BRIEF COMMUNICATION

INCOMPLETE INACTIVATION OF SODIUM CURRENTS IN NONPERFUSED SQUID AXON

JONATHAN J. SHOUKIMAS AND ROBERT J. FRENCH, *Section on Neural Membranes, Laboratory of Biophysics, IRP, NINCDS, National Institutes of Health Woods Hole, Massachusetts 02543, and the Marine Biological Laboratory Woods Hole, Massachusetts 02543 U.S.A.*

ABSTRACT Perfused squid axons in which K-conductance is blocked show, under voltage clamp, incomplete inactivation of the sodium conductance. The presence of this phenomenon in nonperfused axons was found by comparing membrane current records before and after tetrodotoxin addition to the bathing solution. Sodium currents in nonperfused axons are comparable in behavior at positive potentials to those seen in Cs-perfused axons.

INTRODUCTION

The voltage clamp experiments of Hodgkin and Huxley (1952 *a*) showed that in nonperfused squid axons the sodium conductance after an initial increase from rest level undergoes an inactivation for sustained depolarizations. However, more recent experiments with axons that have been perfused with artificial solutions have shown a maintained sodium conductance at potentials more positive than 0 mV. These observations have been made for perfusion with Cs ions (Adelman and Senft, 1966 *a*), for perfusion with NaF (Adelman and Senft, 1966 *b*; Chandler and Meves, 1970 *a*) and more recently for perfusion with TEA (tetraethylammonium ion) (Yeh and Narahashi, 1977) and TMA (tetramethylammonium ion) (Bezanilla and Armstrong, 1977; Oxford and Yeh, 1979). Both Adelman and Senft (1966 *a*) and Chandler and Meves (1970 *a*) have attributed the lack of complete inactivation to a perfusion dependent effect.

A direct influence of internal ionic composition upon the inactivation behavior of the sodium conductance has some important implications for possible mechanisms underlying "gating" of the channel. Therefore, we tested the hypothesis that the development of an incomplete inactivation is dependent upon internal perfusion. A brief description of our findings has been presented elsewhere (Shoukimas and French, 1978).

METHODS

The experiments described in this paper were performed on the giant axon of *Loligo pealei* under voltage clamp conditions. The axial wire voltage clamp was of conventional design and employed series resistance compensation. Membrane currents were first digitized (ten-bit resolution) after appropriate

filtering and all records shown were corrected for linear leakage and capacitative components by addition of current records from oppositely directed pulses (two hyperpolarizing pulses of one-half the depolarizing pulse amplitude were employed). In some experiments the axon was perfused by the use of a cannula that is concentric with the axial wire.

In all experiments the measured temperature in the chamber was 6° ± 0.05°C. The axon was held at −80 mV for the duration of each experiment. A 10-ms prepulse to −120 mV preceeded each test pulse. Potentials are inside minus outside and are nominal values. No correction was made for junction potentials. Both external and internal solutions had a pH of 7.2. The 400 mM Cs internal solution has the following composition: 400mM CsF, 50 mM Na glutamate, 50 K_2HPO_4, and 105 mM sucrose. Artificial seawater (ASW) contained 430 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM MgCl, and 10 mM Tris-hydroxymethylaminomethane-HCl (Tris).

RESULTS

To test the hypothesis that the sodium conductance does not fully inactivate in nonperfused axons, we conducted the following experiment. Nonperfused axons, bathed in ASW, were pulsed to 0, +80, and +120 mV. Total membrane current was recorded at each potential.

