Potassium Current Kinetics in *Myxicola* Axons

Effects of Conditioning Prepulses

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ABSTRACT In *Myxicola* giant axons the time constants for activation of the potassium conductance (G_K) after prepulses less depolarized than a test pulse are comparable to the time constants for turn off of G_K after prepulses more depolarized than the same test pulse. The absolute magnitude of the steady-state level of G_K is also independent of prepulse amplitude in *Myxicola*. The results are contrasted with recent observations on voltage-clamped frog nodes.

INTRODUCTION

In voltage-clamped *Rana* nodes (Palti et al., 1976) the time constants for activation of the potassium conductance (G_K) are strongly dependent on the sign of a conditioning prepulse relative to the test pulse. For prepulses less depolarized than the 20 mV test pulse used, the derived values for τ_n were 5–10 times larger than for prepulses more depolarized than 20 mV. These effects were not strongly dependent on either prepulse duration or external [K^+].

Because of the theoretical implications of this result vis à vis Hodgkin-Huxley kinetics (Palti et al., 1976), we wished to determine whether similar behavior could be observed in voltage-clamped *Myxicola* giant axons where there is already a considerable body of evidence for anomalous sodium channel kinetics (see Schauf, 1976, for references). The result suggests that in *Myxicola* the K^+ channel is relatively well behaved and that there is no need, at present, to greatly modify the existing (Goldman and Schauf, 1973) kinetic description of this preparation.

METHODS

Myxicola giant axons were voltage clamped by methods previously described (Goldman and Schauf, 1973) with compensated feedback at temperatures of 3–6°C. The axon was externally bathed in either artificial seawater (ASW: 430 mM Na^+; 50 mM Mg^{++}; 10 mM Ca^{++}; 10 mM K^+; 20 mM Tris, pH 7.8 ± 0.05) or a solution in which [K^+] had been increased via substitution for Na^+. Tetrodotoxin (10^{-6} M) was added to all solutions after series resistance compensation. Pulse protocols and analysis were similar to those employed by Palti et al. (1976) except

where noted in the text. The data were generally leak corrected by using hyperpolarizing pulses of equal magnitude and assuming a constant leak conductance. In a few cases, however, the analysis was performed directly on total membrane current with no significant effect on the measured K^+ time constants.

RESULTS

In the first series of experiments axons were held at their ASW resting potentials of −70 mV to −60 mV. Potassium currents at a variety of test pulses (V_t) between −40 mV and +60 mV were then recorded after either no prepulse or 10–100-ms prepulses between −160 mV and +100 mV. Two typical families of K^+ currents for test pulses to −30 mV (lower curves) and −1 mV (upper curves) are shown in Fig. 1. In both cases, as the prepulse potential is made more depolarized so that

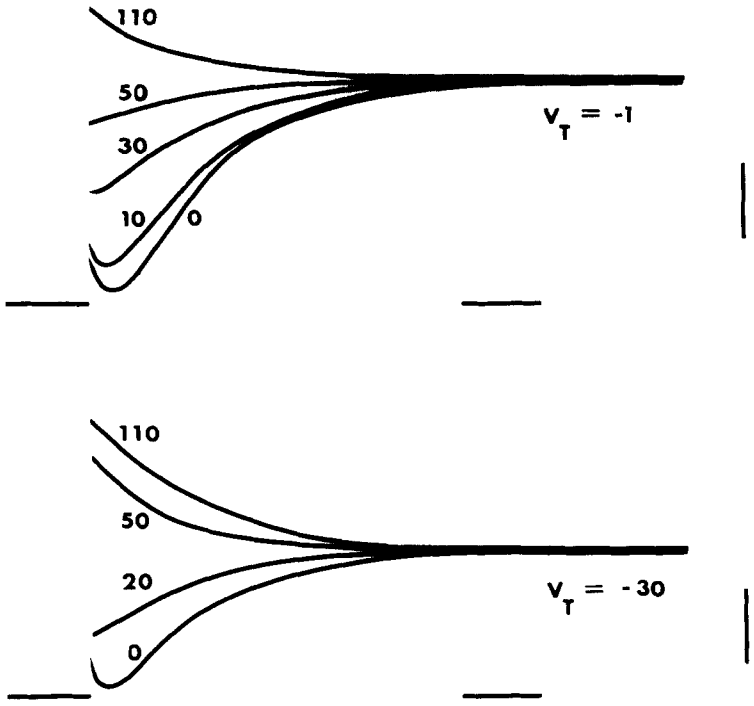

FIGURE 1. Membrane K^+ currents during depolarizations to −30 mV (lower records) and −1 mV (upper records) after 20 ms prepulses of the indicated amplitudes from holding potentials of −60 mV and −61 mV, respectively. The records are from different axons. Current and time calibrations are 0.15 mA/cm^2 and 2 ms. Temperature 5°C.

