ACTIVITY-DEPENDENT CALCIUM TRANSIENTS IN CENTRAL NERVOUS SYSTEM MYELINATED AXONS REVEALED BY THE CALCIUM INDICATOR FURA-2

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ABSTRACT Optical measurements from rat optic nerve, loaded with the new Ca^{2+} indicator Fura-2, provide the first evidence for the presence of activity-dependent fast intracellular [Ca^{2+}] transients in mammalian central nervous system (CNS) myelinated axons. The results suggest that voltage-dependent Ca^{2+} channels are present in some of the myelinated axons. Optical measurements from axons stained with anterogradely transported voltage-sensitive dye suggest the presence of Ca^{2+}-dependent potassium conductances in these axons. This report also demonstrates that Fura-2 can readily detect changes in [Ca^{2+}] inside cells as a result of electrical activity, and establishes its suitability for measurements of intracellular Ca^{2+} transients in the millisecond time domain.

INTRODUCTION

The physiology of myelinated axons has been the subject of extensive investigation both because of its significance to the overall function of the nervous system and its relevance to pathological conditions in the central nervous system (CNS). However, previous electrophysiological investigations failed to detect the presence of voltage dependent Ca^{2+} channels in CNS myelinated axons (e.g., Foster et al., 1982). Recent investigations of axon-glia interactions in rat optic nerve in which voltage-sensitive dyes were used have suggested, but not conclusively shown, the presence of voltage-sensitive Ca^{2+} conductance and Ca^{2+} dependent K^+ conductance in the axolemma of myelinated axons (Lev-Ram and Grinvald, 1986). Results from skate cerebellar slices are also consistent with, but not direct evidence for, similar channels in the nonmyelinated parallel fibers (Konnerth et al., 1987). An expanded repertoire of voltage and ion-sensitive conductances would imply that the electrical properties of vertebrate and mammalian CNS axons are considerably more complex than previously thought. To detect Ca^{2+} entry into myelinated axons during action potentials, the present experimental strategy was to load axons with a Ca^{2+} indicator, Fura-2. This indicator is a fluorescent tetracarboxilate chelator that exhibits a spectral shift in excitation maxima upon binding Ca^{2+}. Unlike Arsenazo III (Brown et al., 1975), Fura-2 can be loaded into cells simply by incubating them with the permeant ester derivative Fura-2 AM. This compound is subsequently cleaved by intracellular enzymes to yield the impermeant anionic indicator, which remains trapped inside the cells (Grynkiewicz et al., 1985, Poenie et al., 1985). Although there are several reports about the use of Fura-2 (e.g., Williams et al., 1985; Connor, 1986), measurements with Fura-2 in the millisecond time domain, or the detection of [Ca^{2+}] transients in response to single action potentials, have not yet been reported. The present results were already presented in a preliminary form (Lev-Ram, 1985).

METHODS

56 Sprauge-Dawly rats were used. The dissection and maintenance of isolated optic nerves has been described elsewhere (e.g., Foster et al., 1982; Connors et al., 1982; Lev-Ram and Grinvald, 1986). Successful loading of the axons of this in vitro CNS preparation with Fura-2 required a different protocol from that used for loading single cell preparations. Nerves were loaded by incubating them in a normal Krebs solution containing 20–50 μM of Fura-2 AM, dissolved in dimethyl sulphoxide (DMSO); the final DMSO concentration was <1%. Incubation time was 1.5–2 h. To wash the extracellular indicator and to allow for the enzymatic cleavage of the ester, the staining solution was replaced with a normal solution and the nerve incubated for another 1–2 h. The temperature was 34°–37°C. The electrical behavior of stained and unstained nerve was very similar, but upon close examination of the time course of the compound action potential, it appeared that components having different conduction velocity were slightly better resolved in the loaded nerves.

