Journal of Physiology (1989), **412**, *pp.* 135–154
With 11 *text-figures*
Printed in Great Britain

CHARACTERIZATION OF Ca^{2+} AND K^+ CURRENTS IN THE HUMAN JURKAT T CELL LINE: EFFECTS OF PHYTOHAEMAGGLUTININ

BY GILLES DUPUIS*, JOSÉE HÉROUX* AND MARCEL D. PAYET†‡

*From the *Departments of Biochemistry and †Physiology and Biophysics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4*

(*Received 1 June 1988*)

SUMMARY

1. Inward and outward currents were recorded in the human Jurkat T cell line using the whole-cell configuration of the patch-clamp technique.

2. The transient outward current was activated at membrane potentials positive to −60 mV. The activation time constant–voltage relationship decreased from 17 ms to 2 ms for membrane potentials ranging from −40 to +40 mV. The inactivation phase could be fitted by a single-exponential function and the inactivation time constant decreased from 250 ms to 150 ms for membrane potentials ranging from −20 to +100 mV.

3. The steady-state inactivation–voltage relationship showed a mid-point potential of $-32 \pm 2{\cdot}6$ mV, and the slope factor was $10{\cdot}8 \pm 1{\cdot}8$ mv ($n = 3$).

4. The calcium ionophore A23187 provoked a decrease in the amplitude of the outward current, suggesting a dependence of this current on the cytosolic concentration of Ca^{2+}.

5. The K^+ outward current was blocked by tetraethylammonium (TEA, Michaelis–Menten constant (K_m), 6 mM) and by the calcium channel blockers Ni^{2+}, Co^{2+}, Mn^{2+} and Cd^{2+}.

6. Forty per cent ($n = 120$) of the patched Jurkat cells displayed an inward current. In a physiological medium containing Ca^{2+} (2·2 mM), the inward current threshold voltage was −60 mV, the maximum current was observed at −40 mV and the zero current voltage was positive to +20 mV. At negative membrane potentials, the time required to reach 50% of the maximum amplitude was 60 ms and grew shorter with increasing depolarization, reaching a value of 5 ms at −5 mV. The inactivation of the inward current was very slow and the time constant varied from 1200 ms at −35 mV to approximately 250 ms for potentials positive to −10 mV.

7. The current availability had a value of one for potentials negative to −50 mV and zero for potentials positive to −15 mV. The mid-point potential was $-31 \pm 3{\cdot}4$ mV and the slope factor was $3{\cdot}3 \pm 0{\cdot}2$ mV ($n = 3$).

8. The inward channels were permeable to Sr^{2+}, but were blocked by classical Ca^{2+} channel inhibitors such as Co^{2+}, Mn^{2+} and Ni^{2+}.

9. *Phaseolus vulgaris* phytohaemagglutinin (PHA), an inducer of interleukin-2 production in Jurkat cells, increased the inward current amplitude by 32 ± 20%

‡ To whom reprint requests should be addressed.

($n = 4$). This increase was concomitant with a decrease ($45 \pm 12\%$) in the amplitude of the outward current, but only when the current was carried by Ca^{2+}. In the case of cells that did not show a Ca^{2+} inward current, the amplitude of the outward current was increased by $17{\cdot}6 \pm 13\%$ ($n = 5$) at -20 mV.

10. Because PHA stimulation of interleukin-2 production by Jurkat cells depends on an increase in $[Ca^{2+}]_i$, we suggest that this response depends, at least in part, on the stimulation of voltage-dependent Ca^{2+} channels.

INTRODUCTION

The addition of stimulants such as mitogens, monoclonal antibodies or antigens to lymphocytes cultured *in vitro* results in the perturbation of a series of metabolic processes and cell division (MacDonald & Nabholz, 1986; Cambier & Ransom, 1987; Linch, Wallace & O'Flynn, 1987). Significant changes in the rate of cation transport are amongst the earliest detectable events and such changes are related to subsequent lymphocyte proliferation (Kaplan, 1978; Deutsch & Price, 1982; Owens & Kaplan, 1982). Ionic channels must therefore play a determining role in triggering the cascade of events leading to mitosis. The patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) has been used to obtain evidence suggesting that a transient outward K^+ flux may play a key role in cellular response, including lymphocyte activation (DeCoursey, Chandy, Gupta & Cahalan, 1984; Fukushima, Hagiwara & Henkart, 1984; Matteson & Deutsch, 1984; Cahalan, Chandy, DeCoursey & Gupta, 1985). In addition, the use of fluorescent calcium chelators (Tsien, Pozzan & Rink, 1982) has demonstrated that an increase in $[Ca^{2+}]_i$ is an essential part of the triggering signal of lymphocyte stimulation (Weiss, Imboden, Shoback & Stobo, 1984; Weiss, Imboden, Hardy, Manger, Terhorst & Stobo, 1986; Alcover, Ramarli, Richardson, Chang & Reinherz, 1987; Gelfand, Mills, Cheung, Lee & Grinstein, 1987). Michell (1975) originally proposed that Ca^{2+} channels may be responsible for Ca^{2+} entry into the cells, and unitary inward currents, of which the probability of opening is increased by lectins, have recently been recorded (Kuno, Goronzy, Weyand & Gardner, 1986; Kuno & Gardner, 1987). However, macroscopic Ca^{2+} inward currents in human T cells have not been clearly characterized yet, nor has the effect of phytohaemagglutinin (PHA) on this current been studied.

In the present report, we have used the whole-cell configuration of the patch-clamp technique to investigate the electrical properties of leukemic human Jurkat T cells (Schneider, Schwenk & Bornkmann, 1977) and the effect of PHA, a mitogenic lectin that induces IL-2 production in these cells (Gillis & Watson, 1980; Dupuis & Bastin, 1988). Our results show the existence of a voltage-dependent K^+ channel characterized by a rapid activation and a slow inactivation. The properties of this channel are similar to those described for the well-known delayed rectifier K^+ current of nerve and muscle cells. The current amplitude of the K^+ channel is decreased in the presence of the Ca^{2+} ionophore A23187. We also present data that suggest the presence of a voltage-dependent inward current that can be conclusively attributed to Ca^{2+} ions. PHA consistently increased the inward current with a concomitant decrease in outward K^+ current. Our data suggest that the previously reported increase in PHA-dependent $[Ca^{2+}]_i$ in Jurkat cells (Weiss *et al.* 1986) is due, at least

in part, to voltage-dependent Ca^{2+} channels, whereas outward K^+ current is indirectly mediated through a $[Ca^{2+}]_i$ increase during the early phase of stimulation. In support of this interpretation, PHA increased slightly the outward current in Jurkat cells that did not show a Ca^{2+} current, in agreement with reports of Cahalan *et al.* (1985) and Decoursey *et al.* (1984) in the case of peripheral blood T cells.

METHODS

Materials

Bovine serum albumin (BSA), antibiotics and poly-L-lysine were purchased from Sigma Chemical Co. (St Louis, Mo, USA). Fetal bovine serum and culture media came from Flow Laboratories (Montreal). Gey's balanced salt solution (GBSS) and RPMI 1640 medium were from Gibco (Grand Island Biologicals, Grand Island, NY, USA). PHA from *Phaseolus vulgaris* (red kidney bean) was prepared in our laboratory (Dupuis & Bastin, 1988). It showed one Coomassie Brilliant Blue-positive band by sodium dodecylsulphate–polyacrylamide gel electrophoretic analysis (M_r 33000).

Cell cultures

Jurkat 77 6·8 cells were kindly provided by Dr. K. A. Smith (Dartmouth Medical School, Hanover, NH, USA) and maintained in RPMI 1640 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml) and garamycin (40 μg/ml). The medium was supplemented with 10% heat-inactivated (56 °C for 20 min) fetal bovine serum.

