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Characterization of K^+ Currents in Rat Malignant Lymphocytes (Nb2 Cells)

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Summary. Membrane K^+ currents of malignant lymphocytes (Nb2 cells) were studied with the whole-cell patch-clamp method. Upon depolarization, K^+ currents activate with a delay and follow a sigmoid time course, resembling other delayed rectifier K^+ currents present in nerve and muscle cells. The activation time constant of these currents is voltage dependent, increasing from 1 msec at +90 mV to approximately 37 msec at −30 mV. The fractional number of open channels has a sigmoid voltage dependence with a midpoint near −25 mV. Deactivation of K^+ currents in Nb2 cells is voltage dependent and follows a simple exponential time course. Time constant of this process increases from 5 msec at −115 mV to almost 80 msec at −40 mV. The relative permeability of K^+ channels to different monovalent cations follows the sequence: K^+ (1) > Rb^+ (0.75) > NH_4^+ (0.11) > Cs^+ (0.07) > Na^+ (0.05). Inactivation of K^+ currents is a biexponential process with time constants of approximately 600 and 7,000 msec. Inactivation of K^+ currents in Nb2 cells is not a voltage-dependent process. The steady-state inactivation curve of K^+ currents has a midpoint near −40 mV. Following a 500-msec voltage pulse, inactivation of K^+ currents recovers with a simple exponential process with a time constant of 9 sec. Short duration (~50 msec) voltage-clamp pulses do not induce significant inactivation of these currents. K^+ currents in malignant lymphocytes do not display the phenomenon of cumulative inactivation as described for other delayed rectifier-type K^+ channels. Application of a train of voltage pulses to positive potentials at different frequencies induces a moderate decrease in peak outward currents. The use of substances (N-bromoacetamide, trypsin, chloramine-T, and papain) that remove the inactivation of Na^+ and K^+ currents in other cells are not effective in removing the inactivation of K^+ currents present in this lymphoma cell line. Significant differences were found between the characteristics of K^+ currents in this malignant cell line and those present in normal lymphocytes. Possible physiological implications for these differences and for the role of K^+ currents in the proliferation of normal and malignant lymphocytes are discussed.

Key Words lymphocytes · membrane permeability · malignant cells · K^+ currents

Introduction

The application of the patch-clamp technique to lymphocytes of different origins has led to the discovery and characterization of K^+ currents present in these cells (*cf.* Gardner, 1990; Lewis & Cahalan, 1990). K^+ currents in lymphocytes resemble the delayed rectifier current present in the membrane of nerve and muscle cells. Following a step depolarization in membrane potential, lymphocyte K^+ currents activate with a characteristic sigmoid time course and then inactivate almost completely with a time constant of 100–300 msec (DeCoursey et al., 1984; Fukushima, Hagiwara & Henkart, 1984; Matteson & Deutsch, 1984; Cahalan et al., 1985; Deutsch, Krause & Lee, 1986). The discovery of these K^+ currents raised the question of their possible modulatory role in the physiological or pathophysiological events associated with lymphocyte activation.

While the precise molecular mechanisms by which K^+ currents interfere with lymphocyte activation are not known, considerable experimental evidence suggests a link between these different phenomena. It was demonstrated that mitogenic lectins shift the voltage dependence of K^+ currents to more negative transmembrane potentials and increase K^+ conductance (DeCoursey et al., 1984; Cahalan et al., 1985; Schlichter, Sidell & Hagiwara, 1986; DeCoursey et al., 1987). In addition, interleukin-2 and tumor-promoting agents (phorbol esters) induce lymphocyte proliferation and an increase in K^+ conductance (Deutsch et al., 1986; Lee et al., 1986). Because cAMP and isoproterenol decrease K^+ currents in lymphocytes, it was suggested that the ionic channels responsible for these currents could be modulated by intracellular messengers during the process of lymphocyte activation (Choquet et al., 1987; Soliven & Nelson, 1990). On the other hand, different K^+ channel blockers inhibit lymphocyte mitogenesis (DeCoursey et al., 1984; Sabath et al., 1986; Dos Reis, Nobrega & Persechini, 1988; Amigorena et al., 1990). Studies with K^+ channel blockers have demonstrated a reasonable quantitative agreement between block of K^+ currents and inhibition of lymphocyte proliferation. In addition to these observations, the electrophysiological prop-

erties of K^+ currents seem to vary with populations of lymphocytes that display different patterns of proliferation (Chandy et al., 1986; Lewis & Cahalan, 1988).

I reasoned that if K^+ current activation is an essential step in triggering lymphocyte proliferation, it should be possible to detect alterations in this conductance in malignant lymphocytes in relation to that present in normal T-cells. Since malignant cells proliferate extensively, it is conceivable that changes in activation, deactivation, selectivity and/or inactivation of K^+ currents could exert some modulatory function in the growth of lymphomas. The main goal of this study was to characterize the properties of a K^+ current in malignant lymphocytes. I describe that this current shares many common properties but also shows important differences in relation to K^+ currents previously studied in normal nonmalignant lymphocytes.

Materials and Methods

Lymphoma Cell Line

In this study, a lymphoma cell line (Nb2 cells) was used as an experimental model. Nb2 cells originate from a lymph node of an estrogenized male Noble (Nb) rat (Noble, Hochachka & King, 1975; Gout, Beer & Noble, 1980; Noble, Beer & Gout, 1980). An interesting characteristic of these cells is that lactogenic hormones induce their proliferation both in vivo and in vitro conditions (Gout et al., 1980; Noble et al., 1980). Proliferation of Nb2 cells has been used in different laboratories as a convenient bioassay for prolactin. These cells were characterized by different immunochemical and enzymatic techniques to identify membrane, nuclear, and cytoplasmic markers. Nb2 cells possess surface immunoglobulins characteristic of T-lymphocytes (Fleming et al., 1982).