The optical recording techniques for the Fura-2 signals are similar to those used previously, with voltage-sensitive dyes, to detect small and fast signals from single cells and their processes (Grinvald et al., 1982, 1983, 1987; Grinvald, 1985). The following modifications were introduced: (*a*) an inverted microscope (model IM-35; Carl Zeiss, Inc., Thornwood, NY) and a 32× objective (E. Leitz, Inc., Rockleigh, NJ) having a 0.6 numerical aperture were used. (*b*) A 150-W Xenon lamp equipped with a negative feedback stabilizing circuit (Pine, J., personal communication) was employed. Successful measurements were made also with a 100-W mercury lamp, however, arch wandering in that lamp gave rise to noisier

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recordings. (*c*) To correct the time course of the optical signal for the bleaching and imperfections of the apparatus response (e.g., drift of the analog sample-and-hold circuit, time-dependent change in filters transmission or reflection), one trial with a stimulus to the nerve was subtracted from another trial without a stimulus. To avoid the accumulation of systematic errors, the order of these two trials was changed after each pair of trials. The flat trace shown at the bottom of Fig. 1 *B* demonstrates the validity of this procedure. Bleaching and photodynamic damage appear to be insignificant in the present experiments.

To measure the voltage changes associated with the Ca^{2+} transients in this small axon, optical measurements from stained axons (and not from the surrounding glia) were also performed, using a voltage-sensitive dye (RH-461). This new approach has been described recently (Grinvald et al., 1987) and was used with two modifications: a tungsten-halogen lamp, rather than the mercury lamp was used; an alternating current coupling with a time constant of 300 ms was used, rather than the sample-and-hold circuit. The specific labeling of axons with voltage-sensitive dyes was achieved by intraocular injection of high concentrations of RH-461. A few days were required for proper uptake by the ganglia cells and the anterograde labeling of the axons. In such stained nerves the amplitude of the electrically recorded compound action potential was 40–60% relative to that of unstained axons. This observation suggests that several ganglia cells and/or their axons did not survive the present labeling procedure, presumably because of the high concentration of the dye in the injected eye. However, the time course of the compound action potential was similar to that of control nerve, suggesting that the remaining functional axons were not significantly modified (as shown by all of the present experimental manipulations with drugs or ions). Bleaching and photodynamic damage limited the duration of the optical measurements with the voltage-sensitive dye to three to four reliable experiments (60 trials averaged in each).

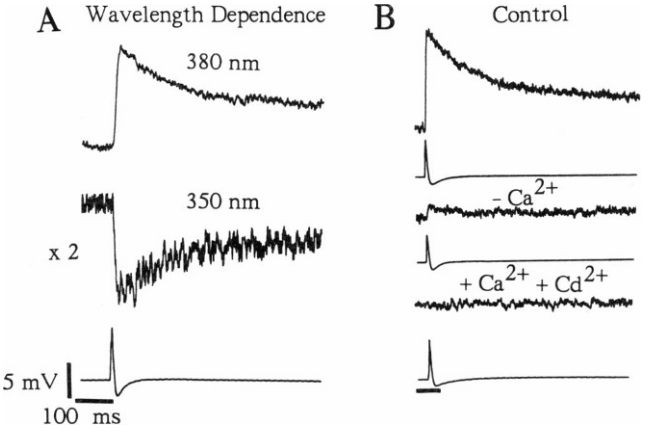

FIGURE 1 Detection of Ca^{2+} transients from myelinated rat optic nerve loaded with Fura-2. (*A*) The wavelength-dependent polarity of the flourescence signal. *Top:* the result obtained with a 380-nm excitation filter (30 nm band width). *Middle:* the signal obtained with a 350-nm excitation filter (band width 20 nm). The time-constant for the optical amplifier was 2.25 ms. *Bottom:* the extracellularly recorded compound action potential recorded with a suction electrode (band width 0.1 Hz-to-10 kHz; a 2.25-ms filter would delay the time-to-peak by 3–5 ms). Unless otherwise stated, the nerve was bathed in a normal Krebs solution containing 1 mM 4-AP. 30 trials were averaged in each experiment. Horizontal calibration bars: 100 ms (10 ms for the insets). (*B*) The fluorescence signal is reduced upon reducing the $[Ca^{2+}]$ and is abolished by Cd^{2+}. *Top:* the fluorescence signal in Krebs solution containing 4-AP. *Middle:* the fluorescence signal 10 min after the nerve was incubated in a Ca^{2+}-free Krebs containing 2.4 mM Mg^{2+} (no EGTA). *Bottom*: the fluorescence signal in normal solution that also contained 0.2 mM Cd^{2+} and 4-AP.

