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DIFFERENTIAL EFFECTS OF LOW AND HIGH CONCENTRATIONS OF 4-AMINOPYRIDINE ON AXONAL CONDUCTION IN NORMAL AND INJURED SPINAL CORD

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Abstract—Blockade of potassium channels with the drug 4-aminopyridine has been shown to effect recovery of action potential conduction in myelinated axons under a variety of pathological conditions, but the mechanism and significance of this phenomenon are not completely understood. This study examined the effects of a range of 4-aminopyridine concentrations on conduction in an experimental model of chronic spinal cord injury in guinea-pigs, using sucrose-gap recording from isolated spinal cord strips. The amplitude of the compound action potential increased in response to bath application of 4-aminopyridine, with a threshold between 0.5 and 1 μM and the peak response between 10 and 100 μm. Conduction was suppressed at concentrations of 1 and 10 mM. Uninjured white matter showed no effect on the compound potential of 4-aminopyridine below 1 mM, but there was a similar suppression at concentrations above 1 mM, accompanied by marked membrane depolarization. Peripheral nerve showed only slight action potential suppression and depolarization in the presence of 10 mM 4-aminopyridine.

The sensitivity of injured axons to 1 μM 4-aminopyridine is consistent with the hypothesis that some beneficial effects of the drug seen in patients with spinal cord injury are related to improved conduction in myelinated axons, since cerebrospinal fluid levels of 4-aminopyridine should approach this concentration following clinical systemic doses, although it remains likely that synaptic effects also play a role. The blockade of action potential conduction produced by much higher levels of 4-aminopyridine *in vitro* is possibly a consequence of interference with the resting potential mechanism of the axon membrane, which appears to differ between central and peripheral nerve fibers. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: potassium, demyelination, action potential, guinea-pig, spinal cord trauma.

Rapidly activating, voltage-dependent potassium channels in myelinated axons appear to play a relatively subtle role in the normal physiology of the action potential, compared with the essential part that they play in repolarization of unmyelinated fibers.[26] The low capacitance conferred by the myelin sheath allows rapid, passive repolarization. At least in large-caliber axons, fast potassium channels appear to be located primarily in the internodal axolemma,[1,3,15,48] where they experience little depolarization during passage of a single action potential. However, under conditions where the myelin sheath fails to shield the capacitance of the internode, these channels are in a position to contribute significant shunting of the action current.

Blockade of voltage-dependent potassium channels with 4-aminopyridine (4-AP) has been shown in animal studies to restore action potential propagation in nerve fibers with conduction deficits associated with chemical demyelination,[14,37,45] thermal[19] and mechanical damage.[5] The drug also appears to improve neurological function in a range of clinical studies of conditions in which CNS demyelination is a factor.[17,24,25,40,41,47] Interpretation of these studies and parallel work in intact animals is complicated, since blockade of potassium channels will have widespread effects in the nervous system. There is also an apparent disparity between drug concentrations that are effective in laboratory studies and the much smaller tissue concentrations that can be achieved safely with systemic administration in human subjects. This disparity provided the basis for a recent statement that "the dominant effect of aminopyridines in (multiple sclerosis) may be through their well-documented ability to potentiate synaptic transmission." [22] The implication that effects on conduction play a minor role, or none at all, in these clinical contexts is open to debate[16] and the underlying physiology deserves further examination.

Previous studies from this laboratory examined action potential conduction in chronically injured spinal cords of cats, isolated *in vitro*[4] and the effects of 4-AP on conduction block in such tissue.[5] The technique of intra-axonal recording, used in those studies, was too unstable to allow extensive dose–response analysis, but 4-AP in concentrations of 0.1

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Abbreviations: ANOVA, analysis of variance; 4-AP, 4-aminopyridine; CSF, cerebrospinal fluid.

or 1 mM was shown to increase the temperature of conduction block to more normal physiological levels in some fibers. These drug concentrations were based on earlier *in vitro* studies, and chosen with the expectation that relatively high concentrations would be most effective. The present study used a sucrose-gap technique for recording compound action potentials in chronically injured guinea-pig spinal cords, isolated *in vitro*. This arrangement allowed for stable, prolonged recording of action potential conduction and examination of the effects of a wide range of drug concentrations, including those relevant to clinical experience. The results were previously summarized in abstract form.[38]

EXPERIMENTAL PROCEDURES

Animals and injury technique

Studies were carried out using 22 adult, female, Hartley strain guinea-pigs (Sasco) and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. The technique of injury to the spinal cord has been described elsewhere in full.[6] In brief, guinea-pigs were anesthetized for sterile surgery by intramuscular injection of ketamine HCl (60 mg/kg), xylazine (10 mg/kg) and acepromazine maleate (0.6 mg/kg), supplemented as required. A T12 dorsal laminectomy exposed the spinal cord, with dura intact. A special tool, constructed from watchmakers' forceps, was inserted on either side of the spinal cord and used to compress it to a thickness of 1.2 mm, over a length of 5 mm, for 15 s. The wound was closed in layers and the animals allowed to recover. The animals were maintained in individual cages, with free access to food and water. No problems of urinary retention following spinal cord injury have been encountered in this species. Spinal cords were isolated for recording between one and six months postinjury (average 2.8 months).