Cell Culture

Nb2 cells were grown in Fischer's medium supplemented with 10% fetal calf serum, penicillin (50 μg/ml), and streptomycin (50 μg/ml). All culture media were purchased from GIBCO (Grand Island, NY). Under these conditions, Nb2 cells proliferate continuously and cell splitting is required once or twice a week. As I wish to explore differences in the electrophysiological characteristics of these cells in the future under stationary and nonstationary conditions, the Nb2 cells used in this study were deprived of calf serum for 24 or 48 hr prior to electrophysiological experiments. It was demonstrated that in the absence of serum, Nb2 cells do not proliferate (Gout et al., 1980; Wang, Walker & Cukierman, 1991).

Electrophysiology

The whole-cell patch-clamp method (Hamill et al., 1981) was used for voltage clamping and measuring transmembrane currents in Nb2 cells. A patch-clamp amplifier and commercially available hardware and software were used to record, digitize, and analyze membrane currents (Axon Instruments, Foster City, CA). Different sampling rates were utilized depending on the specific voltage-clamp pulse protocol. Membrane currents were filtered at 10 kHz with an 8-pole low pass Bessel filter (Frequency Devices, Haverhill, MA).

Two different types of glass were used for manufacturing patch pipettes: Corning #7052 and Corning 7040 (Garner Glass, Claremont, CA). Patch pipettes were pulled using a two-stage puller (Narishige Instruments, Tokyo). Before fire polishing, pipette tips were immersed in a silicone solution (Sigmacote, Sigma, St. Louis, MO) for approximately 5 sec. Upon fire polishing, the residual heptane in the pipette tip evaporated. This method of decreasing the glass electrode capacitance was found to be simpler and as effective as the more conventional coating method using Sylgard®. The method used also offers the advantage of coating both sides of the electrode with films of silicone. When filled with internal solutions (*see below*), patch pipettes had resistances ranging from 1 to 3 MΩ.

In the majority of experiments, series resistance compensation (60–80%) was used. However, it was observed that series resistance compensation did not significantly alter the current recordings. K^+ current activation is relatively slow and Nb2 cells do not possess high current densities. Without series resistance compensation, a typical peak current of 2 nA would have introduced a voltage error of approximately 2–6 mV.

Nb2 cells adhere to the bottom of the recording chamber. A single Nb2 cell was approached with a patch pipette to which a continuous train of 1-mV pulses was delivered. Pipette current was monitored continuously on an oscilloscope screen. When an approximately 20–25% drop in pipette current was observed ('close' proximity to the cell membrane), mild suction was applied to the pipette interior resulting in the formation of a membrane seal with resistances ranging from 1 to 50 GΩ. On several occasions, a spontaneous high resistance seal was formed by simply approaching the cell. A slightly more intense suction applied to the interior of the pipette resulted in the breaking of the membrane patch under the tip of the electrode (whole-cell configuration).

Cell capacitance was calculated by integrating the area of the transient capacitive current following a voltage step from -80 to -70 mV and by dividing this area by the voltage step (10 mV). Cell membrane capacitance was then compensated. A good agreement for the value of cell capacitance was found between this method and by simply reading the value of cell capacitance from the capacitance compensation dial of the amplifier. However, the values for membrane capacitance reported were derived from the integration method.

The recording of membrane currents started 10–15 min after the whole-cell configuration was attained. Nb2 cells remained stable and responsive for variable periods of time (30 min–2 hr).

Curve Fitting

Fitting of experimental points to different exponential functions (including Boltzmann distributions) was done using nonlinear square methods based on a Marquardt-Levenberg algorithm. The overall quality of fit was determined by three different parameters: standard deviation of the fit, degree of dependency between different parameters present in the equation, and a small value for residuals (difference between the fitted curve and dependent variable values, *see* Schreiner et al., 1985).

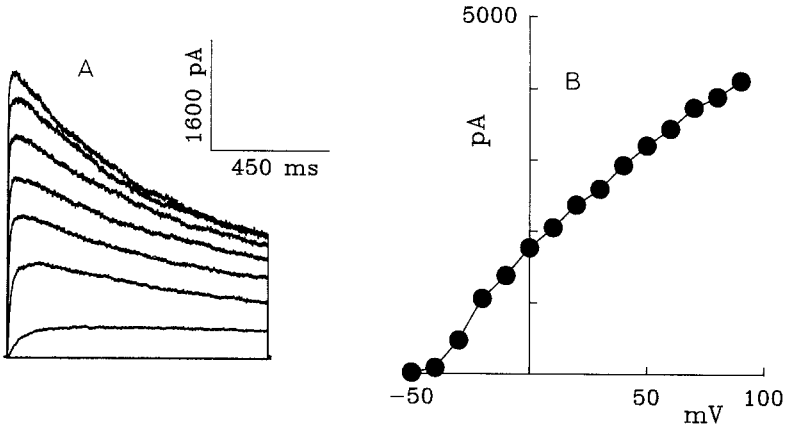

Fig. 1. Peak K^+ current-voltage relationship in an Nb2 lymphocyte. (*A*) Superimposed membrane currents following voltage-clamp pulses from −80 to −50 mV, and thereafter to +90 mV in 20-mV steps. Voltage pulses delivered every 30–40 sec. (*B*) Peak outward currents are plotted against voltage steps. Cell capacitance, 3.3 pF, series resistance compensation of 80% for an electrode resistance of approximately 1 MΩ.

Solutions

In most experiments the patch electrode contained (in mM/liter): KCl 145; $MgCl_2$ 2; K_2EGTA 10; and HEPES 5 (pH = 7.20 with KOH). In some experiments KCl was substituted for K-aspartate or KF. No significant improvement in cell stability or viability was noticed using these different salts.

Different extracellular solutions were used in this study. The basic Ringer solution contained (in mM/liter): NaCl 160; KCl 5; $CaCl_2$ 2; $MgCl_2$ 1; and HEPES 5 (pH = 7.40 with NaOH). K-Ringer solution contained (in mM/liter): KCl 165; $CaCl_2$ 2; $MgCl_2$ 1; and HEPES 5. Na, NH_4, and Cs-Ringer solutions had the same composition as K-Ringer with all KCl replaced by the appropriate chloride salt. In these solutions, the pH was adjusted with the appropriate cation hydroxide.