RESULTS AND DISCUSSION

Measurements in the millisecond time domain with Fura-2 have not yet been reported, therefore it was necessary to establish the feasibility of such experiments. Fig. 1 illustrates that fast fluorescence signals were recorded from electrically active nerves with excellent signal-to-noise ratios. Electrical stimulation of the nerve evoked a compound action potential recorded electrically with a suction electrode, and a decrease in the fluorescence intensity was observed at an excitation wavelength of 380 nm. The fractional change in fluorescence intensity was 2×10^{-3}. An excitation wavelength of 350 nm produced a signal with opposite sign (an increase in fluorescence). The similar time course of the two signals suggested that the same process underlying the fluorescence decrease at 380 nm caused a fluorescence increase at 350 nm. Based on the steady state measurements with this indicator (Grynkiewicz et al., 1985), this wavelength dependence indicates a transient intracellular increase in cytosolic $[Ca^{2+}]$. (In all the figures the fluorescent signals are inverted; a fluorescence decrease [380 nm] is plotted as a positive signal because it corresponds to an intracellular $[Ca^{2+}]$ increase.) The wavelength dependence of the fluorescence signals further indicated that they could not arise from activity-dependent, light-scattering signals, because light-scattering signals never show a wavelength-dependent reversal of the signal direction. In addition, no signals were observed if electrical activity was blocked with tetrodotoxin, or from unstained nerves. These controls indicated that the signals do not result from a stimulus artifact.

To test if the indicator signal was indeed due to Ca^{2+} entry via voltage-sensitive Ca^{2+} channels, we performed two standard controls (e.g., Ahmed and Connor, 1979; Gorman and Thomas, 1980; Stockbridge and Ross, 1984; Ross et al., 1986): lowering the extracellular $[Ca^{2+}]$, and using a blocker of the voltage-dependent Ca^{2+} channel. 10 min after replacement of the normal Krebs solution with a Ca^{2+}-free solution (EGTA was not used) only a small signal remained (Fig. 1 *B*, middle trace). It is likely that most of the remaining signal is due to incomplete replacement of the Ca^{2+}; replacement of all of the free Ca^{2+} required a longer time; 10 min later the fluorescence signal disappeared, but the electrically recorded compound action potential was reduced to ~40% of control. Subsequently, when this nerve was washed with normal Krebs solution, a recovery of the electrical and fluorescence signal was observed (not shown). Superfusion of this nerve with a solution containing both 0.2 mM Cd^{2+} (a blocker of the voltage-sensitive Ca^{2+} channel; for review see Hagiwara and Byerly, 1981) and the normal $[Ca^{2+}]$, abolished the fluorescence signal (Fig. 1 *B*, bottom).

To optimize the signal size we tested different concentrations of Fura-2 AM and a variety of incubation times. Several considerations suggested that loading the my-

elinated optic nerve axons with Fura-2 should require considerably higher concentrations than those used for loading dissociated single cells. The rat optic nerve contains several hundred thousand densely packed, small myelinated axons, and densely packed glial cells, both of which probably form a significant diffusion barrier. In addition, the permeant indicator presumably enters the axons primarily through the nodes, rather than via the multiple lamella of the myelin sheath. Once the dye enters the axons it should be considerably diluted by diffusion away from the nodal region. As expected, with 2 μM of Fura-2 AM, acceptable signal-to-noise ratios were observed only in nonmyelinated neonatal axons. In mature myelinated nerve signals were also observed, but only after extensive signal averaging. A much better signal-to-noise ratio was obtained by increasing the concentration of Fura-2 AM (20 to 50 μM) during the incubation period (this was similar to the behavior of voltage-sensitive dyes; staining the rat optic nerve required 50–100-fold higher concentration than those used to stain dissociated cells in culture; Grinvald et al., 1982). When the nerve was loaded with the indicator for 2 h using an extracellular concentration of 100 μM, large signals could be recorded without signal averaging (Fig. 2 *A*). The time course of the fluorescence signal was similar to that observed with a 50-fold lower concentration. In most of the axons, the electrical activity recorded from nerves stained with the higher concentration of the permeant indicator did not reveal any large changes in amplitude, shape, or conduction velocity of the action potential, indicating that this staining procedure did not significantly alter the physiological properties of these axons. In addition, the fractional change in fluorescence recorded at 380 nm was not much smaller than that recorded with a much lower concentration of Fura-2 (compare Figs. 1 *A* and 2 *A*). The opposite result is expected if the axons were considerably overloaded with Fura-2.