Isolation of spinal cord

The technique for isolation of the spinal cord was similar to that used previously for rats.[5,11] In brief, guinea-pigs were anesthetized and perfused through the heart with approximately 500 ml of oxygenated Krebs' solution (NaCl 124 mM, KCl 2 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.3 mM, $CaCl_2$ 1.2 mM, dextrose 10 mM, $NaHCO_3$ 26 mM, sodium ascorbate 6 mM, equilibrated with 95% O_2, 5% CO_2) at room temperature. The spinal cord was rapidly exposed by excision of the vertebral column over ice, then the vertebral laminae cut away as a continuous sheet by cutting through the pedicles on either side with fine bone-cutters. The spinal roots were cut, the cord was removed carefully from the spinal canal and placed into cold Krebs' solution. Subdivision of the spinal cord into four separate longitudinal tracts was carried out on a cooled Plexiglas block. Laid on its dorsal surface, the ventral fissure was opened along the length (except in the region of the scar tissue around the chronic lesion) by pulling gently on the midline pia-arachnoid with two pairs of watchmakers' forceps. The dorsal column was then divided into left and right halves with a scalpel blade, which was also used to cut through the sagittal midline of the injured part of the cord. The two lateral halves of the cord were then subdivided longitudinally, using a scalpel blade, to make ventral and dorsal white matter strips, which were placed into 15 ml Krebs' solution, bubbled with oxygen at room temperature. The peripheral sciatic nerves were removed by careful dissection and supported in a similar manner to the spinal cord strips for subsequent recording.

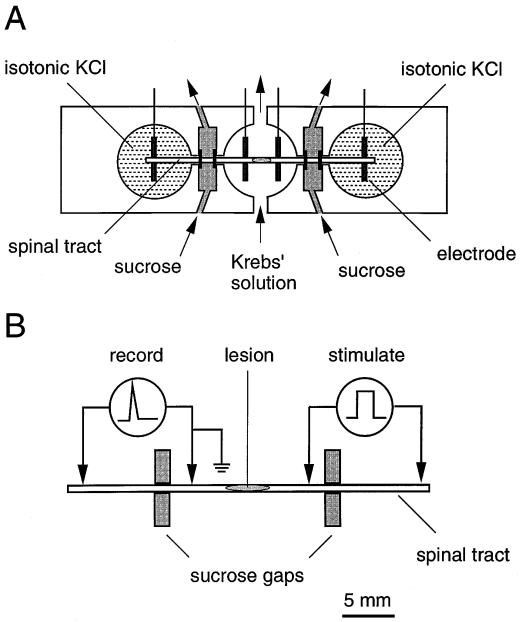

Fig. 1. Diagram of the recording arrangement. (A) The isolated spinal cord tract is shown mounted in the apparatus, with the chronic lesion site placed in the middle of the central well, which is continuously perfused with oxygenated Krebs' solution. The two ends of the tract were placed in separate wells filled with isotonic KCl, which was divided from the central well by narrow channels filled with flowing isotonic sucrose solution. The tract was sealed at both edges of each sucrose gap with shaped coverslips held in place with mineral grease. (B) The electrical stimulating and recording arrangement is shown in this scaled diagram. The 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 recorded at the left-hand gap, using a bridge amplifier.

Sucrose-gap recording

The recording arrangement is illustrated in Fig. 1. An isolated strip of white matter, approximately 35 mm long, was laid in the recording chamber, with the injured or central part of the tissue in a central well, superfused with Krebs' solution (2 ml/min), which could be changed rapidly to superfusion with Krebs' solution containing the drug. The two ends of the strip of white matter were isolated in separate wells containing 120 mM potassium chloride by sucrose-gap channels that contained flowing isotonic sucrose solution (1 ml/min), and which were sealed along their edges with mineral grease, as indicated. Silver/silver chloride wire electrodes were present in the two end wells and in the central superfusion chamber for stimulation and recording of conduction in the white matter strips. The whole apparatus was constructed on top of a Peltier plate (Cambion) that allowed control of the temperature of the chamber, and temperature was recorded with small thermocouples that could be placed at any point in the chamber with a micromanipulator. The tissue was maintained at 37°C throughout the recording procedures.

Stimulation was in the form of 0.1-ms constant current unipolar pulses, delivered to the stimulating electrodes through a stimulus isolation unit (WP Instruments). Recordings were made using a Neurodata Instruments bridge

amplifier and Neurocorder, for digital data storage on videotape. Subsequent analysis was performed using Labview® software (National Instruments) on a Macintosh computer.

The compound potential was recorded continuously at a stimulation frequency of 0.7 Hz. Stimulation current intensity was set to produce maximal compound action potential amplitude. To measure changes in compound potential amplitude, 10 responses were averaged before drug application, at approximately 30 min postdrug application, and at 30 min or more after washing with normal Krebs' solution. The amplitude change was defined as the difference in amplitude during the drug application and the average of the amplitude before drug application and after washing with normal Krebs' solution. The 4-AP (Sigma) was dissolved in the same Krebs' solution used for normal superfusion, and the pH of the solution was restored to 7.3, where necessary, by addition of a small amount of 0.1 N hydrochloric acid.

