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Conditioning Prepulses and Kinetics of Potassium Conductance in the Frog Node

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Summary. The kinetics of potassium conductance were analyzed in response to voltage-clamp steps with holding potential (−75 mV) as initial condition and after a positive prepulse towards +45 mV of 10-msec duration. As the potassium reversal potential E_K altered during potassium current flow, a method to obtain the conductance independent of E_K was used. Conductance kinetics at 15 °C were analyzed according to the Hodgkin-Huxley (HH) model. The time constant of potassium activation, with holding potential as initial condition, is a monotonous decreasing function of membrane potential. Its value of *ca.* 9 msec at −50 mV decreases to 1 msec at +30 mV. Changes in E_K did not affect the voltage dependency of this time constant. The time constant of potassium deactivation, i.e. the off-response following a 10-msec prepulse towards +45 mV, shows a completely different voltage dependency. At a membrane potential of −90 mV it is approximately 2 msec and gradually increases for more positive voltages towards a maximum value of about 6 msec, that is reached between −5 and 0 mV. At still larger values of membrane voltage this time constant starts to fall again. It is concluded that a HH-model, as applied for a single population of potassium channels, has to be rejected. Computer simulations indicate that an extension to two populations of independent potassium channels, each with HH-kinetics, is also inconsistent with the observed results.

Key Words node of Ranvier · voltage clamp · relaxation analysis · potassium accumulation · potassium conductance kinetics

Introduction

In a voltage-clamp step potassium currents activate sigmoidal with time, whereas the deactivation shows an exponential decay. To account for these phenomena Hodgkin and Huxley formulated in 1952 a mathematical model for the axonal membrane currents, consisting of a set of empirical equations. In their model the potassium conductance g_K is proportional to n^x, usually with x equal to 2, 3 or 4, and where n is obtained from

$$dn/dt = \alpha_n(1-n) - \beta_n n. \quad (1)$$

In this equation n is a dimensionless parameter, that can vary between 0 and 1, while α_n and β_n are voltage-dependent rate constants. Usually this equation is interpreted in terms of conducting units or "channels," that consist of x independent gating particles, occurring in either one of the two states: "open" or "closed." The parameter n then is the fraction of gating particles in the open position, while α_n and β_n are the rate constants, determining the transitions between open and closed state. Since these rate constants depend on the momentary voltage only, the time constant of a conductance change, $\tau_n(=1/(\alpha_n+\beta_n))$, should not depend on the initial conditions.

However, a dependency of β_n (and therefore of τ_n) on the preceding membrane voltage was suggested by Frankenhaeuser (1963). Later Palti, Ganot and Stämpfli (1976) confirmed for a single test voltage, that τ_n is a function of conditioning prepulse.

Early investigations on potassium conductance kinetics in the frog node have been based on the analysis of potassium currents, assuming a constant reversal potential E_K (Dodge, 1963; Hille, 1967). Observations of Meves (1961), Ulbricht (1963) and Vierhaus and Ulbricht (1971) indeed indicate no changes in E_K at all. However, according to Palti, Stämpfli, Bretag and Nonner (1972) E_K remains not always constant in the node. With sensory fibers they observed a change in E_K that depended on duration and amplitude of the potassium current, whereas such a shift did not occur in motor fibers. In a more elaborate study Dubois and Bergman (1975) confirmed these E_K shifts for sensory fibers. Also Atwell, Dubois and Ojeda (1980) concluded that E_K is not constant "in large nerve fibers." Moreover, in their opinion the prepulse dependency of τ_n, as observed by Frankenhaeuser, is merely an effect of the E_K shift on potassium currents, as previously suggested by Dubois and Bergman (1975). These discrepancies may be

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explained by differences in the type of fiber used (motor or sensory), but could also result from variations among subspecies. Such variations have been registered among several types of *Rana pipiens* by Armstrong and Hille (1972).

In this study the kinetics of potassium conductance, following different initial conditions, have been analyzed. In order to eliminate the effects of E_K shifts, the potassium conductance has been assessed by means of a double-pulse method.

Materials and Methods

Single myelinated motor fibers were dissected from the N. ischiadicus of the frog *Rana esculenta*. The axons were mounted on a macrolon membrane chamber and voltage clamped as described by Van den Berg and Rijnsburger (1980). The nodes were externally perfused with Ringer's (in mM: 115 NaCl, 2.5 KCl, 2.0 $CaCl_2$, 0.5 $MgCl_2$, 5 Tris-HCl, pH 7.4) at 15 °C. The cut axoplasm ends were immersed in 120 mM KCl. Holding potential was set to the value at which sodium inactivation parameter h was equal to 0.7 (approximately -75 mV; membrane voltage E on an absolute scale). Subsequently TTX (300 nM) was added to completely block the sodium currents.