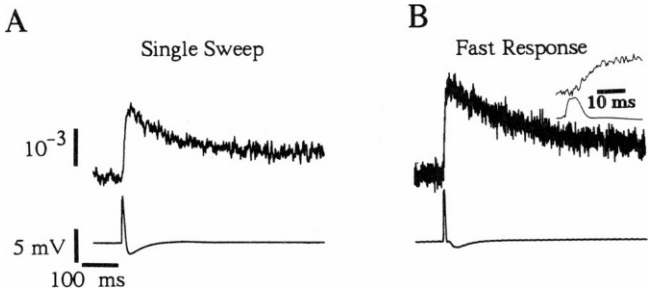

FIGURE 2 Sensitivity and time resolution of the Fura-2 signal. (*A*) Single sweep detection of a Ca^{2+} transient from a highly stained nerve. The scale for the size of the fluorescence signal shows the fractional change in fluorescence intensity (not corrected for stray light and autofluorescence, which in most experiments was 20–60% of the light reaching the photodetector). (*B*) Measurement of the rise time of the Fura-2 signal. Recording of the transient with detectors having 500-μs rise time. 40 trials were averaged. The inset shows this result on an expanded time scale.

To further characterize the suitability of Fura-2 for measurements of fast Ca^{2+} transients we measured the rise time of the fluorescence signal. Fig. 2 *B* illustrates the results of a measurement of the signal performed with a selected time-constant of 500 μs for the amplifier. The inset shows the rise time of the Ca^{2+} transient on an expanded scale. We have observed rise times as fast as 7–10 ms (10–90%, after the correction for the amplifier response function). Since the real rise time of the change in intracellular [Ca^{2+}] is unknown, the indicator response time could be even faster than 7 ms.

Bath application of Fura-2 AM could stain axons and glial cells. Therefore, a priori, the signal may have originated from either active axons, glial cells depolarized by axonal activity (e.g., Orkand et al., 1966; Baylor and Nicholls, 1969; Connors et al., 1982; Lev-Ram and Grinvald, 1986), or from both. The origin of the Ca^{2+} transient was investigated by exploiting several experimental conditions which may either enhance or block activity-induced Ca^{2+} entry into axons, and which also have a predictable effect on activity-dependent depolarization of glial cells (e.g., Lev-Ram and Grinvald, 1986). Incubation with 4-AP (a blocker of some voltage-sensitive K^+ channels), considerably prolonged the compound action potential recorded electrically. Prolonged depolarization of the axons should enhance Ca^{2+} entry via putative Ca^{2+} channels in the axolemma, and indeed the observed Fura-2 signal was about threefold larger in the presence of 4-AP (Fig. 3 *A*). The presence of 4-AP also revealed a distinct second slow component. The fast (~10–15 ms rise time; 150–200 ms decay time) component probably originates from axons, because its rise time and initial decay time are significantly faster than the corresponding parameters for

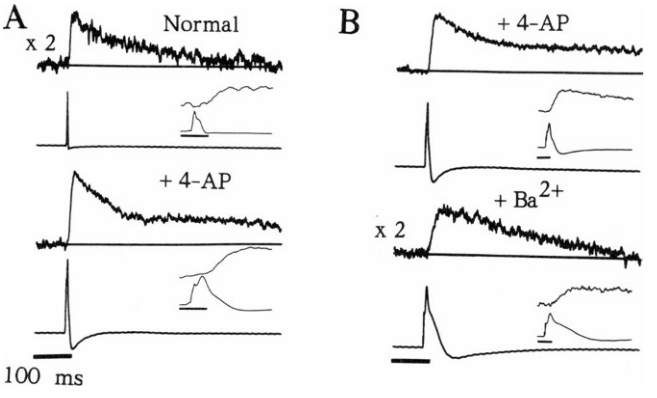

FIGURE 3 Separation of the two components of the Ca^{2+} transient. (*A*) 4-AP increased the amplitude of the calcium transient and revealed a second slow component. *Top:* the calcium transient in normal Krebs solution shown on a twofold expanded scale relative to the bottom. *Bottom:* 10 min after the application of 2 mM 4-AP. (*B*) Barium ions reduced the size of the calcium transient and abolished the slow component. *Top:* the calcium transient in normal solution containing 4-AP. *Bottom:* 15 min after the application of the same solution, with 2.4 mM Ba^{2+} added. The vertical scale was expanded by a factor of 2 relative to the top.

