

I. Ion Channels in Human THP-1 Monocytes

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Abstract. The THP-1 human monocytic leukemia cell line is a useful model of macrophage differentiation. Patch clamp methods were used to identify five types of ion channels in undifferentiated THP-1 monocytes. (i) Delayed rectifier K⁺ current, I_{DR} , was activated by depolarization to potentials positive to -50 mV, inactivated with a time constant of several hundred msec, and recovered from inactivation with a time constant ~21 sec. I_{DR} was inhibited by 4-aminopyridine (4-AP), tetraethylammonium (TEA⁺), and potently by charybdotoxin (ChTX). (ii) Ca-activated K⁺ current (I_{SK}) dominated whole-cell currents in cells studied with 3–10 μ M [Ca²⁺]_i. I_{SK} was at most weakly voltage-dependent, with reduced conductance at large positive potentials, and was inhibited by ChTX and weakly by TEA⁺, Cs⁺, and Ba²⁺, but not 4-AP or apamin. Block by Cs⁺ and Ba²⁺ was enhanced by hyperpolarization. (iii) Nonselective cation current, I_{cat} , appeared at voltages above +20 mV. Little time-dependence was observed, and a panel of channel blockers was without effect. (iv) Chloride current, I_{Cl} , was present early in experiments, but disappeared with time. (v) Voltage-activated H⁺ selective current is described in detail in a companion paper (DeCoursey & Cherny, 1996. *J. Membrane Biol.* **152**:2). The ion channels in THP-1 cells are compared with channels described in other macrophage-related cells. Profound changes in ion channel expression that occur during differentiation of THP-1 cells are described in a companion paper (DeCoursey et al., 1996. *J. Membrane Biol.* **152**:2).

Key words: THP-1 — Ion channels — Phagocytes — K⁺ channels — Macrophages — Monocytes — Cellular differentiation

Introduction

THP-1 is a human monocytic leukemia cell line (Tsuchiya et al., 1980). When stimulated with phorbol ester, THP-1 cells differentiate into adherent macrophage-like cells which mimic native human macrophages with regard to morphological characteristics, expression of membrane antigens, receptors, and oncogenes, and production of secretory products (Auwerx, 1991). Compared to other human myeloid cell lines such as HL-60, U937, KG-1 or HEL, differentiated THP-1 cells more closely resemble native monocyte-derived macrophages (Auwerx, 1991), and therefore are a widely studied and useful model of cellular regulation and differentiation in the monocytic lineage.

The expression of ion channels in monocytes, macrophages, and related cell lines changes, sometimes dramatically, during the course of cell maturation, differentiation, or activation (reviewed by Gallin, 1991). This suggests that ion channels play important roles in cellular regulation of differentiation and of function. Although several studies have explored the effects of ion channel blockers on functional properties of THP-1 cells (Pellassy, Cattani & Aussel, 1992; Gallin, Mason & Moran, 1994; Crutchley, Conanan & Que, 1995), at present there is little information on what types of channels are present and their pharmacological sensitivity. Two types of K⁺ channels, probably corresponding with I_{DR} and I_{SK} , have been reported in THP-1 cells (Grygorczyk & Rodger, 1993), and we have presented preliminary accounts of this work (Kim, DeCoursey & Silver, 1994; Kim et al., 1995). Here we identify seven main types of ion channels in either undifferentiated or differentiated THP-1 cells. Four are K⁺ selective, one each is H⁺ or Cl⁻ selective, and one is nonselective among small cations. Five types of ion channels are expressed by undifferentiated THP-1 cells, of which four are described in this

paper. We compare the properties of these channels with those described previously in other macrophages and related cells. The properties of H^+ currents and changes which occur during differentiation are described in the following paper (DeCoursey & Cherny, 1996). The final paper in the series describes two additional K^+ channels present only in differentiated THP-1 cells and delineates changes in ion channel expression and corresponding ion channel mRNA levels that occur during differentiation (DeCoursey et al., 1996). The proposed functions of ion channels in THP-1 and other macrophage-related cells are discussed in the next two papers.

