CHARACTERISTICS OF SODIUM TAIL CURRENTS IN *MYXICOLA* AXONS

COMPARISON WITH MEMBRANE ASYMMETRY CURRENTS

C. L. SCHAUF, J. O. BULLOCK, AND T. L. PENCEK, *Departments of Physiology and Neurological Sciences, Rush Medical College, Chicago, Illinois 60612 U.S.A.*

ABSTRACT Sodium currents after repolarization to more negative potentials after initial activation were digitally recorded in voltage-clamped *Myxicola* axons compensated for series resistance. The results are inconsistent with a Hodgkin-Huxley-type kinetic scheme. At potentials more negative than −50 mV, the Na^+ tails show two distinct time constants, while at more positive potentials only a single exponential process can be resolved. The time-course of the tail currents was totally unaffected when tetrodotoxin (TTX) was added to reduce g_{Na} to low values, demonstrating the absence of any artifact dependent on membrane current. Tail currents were altered by $[Ca^{++}]$ in a manner consistent with a simple alteration in surface potential. Asymmetry current "off" responses are well described by a single exponential. The time constant for this response averaged 2.3 times larger than that for the rapid component of the Na^+ repolarization current and was not sensitive to pulse amplitude or duration, although it did vary with holding potential. Other asymmetry current observations confirm previous reports on *Myxicola*.

INTRODUCTION

In *Myxicola* giant axons activation and inactivation of the sodium conductance do not appear to be determined by two independent, first-order processes. This conclusion results from detailed measurements of two-pulse inactivation and reactivation (Goldman and Schauf, 1972; Schauf, 1974, 1976) and comparison of these data with the time-course of the decline in sodium conductance during maintained depolarizations (Goldman and Schauf, 1973; Schauf and Davis, 1975; Schauf et al., 1976*b*). As a minimum, it appears that the turn-off of conducting channels may represent a different process than inactivation by changes in initial conditions. However, as yet no measurements have been made in *Myxicola* of the decline in sodium current after repolarization of the membrane to more negative values at a time when g_{Na} is high (tail currents).

Such measurements would be of particular interest since the Hodgkin-Huxley (1952) kinetics, based on the existence of independent activation (m) and inactivation (h) processes with $g_{Na} = m^3 h \bar{g}_{Na}$, makes specific predictions concerning this process. Consider the situation shown in Fig. 1, where the amplitude (V_1) of P_1 is adjusted so repolarization occurs when g_{Na} is near its maximum value. At this point the m variable is approximately unity and the h variable is only slightly less than one (call it h_1).

When the potential is changed from V_1 to V_2, the m variable will rapidly decrease to its steady-state value $m_2 = m_\infty(V_2)$. Simultaneously h will begin to change from its value h_1 at the end of P_1 to its steady-state value $h_2 = h_\infty(V_2)$. At all repolarization potentials V_2 the time constant $\tau_m(V_2)$, describing the rate of change of the m variable, is much smaller than the time constant $\tau_h(V_2)$, describing the time-course of h. Two regions showing qualitatively different behavior may be distinguished. For repolarization potentials that are large and negative (region B), the steady-state values of m and h are zero and one, respectively ($m_2 = 0$; $h_2 = 1$). Since $\tau_m(V_2) \ll \tau_h(V_2)$, g_{Na} will approximate a simple exponential decay of I_{Na} with a time constant $\tau_m(V_2)/3$. That is to say, g_{Na} will rapidly decrease as m decreases from approximately 1 to 0, but the change in h will not be observable since $g_{Na} = m^3h$ and m is already 0. However, for repolarization potentials where the steady-state value of m is not zero and $h_2 > 0$ (for example in the range of -30 to -10 mV, region A), the result will be more complex. Since even here $\tau_m(V_2) \ll \tau_h(V_2)$, the initial event will be a rapid decrease in m to its equilibrium value m_2 with several exponential components ($3\tau_m$, $2\tau_m$, τ_m). But since $m_2 \neq 0$, there still will be some remaining sodium current, which will then proceed to inactivate slowly with a time constant $\tau_h(V_2)$, and thus the measured tail currents should show both an initial rapid and slow decay phase.

We have studied voltage-clamped *Myxicola* axons under such conditions and find that experimentally the behavior of the sodium tail currents is exactly the inverse of this prediction, showing departures from simple exponential kinetics at large negative values of V_2, but not at potentials between -40 and 0 mV. Furthermore, comparison of repolarization with activation of g_{Na} over the range of -40 to 0 mV suggests that models in which the activation of g_{Na} is an algebraic function of some first-order variable are untenable.

Recently there have been a number of attempts to compare the time-course of Na^+ tails in squid axons and frog myelinated nerve with the behavior of membrane asymmetry currents (Armstrong and Bezanilla, 1973, 1974; Bezanilla and Armstrong, 1975, 1976; Keynes and Rojas, 1974, 1976; Meves, 1974; Rojas, 1976). In view of the nature of the tail currents in *Myxicola* at large negative potentials, we have examined the behavior of the asymmetry current in comparable experiments. The results support the need for a new systematic model to relate asymmetry currents and the temporal behavior of the sodium conductance.

METHODS

Intact *Myxicola* axons were voltage-clamped using the basic techniques previously described (Binstock and Goldman, 1969). The artificial sea water (ASW) had the composition: 430 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, and 20 mM Tris. In some instances, however, $MgCl_2$ was eliminated and $[Ca^{++}]$ varied by substitution for osmotically equivalent amounts of Tris to examine the effects of changes in membrane surface potential (Schauf, 1975). In other experiments $[Na^+]$ was reduced by Tris substitution and/or tetrodotoxin (TTX) was added to study the dependence of tail currents on the absolute magnitude of I_{Na}. The pH in all experiments was maintained at 7.8 $\pm$ 0.1, at temperatures ranging from 2 to 12°C.

The measurement of sodium tail currents on repolarization to more negative values after some

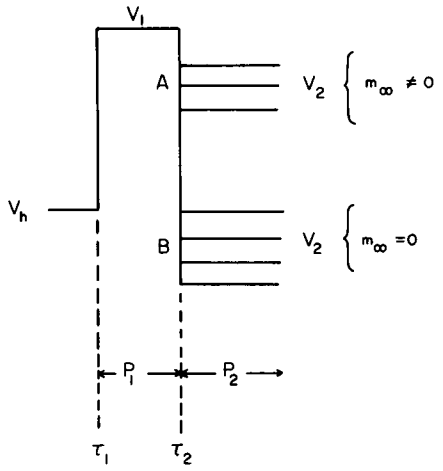


FIGURE 1

FIGURE 2

FIGURE 1 Pulse protocol for measurement of Na^+ tail current.

FIGURE 2 Membrane currents recorded during P_1 and P_2 (Fig. 1) in ASW (upper record) and 1 μM TTX (middle record), and the result of digital subtraction of the TTX record from that obtained in ASW (lower record). Current and time scales are 1.5 mA/cm^2 and 0.25 ms, respectively. Holding potential was −100 mV. Temperature was 5°C.

initial activation of g_{Na} is difficult, due to the multiple problems of overlapping capacity currents, time-dependent early leak, and imperfect compensation for membrane series resistance. The first two obstacles can be overcome by digital recording of membrane current combined with the use of TTX to obtain nonsodium current contributions to total currrent, if proper controls are present to insure that use of TTX does not by itself introduce any error. Imperfect series resistance compensation, as well as problems associated with edge effects or inadequate spatial control, would produce a situation in which the behavior of the system would be exquisitely sensitive to the absolute magnitude of membrane sodium current. Thus, these two errors can be detected by reducing the sodium conductance to low values and comparing the

temporal behavior to that at maximum current. If the temporal behavior is unchanged, then such sources of error cannot affect the results. Because of the close association between these control procedures and the presentation of the results themselves, detailed consideration of such data will be given in the following section.