Nb2 cells were perfused continuously (chamber volume = 200 μl) with extracellular solutions at room temperature (21–23°C).

Different protein reagents, whose concentrations will be mentioned in the text, were used in this study: chloramine-T, N-bromoacetamide, N-bromosuccinimide, papain, and TPCK-treated trypsin were purchased from Sigma (St. Louis, MO).

Results

Activation and Deactivation of K^+ Currents in Nb2 Cells

Figure 1*A* shows superimposed membrane currents in response to 1-sec depolarizing voltage-clamp pulses from −80 to −50 mV and thereafter to +90 mV in 20-mV steps. Outward membrane currents are activated between −40 and −30 mV. Activation of these currents proceeds with a characteristic delay and sigmoid time course. Once this current attains its peak value, it slowly inactivates. Because the inactivation and recovery of inactivation are relatively slow processes (*see below*), voltage-clamp pulses were delivered at a slow rate (once every 30–40 sec). The peak current-voltage relationship illustrated in Fig. 1*B* shows that this outward current displays a moderate inward rectification for large membrane depolarizations.

From the experimental results of Fig. 1, it appears that as the membrane potential becomes more positive, the fractional number of channels in the open configuration increases. In order to quantify this phenomenon, it is necessary to divide the peak currents illustrated in Fig. 1 by the maximum current observed at each membrane potential. Figure 2*A* illustrates the method for determining the maximum current at different membrane voltages. A 25-msec voltage-clamp pulse was applied every 40 sec bringing the membrane potential from −80 to +70 mV. The implicit assumption in this protocol is that all ion channels responsible for this outward current are open at this large positive voltage (*see below*). Following this short voltage step, the membrane potential was hyperpolarized to different levels and 'instantaneous' tail currents were measured within 0.5–1 msec after changing the membrane potential. Superimposed current recordings in response to voltage steps from +70 to +40, +10, −20, and −50 mV are shown in Fig. 2. At a given voltage, the number of open channels is proportional to the peak current (values shown in Fig. 1) divided by the 'instantaneous' value of tail current at the same voltage. These values (fractional number of open channels) were plotted against voltage in Fig. 2. The fractional number of open channels is voltage dependent with a midpoint around −25 mV (*see* legend to Fig. 2), and all channels seem to be fully activated at membrane potentials more positive than +20 mV. The average midpoint of the fractional number of open channels in nine different cells was −27 ± 3 mV (mean ± SEM).

In Fig. 3, the voltage dependence of the kinetics of activation and deactivation of outward currents in Nb2 cells was studied. The activation of outward

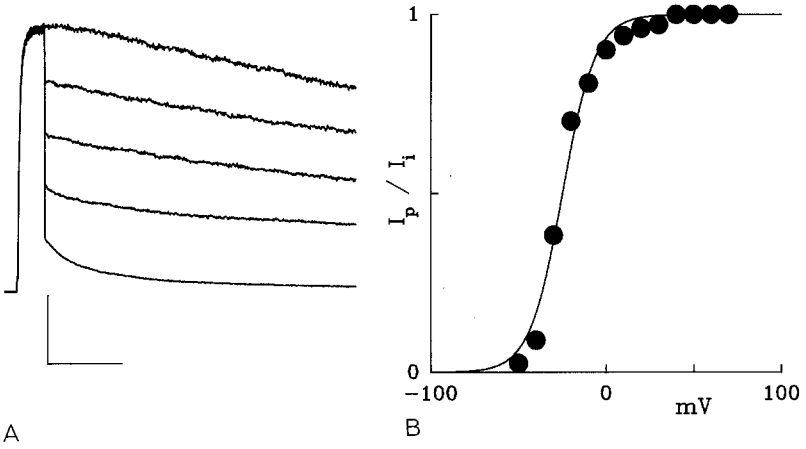

Fig. 2. Gating curve of K^+ currents from an Nb2 lymphocyte. (*A*) Superimposed membrane currents in response to a short pulse from −80 to +70 mV. Tail currents are shown in response to voltage steps from +70 to +40, +10, −20, and −50 mV. (*B*) Gating curve of K^+ currents (*see* text). Experimental points were fitted to equation $I_p/I_i = \{1 + \exp[(V - V_{0.5})/k]\}^{-1}$, where I_p is the peak current defined in Fig. 1, I_i is the 'instantaneous' tail current at the same voltage as I_p (*A*), $V_{0.5} = -24.8$ mV, and $k = -9.39$ mV. Calibration marks for current recordings are 1,000 pA (vertical), and 80 msec (horizontal). Same cell as in Fig. 1.

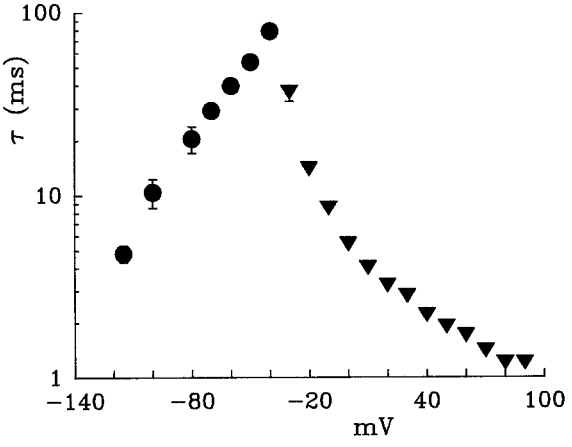

Fig. 3. Dependence of time constants of activation (triangles) and deactivation (circles) on membrane potential. Deactivation time constants are the mean ± SEM of seven different cells. Activation time constants were determined in 46 different cells. Error bars are omitted when smaller than symbols. Activation time constants were obtained by fitting the onset of activation to Eq. (1) described in text. Tail current decay follows a simple exponential process whose time constant is plotted in this figure as a function of voltage.

