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Alterations in a Voltage-Gated K^+ Current during the Differentiation of ML-1 Human Myeloblastic Leukemia Cells

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Summary. A voltage-gated K^+ current has been identified in ML-1 human myeloid leukemia cells, with the use of the whole-cell patch-clamp technique. ML-1 cells proliferate in tissue culture as immature myeloblasts and can be induced to differentiate to nonproliferative monocyte/macrophages. In the myeloblastic cells, activation of the K^+ current occurs upon depolarization of the membrane potential to above −40 mV; inactivation of this current is also voltage dependent and follows a simple exponential time course with a time constant (T_i) of 900 msec at 0 mV. The current is inhibited by 4-aminopyridine (IC_{50} of 80 μM at 0 mV), but is much less sensitive to tetraethylammonium of Ba^{2+}. In cells exposed to the differentiation-inducer 12-O-tetradecanoylphorbol-13-acetate (TPA), dramatic alterations in the K^+ current occur: upon exposure to 10 nM TPA during whole-cell recording, the amplitude of the voltage-activated current initially increases (within 4 min) and later decreases (at approximately 30–50 min). Upon addition of 0.5 nM TPA to cells in tissue culture, the current shows suppressed activation and accelerated inactivation in the early stages of differentiation (10-fold decrease in T_i at approximately 7 hr) and is completely suppressed in the later stages (3 days). Thus, this voltage-gated K^+ current is suppressed early in the induction of differentiation and associated loss of proliferation in myeloid ML-1 cells exposed to TPA; this parallels the fact that channels of a similar type are activated upon the stimulation of proliferation in lymphoid cells exposed to mitogens. Taken together, these findings suggest a role for voltage-gated K^+ channels in cell proliferation, and for their suppression in the loss of proliferation that accompanies differentiation.

Key Words ML-1 cells · K^+ current · cell differentiation · patch clamp

Introduction

Cell differentiation is critical for the development of virtually all tissues, as well as for the maintenance of their physiological properties. An understanding of the signals and molecular mechanisms involved in differentiation is thus of fundamental biological significance. Differentiation is frequently aberrant in cancer cells; hence, an understanding of the molecular events involved may also have clinical relevance. These events can be studied in cell lines that can be induced to differentiate in vitro (e.g., hematopoietic cell lines). The ML-1 human myeloid leukemia cell line is such a system. ML-1 cells proliferate in tissue culture as immature myeloblasts. They can be induced to differentiate along the monocyte/macrophage pathway by exposure to the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (Craig & Bloch, 1984; Craig et al., 1984; Kozopas, Buchan & Craig, 1990). Differentiation involves changes in cell morphology and function, as well as an increase in cell volume and in cytoplasmic/nuclear ratio. Differentiating phenotype appears after about one to three days of exposure to TPA; it is accompanied by a loss of the capacity for cell proliferation (Craig & Bloch, 1984; Kozopas et al., 1990). In ML-1 and other cells, TPA is thought to initially interact with protein kinase C (PKC) (Guan, Hromchack & Bloch, 1989). Less well understood, however, are the subsequent chains of events, initiated at the membrane and transmitted through the nucleus, that result in the induction of differentiation and loss of proliferation.

Differentiation in several other myeloid systems is associated with changes in K^+ channels. For example, immature, promyelocytic HL-60 cells contain a slow-inactivated K^+ channel (Wieland et al., 1987), whereas HL-60 cells induced with macrophage-colony-stimulating factor contain an inwardly rectifying K^+ channel (Wieland, Chou & Gong, 1990). Similarly, human monocyte-derived macrophages (HMDM) at early stages contain a delayed outward-rectifying K^+ current, whereas at later stages they contain both a Ca^{2+}-activated and an inward-rectifying K^+ current (Gallin, & McKinney, 1985; McCann, Keller & Guyre, 1987; Gallin & Sheehy, 1988; Nelson, Jow & Jow, 1990; Nelson, Jow & Popovich, 1990). U-937 promonocytic cells

also contain a voltage-gated K^+ channel, which has a single channel conductance different from that found in HL-60 or HMDM cells (McCann et al., 1987). Lymphocytes likewise contain voltage-gated K^+ channels; these channels are sensitive to 4-aminopyridine (4-AP) and their activity has been linked to the mitogen-induced stimulation of proliferation (DeCoursey et al., 1984; Grinstein & Dixon, 1989). Thus, because of their importance in other systems, we decided to investigate the role of K^+ channels in the differentiation and associated loss of proliferation in ML-1 cells.