Data recording for both sodium tail and asymmetry currents was via a Nicolet 535 signal averager (Nicolet Instrument Corp., Madison, Wis.), equipped for bulk data storage on magnetic tape and automatic cycling of memory. The instrument performed a 12-bit analog-to-digital conversion every 10 μs and was equipped with a 1,024-word memory. Because of the 12-bit resolution of the A/D converter, we could accurately record asymmetry currents (30–50 points, see Fig. 10) without blanking out any portion of the current record. Although asymmetry currents could be resolved in single sweeps, we generally separately recorded the responses to 8–16 paired depolarizing and hyperpolarizing pulses. The only exceptions were with pulse amplitudes less than $\pm$ 60 mV, where up to 64 sweeps were recorded.

Pulses were provided by a series of Ortec (Ortec Inc., Oak Ridge, Tenn.) pulse generators, modified so that they could be individually trimmed to insure precise equality of depolarizing and hyperpolarizing pulses. Tests for linearity were run routinely before and after each experiment with an appropriate equivalent circuit to insure that no asymmetries were being introduced by components external to the axon.

Measurements of membrane asymmetry currents were made in intact axons exposed to a Na^+-free solution containing 1 μM TTX and 10 mM 4-aminopyridine to block both Na^+ and K^+ currents. The latter drug at this concentration has been shown to be a highly specific and effective K^+ blocker for depolarizations that are not too large (Schauf et al., 1976*a*) and has been previously used by Rudy (1976) in initial studies of asymmetry currents in *Myxicola*.

It should be noted that intact *Myxicola* axons exposed to 1 μM TTX and 10 mM 4-aminopyridine were highly stable. Approximately 80% of all dissected axons had action potentials in excess of 100 mV after electrode insertion. In 75% of these axons, membrane asymmetry currents could be continuously recorded for 2–3 h with less than a 10% decrease in magnitude of the response to pulses of $\pm$100 mV.

Over this period of time the individual responses to depolarizing and hyperpolarizing pulses also remained invariant with no evidence of the progressive change in time-dependent leakage current described by Keynes and Rojas (1976). In the remainder of the axons experiments were terminated when asymmetry currents in control $\pm$100 mV pulses decreased by more than 10%.

Slow sodium inactivation in *Myxicola* axons (Rudy, 1975; Schauf et al., 1976*b*) has been reported to affect the magnitude of asymmetry currents when pulses are given more frequently than once every 10 s. (Rudy, 1976). Although qualitatively we also found that rapid (>1/s) pulsing produces an equal decline in both sodium current and asymmetry current, we were unable to detect appreciable effects (less than 10%) on asymmetry current at frequencies less than 1/s. Also at higher frequencies, although the peak amplitude of the asymmetry current at the termination of the pulse (off response) could be substantially decreased, the time constant did not vary by more than 10%. Thus, we generally chose to take data approximately every 2 s.

RESULTS

Sodium Repolarization Currents

Measurements of the kinetics of sodium tail currents after membrane repolarization were made using the following pulse schedule. The axon was held at -100 mV. The sodium conductance was then activated by a 700-μs depolarization to +20 mV, chosen

so that at the end of this pulse g_{Na} would only have slightly declined from its maximum value. This pulse will be termed P_1 (See Fig. 1). Immediately after P_1, the membrane is repolarized to a more negative potential, V_2, for 5 ms. This pulse will be called P_2. Currents in ASW were recorded digitally during P_1 and P_2 with values of the repolarization potential (V_2) ranging from 0 to −160 mV in 10 mV increments. Nonsodium currents were obtained by immediate application of 1 μM TTX and repetition of the identical pulse schedules, a procedure made possible by the lack of significant drift in the currents recorded from intact *Myxicola* axons and justified further below. Subtraction of the records obtained in TTX digitally from those recorded in ASW yields membrane sodium current uncontaminated by displacement currents or nonsodium ionic currents.

The results of this procedure are illustrated in Fig. 2 for an axon in which P_1 activated a sodium conductance of normal magnitude and in which series resistance compensation was used. The repolarization potential during P_2 was −70 mV. At the top is the current recorded in ASW, while the middle record was obtained 20 min later in 1 μM TTX. The rapid phase of the membrane displacement current lasts approximately 20 μs, so that in TTX at the 10-μs digitizer resolution a single point is obtained on the rising phase of the displacement current while two points appear on the falling phase. This is followed (in TTX) by the early time-dependent leakage current. (Note that two of the points at the start of P_1 were off scale in both records as these were photographed to concentrate on the transients at the end of P_1.)

The bottom record of Fig. 2 illustrates the result of digital subtraction of the TTX record from that recorded in ASW. The temporal behavior of the sodium tail current is obviously only very slightly influenced by the TTX subtraction procedures. In this particular case the repolarization current could be described as a sum of two exponential processes with time constants of 78 and 390 μs. For repolarization potentials of −160 mV (see below), the fast component could have a time constant as short as 35 μs and in these cases the subtraction of two or three points for the fast displacement current could have some slight effect on the time-course of the sodium tails. We therefore regard values for the repolarization time constant shorter than 40 μs (8–10 points which can be used to derive τ_1) with some caution.

In Fig. 3 we have provided a series of (TTX subtracted) sodium currents during P_1 and P_2 for repolarization potentials of −10, −20, −30, −50, −70, −100, and −130 mV (same axon as Fig. 2). For values of V_2 more positive than −40 mV, the Na^+ tail currents during P_2 are adequately described by a single time constant. A representative semilogarithmic plot of the current during P_2 with $V_2 = -30$ mV is shown for normal g_{Na} in the upper curve of Fig. 8. There is no sign of the initial rapid decrease in g_{Na} expected on the basis of the Hodgkin-Huxley (1952) kinetics and corresponding to the rapid closure of those "m" gates not normally open at this repolarization potential. At more negative values of the repolarization potential, the currents during V_2 cannot be described as single exponentials, but rather appear to be the sum of at least two exponential processes, as indicated by the solid symbols and lines in Fig. 6 for $V_2 = -80$ mV (see also the later discussion of Figs. 9 and 10). In this range, $m_\infty(V_2) = 0$ in the

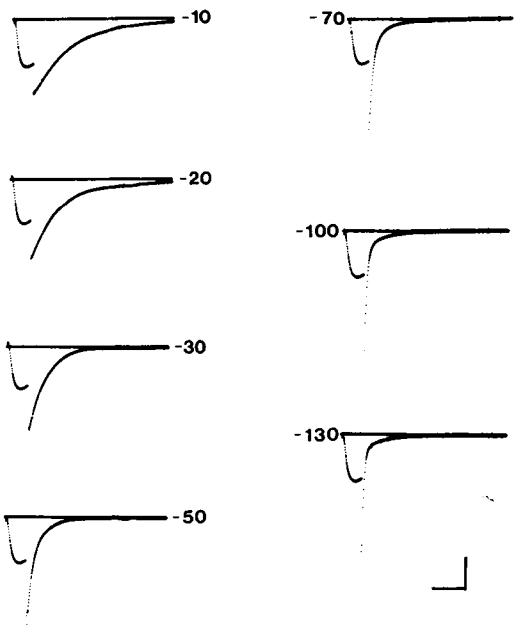


FIGURE 3 Sodium tail currents on repolarization to the indicated potentials (V_2 in Fig. 1) after a 700-μs depolarization to +20 mV. Current and time scales are 0.75 mA/cm^2 and 1.0 ms, respectively. Temperature was 5°C. The records are the result of digital subtraction of corresponding records in ASW and 1 μM TTX to yield sodium currents. (Note that in some of the records occasional points did not carry through the photographic process.) The horizontal line corresponds to zero current.

Hodgkin-Huxley kinetics, and the decay of the sodium conductance should be a single exponential with a time constant $\tau_m(V_2)/3$.