Patch-clamp experiments

These were performed with Jurkat 77 6·8 cells resting in a Petri dish that had been coated 24 h earlier with poly-L-lysine (0·1 mg/ml) dissolved in GBSS and washed with GBSS and BSA (1%). The bath solution (GBSS) contained (mM): NaCl, 120; $CaCl_2$, 2·12; KCl, 5; $MgCl_2$, 2·2; $MgSO_4$; 0·6; D-glucose, 5·6; HEPES, 39; at pH 7·4. Experiments were carried out at 20 °C. The patch electrodes were filled with (mM): KCl, 120; NaCl, 20; $CaCl_2$, 1; EGTA, 11; HEPES, 5; at pH 7·3. Where appropriate, KCl was replaced by an equimolar concentration of CsCl to suppress outward current. The pipette resistance (Pyrex glass, Corning No. 7740) ranged from 4 to 8 MΩ, and the seal resistance varied between 10 and 50 GΩ. Once the seal was established, the cell membrane was ruptured by applying negative pressure. Series resistance was corrected empirically until the rigging point was attained. Experiments were performed using an Axopatch 1-B amplifier. Currents were low-pass filtered (1 kHz) and digitalized on-line via a PDP 11/23 computer. After whole-cell configuration was achieved, the system was left for 10–15 min to allow the voltage dependency of various parameters to stabilize. The cells were then stimulated at a low rate (1 stimulus/30 s) to avoid accumulation of inactivation. The leak current was subtracted from the current–voltage curves.

RESULTS

Whole-cell currents

One example of currents, recorded from a single Jurkat T cell maintained in GBSS, is illustrated in Fig. 1*A*. In this case, the holding potential (HP) was −80 mV. As shown in the figure, a membrane potential less negative than −40 mV activated an inward current which was followed by an outward current that increased with further membrane depolarization. The current–voltage relationship, measured at the peak of either the inward or the outward currents, is shown in Fig. 1*B*. The threshold voltage was observed at approximately −40 mV, whereas the maximum current was obtained at a potential of −20 mV. The current is directed outward for potentials positive to +5 mV. These results indicated that outward as well as inward currents could be recorded in the human Jurkat T cell line.

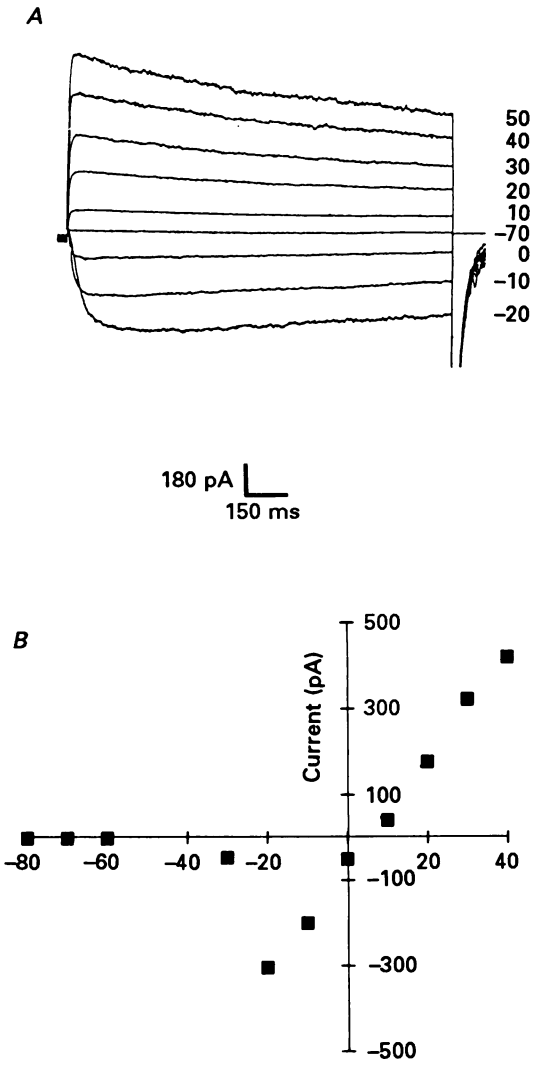

Fig. 1. Global current measured by patch clamp in a Jurkat T cell. *A*, dependency of inward and outward currents on membrane potential. Responses were recorded as a function of depolarizing pulses at −70, −20, −10, 0, 10, 20, 40 and 50 mV. The HP was −80 mV. Depolarizing pulses were applied at 30 s intervals. *B*, current–voltage relationship in Jurkat cells. The current amplitude was measured at the inward and outward peak values.

Outward current

The outward current was studied in cells that showed no inward current. From an HP of −80 mV, the outward current began to be activated for membrane potentials positive to −60 mV. Data shown in Fig. 2*A* indicate that the current was rapidly activated and then was slowly inactivated to a non-zero steady state. The current-

voltage relationship is shown in Fig. 2*B*. In this case, the amplitude was measured at the peak of the outward current after subtracting the leak current.

Kinetics of activation

Current traces plotted on an expanded time scale showed that the activation phase was sigmoidal and that the rate of activation increased with membrane depolarization (Fig. 3*A*, inset). Kinetic analysis of this current was performed for various

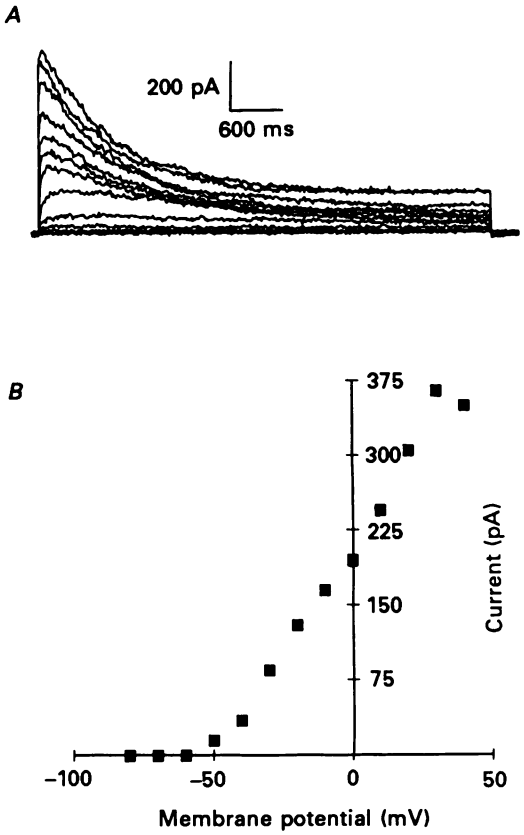

Fig. 2. Outward K^+ current in Jurkat T cells. *A*, time course profile of the K^+ current in a single cell. Depolarizing pulses of 2·8 s duration were applied at 1 min intervals from −80 mV to +40 mV, by 10 mV increments. The current was recorded 10 min after the whole-cell configuration patch was established. *B*, current–voltage relationship for the outward K^+ current, after subtracting the leak current. The HP was −80 mV.

voltage values. The activation phase was fitted using the following equation (Hodgkin & Huxley, 1952),

$$I_t = I_{\max}(1-e^{-t/\tau_n})^x, \tag{1}$$

where $I_{\max}$ is the maximum K^+ current, τ_n is the activation time constant and, x is the exponent of the activation process. We found that the best fit of the experimental data was for $x = 4$. Figure 3*A* shows results obtained with four different Jurkat cells.