Potassium currents were described according to:

$$I_K = g_K(E - E_K). \tag{2}$$

To obtain g_K as a function of time, uncontaminated by changes in E_K, the following (double-pulse) method was used. A voltage step towards a given membrane voltage (E_1) is applied to the membrane. At $t = t_o$ the membrane potential is clamped at a voltage E_2 (usually with $E_2 < E_1$), resulting in a step change of the membrane current (ΔI). Now the potassium conductance at $t = t_o$, $g_K(t_o)$, is given by

$$g_K(t_o) = \Delta I/(E_1 - E_2) - g_L. \tag{3}$$

where g_L is the leakage conductance. Measurements at different values of t_o allow the reconstruction of g_K at voltage E_1 as a function of time. The leakage conductance was estimated in a similar way with a 10- to 30-msec prepulse towards -110 mV, followed by a step to -150 mV. From the potassium current amplitude also estimates of $E_K(t_o)$ can be calculated, assuming E_L remains constant.

To study the effect of initial conditions the voltage pulse E_1 was preceded either by the holding potential or by a 10-msec voltage step towards $+45$ mV. For the reconstruction of a conductance change measurements with 15 to 25 different values of t_o were made. The membrane currents were digitized with a Biomation 802 and stored on disk by computer (PDP 11/34), following low-pass filtering with a cut-off frequency f_c = sampling frequency/4. For analysis (on a PDP 11/70) the nearly exponential tail currents were linearly extrapolated from 0.4 to 1.2 msec following semilog plotting versus time (Dubois & Bergman, 1975). Corrections for capacitive transients were not performed. Their effects will be evaluated in the Discussion.

The time course of $g_K(t)$ at a given membrane potential (E_1) was fitted according to the Hodgkin-Huxley equation:

$$g_K = n^x \bar{g}_K \quad \text{with } n = n_\infty - (n_\infty - n_o)\exp(-t/\tau_n). \tag{4a}$$

As τ_n is the quantity of interest, the following transformation was performed to reduce the number of parameters:

$$g_K = r^x g_{K,\infty} \quad \text{with } r = 1 - (1 - r_o)\exp(-t/\tau_n) \quad \text{and} \quad g_{K,\infty} = n_\infty^x \bar{g}_K \quad \text{and} \quad r_o = n_o/n_\infty. \tag{4b}$$

To investigate the dependency of the time constant on the exponent, x has been varied.

Estimates of the potassium conductance were obtained at membrane voltages between -70 and $+100$ mV according to Eq. (3), with t_o long enough to reach a (pseudo) steady state (10–50 msec). The magnitude of the voltage steps ($E_1 - E_2$) varied between 40 and 140 mV.

Results

Potassium Reversal Potential

In 80 nodes (not all used to analyze kinetics) the stability of E_K was checked. In only 3 nodes the changes in E_K remained negligible during voltage steps (of 15-msec duration) towards $+20$ to $+40$ mV.

During potassium activation both conductance and current are an increasing function of time (Fig. 1*a*). During potassium deactivation the conductance decreases, whereas the current is not necessarily decreasing with time. As shown in Fig. 1*b* this different behavior during deactivation is consistent with a restoration of E_K towards a more negative value with the decreased potassium current flow.

Repolarization measurements were not performed in the experiments where E_K remained constant. Therefore all data on the kinetics of g_K in the off-response were obtained by means of the double-pulse method.

Instantaneous I–E Curve

Application of the g_K formalism, as in Eqs. (2) and (3), is only allowed when the instantaneous $I-E$ curve is a straight line. It proved that the instantaneous change in membrane current (ΔI) indeed increased almost linearly with the applied change in voltage (ΔE). However, after a prepulse of 10 msec towards $+45$ mV a small deviation from linearity was observed with small voltage steps. Estimates for g_K, obtained with ΔE less than 20–40 mV, were slightly smaller than those obtained with larger voltage steps. Further increase in ΔE (above approximately 130 mV) eventually led again to smaller estimates for the K^+ conductance. The latter became especially apparent, when the membrane voltage was switched back towards