Materials and Methods

CELL PREPARATION

THP-1 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were cultured in suspension in RPMI medium supplemented with 0.29 mg/ml glutamine, 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY), 100 U/ml of penicillin, 100 μ g/ml streptomycin, and 0.25 μ g/ml Fungizone (Amphotericin B, Gibco). Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Every 2–3 days about half of the media was replaced with fresh media, and once per week the cells were removed, centrifuged at 1800 rpm for 10 min at 4°C in a Sorvall RT6000 refrigerated centrifuge with a Sorvall H1000B rotor. The cell pellet was resuspended in fresh media at $1-2 \times 10^6$ cells/ml.

In early experiments, the cells and media were simply diluted with fresh media without centrifugation. Using this procedure THP-1 cells expressed either I_{DR} or I_{IR} , in contrast with the studies reported here in which control cells expressed only I_{DR} . We interpreted this behavior to indicate that the media has become contaminated by the accumulation of some substance (e.g., endotoxin from occasional bacteria) which inadvertently activated the cells. After we adopted the more stringent procedure of passaging the cells by centrifugation and removal of all media each week, the behavior of the cells became quite consistent. The ~200 cells studied before adopting the new procedure have been excluded from this study.

ELECTROPHYSIOLOGY

Micropipettes were pulled in several stages using a Flaming Brown automatic pipette puller (Sutter Instruments, San Rafael, CA) from EG-6 glass obtained from Garner Glass (Claremont, CA). Pipettes were coated with Sylgard 184 (Dow Corning, Midland, MI) and heat-polished to a tip resistance measured in Ringer's solution of typically 1–5 M Ω . Pipette and the initial bath solutions were filtered at 0.1 μ m (Millipore, Bedford, MA). The current signal from the patch clamp (Axopatch-1A, Axon Instruments, Burlingame, CA, or List EPC-7, Darmstadt, Germany) was digitized and stored in computer files for offline analysis using Indec Laboratory Data Acquisition and Display Systems (Indec Corporation, Sunnyvale, CA). Experiments were done using the whole-cell, on-cell, and excised patch configurations (Hamill et al., 1981). Inside-out patches were formed by lifting the pipette into the air briefly and returning it to a bath solution with low free Ca^{2+} . Experiments are done at room temperature (20–23°C).

THP-1 cells are nonadherent. An aliquot of cells was added to the recording chamber and allowed to settle. With maintained weak

positive pressure, the pipette was placed on or near a cell and then suction was initiated.

SOLUTIONS

Solutions were varied according to the ion channel studied and are listed in the Table. Most salts and buffers and apamin were purchased from Sigma Chemical (St. Louis, MO). Methanesulfonate ($MeSO_3^-$) salts were prepared by titrating methanesulfonic acid (Aldrich Chemical, Milwaukee, WI) with the appropriate cation hydroxide to make a stock solution at 1 M, from which the solutions were prepared. Measurements of the relative permeability of Tl^+ were made in $TlNO_3$, bracketed between and compared with KNO_3 solution, because $TlCl$ is insoluble. Charybdotoxin was purchased from Bachem (King of Prussia, PA). Ca^{2+} -activated K^+ currents were examined using solutions buffered to various free Ca^{2+} concentrations, $[Ca^{2+}]$, by adding various amounts of $CaCl_2$ to 10 mM EGTA, assuming apparent binding constants of 0.216 M for Mg^{2+} and 3.76×10^{-7} M for Ca^{2+} (derived from Martell & Smith, 1974). The $[Ca^{2+}]$ of these solutions measured with a Ca^{2+} -sensitive electrode (F2002 Ca^{2+} Selectrode, Radiometer, Copenhagen, Denmark) using the Radiometer Ion 83 Ion Meter agreed very roughly with the calculated value except at high $[Ca^{2+}]$. In the text we give the nominal values for $[Ca^{2+}]$ because it is not clear whether the measured values were necessarily more accurate. In any case, the solutions with higher $[Ca^{2+}]$ should be considered quite approximately determined because they are above the optimal range of Ca^{2+} buffering by EGTA. Liquid junction potentials between the pipette solution and the bath, and between the bath and the bath electrode were calculated or measured and were corrected in derived data (e.g., current-voltage plots) but not in raw current records.

