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Incomplete Inactivation of Voltage-dependent K^+ Channels in Human B Lymphoma Cells

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Abstract. The voltage-dependent K (K_V) channel in Daudi human B lymphoma cells was characterized by using patch-clamp techniques. Whole-cell voltage-clamp experiments demonstrated that cell membrane depolarization induced a transient (time-dependent) outward current followed by a steady-state (time-independent) component. The time-dependent current resembled behavior of the type *n* channel, such as use dependence and a unique blockade by tetraethylammonium (TEA). Both time-dependent and time-independent currents were blocked by quinine with a similar IC_{50} (14.2 μM and 12.6 μM). Treatment with antisense oligonucleotide of human Kv1.3 gene significantly reduced both currents by 80%. Single-channel experiments showed that only one type of K_V channel was recorded with a unitary conductance of approximately 19 pS. Consistent with whole-cell recordings, the channel activity in cell-attached patches remained in response to prolonged depolarization, and the remaining channel activity was blocked by quinine, but not TEA. Channel activity was scarcely seen in cell-attached patches after antisense treatment. Whole-cell current-clamp data showed that TEA, which blocks only the time-dependent current, caused a slight decrease in the membrane potential. In contrast, quinine and antisense, which block both time-dependent and -independent currents, strongly reduced the membrane potential. These data together suggest that the K_V channel in Daudi cells does not completely inactivate and that the remaining channel activity due to this incomplete inactivation appears to be primarily responsible for maintaining the membrane potential.

Key words: Daudi cells — Patch-clamp techniques — Type *n* channels — Tetraethylammonium — Quinine — Cell membrane potential

Introduction

Voltage-dependent potassium (K_V) channels were initially considered to be unique ion channels expressed in excitable cells. Surprisingly, studies during the past two decades have demonstrated that K_V channels are also expressed in nonexcitable lymphocytes. Three types of K_V channels named type *n*, *n′*, and *l* were characterized in T lymphocytes by using patch-clamp techniques (Decoursey et al., 1987; Grissmer et al., 1992; Lewis & Cahalan, 1988a; 1988b). A gene encoding the type *n* channel has been cloned and classified as Kv1.3 of the *shaker* superfamily. Its mRNA has been expressed in *Xenopus* oocytes, producing a functional K_V channel that resembles the native type *n* channel in lymphocytes (Attali et al., 1992; Cai et al., 1992; Douglass et al., 1990; Grissmer et al., 1990). The molecular structure of this gene product has been described, and appears to be a homotetramer of Kv1.3 subunits (Cahalan & Chandy, 1997). The type *n* channel appears to be also expressed in B lymphocytes (Sutro et al., 1989; McCann, McCarthy & Noelle, 1990). However, whether Daudi human B lymphoma cells express the type *n* channel and how the channel behaves remain to be determined.

Two types of evidence suggest that the type *n* K_V channel plays an important role in regulating lymphocyte proliferation. First, both T and B lymphocyte proliferation is attenuated after blockade of K_V channels (DeCoursey et al., 1984; Amigorena et al., 1990b; Wang et al., 1992; Kalman et al., 1998). Sec-

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Abbreviations: K_V channels, voltage-dependent potassium channels; TEA, tetraethylammonium; HEPES, *N*-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N′*,*N′*-tetraacetic acid; E_K, the equilibrium potential of potassium; P_K/P_{Na}, the permeability to potassium versus sodium; P_o, open probability

ond, the density of K_V channels in T lymphocytes is increased following mitogenic stimulation (Attali et al., 1992; Cai et al., 1992). K_V channel activity is significantly enhanced in B lymphocytes after stimulation with the bacterial-derived mitogen, LPS (Amigorena et al., 1990a). Recent studies have demonstrated that K_V channel activity is suppressed by stimulation of the apoptotic receptor, APO-1/Fas (CD95) (Szabo et al., 1996). The roles of K_V channels in cell proliferation and apoptosis lead to a hypothesis that K_V channel activity might be elevated in malignant lymphocytes. An understanding of the role K_V channels may play in cell proliferation and apoptosis is expected to provide important information for malignant transformation and therapeutic strategies. Therefore, we have chosen the Daudi cell line as a cell model to examine how K_V channels behave in malignant B cells. The present study shows that a K_V channel is highly expressed in Daudi cells. A full characterization with both patch-clamp techniques and antisense methodology demonstrates that this channel belongs to the Kv1.3 gene product and does not fully inactivate in response to prolonged depolarization. The remaining channel activity resulting from this incomplete inactivation appears to be more important for maintaining the membrane potential than for the transient channel activity.

Materials and Methods

Cell Culture

Daudi Human B lymphoma cells were purchased from American Type Culture Collection. The cells were continuously cultured in RPMI 1640 media (Gibco BRL Co.) with 10% fetal bovine serum (Gibco BRL Co.) in the incubator at 37°C with a constant CO_2 of 5%.

