Symptomatic Therapy for Demyelinating Diseases: The Role of Basic Science

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Introduction

Aberrant impulse conduction in demyelinated axons in the central nervous system cause symptoms typical of multiple sclerosis (MS) and related diseases including blind spots (scotomas); loss of motor control; absence of sensation; and parethesias ("ting-lings"). Conduction block is the major reason for loss of function (44); while paresthesias arise from an increased excitability of demyelinated, conducting fibers (27). The pathophysiology of demyelinating disease can be understood in terms of the conduction safety factor, defined as the ratio of the current generated during an action potential to the minimum (threshold) amount needed to maintain conduction. A normal safety factor is 6-7 and conduction block occurs if it falls below 1. Local anesthetics inhibit pain by reducing action potentials (and safety factor); while in MS there is short-circuiting of current in demyelinated nerves.

In MS frequent, spontaneous exacerbations (new symptoms and/or worsening of existing ones) and remissions occur because demyelinated fibers are delicately balanced with conduction safety factors near one (44). Conduction block takes place if an event (such as inflammation) decreases safety factor; while conduction is restored when the safety factor increases. Many patients (60-80%) are strikingly sensitive to small changes in body temperature. Fever causes a worsening similar to natural exacerbations and cooling results in improvement (33,36,46,55). Temperature-sensitivity arises because heating shortens action potentials and blocks borderline fibers; while cooling lengthens action potentials and can restore conduction in blocked fibers (12,39,40,41,44). The effect of cooling suggests that the symptoms of MS might also be relieved by drugs that increase the conduction safety factor.

Mathematical simulations were used to understand how temperature, calcium, and drugs affect conduction in MS (44). Normal nerve models (11,17,20) were modified to describe demyelination by altering the cable properties for a single fiber (29,47). Temperature effects were calculated by scaling ion channel rate constants by known amounts (18,22), while the action of calcium was obtained from the Ca^{++}-dependence of the same rate constants. The results agreed with observations on normal and demyelinated nerve fibers (3,12,13,38,41). For example, reducing calcium decreases threshold, increases the safety factor and causes transient improvements in MS (13).

There are many K^+ channels (15,16,17,18). The "classical" K+ channel identified by Hodgkin and Huxley (22) in found at nodes in nonmammalian myelinated nerves. In normal mammalian fibers, these K+ channels are excluded from nodes and the action potential is insensitive to K+ channel blockers (7,8,26). Potassium channels in the paranodes and internodes of mammalian nerve are exposed in demyelination, rendering them sensitive to K+ channel blockers (8,9.10,26,42,43,49). Impulses in developing and regenerated fibers are prolonged by K+ channel blockers (27,28,42,57). However, this does not localize K+ channels to nodes, it only shows they contribute to a nerve impulse. For example, mammalian and nonmammalian myelinated fibers may differ in the electrical or chemical accessibility of paranodal and internodal K+ channels.

In addition to cooling and lowering calcium, mathematical simulations indicated conduction in demyelinated nerve could be improved pharmacologically by blocking K+ *channels and by slowing closing (inactivation) of Na+* channels, because both prolong action potentials and increase safety factor (44). For example, the K+ channel blocker tetraethylammonium (TEA), should restore conduction. In animals TEA causes improvement (5,6,38), but it cannot be used in MS because of its side effects. The drug 4-aminopyridine (4-AP) also blocks nodal K+ channels and restores conduction in demyelinated animal models (5,6,14,16,25,26,38,40,45,51). Since 4-AP is used to treat myasthenia (30,31,32), it was natural to study its effect in MS.