DATA ANALYSIS

Fitting digitized currents, $I(t)$, with a single exponential was done by adjusting by eye the amplitude, A , time constant, τ , and steady-state current, I_{SS} , of a curve drawn according to: $I(t) = A(\exp(-t/\tau)) + I_{SS}$, which was superimposed on the data points. Boltzmann functions were fitted to conductance data by nonlinear least squares: $g/g_{max} = \{1 + \exp[(V - V_{1/2})/V_{slope}]\}^{-1}$, allowing g_{max} , $V_{1/2}$, and V_{slope} to vary. Dose-response curves were fitted by eye by superimposing on the data points a curve drawn according to: $I/I_{max} = (1 + [drug]/K_i)^{-1}$, where $[drug]$ is the concentration of blocker, and K_i is the dissociation constant, or half-blocking concentration.

Results

DELAYED RECTIFIER K^+ CURRENT (I_{DR})

Figure 1 illustrates whole-cell currents in THP-1 monocytes dominated by delayed rectifier currents, I_{DR} . A family of voltage-clamp currents in Ringer's solution is shown in Fig. 1A. The corresponding peak and steady-state current-voltage relations in Fig. 1B show that K^+ currents in this cell were activated by depolarizing pulses positive to -40 mV. Activation was half maximal at -20 ± 4.4 mV (mean \pm SD, 7 cells), determined by fitting a Boltzmann function (Materials and Methods) to the chord conductance-voltage data generated assuming a reversal potential of -86 mV. In cells studied with iso-