Patch-Clamp Techniques

Immediately before use, Daudi cells were thoroughly washed with NaCl bath solution (*see below*) and transferred into the patch chamber mounted in the stage of a Leitz inverted microscope. Whole-cell and cell-attached configurations were established with polished micropipettes with a tip resistance of 2.5–5 MΩ. Only the patches with seal resistance above 10 GΩ were used for the experiments. In whole-cell recordings, the voltage-clamp mode was used for monitoring changes in current and the current-clamp mode was used for monitoring changes in the membrane potential. A voltage-step protocol from −100 mV to +60 mV at a holding potential of −60 mV in increments of 20 mV was used. The voltage pulses were separated by an interval of either 30 sec or 1 sec to test use-dependence of the lymphocyte K_V channel. For the special purpose of testing a putative incomplete inactivation, a protocol with duration of 30 sec was used in some experiments. A voltage-ramp protocol from −120 mV to +60 mV at a holding potential of −60 mV was also used to quickly get the current-voltage relationship. After the characterization of macroscopic currents by whole-cell recordings, single-channel analysis was also accomplished in the cell-attached mode. Whole-cell currents filtered at 3 kHz and single-channel events filtered at 1 kHz were recorded on video tape with a modified Sony PCM video converter (Vetter Instruments). Prior to digitization with pClamp 6 software (Axon Instruments), whole-cell current records were filtered at 1 kHz and single-channel events were filtered at 0.5 kHz. For cell-attached experiments, P_o, the open probability, was used to represent the channel activity. P_o was calculated from single-channel records of 2-min duration using pClamp 6 software, as previously described (Ma & Ling, 1996).

Preparation and Use of Antisense Oligonucleotide of Human Kv1.3 Channel

A 25-mer antisense oligonucleotide directed against the first 25 nucleotides of human Kv1.3 transcript beginning with the initiation codon AUG was selected. A search of the Gene Bank revealed only limited similarity of these 25-mers with sequences of other K^+ channels. Using this sequence, phosphothiorate antisense oligonucleotide was synthesized for us by Research Genetics Inc. The corresponding sense oligonucleotide was also synthesized to serve as a control. The lipid transfection agent, FuGene, was used to accelerate the uptake of oligonucleotides by cells. Cells were pretreated with serum-free medium containing 1 μM either sense or antisense oligonucleotides against the Kv1.3 channel. Patch-clamp experiments were performed in the cells with similar size during the period from 24 hr to 48 hr after the treatment.

Chemicals and Solutions

Most chemicals were obtained from Sigma (St. Louis, MO). CTX and quinine were purchased from Research Biochemicals International Co. NaCl bath solution for whole-cell and cell-attached experiments contained (in mM): 145 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, at a pH of 7.4. TEA bath solution contained (in mM): 140 (or 10, 25, 50) TEA-Cl, 5 (or 135, 120, 95) NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, at a pH of 7.4. KCl bath solution contained (in mM): 140 KCl, 10 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, at a pH of 7.4. Pipette solution for whole-cell recordings contained (in mM): 140 KCl, 1 $MgCl_2$, 5 ATP-Na_2, 10 HEPES and 50 nM free Ca^{2+} (after titration with 2 mM EGTA), at a pH of 7.2. For cell-attached recordings, NaCl bath solution without or with K^+ channel blockers was used for filling patch pipettes.

Statistical Analysis

Since both whole-cell current and single-channel activity are variable from patch to patch, paired *t* tests or analyses of variance for multiple comparisons were used for statistical analysis. In most cases, comparisons were made in the same patch before and after experimental manipulations.

Results

Time-dependent and -independent K_V Currents in Daudi Human B Lymphoma Cells

Whole-cell recordings demonstrated that cell membrane depolarization induced a family of outward currents with a transient (time-dependent) component, followed by a steady-state (time-independent) component (Fig. 1*A*). The voltage-step protocol was

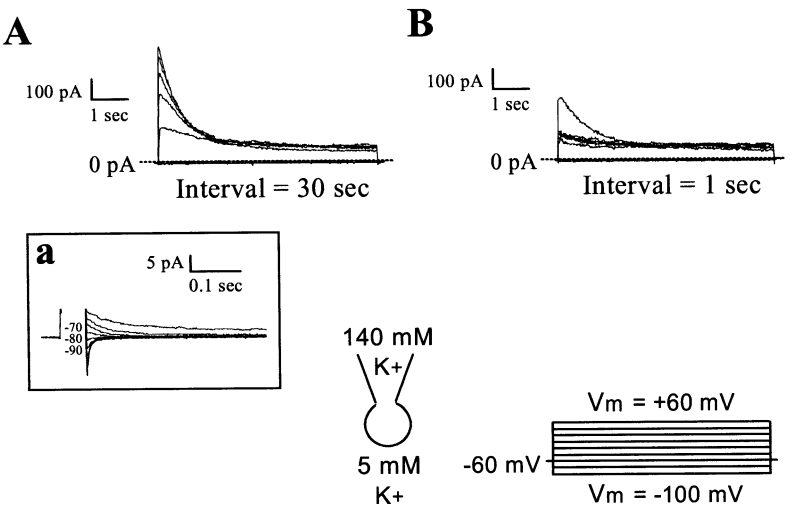

Fig. 1. Time-dependent and time-independent outward currents in Daudi cells. (*A*) A family of whole-cell outward currents was induced by a voltage-step protocol with an interval of 30 sec. A time-dependent current was followed by a time-independent component. (*B*) The time-dependent current was recorded when the cell membrane was depolarized to −20 mV. The traces induced by subsequent voltage steps (0, +20, +40 and +60 mV) were superimposed when the voltage-steps were separated by an interval of 1 sec. *Inset panel a*. Tail-current analysis shows that the reversal potential of the time-dependent current is approximately −80 mV. To eliminate involvement of Ca^{2+}-activated K^+ channels, intracellular free Ca^{2+} was titrated with EGTA in the pipette solution to a concentration of 50 nM, which is much below the threshold for activating Ca^{2+}-activated K^+ channels.