Histology

At the end of the experiment, the spinal cord strips were fixed by immersion in 5% glutaraldehyde in phosphate buffer. Transverse and longitudinal blocks were cut from the center and immediate surroundings of the lesion site, respectively. These were washed in buffer, postfixed in 1% osmium tetroxide, dehydrated and embedded in plastic. Semithin (1 μm) sections from these blocks were stained with 1% Toluidine Blue, observed and photographed with the light microscope.

RESULTS

The chronically injured animals were characterized by a narrowing of the diameter of the spinal cord over the length of the compression site. Toluidine Blue-stained sections through the center of the lesion showed similar histopathology to previous descriptions of this model [6,7,8]. Large numbers of axons survived in the subpial rim of tissue but there was extensive disturbance of the parenchymal architecture, including gliosis, fibrosis, demyelination, partial remyelination and invasion of Schwann cells. In transverse sections (Fig. 2A,B), complete demyelination could be detected, but was scattered and infrequent, although the myelin sheaths that were present were frequently abnormally thin, as in previous studies.[6,9] Surviving nerve fibers were present at the center of the injury, even in spinal cords from which no compound action potentials could be recorded (Fig. 2B). In longitudinal sections, there was evidence of significant paranodal asymmetry in many nerve fibers, with thinly myelinated or completely demyelinated paranodal regions (Fig. 2C,D). Such changes occurred to different extents within different tracts of the same spinal cords as well as varying in overall predominance between animals. The thinning and fibrosis of the lesion center facilitated accurate identification of the injury site.

With the lesion site at the center of the perfusion chamber, conduction of action potentials across the chamber could be detected in 15 out of 22 cords. Conduction was always recorded in strips from uninjured animals and in uninjured parts of the spinal cord of those with injury. The compound potentials recorded from injured segments were smaller in amplitude than in uninjured tissue, and tended to be of longer latency, but similar in form, with a simple monophasic action potential. The compound potentials of injured and uninjured cords were similar to intra-axonal recordings of myelinated axons in mammalian spinal cord.[4,5,11] This similarity included a relatively slow repolarization phase and the presence of long-lasting, depolarizing afterpotentials.

Application of 4-AP in the bathing solution at concentrations up to 100 μM had little effect on the amplitude of compound action potentials in uninjured tissue (Fig. 3A), although concentrations of 100 μM and higher increased the amplitude of the depolarizing afterpotential, and this reversed only slowly on washing. In injured spinal cords, application of 4-AP at concentrations between 1 and 100 μM produced substantial increases in amplitude of the compound potential (Figs 3B, 4), which were fully reversible by washing with normal Krebs' solution. The increase in amplitude of the compound potential was not related to a change in waveform, as could be seen when the amplitudes were digitally normalized (Fig. 3C). The proportional increase in amplitude of the compound potential in injured spinal cords with 10 μM 4-AP correlated inversely with the initial amplitude of the compound potential (Fig. 5). The increase averaged 40% across all cords (Fig. 4) but, in spinal cords with small initial responses, it ranged up to 103% (Fig. 5). The threshold for effect of 4-AP on response amplitude fell in the range of 0.5–1 μM. There was no obvious relation between the time postinjury and the response to 4-AP in this set of animals, between one and six months postinjury (data not shown).

At 1–10 mM, 4-AP produced a concentration-dependent depression of the action potential amplitude, which was similar in both injured and uninjured spinal cord (Figs 4, Fig. 6). This decrease in amplitude of the compound potential was accompanied by a marked increase in the amplitude of the depolarizing afterpotential, and by a characteristic baseline depolarization (Fig. 7). By comparing the size of this shift with the depolarization achieved by cutting the spinal cord at the edge of the sucrose gap, or by perfusing isotonic potassium chloride in the recording chamber, the axonal membrane depolarization produced by 10 mM 4-AP was calculated to be approximately 25 mV. Peripheral nerve from the same animals showed much smaller changes in action potential amplitude, afterpotential and membrane potential in response to 10 mM 4-AP (Fig. 7). The depression of conduction and the increase in depolarizing afterpotential were not fully reversible by washing in nerve or spinal cord (Fig. 6).

DISCUSSION

These experiments have confirmed the enhancement of action potential conduction in chronically