In this report, the whole-cell patch-clamp technique was used to study a voltage-gated K^+ current in ML-1 cells. The K^+ current in these myeloid cells was found to have many of the characteristics of a voltage-gated K^+ channel in lymphoid cells. The channel activity was profoundly influenced by the differentiation-inducer TPA. Paralleling the findings in lymphocytes where increased channel activity is associated with mitogen-stimulated proliferation (DeCoursey et al., 1984; Lee et al., 1986), decreased activity in ML-1 cells is associated with the loss of proliferation that occurs during TPA-induced differentiation. These results suggest that the alteration in K^+ channel activity, a membrane event occurring upon the application of TPA, may represent a link in the transmission of the signal for induction of differentiation and loss of proliferation.

Materials and Methods

Growth and Differentiation of Myeloid ML-1 Cells

ML-1 cells were grown in RPMI 1640 medium containing 25 mM HEPES buffer (GIBCO, Grand Island, NY), as described previously (Craig & Buchan, 1989). The culture medium was supplemented with 7.5% heat-inactivated fetal bovine serum (FBS).

For the induction of cell differentiation, ML-1 cells were exposed to TPA as described previously (Kozopas et al., 1990). Cells were suspended (3×10^5 cell/ml) in RPMI 1640 medium containing 25 mM HEPES buffer, 0.3% FBS and 0.5 nM TPA. After 3 hr, cells were washed as described previously (Kozopas et al., 1990) and resuspended in RPMI 1640 medium containing 25 mM HEPES buffer and 7.5% FBS. Upon exposure of ML-1 cells to TPA, differentiated phenotype appears on about days 1–3.

Patch-Clamp Studies in the Whole-Cell Configuration

Whole-Cell Patch Clamp

ML-1 cells were removed from the tissue culture medium and resuspended in 10 ml Ringer solution (*see* Solutions). Whole-cell patch clamp was performed in a continuously perfused bath chamber with a volume of 0.5 ml. Experiments were carried out at room temperature (22°C). After addition of 2–3 drops of suspended cells into the chamber, single, isolated cells were studied by using an EPC7 patch-clamp amplifier (List, Electronics) grounded with a Ag/AgCl/Cl agar bridge. Data were prefiltered at 1 kHz through an 8-pole Bessel low-pass filter (Frequency Devices) and digitized at 20 kHz directly into a 386/PC computer using pCLAMP software (Axon Instruments) for subsequent analysis using Clampex and Clampfit programs.

For whole-cell patches, pipettes (VWR, Micro-hematocrit capillary tube) were manufactured by a two-stage vertical puller (Narishige PP-83) and polished by a glass-coated heat filament. Pipette resistances varied between 2–4 MΩ when the pipette was filled with 150 mM KCl saline. The pipette was attached onto the cell and a tight seal was formed by application of a gentle suction with an input resistance of 50–100 GΩ. The whole-cell model was then established by an additional suction until the membrane was broken as indicated by the appearance of the capacitance transient current. The input resistance was measured 5–10 min after the membrane was disrupted, which allowed the pipette solution to uniformly diffuse into the intracellular compartment. ML-1 cells have very high whole-cell input resistance between 5–10 GΩ.

Cancellation of Whole-Cell Capacitance Transient

The transient capacitance current was cancelled by using the C-SLOW and G-SERIES controls. Adjustment of the C-SLOW control narrowed the transient current, and then a further adjustment of the G-SERIES control was usually satisfactory to cancel the transient capacitance current. In whole-cell experiments on ML-1 cells, the value for whole-cell capacitance was approximately 7 pF.

Solutions

Whole-cell patch clamp experiments were performed in asymmetrical K^+ solutions. The pipette solution (the intracellular solution) was composed of the following (in mM): 140 KCl, 2 Mg-ATP, 10 EGTA, and 10 HEPES (titrated with KOH to pH 7.2). The Ca^{2+} concentration was calculated by using a Ca^{2+}-selective electrode. The final Ca^{2+} concentration was titrated to 10 nM by addition of $CaCl_2$. For those experiments testing the effect of intracellular Ca^{2+} on the channel, the intracellular Ca^{2+} concentration was varied from 7 nM to 1 μM. The bath solution was composed of (in mM): 140 NaCl, 2 KCl (or varied KCl concentration isotonically replacing the NaCl for selectivity experiments), 1 $CaCl_2$, and 10 HEPES (adjusted with NaOH to pH 7.4). Ringer solution contained (in mM): 140 NaCl, 2.3 K_2HPO_4, 0.4 KH_2PO_4, 1.3 $CaCl_2$, 1.2 $MgCl_2$-$6H_2O$, 5 glucose, and 10 HEPES (pH 7.4); 12-O-tetradecanoylphorbol-13-acetate (TPA), 4-aminopyridine (4-AP), tetraethylammonium (TEA), and other reagents were freshly added into the solution shortly before each experiment.