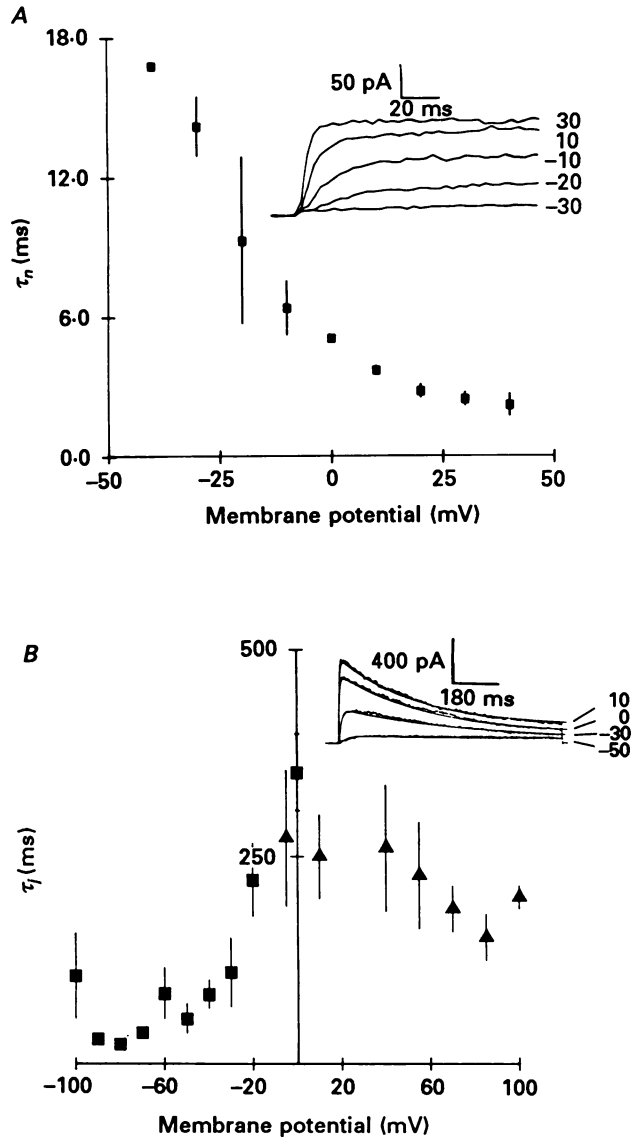

Fig. 3. Kinetics of the outward K^+ current in a Jurkat T lymphocyte. *A*, activation phase: the activation phase is illustrated in the inset. The time constant of the activation phase (τ_n) is plotted as a function of the membrane potential. Each point is the mean of four different experiments. The bars represent S.E.M. *B*, inactivation phase: the inactivation phase was fitted with a single-exponential function. The time constant (τ_j) is plotted as a function of the membrane potential. The values were obtained from the tail current (■) and from the inactivation phase of the current (▲). Each point is the mean of four to five experiments. The bars represent S.E.M. Inset: fitted curves obtained using the following equation,

$$I_t = I_{max}(1 - e^{-t/\tau_n})\, e^{-t/\tau_j} + I_1,$$

where I_{max} is the maximum current at a given potential, τ_n and τ_j are the time constants of the activaction and inactivation process, and I_1 is a time-independent leak current.

It was found that τ_n was voltage-dependent and decreased from 17 ms at −40 mV to 2 ms at +40 mV.

Kinetics of inactivation

As shown in Fig. 2*A*, the outward current was inactivated on sustained membrane depolarization. The inactivation phase of the current was fitted by a single-exponential function and the inactivation time constant (τ_j) was plotted for various

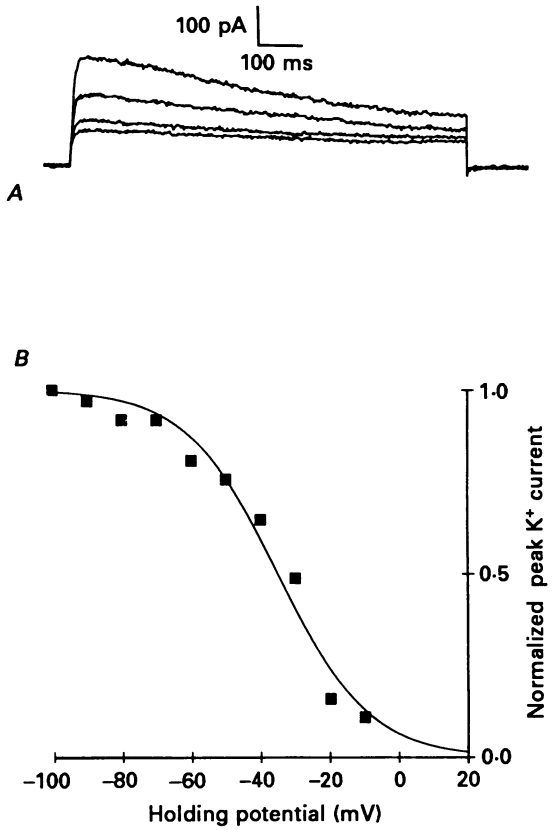

Fig. 4. Inactivation of the outward K^+ current in a Jurkat T cell. *A*, time-dependent accumulation of the current elicited by a depolarizing pulse to 0 mV from an HP of −80 mV. The pulse was applied every 10 s. *B*, voltage dependence of the steady-state inactivation. The current elicited by a pulse to +10 mV was recorded for various HPs that were maintained for 2 min to allow for the equilibrium to be reached. The normalized peak current is plotted as a function of the HP. The continuous line is fitted to the experimental data using eqn (2), where the values for V_k and k are −35 mV and 13 mV, respectively.

membrane potentials. Results from these experiments are shown in Fig. 3*B* (▲). In the case of potentials ranging between −20 and +100 mV, τ_j decreased from 250 ms to 150 ms. The deactivation time constant of the tail current was obtained by stepping the membrane potential from −80 mV to −10 mV with a brief pulse, followed by a test pulse applied at various levels. The decay of the tail current was

fitted by a single-exponential function. The time constant of the tail current (τ_{tail}) was plotted as a function of the test pulse voltage as shown in Fig. 3*B* (■).

The inactivation of the outward current was measured as a function of time intervals between stimulation or under steady-state conditions. The accumulation of the conductance inactivation was tested by stepping the membrane to +10 mv from an HP of −80 mV, at a frequency of 1 stimulation/10 s. Under these experimental

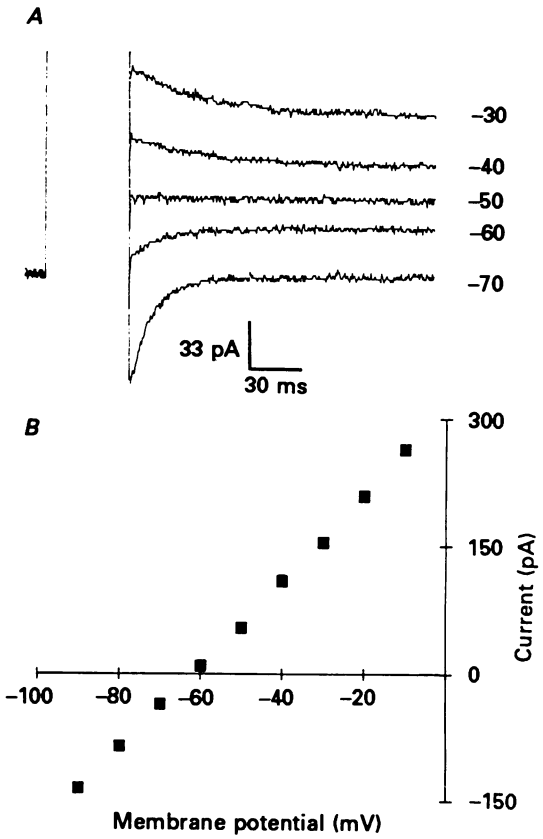

Fig. 5. Zero current voltage of the outward K^+ current in a Jurkat T cell. *A*, the tail currents were obtained by stepping the membrane potential to +20 mV and then repolarizing to different levels. *B*, current–voltage relationship for the tail current. The current amplitude is plotted as a function of the repolarizing potential. The zero current voltage was estimated to be −61 mV.

conditions, the current amplitude decreased rapidly, as shown in Fig. 4*A*. The voltage dependence of the steady-state inactivation of the outward current was determined by varying the HP and recording the current at a constant membrane potential that brought about full current activation. The time interval between each sweep was 2 min. The inactivation–voltage curve obtained under these conditions is shown in Fig. 4*B*. The curve (continuous) was traced according to the Boltzmann equation,