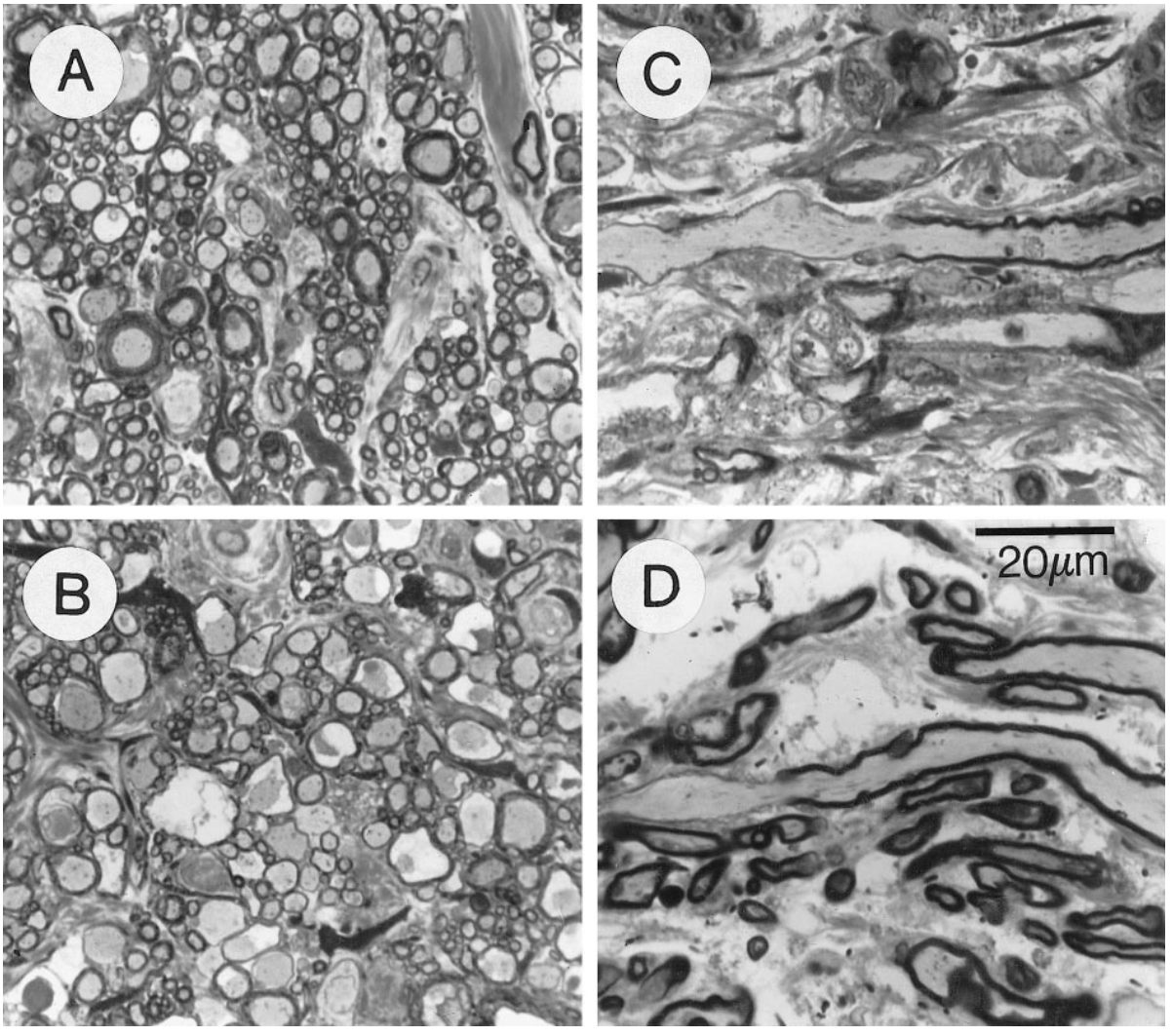

Fig. 2. Photomicrographs of Toluidine Blue-stained, 1-μm sections through the center of the lesion site in four different spinal cord tracts. (A) Transverse section through an isolated tract which showed a substantial increase in compound potential amplitude with 10 μM 4-AP at four months postinjury. Many of the axons are moderately thinly myelinated, while others show relatively normal sheath thickness. (B) A similar section from an isolated tract which showed no detectable compound potential at six months postinjury. Most of the axons have very thin myelin sheaths and a few are completely demyelinated. (C) Longitudinal section from a tract isolated at six months postinjury, showing a striking asymmetry in the myelin thickness on either side of a node of Ranvier. (D) A similar section from another tract at six months postinjury, with thin myelination on one paranode and Schwann cell myelination of greater thickness on the next. The tracts shown in (C) and (D) both showed increased compound potential amplitudes in response to 10 μM 4-AP.

injured mammalian spinal cord white matter by 4-AP that was seen previously in a small number of intracellular recordings.[5] The increase in amplitude of the maximal short-latency compound potential is interpreted as an increase in the number of axons that conduct through the lesion site. This interpretation is based on factors in addition to its consistency with single-fiber studies. First, there was no change in the poststimulus delay, peak latency or overall waveform of the response (Fig. 3B,C), which would have been expected if there was simply an increase in duration of the action current in the vicinity of the recording gap, a summation of repetitive action potentials in response to a single stimulus, or a change in the conduction velocity and synchronization of fibers. Second, the injured area of the spinal cord was restricted to the middle of the central chamber, the axons in proximity to the recording gap were essentially uninjured, with normal morphology, and 1–100 μM 4-AP had no effect on the amplitude of the compound potential in uninjured parts of the same spinal cords (Fig. 3A).