designed to have a relatively long duration of 6 sec to allow simultaneous study of both components. The time-dependent component was cumulatively inactivated by subsequent voltage steps separated by an interval of 1 sec (Fig. 1*B*), which is consistent with the use-dependent behavior of the type *n* K^+ channel, as previously demonstrated in both T and B lymphocytes. Tail-current analysis revealed that the reversal potential of the time-dependent current was approximately −80 mV (inset panel *a*), which is close to the potassium equilibrium potential (E_K) of −84 mV calculated by the Nernst equation according to the concentrations of bath and pipette K^+. Ion substitution experiments were performed as shown in Fig. 2*A*. The replacement of 140 mM Na^+ in the bath with 140 mM K^+ shifted the reversal potential of either time-dependent or -independent currents to 0 mV, suggesting that both of them are carried by K^+ (Fig. 2*B*). The threshold potentials for activating the time-dependent and -independent components were similar, namely approximately −40 mV. The current-voltage relationship was also achieved by using a voltage-ramp protocol (Fig. 2*C*). When a pipette solution contained (in mM) 140 KCl and 10 NaCl and a bath solution contained (in mM) 50 KCl and 100 NaCl, the reversal potentials of these two currents appeared to be similar and less than −25 mV (*data not shown*). The permeability to potassium versus sodium (P_K/P_{Na}) was calculated using the Goldman-Hodgkin-Katz equation. The P_K/P_{Na} was greater than 50:1.

"Crossover" Blockade by TEA and Parallel Blockade by Quinine

Fig. 3*A* and *B* showed that 25 or 140 mM TEA in the bath reversibly reduced the time-dependent current. TEA, as an open channel blocker, blocks K_V channels in human T lymphocytes. Since TEA also prevents the inactivation of the channels, the whole-cell current trace after application of TEA crossed over the control current trace (Grissmer & Cahalan, 1989). This unique "crossover" blockade was also seen in Daudi cells when the membrane potential was depolarized to +60 mV (Fig. 3*b1*). However, the "crossover" was not seen when the membrane potential was depolarized to a lower level of +20 mV (Fig. 3*b2*), indicating that prevention of channel inactivation by TEA appears to be dependent of the voltage across the membrane. Inhibition of the time-dependent current by TEA was dose-dependent and had an IC_{50} of 46.9 mM (Fig. 3*C*). When the bath contained 10, 25, 50, or 140 mM TEA, the peak amplitude of the time-dependent current was reduced by 13%, 39% ($P < 0.05$; $n = 5$), 69% ($P < 0.05$; $n = 5$), or 77% ($P < 0.01$; $n = 12$), respectively. Conversely, the amplitude of the time-independent current was not significantly changed after application of TEA to the bath. The slight increase in the time-independent current was observed, presumably resulting from the effect of TEA on the rate of channel inactivation. In contrast to TEA, application of either 10 or 100 quinine (in μM) to the bath reversibly inhibited both time-dependent and time-independent currents (Fig. 4*A* and *B*). Inhibition of these two currents was dose-dependent and had a similar IC_{50}, which is 14.2 μM for the time-dependent current and 12.6 μM for the time-independent current (Fig. 4*C*). Application of 1, 10, 30, or 100 μM quinine to the bath decreased time-dependent current by 5%, 33% ($P < 0.01$; $n = 6$), 75% ($P < 0.01$; $n = 5$), or 90% ($P < 0.001$; $n = 8$) and time-independent current by 7%, 39% ($P < 0.01$; $n = 6$), 81% ($P < 0.01$; $n = 5$), or 93% ($P < 0.001$; $n = 8$), respectively. These results indicate that the time-independent current may represent incomplete inactivation of time-dependent K^+ current.