$$j_\infty = 1/[1+\exp(V-V_k)/k], \tag{2}$$

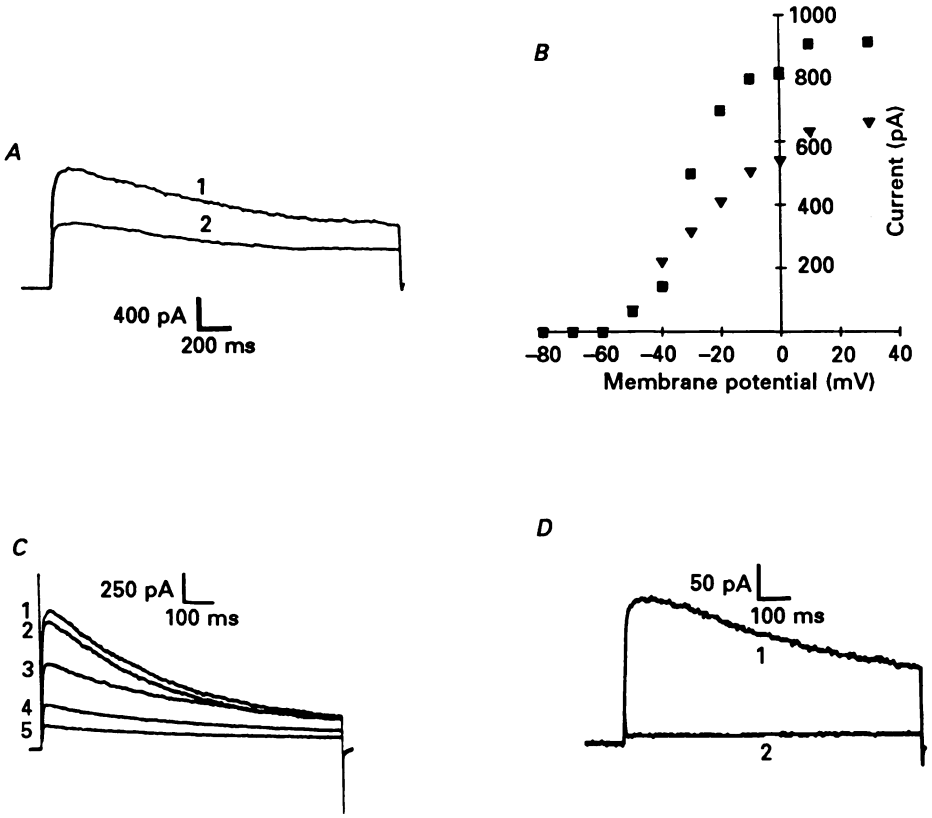

Fig. 6. Effect of the A23187 ionophore on K^+ current in a Jurkat T lymphocyte. *A*, responses to a depolarizing voltage at −10 mV were recorded (1) before and (2) 12 min after the addition of A23187 (1 μM). The HP was −80 mV. *B*, current–voltage relationship of the K^+ current before (■) and after (▼) the addition of A23187 (1 μM). Depolarizing pulses were applied from an HP of −80 mV. *C*, blockage of the outward current by TEA. TEA was added, at various concentrations, to Jurkat cells bathed in GBSS. The current was measured after attaining steady state (5 min). Cells bathed in GBSS (1, control) and in the presence of TEA (mM): 1 (2) 5, (3), 9 (4) and 13 (5). The HP was −80 mV and the membrane potential was −20 mV. *D*, blockage of the outward current 2 min after the addition of Ni^{2+} (1 mM). The HP was −80 mV and the membrane potential was +20 mV.

where V is the membrane potential, V_k is the mid-point potential, and k is the slope factor. The values of the different parameters were obtained by a non-linear least-squares method. Using three different cells, these values were $-32 \pm 2{\cdot}6$ and $10{\cdot}8 \pm 1{\cdot}8$ mV, respectively.

Zero current voltage

The zero current voltage of the outward current was not easily determined from the current-voltage relationship (Fig. 1*B*), and the tail current method was used. Figure 5*A* shows tail currents observed when cells were placed in GBSS containing 5·4 mM-KCl. From an HP of −80 mV, a brief pulse to +10 mV elicited an outward current, after which the cell was plotted as a function of the repolarizing voltage and

the results are shown in Fig. 5*B*. It was estimated that the zero current voltage had a value of −61 mV. An average of −61·3±3·5 mV was found ($n = 3$).

Sensitivity to Ca^{2+}

Several groups have reported the sensitivity of K^+ channels to variations in Ca^{2+} concentrations in a number of somatic cells (Meech, 1978). We therefore asked the

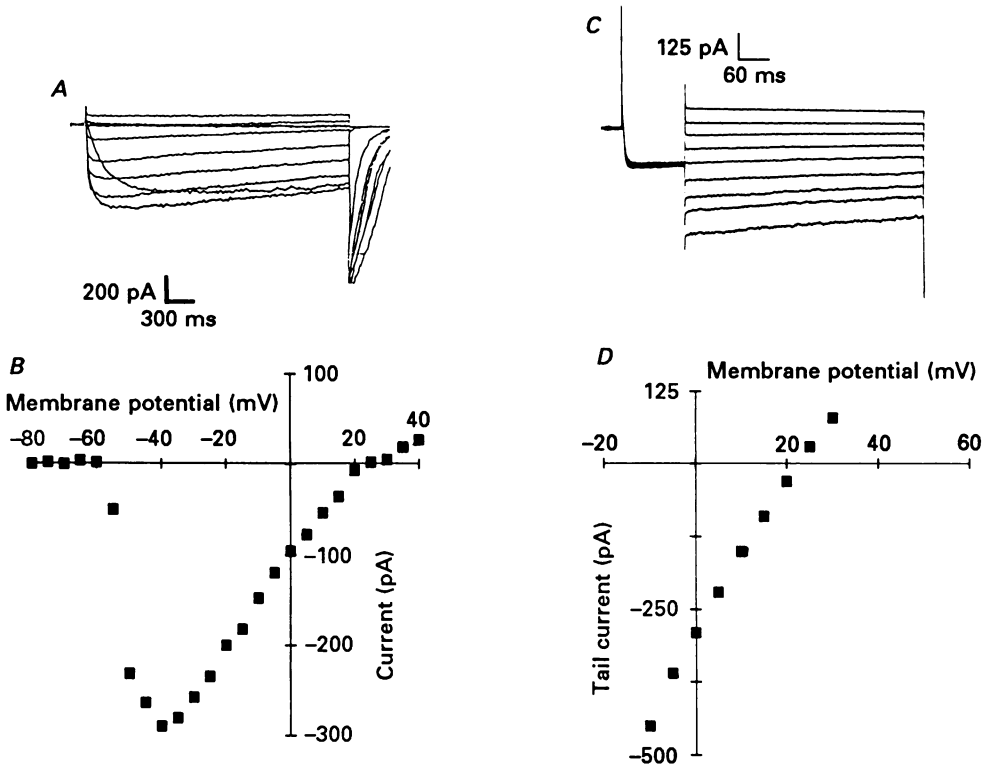

Fig. 7. Slow inward current in Jurkat T cells. The current was recorded with CsCl-filled pipettes in order to block the outward current. The external medium contained 2·2 mM-$CaCl_2$. *A*, current traces obtained for depolarizing pulses from −80 mV to +20 mV, in 10 mV increments. The HP was −80 mV. *B*, current–voltage relationship for the slow inward current. The current starts to be activated at −60 mV and zero current voltage is found at +25 mV. The HP was −80 mV. *C*, tail currents were obtained by stepping the membrane potential to +10 mV and then applying a repolarizing pulse from −10 mV to +30 mV, in 5 mV increments. *D*, current–voltage relationship for the tail current.

question whether this was the case for Jurkat cells. $[Ca^{2+}]_i$ was increased by incubating the cells with the well-known ionophore A23187 and the results are shown in Fig. 6*A*. A significant decrease in the current amplitude was evident after 10 min of incubation. Similar results were obtained with six different patched cells, where the current amplitude was decreased by 31±16%. Current–voltage relationships in the case of cells incubated in the presence (▼) or absence (■) of A23187 are shown in Fig. 6*B*. At 0 mV, the amplitude of the current was decreased by approximately 34%.

The possibility of a direct effect of A23187 on K^+ current was assessed by incubating the cells in a Ca^{2+}-free medium. In this instance, the current amplitude was not affected or was slightly increased (data not shown).

