ACTIVATION-INACTIVATION COUPLING IN *MYXICOLA* GIANT AXONS INJECTED WITH TETRAETHYLAMMONIUM

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ABSTRACT *Myxicola* giant axons internally injected with tetraethylammonium chloride to block potassium currents were examined under voltage clamp. The sodium inactivation time constants obtained from the decline in I_{Na} during step depolarizations were substantially smaller than those obtained using conditioning prepulses to the same potentials and the ratios agreed with previous observations using TTX. Inactivation shifts were also measured and found to be comparable to previous results.

There exist at present a variety of voltage-clamp observations which are quite inexplicable in terms of the classical Hodgkin-Huxley (1952) kinetics. Perhaps the most striking anomaly is the larger time constants of sodium inactivation obtained from the effects of conditioning prepulses compared to those obtained from the decline in I_{Na} during step depolarizations to the same potentials. First measured in *Myxicola* (Goldman and Schauf, 1973; Schauf and Davis, 1975) it has also been observed in detailed studies of lobster kinetics (Oxford and Pooler, 1975) and its presence in squid was briefly noted by Moore and Cox (1976). Nevertheless, recent measurements using voltage-clamped frog nodes (Chiu, 1976) failed to detect these differences. Other anomalies are also variable from one preparation to another. The "inactivation shift" (Hoyt and Adelman, 1970) is quite prominent in *Myxicola* (Goldman and Schauf, 1972) and certain crab axons (Connor, 1976), but not in other crab axons characterized by the ability to fire repetitively (Connor, 1976). Delays on development of inactivation are clearly observed in *Myxicola* (Goldman and Schauf, 1972; Schauf and Davis, 1975) and frog node (Peganov, 1973), but less consistently in squid (Armstrong, 1970; Chandler et al., 1965). Delays on removal of inactivation have only been investigated in *Myxicola* (Schauf, 1974) and nodes (Chiu, 1976).

It has been suggested that some of these differences might be due to the experimental methods employed, specifically the common use of tetrodotoxin (TTX) to obtain nonsodium currents and subsequent subtraction from total membrane currents to yield the records of interest. Though seemingly not a likely explanation in view of the magnitudes of the effects seen, the lack of a time constant separation in frog nodes (Chiu, 1976) led us to reinvestigate these anomalies in *Myxicola* axons in which the potassium currents have been eliminated by internal injection of tetraethylammonium (TEA) chloride.

Aside from the injection of 1–2 μl of 250 mM TEA (Armstrong and Binstock, 1965) into axons with diameters between 550 and 700 μm, and the elimination of the use of TTX in favor of the direct recording of I_{Na}, methods of procedure were comparable to those reported previously (Goldman and Schauf, 1972, 1973; Schauf and Davis, 1975, Schauf, 1976). It should be noted that the estimated internal TEA concentrations of 50–100 mM were capable of completely eliminating time-dependent outward currents at membrane potentials more negative than +20 mV, leaving only leakage current. However, at more depolarized potentials measurements in the presence of TTX indicated that there was a component of I_K which was insensitive to the highest TEA concentrations used. This may be related to a similar partial insensitivity of *Myxicola* K^+ channels to externally applied aminopyridines (Schauf et al., 1976). With both treatments there is a shift in the $G_K(V)$ curve in the depolarized direction combined with a large decrease in $\bar{G}_K$, although quantitatively TEA^+ is a more effective inhibitor. In any case, all voltage steps were maintained at levels at which residual K^+ currents were insignificant.