currents could be well described by an equation of the form

$$I_K = A \cdot [1 - \exp(-t/\tau_a)]^3 \qquad (1)$$

where A is an arbitrary factor, t is time, and τ_a is the activation time constant. The voltage dependence of τ_a is plotted in Fig. 3 (triangles). As the membrane potential increases, activation of outward currents in Nb2 cells becomes monotonically faster. At −30 mV, τ_a is approximately 37 msec while at +90 mV, this value decreases to 1 msec. In principle, a complete description of the activation of K^+ currents should also take into consideration the inactivation component of these currents. However, because K^+ currents in this lymphoma cell line inactivate with time constants several orders of magnitude larger than activation (*see below*), neglecting the inactivation process does not affect the determination of τ_a. In the worst case, an error of approximately 4% arises as a consequence of neglecting inactivation during the determination of τ_a.

The time constants of deactivation of outward currents as a function of membrane potential are plotted in circles in Fig. 3. Following a 25-msec voltage-clamp step to +70 mV, the membrane potential was stepped to different voltage levels (*see* Fig. 2*B*) and time constants for the relaxation of tail currents were determined. The decay of these tail currents follow a simple exponential time course. The time constant of this process is also monotonically dependent on the membrane potential, increasing from 5 msec at −115 mV to almost 80 msec at −40 mV.

Permeation Properties of a K^+ Conductance System in Nb2 Cells

Figure 4 shows superimposed tail current recordings in Na-Ringer (Fig. 4*A*), and in NH_4-Ringer (Fig. 4*B*) solutions from the same malignant lymphocyte. Voltage-clamp steps (25 msec) were delivered to +70 mV every 35 sec (currents in response to this pulse are off the scale). Following this pulse, hyperpolarizing voltage steps to −40, −55, −70, −85, and −100 mV (Fig. 4*A*), and to −25, −40, −55, −70, and −100 mV (Fig. 4*B*) were applied. Two observations are apparent from this figure: (i) NH_4^+ flows through these channels more effectively than Na^+ does. Compare the size of tail currents in the two different panels of this figure; (ii) The reversal

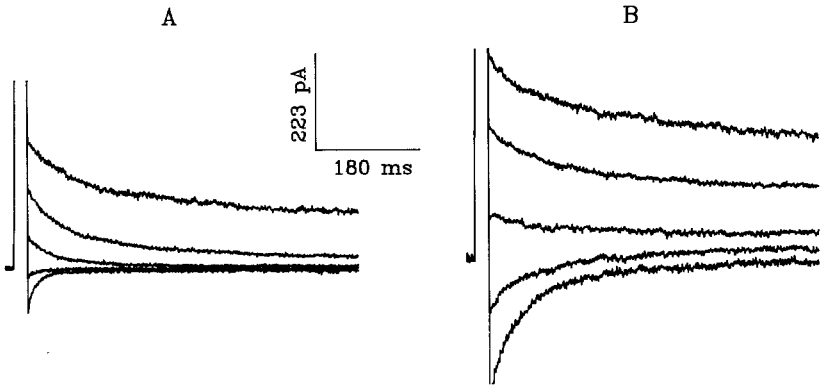

Fig. 4. Reversal potentials in Na-Ringer (*A*) and NH_4-Ringer (*B*) solutions. Outward currents were activated by a pulse from −80 to +70 mV every 35 sec (currents corresponding to these voltage steps are off scale). Following this 25-msec pulse, membrane potential was stepped to −40, −55, −70, −85, and −100 mV (*A*) and to −25, −40, −55, −70, and −100 mV (*B*). Same cell for both panels. Cell capacitance, 4.7 pF; 70% series resistance compensation. Electrode resistance, 3 mΩ.

Table 1. Reversal potentials determined in different Ringer solutions (mean ± SEM)[a]

	V_{rev} (mV)	n	P_X/P_K[b]
K	1.3 ± 0.1	3	1.00
Rb	−7.3 ± 0.3	3	0.75
NH_4	−56.3 ± 1.4	4	0.11
Cs	−66.7 ± 2.1	3	0.07
Na	−77.3 ± 2.6	5	0.05

[a] Reversal potentials were determined in Na, K, Rb, Cs, and NH_4-Ringer solutions in different cells.
[b] Relative permeabilities were calculated from the Goldman-Hodgkin-Katz equation, $V_{rev} = RT/F \cdot \ln P_X/P_K \cdot [X]_o/[K]_i$.

potential in NH_4-Ringer solution is depolarized in relation to the one obtained in a Na-Ringer solution.

Similar experiments to the one illustrated in Fig. 4 were performed in different Ringer solutions containing as the major monovalent cation K^+, Rb^+ or Cs^+. The measured reversal potentials with these different extracellular solutions are shown in the Table. The relative permeabilities of this major (if not only) ionic current in Nb2 cells increase in the following order: Na^+ (0.05) < Cs^+ (0.07) < NH_4^+ (0.11) < Rb^+ (0.75) < K^+ (1). It should be pointed out that considerable tail inward currents were observed in different monovalent Ringer solutions with the exception of Cs-Ringer (*results not shown*). In Cs-Ringer solutions, the 'instantaneous' current-voltage relationship displays a strong inward rectification. This suggests that Cs^+ is able to bind very tightly to a site in the conduction pathway of the pore while having a considerable permeability (Cukierman, Yellen & Miller, 1985).