Patients improve within minutes after receiving low does of 4-AP (23,50). The reason 4-AP affects demyelinated nerve fibers is that paranodal and internodal K+ channels are exposed and are 4-AP sensitive. However, the plasma levels of 4-AP may be as low as 1 μM in MS patients and micromolar amounts of 4-AP do not affect K+ channels in nonmammalian nerve fibers (16,25). The beneficial effect of 4-AP in mammalian systems would be expected if paranodal and internodal K+ channels were more sensitive than nodal channels. To compare nodal and non-nodal K+ channels one needs to study both populations in one fiber because K+ currents vary and 4-AP access may differ. In frogs the density of nodal and paranodal K+ channels depends on diameter (49). Large fibers have nodal K+ channels and the exposure of the paranode by demyelinating agents does not produce much of a change in K+ current. Small fibers lack nodal K+ channels, so that K+ currents are only seen following LPC demyelination. We took advantage of this size-dependence to show that the paranodal and internodal K+ channels exposed by demyelination are more sensitive to 4-AP than K+ channels present at the nodes.

Methods

Studies of Single Nerve Fibers

Frog fibers 8-10 μM in diameter (small) or 16-18 μM (large) were voltage-clamped by standard techniques (15,37,49). The external solution contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 5 mM Tris (pH of 7.30; 18-20 °C). Fibers were held at -80 mV and depolarized to voltages between -40 mV and $+100$ mV. Currents were recorded digitally and converted to conductances. Tetrodotoxin (1 μM) was used to reversibly block Na+ currents when K+ channels were being studied. Internodal resistance, resistance of the vaseline seals, nodal leakage current, nodal capacity, and the Na+ equilibrium potential were frequently measured. Continuous monitoring is important because internodal resistance gives membrane current; nodal capacitance indicates the nodal area; and internal Na+ can block K+ channels.

In untreated large and small fibers the capacity transient is exponential. To disrupt the paranode and expose paranodal and internodal K+ channels 1% lysolecithin (LPC) was applied for 1-2 minutes (49). Following LPC treatment the capacity transient had fast and slow components. The time constant (40-60 μsec) of the fast component was the same as prior to LPC, while the slow component had a time constant of 1 msec becaue the paranodal capacitance is being charged via a large series resistance. The integral of the slow component varied depending on the paranodal membrane exposed. Untreated large fibers had a nodal capacity of 1.4-1.6 pF and a nodal resistance of 34-46 megohms. Treatment with LPC elevated the capacitance (up to 55 pF); by an amount that depended on fiber diameter (49) but did not change the magnitude or time course of the Na+ current. In most cases LPC increased the nodal capacitance without any visible change, suggesting that LPC can disrupt the paranodal junction.

Animal Models of Demyelination

In peripheral nerve fibers the immediate reaction to a breach of the perineural sheath is herniation of the underlying fibers. Nerve fibers in this "perineural window" demyelinate in 3-6 weeks and later remyelinate (37). Demyelinated nerve trunks can be removed and mounted in a recording chamber to measure the number of conducting fibers (37). If the temperature of the recording pool is increased, many borderline conducting fibers are blocked, reducing the size of the compound action potential. If one subsequently applies drugs to the recording pool, any increase in amplitude of the compound action potential indicates restoration of conduction. This sytem is a sensitive way to determine the effect of pharmacological agents.

Clinical Studies of 4-Aminopyridine

Since hyperthermia reversibly blocks borderline-conducting demyelinated nerve fibers, temperature-sensitive MS patients are most likely to respond to drugs. Patients with thermally labile neurological deficits were selected using a water bath or a temperature controlled NASA astronaut space suit. Elevation of body temperature to 100-102 °F was sufficient to bring out latent signs. Paients also met the following criteria: (a) definite diagnosis of MS, (b) males not older 47, and (c) no history of other disease. Visual function, neurological exams, and vital signs (temperature, blood pressure, pulse, respiration, EKG, blood chemistries, and urine analysis) were monitored before; at intervals during administration of 4-AP or placebo; and until any changes observed reversed to pre-treatment levels.