Fig. 1 shows typical records of total membrane current in response to step depolarizations of 10–70 mV in the presence of TEA, after correction for a linear leakage current. Each clamp step was preceded by a 100 mV hyperpolarization lasting 100 ms. Series resistance compensation was employed. The time constants for sodium inactivation derived from these and similar records (τ_h^s) are plotted as the open symbols in Fig. 2. The same axons were then subjected to procedures in which test pulses of 60–70 mV followed conditioning prepulses of variable amplitude and duration. The prepulse inactivation time constants (τ_h^p) were determined and plotted in Fig. 2 as the filled symbols (Schauf and Davis, 1975). There is no significant difference between

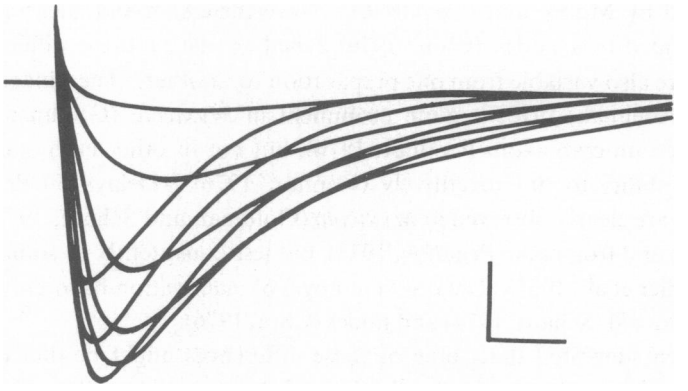

FIGURE 1 Membrane currents in an axon injected with 1 μl of 250 mM TEA chloride to give a final concentration of approximately 75 mM. The currents correspond to depolarization of 10, 20, 30, 35, 40, 50, 60, and 70 mV from a holding potential of −60 mV. Leakage current was subtracted using a signal averaging computer and the response to the equivalent hyperpolarizing pulse. Current and time calibrations are 0.3 mA/cm^2 and 1 ms, respectively. Temperature of 5°C.

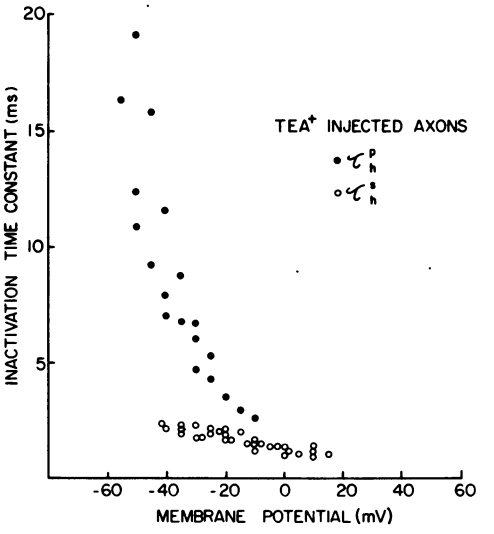

FIGURE 2 Inactivation time constants during step depolarizations (open symbols) and as derived from prepulse experiments (solid symbols) as a function of membrane potential. See Goldman and Schauf (1973) and Schauf and Davis (1975) for details of computational procedures.

these data and those previously reported (Goldman and Schauf, 1973; Schauf and Davis, 1975). Inactivation delays, although not studied in detail, also seemed comparable to previous observations.

The only remaining uncertainty in the interpretation of these records would appear to be the matter of adequate spatial control of membrane potential. Two experimental procedures have been performed to investigate this possibility. In the first some of the experiments of Cole and Moore (1960) on squid axons were repeated on *Myxicola*. After insertion of a piggyback electrode from one end of the axon, an exploring micropipette was inserted longitudinally from the opposite end and the potential recorded while the axon was being clamped. With the tip of the axial wire extending 1 mm beyond the chamber guard electrodes, the records of membrane potential obtained from the exploring electrode at all points in the chamber up to 1 mm inside the (4.5 mm) lateral guards were superimposable on the records obtained from the micropipette normally positioned in the center of the axon over the entire potential range of interest.

In more recent experiments (Schauf and Davis, 1975) we had occasion to obtain records of $I_{Na}(v, t)$ during step depolarizations at various times following exposure of the axons to tetrodotoxin prior to complete block. The time constants of decay of I_{Na} under conditions in which G_{Na}^{Max} had been reduced to as little as 20% of its initial value were identical to those obtained prior to application of TTX. If spatial control had not been adequate this result would not have been anticipated.