axonal-activity–induced depolarization of glial cells, recorded electrically (e.g., Orkand et al., 1966; Baylor and Nicholls, 1969) or optically (Lev-Ram and Grinvald, 1986; Konnerth and Orkand, 1986; Konnerth et al., 1987). The slow second component could be attributed to slower processes of intracellular buffering of the abnormal increase in Ca^{2+} in the axons. Single neurons filled with Arsenazo III also exhibit slow components and these have been attributed to slower processes of intracellular Ca^{2+} buffering or extrusion (e.g., Gorman and Thomas, 1980; Stockbridge and Ross, 1984; Ross et al., 1986). However, an enhanced slow depolarization of glial cells in the presence of 4-AP is also expected if enhanced Ca^{2+} entry into the axons produces a larger K^+ efflux via putative Ca^{2+}-dependent K^+ channel in axons (Lev-Ram and Grinvald, 1986; Konnerth et al., 1987). Therefore, at least part of the second slow component of the Fura-2 signal could have originated from a slow increase in intracellular Ca^{2+} in depolarized glial cells, as discussed later.

Three additional experiments revealed only a fast component that probably had originated only from axons. First, barium ions often block both voltage-dependent K^+ channels and Ca^{2+}-dependent K^+ conductance (for review see Lattore and Vergara, 1984) and also depolarize glial cells in this preparation (Ransom, B., personal communication). Therefore, in the presence of Ba^{2+}, it is unlikely that a $[Ca^{2+}]$ transient from glia would be evoked by axonal activity. The addition of Ba^{2+} to a Ca^{2+}-containing 4-AP solution eliminated the slow component, but a smaller fast component was still observed (Fig. 3 *B*). The fluorescence signal observed in the presence of Ba^{2+} and Ca^{2+} could be attributed to either Ca^{2+} or Ba^{2+} entry into axons or both. (In spectrophotometric titration experiments, we found that Fura-2 also binds Ba^{2+} ions, but the isosbestic point shifted from 359 to ~382 nm. Because an excitation wavelength of 380 was used in the nerve experiments, it is likely that much of the observed fluorescence transient in Fig. 3 *B* is due to residual Ca^{2+} entry or intracellular Ca^{2+} displacement, rather than Ba^{2+} entry.) Second, a large glial depolarization was not detected (Lev-Ram and Grinvald, 1986) in the nonmyelinated optic nerves (Foster et al., 1982) of neonatal rats, because in 4–6 d postnatal potassium accumulation was <0.25 mM (Connors et al., 1982). Therefore, it was not surprising that with Fura-2 only a fast Ca^{2+} signal was observed (Fig. 4 *A*). The third evidence for the axonal origin of the fast component came from experiments in which only the axons were selectively loaded with the Ca^{2+} probe by injecting a concentrated Fura-2 solution into the eye (under Nembutal anesthesia), and allowing 10–36 h for anterograde labeling of ganglion cell axons exclusively, rather than surrounding glial cells. Although this procedure only faintly stained the axons, in 4 of the best stained nerves (out of 20) we recorded only fast signals (Fig. 4 *B*). While the signal-to-noise ratio in these experiments was poor, the small signals could not be attributed to a scattering-dependent fluorescence change because such signals were not observed from unloaded axons which had nearly equal autofluorescence.

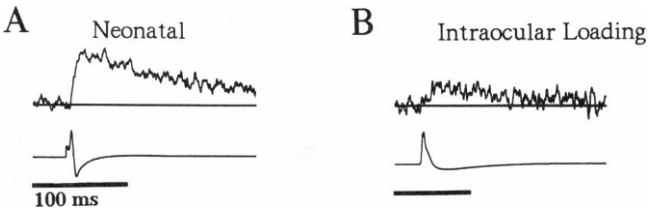

FIGURE 4 Detection of the fast components from neonatal nerves or mature nerves in which axons were selectively loaded with Fura-2. (*A*) The slow component is absent in 4-d postnatal nerve. (Neonatal nerves were loaded with 1–2 μM of Fura-2.) Note that the time scale is expanded relative to other figures. (*B*) The fast component was observed from selectively loaded axons. The axons were selectively loaded by Fura-2 injection (20 μl of 10 mM Fura-2 in DMSO) into the eye 24 h before the experiment. 200 trials were averaged. In this experiment the contribution of Fura-2 fluorescence to the overall light reaching the photodetector was <10%, and therefore the signal-to-noise ratio was relatively poor. The measurement was done in the presence of 1 mM 4-AP.