Visual acuity, visual fields, and critical flicker fusion (CFF) were monitored. To measure acuity, two lines 0.14 minutes in width were displayed on an oscilloscope screen 0.3-3.0 meters from the patient, depending on the severity of the baseline visual impairment. The lines were initially superimposed and separated at constant velocity until a patient signaled two lines. The angular separation between the lines indicates acuity and the results of 5 trials were averaged for each eye. Central visual fields were monitored with the Goldman Perimeter. A light turned ON and OFF at low frequency is perceived as flickering. As frequency increases, a point is reached where the flicker disappears and a steady light is seen (the CFF). Five trials were averaged for each eye. Neurological exams were videotaped to qualitatively assess a broad spectrum of function. Non-visual neurological symptoms were rated on a scale of 0-6 with grade 0 representing normal performance and grade 6 a deficit so severe that the function was nearly or completely absent (e.g., inability to walk). Changes of two or more grades were considered significant, but improvement was only noted if the change in neurological function reversed.

The study was blinded for the patient. The neurologist administering 4-AP was not blinded to ensure appropriate management of adverse reactions. However, the overall evaluation of 4-AP was blinded because a technician not knowing if 4-AP was given obtained data on visual function; while the videotaped neurological exams were evaluated by a neurologist unaware of whether drug of placebo had been administered.

Results

Restoration of Impulse Conduction by 4-AP in Demyelinated Nerve

Figure 1 illustrates the compound action potential recorded from demyelinated frog sciatic nerves and is similar to results previously reported (38). Trace A was obtained at 25 °C and B at 28 °C. The integral of trace B is 22% of the integral of trace A, suggesting that 80% of the fibers are blocked by a 3 °C temperature elevation. Trace C was obtained 5 minutes after the application of 0.5 mM 4-AP to the recording pool and indicates a nearly complete restoration of conduction to the blocked fibers. Several other drugs were tested. Those increasing the amplitude of the compound action potential were

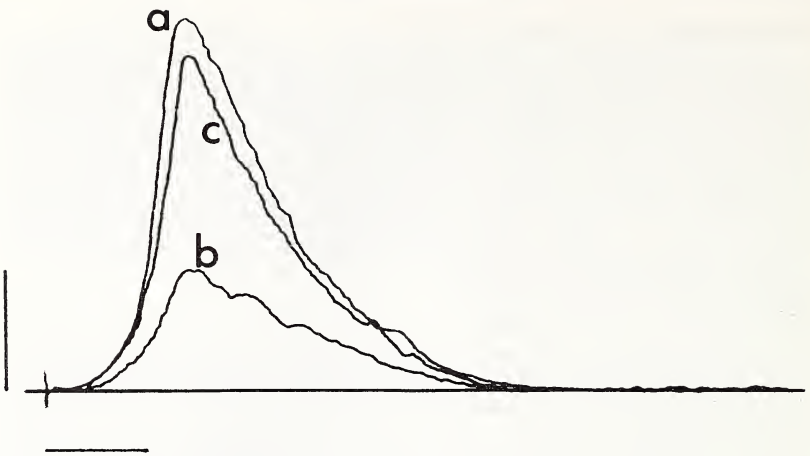

FIGURE 1. Compound action potentials in a frog sciatic nerve 26 days following perineural window-induced demyelination. Trace A was obtained at 25 °C and trace B at 28 °C in normal Ringer's solution. Trace C shows the compound action potential 5 minutes after adding 0.5 mM 4-AP to the recording pool. Voltage and time calibrations are 50 uV and 1.0 msec respectively.

the K+ channel blockers tetraethylammonium (TEA) and gallamine; and three inhibitors of Na+ inactivation including scorpion venom, n-bromoacetamide, and amantadine. The first four compounds have been investigated previously (6,38,41,48). Amantadine is primarily an antiviral drug, but is also used to treat Parkinson's disease. Studies (in progress) of voltage-clamped *Myxicola* giant axons have demonstrated that amantadine selectively slows Na+ inactivation, particularly at negative membrane potentials.

Differential Effects of 4-AP on Nodal and Paranodal K+ Channels

Three classes of frog myelinated nerve fibers were examined: (1) small fibers that normally lack K+ currents, but where large K+ currents can be induced by LPC, (2) large fibers in which LPC produces no more than a 10% increase in K+ current, and (3) intermediate-sized fibers where LPC treatment can double the amount of K+ current. Figure 2 shows that 4-AP selectively blocks the paranodal K+ channels exposed by LPC in small fibers. The left-hand records were obtained from untreated large (top) and small (bottom) fibers with Na+ equilibrium potentials of +52 mV and +54 mV respectively. Large fibers had a K+ conductance which averaged 30% of the Na+ conductance, while small fibers had only inward Na+ currents.