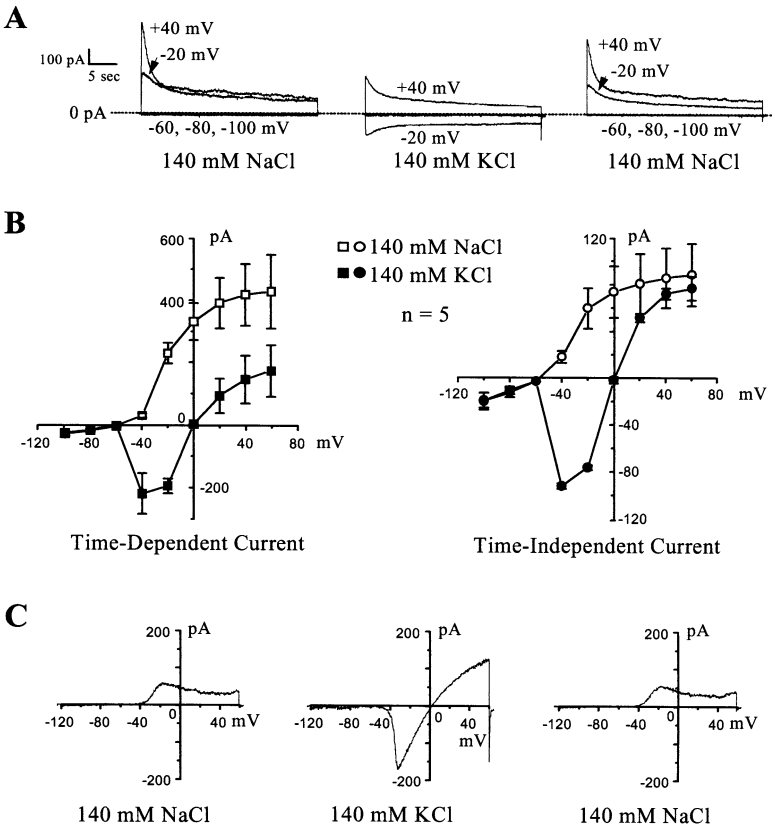

Fig. 2. A parallel shift of reversal potential of time-dependent and time-independent currents. (*A*) Representative whole-cell current traces show that replacement of NaCl bath solution with KCl bath solution in the patch chamber reversibly shifted the reversal potential of both time-dependent and time-independent currents. (*B*) Summary plots of current-voltage relationship of both currents before and after the substitution of Na^+ for K^+. The time-dependent and -independent currents in response to a voltage-step protocol were measured at peak points and at 4000 msec, respectively. The time-dependent current represents the peak amplitude, subtracted by the amplitude of the time-independent current at 4000 msec. (*C*) A shift of the reversal potential after the substitution of Na^+ for K^+ was shown by a voltage-ramp protocol.

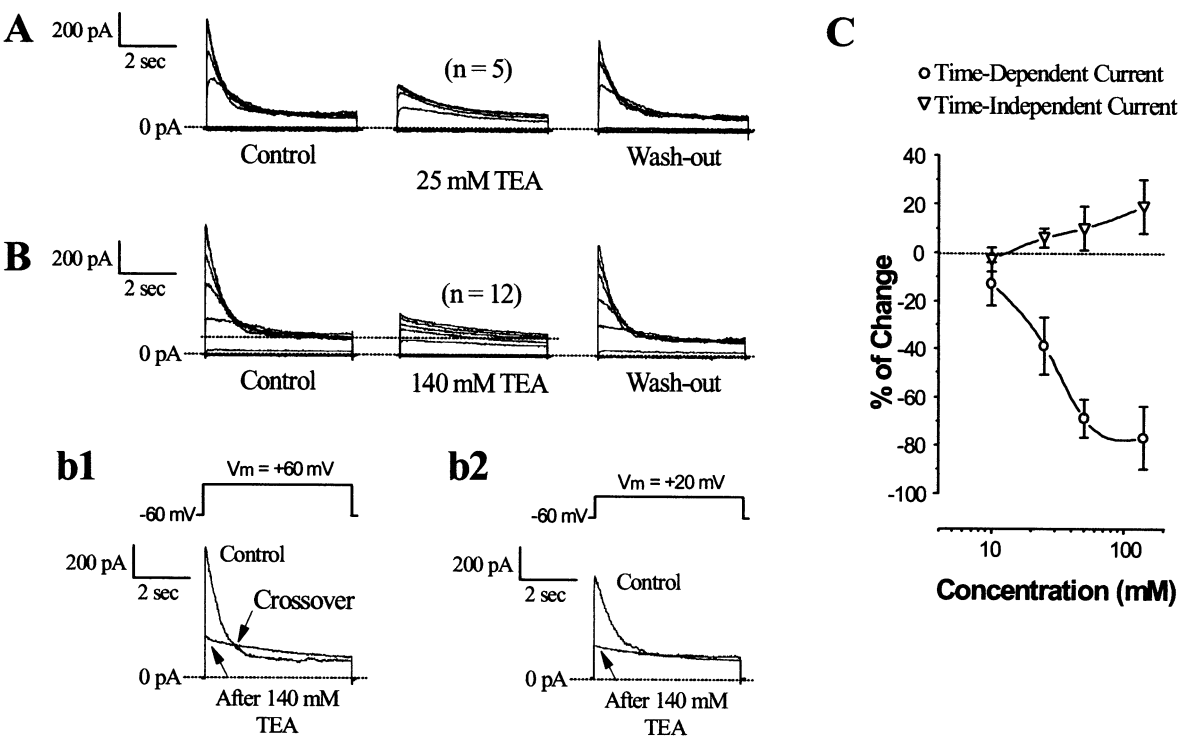

Fig. 3. Blockade of time-dependent current by TEA. Replacement of NaCl bath solution with TEA bath solution containing either 25 mM (*A*) or 140 mM TEA (*B*) in the patch chamber reversibly reduced the time-dependent current. The time-independent current in response to a voltage pulse of +60 mV was slightly enhanced when the bath contained 140 mM TEA, leading to a "crossover" blockade (*b1*). However, no enhancement was observed when using a voltage pulse of +20 mV (*b2*). (*C*) Summary plot of % changes in both currents (at +60 mV) when bath contained 10, 25, 50, or 140 TEA (in mM).