The results show a threshold for recovery of conduction in chronically injured cord at 4-AP concentrations of 0.5–1 μM, a peak at 10–100 μM and a reversal to active suppression at 1–10 mM. Previous

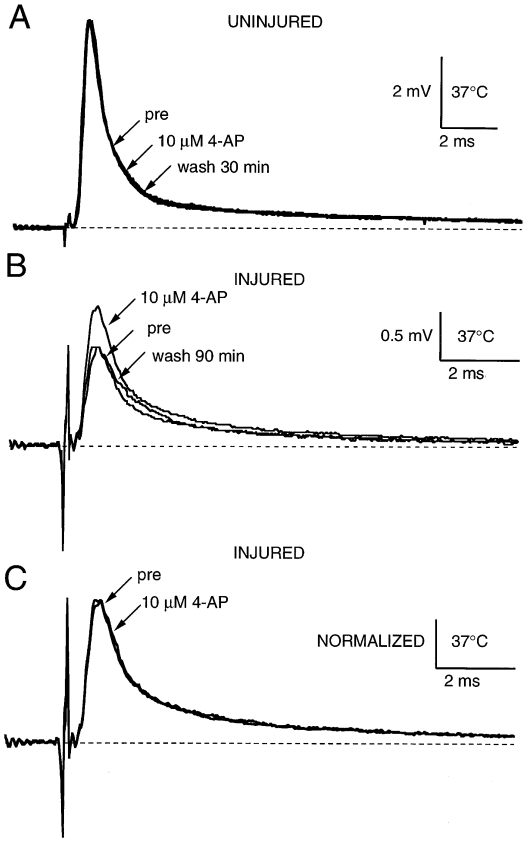

Fig. 3. (A) Three superimposed, averaged compound potential recordings from an uninjured strip of spinal cord white matter. The maximal response consists of a monophasic action potential, followed by a depolarizing afterpotential which is similar in form to intracellularly recorded action potentials in single fibers. The form and amplitude of the potential were unchanged by 30-min exposure to 10 μM 4-AP or by 30-min wash in normal Krebs' solution. (B) A similar set of recordings for a strip of white matter from a spinal cord injured four months previously. In this case, the amplitude of maximal response increased during exposure to 10 μM 4-AP and returned to its original value after washing in Krebs' solution. Slight modification of the depolarizing afterpotential remained even after 90 min of washing. (C) Superimposition of the compound potentials recorded before and after 4-AP in (B), but with the vertical scales normalized to the peak. This shows no change in the shape of the potential accompanying the large increase in amplitude.

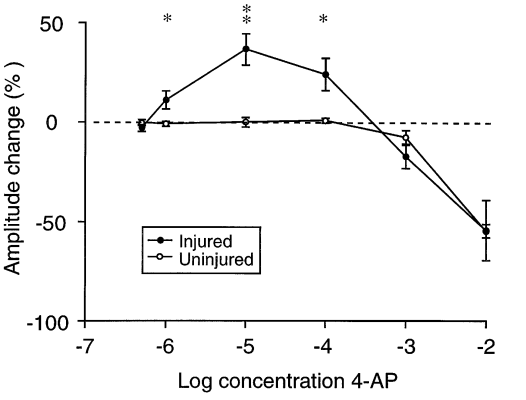

Fig. 4. Graph showing the relation between 4-AP concentration and the mean (±S.E.M.) change in compound potential amplitude recorded in injured and uninjured spinal cord strips. The plots were derived from a total of 40 experimental and 34 control measurements. At 0.5 μM there was no significant change in amplitude and no difference between injured and uninjured cords. At 1 and 10 mM there was a significant depression of compound potential amplitude, with no difference between injured and uninjured. At 1, 10 and 100 μM a significant increase in amplitude occurred in the injured but not the uninjured tissue. The two groups differed significantly at these three concentrations (ANOVA, $*P<0.05$; $**P<0.005$).

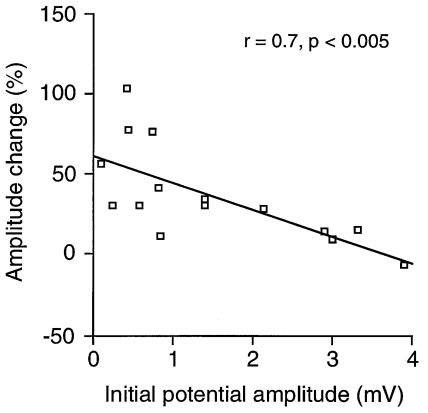

Fig. 5. Scattergram showing the maximal compound action potential amplitude change observed with 10 μM 4-AP plotted against the initial compound potential amplitude, for 15 different isolated tracts. There was a significant negative correlation between the two measurements.

studies of 4-AP effects on axonal conduction block have employed much higher concentrations of the drug, but mostly without establishing the concentration–response characteristics. One other report specifically points to a much lower sensitivity to 4-AP in demyelinated axons.[22] Different types of axonal pathology may be associated with different sensitivity to potassium channel blockade. That interpretation is supported by our own finding, using identical techniques in acute traumatic injury, where enhancement of conduction appears to require higher concentrations of 4-AP (unpublished observations). The morphological deficits in acute and chronic injury are quite different. There is no equivalent in the first few hours or days from injury of the irregular thin myelination or aberrant Schwann cell myelination of central axons seen in chronically injured tissue[6,10,13] (see also Fig. 2). In addition, there may be species differences in the biophysical characteristics of myelinated axons and responses to drugs.