Variability of K^+ Current Density in Nb2 Cells

Considerable variation in the magnitude of K^+ currents in different Nb2 cells was found. To investigate whether this variability correlates with cell size, peak K^+ currents in response to a +50-mV voltage step were normalized in relation to membrane capacitance. There is a poor relationship between cell size and peak K^+ currents (*results not shown*). In 37 different cells, the average values for membrane capacitance and peak currents (for a +50-mV pulse) were 4.3 ± 0.3 pF and 1,671 ± 330 pA, respectively. Quiescent Nb2 cells have an average diameter of 9.5 ± 1.2 μm. Assuming that Nb2 cells are perfectly spherical, a total membrane surface area would be 2.8×10^{-6} cm^2. If a typical value of 1 $\mu F/cm^2$ is assumed for the specific membrane capacitance, an average value of 4.3 pF is larger than would be expected. However, electron microscopic observations of Nb2 cells revealed the presence of numerous and significant membrane invaginations (Fleming et al., 1982), leading to an effective increase in the membrane surface area.

Inactivation of K^+ Currents

The inactivation of K^+ currents in malignant lymphocytes is a biexponential process with a fast (~700-msec) and slow (~8-sec) time courses (*see below*). Before analyzing in more detail the inactivation behavior of these K^+ currents, it is important to address the possibility of significant accumulation of K^+ ions near the extracellular side of the membrane. To test this, the reversal potential of 'instantaneous' tail currents were measured at the end of 40- and a 6,000-msec voltage-clamp pulses to +70 mV. Reversal potentials measured with these two different pulse protocols were not different (*results not shown*), suggesting that no significant accumulation of K^+ is taking place near the membrane.

Figure 5 shows K^+ currents at the indicated voltage steps from a −80-mV holding potential. Circles in each panel represent the least-squares fit to the current following an equation of the form:

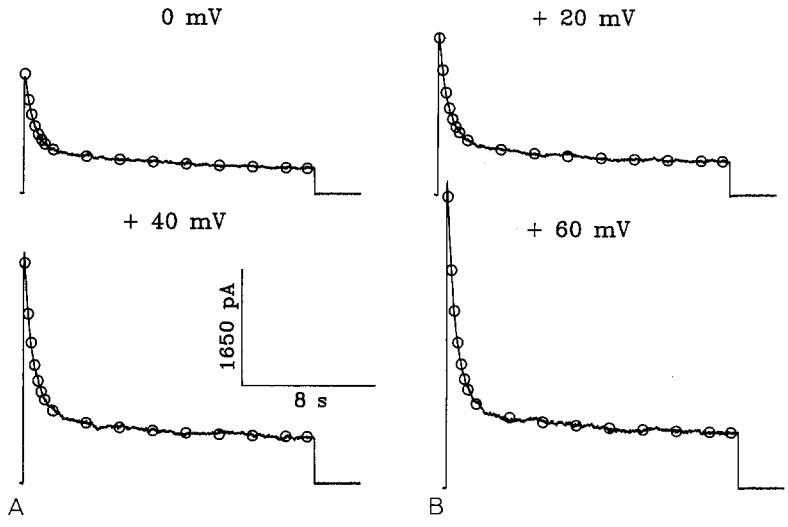

Fig. 5. Outward currents in response to long-duration pulses. Membrane potential was stepped from −80 mV to the indicated voltages in each panel. Circles are the best fit to Eq. (2) indicated in the text. Inactivation time constants are: 534 and 9640 msec (at 0 mV), 528 and 5574 msec (20 mV), 524 and 5632 msec (40 mV), and 515 and 6626 msec (60 mV). Although some variation in τ_2 was observed, this time constant did not show consistent dependence on the value of membrane potential (*see* Fig. 7 for averaged values).

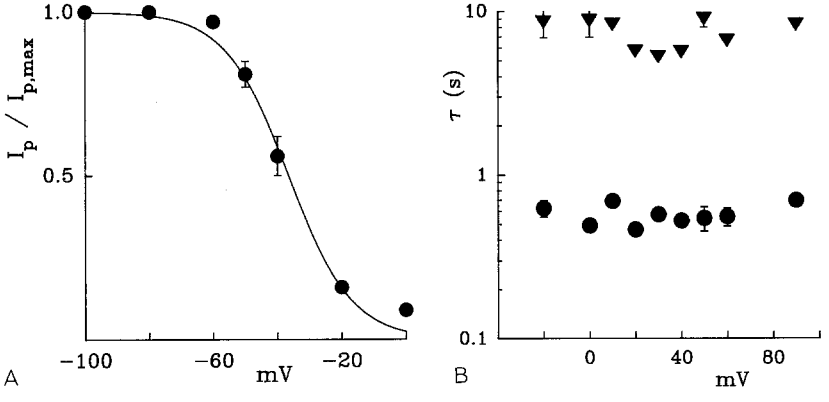

Fig. 6. (*A*) Steady-state inactivation of K^+ currents in Nb2 cells. Relative peak currents are plotted against the holding membrane potential. The holding membrane potential was maintained for 4560 sec prior to the application of a +60-mV pulse. Mean ± SEM for five experiments are shown. Points were fitted to $I_p/I_{p,max} = \{1 + \exp[(V - V_{0.5})/k]\}^{-1}$ where $V_{0.5} = -37$ mV and $k = 9.8$ mV. (*B*) Voltage dependence of fast (circles) and slow (triangles) components of K^+ current inactivation in Nb2 cells. Each point represents the mean ± SEM of a minimum of three and a maximum of five different cells. The determination of time constants are explained in the text and in the legend to Fig. 5.

$$I_K = A \cdot \exp(-t/\tau_1) + B \cdot \exp(-t/\tau_2) \quad (2)$$

where A and B are scaling factors, t is time, and τ_1 and τ_2 are different time constants. In this figure, τ_1 has values of 534, 528, 524, and 515 msec for voltage-clamp pulses to 0, +20, +40, and +60 mV, respectively. τ_2's were considerably longer: 9,640, 5,574, 5,632, and 6,626 msec for the same voltage-clamp steps, respectively. At this point, one might ask why not consider the inactivation of these K^+ currents an incomplete process and assume that said inactivation follows a simple exponential time course with a time constant between τ_1 and τ_2. The experiments shown in the next figure suggest that K^+ currents in Nb2 cells can inactivate almost completely. Figure 6*A* illustrates the results of different experiments where the holding voltage was set at different levels (−100 to 0 mV) for 45–60 sec prior to the application of a +60-mV voltage-clamp pulse. The relationship between K^+ peak currents at a given holding voltage and the maximum observed peak current is plotted against the holding membrane potential. The experimental points were fitted by a simple Boltzmann distribution (*see* legend to figure) with a midpoint of −37 mV. Figure 6*B* shows the voltage dependence of both slow and fast time constants of inactivation of K^+ currents in Nb2 cells. Contrary to the activation and deactivation processes, inactivation of K^+ currents in this lymphoma cell line is not a voltage-dependent process.