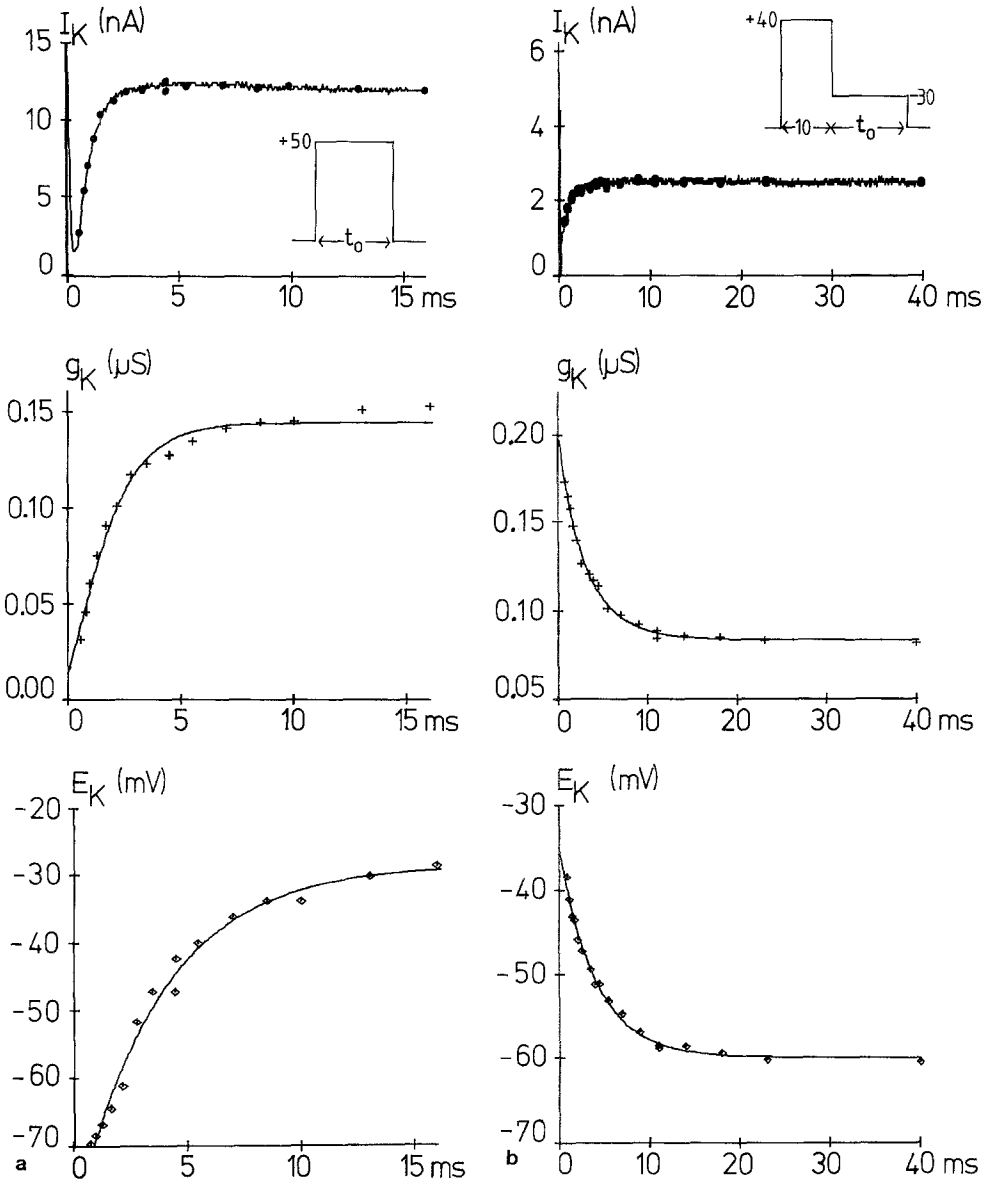

Fig. 1. (*a*) *Top:* potassium current during voltage step from holding potential towards +50 mV (inset). *Middle:* potassium conductance during same voltage step, calculated through Eq. (3). Time constant of the fitted curve [$x=4$, Eq. (4b)] is 1.39 msec. *Bottom:* estimates of E_K obtained in same voltage step. Line drawn according to single exponential with time constant 3.70 msec. The discrepancy between current and conductance is consistent with the E_K shift observed, as illustrated by the I_K values (top: •), reconstructed from g_K (middle) and E_K (bottom). (*b*) As in part *a* but with voltage step towards −30 mV, that was preceded by a 10-msec prepulse towards +40 mV (inset). Time constants are, respectively, 3.66 msec for the conductance deactivation (middle) and 4.05 msec for the E_K change (bottom)

a value below −100 mV. Changes in (potassium) tail currents then became too fast to extrapolate properly. Similar observations on instantaneous $I-E$-curves were made after prepulses of smaller amplitude.