The sensitivity of vertebrate neuronal potassium channels to 4-AP has been explored in a wide variety of physiological preparations, although few studies have examined a full range of concentrations. The response characteristics seen in this study appear most comparable to the fast delayed rectifier of the amphibian node of Ranvier which was shown to be

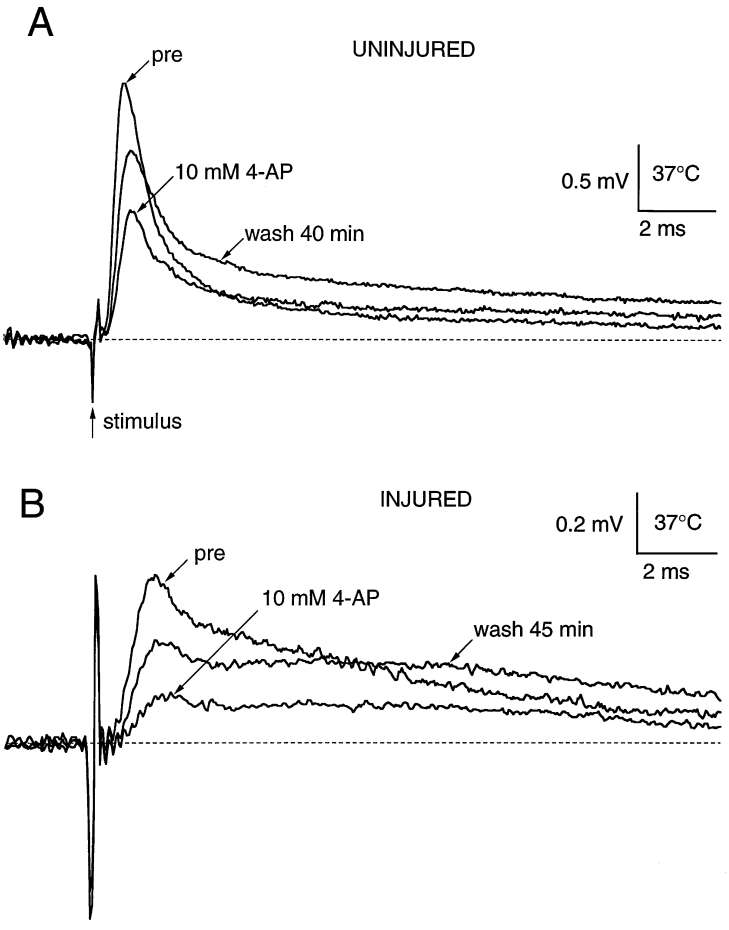

Fig. 6. Recordings similar to those in Fig. 3, showing the effect of 10 mM 4-AP on (A) uninjured, and (B) chronically (4.5 months) injured spinal cord tracts. Note that the depression of the compound potential amplitude seen with 4-AP application was partially reversible with prolonged washing, whereas the increase in depolarizing afterpotential amplitude was not readily reversed.

50% blocked by 10 μM 4-AP, with a threshold for block between 10^{-7} and 10^{-6} M,[18] although it has been noted that the latter measurements were performed under conditions that make the data difficult to relate to normal physiology.[2] Similar fast potassium channels appear to be present in the paranodal region of the mammalian axon.[15] Increased quantal content at the frog and mouse neuromuscular junction is measurable in the presence of 4-AP between 1 and 5 μM, with much more powerful effects above 5 μM.[31,32] A slowly inactivating K^+ current in mammalian nodose ganglion neurons is blocked by 4-AP in the range of 1–30 μM.[39] By contrast, delayed rectifier currents in a variety of other neurons are blocked only by 4-AP concentrations in excess of 1 mM, and neuronal A-currents are usually much less sensitive to 4-AP, with K_d values typically in the range of 1–2 mM.[34,35] These considerations raise the possibility that the differential sensitivity of different types of axonal conduction block to 4-AP may represent the participation of distinct types of potassium channel, and is perhaps related to differences in distribution of those channels within the axon, and the specific morphological reorganization that may be involved in the underlying pathology.

The current interpretation of potassium channel distribution in myelinated fibers is that a population of fast-activating channels, blocked by millimolar concentrations of 4-AP, is present, principally in the internode, perhaps concentrated in the paranodal region, while the more classical, tetraethylammonium-sensitive delayed rectifier is present in the nodal membrane and perhaps the internode as well.[1,3,48] The present data indicate the existence of a subdivision of the fast channel on the basis of a higher sensitivity to 4-AP, similar to that described in the frog.[18] The distinct response to low concentrations of 4-AP seen here in chronic injury might result from molecular changes in the channel itself or simply be a reflection of normal physiology that is revealed by the consequences of structural alterations in the myelin sheath.