the K^+ currents eventually are transformed into tail currents, the apparent time constant does not appear to vary greatly, at least within the limits of visual inspection. Also it is clear that the steady-state potassium current during each test pulse is quite independent of the amplitude of the conditioning potential, an observation which remained true over the entire range of prepulse potentials and durations used.

In Fig. 2 we have plotted values for

$$n(\infty) - n(t) = \frac{1}{G_K(V - V_K)}[I_K(\infty)^{1/2} - I_K(t)^{1/2}]$$

taken from the upper curves of Fig. 1 semilogarithmically as a function of time (Palti et al., 1976). As previously noted (Goldman and Schauf, 1973), satisfactory fits to the observed K^+ currents could only be obtained over the entire voltage range by assuming that the fraction of open channels was proportional to n^2. Nevertheless we made an attempt to force fits to the $I_K(V, t)$ data using powers of

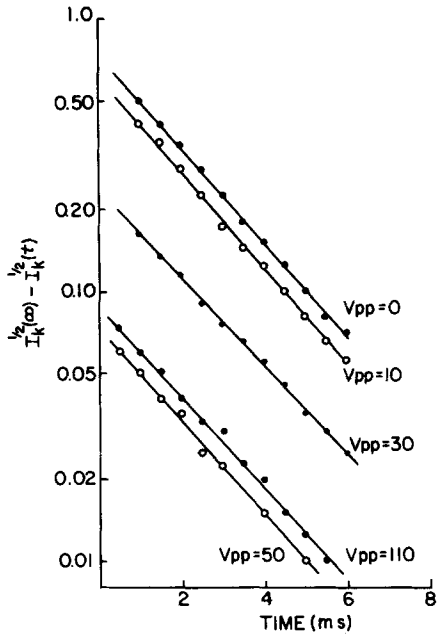

FIGURE 2. Values of $I_K^{1/2}(\infty) - I_K^{1/2}(t)$ from the upper curves of Fig. 1 plotted semilogarithmically as a function of time.

1 and 4 in order to see if our general conclusions might be altered. In no case could the time constants for prepulses depolarized relative to V_t be made to be systematically different from those obtained with hyperpolarized prepulses.

The time constants derived from the data of Fig. 2 and additional plots for a variety of other test pulses were then plotted as a function of prepulse potential as in Fig. 3. It is clear that for test pulses in this axon between −21 mV and +59 mV there is no systematic change in the potassium time constant as the prepulse potential passes through V_t (arrows). Our entire set of data on five axons is provided in another form in Table I. Here the values of τ_n for a given test pulse were averaged for prepulse amplitudes both depolarized and hyperpolarized relative to V_t and a statistical analysis was carried out between the two sets of data.

In the majority of cases there was no significant difference between the τ_n values obtained with prepulses depolarized and hyperpolarized relative to V_t (P

> 0.05). In one case (axon 75M21, $V_t = -21$) there was a marginally significant decrease in τ_n for prepulses depolarized to V_t, and in three other instances there were clear decreases. In one axon (75M69, $V_t = -10$) there was a significant increase in τ_n for $V_{pp} > V_t$. Even when τ_n difference was observed, however, it was clearly minor compared to the magnitude of the effects observed by Palti et al. (1976).