In large fibers, application of 20 μM 4-AP decreased the K+ current by 45-50% at all voltages, but did not affect the nodal Na+ current (top center records in Figure 2). At 1 mM, 4-AP completely eliminated the K+ current, again without altering the Na+ current (or the nodal capacitance, internodal resistance, or Na+ equilibrium potential). The filled circles in Figure 3 show the dose-response curve for 4-AP obtained in separate experiments on two large fibers. The solid line was calculated assuming a single 4-AP binding site with a dissociation constant of 20 μM and provides an adequate fit over the entire concentration range tested.

Treatment with LPC causes a large increase in the K+ current in small fibers without any change in the Na+ current or Na+ equilibrium potential (bottom center records in Figure 2). These LPC-induced K+ currents were associated with a 14-fold increase

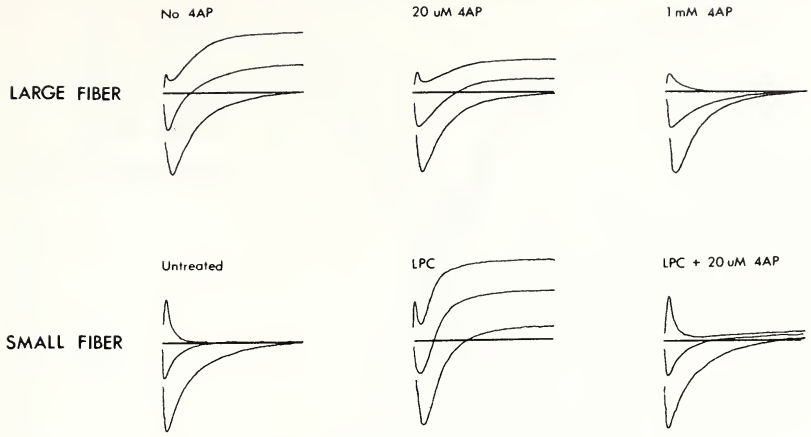

FIGURE 2. The effect of 4-AP on currents at single nodes of Ranvier for depolarizations to −30 mV, +20 mV, and +70 mV in normal and lysolecithin (LPC) treated fibers. The records in the upper portion of the Figure were from a 17 μM fiber, while those at the bottom were from a 9 μM fiber. The large fiber has a 4-AP sensitive K+ current (top center and right-hand records). Untreated small fibers lack K+ currents (lower left-hand record), but K+ currents appear following LPC (lower center). The LPC-induced K+ currents are completely blocked by 20 μM 4-AP (lower right), while the same concentration of 4-AP only produces a 50% inhibition of nodal K+ currents in untreated large fibers (top center records). The current calibration is 16 nA for the large fiber and 8 nA for the small fiber. The time scale is 2 msec for both.

in the nodal capacitance measured by integrating the area under the total capacity current (fast and slow components). In LPC-treated small fibers, 20 μM 4-AP completely eliminated the K+ current (bottom right records in Figure 2) and the dose response curve for two different small fibers is shown by the open circles in Figure 3. The behavior of these fibers is consistent with a decrease in the 4-AP dissociation constant from 20 μM to 4-5 μM, so that paranodal K+ channels exposed by LPC are 4-5 times more sensitive to 4-AP than are nodal K+ channels. Although the sensitivity to 4-AP differs, both nodal and paranodal K+ channels have a similar voltage- and time-dependent activation (not illustrated).

While paranodal K+ channels are preferentially blocked by 4-AP, they are less sensitive to TEA. Figure 4 shows that the dissociation constant for TEA on K+ channels in large fibers (filled circles and the solid line) is 0.5 mM, while that for paranodal K+ channels in small fibers is 5.0 mM. A decreased sensitivity of non-nodal K+ channels to TEA has previously been attributed to limited drug access (7,8,9,10). However, the fact that paranodal channels are more sensitive to 4-AP suggests that the differential response to TEA may also be real.