These results appear to be exactly contrary to the predictions of the Hodgkin-Huxley (1952) kinetics, and so one is particularly concerned to eliminate all possible sources of error experimentally. To rule out any possibility that nonsodium currents had changed between the time when initial recordings were made in ASW and the time they were repeated in TTX, we adopted the following procedures: Currents during P_1 and P_2 were recorded in ASW (sometimes containing 10 mM 4-aminopyridine to block I_K) for $V_2 = -100$ mV (holding potential -100 mV). Next, we recorded the current during a step from -100 to -220 mV. During this pulse the capacity and leakage currents should have been similar to those during the step from +20 to -100 mV, except for the presence of a small asymmetrical component of membrane displacement current and a possible contribution due to nonlinear leak. The currents recorded at -220 mV were subtracted digitally from those measured at -100 mV and the result compared to that obtained by subsequent addition of TTX as a means of obtaining nonsodium currents. A similar procedure was then followed for other repolarization potentials between -140 and -40 mV.

Fig. 4 illustrates the results of this comparison for repolarization potentials of -40 and -100 mV. In both cases there was no significant difference in the time-course of

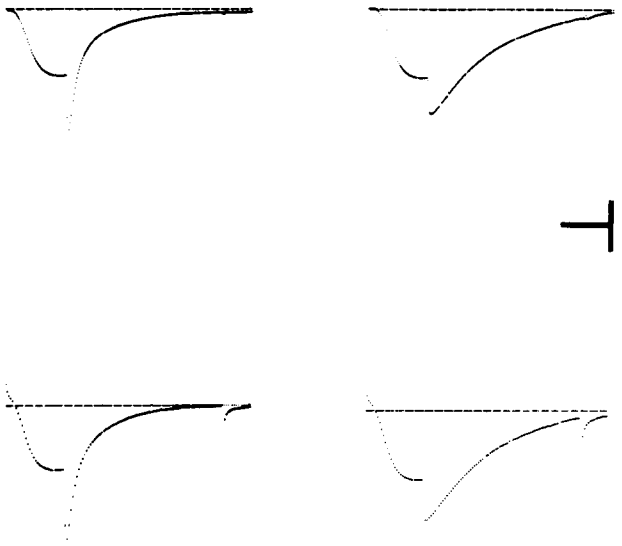


FIGURE 4 Membrane sodium currents during P_1 and P_2 (see Fig. 1) obtained as the difference in currents recorded in ASW and in the presence of 1 μM TTX (upper records), compared to those obtained by adding the displacement currents recorded during equivalent hyperpolarizing pulses (lower records; see text). Records on the left were obtained in ASW with $P_1 = +20$ mV and $P_2 = -100$ mV, while those on the right are from an axon in ASW + 10 mM 4-aminopyridine with $P_1 = +20$ mV and $P_2 = -40$ mV. Digitizing rate is 10 μs/point in the upper records and 20 μs in the lower records. Current and time scales are 1.5 mA/m^2 and 0.5 ms, respectively. Temperature was 5°C.

the repolarization current measured by the use of TTX subtraction and paired pulses. (Note the slower digitizing rate used in the lower records). In particular, the slow component of the Na^+ tail current was completely unaltered, providing compelling evidence that it was not an artifact produced by any changes in membrane currents during the interval in which TTX was blocking I_{Na}. Similar conclusions were reached for all other repolarization potentials.

It should be noted that in Fig. 4 the paired pulse procedure was carried out in one case in ASW and in the other in the presence of 10 mM 4-aminopyridine. In general, the use of this agent did not seem to affect the time-course of any component of the sodium conductance even when applied at concentrations large enough to cause a significant reduction in $\bar{g}_{Na}$. This is illustrated by the records in Fig. 5 obtained in ASW and after addition of 20 mM 4-aminopyridine, the peak conductance in this case being reduced by approximately 15%. This is particularly important in that most of the asymmetry current measurements were carried out in 10 mM 4-aminopyridine, and even though tail currents were measured in these axons under similar conditions, a differential effect of this drug would have been a possibility (see also Fig. 14).

Because our data are recorded digitally rather than being read off photographic film, and because of the above arguments, we feel we can accurately subtract displacement currents and time-dependent nonsodium currents by the use of TTX. Thus, the only remaining uncertainty is provided by the possibility that our measurements were sig-

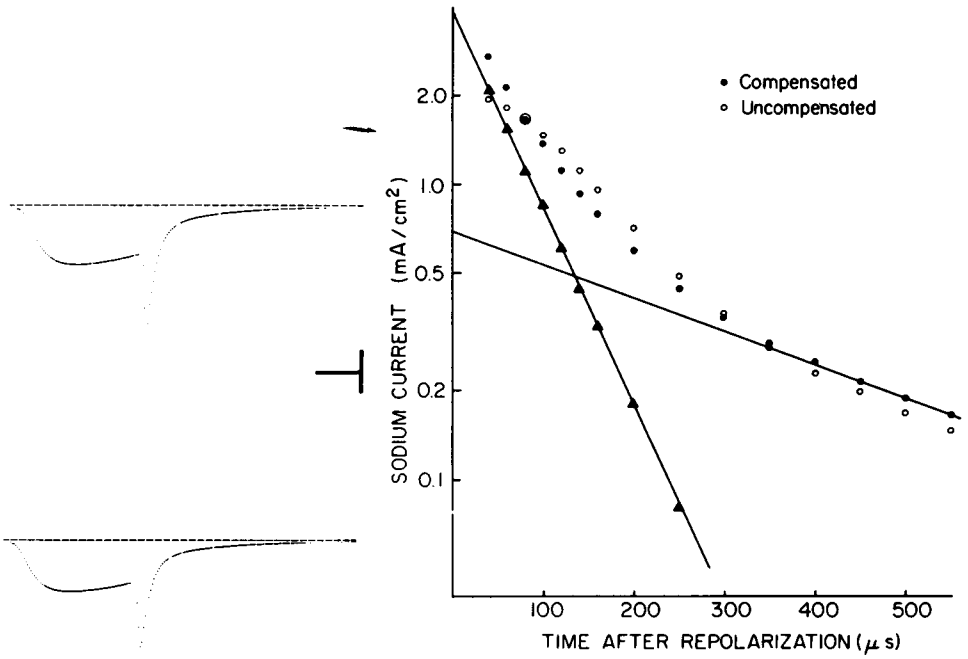


FIGURE 5

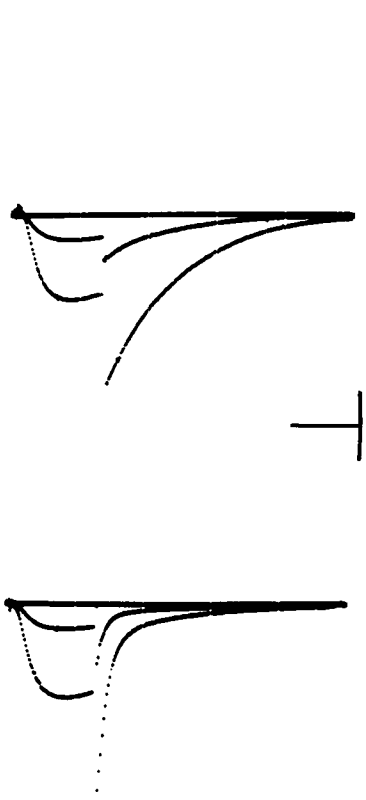
FIGURE 6

FIGURE 5 Sodium currents during P_1 and P_2 (see Fig. 1) as recorded in ASW (above) and ASW +20 mM 4-aminopyridine (below). In this experiment P_1 = +20 mV and P_2 = −100 mV. Current and time scales are 1.5 mA/cm^2 and 0.25 ms, respectively. Temperature was 4°C.

FIGURE 6 Semilogarithmic plot of sodium tail currents on repolarization to −80 mV after initial depolarization to +20 mV for 700 μs in axon in which the series resistance was either compensated by our standard criteria (filled circles) or deliberately left uncompensated (open circles). In the former case the solid triangles illustrate the result of subtracting out the slow exponential component. The solid lines are the least-squares fits to the data, obtained by series resistance compensation, and correspond to τ_1 = 56 μs and τ_2 = 385 μs. Temperature was 5°C.

nificantly affected by errors in compensation for membrane series resistance, and investigation of this question formed a major component of our effort.