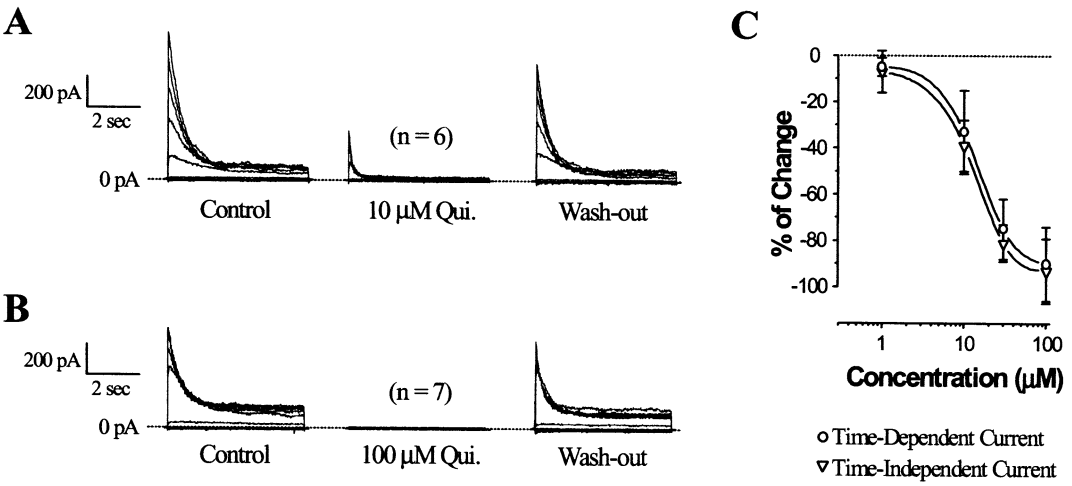

Fig. 4. Blockade of time-dependent and time-independent currents by quinine. Application of either 10 μM (*A*) for 100 μM quinine (*B*) to the bath reversibly reduced both time-dependent and time-independent currents. (*C*) Summary plot of % changes in both currents (at +60 mV) after addition of 1, 10, 30, or 100 quinine (in μM) to the bath. The concentrations that cause 50% inhibition (IC_{50}) are 14.2 μM for the time-dependent current and 12.6 μM for the time-independent current.

Parallel Inhibition of Time-dependent and -independent K^+ Currents by Antisense Oligonucleotide against the Kv1.3 Gene

To test whether time-dependent and -independent K^+ currents represent the channel activity from the Kv1.3 channel, Daudi cells were pretreated with antisense oligonucleotide against the Kv1.3 channel. In contrast to the current under control conditions, K_V currents in Daudi cells were significantly reduced after the antisense treatment: both time-dependent and -independent currents were reduced in parallel by approximately 80%. However, treatment with sense oligonucleotide did not show any significant effect on the K_V current (Fig. 5*A* and *B*). These data suggest that the time-dependent and -independent currents represent a single type of K_V channel, which is the Kv1.3 channel.

Single-Channel Recordings of the K_V Channel in Daudi Cells

To further eliminate the possibility that the time-independent current arises from another K_V channel subtype, single-channel analysis was performed. Cell-attached experiments demonstrated that the time-dependent K_V channel appeared to have an inefficient inactivation in response to prolonged membrane depolarization (Fig. 6*A*). A summation of 41 responses induced by a depolarizing voltage pulse in 11 cell-attached patches showed that the summarized single-channel current resembled the macroscopic current observed by whole-cell recordings (Fig. 6*B*). These data further suggest that the lack of inactivation accounts for the time-independent component observed in the above whole-cell experiments. If the K_V channel in Daudi cells does not fully inactivate, the channel should be detected in continuous recordings, which was confirmed by the data shown in Fig. 7. The K_V channel events in the same cell-attached patch of "Cell 1" in Fig. 6*A* were continuously recorded at different applied pipette potentials (Fig. 7*A*). When the patch pipette was filled with KCl bath solution, the channel currents were reversed (inset panel *a*), which is consistent with the results from ion substitution experiments performed in the whole-cell configuration. The unitary conductance is approximately 19 pS at the range between +40 and +80 mV (Fig. 7*B*), which is consistent with a previous report in normal human B lymphocyte (Partiseti et al., 1992). To confirm that the sustained channel activity results from an incomplete inactivation of the Kv1.3 channel, the sensitivity to K^+ channel blockers and antisense treatment was examined. In comparison with control conditions without any K^+ channel blockers in the patch pipette (first trace in Fig. 7*C*), the remaining K_V channel activity in response to a constant depolarization ($-V_{pipette}$ = +80 mV) was not significantly changed when patch pipettes contained 140 mM TEA (second trace in Fig. 7*C*). In contrast to the effect of TEA, channel activity was significantly reduced when patch pipettes contained 100 μM quinine (third trace in Fig. 7*C*). Furthermore, channel activity was scarcely seen in cell-attached patches after the cells were pretreated with antisense oligonucleotide of the Kv1.3 gene (fourth trace in Fig. 7*C*). However, sense oligonucleotide did not affect the channel expression (fifth trace in Fig. 7*C*). The mean P_o was also compared between each group of experiments (Fig. 7*D*). When patch