The differences between estimates of a particular conductance value may amount up to 15% of the largest estimate obtained, if the total conductance approximately equals g_L and ΔE is less than 20–30 mV. For larger g_K and intermediate values of ΔE (50–130 mV) the differences are generally not more than a few percent, i.e. in the order of the measuring error. These small deviations from linearity could not be explained by the constant field equation (Goldman, 1943), as this would predict a pronouncedly curved $I-E$ curve (*cf. also* Dubois & Bergman, 1977).

To prevent any possible bias, however, only conductance measurements obtained in the same voltage step, were used for the kinetic analysis.

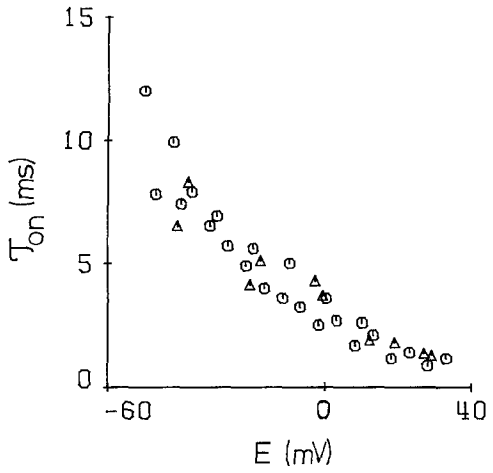

Fig. 2. Time constant (τ_{on}) obtained from fits on potassium activation according to Eq. (4b) with $x=4$. Conditioning voltage was holding potential; τ_{on} was obtained from potassium currents (o), when E_K remained constant (3 nodes) and from conductance (△), in case E_K shifted (2 nodes)

Potassium Activation Kinetics (On-Responses)

Analysis Assuming a Single Population of Channels. Activation curves were fitted according to Eq. (4b). To prevent effects of slow "capacitive transients", the very first part was omitted up to 0.4–2.4 msec, depending on E. In the curve fits systematic deviations were observed, reflecting a relative slow phase in the increase of the conductance. In the potassium currents this relative slow phase is usually masked by E_K shifts. The contribution of an additional very slow component (up to 50–300 msec) has been largely eliminated, since the analysis has been restricted to the first 8–50 msec, thereby covering 90–95% of the conductance change.

The time constant (τ_{on}) has a maximum value for the smallest depolarizations, where potassium activation can still be analyzed (i.e., around

Table 1.

E (mV)	$x=1$			$x=2$			$x=4$		
	τ(msec)	SD	rRSS	τ(msec)	SD	rRSS	τ(msec)	SD	rRSS
(*a*)[a]									
−42	12.9	(0.41)	0.93	10.6	(0.31)	0.97	9.80	(0.28)	1.0
−32	8.93	(0.06)	0.75	7.03	(0.08)	0.84	6.45	(0.08)	1.0
−22	7.18	(0.04)	1.30	5.36	(0.04)	0.71	4.93	(0.04)	1.0
−12	5.71	(0.03)	1.33	4.10	(0.03)	0.66	3.77	(0.03)	1.0
− 2	4.06	(0.04)	2.01	2.74	(0.02)	0.66	2.53	(0.03)	1.0
8	2.84	(0.03)	3.32	1.83	(0.02)	0.68	1.71	(0.02)	1.0
18	2.17	(0.05)	4.54	1.29	(0.01)	0.79	1.13	(0.02)	1.0
28	1.64	(0.04)	6.05	1.02	(0.01)	1.03	0.85	(0.01)	1.0
(*b*)[b]									
−41	8.92	(1.69)	0.98	7.14	(1.27)	0.99	6.49	(1.13)	1.0
−21	5.86	(0.34)	0.44	4.59	(0.35)	0.79	4.13	(0.35)	1.0
− 1	4.77	(0.44)	0.57	3.78	(0.41)	0.85	3.42	(0.40)	1.0
19	3.01	(0.18)	0.97	2.01	(0.17)	0.89	1.79	(0.16)	1.0
29	2.75	(0.22)	2.13	1.58	(0.09)	0.86	1.28	(0.14)	1.0
(*c*)[c]									
−85	0.95	(0.10)	1.11	1.66	(0.40)	1.05	2.49	(1.10)	1.0
−60	1.77	(0.20)	1.14	2.21	(0.27)	1.04	2.51	(0.33)	1.0
−46	1.99	(0.14)	1.11	2.33	(0.17)	1.03	2.53	(0.19)	1.0
−31	2.88	(0.13)	1.30	3.26	(0.14)	1.09	3.48	(0.15)	1.0
−17	3.81	(0.39)	1.17	4.19	(0.42)	1.06	4.39	(0.44)	1.0
− 7	5.01	(0.45)	1.10	5.38	(0.49)	1.03	5.58	(0.51)	1.0