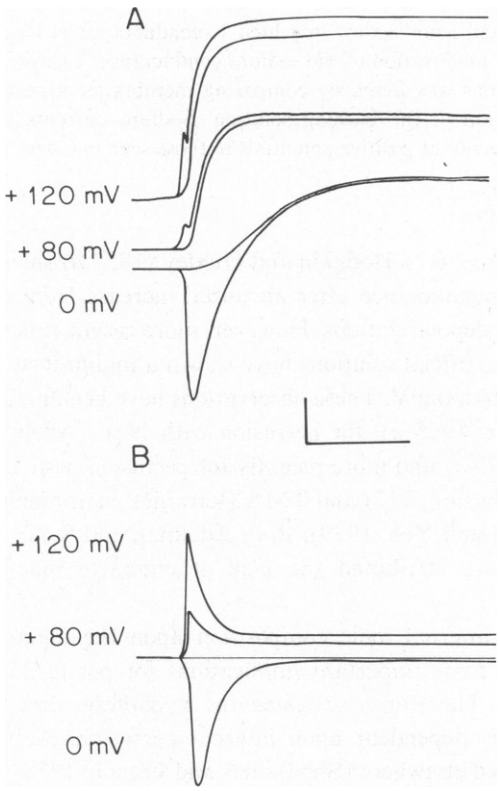

FIGURE 1 Membrane currents from a nonperfused axon. (*A*) Tracings of original data records; at each voltage (indicated to the left of the record pair) currents were recorded before and after addition of TTX. Records taken before TTX addition show initial surge of sodium current. (*B*) Difference between records shown in *A* at each potential. Records are positioned so that zero current for each potential falls on the thin line in the figure. Vertical scale: (*A*) 2 mA/cm² for 0 mV; 4 mA/cm² for +80 and +120 mV. (*B*) 2 mA/cm² for all records. Horizontal scale: 10 ms for all records.

Then, tetrodotoxin (TTX) at a final concentration of 1.0 μM was added to the bath and the pulse sequences and current measurements repeated. An average time of 9.5 min elapsed between sets of measurements. The difference between the current records with and without TTX is the contribution of sodium conductance to the total current. Data records before and after TTX addition, as well as the difference records, are shown in Fig. 1. Leakage currents measured with the hyperpolarizing pulses showed no increase from the beginning of the experiment to its completion.

The difference records show an initial surge of current, inward for the pulse to 0 mV, outward for the pulses to +80 and +120 mV. The initial surge of current is followed by a decline to some steady-state level. It is clear from these records that the currents do not completely inactivate at either of the positive potentials.

For the sake of comparison, axons, perfused with the 400 mM Cs solution, were studied with the same pulse protocol. Internal Cs has been shown to effectively block potassium currents (Figs. 5–15 of Adelman, 1971; Bezanilla and Armstrong, 1972; French, Shoukimas and Mueller, 1978). Current records obtained from a typical axon are shown in Fig. 2. As in the nonperfused axon, these currents also do not completely inactivate at positive potentials. Both the peak and steady state currents are eliminated by addition of TTX to the bath and have very nearly the same reversal potential (+52 and +55 mV, respectively, for peak and steady-state currents; experiment not shown here). From our data and other evidence cited in the Introduction it seems well established that the sodium conductance does not ever fully inactivate at any potential.

To compare the inactivation behavior of the sodium conductance between the two experimental conditions, we have calculated the ratio of the sodium current at 25 ms after the onset of the test pulse to the peak value. The ratio of current at 25 ms to peak gives a measure of inactivation at these voltages where the h parameter of the Hodgkin-Huxley model would be at or very close to steady-state. Table I shows that sodium inactivation has similar characteristics in both intact and Cs perfused axons. Because of the variance between axons in the calculated ratio for nonperfused axons, it is not possible to accurately compare the

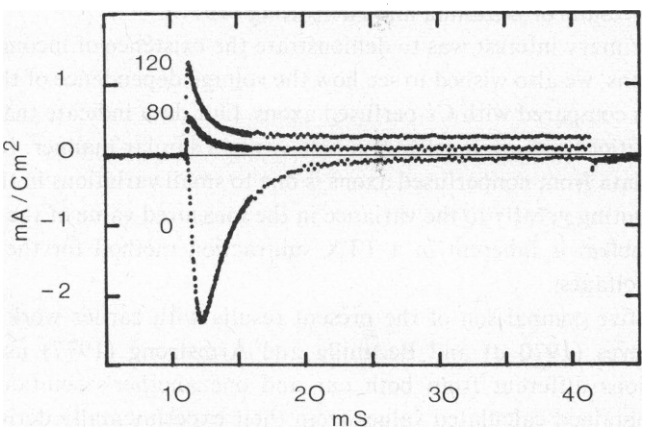

FIGURE 2 Sodium currents from an axon perfused with Na and Cs. Zero current indicated by thin line through origin. Numbers to left of each record indicate test potential. Time scale refers to initiation of data collection; pulse origin at 11 ms.