Steady-state sodium inactivation curves were also determined using pairs of test pulses on an approximately linear region of the negative limb of the sodium current-voltage relation (Goldman and Schauf, 1972). A typical result is shown in Fig. 3 for

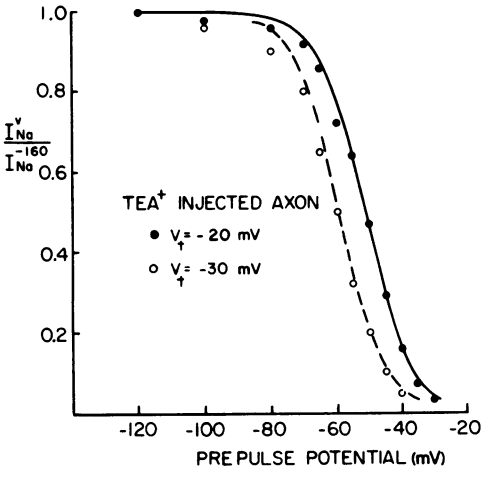

FIGURE 3

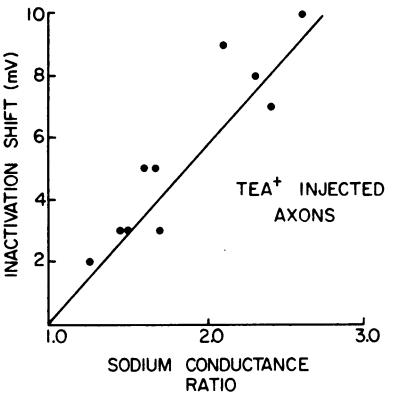

FIGURE 4

FIGURE 3 Steady-state Na^+ inactivation curves for test pulses of -30 mV (G_{Na}^{peak} = 7.1 mmho/cm^2) and -20 mV (G_{Na}^{peak} = 16.0 mmho/cm^2) in an axon with maximum G_{Na} of 35.0 mmho/cm^2. Solid line translated by +8 mV from the dashed line (see text).

FIGURE 4 Inactivation shift as a function of the peak sodium conductance ratio during pairs of test pulses on a linear region of the negative limb of the $I_{Na}(V)$ relation. The solid line corresponds to data previously obtained using TTX (Goldman and Schauf, 1972).

two test pulses at which the peak G_{Na} differed by a factor of 2.34. The two curves are solutions of the expression

$$h_\infty = [1 + e^{(V - V_h)/k_h}]^{-1}$$

for k_h = 7.0 mV and V_h = -59 mV (dashed curve) or -51 mV (solid curve). In Fig. 4 we have plotted for all axons the magnitude of the change in V_h as a function of the ratio of the peak sodium conductance during the two test pulses. The solid line corresponds to the best fit for previous observations using TTX (Goldman and Schauf, 1972). Values for k_h in these studies ranged from 6.0 to 7.0 mV and were insensitive to the magnitude of the test pulse. No change in the shape or position of the steady-state inactivation curve occurred when test pulses were taken in a range in which G_{Na} was saturated (Goldman and Schauf, 1972).

We conclude that the use of tetrodotoxin and subtraction procedures by us and others does not significantly contribute to the determination of anomalies in the kinetic features of the sodium conductance, and that the differences in the behavior of various preparations reflect some quantitative or qualitative variation in fundamental organization. Unfortunately, we could not easily compare sodium inactivation kinetics in the presence of TEA^+ with those derived using TTX on the same axons. In *Myxicola* recovery from TTX takes up to 30 to 60 min and is often still incomplete. Although intact axons are extremely stable, which may account for our ability to use TTX as routine to obtain sodium currents, perfused (rather than injected) axons tend

to deteriorate sufficiently rapidly so that one could not be confident in comparing observations following TEA removal. Nevertheless, we feel the close quantitative agreement between these and previous data provides compelling evidence that TTX subtraction does not introduce appreciable error in *Myxicola*.

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