[a] Conditioning voltage: holding potential. Activation time constant (τ) is given as a function of membrane voltage (E) for different values of the exponent x [Eq. (4b)]. Between brackets the standard deviation, as obtained from the fits on the potassium currents from a single node (E_K constant). The relative residual sum of squares (rRSS) is used to compare the "goodness of fit" for different x, and is taken unity for $x=4$.

[b] Conditioning voltage: holding potential. Activation time constant from fits on potassium conductance of single node, calculated through Eq. (3) in case E_K changed. Further as in part *a*.

[c] Conditioning potential: +45 mV (10 msec). Deactivation time constant (τ). Further as in part *b*.

-50 mV). For higher membrane voltages τ_{on} decreases monotonously. At $+30$ mV for example its value was approximately one order of magnitude smaller than at -50 mV. The values for τ_{on}, obtained from fits on potassium conductance, show good agreement with those obtained from fits on potassium currents, when E_K remained constant. In Fig. 2 τ_{on} is plotted as a function of membrane voltage for $x=4$.

Time constants obtained from potassium currents, when E_K changed, tend to be smaller by a factor between 1 and 2.

Lowering the value of x [Eq. (4)] has the following effects:

a) Larger values for τ_{on} are obtained, though the dependency on membrane potential remains qualitatively the same (Table 1 *a, b*).

b) The residual sum of squares (RSS) becomes smaller (Table 1 *a, b*).

However, the optimum value for x appears to be membrane potential dependent, as it increases from about 0.8 at -50 mV to a value of at most 2.5 with larger depolarizations.

Analysis Assuming Two Populations of Channels. Introduction of two populations of potassium channels, each according to Eq. (4b), leads to an appreciable improvement in the fits. However, as applied to the conductance measurements at different t_o, the values of the parameter estimates usually scattered considerably. More accurate results could be obtained in the cases, where potassium currents could be fitted directly, i.e. when E_K remained constant. In these cases a larger number of points (>200), with higher precision was available for the analysis. The results are given in Fig. 3 *a* and *b*.

Again the time constants are monotonously decreasing with membrane voltage. Maximum relative conductances were, respectively, 0.72 and 0.28 (SD = 0.05; three nodes) for the fast (I) and slow (II) potassium system. Fits made with x_I and x_{II} free did not show clear optima as a function of membrane potential for either exponent. Assuming $x_I=x_{II}=4$, however, gave fits that, on visual inspection of the residuals, were not better or worse than those obtained with both exponents free.

Potassium Deactivation Kinetics (Off-Responses)

Analysis Assuming a Single Population of Potassium Channels. Potassium kinetics of the deactivation process, analyzed according to Eq. (4b), exhibits a behavior that is in marked contrast to kinetics of the activation process. At a membrane potential of -90 mV the time constant (τ_{off}) equals approximately 2 msec (when $x=4$) and increases for more positive values of E (Fig. 4). Between -5 and 0 mV a maximum of approximately 6 msec is reached. In still larger depolarizations the time constant falls again. As the method is based on the decrease from a nearly maximum conductance at $+45$ mV towards a new steady level, it is readily seen that this decrease becomes small at positive test voltages. Hence it was impossible to obtain reliable data beyond $+15$ mV.

Also in the off-response a very slow contribution to the conductance change could be observed, that became more apparent with larger depolarizations. This could interfere with the off-response as follows. After a relative fast decrease in the g_K, conductance could increase very slowly again. Due to this slow process, apparently the same as in activation, the rather steep decrease in τ_{off} may be somewhat overestimated.

