RESEARCH COMMUNICATIONS

SODIUM CURRENTS IN *MYXICOLA* AXONS

NONEXPONENTIAL RECOVERY FROM THE INACTIVE STATE

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In both *Dosidicus* (Armstrong, 1970) and *Myxicola* (Goldman and Schauf, 1972) axons subjected to two-pulse experiments, sodium inactivation does not develop according to the simple exponential time course predicted by the Hodgkin-Huxley equations (Hodgkin and Huxley, 1952 *a*, *b*). Rather, there is a brief initial delay during which increasing the duration of a depolarizing prepulse from zero causes no decrease in the peak sodium current measured during a subsequent test pulse.

The converse experiment, namely completely inactivating the sodium channel by a large depolarizing pulse and then allowing the system to reactivate at the holding potential, has not been done using very short recovery times. Again, Hodgkin-Huxley kinetics predict a strictly exponential recovery from inactivation. Furthermore, the observation that there exist delays on development of inactivation does not require similar deviations on removal of inactivation. Experimental evidence on this point would serve to further restrict possible kinetic models of the sodium channel.

Myxicola giant axons were voltage clamped by methods previously described (Binstock and Goldman, 1969). Compensated feedback was employed throughout. Artificial sea water (ASW: 430 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$) was buffered with 5 mM Tris to pH 8.0, usually at a temperature of 5°C.

12 axons were examined for evidence of delays on the removal of inactivation. A depolarizing pulse sufficient to completely inactivate the sodium conductance (a 40–60 mV depolarization lasting 100 ms) was followed by a return to the holding (resting) potential for a variable period of time (recovery time), then by a 70 mV test pulse. The peak sodium current during the test pulse was determined by sub-

tracting from the records obtained in ASW the comparable current records observed in a solution containing 10^{-6} M tetrodotoxin. The ratio of the peak sodium current for a particular recovery time to that observed when the recovery time was 200 ms was then computed and plotted as a function of the recovery time. Note that each determination was bracketed by measurements with a 200 ms recovery time to correct for any deterioration of the sodium system with time. In two experiments the sodium equilibrium potential was measured after times of 1, 2, 4 and 200 ms. No significant change in E_{Na} could be observed.

Fig. 1 shows the result of a typical experiment. The solid symbols represent experimental measurements, while the solid line is the calculated best exponential fit to the data for recovery times in excess of 5 ms. The dashed line is drawn by eye.

Clearly, for short recovery times the process is sigmoidal rather than exponential. Comparable results were seen in all axons, as shown in Fig. 2. Here, in order to combine the results obtained from all experiments, the following procedure was used. For a given axon the best exponential fit to the data for recovery times in excess of 5 ms was determined by a regression analysis. Then the observed currents at recovery times less than 5 ms were divided by the currents extrapolated from the exponential fit (expected current). Thus, ratios less than unity imply the existence of deviations from simple exponential behavior.

For recovery times of less than 1 ms, Fig. 2 shows that little or no sodium current is observed, at 1 ms currents are approximately one-third of the expected values, and deviations do not disappear completely until recovery time is of the order of 4 ms.

These experiments have significant implications for the construction of models of

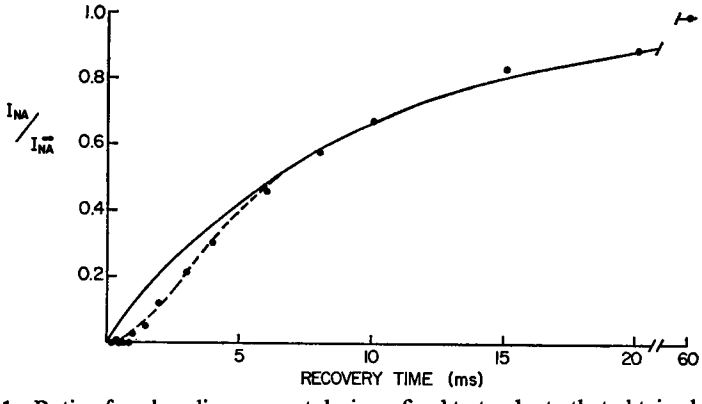

FIGURE 1 Ratio of peak sodium current during a fixed test pulse to that obtained with a recovery time at the holding potential of 200 ms, plotted as a function of recovery time (see text). For this axon the holding (resting) potential was −66 mV. A prepulse to −6 mV lasting 100 ms completely inactivated the sodium conductance. An 8 ms test pulse to +4 mV was used to assay the rate of recovery from inactivation at the holding potential. Temperature was 6.5°C. The solid line corresponds to a simple exponential recovery with a time constant of 9.25 ms and represents the best fit to the data for recovery times in excess of 5 ms. The dashed line is drawn by eye.

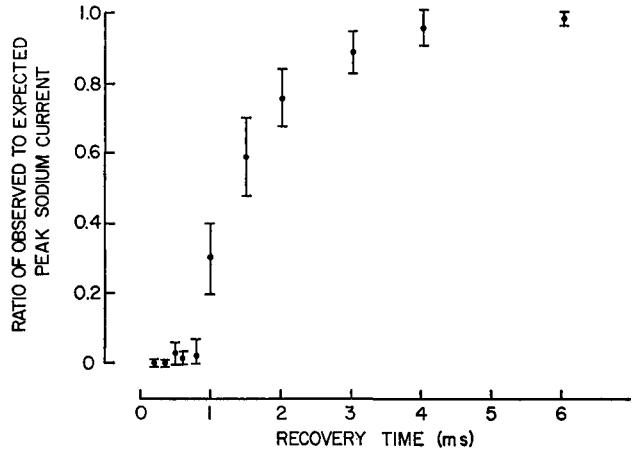

FIGURE 2 Pooled data from all axons examined. The ratio of experimentally observed currents (e.g., solid symbols in Fig. 1) to those calculated from the best exponential fit to the data for recovery times in excess of 5 ms (e.g., solid line of Fig. 1), as a function of recovery time. Ratios less than unity imply increasing deviations from simple exponential behavior. The solid symbols and vertical bars correspond to the mean ratio and standard error of the measurements at a particular recovery time for all axons. For the shortest recovery times of 0.2 and 0.4 ms, observed currents were zero in all cases.

the sodium channel. The kinetic model of Moore and Jakobsson (1971), for example, is based on a calcium binding hypothesis, and unless modified would predict there to be delays on the development, but not the removal, of inactivation. The absence of delays on the removal of inactivation would seem to be expected for all models in which the inactive state is kinetically an end state of the system and all rate constants are first order.

Though the Frankenhauser-Hodgkin space (Frankenhauser and Hodgkin, 1956) in *Myxicola* axons is substantially larger than that observed for squid axons (Binstock and Goldman, 1971), significant potassium accumulation may still occur during the depolarizing prepulses used here, and such an increase in external potassium concentration might have an inactivating effect on the sodium conductance (Adelman and Palti, 1969). The change in $[K^+]_o$ calculated from the data of Binstock and Goldman (1971) for the largest prepulses used is a maximum of 10 mM, and in two experiments on *Myxicola* axons, such an increase produced a 10–15 % inhibition of the peak sodium current following large hyperpolarizing prepulses, values comparable to those obtained by Adelman and Palti (1969) on squid axons. Clearly this could not account for the large deviations from simple exponential behavior seen in Fig. 2. Moreover, the expected time constant for washout of excess K^+ is long relative to the time scale of the effects reported here (Frankenhauser and Hodgkin, 1956).

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