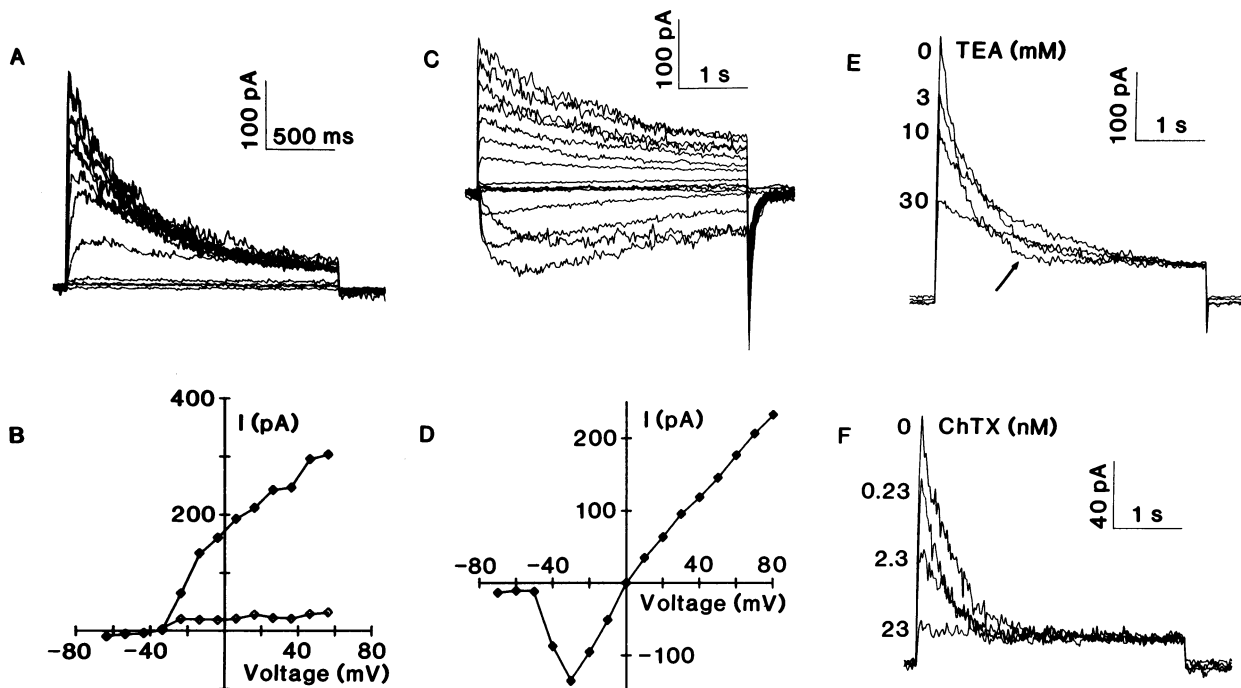


Fig. 1. Properties of I_{DR} . (A) A family of superimposed delayed rectifier K^+ currents in a THP-1 cell in Ringer's solution, with KCl solution in the pipette. Depolarizing pulses 2 sec in duration were applied from a holding potential, V_{hold} , of -80 mV, with an interval of 23 sec between pulses. Illustrated pulses are from -60 to $+60$ mV in 10 mV increments (before correction for liquid junction potentials). Filter 2 kHz. (B) Current-voltage relationship for the cell in part A. Peak current (\blacklozenge) and current at the end of the 2-sec pulses (\blacklozenge) are shown, with the points connected by straight lines. (C) A family of voltage-clamp currents in a cell bathed in isotonic K^+ Ringer's solution. From $V_{hold} = -80$ mV, 4-sec pulses were applied in 10 mV increments at 47-sec intervals from -70 to $+70$ mV. Filter 2 kHz. (D) Peak current-voltage relationship for the experiment shown in C. (E) State dependent block of I_{DR} by TEA^+ . Currents recorded during identical 4-sec pulses to $+20$ mV are superimposed. The indicated concentration of TEA^+ (in mM) was added to the Ringer's solution, with $KMeSO_3$ in the pipette. The cell was "rested" for >60 sec before each pulse. Note that TEA^+ slowed the decay of current, and that the control current (arrow) crosses over the others. (F) Block of I_{DR} by ChTX. Superimposed are currents during 4-sec test pulses to $+20$ mV, with >60 sec at $V_{hold} = -80$ mV before each pulse.

tonic K^+ Ringer's in the bath (Fig. 1C), large inward K^+ currents were observed between -50 mV and 0 mV and outward currents at more positive potentials. The peak current-voltage relationship in Fig. 1D for this experiment reversed near 0 mV in symmetrical K^+ solutions, consistent with this conductance being K^+ selective. The average reversal potential, V_{rev} , in 4.5 mM $[K^+]_o$ was -85.3 ± 3.9 mV (mean \pm SD, $n = 6$ cells), measured using tail currents, and in 150 mM $[K^+]_o$ was $+1.6 \pm 0.9$ mV ($n = 5$). The observed shift of 86.9 mV is close to the 88.6 mV calculated from the Nernst equation for a perfectly K^+ selective channel.

Blockers

Several K^+ channel inhibitors were tested to compare the sensitivity of the delayed rectifier in THP-1 monocytes with that of K^+ channels in other cells. Figure 1E shows inhibition by external TEA^+ . The peak current was reduced with $K_i = 12.5 \pm 2.5$ mM (mean \pm SD, $n = 5$). TEA^+ slowed the inactivation of I_{DR} with the result that the currents cross over with increasing TEA^+ concentra-

tions. This slowing of inactivation has been interpreted to mean that open channel block by TEA^+ interferes with channel inactivation (Grissmer & Cahalan, 1989a). A similar phenomenon was observed in alveolar epithelial K^+ currents (Jacobs & DeCoursey, 1990). Bath application of 100 μ M 4-AP abolished I_{DR} (data not shown). Figure 1F shows inhibition by charybdotoxin, ChTX, a toxin extracted from *Leiurus quinquestratus* scorpion venom. ChTX appeared to scale down the currents without affecting the kinetics, with a mean K_i of 1.7 ± 0.5 nM ($n = 3$).

Inactivation of I_{DR}

Delayed rectifier K^+ currents inactivated during long depolarizing pulses (Fig. 1A). Inactivation was substantial but not complete in Ringer's solution during 2-sec pulses (open symbols in Fig. 1B), and appeared to be slower and less complete in K^+ Ringer's solution (Fig. 1C), consistent with effects of $[K^+]_o$ on delayed rectifier K^+ channels in other cells (Ruben & Thompson, 1984; Kostyuk & Martynyuk, 1988; Grissmer & Cahalan, 1989b; De-