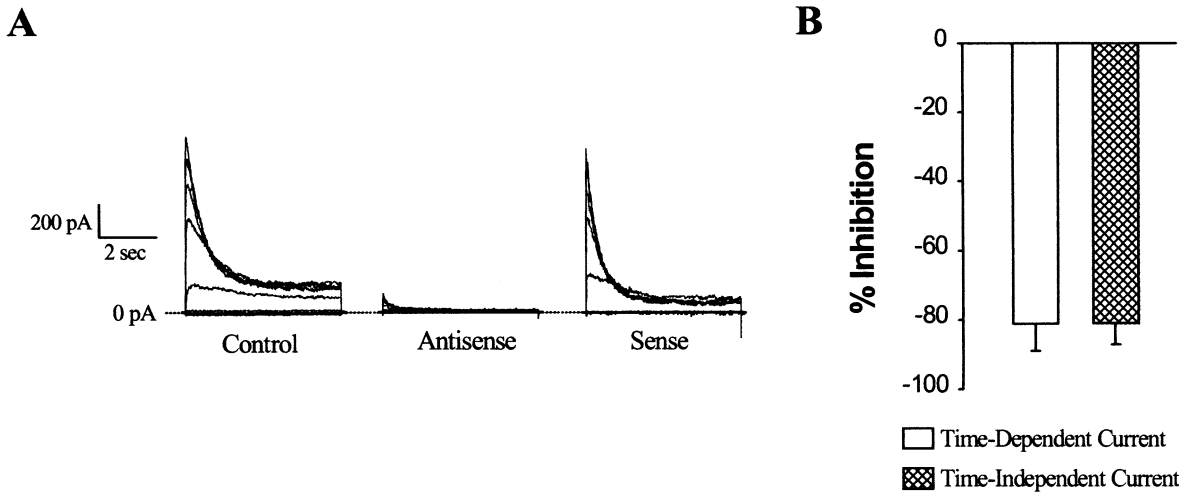

Fig. 5. K_V currents under control conditions and after treatment with antisense or sense oligonucleotide of the Kv1.3 channel. (*A*) Representative traces of whole-cell currents. (*B*) Percent inhibition of time-dependent and -independent currents by antisense oligonucleotide.

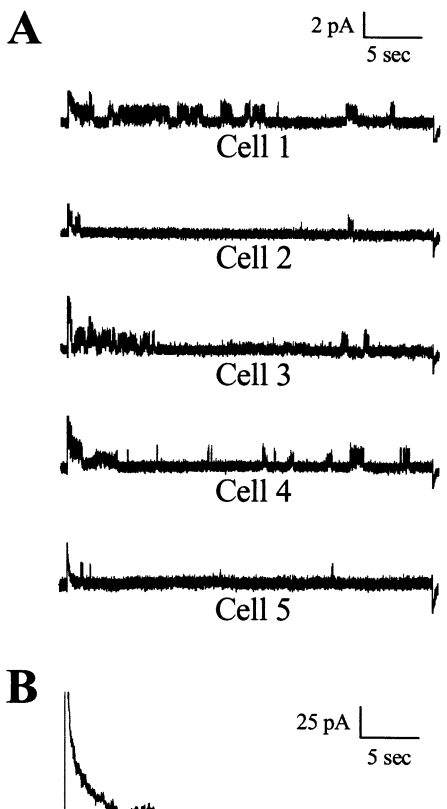

Fig. 6. Single-channel recordings of a K_V channel in Daudi cells. A voltage-step protocol was used, in which pipette potential was stepped down to −80 mV from a holding pipette potential of 0 mV and was clamped for 30 sec. (*A*) Each trace represents a cell-attached recording from an individual cell. (*B*) Summary plot of 41 responses induced by the voltage-step protocol from 11 cells (including from the 5 cells shown in *A*). Upward single-channel events represent channel openings, indicating K^+ efflux. The traces were filtered at a corner frequency of 500 Hz.

pipettes contained 140 mM TEA, the P_o was not significantly changed (0.11 ± 0.08 vs. 0.13 ± 0.07). When patch pipettes contained 100 μM quinine, the P_o was decreased, from 0.11 ± 0.08 to 0.02 ± 0.03 ($P < 0.01$). After treatment with antisense oligonucleotide, only 1 out of 10 cell-attached patches contained channel activity with a P_o as observed under control conditions. The mean P_o (0.02 ± 0.07) of these 10 patches was significantly lower than the control level (0.11 ± 0.08) ($P < 0.01$), indicating that channel density was reduced. These single-channel data confirmed that the time-independent whole-cell current represents remaining activity of a single type of K_V channel, which is the Kv1.3 channel.

Effects of K^+ Channel Blockers and Antisense Oligonucleotide on the Membrane Potential

Since the remaining K_V channel activity provides a constant pathway for K^+ efflux, we hypothesized that the defect inactivation of the K_V channel might play an important role in maintaining lymphocyte membrane potential. To test this hypothesis, the membrane potential was simply monitored by the current-clamp mode under the whole-cell configuration. After patch formation, the membrane potential (MP) was gradually decreased from an initial level of −57 ± 9 mV ($n = 20$). Within 5 min MP reached a steady level of approximately −40 mV, and was maintained at this level for more than 1 hr. All the following experiments were performed after MP had reached the steady level. 140 mM TEA in the bath slightly reduced the membrane potential from −43 ± 5 mV to −36 ± 10 mV ($P < 0.01$; $n = 20$). However, the membrane potential was strongly decreased by application of 100 μM quinine, from −44 ± 6 mV to