In a few experiments 4-AP and TEA were applied to intermediate-sized fibers. Untreated 12-14 μM fibers had resting K+ conductances of 0.10-0.15 $\times$ 10^{-7} S and we the dissociation constants for 4-AP and TEA were 20 μM and 0.5 mM respectively, identical to those in large fibers. When LPS was applied to intermediate fibers the K+ conductance increased to 0.22-0.31 $\times$ 10^{-7} S, but there was no change in Na+ currents. The dissociation constant for 4-AP on the LPC-induced incremental K+ conductance was 4 μM, while that for TEA was 3-5 mM. Paranodal K+ channels in intermediate

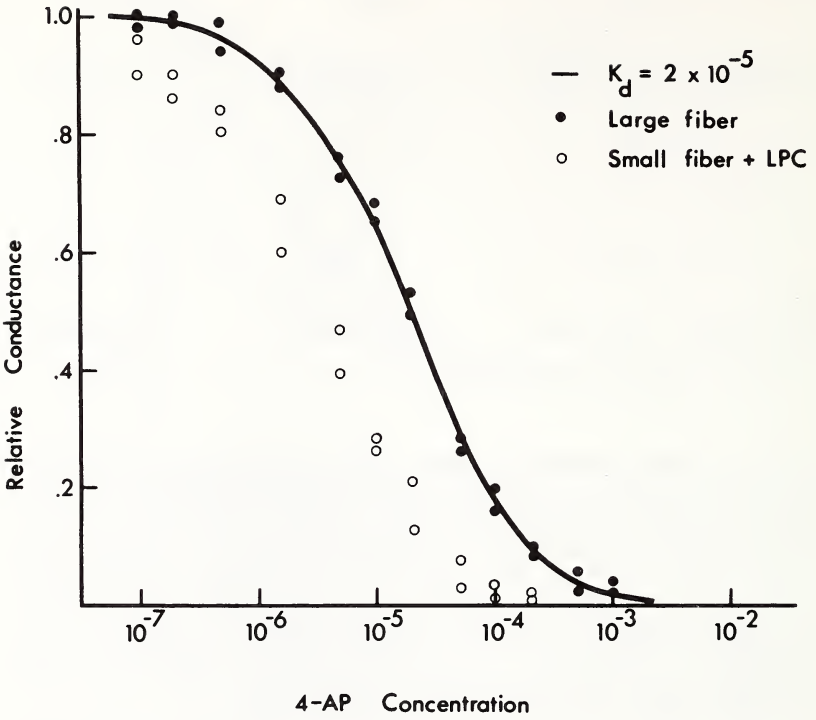

FIGURE 3. Dose-response curves for 4-AP in two different large (filled circles) and small (open circles) fibers. The ordinate is the K + conductance in the presence of 4-AP relative to that prior to drug application. The solid line was calculated assuming a single binding site with a dissociation constant of 20 μM.

fibers thus resemble those in small fibers, while nodal channels have the same pharmacological specificity as nodal K + channels in large fibers.

Effects of 4-AP in Multiple Sclerosis

In the clinical studies 4-AP was given intravenously (7-35 mg in 1-5 mg doses every 10-60 minutes over 1.5-3.5 hours) to 12 temperature-sensitive male MS patients and 5 normal men; and orally (5 mg capsules in divided doses to a total of 10-25 mg) to 9 MS patients and 6 normal controls. Nine additional male MS patients comprised a blinded control group receiving 0.9% NaCl intravenously or oral placebo. No changes occurred in vital signs, ECG, or EEG in any of the 33 individuals studied. All subjects who received 4-AP had transient peri-oral paresthesias, while those receiving placebo had no symptoms. In subjects receiving 4-AP mild dizziness sometimes occurred at 30-35 mg. This reversed 60 to 90 minutes after discontinuing 4-AP, but no subject received additional 4-AP if such symptoms developed.

