INTERNAL CESIUM ALTERS SODIUM INACTIVATION IN *MYXICOLA*

C. L. SCHAUF AND J. O. BULLOCK, *Departments of Physiology and Neurological Sciences, Rush University, Chicago, Illinois 60612*

ABSTRACT When *Myxicola* giant axons are internally dialyzed with Cs^+ as the sole cation, the time-course of prepulse inactivation is selectively accelerated compared to its rate with K^+ dialysis in the same axons. This decrease in τ_h^p occurs without any change in the magnitude or time-course of I_{Na} during step depolarizations and results in τ_h^p/τ_h^s ratios near unity over most of the potential range in Cs^+ dialyzed axons.

INTRODUCTION

In voltage-clamped *Myxicola* giant axons the time constant (τ_h^s) of sodium inactivation during a maintained depolarization has been shown to be substantially smaller than the time constant (τ_h^p) of inactivation produced by conditioning prepulses for potentials more negative than 0–10 mV (Goldman and Schauf, 1973; Schauf and Davis, 1975). This difference has been shown to be quantitatively unchanged by procedures that alter the absolute magnitude of inward current (Bullock and Schauf, 1978), suggesting that it is unlikely to be due to incomplete series resistance compensation or spatial nonuniformity. It has also been detected in lobster axons (Oxford and Pooler, 1975), but has not been seen in careful studies of perfused squid giant axons (Bezanilla and Armstrong, 1977). We report here studies that demonstrate that the internal dialysis of *Myxicola* with Cs^+ reduces or eliminates the τ_h^s/τ_h^p difference due to an acceleration of prepulse inactivation, without any effect on either τ_h^s or the absolute magnitude of sodium conductance.

METHODS

Methods for simultaneous voltage clamp and internal dialysis of *Myxicola* axons were as previously reported (Bullock and Schauf, 1978). The internal solutions used in this study were as follows: K^+ dialysis-560 mM K^+, 50 mM F^-, 30 mM $HPO_4^=$, 450 mM glutamate; K^+ + tetraethylammonium (TEA^+) dialysis—same but 40 mM TEA^+ added; Cs^+ dialysis—610 mM Cs^+, 450 mM glutamate, 50 mM F^-. The internal pH was maintained at 7.30 ± 0.05 with 1 mM Hepes buffer. External solution was K^+ free artificial seawater (ASW; Schauf et al., 1977) at pH 7.8. Axons were held at −80 mV and compensated for series resistance. The

adequacy of compensation was tested by repeating all protocols in low Na^+ (20% of normal using Tris substitution) and observing whether $I_{Na}(t)$ records differed by only a scaling factor at all potentials (Schauf et al., 1977). Membrane potentials were corrected for liquid junction potentials measured in the different internal solutions by moving the internal electrode from the external solution to samples of the internal solutions coupled via a saturated KCl-agar bridge. For the K^+ solution this correction was 4.5 mV whereas for the Cs^+ solution it was 5.5 mV.

In general the procedure was to record membrane sodium currents both during a series of depolarizing voltage steps and during a fixed test step to 0 mV after conditioning pulses of variable amplitude and duration, first in axons dialyzed with $K^+ + TEA^+$ and then in the same axons 20 min after switching the internal dialysate to the Cs^+ solution. Thus all data shown here directly illustrate a differential effect of Cs^+ on Na^+ inactivation over a brief period of time in a particular axon.

RESULTS

The basic observations are illustrated in Fig. 1. In Fig. 1 A we show the sodium currents measured using step depolarizations first during dialysis with $K^+ + TEA^+$ and