The possibility that part of the slow component (e.g., Fig. 3 *A*) originated from the surrounding glial cells warrants discussion. Ca^{2+} channels have been described in astrocytes in vitro (MacVicar, 1984). However, in mature rat optic nerve, the average change in extracellular $[K^+]$ induced by a single action potential is <0.1 mM (Connors et al., 1982), and therefore the potassium-dependent depolarization of the astrocytes is much too small to activate known voltage-dependent Ca^{2+} channels in astrocytes. Even though the average increase in extracellular potassium concentration was small, the oligodendrocytes, which are also sensitive to potassium accumulation (Kettenmann et al., 1983), may experience a much larger local increase in the potassium concentration and depolarize significantly more than astrocytes. This is expected because of the minute volume of the extracellular space at the paranodal region (Schnapp et al., 1976), and if K^+ channels are localized there (e.g., Chiu and Ritchie, 1981). Therefore, the present data do not rule out the interpretation that at least part of the slow calcium transient originated in the oligodendrocyte paranodal loops. Such hypothetical intracellular Ca^{2+} increase in oligodendrocytes may be attributed to (*a*) Ca^{2+} entry via voltage-sensitive Ca^{2+} channels in oligodendrocytes and (*b*) release from intracellular Ca^{2+} stores in response to K^+ depolarization. Proof of Ca^{2+} entry into oligodendrocytes, or differentiation between *a* and *b* above, could not be obtained in these experiments, and will require experiments on single cells or simpler preparations.

The present experiments provide evidence for voltage dependent Ca^{2+} transients in some axons of the rat optic nerve. The pharmacological and ionic substitution experiments suggest, but do not prove, that voltage-dependent Ca^{2+} channels are involved. Why those channels were not observed before in the previous studies of myelinated axons

is an intriguing question. If such channels are concentrated at the paranodal region, their detection would be difficult with conventional approaches. In addition, myelinated axons of different diameters have different biophysical properties and a different regional distribution of ionic channels (Foster et al., 1982; Smith and Schauf, 1981). Thus, it is possible that only the small diameter ($<0.3\ \mu m$) myelinated axons have Ca^{2+} channels (they are also the most sensitive to 4-AP [Foster et al., 1982]).

The average amount of Ca^{2+} entry into individual components of a heterogeneous preparation (such as the rat optic nerve) cannot be quantified with the present technique, since intracellular Fura-2, in cells or "compartments" that do not experience any Ca^{2+} transients, produce an undefined, inestimable fluorescence background (Grynkiewicz et al., 1985). This is not a unique limitation of the present approach; it has been emphasized (Simon and Llinas, 1985) that large and highly localized changes in intracellular Ca^{2+} may be detected as small, spatially and temporally averaged changes with any of the alternative approaches for measuring fast Ca^{2+} transients. The density of these Ca^{2+} channels is not large enough to support a Ca^{+} action potential in the presence of tetrodotoxin (TTX) and their role in the function of myelinated axons remains to be investigated.

Our results do not rule out the possibility that the activity-dependent intracellular [Ca^{2+}] increase is mediated by some intracellular exchange mechanism that depends also on the extracellular [Ca^{2+}] and is blocked by Cd^{2+} or Ba^{2+}. In any case this intracellular [Ca^{2+}] increase in the axons may activate a Ca^{2+}-dependent K^{+} conductance, as suggested by previous studies in which voltage-sensitive dyes were used for external staining of heterogeneous preparations (Lev-Ram and Grinvald, 1986; Konnerth et al., 1987). In the present investigation, additional indirect evidence for the existence of a Ca^{2+}-dependent K^{+} conductance in some of the myelinated axons of rat optic nerve was obtained. Axons were selectively stained with an anterogradely transported voltage-sensitive dye (RH-461) that was injected intraocularly (100 μl of 50 mM dye in DMSO). Measurements were performed 4–10 d after the injection to allow for slow uptake of the dye by ganglion cells and anterograde diffusion along optic nerve axons. With this intracellular dye the optical signals are also linearly related to the membrane potential change, yet the signals have an opposite polarity relative to signals obtained with extracellular staining (Gupta et al., 1981; Grinvald et al., 1987). Upon stimulation of the nerve, the fast optical signal corresponding to the compound action potential recorded electrically was also recorded optically (Fig. 5, top). When the compound action potential was prolonged with 1 mM 4-AP, a prominent slow negative component (hyperpolarization) was detected (arrows in Fig. 5 *A*, middle and 5 *B*, top) and was blocked by adding 1 mM Ba^{2+} (Fig. 5 *A*, bottom) or Cd^{2+} to the solution (Fig. 5 *B*, bottom). These results