Lowering the value of the exponent (Eq. 4b) now has effects opposite to those observed on potassium activation:

a) Smaller values for x give lower estimates for the time constant (Table 1 *c*). This becomes especially apparent for membrane voltages below

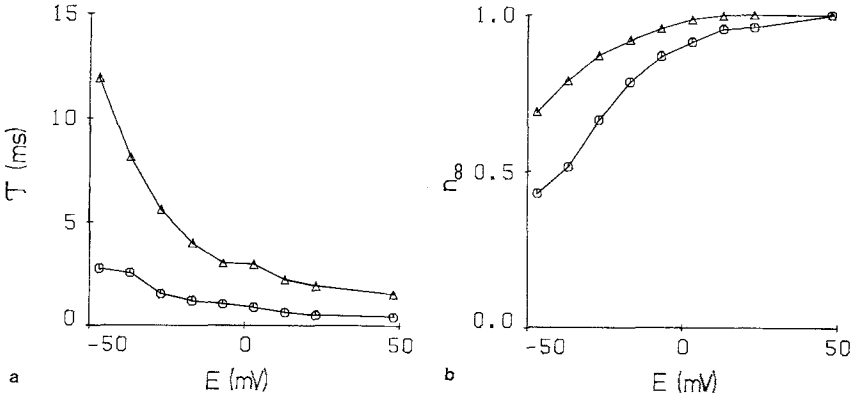

Fig. 3. (*a*) The estimated values of τ_I (o) and τ_{II} (Δ) were obtained from fits on potassium current activation, when E_K was constant. Either population described by Eq. (4b), with $x_I = x_{II} = 4$. (*b*) The estimates of $n_{\infty,I}$ (o) and $n_{\infty,II}$ (Δ) were obtained from the same fits as in part *a*

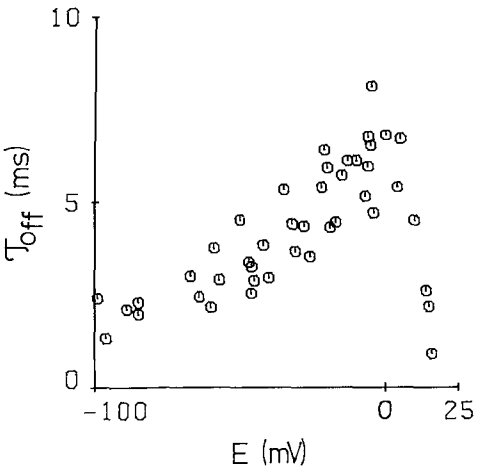

Fig. 4. Time constant (τ_{off}) obtained from fits on deactivation of potassium conductance according to Eq. (4b), with $x=4$. Conditioning prepulse (10 msec) was approximately +45 mV (8 nodes)

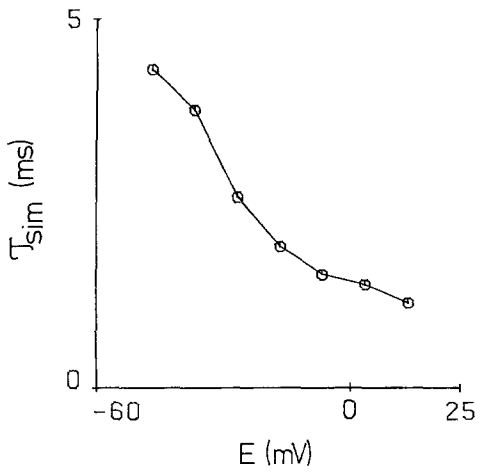

Fig. 5. Time constant (τ_{sim}) was obtained from fits on simulated deactivation curves of potassium conductance. Parameters used to simulate off-responses were obtained from analysis of potassium activation assuming two populations of channels (same node as in Fig. 3)

−50 mV, where progressively smaller end values for g_K are obtained. In case of $x=4$ then also the standard deviations of the estimate begin to increase up to about 50–100% of the estimated value.

b) Larger values of x give better fits. Considering $1 \leqq x \leqq 4$ the smallest RSS was usually obtained for $x=4$. (Table 1*c*). Optimum values for x could only rarely be obtained, when x was restricted to values smaller than 50.

Analysis Assuming Two Populations of Potassium Channels. Fits made, assuming two sets of independent potassium channels (each according to Eq. 4b with $x=4$), again gave estimates with large standard deviations. Results obtained in different experiments also matched poorly. Moreover, effective reduction to one population could result. Unfortunately deactivating potassium currents had not been recorded in the experiments where E_K remained constant. Therefore the following method was used to verify whether kinetics of potassium deactivation are consistent with activation kinetics when assuming two populations of channels. Using the parameter estimates obtained in fits on potassium activation according to two populations of channels (Fig. 3), off-responses were calculated in the time range normally analyzed. To this end initial values for $n_{o,I}$ and $n_{o,II}$ were taken equal to 1−consistent with a 10-msec prepulse towards +45 mV. These calculated curves were then fitted with a single time constant. If indeed two populations of Hodgkin-Huxley potassium channels would exist, the simulated time constant (τ_{sim}) now should match the observed τ_{off}. As illustrated in Fig. 5 this is clearly not the case. Instead, the time constant obtained in the simulations is a decreasing function of membrane voltage.