Series resistance was estimated from the initial response of the current-clamped membrane to step changes in applied current after correcting for the finite rise time of the current pulse by the method of Binstock et al. (1975). The amount of series resistance compensated for was directly measured from the dip in the command pulse records (Goldman and Schauf, 1972) and adjusted to have a residual uncompensated component ranging from 0 to 3 Ω-cm^2. The response of the membrane under voltage clamp during a repolarization to −80 mV after initial g_{Na} activation is shown as a semilogarithmic plot in Fig. 6 for both the compensated and uncompensated case. It is evident that at this potential, in the absence of series resistance compensation, the tail currents are not adequately described as a sum of two exponential terms (open



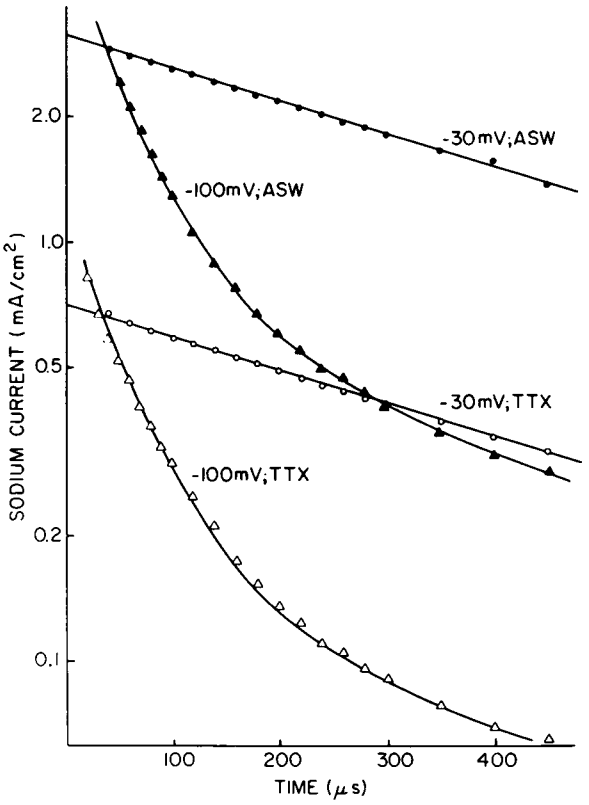


FIGURE 7

FIGURE 8

FIGURE 7 Sodium tail currents on repolarization to −30 mV (top) and −100 mV (bottom) after a 700-μs depolarization to +20 mV in normal ASW (lower curves) and in the presence of 50 nM TTX (upper curves). The horizontal lines correspond to zero current. The sodium conductance during the initial depolarization was reduced by 77% in the presence of TTX. Current and time scales are 0.75 mA/cm^2 and 0.5 ms, respectively. Note that this is the same axon as shown in Fig. 3.

FIGURE 8 Semilogarithmic plots of the data in Fig. 7. The solid lines were fit by eye to the data in ASW and then translated vertically to fit the data in 50 nM TTX.

circles; note the concave downward curvature at the extreme left). The initial current is lower than in the compensated case and the effective decay constant becomes more rapid over the first 150 μs as the decline in I_{Na} causes an effective membrane hyperpolarization. At times over 300 μs, however, where the current has been reduced to less than 10% of its initial value, the results coincide, showing the slow phase of tail currents to be independent of series resistance compensation. With compensation the decay of I_{Na} at this potential is well described as the sum of two exponential processes (solid lines in Fig. 6). Similar kinds of deviations were observed in the absence of series resistance compensation at all other potentials examined in this study.

Since series resistance errors are directly proportional to the magnitude of current

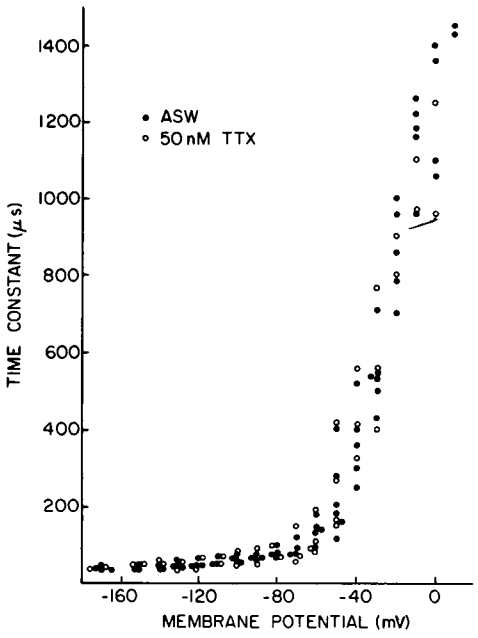


FIGURE 9

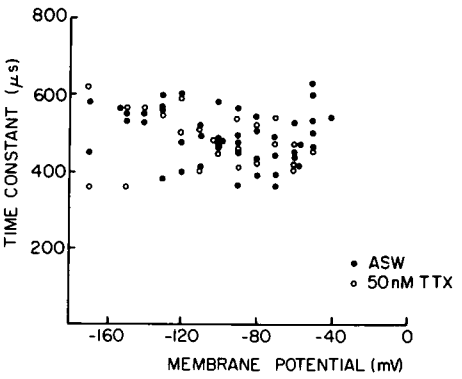


FIGURE 10

FIGURE 9 Time constants for the rapid decline in sodium current on repolarization (τ_1) as a function of membrane potential in a series of axons in which responses were recorded both in ASW (•) and in 50 nM TTX (○). Note that for $V \geq -40$ mV two distinct exponential processes could not be distinguished and only a single time constant was available. Temperature was 4–6°C.

FIGURE 10 Time constants for the slow decline in sodium current on repolarization (τ_2) as a function of membrane potential in the same set of axons as in the previous figure.

flow, to insure that the applied series resistance compensation was in fact adequate, we next carried out procedures in which we measured the kinetics of repoloarization at various absolute levels of I_{Na} in the same axon. Fig. 7 shows the results of adding 50 nM TTX to the external ASW for repolarization potentials (V_2) of −30 mV (top) and −100 mV (bottom). The sodium conductance during the first pulse was reduced to 23% of its initial value and, as can be seen from the semilogarithmic plots of Fig. 8, there was no significant change in the time-course of the measured tail currents. This was not the case if the membrane was deliberately left undercompensated. The data remained insensitive to alterations in absolute g_{Na} down to values as low as 8% of the ASW conductance, thus demonstrating that our measurements were not subject to any artifact dependent on membrane current.

The complete data obtained from an analysis of records comparable to those of Fig. 8 is shown as a function of repolarization potential in Figs. 9 and 10, both for measurements in ASW (filled circles) and for the same axons in ASW containing 50 nM TTX (open circles) to reduce g_{Na} to 10–20% of its initial value. At repolarization potentials more depolarized than −50 mV, only a single relaxation process could be

resolved. For other potentials the data was described by two exponential components. Over the range where two processes could be resolved, the slower relaxation process (Fig. 10) was essentially independent of membrane potential, generally varying between 400 and 600 μs. Since for potentials more positive than -50 mV only a single time constant can be resolved and since it becomes larger with increasing potential, we have assumed that this represents the behavior of the fast, voltage-dependent component. Therefore in Fig. 9 we have combined measurements of the faster time constant for $V_2 < -50$ mV with the single time constant characteristic of $V_2 > -50$ mV. The resultant composite of time-constant data decreases monotonically from 1.0–1.4 ms at 0 mV to 30–50 μs at -160 mV. The scatter is quite large in the vicinity of -50 mV because of frequent uncertainty in the separation of the two components in this region. In neither figure do the determinations appear to be affected by the use of TTX to reduce g_{Na}, leading to the conclusion that the result is independent of the absolute magnitude of membrane current, and therefore not likely to be contaminated either by any significant series resistance artifact, or by any contributions from areas of axon outside the guard electrodes.