Results

Voltage Dependence of the K^+ Current in ML-1 Cells

An outward K^+ current was identified in ML-1 cells (uninduced cells at the immature, proliferative stage). The activation of this current was voltage

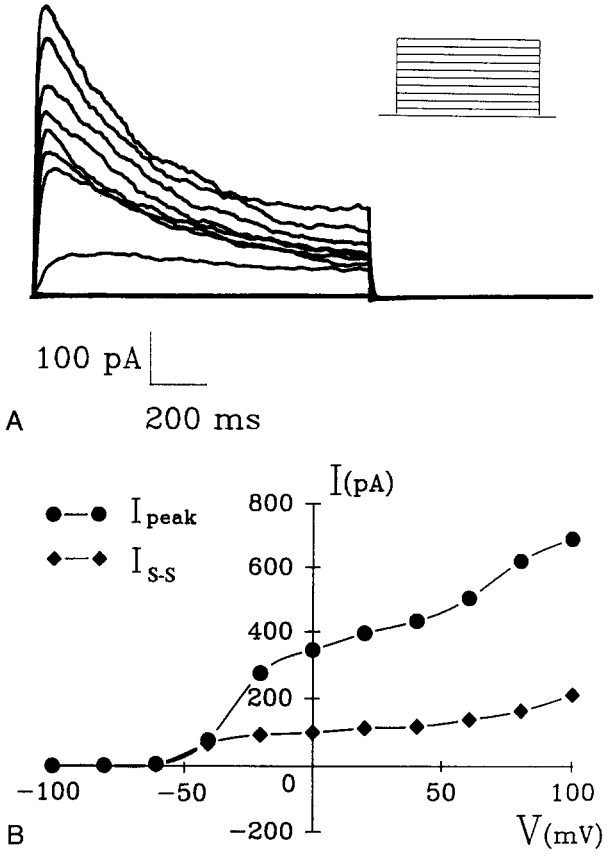

Fig. 1. Voltage activation of whole-cell currents in ML-1 cells. (*A*) Voltage-gated K^+ currents were evoked by the pulse protocol shown in the insert. This pulse protocol depolarized the membrane potential from a holding potential of -100 to $+100$ mV in 20-mV increments. (*B*) The amplitudes of the peak current (I_{peak}) and the steady-state current (I_{s-s}) were plotted as a function of the membrane potential. Peak current was measured at peak amplitude and steady-state current was measured 1,100 msec after the onset of activation.

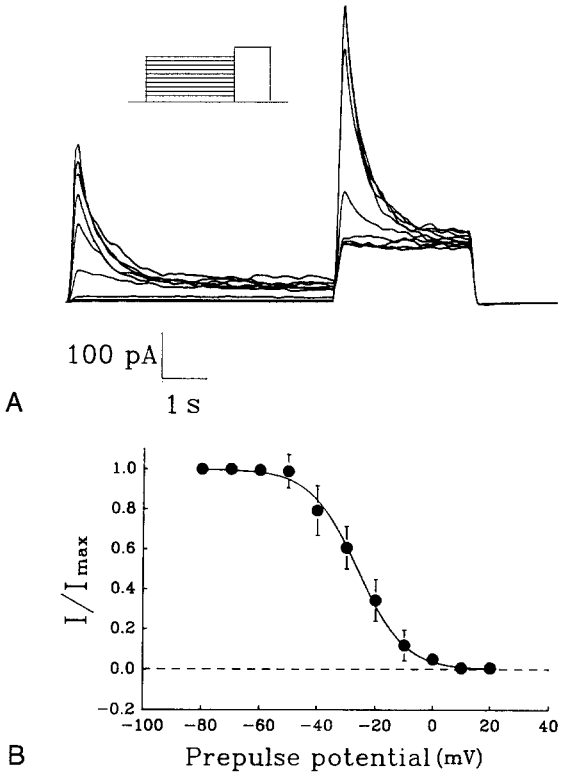

Fig. 2. Effects of prepulse depolarization on the inactivation of the whole-cell current in ML-1 cells (*A*) Whole-cell currents were elicited by applying the prepulse protocol shown in the insert. The membrane potential was held at -100 mV, and prepulse voltages of -80 to $+20$ mV in 10-mV increments were applied for 6,000 msec before each command depolarization to $+40$ mV. (*B*) Peak current in response to the command voltage was plotted as a function of prepulse potential. The points shown are the mean values ± SE of five experiments. The points were fitted by a Boltzmann distribution (Eq. 1).

dependent. Current traces recorded from a single suspended cell are shown in Fig. 1*A* The outward current was activated by depolarization of the membrane potential to -40 mV, increased current being observed upon depolarization to more positive membrane potentials. The current-voltage (*I-V*) relationship is shown in Fig. 1*B*. The average amplitude of the peak current upon depolarization to a membrane potential of $+60$ mV was 435 ± 58 pA (n = 46). The average cell capacitance was 7.5 ± 0.5 pF (n = 27). Intracellular Ca^{2+} concentrations ranging from 7 nM to 1 μM did not affect the peak current (n = 3, *data not shown*).