These calculations were repeated, using parameters obtained by Ilyin et al. (1980), who analyzed potassium currents in single nerve fibers from *Rana ridibunda* according to two populations of potassium channels. Also with their data a similar result for τ_{sim} was obtained.

"Steady"-State Conductance

The steady-state values of potassium conductance (at 10–50 msec) were slightly influenced by the positive prepulse. Somewhat smaller values for g_K were obtained, when holding potential was the initial condition.

This effect, however, was often too small to detect with a prepulse of 10-msec duration. Differences (though never exceeding 7–9% of $\bar{g}_K$) became smaller, when the test potential E_1 came close to +45 mV. Increasing the prepulse duration usually increased the differences slightly, for prepulses up to 50–100 msec.

Long depolarizing voltage pulses (beyond 100–600 msec) had two other effects:

a) Inactivation of the potassium conductance started to develop, though slower than the decrease of the potassium current. Formally this is represented by an extension of Eq. (2) with a multiplication factor k with $0<k\leqq 1$ (Schwarz & Vogel, 1971).

b) Following an increasing inactivation at the

test voltage a shift in the current at holding potential was observed, that was more pronounced following larger depolarizations. In addition, its amplitude and time to recover increased proportionally to the duration of a given test pulse (up to tens of seconds). Application of an additional voltage step from holding potential showed that leakage or potassium conductance or both had increased during this shift in holding current.

Discussion

The effects of potassium current flow on E_K appear to be considerable. In spite of variation between different nodes it was not uncommon for E_K to change from an estimated value of -80 mV (at rest) towards a value between -45 and -20 mV during a 15-msec voltage step towards $+60$ mV. According to Adelman, Palti and Senft (1973) and Dubois and Bergman (1975) shifts in E_K result from a changing potassium concentration ($[K^+]_o$) outside the nerve membrane, assuming that E_L is constant. Accumulation of the ions carried by leakage conductance, however, cannot be excluded as yet. As concluded by Dodge (1963) and supported by Hille (1973) and by Van den Berg and Rijnsburger (1980), leakage current is mainly carried by potassium ions. If so, E_L will change as well during potassium current flow. Against this plead the observations of Stämpfli (1959), Bromm and Esslinger (1974) and Atwell et al. (1980), that E_L is insensitive to potassium ion concentration changes. In any case, by the method used in the present investigation, the effects of all changing reversal potentials have been eliminated from potassium kinetics. However, direct effects of a changing $[K^+]_o$ are not eliminated. According to Dubois and Bergman (1977) potassium conductance increases as a Michaëlian function of $[K^+]_o$ for inward-going currents at constant membrane voltage. Moreover, direct effects of $[K^+]_o$ on the kinetics have been reported by Dubois (1981*a*) and Swenson and Armstrong (1981). On the other hand the results in this paper show that the time constants obtained on potassium conductance activation, with changing E_K, are not different from those obtained on potassium current activation, in case E_K is constant. Therefore the effects of the rapid changes in $[K^+]_o$ appear to be negligible, whereas prolonged application of high $[K^+]_o$ results in a modification of both potassium conductance and kinetics.

The applied method may have been slightly hampered by the occurrence of a small, slow transient current $I_{c'}$. According to Hille (1967), $I_{c'}$ is related to an increase of the membrane capacity, resulting from withdrawal of myelin, as would happen after a long experiment. However, in potassium activation $I_{c'}$ is visible from the beginning of the experiments, immediately after the dissection and mounting of the nerve fiber. It can also be seen in the potassium currents, measured by Palti et al. (1976) and Begenisich (1979). Capacitive transients, apparently including $I_{c'}$, are often subtracted from the membrane currents with an analog circuit that generates the sum of two or three exponentially decaying transients, proportional to the applied voltage step (Atwell et al., 1980; Conti, Neumcke, Nonner & Stämpfli, 1980). A similar procedure has been applied to the digitized currents. Fitting the corrected data according to Eq. (4b) revealed that the parameters changed with 3–10% only. Values of g_L, however, decreased with 20–30%.

An important condition for the validity of the double-pulse method is linearity of the instantaneous $I-E$ curve. In contrast to results of Dubois and Bergman (1977) we see that the instantaneous $I-E$ curve is not strictly linear for small ΔE. The deviation, however, is small. Moreover, the time constants, obtained from potassium currents, in case E_K was constant, agree satisfactorily with those from fits on conductance, when E_K changed (cf. Fig. 2). Therefore, possible effects of this non-linearity on the analysis appear to be negligible as well.