Channel blocking

Various concentrations of tetraethylammonium (TEA) were used to block the outward current. Figure 6*C* shows the sensitivity of the K^+ current to TEA, after a

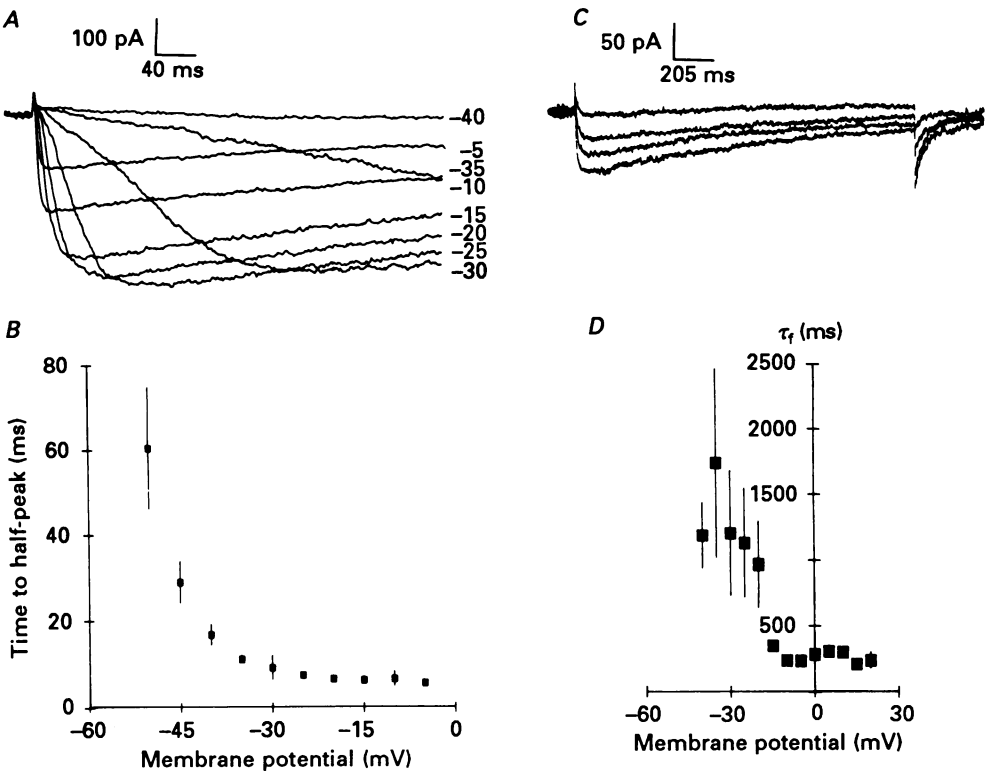

Fig. 8. Kinetics of the slow inward current in a Jurkat T cell. *A*, activation phase of the slow inward current plotted on an expanded time scale. The HP was −80 mV. *B*, time to half-peak plotted as a function of the membrane potential. Values are the mean of four different experiments. The bars represent S.E.M. *C*, inactivation phase of the slow inward current. Membrane potentials were stepped from −40 to −25 mV in 5 mV increments. The HP was −80 mV. *D*, time constant of inactivation (τ_f) plotted as a function of the membrane potential. Values are the mean of six different experiments. The bars represent S.E.M.

5 min perfusion following steady state. In this cell, the TEA concentration required to reach 50% inhibition was 6 mM. Three other experiments gave similar results. In addition, the outward K^+ current was rapidly blocked by incubating Jurkat cells (2 min) in the presence of Ni^{2+} (Fig. 6*D*), a specific Ca^{2+} channel inhibitor (Tsien, Hess, McCleskey & Rosenberg, 1987). Similar results were obtained in the case of other inhibitors such as Co^{2+} ($n = 4$), Mn^{2+} ($n = 3$) or Cd^{2+} ($n = 7$); these data are not shown.

Inward current

A voltage-dependent inward current was recorded in 40% of Jurkat cells (n = 120), whether outward current was present or inhibited by using Cs^+-loaded pipettes.

Current–voltage relationships and reversal potential

These experiments were carried out on cells incubated in the presence of Ca^{2+} (2·2 mM) and with pipettes filled with CsCl (130 mM) in order to block the outward

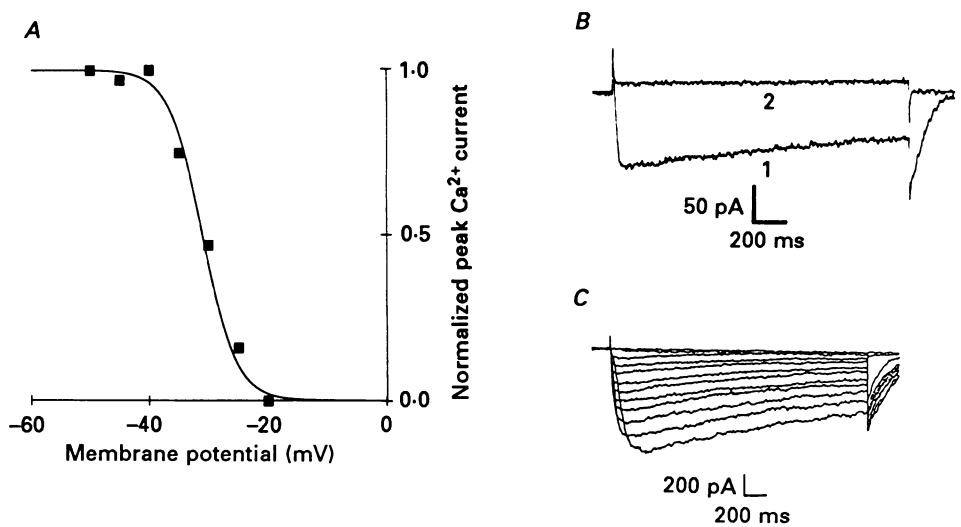

Fig. 9. Voltage dependence of the steady-state inactivation of the inward current in a Jurkat T cell. *A*, the membrane potential was stepped to −10 mV and the HP was varied. The normalized peak currents are plotted *versus* the HPs. The continuous line was drawn according to the Boltzmann function, where $V_k = -31$ mV and $k = 2{\cdot}8$ mV. *B*, blocking effect of Cd^{2+} (1 mM) on the slow inward current. Tracings represent experiments carried out before (1) and 2 min after (2) addition of Cd^{2+}. *C*, currents recorded with cells placed in GBSS containing Sr^{2+} (2·2 mM). Membrane potentials were varied from −80 mV to −10 mV, in 5 mV increments. The HP was −80 mV.

current. A family of current traces obtained for various depolarizations from an HP of −80 mV is shown in Fig. 7*A*. Tracings show an obvious rapid current activation and a slow inactivation that is a function of time. The current–voltage relationship shows that the threshold voltage was near −60 mV, whereas the peak of the maximum current was found at approximately −40 mV and the zero current voltage was positive to +20 mV (Fig. 7*B*).

The tail current method was used to determine the zero current voltage, as described above in the case of the outward current. Results are shown in Fig. 7*C*. The amplitude of the tail current was plotted as a function of the repolarizing voltage and had a value of zero at +23·6 mV (Fig.7*D*). Six similar experiments gave a mean value of 19·0±4·1 mV.

Kinetics of the inward current

Figure 8*A* shows current traces on an expanded time scale. In the case of negative membrane potentials, the current activates very slowly and activation becomes faster with depolarization. An estimate of the activation rate was obtained by determining the time needed to reach 50% of the maximum amplitude (rise time to half-maximum current, $t_{\frac{1}{2}}$) at a given membrane potential (Cahalan *et al.* 1985). The results are shown in Fig. 8*B* ($n = 4$). When the membrane potential was negative (−50 mV), $t_{\frac{1}{2}}$ was 60 ms and grew shorter with increasing depolarization. For instance, at −5 mV $t_{\frac{1}{2}}$ was 5 ms.

Inactivation of the inward current was very slow and was completed only after approximately 2–3 s, as shown in Fig. 8*C*. The inactivation phase of the current was fitted with a single-exponential function and the inactivation time constant τ_f was plotted as a function of the membrane potential (Fig. 8*D*).

Steady-state inactivation

Steady-state inactivation experiments were performed by changing the HP and measuring the inward current elicited by stepping the membrane to −10 mV. For each HP studied, there was a waiting period of 2 min to allow the system to reach equilibrium. The normalized peak currents were plotted against HP values and the results are shown in Fig. 9*A*. The curve (continuous) was drawn according to the Boltzmann equation, using the parameters $V_k = -31$ mV and $k = 2{\cdot}84$ mV. From three experiments we found that V_k had a value of $-31 \pm 3{\cdot}4$ mV and k had a value of $3{\cdot}3 \pm 0{\cdot}2$ mV.

Channel blockers and selectivity

Inorganic ions are known to block the inward Ca^{2+} current in a variety of cells (Tsien *et al.* 1987). In the case of Jurkat cells, we observed that Co^{2+} (3 mM) and Mn^{2+} (3 mM) completely inhibited the inward current after 2–3 min of incubation (data not shown). In Fig. 9*B* we present data on the inhibition of the inward current by Cd^{2+} (1 mM). Clearly, the inward current was totally inhibited after a 2 min incubation period.