Ten of 12 MS patients receiving intravenous 4-AP mildly to markedly improved ($X^2 = 10.12$; $P < 0.002$). Vision improved in 7 ($X^2 = 8.57$; $P < 0.0004$), oculomotor functions in 5 ($X^2 = 5.08$; $P < 0.024$), and motor function in 5 patients ($X^2 = 5.08$; $P < 0.024$). The improvements developed at doses as low as 2 mg and reversed 2-4 hours following the last dose. In the oral studies all 9 patients improved. No changes were seen

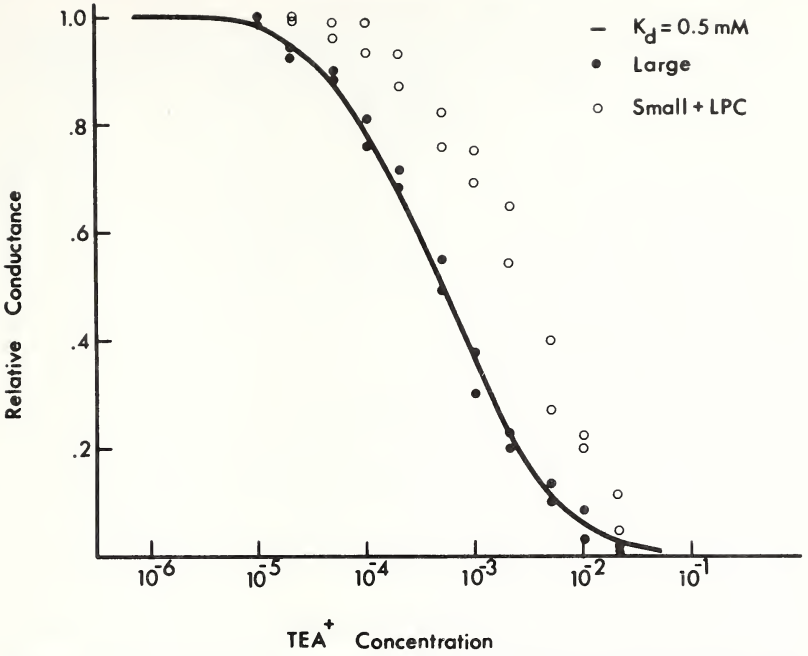

FIGURE 4. Dose-response curves for tetraethylammonium (TEA) in two large (filled circles) and small (open circles) fibers. The ordinate is the K+ conductance in the presence of TEA relative to that prior to drug application. The solid line was calculated assuming a single binding site with a dissociation constant of 0.5 mM.

in the MS patients receiving placebo. Table 1 summarizes the results of 4-AP administration. Blank spaces indicate a neurological function was not tested, either because it was normal or was not temperature-sensitive.

Figure 5 shows the effect of 4-AP on gait and visual signs. Improvement of gait (part A) began within 25 minutes after 4-AP at a dose of 2 mg and peaked at 65 minutes at a total dose of 5 mg. Reversal occurred gradually and was complete 2 hours and 40 minutes after the last dose. In MS patients CFF frequency is typically lower than the normal frequency of 40-50/sec. In another patient CFF frequency was 22/sec before 4-AP administration. After 10 mg of 4-AP, CFF rose to 45/sec and the effect gradually reversed to baseline 4 hours, 45 minutes after peak improvement. Static quantitative perimetry showed a similar improvement of visual fields (not illustrated). Before 4-AP the patient had a large central blind spot, but 25 minutes after receiving 15 mg of 4-AP the visual fields were nearly normal. The improvement was transient and the scotoma redeveloped 2 hours and 30 minutes later.