Potassium accumulation can of course occur in *Myxicola* axons, particularly for large depolarizing prepulses. Conceivably, a shorter τ_n for $V_{pp} > V_t$ could then

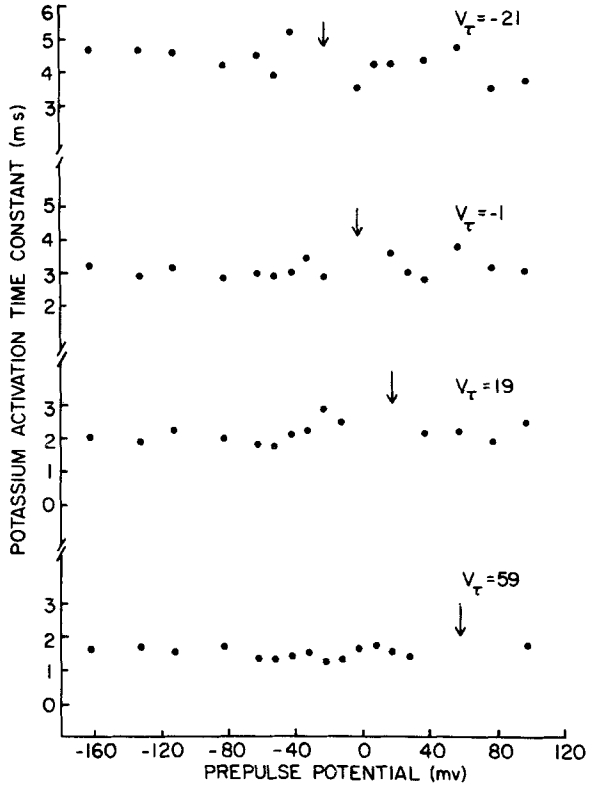

FIGURE 3. Potassium activation time constants (τ_n) determined at test pulses of −21, −1, 19, and 59 mV as a function of prepulse amplitude. Test pulses are indicated by arrows. Data from least-squares fits to plots similar to those shown in Fig. 2. Temperature 5°C. Holding potential −61 mV.

be masked by a slower dissipation of accumulated K^+, causing an effective increase in $V - E_K$ as a function of time for $V_{pp} > V_t$. This possibility was examined in several ways.

In some experiments axons were exposed to an ASW in which $[K^+]$ was elevated to 50 mM, where the normal resting potential is approximately −40 mV, and the protocols were repeated for test pulses of approximately −10 mV, where G_K is still not fully activated. The result is illustrated by the data in Table II taken from one of several axons examined. Values for τ_n were recorded first

in normal ASW with the axon held at −60 mV, then in 50 mM K^+-ASW with the axon held either at its new resting potential (−40 mV) or at −60 mV. The same set of absolute test potentials were used in all cases. Not only was there no significant difference in the τ_n values for $V_{pp} > V_t$ compared to $V_{pp} < V_t$, but also there was no difference in the values of τ_n obtained at the same V_t in 50 mM K^+ compared to those in 10 mM K^+. Since the same outward K^+ current during V_{pp} would produce a smaller relative change in E_K at 50 mM K^+ than at 10 mM K^+, this result suggests that K^+ accumulation did not significantly affect the data.

TABLE I

EFFECTS OF PREPULSE AMPLITUDE ON POTASSIUM TIME CONSTANTS

		τ_n (ms)		
Axon	V_t (mV)	$V_{pp} < V_t$	$V_{pp} > V_t$	P
75M20	−20	5.1±0.3 (5)*	4.9±0.5 (7)	NS
	0	3.1±0.2 (6)	3.3±0.5 (6)	NS
	+20	2.2±0.3 (8)	2.5±0.5 (4)	NS
75M21	−21	4.6±0.5 (5)	4.0±0.4 (6)	<0.04
	−1	3.1±0.2 (7)	3.2±0.4 (6)	NS
	19	2.2±0.4 (8)	2.2±0.2 (4)	NS
	59	1.5±0.2 (12)	0.72 (1)	NS
	79	1.4±0.1 (12)	–	–
75M58	−30	17.3±2.7 (3)	13.1±2.5 (5)	NS
	−20	8.7±0.5 (6)	5.8±0.7 (5)	<0.0001
	−10	7.1±0.5 (7)	5.6±0.8 (5)	<0.003
	+10	5.2±1.2 (8)	6.0±0.7 (3)	NS
75M68	−40	13.2±0.4 (4)	7.7±2.2 (4)	<0.003
	−30	8.4±2.0 (5)	7.2±1.7 (5)	NS
75M69	−30	5.8±0.8 (4)	6.2±0.4 (5)	NS
	−20	6.3±0.6 (5)	6.5±1.3 (5)	NS
	−10	5.9±1.0 (6)	9.5±0.6 (3)	<0.001

* Mean ± SD. Numbers in parenthesis indicate number of different prepulses used.