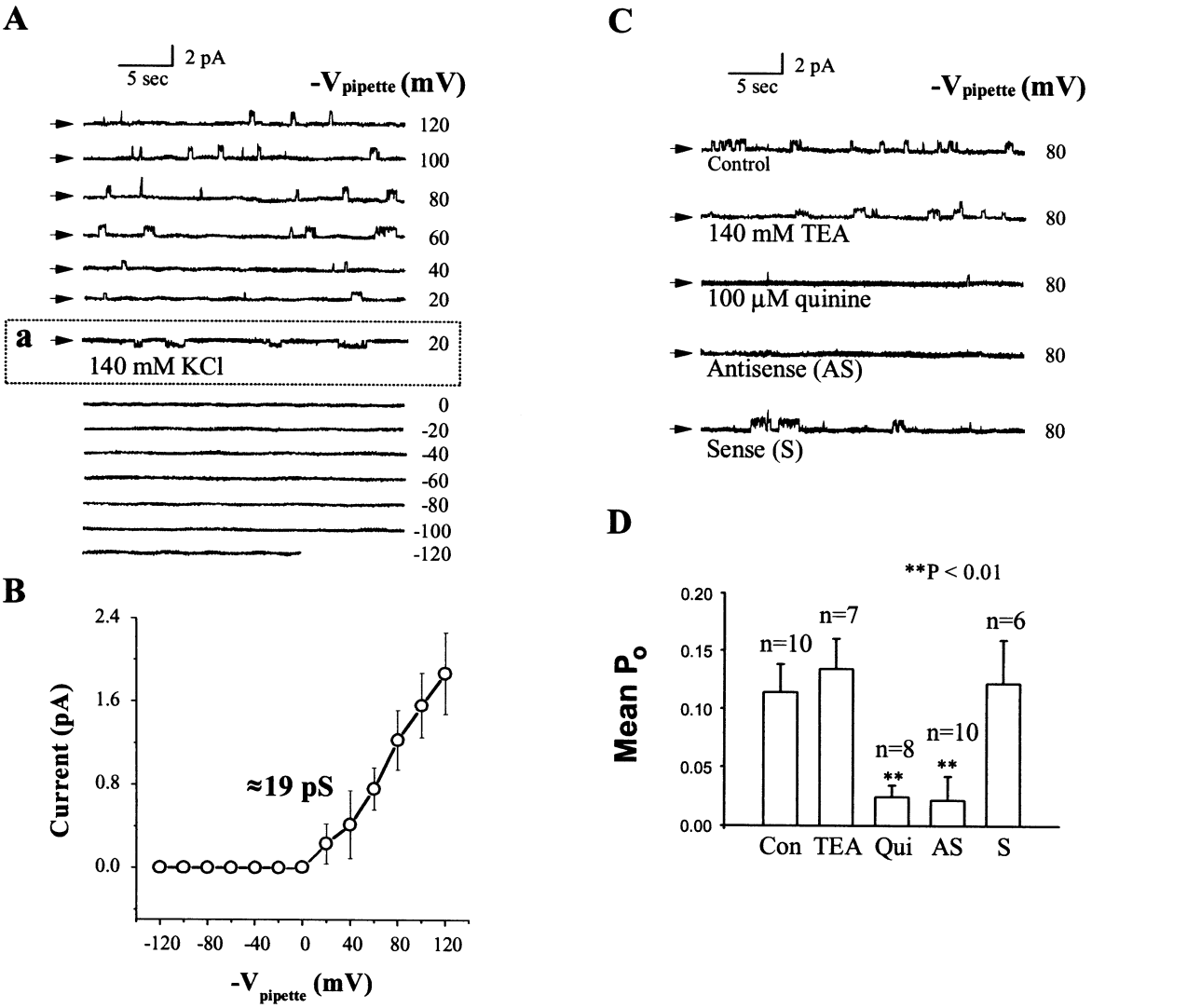

Fig. 7. Continuous cell-attached recordings of the K_V channel. (*A*) Single-channel events versus different pipette potentials in the same cell-attached patch of "Cell 1" shown in Fig. 5*A*. Inset panel *a* shows the reversed channel events when the patch pipette contained KCl bath solution. (*B*) Summary plot of current-voltage relationship, which reveals that the unitary conductance was 19 pS ($n = 7$). (*C*) Representative current traces without (control) and with either 140 mM was TEA or 100 μM quinine in patch pipettes or when cells were pretreated with antisense or sense oligonucleotides. (*D*) Summary plots of P_o under above conditions. Traces were filtered at 100 Hz for display, however, P_o was calculated at a corner frequency of 500 Hz. Arrows indicate the state that channels are closed. Upward single-channel events represent channel openings, indicating K^+ efflux.

-7 ± 2 mV ($P \ll 0.0001$; $n = 11$), and reversed to -46 ± 8 mV by washing quinine out of the bath. Treatment of cells with antisense oligonucleotide reduced the membrane potential from -40 ± 3 mV to -11 ± 5 mV ($P \ll 0.0001$; $n = 8$). In contrast, sense oligonucleotide did not change the membrane potential (Fig. 8*A*). Furthermore, quinine reduced the membrane potential with an IC_{50} of 14.8 μM, which matches the IC_{50} for the effect of quinine on K_V currents (Fig. 8*B*). These data together suggest that the time-independent component of K_V currents appears to be more important in maintaining the membrane potential than the time-dependent component.

Discussion

Using patch-clamp techniques, we demonstrate that cell membrane depolarization induces a time-dependent current followed by a time-independent component in Daudi human B lymphoma cells. Ion substitution experiments indicate that both currents are carried by K^+. The time-dependent current resembles all the properties of the type *n* K_V channel, which was characterized in T lymphocytes (Lewis & Cahalan, 1988b). The pharmacological profile shows that the time-dependent current can be blocked by both TEA and quinine. In contrast, the time-independent current is blocked by quinine, but not TEA.