It is well established that Ca^{2+} channels are permeable to divalent cations such as Sr^{2+} (Tsien *et al.* 1987). Substitution of Ca^{2+} for an equimolar concentrations of Sr^{2+} did not change the kinetics or the amplitude of the inward current in Jurkat cells (Fig. 9*C*). These observations suggest that, in the case of Jurkat cells, Ca^{2+} channels appear to be equally permeable to Ca^{2+} and Sr^{2+} ions.

Effects of PHA on the inward and the outward currents

Since we had observed that the outward current amplitude decreased when $[Ca^{2+}]_i$ was augmented (Fig. 6) and since PHA induces increased $[Ca^{2+}]_i$ (Weiss *et al.* 1986) in Jurkat cells, we investigated the possibility that the effect of the lectin on the outward current amplitude may involve Ca^{2+} channels. Experiments were performed on the global ionic current and results are shown in Fig. 10. In the case of control experiments (absence of PHA, cells in GBSS containing 2·2 mM-Ca^{2+}) a membrane

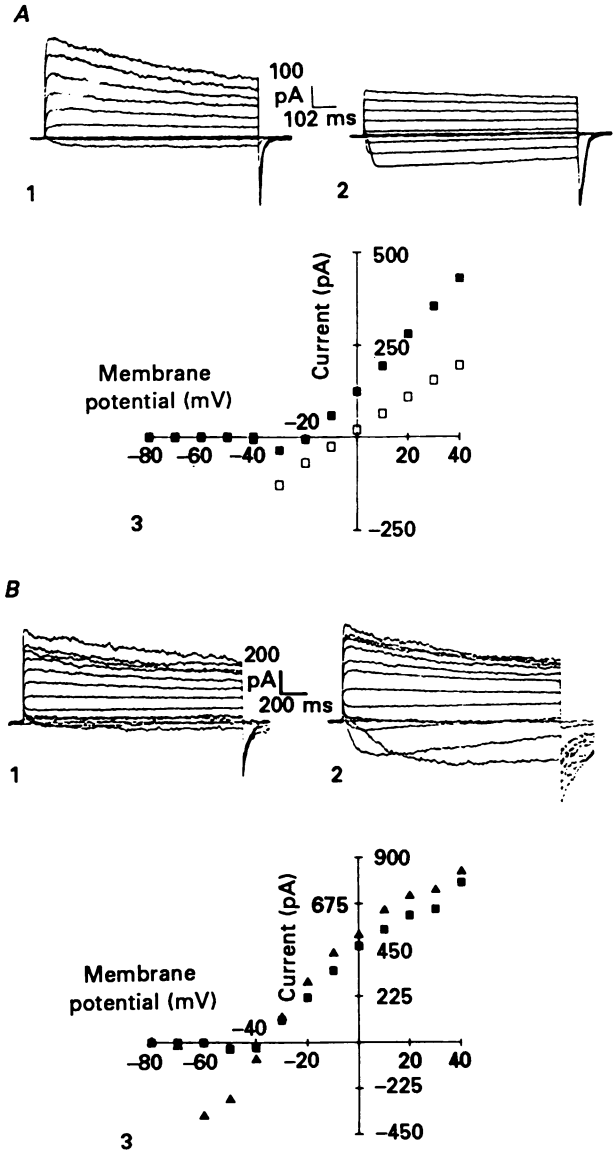

Fig. 10. Representative examples of the effects of PHA on the inward and outward currents of Jurkat T cells. *A*, effect of PHA on the global current. Jurkat T cells were bathed in GBSS medium containing Ca^{2+} (2·2 mM): (1) current traces obtained under control (absence of PHA) conditions. Membrane potentials were varied from −40 mV to +30 mV, in 10 mV increments. The HP was −80 mV, (2) currents recorded in the same cell after addition of PHA (10 μg/ml), (3) current–voltage relationships for the global current before (■) and after (□) the addition of PHA (10 μg/ml). *B*, effect of PHA on the global current of a Jurkat T lymphocyte bathed in GBSS containiung Sr^{2+} (2·2 mM): (1) current traces obtained under control (absence of lectin) conditions. Membrane potential was varied from −80 mV to +30 mV, in 10 mV increments. The HP was −80 mV, (2) currents recorded in the same cell after the addition of PHA (10 μg/ml), (3) current–voltage relationships for the global current before (■) and after (▲) the addition of PHA (10 μg/ml).

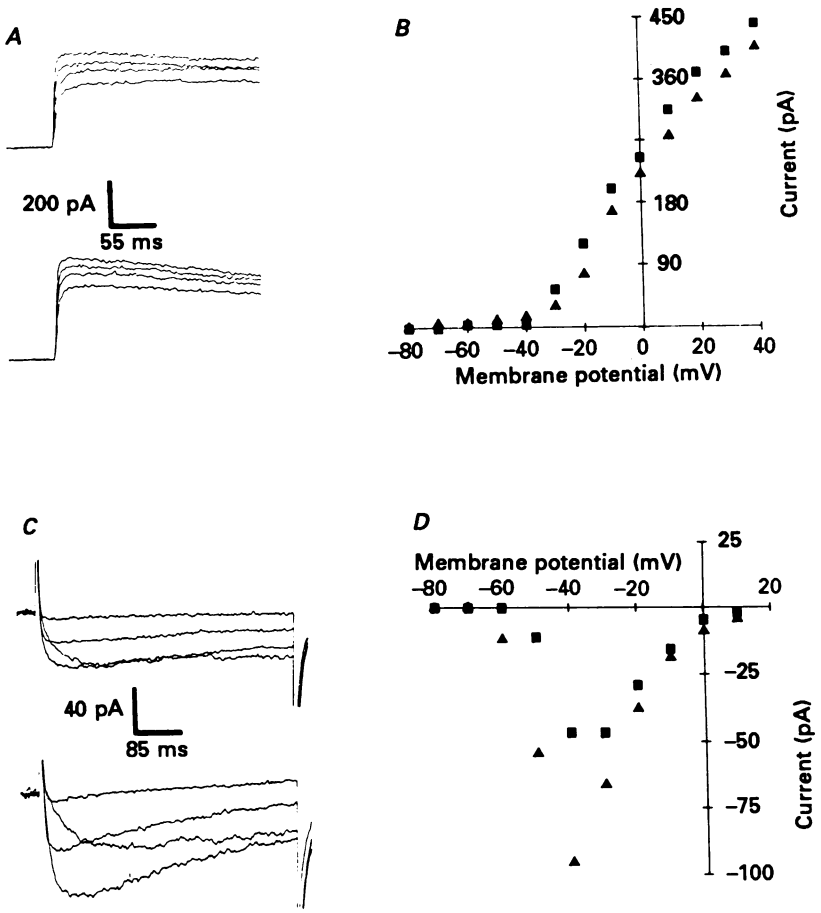

Fig. 11. Effects of PHA on the outward current in a Jurkat cell. *A*, the outward current was recorded in a Jurkat T cell that did not display any inward current. Data are presented for the control (absence of PHA) and experimental conditions (PHA, 10 μg/ml). The HP was −80 mV, and membrane potentials were 10, 20, 30 and 40 mV, respectively. *B*, current–voltage relationships before (▲) and after (■) addition of PHA (10 μg/ml). Effects of PHA on the inward current in a Jurkat cell. *C*, experiments were carried out under conditions in which the outward current was blocked (CsCl in the patch electrode). The upper part of the panel represents control conditions (absence of PHA) and the lower part experimental conditions (PHA, 10 μg/ml). The HP was −80 mV and the membrane was depolarized to −50, −40, −30 and −20 mV. *D*, current–voltage relationships before (■) and after (▲) addition of PHA (10 μg/ml). The leak current was subtracted.

potential positive to −50 mV elicited inward currents from an HP of −80 mV (Fig. 10*A*1). When the potential was more positive than −20 mV, outward currents were recorded, as shown in Fig. 10*A*. Addition of PHA (10 μg/ml) induced two obvious major changes in the current trace pattern: an increase in the inward current amplitude and a decrease in the outward current amplitude (Fig. 10*A*2). These changes are summarized in Fig. 10*A*3 where the respective current–voltage relationships are illustrated. In studies with eight different cells, the outward current was decreased by 45±12%.