My results confirm the conclusions of Palti et al. (1976). Potassium conductance kinetics show a clear dependency on prepulse conditions. Their values for τ_n obtained in normal Ringer's, however, are most likely contaminated by changes in E_K. Their results in 80 mM KCl are probably not much hampered by this complication. The average value for τ_{off} at -50 mV agrees reasonably well with the data presented here. By extending the observations of Palti et al. (1976) it is concluded that τ_n depends on initial conditions for the whole range of membrane potentials, at least from -50 to $+15$ mV. So far in standard HH-analysis τ_n has been obtained at membrane voltages below -50 mV from off-responses. It is therefore clear, that the "classical" plot of τ_n as a function of membrane potential, with a maximum at -50 mV, merely results from the combination of two methods.

The suggestion has been made by Palti et al. (1976) that more than one type of potassium channel may explain their observed prepulse dependency of τ_n. Similar conclusions were drawn by Ilyin et al. (1980) on *Rana ridibunda*. Applying

a high potassium concentration outside the node, the latter could fit both activating and deactivating potassium currents, without observing a change in the time constants. Results presented here tend to support the hypothesis in respect to potassium activation, as fits improve considerably following the introduction of a second population of potassium channels. However, in contrast to the findings of Ilyin et al. (1980), my results indicate an inconsistency in the HH-kinetics of the two populations, when comparing activation and deactivation. Remarkably, the differences with Ilyin et al. (1980) appear to be restricted to the observations on potassium deactivation, as the parameters on activation (Fig. 3*a, b*) agree fairly well. Since their measurements were performed in 128 mM KCl, the different observations could result from the effects of a prolonged application of high $[K^+]_o$ (Dubois, 1981*a*; Swenson & Armstrong, 1981).

Recently, Dubois (1981*b*) suggested the existence of three different populations of potassium channels. His third – and slowest – population hardly plays a role in the kinetic analysis presented, as its time constant has a value between 20 and 80 msec (assuming a Q_{10} of 2.5). Moreover, its contribution to the overall conductance is less than 15% in 117 mM K^+ outside the node. In normal Ringer's this fraction appears to be even smaller (Dubois, 1981*b*). Nevertheless, as mentioned in the Results, a very slow conductance increase could be noticed in test pulses towards positive membrane voltages, especially when duration was increased. As the two remaining "fast" populations of potassium channels lack a kinetic description, further comparison is unfortunately not possible.

The effects of a short (10–50 msec) conditioning positive prepulse on "steady"-state values of potassium conductance were small, but systematic. Since Palti et al. (1980) observed similar prepulse-induced shifts, using 80 mM KCl outside the node, this increase cannot be explained by a raised external potassium concentration. Instead a slow phase in the activation and deactivation of potassium conductance appears to be responsible (Dubois, 1981*b*). The more pronounced increase in conductance at holding potential, following long lasting voltage pulses, cannot be explained by Dubois' population of slow channels. Instead it apparently reflects the slowly developing effect of a prolonged high-potassium concentration outside the node. As shown by Dubois (1981*a*), and supported by preliminary observations in 115 mM KCl, potassium conductance increases around holding voltage with increased $[K^+]_o$. Since Dubois (1981*b*) observed no such phenomena following long lasting depolarizations (using 117 mM KCl), this is consistent with a slowly developing action of increased $[K^+]_o$.

As the results of the kinetic analysis give no evidence for the existence of more than a single population of (fast) channels, alternative ways to describe the conductance of one population of identical channels will be considered.

1. Potassium conductance results from a multistate process: conditioning voltage steps will influence the relative occupancy of the respective states, thereby effecting kinetics in the conductance change, that depend on previous conditions (Palti et al., 1976; Neher & Stevens, 1977).

2. Potassium conductance obeys Eq. (4a), with an additional voltage-dependent relaxation of the rate constants, that is, e.g., described similar to the time-dependent relaxation of n (Clay & Shlesinger, 1982). This type of relaxation could result from voltage-dependent changes in the lipid membrane: the channel proteins sense a changed surrounding.

3. Nonlinear differential equations describe the potassium conductance. An example of such a description is given by Tille (1965).

4. Finally, higher order differential equations may be considered.

Much more experimental work is needed before discrimination between these alternatives will be possible. In this respect fluctuation analysis may provide information to narrow down the possible models.

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