The voltage-dependent inactivation of this K^+ current was next characterized using prepulse experiments. The traces in Fig. 2*A* show the current activated by the prepulse voltage (the first amplitude) and by the command depolarization (the second amplitude). The peak current amplitude activated by the command depolarization was measured in the absence of prepulse voltage (I_{max}) and in the presence of prepulse voltage (I). The fractional peak current (I/I_{max}) was plotted as a function of prepulse voltage, as shown in Fig. 2*B*. The data points in Fig. 2*B* were fitted by a theoretical curve calculated from the Boltzmann distribution:

$$P(V) = I/I_{max} = [1 + e^{(V - V_{1/2})/\tau}]^{-1} \quad (1)$$

where $P(V)$ is the fraction of the voltage-dependent current remaining active following a prepulse to membrane potential V, $V_{1/2}$ is the prepulse voltage causing a 50% current inactivation, and τ is the slope per e-fold change of the prepulse voltage. Values obtained from the best fit of the Boltzmann distribution are -26 mV for $V_{1/2}$ and 8 mV for γ. Overall, the results in Figs. 1 and 2 demonstrate the presence

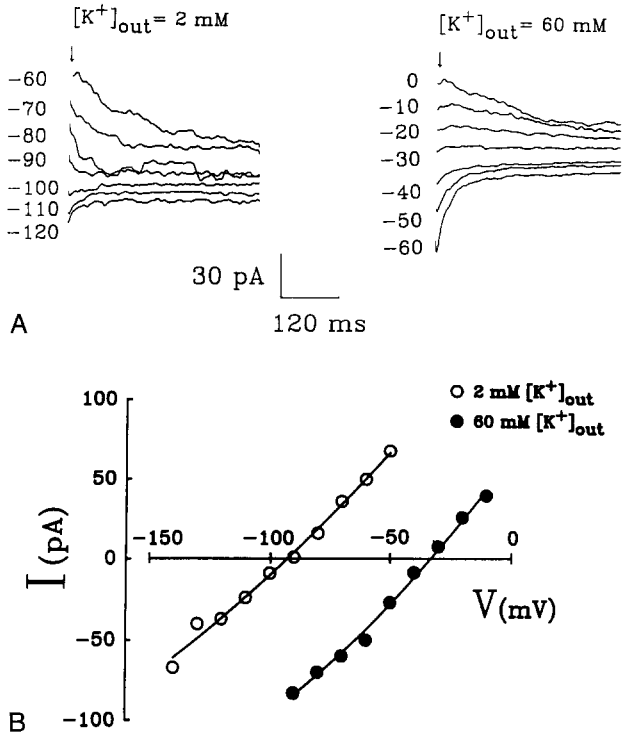

Fig. 3. Ion selectivity of the whole-cell current in ML-1 cells as determined by current relaxation recording reversal potentials. (A) Tail currents were recorded in different extracellular K^+ concentrations. The membrane potential was first depolarized to +40 mV from a holding potential of −100 mV for 100 msec; it was then repolarized to the voltages indicated at the left side of each current trace. (*B*) *I-V* relationships of the tail currents in panel *A*. The reversal potentials estimated from the *I-V* curves were −90 mV with an extracellular K^+ concentration of 2 mM and −32 mV with an extracellular K^+ concentration of 60 mM.

of an outward K^+ current that exhibits voltage dependence of both activation and inactivation in ML-1 cells (at an immature, proliferative stage).

Ion Selectivity of the K^+ Current in ML-1 Cells

The ion selectivity of the K^+ current in ML-1 cells was determined by relaxation potential experiments. Tail currents of the K^+ current were recorded in normal Ringer solution (K^+ concentration of 2 mM) and in a bath solution containing 60 mM K^+, as shown in Fig. 3*A*. The *I-V* relationships under these two different conditions were plotted, as shown in Fig. 3*B*. The reversal potential was between −90 to −100 mV when measured in Ringer solution. The reversal potential was shifted to between −30 to −40 mV when the extracellular K^+ concentration was increased to 60 mM. This represents a shift of approximately 60 mV toward the positive potential when extracellular Na^+ was substituted with K^+. Under the conditions of whole-cell patch clamp, the intracellular K^+ concentration is considered to be equivalent to that in the pipette solution (K^+ = 140 mM). The selective permeability of the K^+ channel to K^+/Na^+ ions can then be estimated using a function derived from the Goldman-Hodgkin-Katz equation:

$$\Delta V_{rev} = RT/ZFe\,\{([K]_{io} + P_{Na}/P_K[Na]_{io})/ ([K]_{xo} + P_{Na}/P_K[Na]_{xo})\} \quad (2)$$

where ΔV_{rev} is the change of the reversal potential when extracellular Na^+ is replaced with K^+ ions, Z is the valence, F is Faraday's constant, R is the gas constant, T is the absolute temperature, $[K]_{io}$ and $[Na]_{io}$ are the initial extracellular K^+ and Na^+ concentrations, and $[K]_{xo}$ and $[Na]_{xo}$ are the extracellular K^+ and Na^+ concentrations after the replacement of Na^+ with K^+ (Fig. 3). The permeability ratio (P_{Na}/P_K) of the K^+ channel in ML-1 cells was calculated to be 0.008. Thus, this channel appears highly selective for K^+ as compared to Na^+.

Blockade of the K^+ Current in ML-1 Cells

Various K^+ channel blockers were tested for their effects on the K^+ current in ML-1 cells. We found 4-AP to reduce the amplitude of this current. Perfusion of 100 μM 4-AP in the bath solution inhibited the K^+ current by 66% (± 4%, n = 4) at a membrane potential of −20 mV and by 55% (± 3%, n = 4) at +60 mV (Fig. 4*A*).

The fractional block of the K^+ current by 4-AP was calculated as ($1-I/I_0$), where I and I_0 are the amplitudes of the current measured in the presence (I) and in the absence (I_0) of the blocker. The data points in Fig. 4*A* were fitted by the dose-dependent saturation function:

$$I/I_0 = [B]/(K_i + [B]) \quad (3)$$

where [*B*] is the concentration of the blocker and K_i represents the concentration giving half-current inhibition (IC_{50}). The IC_{50} values calculated from the theoretical fitted curves were plotted as a function of the membrane potential as shown in Fig. 4*B*. These data indicate that the blocking effect of 4-AP is voltage dependent and is more pronounced at more negative membrane potentials. The slope per 100 mV change of the membrane potential is 80 μM. In contrast to 4-AP, neither barium (2 mM) nor TEA (2 mM) had a significant inhibitory effect on the K^+ current (*data not shown*).

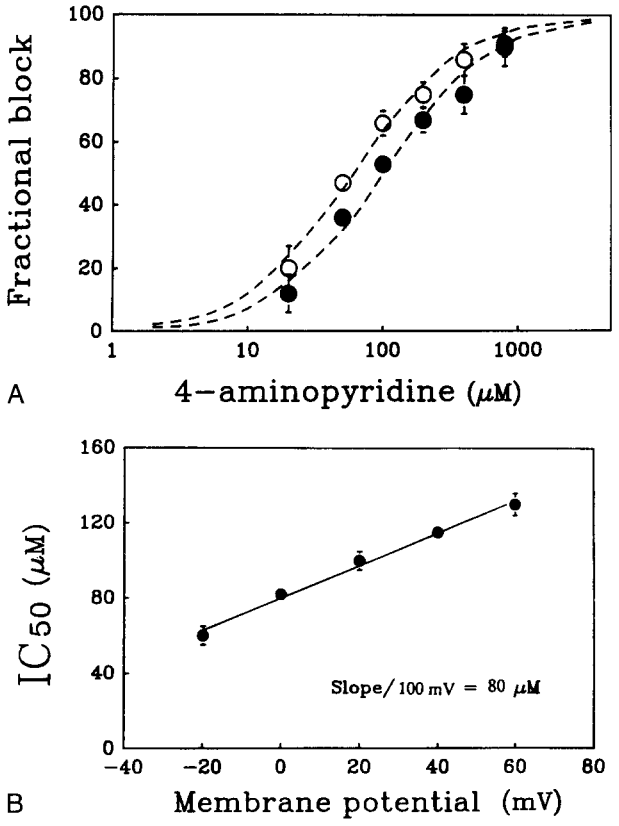

Fig. 4. Inhibition by 4-aminopyridine (4-AP) of the whole-cell current in ML-1 cells. (*A*) The fractional block of the K^+ current by external 4-AP was plotted as a function of 4-AP concentration at membrane potentials of −20 and +60 mV. Data were collected from four independent experiments and fitted with dashed curves calculated from Eq. (3). (*B*) The half-blocking concentration (IC_{50}) of 4-AP was plotted as a function of the membrane potential. The values for the IC_{50} were obtained from the theoretical curves calculated using Eq. (3).

