COMPARISON OF TWO-PULSE SODIUM INACTIVATION WITH REACTIVATION IN *MYXICOLA* GIANT AXONS

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ABSTRACT Values for the time constant of reactivation of the sodium conductance following depolarizations sufficient to completely inactivate G_{Na} have been compared over a 15 mV range of membrane potential with the time constants of inactivation during a depolarizing prepulse. Over this range the reactivation time constants were consistently 30–50% larger than the inactivation time constants determined simultaneously at the same potential in the same axon. The data suggests that inactivation and reactivation do not occur by identical mechanisms, and therefore implies that there are at least three kinds of experimental procedures necessary to fully characterize the sodium inactivation process in any particular system.

INTRODUCTION

In both *Myxicola* axons (Goldman and Schauf, 1973; Schauf, 1973; Schauf and Davis, 1975) and lobster axons (Oxford and Pooler, 1975) inactivation time constants determined from the decay of sodium current during a maintained depolarization (τ_h^i) are substantially smaller than the inactivation time constants determined at the same membrane potential using conditioning prepulses (τ_h^p). This comparison is of course limited to potentials depolarized relative to the resting potential and suggests that the processes being examined when open channels become inactivated (τ_h^i) is not the same as that occurring during a depolarizing prepulse to the same potential (τ_h^p).

Conditioning pulse procedures however yield values of τ_h^p over a wide range of membrane potential since in most systems the sodium channels are partially inactivated at the normal resting potential and one can measure the rate of recovery from this resting inactivation at hyperpolarized membrane potentials (Hodgkin and Huxley, 1952; Goldman and Schauf, 1972). However, the previous inequality of τ_h^i and τ_h^p for depolarized potentials raises the possibility that the process of recovery from inactivation may not be identical with the process of inactivation during a prepulse. This question has not been experimentally examined, rather a single $\tau_h^p(V_c)$ relation has been assumed to be defined by the combination of depolarizing and hyperpolarizing prepulses (Hodgkin and Huxley, 1952; Goldman and Schauf, 1972).

Let τ_h^{RE} denote those values of τ_h^p determined by reactivating the sodium system from a partially inactivated state. Over a narrow range of membrane potentials, basically bounded by h_∞ values between 0.2 and 0.8 in the classical Hodgkin-Huxley (1952) for-

malism, it is possible to measure reliably both τ_h^I and τ_h^{RE} in the *same* axon at the *same* potential. To measure τ_h^I the membrane is initially hyperpolarized to remove all resting inactivation and insure constant initial conditions, then depolarized to the potential V_c for a variable period of time, and the state of the sodium system is assayed by a large test pulse. To determine τ_h^{RE} the sodium system is first completely inactivated by a moderate depolarization following which the membrane is returned to a less depolarized potential V_c. Again a large test pulse is used to determine the availability of the sodium system. According to Hodgkin-Huxley kinetics, these time constants should be equal to one another. The following report examines this relationship experimentally in *Myxicola* giant axons.

METHODS

Myxicola axons were voltage clamped by methods previously described (Binstock and Goldman, 1969). Compensated feedback was used throughout. Artificial seawater (ASW: 430 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$) was buffered with 10 mM Tris to pH 7.8 ± 0.05 at a temperature of 5°C.

In order to rapidly and reliably obtain nearly simultaneous values for both τ_h^{RE} and τ_h^I in the same axon the following procedure was adopted. The axon was held at its normal resting potential. In the first clamp sequence a 100 mV hyperpolarization, 100 ms in duration (sufficient to remove all resting sodium inactivation) was followed by stepping to a potential V_c ($-55\text{ mV} < V_c < -40\text{ mV}$) for a period of time Δt to allow inactivation to occur. The state of sodium conductance was then assayed by recording the membrane current during a fixed test pulse, usually near +20 mV. This was then followed by a second clamp sequence in which the initial step was a 30–60 mV depolarization of the membrane for 100 ms (sufficient to completely inactivate G_{Na}) and the subsequent values of V_c, Δt and test pulse were identical to those in the first sequence.

The paired procedure just described was then repeated for randomized values of Δt between 1.0 and 100 ms, thus defining the time constants τ_h^I and τ_h^{RE} at the potential V_c. Frequent applications of an initial hyperpolarization with $\Delta t = 0$ served to insure that there was no significant deterioration of the preparation, and provided this was the case the entire procedure was repeated for another value of V_c. Once the range $-55\text{ mV} < V_c < -40\text{ mV}$ was explored, values of τ_h^I and τ_h^{RE} were obtained at a few potentials outside the region of overlap in order to fully characterize each axon for comparison with previous data (Goldman and Schauf, 1972).

Finally, the axon was exposed to 10^{-6} M tetrodotoxin and the complete protocol repeated to obtain the nonsodium contribution to each of the current records. Subsequent data analysis was then identical to that used in previous studies (Goldman and Schauf, 1972; Schauf, 1974; Schauf and Davis, 1975).