In Fig. 7, the recovery of inactivation following a 50-msec (open symbols) or 500-msec (filled symbols) voltage step from −80 to +30 mV was studied. These experiments consisted of applying a pair of pulses separated by different time intervals. Pulse pairs were delivered once every 40–50 sec. This figure shows that during the first 50 msec, inactivation does not occur at a significant level. With longer pulse durations (500 msec), a significant amount of inactivation developed. The recovery of inactivation

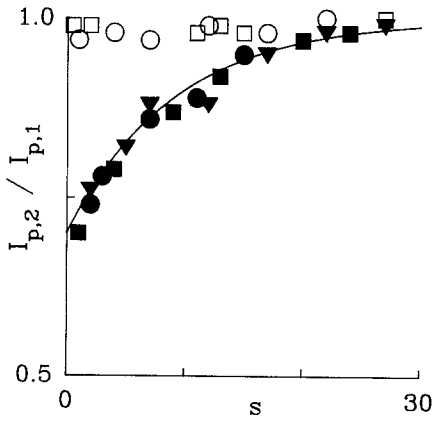

Fig. 7. Recovery of inactivation. Identical 50-msec (open symbols) or 500-msec (filled symbols) pairs of pulses separated by a variable interval were delivered every 40 sec. Voltage steps were from −80 to +30 mV. Different symbols correspond to different cells (same open and filled symbols are from the same cell). Filled symbols were fitted to $I_{p,2}/I_{p,1} = 1 - 0.30 \cdot \exp(-t/8.97)$, where $I_{p,2}$ and $I_{p,1}$ are the peak currents for the second and first pulses, respectively, and t is time. Notice that 50-msec pulses did not induce significant current inactivation.

followed a simple exponential process with a time constant of approximately 9 sec (*see* legend).

Different preparations display a frequency-dependent increase in K^+ current inactivation (Aldrich, Getting & Thompson, 1979; Aldrich, 1981). This phenomenon, termed cumulative inactivation, produces a current pattern in which the peak current during a second voltage-clamp pulse is considerably smaller than the current at the end of the preceding pulse. Conditions for the development of cumulative inactivation include a relatively fast onset of inactivation associated with a slow recovery from inactivation. These characteristics were demonstrated in normal lymphocytes and, indeed, these cells show cumulative inactivation (Cahalan et al., 1985). Do K^+ currents in Nb2 cells have cumulative inactivation? In Fig. 8*A*, superimposed current recordings in response to voltage-clamp pulses delivered at a rate of 1 Hz from −80 to +60 mV are shown. Numbers beside each trace identify the pulse number in a train of 10 consecutive pulses. The peak current in recording 2 (or 3) is higher than the current at the end of pulse 1 (or 2). Even the peak current in pulse 10 is larger than the current at the end of the third pulse. K^+ currents in this lymphoma cell line do not accumulate inactivation. Figure 8*B* shows the temporal evolution of peak currents (relative to the peak current in response to the first pulse) at three different frequencies of stimulation (squares, 0.063 Hz; triangles, 0.25 Hz; circles, 1 Hz).

Is It Possible to Remove the Inactivation of K^+ Currents in Nb2 Cells by Chemical Means?

Inactivation of Na^+ currents can be removed with different proteolytic enzymes, specific amino acid reagents or different alkaloids (Armstrong, Bezanilla & Rojas, 1973; Oxford, Wu & Narahashi, 1978; Rudy, 1978; Catterall, 1980; Gonoi & Hille, 1987; Brown, 1988; Cukierman, 1991). The inactivation of K^+ currents in GH3 cells can also be removed with some of these agents (May & Oxford, 1986; Matteson & Carmeliet, 1988; Oxford & Wagoner, 1989). I asked whether the inactivation of K^+ currents in Nb2 cells can be removed by chemical treatments. Figure 9 shows recordings of K^+ currents in response to depolarizing voltage steps from −80 to +60 mV in control conditions (panels *A* and *C*, approximately 1-min after the whole-cell configuration was attained) in two different cells. In Fig. 9*A* and *B*, the patch pipette contained 50 μM N-bromoacetamide (NBA) from the beginning of the whole-cell clamp. The recording in Fig. 9*B* was obtained 10 min after 1 mM NBA was added to the extracellular solution. In this and in two other experiments, NBA did not remove the inactivation of K^+ currents in Nb2 cells. A small but consistent acceleration of inactivation in the presence of NBA was noticed in this as well as in two other experiments. In Figs. 9*C* and *D*, papain (0.3 mg/ml) was present in the pipette solution since the whole-cell clamp configuration was attained. As with NBA, papain did not remove the inactivation of these K^+ currents. Experiments with trypsin (0.3 mg/ml) and chloramine-T (1 mM) inside the pipette were also performed with negative results.