In one set of experiments for test pulses of −21 mV and −1 mV, E_K was determined both at the end of the various prepulses and in the resting state by observation of the voltage dependence of K^+ tail currents (Binstock and Goldman, 1971). During the most depolarized test pulse the increase in E_K was approximately 8 mV, which is insufficient to have greatly affected the determination of τ_η during V_t even when one assumes a very short time constant for washout of accumulated K^+.

The data of Table I were determined by a variety of prepulse durations ranging from 10 to 100 ms, in some cases for the same prepulse and test pulse amplitude. If potassium accumulation were a significant factor τ_n values would be expected to systematically increase with increasing prepulse duration (and amplitude) for $V_{pp} > V_t$. Such did not appear to be the case.

DISCUSSION

In voltage-clamped *Rana* nodes, Palti et al. (1976) found that for $V_{pp} > V_t$ values for τ_n were consistently an order of magnitude smaller than for $V_{pp} < V_t$. This difference in τ_n was independent of the choice of the power in the expression $g_k = g_k n^x$, and was observed both in normal Ringer solution and in solutions with up to 80 mM K^+. In high [K^+] solutions values for τ_n were consistently somewhat larger, particularly for prepulses more depolarized than V_t, so that the overall differences were reduced. However, values for τ_n for $V_{pp} > V_t$ were still three to five times smaller than for $V_{pp} < V_t$. There was relatively little effect of prepulse duration on the τ_n separation either in Ringer or in high [K^+]. Finally, Palti et al. (1976) noted some effect of prepulse potential on the steady-state K^+ current during the subsequent test pulse.

None of this behavior seems to consistently exist in voltage-clamped *Myxicola* axons in normal seawater (Table I) or in high [K^+] (Table II). In the majority of

TABLE II

EFFECTS OF PREPULSE AMPLITUDE AND [K^+] ON POTASSIUM TIME CONSTANTS

			τ_n (ms)		
[K^+] (mM)	V_H (mV)	V_t (mV)	$V_{pp} < V_t$	$V_{pp} > V_t$	P
10	−60	−20	3.89±0.89 (5)	4.30±0.58 (6)	NS
		−10	3.50±0.54 (6)	3.58±0.36 (5)	NS
		0	3.19±0.31 (6)	3.93±0.66 (4)	NS
50	−40	−20	3.90±0.37 (3)	3.70±0.45 (3)	NS
		0	3.05±0.65 (7)	3.55±0.73 (4)	NS
50	−60	−20	3.79±0.28 (4)	3.90±0.10 (3)	NS
		0	3.19±0.45 (7)	3.55±0.37 (4)	NS

cases (18 experiments) values of τ_n were independent of prepulse amplitude. In the four experiments in which a significant decrease in τ_n was observed for $V_{pp} > V_t$ the magnitude of the decrease was less than 40%. We observed no effect of [K^+] on the magnitude of τ_n for either range of prepulse potentials used (Table II) and values for the steady-state K^+ current during V_t were consistently independent of prepulse potential (Fig. 1).

It is becoming increasingly apparent as more excitable systems are evaluated under voltage clamp that real differences exist in the kinetic behavior of various preparations. Thus *Myxicola* axons, which show departures from Hodgkin-Huxley sodium channel kinetics to a greater degree than other systems (see Schauf, 1976) seem relatively well-behaved in terms of their potassium kinetics. Frog nodes, which do not seem to exhibit the large differences in inactivation time constants seen in *Myxicola* (Chiu, 1976), have K^+ channel kinetics (Palti et al., 1976) quite inconsistent with those of Hodgkin and Huxley (1952). Whether such differences in behavior reflect the presence of distinct channel components, or simply result from relatively small quantitative variations in the relevant rate constant remains a problem for future investigation.

This work was supported in part by the Morris Multiple Sclerosis Research Fund, by the National Multiple Sclerosis Society (921-A-1), and by a Research Career Development Award to C. L. Schauf (5 K04 NS 00004-02).

Received for publication 16 April 1976.

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