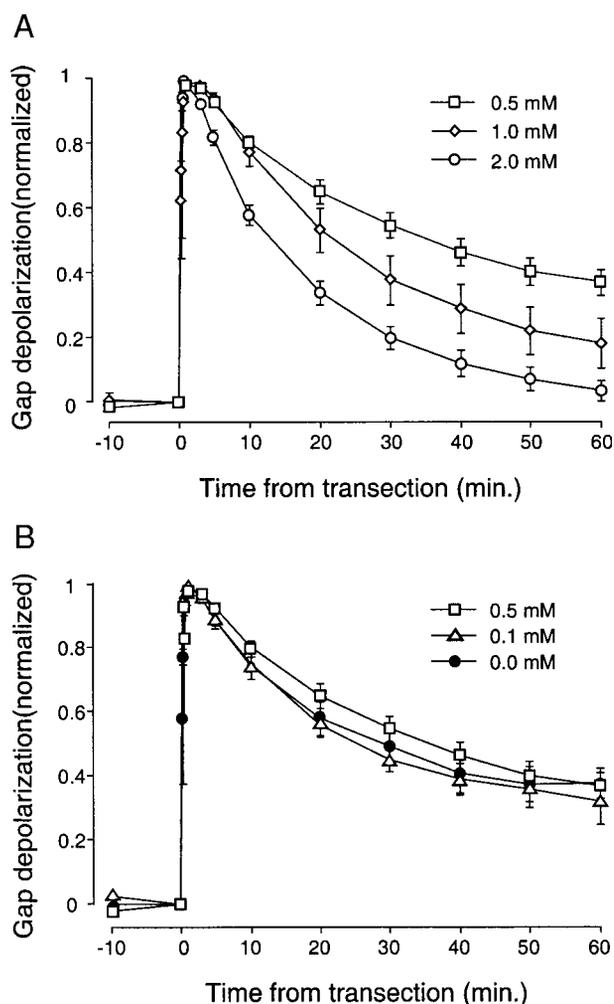


FIG. 4. Graphs, similar to those in Fig. 2B, showing the recovery of gap potential in 5 sets of spinal tracts transected in Krebs' solutions containing different concentrations of  $\text{Ca}^{2+}$  from 2 to 0 mM ( $n = 8, 5, 9, 4, 5$ ). *A*: there was a significant reduction in the rate of recovery of the gap potential with reductions in  $\text{Ca}^{2+}$  activity between 2 and 0.5 mM. *B*: further reduction in  $\text{Ca}^{2+}$  activity from 0.5 to 0 mM produced no significant further reduction in rate or completeness of recovery of the gap potential.

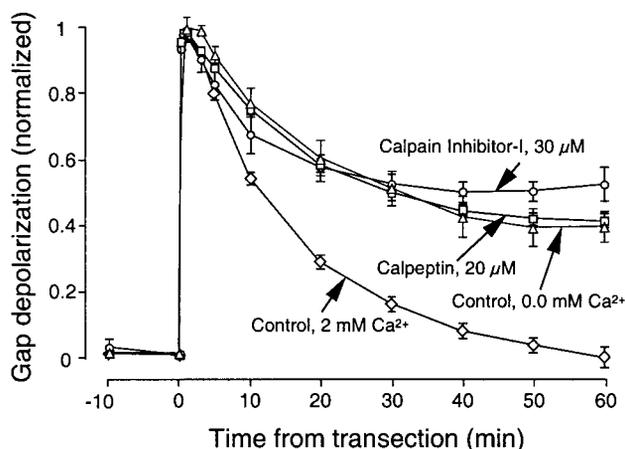


FIG. 5. Graphs, similar to those shown in Fig. 4, illustrating the effects of 30  $\mu\text{M}$  calpain inhibitor-1 ( $n = 8$ ) and 20  $\mu\text{M}$  calpeptin ( $n = 5$ ) on recovery of the gap potential in 2 groups of spinal tracts following transection, compared with a control group ( $n = 10$ ). All 3 groups were maintained in Krebs' solution containing 2 mM  $\text{Ca}^{2+}$ . Data from strips maintained in 0.0 mM  $\text{Ca}^{2+}$  are also shown for comparison.

of sealing in mammalian spinal axons to determine whether the extracellular calcium changes seen in spinal cord trauma might play a role in neurological recovery, as has been suggested, based on observations *in vivo* (Young 1992).