Discussion

Comparative Aspects of K^+ Currents in Normal and Malignant T-Lymphocytes

Activation of K^+ currents in Nb2 cells is similar to K^+ currents present in normal lymphocytes. Upon depolarization, both K^+ currents activate with a delay and follow a sigmoid time course resembling the delayed rectifier currents observed in nerve and muscle cells. The gating curve of K^+ currents in Nb2 cells has a midpoint around −27 mV while normal lymphocytes have a midpoint at more hyperpolarized membrane potentials (−40 mV, Cahalan et al., 1985). The activation time constants of these currents in Nb2 cells are voltage dependent and their values are well within the range previously reported

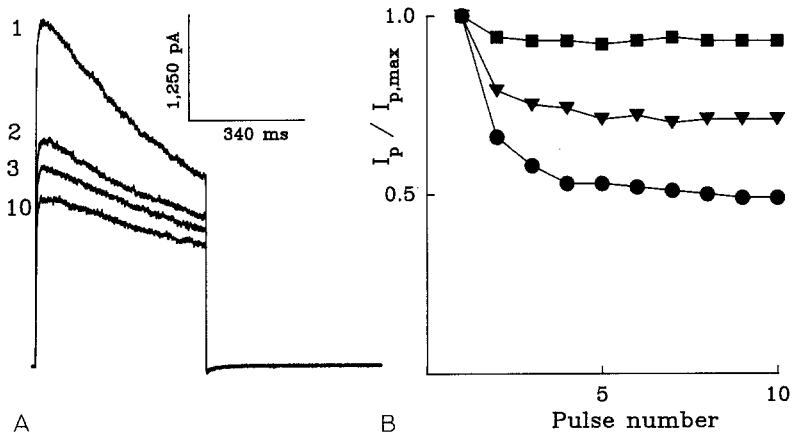

Fig. 8. Effects of stimulation frequency on peak outward K^+ currents. A train of 10 identical voltage pulses was applied at different stimulation frequencies. Recordings show superimposed membrane currents for the 1st, 2nd, 3rd and 10th voltage-clamp pulses (stimulation frequency of 1 Hz). Membrane step depolarizations were from −80 to +60 mV (500-msec durations). Graph shows the relative peak currents (in relation to the peak current produced by the first pulse) as a function of pulse number. Squares, 0.0625 Hz; triangles, 0.25 Hz; and circles, 1 Hz. Cell capacitance, 5.6 pF, series resistance compensation employed (75%), electrode resistance, 2 MΩ.

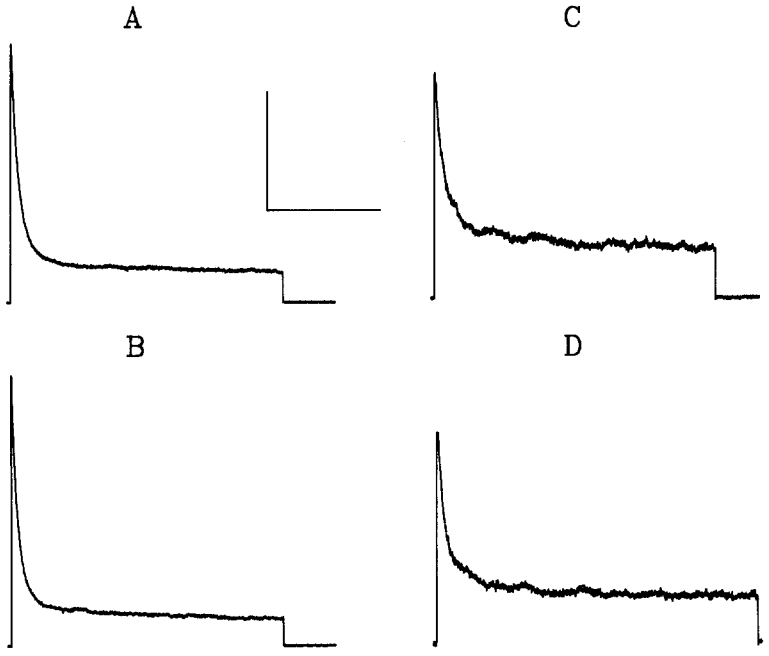

Fig. 9. Effects of NBA and papain on K^+ currents in Nb2 cells. Recordings in *A* and *C* were obtained approximately 1 min after breaking inside the cell (whole-cell clamp configuration). Patch pipettes contained 50 μM NBA (*A* and *B*) or 0.3 mg/ml papain (*C* and *D*). Currents in *B* were obtained 20 min after *A* and 10 min after perfusing the cell with 1 mM extracellular NBA. Notice in this recording a slight acceleration of inactivation in relation to *A*. Membrane currents in *D* were recorded 15 min after *C*. Cell capacitances were 6.2 pF (*A* and *B*) and 5.4 pF (*C* and *D*). No series resistance compensation was used in these recordings. Calibration bars: 7.2 sec (horizontal), vertical: 4.370 pA (*A* and *B*), 400 pA (*C* and *D*).

for normal nonmalignant T-cells (Cahalan et al., 1985; Deutsch et al., 1986).

Deactivation of K^+ currents in Nb2 cells follows a simple exponential decay. The voltage dependence of the time constant is very similar to the n-type K^+ currents described by Lewis and Cahalan (1988) in T-lymphocytes.

From reversal potential measurements, it was found that the relative permeability of K^+ channels in Nb2 cells to different monovalent cations follows the sequence: K^+ (1) > Rb^+ (0.75) > NH_4^+ (0.11) > Cs^+ (0.07) > Na^+ (0.05). This sequence is in excellent quantitative agreement with that reported by Cahalan et al. (1985) in normal T-cells. Thus, K^+ currents in normal and malignant lymphocytes share very similar properties concerning activation, deactivation, and permeability. The average K^+ current density is also similar in both populations of cells. Lymphoma cells have an average K^+ current density of approximately 400 pA/pF (for a +50-mV voltage pulse). This value compares well with the K^+ current density measured in normal lymphocytes (750 pA/pF for a +100-mV voltage clamp pulse, Deutsch et al., 1986).