RESULTS AND DISCUSSION

Fig. 1 summarizes the results of our experiments. The solid symbols represent measurements of reactivation time constants (τ_h^{RE}) beginning from a completely inactivated condition, whereas the open circles represent measurements of inactivation time constants (τ_h^I) beginning from a condition in which there was no inactivation. The solid line is that used by Goldman and Schauf (1972) to fit their nonoverlapping data obtained during displacements from a resting potential at which there was on the average about a 30% inactivation. The present data agree perfectly with the earlier work, the only difference being the extension of reactivation measurements to more depolar-

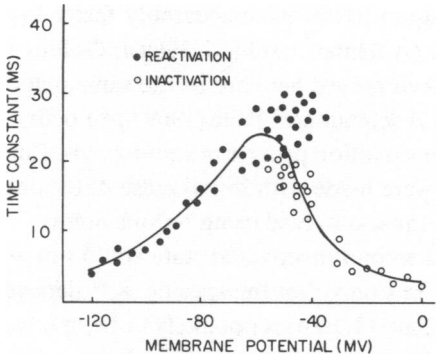

FIGURE 1 Time constants for sodium reactivation following a 30–60 mV depolarizing pulse 100 ms in duration (•) and for sodium inactivation following a 100 mV, 100 ms hyperpolarizing pulse (○), plotted as a function of membrane potential. The solid line is calculated from Eqs. 4, 6, and 7 of Goldman and Schauf (1972). Time constants were calculated by methods described in Schauf (1974) and Schauf and Davis (1975) for reactivation and inactivation, respectively. Temperature was 5 ± 1°C.

ized values. However repetition of the measurements of τ_h^{RE} and τ_h^{I} in the region of nonoverlap was essential since these initial conditions differed from those used by Goldman and Schauf (1972) and some effect was in principle possible.

In these experiments the potential range over which τ_h^{RE} and τ_h^{I} could be reliably determined at the same potential extended from −55 mV to −40 mV. Over this range, values of τ_h^{RE} were consistently 30–50% larger than the values of τ_h^{I} determined simultaneously in the same axon. Although the scatter is quite large, the time constant data remain completely nonoverlapping. Moreover, if the ratio τ_h^{I}/τ_h^{RE} is calculated for each axon the variability is substantially reduced since those axons with relatively large values of τ_h^{RE} also had larger values for τ_h^{I}. During the preparation of this report we learned that inactivation time constant data very similar to that seen in *Myxicola* including the present findings, have recently been obtained in voltage-clamped crab nerve fibers (Connor and Jakobsson, personal communication).

A process of slow sodium inactivation, dependent both on membrane potential and external potassium concentration has been described in squid giant axons (Adelman and Palti, 1969 a, *b*). These workers demonstrated that external potassium ions have an inactivating effect on G_{Na}, which can however be largely counteracted by membrane hyperpolarizations of very long duration. It could be argued that the differences in reactivation and inactivation time constants reported here are the result of driving a significant number of channels into (or out of) such a second inactivated state during the 100-ms depolarizations (or hyperpolarizations) initially applied to these axons.

However, removal of inactivation in *Myxicola* proceeds (except for the initial delay) as a single exponential up to at least 200 ms with no evidence for a second, slower process. Also, the present experiments were done in 10 mM K^+, using depolarizations to inactivate G_{Na} which were too small to produce an appreciable change in E_K. At 10 mM K^+, the slow inactivation process examined by Adelman and Palti (1969 *b*) is a relatively small component of the overall behavior. In addition, the rapid (prepulse)

inactivation process in squid axons is considerably faster than that in *Myxicola* at comparable temperatures (Adelman and Palti, 1969 *a*; Goldman and Schauf, 1972), so that the 100-ms durations employed here are of the same magnitude, relative to τ_h^p, as the 30-ms pulses used by Adelman and Palti (1969 *b*) to define the initial quasi-steady state of the fast sodium reactivation process in squid axons. Finally, in two axons, measurements of τ_h^p and τ_h^{RE} were made with initial pulse durations of 50 ms, and the results were comparable to those obtained using 100-ms pulses.

This is not to say that a second inactivated state could not account for the observed difference in τ_h^p and τ_h^{RE}, but only that the specific K^+ dependent, slow process described by Adelman and Palti (1969 *b*) is not likely to be involved. If the rate constants governing transitions between the usual inactivated state and an "extra-inactivated" state were of the same order of magnitude, there might not be a clear separation between the two processes.

In any case, the results of the present study suggest that prepulse inactivation and reactivation do not occur by identical mechanisms. Since it has previously been established that the process being examined when open channels inactivate is different from that occuring during a depolarizing prepulse (Goldman and Schauf, 1973; Oxford and Pooler, 1975; Schauf and Davis, 1975), it must be concluded that separate measurements of τ_h^s, τ_h^p, and τ_h^{RE} must be made in order to fully characterize the process of sodium inactivation in any particular experimental system.

The author is grateful for the superb technical assistance of Ms. Barbara J. Reed.

This work was supported by the Morris Multiple Sclerosis Research Fund, and by NIH Research Career Development Award 1 K04 NS 00004-01.

Received for publication 14 July 1975 and in revised form 29 August 1975.

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