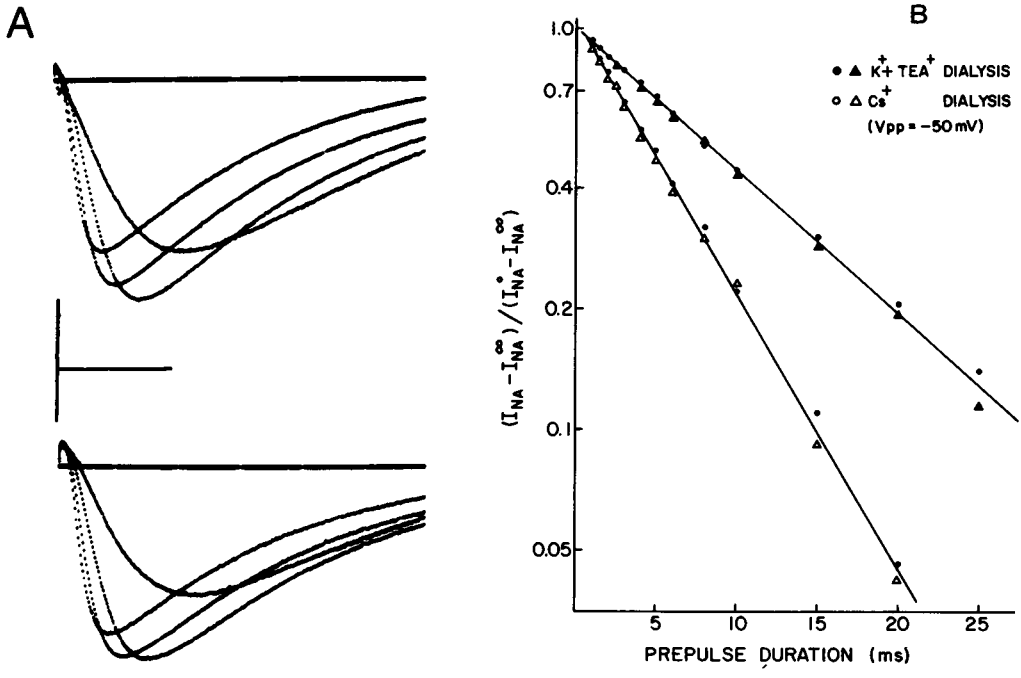

FIGURE 1 A. Membrane currents recorded during step depolarizations to −30, −20, −10, and 0 mV in an axon dialyzed first with $K^+ + TEA^+$ (upper records), then 30 min after switching to Cs^+ dialysis (lower records). Temperature, 5°C. Calibrations are 0.75 mA/cm^2 and 1.0 ms, respectively. B. Time-course of prepulse inactivation with $K^+ + TEA^+$ dialysis compared to that with Cs^+ dialysis. Data from two axons, each of which was first dialyzed with $K^+ + TEA^+$, then with Cs^+. The relative magnitude of peak inward current as a function of prepulse duration is calculated as described in the text. Prepulse potential, −50 mV. The solid lines represent the least squares fit to the data for each internal solution. Temperature, 5°C.

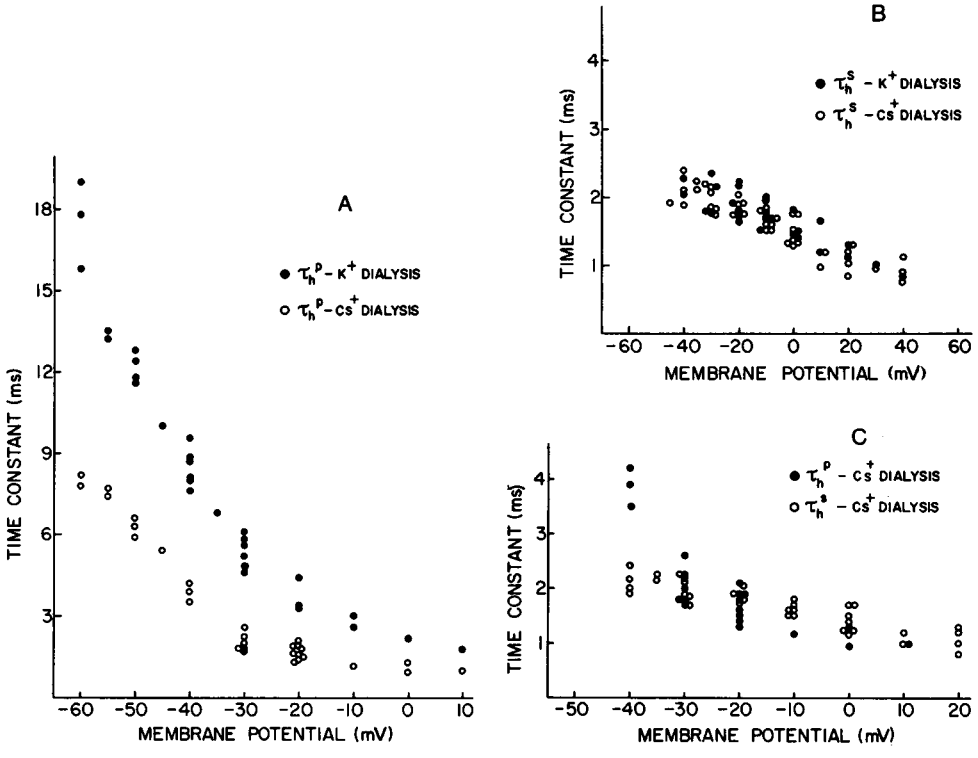

FIGURE 2 Composite I_{Na} inactivation data from five axons first dialyzed with K^+ + TEA^+, then with Cs^+. In B the time constants of inactivation during step depolarizations (τ_h^s) are given in the two solutions, whereas in A the time constants of prepulse inactivation (τ_h^p) are compared. In C we have compared the values of τ_h^s and τ_h^p in Cs^+ dialyzed axons (see text).

then after changing to a Cs^+ dialysate. Semilogarithmic plots of these data (not shown) prove that the time-course of I_{Na} is comparable in both cases (Fig. 2), illustrating that Cs^+ does not appreciably alter the kinetics of inactivation of conducting channels. The small (10%) decrease in inward current in the Cs^+ records probably represents a slight deterioration and was not consistently observed.