Discussion

Administration of 4-AP improves conduction in experimentally demyelinated nerve fibers in animals, while in MS patients it enhances vision, oculomotor, and motor function without serious side effects. The clinical improvements were great enough to represent a functionally significant therapy and the acute protocol used eliminates uncertainties caused by spontaneous fluctuations in the severity of symptoms in MS. The kinetics

TABLE 1. Results of 4-Aminopyridine Administration

Patient	Vision	Oculomotor	Motor	Net Effect
		Intravenous 4-AP		
1	3+	1+	0	Y
2	1+	2+		Y
3	2+		0	Y
4		3+	2+	Y
5			3+	Y
6		0	0	N
7	2+	3+	3+	Y
8	2+			Y
9	0	1+	3+	Y
10	0		0	N
11	2+			Y
12	2+		1+	Y
		Oral 4-AP		
1	2+	2+	1+	Y
2	3+	2+		Y
3	1+	1+	1+	Y
4		2+	3+	Y
5			2+	Y
6		2+	2+	Y
7	3+	1+	2+	Y
8	1+	2+		Y
9	1+	1+	3+	Y

1+ = mild improvement
2+ = moderate improvement
3+ = marked improvement
0 = no improvement
Y = positive effect
N = without effect

of 4-AP have been studied in normal subjects at serum levels of 40 μg/L (53). The half-life of 3.6 $\pm$ 0.9 hours corresponds to the duration of the improvements observed. Studies of myasthenia show 4-AP affects synapses (31,31,32). Synaptic effects probably do not cause improvements in MS because no changes in motor function in normals were seen, and enhancement of vision strongly supports a direct action of 4-AP on demyelinated optic nerves. In experimental lesions 4-AP causes repetitive firing (27). Seizures can be triggered by ectopic activity in axons (21, so MS patients might be at higher risk than normals.

Clinical changes produced by 4-AP most likely are the result of restoration of conduction in blocked demyelinated nerve fibers. Where does 4-AP act and why is it effective at low doses? The results of LPC-treatment in frog fibers provide answers to both questions. Potassium channels are absent from normal mammalian nodes and small frog fibers, but are present in the paranode and internode and become exposed in demyelination (7,8,9,10,26,51). Since 4-AP only affects K+ channels, it must act on demyelinated axons. However, theoretical simulations suggest the crucial safety factor decrease occurs at the junction between the normal region of a myelinated fiber and a demyelinated segment (40,56,57,58,60). If 4-AP were restoring conduction here, it would be doing so by acting at the last normal node. Since normal nodes do not contain K+ channels, an effect of 4-AP would require some change that would expose paranodal K+ channels, such as a loosening of the myelin seal.

Paranodal K+ channels have a four-fold lower dissociation constant for 4-AP than nodal K+ channels. If internodal K+ channels are similar, then 4-AP should preferen-