Similar experiments using a Ca^{2+}-free, Sr^{2+}-containing medium showed that the Sr^{2+} current was increased by the lectin, while the outward current was not affected (Fig. 10*B*1 and *B*2). The current–voltage relationship showed an increase in the range of voltage where the current was inward, but no changes were detected in the outward portion of the curve (Fig. 10*B*3).

Moreover, the effect of PHA was assessed in cells which did not display an inward current. In these cases ($n = 5$), the outward current was increased by $17{\cdot}6 \pm 13\%$ at -20 mV (Fig. 11*A* and *B*). The inactivation time constant τ_j decreased for all of the voltage ranges studied, with specific decreases of $-38{\cdot}5 \pm 9$ and $-23 \pm 4\%$ at 0 and $+40$ mV, respectively.

Figure 11*C* shows the current traces obtained in patched Jurkat cells at various membrane potentials from an HP of -80 mV. In this case, the external GBSS medium contained 2·2 mM-$CaCl_2$ and pipettes were filled with CsCl (120 mM). After addition of PHA (10 μg/ml), the amplitude of the current increased significantly as shown in Fig. 11*C*. The current–voltage relationship is shown in Fig. 11*D*. Kinetic analysis of the inactivation phase of the Ca^{2+} current showed that PHA had no effect on τ_f ($n = 4$, data not shown).

DISCUSSION

The present study demonstrates the presence, in the Jurkat T cell line, of an outward K^+ current as well as an inward Ca^{2+} current that shows time- and voltage-dependent kinetics.

K^+ channels

The outward K^+ current presents activation and inactivation kinetics similar to those previously observed for human peripheral T lymphocytes (DeCoursey *et al.* 1984; Matteson & Deutsch, 1984; Cahalan *et al.* 1985; Bregestovski, Redkozubov & Alexeev, 1986), murine T cells (DeCoursey, Chandy, Gupta & Cahalan, 1987) and murine cytotoxic (Fukushima *et al.* 1984) and helper (Lee, Sabath, Deutsch & Prystowsky, 1986) T lymphocytes.

K^+ channels activated by $[Ca^{2+}]_i$ have been found in neurones and in many excitable cells (Meech, 1978). However, some types of K^+ channels such as those found in human T lymphocytes (Bregestovski *et al.* 1986) or in murine B cells (Choquet, Sarthou, Primi, Cazenaven & Korn, 1987) are blocked by an increase in $[Ca^{2+}]_i$. This also appears to be the case for Jurkat cells as evidenced by the effect of the calcium ionophore A23187 (Fig. 6) or ionomycin (not shown). PHA also reduces the outward K^+ current with a concomitant increase of the Ca^{2+} current (Fig. 11). In contrast, the lectin increased the Sr^{2+} current but this did not affect the K^+ current. We thus postulate that the PHA-dependent decreased K^+ current observed in Jurkat cells is due to a lectin-dependent increase in $[Ca^{2+}]_i$. This proposition is based on (1) the fact that PHA increases $[Ca^{2+}]_i$ in Jurkat cells (Weiss *et al.* 1986), (2) an observable increase in Ca^{2+} current in these cells (Fig. 11), (3) the effect of ionophores on the K+ current (Fig. 6) and (4) the lack of effect of PHA on cells that did not display any inward current. In keeping with this last result, we have found that PHA increased the outward current in cells that did not display an inward current

(Fig. 10*A* and *B*). These observations are in agreement with those of Cahalan *et al.* (1985) who reported that PHA increased the amplitude of the peak conductance by altering the external surface potential (DeCoursey *et al.* 1984). However, Schlichter, Sidell & Hagiwara (1986) have suggested that this phenomenon is not an essential event in the T cell response to PHA.

The potency of TEA to block the delayed outward K^+ current in somatic cells depends on the type of K^+ channels involved. For instance, the concentration needed for 50% inhibition has been found to be 8–16 mM in the case of type n K^+ channels, whereas it is 50–100 μM in the case of type l K^+ channels (DeCoursey *et al.* 1987). In the present study, our data suggest that Jurkat cells posesss type n K^+ channels.

Ca^{2+} channels

The existence of Ca^{2+} channels in lymphoid cells was originally proposed by Michell (1975), but the presence of such channels could not be recorded in human (Matteson & Deutsch, 1984; Cahalan *et al.* 1985) or in cloned murine T lymphocytes (Fukushima *et al.* 1984). However, macroscopic as well as unitary voltage-independent Ba^{2+} currents have been observed in cloned human helper T lymphocytes (Kuno *et al.* 1986) and unitary Ca^{2+} currents have been reported in Jurkat cells (Kuno & Gardner, 1987) and in T lymphocyte tumour cell markers reconstituted in bilayer phospholipid membranes (Pecht, Corcia, Liuzzi, Alcover & Reinherz, 1987).

Our results show conclusively the presence of voltage-dependent Ca^{2+} channels in approximately 40% of the Jurkat cells studied. It is of note to mention that MacCumber & Tucker (1987) have reported that 50% of peripheral human lymphocytes respond to PHA by increasing $[Ca^{2+}]_i$. This observation may be related to the frequency of Ca^{2+} currents observed in the present study.

At a holding potential of -80 mV and in the presence of external Ca^{2+} (2·2 mM), the current starts to be activated at a voltage positive to -60 mV, the maximum current is found near -40 mV and the zero current voltage is observed between $+20$ and $+30$ mV. This low value for the zero current voltage could be due to an outward current of monovalent cations (Cs^+) flowing through the Ca^{2+} channels, as reported by Fukushima & Hagiwara (1985).

Recently, various types of Ca^{2+} channels have been described and they differ in voltage thresholds, steady-state inactivation curves, kinetics and response to pharmacological agents (Nowycky, Fox & Tsien, 1985). Although the current found in Jurkat T cells shows a slow kinetic pattern similar to that of the long-lasting (L-type) channel, the threshold voltage was more negative than that of a typical L-type channel. Further work using channel agonists or antagonists is obviously required to classify the Ca^{2+} channel observed in Jurkat cells.

The observation that a voltage-dependent Ca^{2+} is present in the leukemic Jurkat T cell line whereas it has not been previously reported in peripheral blood T lymphocytes, may suggest that calcium channels are too small in number to be detected as macroscopic current in normal T cells. In addition, cell transformation may be accompanied by an increase in the number of Ca^{2+} channels (Fukushima & Hagiwara, 1983).

Effects of PHA on ionic currents in Jurkat cells

PHA is known to stimulate T lymphocyte from various species to undergo blast transformation and mitosis (Gelfand *et al.* 1987). One of the earliest events that is associated with PHA-dependent lymphocyte stimulation is an increase in $[Ca^{2+}]_i$ and a depolarization of the plasma membrane (Gelfand *et al.* 1987).

With respect to changes in $[Ca^{2+}]_i$, Tsien *et al.* (1982) initially reported that PHA brings about an approximately twofold increase in $[Ca^{2+}]_i$ in porcine lymphocytes. Similar results have been reported by Gelfand, Cheung & Grinstein (1986) in the case of human peripheral T cells and by Weiss *et al.* (1986) in Jurkat T cells. The source of Ca^{2+} involved in such increases has been established. The largest contribution comes from extracellular Ca^{2+} that is responsible for the sustained increase in $[Ca^{2+}]_i$ (Oettgen, Terhorst, Cantley & Rosoff, 1985; Alcover *et al.* 1987; Imboden & Weiss, 1987), whereas, Ca^{2+} mobilization from intracellular stores, probably as a result of inositol 1,4,5-trisphosphate ($InsP_3$) release, accounts for the early rise in $[Ca^{2+}]_i$ (Berridge & Irvine, 1984). It has been postulated that lymphocyte Ca^{2+} channels involved in the cytosolic increase of this ion may be regulated directly by the T cell antigen–receptor complex (Oettgen *et al.* 1985) or indirectly by $InsP_3$ (Kuno & Gardner, 1987). Alternatively, Cahalan *et al.* (1985) have suggested that Ca^{2+} entry into lymphocytes could proceed via K^+ channels. However, reports by Gelfand *et al.* (1986) and data presented in this study do not agree with this hypothesis (Fig. 10). Our observations suggest that cytosolic increases in Ca^{2+} concentration previously observed in Jurkat cells (Weiss *et al.* 1986) may, at least in part, be mediated via voltage-dependent calcium channels. Whether PHA acts directly on such channels or whether the action is mediated through a second messenger such as $InsP_3$ (Kuno & Gardner, 1987) remains to be determined.