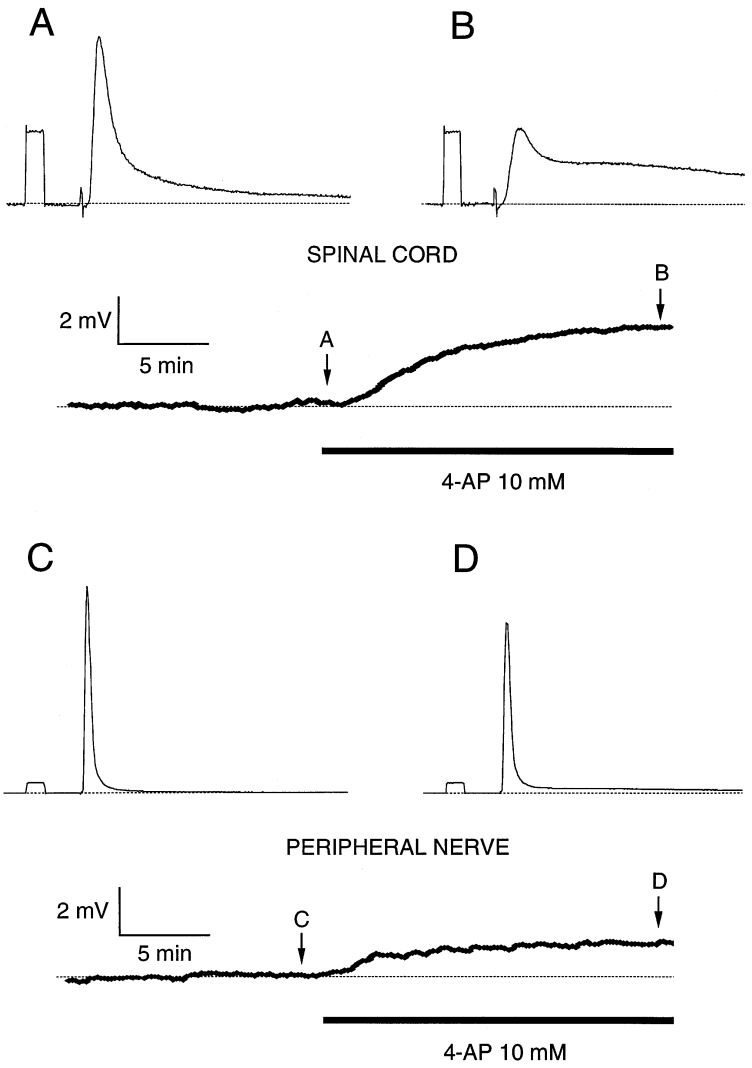

Fig. 7. Recordings, similar to those in Figs 3 and 6, showing sucrose gap recordings from a spinal cord strip (above) and a peripheral sciatic nerve (below). The apparent change in membrane potential with superfusion of 10 mM 4-AP is shown below representative averaged recordings of action potentials, before (A,C) and after (B,D) drug application. The central nerve fibers show a marked depression of the action potential amplitude, a large increase in depolarizing afterpotential, and a strong depolarization (equivalent to approximately 25 mV) following 4-AP superfusion. The peripheral nerve shows a smaller reduction in compound potential amplitude, a small amplitude depolarizing afterpotential, and a smaller membrane depolarization (equivalent to approximately 9 mV) with the same concentration of 4-AP. Spinal cord and nerve were cut at the face of the recording gap at the end of the experiment to measure the equivalent amplitude of the resting membrane potential.

Suppressive effects of higher concentrations

The reduction in compound potential amplitude with millimolar levels of 4-AP was unexpected. Although suppressive effects of higher concentrations on peripheral nerve were reported in early experiments,[30] others have seen no more than a slight depression of extracellularly recorded potentials in peripheral nerves,[14] and this was our own experience using peripheral nerves in the same recording arrangement used here (Fig. 7). A negative effect on central axonal conduction of 5 mM 4-AP is also apparent in data derived from uninjured and acutely injured rat spinal cord, showing a decrease in amplitude and increase in latency of the compound potential.[21] Superfusion of adult rat optic nerve and spinal cord with 1–3 mM 4-AP apparently produced measurable depression of the compound action potential recorded in close proximity to the stimulus.[23,28,29] The concurrent depolarization, seen here in the sucrose-gap recordings (Fig. 7) may be due to blockade of resting potassium conductance by 4-AP, and may partly explain the reduction in amplitude of the compound potential.

Depolarization of the axon would increase the inactivation of sodium channels in the nodal

membrane. In this case, the decrease in amplitude of the compound action potential would result from a decreased amplitude of the action currents themselves, but would not necessarily reflect conduction failure. In fact, higher concentrations of 4-AP may simultaneously improve conduction in injured axons while decreasing the size of the compound potential recorded from the uninjured part of the spinal cord strip. This may explain why 1 mM 4-AP previously was found to improve conduction in some single-fiber recordings, increasing the temperature of conduction block,[5] while similar concentrations tended to reduce the amplitude of the compound action potential in this study.

Methodology

The sucrose-gap technique for recording compound action potentials has proven useful in this context. It provides an effective compromise between intracellular and extracellular recording techniques, with a combination of stability and accurate representation of the underlying action potential. It is also much less susceptible to stimulus artifact than extracellular microelectrode recordings. It is equivalent in most respects to the use of suction electrodes in optic nerve recordings[42] but avoids the mechanical disturbance of the tissue which makes suction electrodes difficult to use with the less compactly structured spinal cord white matter. We chose to use peak amplitude of the compound action potential rather than the integrated amplitude (area under the curve) which has been used elsewhere.[43,44] This is justified by the problem that extension of the duration of the action potential, increase in afterpotential amplitude, or initiation of multiple spikes (all of which may occur with high concentrations of 4-AP) would be expected to increase the integrated amplitude of the potential but would not represent an increase in conducting fibers. As can be seen in Fig. 3B, the increase in amplitude in chronically injured tissues is associated with a well-synchronized, short latency response and is not the result of a temporal distortion of the response at the recording site. Conversely, in Fig. 6A, the effect of higher levels of the drug on the normal action potential is to increase the afterpotential, which would increase the integrated action potential, even though there is clearly depression of the peak amplitude of the response.