Significant differences were found between the inactivation processes of K^+ currents in malignant and normal lymphocytes: (i) Inactivation in normal T-cells follows a simple exponential decay (Cahalan et al., 1985; Deutsch et al., 1986; Choquet et al., 1987), with a voltage-independent time constant ranging from 150 to 300 msec. Also, K^+ currents in normal lymphocytes inactivate almost completely in less than 1 sec. As with normal lymphocytes, the inactivation of K^+ currents in Nb2 cells is voltage independent. However, and most importantly, the inactivation time course of K^+ currents in Nb2 cells is biexponential and is considerably slower than in nonmalignant lymphocytes; (ii) Repetitive applications of short (50-msec) depolarizing voltage-clamp pulses in normal lymphocytes induce a significant amount of inactivation (Cahalan et al., 1985). The same pulse protocol applied to Nb2 cells, does not result in inactivation; (iii) Following a 500-msec voltage-clamp pulse, K^+ currents in malignant lymphocytes recover from inactivation with a simple exponential time course (time constant of 9 sec). In normal lymphocytes the recovery of inactivation is far more complex and slower: it is a biexponential process with time constants of 10 and 400 sec (Cahalan et al., 1985); (iv) Different characteristics for K^+ current inactivation mentioned in items (i)–(iii) above, make cumulative inactivation more likely to occur in K^+ currents present in normal T-lymphocytes than in malignant T-cells. Indeed, K^+ currents in normal lymphocytes display cumulative inactivation (Cahalan et al., 1985); during repetitive depolarizing voltage steps, the peak current at any given pulse is significantly smaller than the current at the end of the preceding pulse. K^+ currents in malignant lymphocytes do not present cumulative inactivation; (v) K^+ currents in malignant lymphocytes have a 50% steady-state inactivation at -37 mV. This value is considerably lower than previously reported for normal T-cells (-64 mV, Cahalan et al., 1985). In summary, inactivation of K^+ currents in malignant as compared to normal T-cells is: (i) slower, (ii) recovers faster, (iii) does not accumulate, and (iv) has a steady-state inactivation curve shifted to more depolarized membrane potentials.

What Is the Possible Role of K^+ Currents in Malignant Lymphocytes? Is There Any Physiological Implication for the Fact that Malignant Lymphocytes Have an Altered Inactivation Process of K^+ Currents in Relation to Normal T-Cells?

The malignant lymphocytes used in this study were originally derived from the lymph node of an estrogenized male rat (Noble et al., 1975, 1980; Gout et al., 1980). A remarkable characteristic of these cells is that their proliferation is strictly dependent on the hormone prolactin. Different early post-receptor responses are associated with prolactin and lectin activation in malignant and normal lymphocytes, respectively. While lectin activation of normal lymphocytes depends on extracellular Ca^{2+} (Alcover et al., 1986), prolactin activation of Nb2 lymphocytes does not (Murphy, Dimattia & Friesen, 1988). Indeed, in normal lymphocytes it is possible to bypass the activation of surface receptors and induce proliferation using phorbol ester and Ca^{2+} ionophores (Truneh et al., 1985; Weiss & Imboden, 1987). The same treatment applied to malignant Nb2 lymphocytes does not result in cell proliferation (Murphy et al., 1988). It is interesting to notice that concanavalin-A and phytohemmaglutinin do not induce stimulation of malignant lymphocytes (Y.F. Wang and A.M. Walker, *personal communication*). These observations suggest that prolactin in Nb2 cells and lectins in normal lymphocytes do not share common biochemical pathways in the proliferation process.

Because different biochemical pathways are involved in the activation of malignant and normal lymphocytes, it is important to address the issue of the physiological significance of K^+ currents for the proliferation of both cell populations. Preliminary observations demonstrated very clearly that different K^+ channel blockers inhibit (or even abolish completely) the prolactin-induced proliferation of this malignant cell line (Wang et al., 1991). Different K^+ channel blockers were utilized (barium, quinidine, tetraethylammonium, and 4-aminopyridine), suggesting that nonspecific effects of these blockers on cell proliferation are unlikely to occur (however, *see* Schell et al., 1987). As with normal lymphocytes, these channels seem to be important for the proliferation process of malignant T-cells. Thus, it is extremely important to notice that K^+ currents apparently modulate the proliferation of distinct populations of lymphocytes that display different patterns of stimulation (*see above*).

Since, (i) extracellular Ca^{2+} is not involved in proliferation of malignant Nb2 lymphocytes as it is in normal ones and, (ii) the essential difference between K^+ currents in normal and malignant lymphocytes is related to the inactivation process, it is important to question whether this process has a special meaning for the proliferation of malignant lymphocytes. If activation of K^+ currents is an essential step in lymphocyte proliferation, it is conceivable that malignant cells could possess a specific molecular mechanism responsible for enhancing these currents. Making the inactivation of K^+ currents slower would result in a sustained enhance-

ment of K^+ fluxes across the membrane. Thus, it is possible that the extensive proliferation activity displayed by malignant lymphomas could be a consequence of (or is favored by) a slow inactivation process of K^+ currents which also has fast recovery kinetics, conditions that do not favor the presence of cumulative inactivation. It would be meaningful to investigate whether substances capable of modulating the inactivation of K^+ currents in different lymphocytes have the capability of modulating cell proliferation.

It is important to evaluate whether the observed differences between K^+ currents in normal and malignant lymphocytes also occur in other normal and malignant cells originating from the same tissue. Meanwhile, normal and malignant lymphocytes can be regarded as good experimental models to further explore the functional meaning of their different electrophysiological behaviors.

Slow Inactivation of K^+ Currents in Nb2 Cells Is Not Removed by Conventional Protein Reagents

The removal of K^+ current inactivation in GH3 cells by NBA and papain was demonstrated (May & Oxford, 1986; Matteson & Carmeliet, 1988; Oxford & Wagoner, 1989). In this study, I found that these substances (and trypsin and chloramine-T, *results not shown*) do not remove the inactivation of K^+ currents in Nb2 cells. Outward K^+ currents in GH3 cells inactivate faster than K^+ currents in Nb2 lymphocytes. These observations suggest that different molecular mechanisms underlie the slow and fast inactivation processes of different K^+ channels. It will be very important to know whether inactivation removers slow or abolish the fast inactivation process of K^+ currents in normal lymphocytes. If this happens to be the case, do inactivation removers induce (or enhance) proliferation of normal T-cells?

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