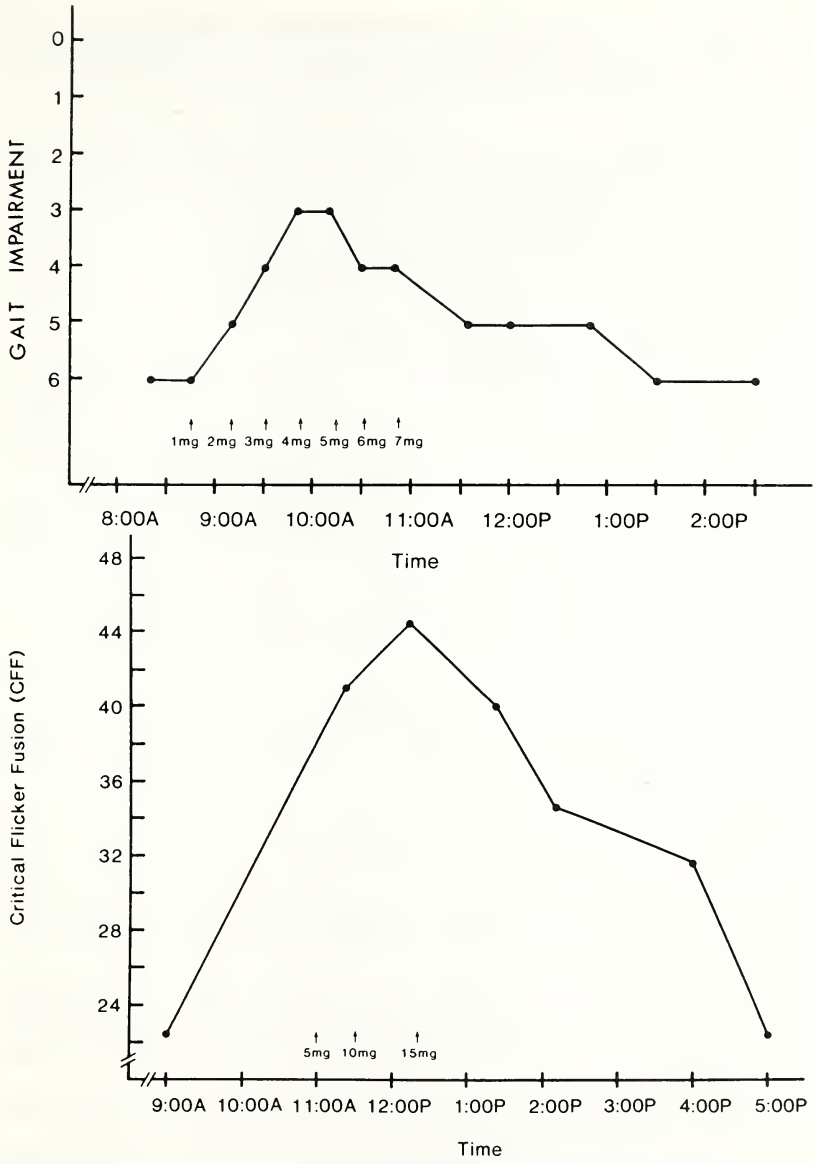

FIGURE 5. Part A illustrates the effect of intraveneous 4-AP on gait in a 43 year old MS patient. Gait is rated on a scale of 0 to 6 with 0 representing normal function and 6 severe impairment. Administration of 4-AP is noted by the arrows and dosages on the abcissa. Note that the improvement produced by 4-AP is transient and returns to baseline levels after cessation of 4-AP therapy. Part B shows the effect of 4-AP on critical flicker fusion (CFF) frequency in a second, 46 year old patient. Again, the administration of 4-AP is noted by the arrows and dosages on the abcissa and the improvement produced by 4-AP is transient.

tially affect demyelinated nerve. The K + channel in nonmyelinated fibers and at synapses resemble nodal K + channels in terms of their 4-AP sensitivity, providing a rationale for the lack of significant side effects at dosages sufficient to restore functional capacity in MS patients.

Several derivatives of 4-AP are potent K + channel blockers. For example, 3,4-diaminopyridine (3,4 DAP) has a dissociation constant of 6 μM in squid axons (24). In two large frog fibers 5 μM 3,4 DAP reduced the K + current by 60-70%, consistent with a 5-6 fold lower dissociation constant for 3,4 DAP compared to 4-AP. In two LPC-treated small fibers 5 μM 3,4 DAP eliminated the K + current. Thus a similar differential sensitivity of nodal and paranodal channels seems to hold for 4-AP derivatives. In myasthenia, 3-4 DAP has fewer side effects (32), perhaps because it traverses the blood brain barrier less rapidly. If so, 3,4 DAP offers the advantage of acting more selectively than 4-AP at MS lesions and should be investigated.

Other drugs need to be examined in the same way as 4-AP. Gallamine blocks K + channels (48) and has a low blood-brain barrier permeability. As a neuromuscular blocking agent in surgery, gallamine is injected at a dose of 1 mg/kg. In unanesthetized subjects 0.7 mg/kg gives minimal surgical relaxation (54) and at this dose a plasma level of 3μM would be expected. In frog myelinated nerve there is a 10% block of K + conductance with 10 μM gallamine (48), so significant K + conductance changes might occur in man. There are also threshold-lowering agents. For example, WR-2721 produces hypocalcemia by inhibiting parathyroid secretion and calcium release from bone (1,19) and should have the same therapeutic effects as oral phosphate (13). A combined administration of a threshold-lowering agent and a K + channel blocker would be an optimal way to produce a maximal increase in conduction safety factor with the least side effect.

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