In several experiments we measured Na^+ tail currents as a function of temperature over the range 2–12 °C and these results are shown in Fig. 11. Both the fast (τ_1) and slow (τ_2) components were sensitive to changes in temperature with a Q_{10} of 2.8, a value comparable to that obtained from measurements of other kinetic features of *Myxicola* axons (Schauf, 1973).

In another series of experiments repolarization currents were measured in Mg^{++}-free solutions containing either 10 or 100 mM Ca^{++}, and these results are summarized in Fig. 12. On the left is shown the conductance-voltage curve for an axon in 10 and 100 mM Ca^{++}. The dashed curve has been shifted by 20 mV in the depolarizing direction from the solid curve. The shift is comparable to values determined in previous experi-

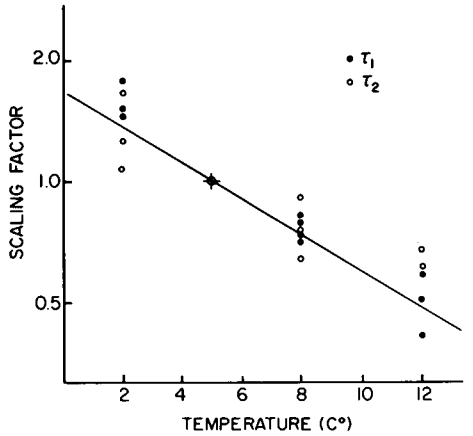


FIGURE 11 Values for the time constants of the rapid (τ_1-filled circles) and slow (τ_2-open circles) decline in I_{Na} on repolarization as a function of temperature. The solid line corresponds to a Q_{10} of 2.8.

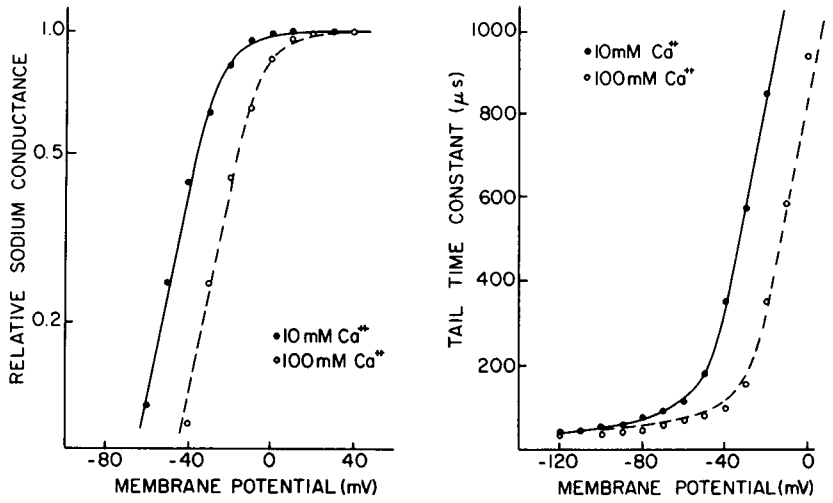


FIGURE 12 Effect of $[Ca^{++}]$ on the voltage dependence of the sodium conductance and on the voltage dependence of the rapid component of the sodium tail current as measured in the same axon. The dashed line has been translated by 20 mV in the depolarizing direction from the solid line fitted by eye to the data in 10 mM Ca^{++}. Temperature was 5°C.

ments (Schauf, 1975). On the right side of the figure the time constant for the rapid component of the repolarization current is plotted for the same axon. Again the dashed curve is translated by 20 mV and is seen to provide an equally satisfactory description of the effect of changes in $[Ca^{++}]$ on the tail currents.

In comparable experiments, Frankenhauser and Hodgkin (1957) found that sodium tail current time constants in low $[Ca^{++}]$ depended to a significant extent on the magnitude of g_{Na} at the time of repolarization. Table I summarizes data on *Myxicola* axons directed at investigating this possibility. Two experimental protocols were used on different axons. In the first, the amplitude (V_1) of P_1 (Fig. 1) was varied, with the duration of P_1 held at 700 μs. For values of P_1 more negative than −10 mV, repolarization occurred well before peak g_{Na} while for values more positive than 30 mV, repolarization occurred after peak g_{Na}. In the second, prepulse amplitude (V_1) remained constant while the duration of P_1 was varied so that repolarization occurred before and after peak g_{Na}. Repolarization potential (V_2) was −100 mV in all cases. While sodium conductance at the end of P_1 varied from 2.6 to 17.0 mmho/cm^2, the measurements of the fast component of the repolarization currents showed no systematic tendency to vary with the amplitude of g_{Na}. Within the limits of experimental uncertainty, similar conclusions applied to the slow component.

Asymmetry Current Measurements

Consider an axon subjected to two pulses of identical duration and equal and opposite in magnitude from a holding potential V_h. Let the first pulse depolarize the membrane to a potential $V_1 = V_h + \Delta V$, while the second hyperpolarizes the membrane to $V_2 = V_h - \Delta V$. The displacement current flowing at the beginning of the pulse

TABLE I

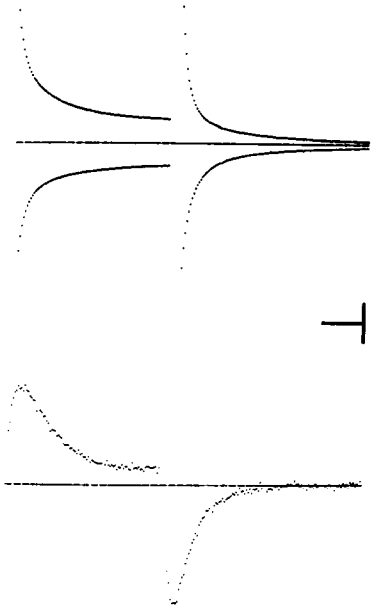
DEPENDENCE OF REPOLARIZATION TIME CONSTANTS IN *MYXICOLA* ON INITIAL VALUES OF SODIUM CONDUCTANCE

Axon	V_p*	t_p*	g_{Na}	τ_1‡
	mV	*ms*	*mmho/cm*^{2}	*μs*
76M29	−30	0.80	10.1	66
	−20	0.80	14.6	69
	−10	0.80	16.9	65
	0	0.80	16.2	62
	10	0.80	13.9	62
	20	0.80	10.4	64
	30	0.80	6.9	60
	40	0.80	4.0	68
	50	0.80	1.6	64
76M30	−30	0.70	3.0	55
	−20	0.70	8.0	60
	−10	0.70	11.3	63
	0	0.70	13.6	57
	10	0.70	14.0	55
	20	0.70	13.8	49
	30	0.70	12.2	52
	40	0.70	10.9	48
	50	0.70	6.0	54
76M31	−10	0.20	2.6	70
	−10	0.30	6.7	75
	−10	0.40	9.0	81
	−10	0.50	10.1	75
	−10	0.60	10.2	72
	−10	0.80	9.5	72
	−10	1.00	8.3	65
	−10	1.20	7.8	65
	−10	1.60	4.3	65
76M32	−10	0.2	4.9	54
	−10	0.3	9.3	51
	−10	0.4	11.5	56
	−10	0.5	12.5	49
	−10	0.6	12.6	53
	−10	0.8	11.8	48
	−10	1.0	10.5	52
	−10	1.2	9.1	55
	−10	1.6	6.9	51

*Prepulse amplitude and duration.