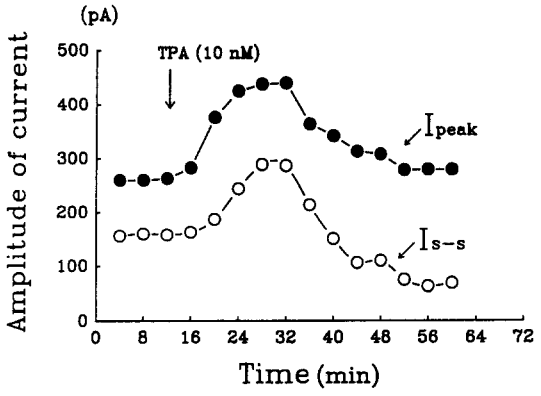

Fig. 5. Effect of TPA on the amplitude of the whole-cell current in ML-1 cells. Outward currents were continuously recorded by depolarizing the membrane potential from a holding potential of −100 mV to +40 mV with a time interval of 4 min. TPA (10 nM) was perfused into the extracellular solution at the time indicated by the arrow. The I_{peak} and the I_{s-s} were measured as described in the legend to Fig. 1.

Changes in the K^+ Current upon Addition of TPA during Whole-Cell Patch Clamp

The K^+ current in ML-1 cells was monitored upon direct addition of TPA to the bath solution during whole-cell recording, as shown in Fig. 5. The amplitude of the voltage-activated current (I_{peak}) increased upon initial addition of TPA. The increase occurred within 4 min and was maximal at about 20/30 min. The amplitude of the evoked current then declined to near baseline levels within 50 min (n = 7). The steady-state current (I_{s-s}) followed a similar time course, but declined to below baseline. This difference between I_{peak} and I_{s-s} suggested that the inactivation phase of the current was accelerated after the application of TPA. The voltage-activated current in control experiments (no TPA) did not change in amplitude during the 60-min observation period (neither I_{peak} nor I_{s-s}, *data not shown*).

Changes in the K^+ Current during TPA-Induced Differentiation of ML-1 Cells

Changes in the K^+ current were also examined during the course of differentiation induced by addition of TPA to ML-1 cells in tissue culture. After exposure to TPA in tissue culture, cells were transferred to the patch-clamp chamber to record the whole-cell current. Normalized K^+ currents, as shown in Fig. 6*A*, were recorded from uninduced cells, cells that were induced but not yet fully differentiated, and differentiated cells, and were plotted as a function of membrane potential in Fig. 6*B*. Voltage activation of the K^+ current was suppressed in cells exposed to TPA. The current amplitude was decreased at 7 hr after the addition of TPA, in agreement with the decrease in current amplitude seen at ~50 min after direct addition of TPA into the patch-clamp chamber (Fig. 5). The average amplitude of the K^+ current recorded from these TPA-induced cells was 300 ± 23 pA at a membrane potential of +40 mV (n = 9), which was 30% lower than the value from uninduced cells. The mean value of cell capacitance measured from the TPA-induced cells was increased by about 36% to 10.2 ± 0.7 pF (n = 17). The voltage-gated K^+ current was further reduced in fully differentiated cells. Cell capacitance was also additionally increased to 36.7 ± 3 pF (n = 7), in accord with the increase in the cell volume (Craig & Bloch, 1984; Craig et. al., 1984). Overall, these data show that TPA can have an initial stimulatory effect on the whole-cell K^+ current (Fig. 5), and that prolonged exposure results in a decline and then loss of channel activity (Figs. 5 and 6).

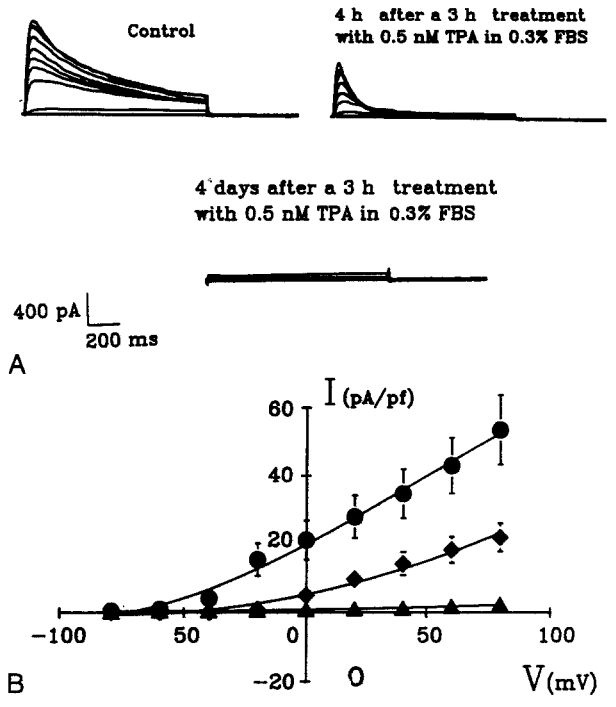

Fig. 6. Changes the in K^+ current during TPA-induced differentiation of ML-1 cells. (*A*) Voltage-gated K^+ currents were recorded from uninduced cells, and cells at various times after induction with TPA. (*B*) *I-V* relationships of these currents were plotted as filled circles for controls (n = 34), filled diamonds for cells at early stages of differentiation (n = 17) and filled triangles for differentiated cells (n = 7). All currents (in pA) were measured at the peak and normalized by dividing by cell capacitance in pF. The pulse protocol used in these experiments was the same as described in the legend to Fig. 1.