We thank Dr K. A. Smith for providing the Jurkat 77 6·8 cells. The work was supported by grants from the Medical Research Council of Canada (M. D. P. and G. D.), the Centre de Recherches Cliniques du C.H.U.S. and 'Challenger 87' summer training programme (J. H.). M. D. P. is a holder of a 'Chercheur Boursier' scholarship from the Fonds de la Recherche en Santé du Québec. We would also like to thank Lyne Bilodeau for technical assistance. This work is dedicated to Dr J. P. Caillé, *in memoriam*.

REFERENCES

ALCOVER, A., RAMARLI, D., RICHARDSON, N. E., CHANG, H.-C. & REINHERZ, E. L. (1987). Functional and molecular aspects of human T lymphocyte activation via T3-Ti and T11 pathways. *Immunological Reviews* **95**, 5–35.

BERRIDGE, M. J. & IRVINE, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312**, 315–321.

BREGESTOVSKI, P., REDKOZUBOV, A. & ALEXEEV, A. (1986). Elevation of intracellular calcium reduces voltage-dependent potassium conductance in human T cells. *Nature* **319**, 776–778.

CAHALAN, M. D., CHANDY, K. G., DECOURSEY, T. E. & GUPTA, S. (1985). A voltage-gated potassium channel in human T lymphocytes. *Journal of Physiology* **358**, 197–237.

CAMBIER, J. C. & RANSOM, J. T. (1987). Molecular mechanisms of transmembrane signaling in B lymphocytes. *Annual Review of Immunology* **5**, 175–199.

CHOQUET, D., SARTHOU, P., PRIMI, D., CAZENAVE, P.-A. & KORN, H. (1987). Cyclic AMP-modulated potassium channels in murine B cells and their precursors. *Science* **235**, 1211–1214.

DeCoursey, T. E., Chandy, K. G., Gupta, S. & Cahalan, M. D. (1984). Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis. *Nature* **307**, 465–468.

DeCoursey, T. E., Chandy, K. G., Gupta, S. & Cahalan, M. D. (1987). Two types of potassium channels in murine T lymphocytes. *Journal of General Physiology* **89**, 379–404.

Deutsch, C. & Price, M. (1982). Cell calcium in human peripheral blood lymphocytes and the effect of mitogen. *Biochimica et biophysica acta* **687**, 211–218.

Dupuis, G. & Bastin, B. (1988). Lectin interactions with the Jurkat leukemic T-cell line: quantitative binding studies and interleukin-2 production. *Journal of Leukocyte Biology* **43**, 238–247.

Fukushima, Y. & Hagiwara, S. (1983). Voltage-gated Ca^{2+} channel in mouse myeloma cells. *Proceedings of the National Academy of Sciences of the USA* **80**, 2240–2242.

Fukushima, Y. & Hagiwara, S. (1985). Currents carried by monovalent cations through calcium channels in mouse neoplastic B lymphocytes. *Journal of Physiology* **358**, 255–284.

Fukushima, Y., Hagiwara, S. & Henkart, M. (1984). Potassium current in clonal cytotoxic T lymphocytes from the mouse. *Journal of Physiology* **351**, 645–656.

Gelfand, E. W., Cheung, R. K. & Grinstein, S. (1986). Mitogen-induced changes in Ca^{2+} permeability are not mediated by voltage-gated K^+ channels. *Journal of Biological Chemistry* **261**, 11520–11523.

Gelfand, E. W., Mills, G. B., Cheung, R. K., Lee, J. W. W. & Grinstein, S. (1987). Transmembrane ion fluxes during activation of human T lymphocytes: role of Ca^{2+}, Na^+/H^+ exchange and phospholipid turnover. *Immunological Reviews* **95**, 59–87.

Gillis, S. & Watson, J. (1980). Biochemical and biological characterization of lymphocyte regulatory molecules. V. Identification of an interleukin 2 producing human leukemia T cell line. *Journal of Experimental Medicine* **152**, 1709–1719.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* 391, 85–100.

Hodgkin, A. L. & Huxley, A. F. (1952). Quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology* **117**, 500–544.

Imboden, J. B. & Weiss, A. (1987). The T-cell antigen receptor regulates sustained increases in cytoplasmic free Ca^{2+} through extracellular Ca^{2+} influx and ongoing intracellular Ca^{2+} mobility. *Biochemical Journal* **247**, 695–700.

Kaplan, J. G. (1978). Membrane cation transport and the control of proliferation of mammalian cells. *Annual Review of Physiology* **40**, 19–41.

Kuno, M. & Gardner, P. (1987). Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T lymphocytes. *Nature* **326**, 301–304.

Kuno, M., Goronzy, J., Weyand, C. M. & Gardner, P. (1986). Single-channel and whole-cell recordings of mitogen-regulated inward currents in human cloned helper T lymphocytes. *Nature* **323**, 269–273.

Lee, S. C., Sabath, D. E., Deutsch, C. & Prystowsky, M. B. (1986). Increased voltage-gated potassium conductance during interleukin 2-stimulated proliferation of a mouse helper T lymphocyte clone. *Journal of Cell Biology* **102**, 1200–1208.

Linch, D. C., Wallace, D. L. & O'Flynn, K. (1987). Signal transduction in human T lymphocytes. *Immunological Reviews* **95**, 137–159.

MacCumber, M. W. & Tucker, R. W. (1987). Studies of intracellular free calcium in human lymphocytes using Fura 2 and digital image analysis. *Annals of the New York Academy of Science* **494**, 230–232.

MacDonald, H. R. & Nabholz, M. (1986). T-cell activation. *Annual Review of Cell Biology* **2**, 231–253.

Matteson, D. R. & Deutsch, C. (1984). K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion. *Nature* **307**, 468–471.

Meech, R. W. (1978). Calcium-dependent potassium activation in nervous tissues. *Annual Review of Biophysics and Bioengineering* **7**, 1–18.

Michell, R. H. (1975). Inositol phospholipids and cell surface receptor function. *Biochimica et biophysica acta* **415**, 81–147.

Nowycky, M. C., Fox, A. P. & Tsien, R. W. (1985). Three types of neuronal calcium channels with different calcium agonist sensitivity. *Nature* **316**, 440–443.

OETTGEN, H. C., TERHORST, C., CANTLEY, L. C. & ROSOFF, P. M (1985). Stimulation of the T3-T cell receptor complex induces a membrane-potential-sensitive calcium influx. *Cell* **40**, 583–590.

OWENS, T. & KAPLAN, J. G. (1982). Increased cationic fluxes in stimulated lymphocytes of the mouse: response of enriched B- and T-cell subpopulations to B- and T-cell mitogens. *Canadian Journal of Biochemistry* **58**, 831–839.

PECHT, I., CORCIA, A., LIUZZI, M. P. T., ALCOVER, A. & REINHERZ, E. L. (1987). Ion channels activated by specific Ti or T3 antibodies in plasma membranes of human T cells. *EMBO Journal* **6**, 1935–1939.

SCHLICHTER, L., SIDELL, N. & HAGIWARA, S. (1986). K channels are expressed early in human T-cell development. *Proceedings of the National Academy of Sciences of the USA* **83**, 5623–5629.

SCHNEIDER, U., SCHWENK, H.-U. & BORNKMANN, G. (1977). Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. *International Journal of Cancer* **19**, 621–626.

TSIEN, R. W., HESS, P., MCCLESKEY, E. W. & ROSENBERG, R. L. (1987). Calcium channels: mechanisms of selectivity, permeation, and block. *Annual Review of Biophysics and Biophysical Chemistry* **16**, 265–290.

TSIEN, R. Y., POZZAN, T. & RINK, T. J. (1982). T-cell mitogens cause early changes in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. *Nature* **295**, 68–71.

WEISS, A., IMBODEN, J., HARDY, K., MANGER, B., TERHORST, C. & STOBO, J. (1986). The role of the T3/antigen receptor complex in T-cell activation. *Annual Review of Immunology* **4**, 593–619.

WEISS, A., IMBODEN, J., SHOBACK, D. & STOBO, J. (1984). Role of T3 surface molecules in human T cell activation: T3-dependent activation results in an increase in cytoplasmic free calcium. *Proceedings of the National Academy of Sciences of the USA* **81**, 4169–4173.