‡Measured during repolarization to −100 mV in 10 mM Ca^{++}.

from V_h to V_1 is slightly greater than the displacement current after the pulse from V_h to V_2 (the "on" response), while at the end of the pulses the displacement current flowing after the step from V_2 to V_h is slightly greater than that flowing after the step from V_1 to V_h (the "off" response). These asymmetry currents have been examined by a variety of investigators to determine the relationship between these responses and the



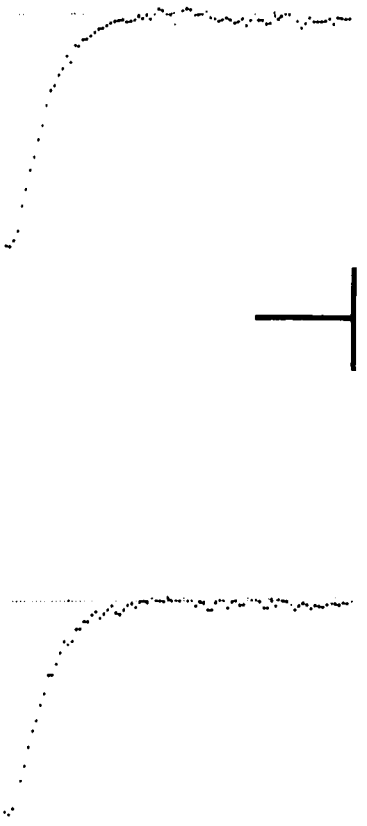


FIGURE 13

FIGURE 14

FIGURE 13 Membrane currents (top) recorded in response to 16 pulses of +100 mV and 16 pulses of −100 mV from a holding potential of −100 mV, and the asymmetry current (bottom) resulting from digital addition of these records. Note that the raw displacement current data have been superimposed so that the responses to +100 and −100 mV correspond in time. Note further that the first 50 μs (five points) of the displacement currents are off scale in this figure, though they did not saturate the signal averager. Outward current is upward. The vertical scale is 0.1 mA/cm^2 for the displacement currents and 12 μA/cm^2 for the asymmetry current. The time scale is 250 μs. Temperature was 5°C.

FIGURE 14 Membrane asymmetry currents off responses recorded in ASW (top) and in the presence of 20 mM 4-aminopyridine. Data is the sum of 16 pulses of −100 mV from a holding potential of −100 mV. Outward current is upward. Current and time scales are 19 μA/cm^2 and 250 μs, respectively. Temperature was 4°C.

kinetics of the sodium channel (Bezanilla and Armstrong, 1976; Neumcke et al., 1976; Rojas, 1976).

Because the kinetics of repolarization in *Myxicola* clearly require more than a single time constant at potentials more negative than −50 mV, it seemed to us that this preparation offered a unique opportunity to determine the extent to which asymmetry currents are directly related to g_{Na}. Asymmetry currents were recorded in intact axons

bathed in a solution containing 40 mM Na^+, 390 mM Tris, 10 mM 4-aminopyridine, and 1 μM TTX (Rudy, 1976). Responses to both depolarizing and exactly matched hyperpolarizing pulses were recorded separately and later either added and averaged or analyzed by alternative methods (e.g. the divided pulse procedure of Armstrong and Bezanilla, 1974). Typical results are provided in Fig. 13 for an axon held at −100 mV and subjected to equal and opposite pulses of 100 mV.

For all pulse amplitudes we found the total charge displaced at the beginning and end of the pulse, calculated from measurements of the time constant for the decline in asymmetry current and the extrapolated value of current at the instant of voltage change to be equal and opposite (Rudy, 1976). Also in agreement with Rudy's (1976) observations of *Myxicola,* the steady-state charge-voltage curve, measured by using the off responses, had a maximum slope in the range 22–26 mV/*e*-fold change in axons in which the limiting slope of the g_{Na} (V) curve was 9–11 mV/*e*-fold, giving ratios averaging around 2.5. The half-maximums of the $Q(V)$ and $g_{Na}(V)$ curves were in the range of −30 to −25 mV, while the half-maximum of the $(g_{Na}/g_{Na}^{max})^{1/3}$ curve was approximately −40 mV. Maximum gating charge movement averaged 10.6 ± 1.4 nC/cm^2, corresponding to 0.34 ± 0.03 nC/mmho when normalized to the peak sodium conductance.

It is possible to record asymmetry currents during displacements from large negative holding potentials (sufficient to delay activation of I_K) in the absence of 4-aminopyridine. Fig. 14 shows the off responses obtained in ASW + 1 μM TTX and in the same axon after addition of 20 mM 4-aminopyridine. There is no apparent difference in the time-course of the responses obtained in either solution. Thus, although asymmetry current measurements in aminopyridine must be cautiously interpreted, these data suggest no fundamental alteration has occurred.

Measurements were made of the time constants (τ_{off}) of decline in membrane asymmetry currents on repolarization to potentials ranging from −140 to −70 mV. Over

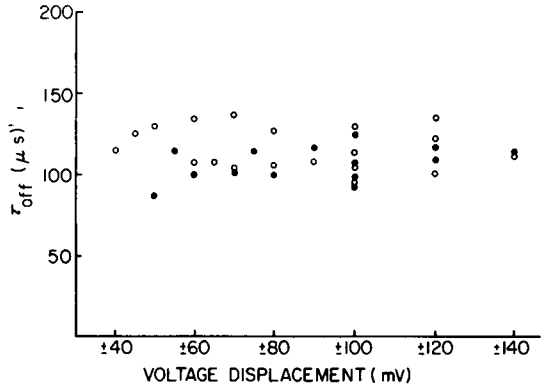


FIGURE 15 Time constant for the asymmetry current off responses at a holding potential of −100 mV as a function of voltage displacement for pulses of equal and opposite magnitude. Data are shown for two different axons as filled and open circles. Temperature was 5°C, pulse duration 1.0 ms.

TABLE II

THE INFLUENCE OF PULSE DURATION ON ASYMMETRY CURRENT TIME CONSTANTS IN *MYXICOLA* AXONS

Axon	V_h	V	t	τ_{off}
	mV	*mV*	*ms*	*μs*
76M39	−100	±100	0.25	152
			0.5	153
			1.0	168
			1.5	150
76M43	−100	±100	0.25	116
			0.5	112
			1.0	115
			1.5	120
76M44	−100	±100	0.25	93
			0.5	98
			1.0	117
			1.5	105
76M84	−100	±100	0.25	101
			0.5	95
			1.0	96
			1.5	98

Temperature ranged from 4 to 6°C.

this range the off responses showed a simple exponential decay, except for the initial 40–60 μs, which was complicated by the usual rising phase characteristic of such records (Bezanilla and Armstrong, 1976). We could detect no second slow decay corresponding to the slow phase of repolarization observed in the Na^+ tail currents. However, since at these potentials the second tail component was 5–10 times slower, with a magnitude at $t = 0$ of 15–20% of the initial magnitude of the fast component, it is possible that the corresponding asymmetry current would have been difficult to detect. There did not seem to be any consistent effect of either the magnitude of the voltage displacement (Fig. 15) or the duration of the pulse (Table II) on the off response time constants.

The comparison between the time constants of decline in I_{Na} on repolarization to −100 mV and the asymmetry current off responses at the same potential in axons in which both responses were measured is given in Tables III and IV. Data shown in Table II and duplicate measurements at particular potentials included as controls are not shown in these Tables. The average at −100 mV in 52 trials with various pulse amplitudes and durations was 121 ± 2.9 μs (SEM), while the average value for the faster phase of the two sodium repolarization current time constants was 52.2 ± 3.1 μs (SEM) in the same axons, giving a ratio of 2.31. Note that this average value for τ_1 was not significantly different from that of 56.4 ± 4.0 μs obtained by including data from seven experiments intended solely to measure sodium tail currents. The scatter in the values for τ_{off} was sufficiently large so that we could not adequately determine a

TABLE III

COMPARISON OF ASYMMETRY CURRENT TIME CONSTANTS WITH THE RATE OF DECLINE OF SODIUM CURRENTS ON REPOLARIZATION IN *MYXICOLA*