#### Gap potential as a measure of sealing

It may appear counterintuitive to study injury mechanisms in isolated tissues of this kind because the tissue is injured several times, once by transection when the spinal cord is removed from the animal, a second time when the ventral white matter strip is cut from the length of isolated cord, and a third when the strip is finally cut or compressed. However, following the initial trauma of isolation, a minimum of 1 h at 20°C and 1 h in 37°C was given to allow the cord to recover, both in terms of metabolism and membrane sealing. Furthermore, the experimental injuries were made at least 10 mm (several length-constants) from the end of the strip, where the isolating transverse cuts were made. Thus the experimental injury was

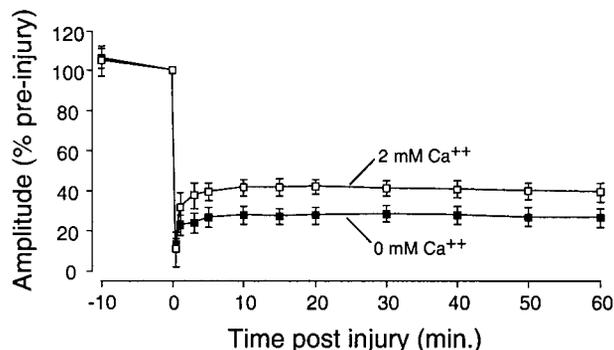


FIG. 6. The response of the compound action potential amplitude to compression injury in 2 groups of spinal tracts ( $n = 8, 8$ ) maintained in Krebs' solution containing 2 and 0 mM  $\text{Ca}^{2+}$ , respectively. The compound potential amplitude was reduced and almost extinguished at the time of compression, and recovered within a few minutes to a new plateau level. The recovered plateau amplitude was approximately 60% larger in the 2-mM  $\text{Ca}^{2+}$  solution compared with the 0  $\text{Ca}^{2+}$  condition. This difference was significant overall and for all time points after 5 min post injury (repeated measures ANOVA, with Fisher's PLSD post hoc comparison).

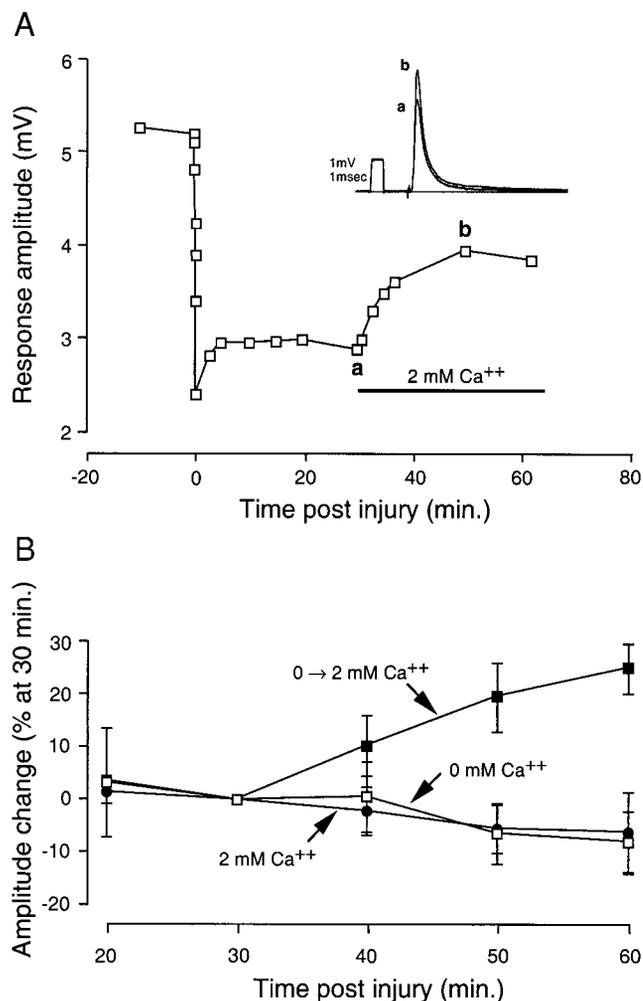


FIG. 7. Response of the compound potential amplitude to changing the bathing solution from 0 mM  $\text{Ca}^{2+}$  to 2 mM  $\text{Ca}^{2+}$  at 30 min after compression injury. A: an individual record of the time course of changes in the compound potential amplitude. Inset shows the increase in amplitude of the compound potential within 20 min of changing to 2 mM  $\text{Ca}^{2+}$  Krebs' solution. B: graphs to show the relative change of amplitude of the compound potential between 20 and 60 min post compression, referenced to the amplitude at 30 min, for 3 groups of tissues: compressed and maintained in 2 mM  $\text{Ca}^{2+}$  ( $n = 8$ ), compressed and maintained in 0 mM  $\text{Ca}^{2+}$  Krebs ( $n = 8$ ), and compressed in 0 mM  $\text{Ca}^{2+}$  and changed to 2 mM  $\text{Ca}^{2+}$  Krebs at 30 min post compression ( $n = 7$ ).

spatially as well as temporally separated from the injuries created during extraction.

A simple experiment confirmed that the injury of extraction was unlikely to affect the characteristics of sealing in the experimental transection. Spinal tracts were transected on one side of the chamber for an initial recording in 2 mM  $\text{Ca}^{2+}$ , then transected again on the other side of the chamber an hour later (switching the recording amplifier to the opposite end of the chamber). The second injury resulted in recovery of compound resting membrane potential with the same characteristics as recovery from the first transection. Therefore it seems unlikely that the response to experimental injury *in vitro* is significantly conditioned by the injury involved in tissue isolation.