Inactivation of the K^+ current was also altered in differentiating cells. The inactivation of the K^+current was greatly accelerated after induction with TPA, as shown by the scale-match technique (Fig. 7*A*). Here, current traces recorded from the TPA-induced cell were enlarged 1.7-fold (labeled *b*) to match those from the control cell (labeled *a*). Time constants of inactivation were obtained by fitting the decay phase of the K^+ current with an exponential function:

$$P(t) = A \cdot e^{(-t/\tau_i)} \quad (4)$$

where τ_i is the inactivation time constant for the K^+ current. This inactivation time constant was plotted as a function of membrane potential, as shown in Fig. 7*B*. Curves representing induced (filled circles) and uninduced (open circles) cells showed the same pattern of voltage-dependent inactivation in the range of -20 to $+40$ mV, with the τ_i being faster at more positive membrane potentials in both cases. However, at every point, the τ_i in induced cells was about 10-fold faster than in uninduced cells. Thus, paralleling the results of addition of TPA to the bath during whole-cell recording, addition of TPA to cells in tissue culture appeared to affect both the activation and the inactivation of the K^+ current.

Discussion

We have identified a voltage-gated K^+ current in myeloblastic ML-1 cells and have found both activation and inactivation of this current to be altered by the differentiation-inducer TPA. Activation of the K^+ current is affected in a biphasic manner: Current amplitude initially increases and then decreases within one hour after the direct application of TPA during whole-cell recording. Current amplitude also decreases in the early stages of differentiation induced by several hours of exposure to TPA in tissue culture. These early decreases in current amplitude are accompanied by an acceleration of inactivation. At later times, for example in the TPA-induced monocyte/macrophages that appear after several days, the current is essentially completely suppressed.

The biphasic effects of TPA on K^+ channel activation are reminiscent of the fact that it initially stimulates, and then downregulates, PKC activity in ML-1 cells (Guan et al., 1989). The alterations in the K^+ current could thus be related to changes induced by PKC, such as changes in protein phosphorylation. Phosphorylation by PKC, or by cAMP-dependent protein kinase (PKA), is known to regulate a variety of ion channels. PKA regulates L-type Ca^{2+} and K^+ channels in pre-B cells, myocytes, and neurons (Bean, Nowycky & Tsien, 1984; Choquet et al., 1987; Tsien et al., 1988; Walsh & Kass, 1988); both PKA and PKC regulate a Cl^- channel in epithelial cells (Li et al., 1988; Hwang et al., 1989). In the case of this Cl^- channel, phosphorylation results in channel activation; this is similar to our finding that direct exposure of ML-1 cells to TPA results initially in K^+ channel activation. In the case of the amiloride-sensitive Na^+ channel in kidney epithelial cells, in contrast, phosphorylation results in a reduction of opening probability (Ling & Eaton, 1989); this is similar to our finding that prolonged exposure of ML-1 cells to TPA results in a suppression of K^+ channel activity.

The K^+ current in ML-1 cells is similar to the type n K^+ channels in T lymphocytes and pre-B cells (DeCoursey et al., 1984; Choquet et al., 1987). Like these channels in lymphocytes and the *Drosophila Shaker* K^+ channel, the current in ML-1 is sensitive to 4-AP, but it is less sensitive to TEA or Ba^{2+}. The K^+ channels in lymphocytes are influenced by mitogens, as well as by 5-hydroxytrypto-