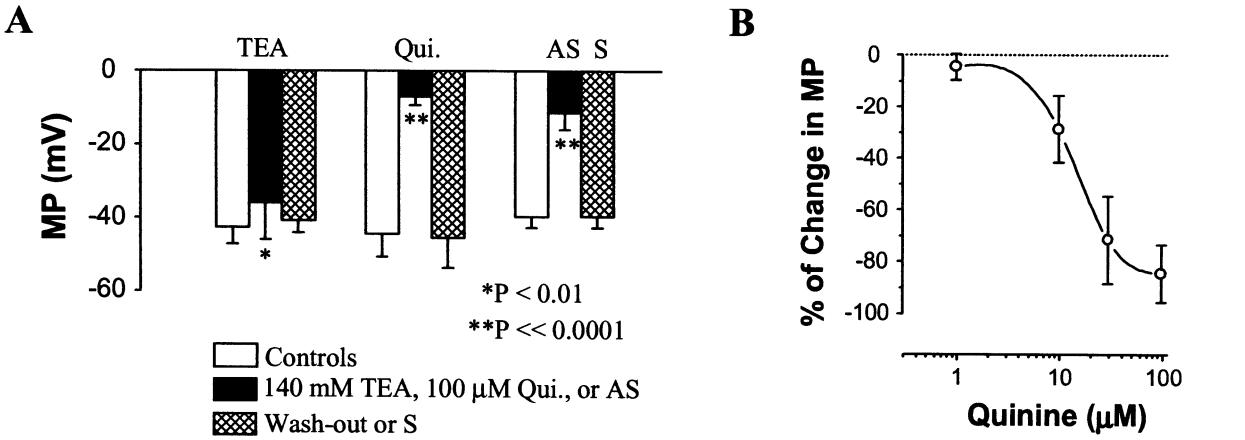

Fig. 8. Effects of TEA, quinine, antisense, or sense oligonucleotides on the membrane potential. (*A*) Membrane potential (MP) under control conditions, after replacement of bath solution with a solution containing 140 mM TEA or addition of 100 μM quinine (Qui.) to the bath, and after washing these two compounds out of the bath. MP was also compared between control cells and cells treated with antisense (AS) or sense (S) oligonucleotide. (*B*) Summary plot of % changes in MP after addition of 1,10, 30, or 100 μM quinine to the bath. The concentration causing 50% inhibition (IC_{50}) of MP is 14.8 μM.

On the basis of the differential blockade by TEA and quinine, we could not simply conclude that the time-independent current is another type of K_V channel. Since TEA, as an open-channel blocker, prevents the inactivation of K_V channels in human T lymphocytes (Grissmer & Cahalan, 1989), we could not, without further determination, eliminate the possibility that the time-independent component actually represents an incomplete inactivation of the same type of K_V channel. By employing the antisense methodology, we show that inhibition of Kv1.3-channel expression by antisense oligonucleotide causes a parallel reduction of both time-dependent and -independent currents. Furthermore, single-channel recordings demonstrate that there is only one type of K_V channel recorded from all 11 cell-attached patches and that the channel activity remains during sustained depolarization. Taking these results together, we conclude that the time-independent component represents an incomplete inactivation of the type *n* K_V channel. Although a non-inactivating K_V channel is found in some primary human T lymphocytes (Lee, Levy & Deutsch, 1992; Verheugen & Korn, 1997), this channel does not occur in the whole population of T cells. Since the type *n* K_V channel may behave differently at a state in which the inactivation does not fully occur, it remains to be determined whether the channel observed by other investigators actually represents the type *n* K_V channel under some circumstances in which the inactivation is not completely developed.

Previous studies suggest that the type *n* K_V channel plays an important role in mediating lymphocyte proliferation (DeCoursey et al., 1984; Matteson & Deutsch, 1984; Amigorena et al., 1990a), presumably by maintaining the membrane potential in order to provide the driving force for Ca^{2+} influx (Lewis & Cahalan, 1995; Cahalan & Chandy, 1997). Recent studies also indicate that the type *n* K_V channel appears to regulate apoptosis of lymphocytes (Szabo et al., 1996), probably also by maintaining the membrane potential (Bortner & Cidlowski, 2001). Therefore, understanding how the membrane potential is regulated by the type *n* K_V channel may provide significant evidence for the mechanism of lymphocyte proliferation and apoptosis. In the present study, we demonstrate that TEA at a dose that significantly inhibits the transient K_V channel activity only causes a slight reduction of the membrane potential, while quinine, which is able to reduce both the transient and steady-state K_V channel activity, strongly decreases the membrane potential. We also demonstrate that specific inhibition of Kv1.3 channel expression by antisense treatment mimics the effect of quinine, indicating that the molecular identity of the channel we observed is the Kv1.3 gene product. These results suggest that the remaining channel activity due to an incomplete inactivation of Kv1.3 channels appears to be more important in maintaining the membrane potential of Daudi cells by providing a constant K^+ efflux. Since previous studies suggest that the maintenance of membrane potential is important for lymphocyte proliferation (Cahalan & Chandy, 1997; Lewis & Cahalan, 1995) and probably is important for preventing cells apoptosis as well (Bortner & Cidlowski, 2001), we speculate that the defective inactivation of the Kv1.3 channel may result in lymphocyte abnormal proliferation and defective apoptosis. Further studies are required to examine the mechanism that causes the Kv1.3 channel to not fully inactivate and the putative role of the incomplete inactivation in mediating Daudi cell malignancy.

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