The basis for the variation in responsiveness of spinal cord tracts from different animals may lie in differences in the severity of original demyelination and the nature of subsequent remyelination. This would be consistent with the fact that the largest proportional increases in amplitude were seen in those cases with the smallest amplitude initial responses, indicating the most severe primary and secondary tissue disruption. Evidence from previous studies correlating morphological and behavioral aspects of this injury model indicate that the proportional extent of secondary damage to myelination and the degree of repair is notably independent of the initial injury, and may depend more on posttraumatic inflammatory responses and other pathophysiological variables.[8,9]

Clinical effects

The present studies address the issue of dose–response for the effect of 4-AP on action potential conduction in chronically injured mammalian spinal cord. This is important for interpreting findings from human subjects and in planning further developments of this approach to symptomatic treatment in spinal cord injury and perhaps multiple sclerosis. The clinical significance of previous laboratory demonstrations of restoration of conduction with 4-AP has been called into question on the basis of experience with a chemically induced (ethidium bromide) demyelination in rats. In those studies, it was found necessary to use 0.1–0.5 mM 4-AP *in vitro* to restore conduction in a proportion of demyelinated axons.[22] Such high concentrations could not be achieved in human subjects, using systemic delivery, without incurring unacceptable toxic effects of the drug. Felts and Smith[22] calculate from various sources that maximal cerebrospinal fluid (CSF) levels in human subjects would be approximately 5 μM, and others have suggested higher concentrations.[16] Recent studies in dogs showed that intravenous bolus injection of 0.5 mg/kg 4-AP gave CSF levels that did not usually exceed 50 ng/ml. In human subjects, infused with lower doses of the drug, plasma levels rose slowly to approximately 100 ng/ml,[24] which would be expected to produce CSF levels no higher than 1 μM.

However, the pharmacokinetics of this drug are still incompletely understood, as evidenced by the observation that CSF levels of the drug peak immediately following a bolus injection and continue to drop steadily, although plasma levels appear to remain five to 10 times higher.[33] This may mean that 4-AP is transferred to a third compartment, more accessible to the CSF than to plasma, perhaps incorporated into the white matter, from where it is subsequently cleared only very slowly, by which point the serum concentration becomes unmeasurable by high-performance liquid chromatographic techniques (<10 nM). Under these circumstances, the microscopic distribution of the drug might become important, and in particular the periaxonal concentration might be different from that in the free CSF.

The results of the current experiments indicate that 4-AP affects conduction in chronically injured axons at concentrations as low as 1 μM. Hence it remains likely that clinical effects of systemically administered 4-AP in humans involve restoration of conduction in damaged axons. This does not mean that effects at the synaptic level do not also play a major role. Profound amplification of synaptic potentials by 0.5–1 mg/kg doses has been shown in anesthetized

cats.[27] Since similar doses given to awake cats have no obvious behavioral effects, the extent to which this synaptic amplification represents antagonism of anesthetic depression is not clear. In the context of synaptic effects, it may be relevant that at least some clinical responses in humans appear to persist well beyond the effective presence of the drug in serum.[24] This may be consistent with the fact that enhanced synaptic transmission in the anesthetized cat persisted undiminished for the full 6 h of experimental recording following a single dose,[27] although serum half-life is expected to be considerably shorter than this.[20,36,46] Given the reversibility of the effect of low doses on axonal conduction shown here, it may eventually be possible to differentiate rapidly reversible and longer lasting effects of 4-AP as dependent on axonal and synaptic mechanisms, respectively. In this regard, it is interesting that prolonged effects have been more apparent in spinal cord injury, which is marked by extensive loss of axons and synaptic input to the spinal cord, than they have in multiple sclerosis, which is principally characterized by axonal demyelination.

Finally, although reversal of conduction block in axons may be a significant factor in the beneficial effects seen with systemic 4-AP, such benefits would not be expected to be optimal within systemically tolerable doses. The greatest increase in conduction was seen with 10 μM 4-AP, a concentration that can only be achieved safely with direct application to the spinal cord lesion, avoiding exposure of the rest of the nervous system. Therefore, intrathecal administration may prove to be the most effective therapeutic approach, as has been suggested previously.[12,33]

CONCLUSIONS

4-AP can overcome conduction block in chronically injured spinal cord axons at concentrations as low as 1 μM, which may be clinically achievable with maximal systemic doses in human subjects. Maximal increase in conduction is seen at 10 μM. There is a paradoxical suppression of conduction in normal and injured spinal tracts with concentrations of 4-AP above 1 mM. This suppression appears to be associated with axonal depolarization. Similar but smaller effects of high concentrations are seen in peripheral nerve.

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