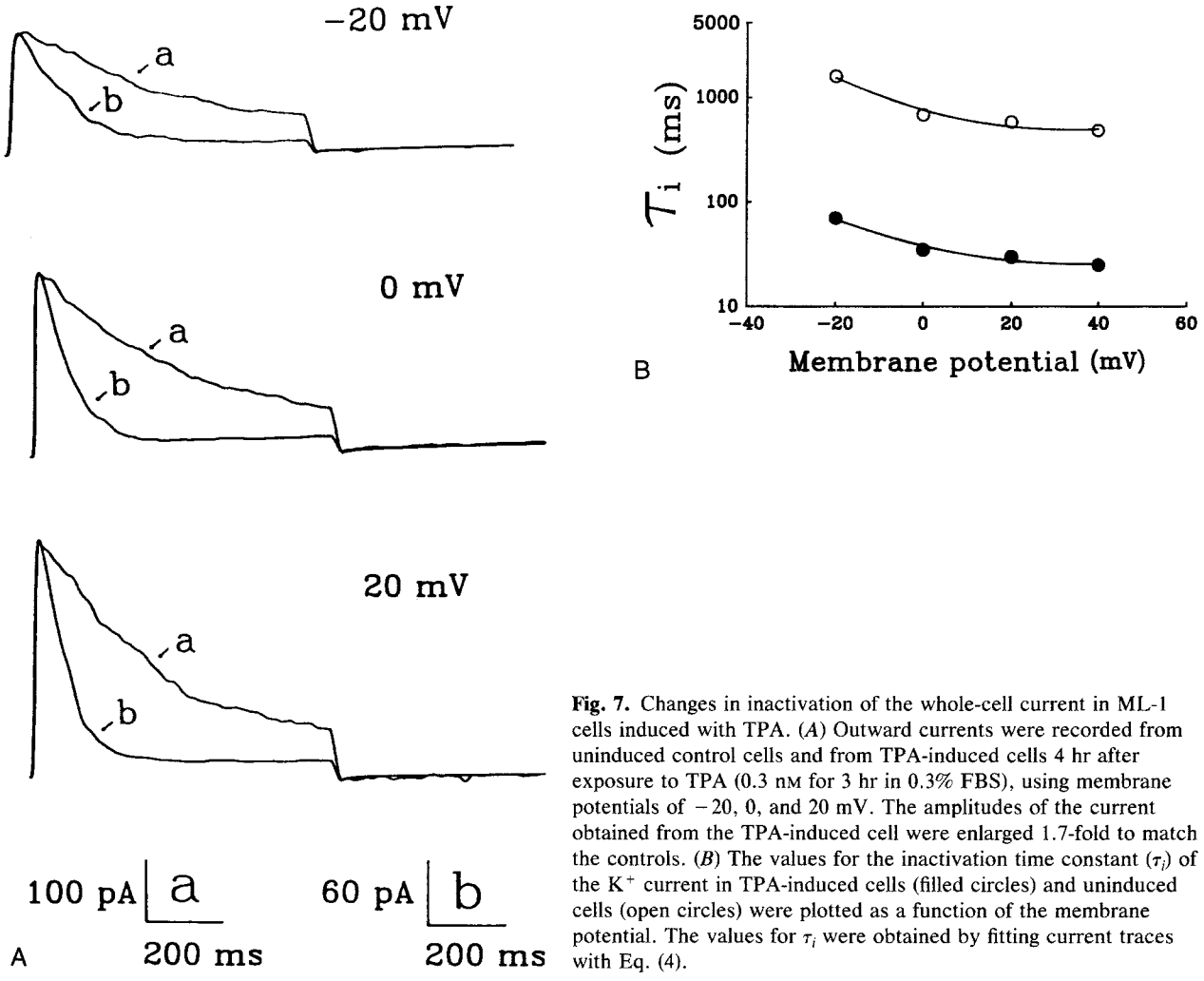

Fig. 7. Changes in inactivation of the whole-cell current in ML-1 cells induced with TPA. (*A*) Outward currents were recorded from uninduced control cells and from TPA-induced cells 4 hr after exposure to TPA (0.3 nM for 3 hr in 0.3% FBS), using membrane potentials of −20, 0, and 20 mV. The amplitudes of the current obtained from the TPA-induced cell were enlarged 1.7-fold to match the controls. (*B*) The values for the inactivation time constant (τ_i) of the K^+ current in TPA-induced cells (filled circles) and uninduced cells (open circles) were plotted as a function of the membrane potential. The values for τ_i were obtained by fitting current traces with Eq. (4).

phan (Choquet & Korn, 1988), substance P (Schumann & Gardner, 1989), intracellular pH (Deutsch & Lee, 1989) and Ca^{2+} (Grissmer & Calahan, 1989). For example, these K^+ channels are activated during the mitogen-induced stimulation of lymphocyte proliferation (Matteson & Deutsch, 1984; Ypey & Clapham, 1984; Amigorena et al., 1986; Lee et al., 1986). Addition of K^+ blockers to suppress channel activation results in an inhibition of mitogenesis (Decoursey et al., 1984; Amigorena et al., 1990); this suggests that channel activation might participate in the stimulation of proliferation. Other studies of this K^+ current in lymphoid cells suggest that the channel might participate in volume regulation (Lee et al., 1988; Deutsch & Lee, 1989). Our results show that the K^+ current in myeloid ML-1 cells is influenced by TPA and is suppressed during the induction of cell differentiation. Future studies will be aimed at determining to what extent, and through what mechanisms, these changes in the K^+ current participate in the transduction of the signal for induction of differentiation and loss of proliferation.

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