Axon	V_h	V	τ_1	τ_2	τ_{off}	τ_{off}/τ_1
	mV	*mV*	*μs*	*μs*	*μs*	
76M35	−100	±100	58	440	112	1.93
76M36	−100	±100	49	390	95	1.94
76M37	−100	±100	83	525	133	1.60
76M39	−100	±60	48	390	135	2.81
	−100	±70			146	3.04
	−100	±80			154	3.21
	−100	±90			164	3.42
	−100	±100			160	3.33
	−100	±120			176	3.67
	−120	±120	40	395	175	4.38
76M41	−80	±80	58	600	115	1.98
	−90	±90	48	630	92	1.92
	−100	±100	49	690	106	2.16
	−120	±120	42	660	115	2.74
76M42	−90	±90	49	520	130	2.65
	−100	±100	42	480	135	3.21
	−120	±120	40	490	120	3.0
76M43	−80	±100	59	510	114	1.93
	−100	±100	48	510	114	2.38
76M44	−70	±70	51	520	110	2.16
	−80	±80	43	530	110	2.56
	−90	±90	47	530	108	2.30
	−100	±100	45	550	115	2.56
	−120	±120	34	550	102	3.0
	−140	±140	37	600	116	3.14
	−100	±40	45	550	116	2.58
	−100	±45			126	2.80
	−100	±50			130	2.89
	−100	±60			116	2.58
	−100	±65			108	2.40
	−100	±70			120	2.67
	−100	±80			117	2.60
	−100	±90			108	2.40
	−100	±100			130	2.89
	−100	±120			129	2.87

Temperatures ranged from 4 to 6°C.

voltage dependence over the range −140 to −70 mV, but in line with work on other preparations (Bezanilla and Armstrong, 1975; Keynes and Rojas, 1976) it is not likely to be very large. Measurements at holding potentials more positive than −70 mV were difficult because of the decreasing size of the asymmetry currents.

In some experiments in addition to recording displacement currents during equal

TABLE IV

COMPARISON OF ASYMMETRY CURRENT TIME CONSTANTS WITH THE RATE OF DECLINE OF SODIUM CURRENTS ON REPOLARIZATION IN *MYXICOLA*

Axon	V_h	V	τ_1	τ_2	τ_{off}	τ_{off}/τ_1
	mV	*mV*	*μs*	*μs*	*μs*	
76M46	−100	±50	43	490	87	2.02
	−100	±55			115	2.67
	−100	±60			100	2.33
	−100	±70			102	2.37
	−100	±75			116	2.70
	−100	±80			99	2.30
	−100	±100			100	2.33
	−100	±120			114	2.65
	−100	±140			115	2.67
76M48	−100	±45	63	540	156	2.48
	−100	±55			114	1.81
	−100	±65			120	1.90
	−100	±75			120	1.90
	−100	±85			156	2.48
	−100	±95			138	2.19
	−100	±105			152	2.41
76M64	−80	±80	75	430	120	1.60
	−100	±100	57	410	104	1.82
	−120	±120	48	410	85	1.77
76M71	−80	±100	65	470	100	1.54
	−100	±100	47	430	102	2.17
76M84	−70	±70	71	490	121	1.70
	−80	±80	64	530	102	1.59
	−90	±90	57	485	99	1.74
	−100	±100	46	520	95	2.07
	−120	±120	39	540	98	2.51

Temperatures ranged from 4 to 6°C.

and opposite voltage steps we held the axon at −160 mV and recorded responses to depolarizations of 10–40 mV. These were then scaled appropriately and subtracted from the responses obtained during larger depolarizing steps starting at −100 mV (Armstrong and Bezanilla, 1974). Analysis of these records produced results identical to those obtained with exactly paired pulses, with only a slight tendency for the rising phase to disappear. In no case could we eliminate the rising phase of the asymmetry currents by this procedure. However, the measurements of time constants and the extrapolation of asymmetry currents to the instant of repolarization were not different from those made using equal and opposite pulses.

Although the data in Tables III and IV (summarized by Fig. 15) show little consistent change in τ_{off} with the magnitude of the voltage displacement (axon 76M39 may be an exception in this regard) and therefore little amplitude dependence of the ratio τ_{off}/τ_1, this ratio does appear to be sensitive to holding potential. In most axons

TABLE V

VARIATIONS OF τ_{off}/τ_1 WITH HOLDING POTENTIAL IN *MYXICOLA*

V_h	τ_{off}/τ_1 (±SEM)	N	P^*
mV			
−70	1.93 ± 0.23	2	<0.08
−80	1.87 ± 0.16	6	<0.01
−90	2.15 ± 0.20	4	<0.025
−100	2.51 ± 0.07	43	<0.05
−120	2.90 ± 0.35	6	—
−140	3.14	1	—

*Compared to −120 by unpaired *t* test.

although τ_{off} does not vary greatly with holding potential (76M64 is an exception), τ_1 decreases at more negative repolarization potentials, resulting in an increase in the ratio τ_{off}/τ_1. This is summarized in Table V. Although the standard errors are relatively large, there are statistically significant differences in the direction to increase this ratio at large negative holding potentials, in agreement with the observations of other investigators (Keynes and Rojas, 1976; Neumcke et al., 1976).

DISCUSSION

According to the Hodgkin-Huxley (1952) kinetics for squid giant axons, the decline in sodium current on repolarization should show both rapid and slow phases for repolarization potentials between about −30 and −10 mV (where the value of m_∞ is between 0 and 1), but only a single time constant for repolarization to large negative potentials where $m_\infty = 0$. Our results show that the kinetic behavior of sodium tails in *Myxicola* is exactly opposite to this prediction. At repolarization potentials less negative than −50 mV the decline in I_{Na} is a single exponential, while at large negative potentials the tail currents clearly have both a fast and a slow component. This behavior is not due to imperfect leak subtraction since it can be demonstrated in experiments in which exposure to TTX is not the means of obtaining nonsodium currents. It is also not due to inadequate compensation of membrane series resistance, since the temporal behavior of the tail currents is unchanged when g_{Na} is reduced to very low values by introduction of increasing amounts of TTX. This observation also rules out any possible contribution of errors dependent on membrane current.

If one attempts to describe *Myxicola* kinetics within the Hodgkin-Huxley framework (Goldman and Schauf, 1973) by ignoring the more subtle anomalous behavior of activation and inactivation, the problem is not resolved since the qualitative predictions of this scheme are essentially identical. It would be of some interest to know the predictions of the generalized second-order description of Goldman (1975) concerning the form of such tail currents, since that description predicts most other departures from classical kinetics observed in this preparation (Goldman and Schauf, 1972, 1973; Schauf, 1974, 1976).

When this repolarization data is taken into account, the sodium kinetics in *Myxicola* do not seem to be consistent with any simple power-law dependence of conductance on some activation variable. The time constant for the decline in I_{Na} on repolarization becomes larger as the potential becomes more positive over the range −40 to 0 mV, while at the same time the time constant for activation of g_{Na}, derived assuming an m^3 dependence, is decreasing (Goldman and Schauf, 1973). The problem is clearly not resolvable simply by a different choice of power, since this would only change the absolute magnitudes of the derived $\tau_m(V)$ curves but not the qualitative nature of their voltage dependence. For this reason we have not attempted to compare time constants for the activation of g_{Na} with the corresponding time constants for the decline in asymmetry current at the beginning of a depolarizing pulse (τ_{on}). Although $\tau_{on}(V)$ is an experimental quantity, and will be described in detail elsewhere, values of $\tau_m(V)$ for Na activation are derived from specific kinetic schemes, none of which seem completely adequate for *Myxicola,* therefore making such a comparison meaningless at present.

Although we have provided compelling evidence that the slow component of the sodium repolarization current is not a measurement artifact, further studies are needed to determine whether it reflects a fundamental kinetic feature of all sodium channels, or whether there might exist some subpopulation of channels, also TTX-sensitive, with different kinetic features not resolvable in other sorts of experimental protocols.