Nerve fibers in isolated spinal cord strips completely sealed to HRP 1 h after transection in Krebs' solution containing 2 mM  $\text{Ca}^{2+}$ . However, the axons were practically completely accessible to HRP one hour following transection in solutions

containing 0.5 mM  $\text{Ca}^{2+}$  or a combination of 2 mM  $\text{Ca}^{2+}$  and 30  $\mu\text{M}$  calpain inhibitor-I. The recovery of membrane integrity for the large molecule correlated well with recovery to baseline of the measured "compound resting membrane potential" (Leppanen and Stys 1997a,b), supporting the use of this potential as an indicator of the recovery of membrane integrity.

The compound resting potential would be expected to relate to ionic permeability changes, and it may therefore provide a measure of membrane permeability changes that are more subtle than the more extensive membrane disruption required for access of HRP. In addition, the initial phase of potential recovery occurred consistently, even in the absence of sealing to HRP or of subsequent return to the original baseline resting membrane potential. The initial phase of recovery of the gap potential therefore seems to be based not on sealing of a proportion of the nerve fibers in the tract, but perhaps on a decrease in the core conductance near the cut end of the fibers. This would be consistent with the kind of constriction that has been seen in morphological studies of giant axons (Krause et al. 1994) and that has been shown to be unaffected by replacement of extracellular  $\text{Ca}^{2+}$  with  $\text{Mg}^{2+}$  (Gallant 1988).

#### *HRP uptake as a measure of sealing*

Changes in intra-axonal staining with HRP were interpreted as measuring axolemmal sealing (sufficient to exclude diffusion of the HRP molecule into the axon). It seems possible that uptake or transport of HRP along the axon could be affected by higher calcium; however, the control for this was to observe HRP transport in white matter exposed to high calcium and HRP immediately after transection. There was no profound reduction in HRP transport when the axons were exposed to HRP before membrane sealing could occur (Fig. 3A). There was also no reduction in the intensity of staining in individual axons exposed at intermediate timepoints, only an increase in the number of axons showing no staining at all (Fig. 3B).

#### *Calcium dependence of sealing following transection*

Resealing of axons in this preparation, recorded by either electrophysiological or histological means, depends on extracellular calcium ion activity in the millimolar range. In solutions with calcium activity below 0.5 mM, sealing does not take place within the duration of the experiment, up to several hours. This observation, combined with the blocking effect of calpain inhibitors on the resealing process, indicates that the sealing of myelinated nerve fibers in the mammalian CNS depends on the activity of millimolar calpain. This is consistent with the selective presence of this form of the enzyme within axons of the CNS (Hamakubo et al. 1986). The mechanism of the role of millimolar calpain is not clear in this context, or in axon sealing in other contexts. It may be that some degree of calpain-dependent rapid axoskeletal degradation is necessary to begin the axolemmal membrane sealing process. It could be imagined that this might involve the need to free the axonal membrane from the rigidity of the axoskeleton to allow sufficient spatial reorganization to bring the cut ends together. The present study did not examine the role of phospholipase-A2 in the sealing process (Yawo and Kuno 1985). Unfortunately, the

available inhibitors of phospholipase-A2 are themselves toxic to normal axons (Xie and Barrett 1991).

Perhaps most importantly, from the aspect of understanding the pathology of human CNS injury, the recovery of action potential conduction in white matter tracts that have been injured by blunt compression is also calcium dependent in this range. Conduction block in some axons injured in low extracellular calcium concentrations appears to be restored when normal calcium activity is returned. This indicates that resealing of critically damaged, but nontransected axons may play a significant role in recovery from blunt contusion injury in the CNS. It seems likely that the membrane damage produced by stretch or compression injury may simply represent on a smaller and more distributed scale the same kind of membrane disruption represented by the more dramatic case of axonal transection.

Membrane sealing is required to halt the progressive cycle of depolarization, calcium and sodium entry, consequent metabolic disruption, and cytoskeletal disassembly that produces "dieback" of cut axons. An important additional role is the repair of more subtle membrane damage, which may produce an increase in nonspecific membrane permeability without immediately breaking axonal continuity. Such critical changes in membrane permeability, induced by stretch or blunt impact, may be responsible for the kind of progressive, axonal breakdown that has been described in "diffuse axonal injury" following brain trauma (e.g., Buki et al. 2000; Povlishock 1992). Similar processes may occur in the injured spinal cord, given the similarity of the mechanical disruption, although no studies have addressed the specific presence of this kind of pathology either in human studies or animal models. Such "occult" injury may be accessible to therapeutic interventions aimed at accelerating membrane sealing and halting the ionic disruption that accompanies the loss of membrane integrity at the site of injury.

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