In Fig. 1 B we have plotted for two axons the time-course of inactivation of I_{Na} during a prepulse to −50 mV as measured using a subsequent test step to 0 mV. The peak inward current during the test step was measured, after being corrected for an assumed linear leak conductance by using records obtained during equal hyperpolarizing pulses, and plotted as a function of prepulse duration. The data were normalized to account for the incomplete inactivation of I_{Na} by calculating $(I_{Na} - I_{Na}^{\infty})/(I_{Na}^{0} - I_{Na}^{\infty})$, where I_{Na} is the peak Na^+ current obtained for a particular prepulse duration, I_{Na}^{∞} is the peak current obtained after a 100-ms prepulse, and I_{Na}^{0} is the peak current obtained with no prepulse. The time constant of slow I_{Na} inactivation is of the order of 1–2 s at potentials between −20 and 0 mV (Schauf et al., 1976*b*), consequently, use of a 100-ms prepulse to define I_{Na}^{∞} should produce no more than a 10% error with much less error for more negative potentials.

The values of I_{Na}^{0} in each axon were comparable with either $K^+ + TEA^+$ or Cs^+ dialysis and the normalized data for a particular dialysate superimpose. However, the time constant of prepulse inactivation was 12.2 ms when the axon was dialyzed with $K^+ + TEA^+$, but only 6.3 ms when the axon was dialyzed with Cs^+. Delays in the development of I_{Na} inactivation are not easily seen in the scale of Fig. 1 B but were comparable to previous data (Schauf and Davis, 1975) and not obviously different in the two solutions.

The results of our analysis of five axons over a full range of potentials are shown in Fig. 2. In Fig. 2 B we have plotted $\tau_h^s(V)$ for the decline in sodium current during a maintained depolarization in axons first dialyzed with $K^+ + TEA^+$, then with Cs^+. The measurements superimpose within experimental error. In Fig. 2 A the values of the prepulse inactivation time constant (τ_h^p) are plotted as a function of prepulse potential. The values of τ_h^p are significantly smaller with Cs^+ as the internal cation, the data being equally well described as either a twofold decrease in $\tau_h^p(V)$ or a 20-mV shift of the $\tau_h^p(V)$ curve in the hyperpolarizing direction. Finally, in Fig. 2 C we have replotted the data so as to compare the values of τ_h^s and τ_h^p in Cs^+ dialyzed axons. Unlike intact (Goldman and Schauf, 1973), TEA^+-injected (Schauf et al., 1976*a*), or $K^+ + TEA^+$-dialyzed axons, there is little or no difference between τ_h^p and τ_h^s (except for the points at −40 mV) as is observed in squid axons Bezanilla and Armstrong, 1977). The steady-state I_{Na} inactivation curve was also measured in these axons, during both $TEA^+ + K^+$ and Cs^+ dialysis, and no significant differences were found.

It should be noted that both TEA^+ and Cs^+ dialysis completely block potassium currents over the potential range examined here. TEA^+ dialysis is insufficient for measurement of asymmetry currents during long pulses to positive potentials (Bullock and Schauf, 1978) because of a 3–5% residual I_K in this range. However, in the presence of TEA^+ or Cs^+ there is no significant K^+ activation during long prepulses to potentials of −10 mV or less, or during shorter test pulses to 0–20 mV.

DISCUSSION

In *Myxicola* giant axons dialyzed with K^+ and with TEA^+ present to block I_K, τ_h^s is significantly smaller than τ_h^p at negative potentials, the ratio τ_h^p/τ_h^s varying from approximately 5.5 at −55 mV to 1.5 at 0 mV in agreement with previous observations (Goldman and Schauf, 1973; Schauf and Davis, 1975; Schauf et al., 1976*a*). Dialysis of the same axons with Cs^+ causes a selective decrease in the magnitude of $\tau_h^p(V)$ such that the ratio τ_h^p/τ_h^s is near unity for potentials of −30 mV or greater, without producing any change in the magnitude or time-course of I_{NA} during step depolarizations or the level of steady-state Na^+ inactivation.

In *Dosidicus* axons injected with TEA^+, as well as in *Loligo* axons perfused with $K^+ + TEA^+$ or with 200 mM TEA, the τ_h^p/τ_h^s ratio is near unity for potentials between −40 and +60 mV (Bezanilla and Armstrong, 1977). However, in intact squid axons under sucrose gap voltage clamp (Moore and Cox, 1976), as well as in lobster giant axons (Oxford and Pooler, 1975), τ_h^p was larger than τ_h^s at most negative

potentials. The results described here demonstrate that sodium inactivation in *Myxicola* can be markedly altered by changes in internal solutions, and suggest the need for caution in interpreting results when comparisons are to be made between systems examined under different internal conditions.

This work was supported by U.S. Public Health Service Research Career Development Award K04NS00004 to C. L. Schauf, the Morris Multiple Sclerosis Research Fund, and by the National Multiple Sclerosis Society.

Received for publication 22 April 1978.

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