Frankenhauser and Hodgkin (1957) found that decreasing [Ca^{++}] increased the time constant of the decline in I_{Na} on repolarization by an amount very much larger than expected from the Ca^{++}-induced shifts in the conductance-voltage relation, and furthermore observed that, particularly in low (22 mM) Ca^{++}, the tail time constant was much larger when repolarization occurred at the time of maximum g_{Na} during the initial depolarizing pulse (P_1 in Fig. 1) than when repolarization occurred earlier or later. Neither of these effects are present in the tail currents in *Myxicola.* The $\tau_1(V)$ curve is shifted along the voltage axis by amounts comparable to the shift in $g_{Na}(V)$ and there is no evidence of a significant dependence of τ_1 on the magnitude of g_{Na}. Such variations as do occur (axon 76M31, Table I) are very small compared to the effects seen by Frankenhauser and Hodgkin (1957). However, although the measurement of tail time constants is very sensitive to the adequacy of series resistance compensation, this is not necessarily the explanation of the entire effect seen by Frankenhauser and Hodgkin (1957), since they observed variations in τ of 300–400% produced by less than twofold changes in sodium currents in compensated axons. Nevertheless, the lack of an effect in *Myxicola* seems to be further evidence of nearly ideal compensation, since it is unlikely that series resistance errors could exactly counteract a real effect of g_{Na} on τ_1.

Previous comparisons of sodium tail currents and corresponding asymmetry currents on squid axons have produced conflicting data. Armstrong and Bezanilla (1974) and Bezanilla and Armstrong (1975, 1976) concluded that the asymmetry current at the end of a pulse, which would activate g_{Na}, declines with approximately the same time-course, although τ_{off} seems slightly (0–30%) larger in most of their records. Keynes and

Rojas (1976) and Rojas (1976), on the other hand, reported that τ_{off} could be as much as 3.5 times as large as the sodium tail time constant if very negative holding potentials (−100 mV) and large pulses were used, although for smaller pulses and less hyperpolarized holding potentials ratios were obtained in the range 1.6–2.0. Similar variations were observed by Neumcke et al. (1976), who further concluded that asymmetry currents "... cannot be considered as a correlate of the Hodgkin-Huxley *m* variable."

In *Myxicola* axons the asymmetry current time constant on repolarization to −100 mV averages 122 μs and is quite independent of pulse amplitude. The absolute magnitude agrees quite well with measurements on squid axons at comparable potentials (Bezanilla and Armstrong, 1975; Keynes and Rojas, 1976). Comparison of τ_{off} with the faster component of the sodium tail current (τ_1) gives ratios (τ_{off}/τ_1) averaging 2.3 with no systematic variation in this ratio with pulse amplitude or duration. This is in contrast to the observations of Keynes and Rojas (1976), in which there was a 40% increase in τ_{off} for pulses of ±160 mV (holding potential −100 mV) compared to that for ±60 mV pulses.

Nevertheless, we agree with the conclusion of these authors and Neumcke et al. (1976) that the ratio τ_{off}/τ_1 tends to become larger at large negative holding potentials, although in our studies it is primarily due to a change in τ_1 rather than τ_{off}. At a holding potential of −100 mV, Keynes and Rojas (1976) observed an average ratio of 2.83, while at −60 mV the average ratio was 1.98. This is in good quantitative agreement with the data of Table V, although the presence of two distinct relaxation processes in the sodium tails and the lack of any consistent slow component in the asymmetry current complicates our comparison while supporting the conclusion of Neumcke et al. (1976), that a systematic model relating g_{Na} to the asymmetry current is needed to interpret such results.

This work was supported by a Research Career Development Award (K04-NS00004) to Dr. Schauf and by the Morris Multiple Sclerosis Research Fund. We are grateful to Dr. Robert S. Eisenberg for his helpful comments regarding the manuscript. Mr. Bullock was supported during the course of this work on U.S. Public Health Service training grant GM780 in the Department of Biophysics and Theoretical Biology of the University of Chicago.

Received for publication 15 October 1976 and in revised form 17 January 1977.

REFERENCES

ARMSTRONG, C. M., and F. BEZANILLA. 1973. Currents related to movement of the gating particles of the sodium channels. *Nature (Lond.).* **242**:459–461.

ARMSTRONG, C. M., and F. BEZANILLA. 1974. Charge movement associated with the opening and closing of the activation gates of the Na channels. *J. Gen. Physiol.* **63**:533–552.

BEZANILLA, F., and C. M. ARMSTRONG. 1975. Kinetic properties and inactivation of the gating currents of sodium channels in squid axon. *Philos. Trans. R. Soc. Lond. B.* **270**:449–458.

BEZANILLA, F., and C. M. ARMSTRONG. 1976. Properties of the sodium channel gating current. *Cold Spring Harbor Symp. Quant. Biol.* **40**:297–304.

BINSTOCK, L., and L. GOLDMAN. 1969. Current and voltage-clamped studies on *Myxicola* giant axons. Effect of tetrodotoxin. *J. Gen. Physiol.* **54**:730.

BINSTOCK, L., W. J. ADELMAN, JR., J. SENFT, and H. LECAR. 1975. Determination of the resistance in series with the membranes of giant axons. *J. Membr. Biol.* **21**:25–47.

FRANKENHAUSER, B., and A. L. HODGKIN. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol. (Lond.).* **137**:218–244.

GOLDMAN, L. 1975. Quantitative description of the sodium conductance of the giant axon of *Myxicola* in terms of a generalized second-order variable. *Biophys. J.* **15**:119–136.

GOLDMAN, L., and C. L. SCHAUF. 1972. Inactivation of the sodium current in *Myxicola* axons. Evidence for coupling to the activation process. *J. Gen. Physiol.* **59**:659–675.

GOLDMAN, L., and C. L. SCHAUF. 1973. Quantitative description of sodium and potassium currents and computed action potentials in *Myxicola* giant axons. *J. Gen. Physiol.* **61**:361–384.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* **117**:500–554.

KEYNES, R. D., and E. ROJAS. 1974. Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. (Lond.).* **239**:393–434.

KEYNES, R. D., and E. ROJAS. 1976. The temporal and steady-state relationships between activation of the sodium conductance and movement of the gating particles in the squid giant axon. *J. Physiol. (Lond.).* **255**:157–189.

MEVES, H. 1974. The effect of holding potential on the asymmetry currents in squid giant axons. *J. Physiol. (Lond.).* **243**:847–867.

NEUMCKE, B., W. NONNER, and R. STAMPFLI. 1976. Asymmetrical displacement current and its relation with activation of sodium current in the membrane of frog myelinated nerve. *Pflügers Arch. Eur. J. Physiol.* **363**:193–203.

ROJAS, E. 1976. Gating mechanisms for the activation of the sodium conductance in nerve membranes. *Cold Spring Harbor Symp. Quant. Biol.* **40**:305–320.

RUDY, B. 1975. Slow recovery of the inactivation of sodium conductance in *Myxicola* giant axons. *J. Physiol. (Lond.).* **249**:22P.

RUDY, B. 1976. Sodium gating currents in *Myxicola* giant axons. *Proc. R. Soc. Lond. B.* **193**:469–475.

SCHAUF, C. L. 1973. Temperature dependence of the ionic current kinetics of *Myxicola* giant axons. *J. Physiol. (Lond.).* **235**:197–205.

SCHAUF, C. L. 1974. Sodium currents in *Myxicola* axons. Nonexponential recovery from the inactive state. *Biophys. J.* **14**:151–154.

SCHAUF, C. L. 1975. The interactions of calcium with *Myxicola* giant axons and a description in terms of a simple surface charge model. *J. Physiol. (Lond.).* **248**:613–624.

SCHAUF, C. L. 1976. Comparison of two-pulse sodium inactivation with reactivation in *Myxicola* giant axons. *Biophys. J.* **16**:245–248.

SCHAUF, C. L., and F. A. DAVIS. 1975. Further studies of activation-inactivation coupling in *Myxicola* axons: insensitivity to changes in calcium concentration. *Biophys. J.* **15**:1111–1116.

SCHAUF, C. L., C. A. COLTON, J. S. COLTON, and F. A. DAVIS. 1976*a*. Aminopyridines and sparteine as inhibitors of membrane potassium conductance: effects of *Myxicola* axons and the lobster neuromuscular junction. *J. Pharmacol. Exp. Therap.* **197**:414–425.

SCHAUF, C. L., T. L. PENCEK, and F. A. DAVIS. 1976*b*. Slow sodium inactivation in *Myxicola* axons: evidence for a second inactive state. *Biophys. J.* **16**:771–778.