TABLE I
RATIO OF I_{Na} AT 25 ms TO PEAK I_{Na}, ±1 SEM

Em	Intact axons $N = 10$	Cs-perfused axons $N = 7$
0	0.04 (±0.01)	0.02 (±0.003)
80	0.57 (±0.15)	0.14 (±0.004)
120	0.45 (±0.10)	0.18 (±0.008)

potential dependence of inactivation for nonperfused versus perfused axons. In the nonperfused axons there is some hint that the sustained currents seen at positive potentials increase with time. However, the increase is small and not clearly significant.

DISCUSSION

The major finding presented in this paper is that over a time scale of tens of milliseconds the sodium conductance does not ever completely inactivate in nonperfused axons. Further, the voltage dependence of inactivation is at least qualitatively similar to that reported for perfused axons in which significant K currents are absent. It is not surprising that this phenomenon was not observed by Hodgkin and Huxley (1952 *a*). In their experiments, sodium inactivation values for a depolarization were determined by a prepulse-test pulse procedure and experimental values were not available for potentials more positive than −30 mV. They chose to describe the voltage dependence of inactivation with a Boltzmann type function (Hodgkin and Huxley, 1952 *b*) which approaches a value of zero for voltages above 0 mV. Although it is now clear that inactivation shows a different voltage dependence than described by this function, the differences, for the purpose of modeling normal membrane action potentials, are probably not significant. This is so because a large sustained sodium conductance occurs only at potentials that are much more positive than those encompassed by the action potential. The potential rather than current direction dependency of inactivation behavior seems well established by the results of Bezanilla and Armstrong (1977).

Although our primary interest was to demonstrate the existence of incomplete inactivation in nonperfused axons, we also wished to see how the voltage dependence of the phenomenon in nonperfused axons compared with Cs-perfused axons. Our data indicate that under these two experimental conditions sodium inactivation behaves in a similar manner. It is likely that the variability in our data from nonperfused axons is due to small variations in the relatively large K currents contributing greatly to the variance in the measured value of the sustained sodium currents. This problem is inherent in a TTX subtraction method for the study of sodium currents at these voltages.

Direct quantitative comparison of the present results with earlier work is difficult. Both Chandler and Meves (1970 *a*) and Bezanilla and Armstrong (1977) used perfusion and superfusion solutions different from both our and one another's solutions. Chandler and Meves (1970 *b*) obtained calculated values from their experimentally derived model for the sum of the two h processes ($h_1 + h_2$) at steady-state as follows (approximate values): at 0 mV, ($h_1 + h_2$) = 0.15; at +80 mV, ($h_1 + h_2$) = 0.38; at +120 mV, ($h_1 + h_2$) = 0.55. Internally,

their axons were perfused with a solution containing 300 mM NaF and, externally, superfused with a K-free solution containing 472.5 mM Na. Bezanilla and Armstrong (1977) made steady-state inactivation measurements for a single pulse and obtained the following values (normalized to maximal peak tail current approximate values): at 0 mV, $I_{ss}/I_{peak} = 0.10$; at +80 mV, $I_{ss}/I_{peak} = 0.40$. Their axons in these experiments were perfused with a sodium-free solution containing 200 mM TMA (tetramethylammonium ion) and superfused with a solution containing 60 mM Na. Both our external and internal solutions (or axoplasm for nonperfused axons) are closer in ionic strength to those of Chandler and Meves. But the absolute amounts of internal sodium and the species of other ions are quite different. The most striking thing about the earlier data is the close agreement between the steady-state inactivation values for the widely different ionic strengths and composition of the two internal solutions, especially at +80 mV, a voltage where the steady-state current to peak current ratio and $(h_1 + h_2)$ should most likely be the same quantities.

In contrast, our value for the I_{ss}/I_{peak} ratio at +80 mV (which is a reasonable approximation of the value of steady-state inactivation) for Cs-perfused axons is about one-half the value reported above. In recent work on the interaction of ions with sodium inactivation, Oxford and Yeh (1979) obtained a value of steady state I_{Na} to peak I_{Na} with Cs-perfusion of 0.13, one that is very close to our own. And it also seems clear from the work of Oxford and Yeh (1979) that the nature of the internal cations has a direct influence on the gating behavior of the sodium conductance inactivation.

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