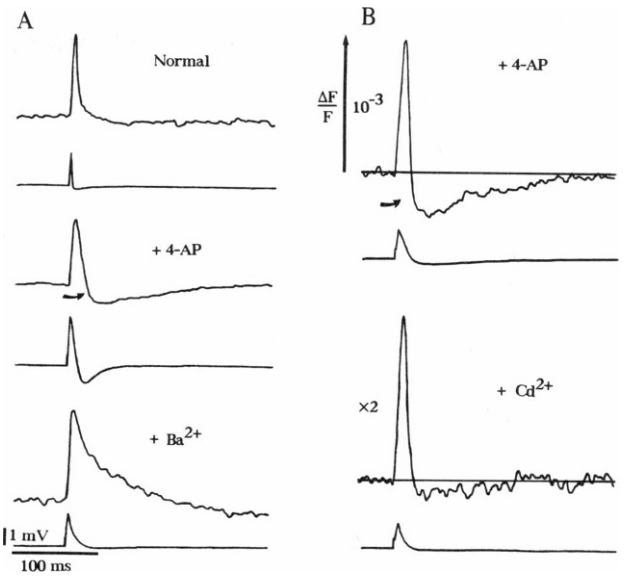

FIGURE 5 Optical recording of the membrane potential changes in axons labeled with intracellular voltage-sensitive dye. (*A*) Detection of a prominent hyperpolarization under conditions that enhance Ca^{2+} entry. Axons were labeled with intracellular voltage-sensitive dye (RH-461) by intraocular injections. *Top:* optical and electrical recordings of the compound action potential in normal Tris solution. *Middle:* optical and electrical recording after the application of 1 mM 4-AP. A prominent hyperpolarization was detected *(arrow). Bottom:* a large hyperpolarization was not observed in the presence of 1 mM Ba^{2+}. (*B*) Cd^{2+} abolished the slow hyperpolarization. *Top:* a large hyperpolarization observed in the presence of 4-AP from another nerve. *Bottom:* the hyperpolarization largely diminished after the application of 1 mM Cd^{2+}. The vertical arrow shows the direction and size of the fractional change in fluorescence. The scale was expanded by a factor of 2 for the fluorescence signal in the presence of Cd^{2+}. (The fluorescence signal was smaller due to bleaching and the reduced excitability in the presence of Cd^{2+}; see Methods.)

support several of the previous interpretations of the data obtained with bath applied voltage-sensitive dyes (Lev-Ram and Grinvald, 1986), and specifically the presence of a Ca^{2+}-dependent K^{+} conductance in the axons. The functional role of such conductance remains to be investigated; it may play an important role in regulating high-frequency repetitive firing of small myelinated axons. Furthermore, a large potassium accumulation after repetitive firing may serve as a signal in the ionically mediated communication between myelinated axons and their supporting oligodendrocytes. This signal could be related to the biochemical dynamics of the structure of myelin basic protein, i.e., its Ca^{2+}-dependent and activity-dependent phosphorylation (e.g., Murray and Steck, 1984).

Finally, a technical comment about fluorescent Ca^{2+} indicators. Fluorescence measurements are much more sensitive than transmission measurements whenever the number of probe molecules is small (Rigler et al., 1974; Waggoner and Grinvald, 1977). Therefore, it is likely that Fura-2 (or improved fluorescent indicators) will prove more useful than Arsenazo III for the valuable measurements of the regional distribution of calcium transients

from single cells and their processes (Stockbridge and Ross, 1984; Ross et al., 1986). Recent reports demonstrated the imaging of steady-state Ca^{2+} gradients in single cells (Williams et al., 1985; Connor, 1986). It is likely that whenever averaging is feasible, a dynamic picture of such gradients, in the millisecond time domain, can also be obtained. It should be also possible to use Fura-2 for real-time optical mapping of neuronal activity associated with intracellular changes or extracellular changes in $[Ca^{2+}]$, in the CNS in vivo and in vitro.

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