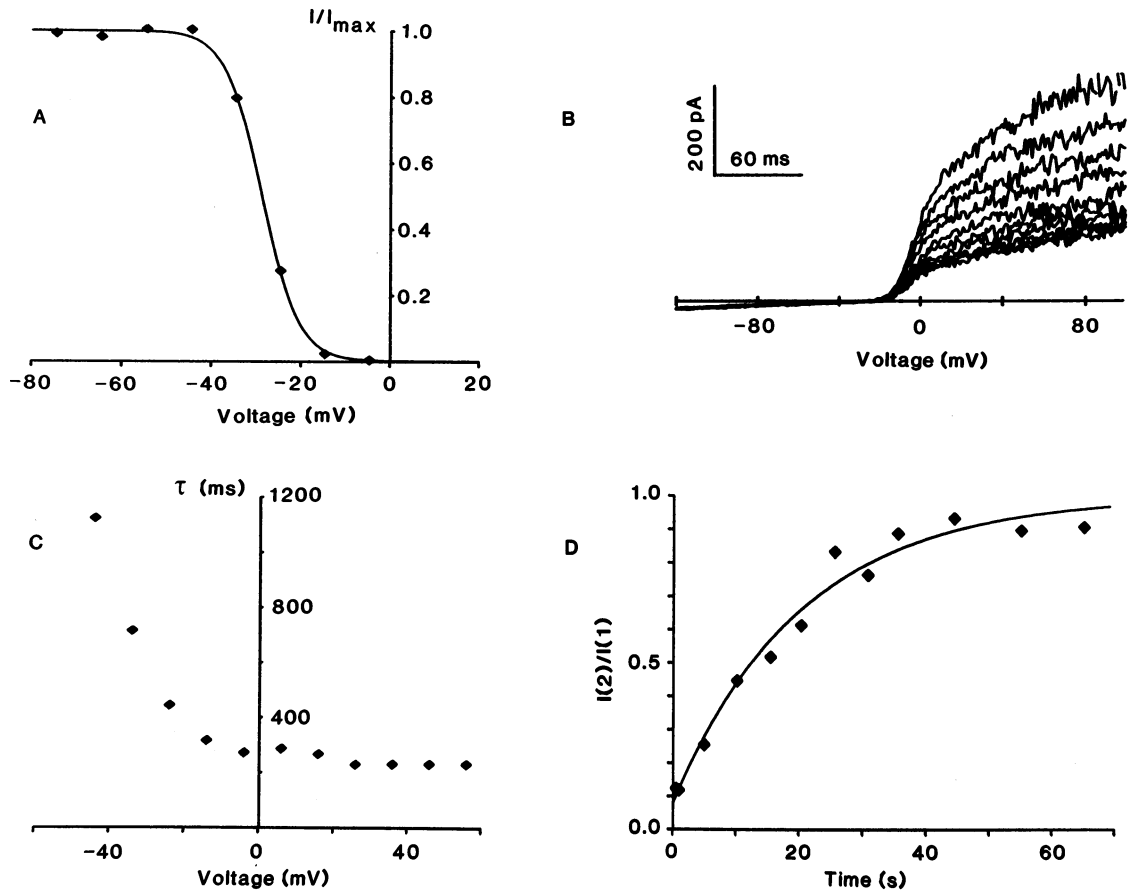


Fig. 2. Inactivation of I_{DR} . (A) Voltage dependence of prepulse inactivation. From $V_{hold} = -80$ mV, 4-sec prepulses to the potential on the abscissae were followed immediately by a test pulse to $+60$ mV. The inactivating current (i.e., the difference between peak and steady-state current) during this test pulse is plotted, normalized to the fitted maximum test current, 770 pA in this cell. The curve shows the fit to a Boltzmann (Materials and Methods) with midpoint $V_{1/2} = -24.1$ mV and slope factor $V_{slope} = 4.15$ mV. The cell was rested for 60 sec at V_{hold} before each pulse. Ringer's solution in the bath, KCl in the pipette. (B) Accumulation of inactivation of delayed rectifier during repeated voltage ramps. After "resting" the membrane at -80 mV for at least 60 sec, the membrane potential was ramped from -120 to $+100$ mV at a rate of 716 mV/sec, and was held for 50 msec at $V_{hold} = -80$ mV and then 60 msec at -120 mV between successive ramps. The outward current was largest during the first ramp, and decreased progressively with repeated ramps. The obvious accumulation of inactivation was characteristic of I_{DR} . (C) Voltage dependence of the time constant of inactivation in a typical cell in Ringer's solution, with KCl in the pipette. The decay of K^+ current during 4-sec depolarizing pulses was fitted with a single exponential. (D) Time course of recovery from inactivation in Ringer's solution, with KCl in the pipette. From $V_{hold} = -80$ mV, pairs of 4-sec pulses to 0 mV were applied with various intervals between pulses. The amplitude of the peak current during the second pulse is plotted, after normalization to the peak current during the first pulse. The data were fitted by nonlinear least-squares to a single exponential to obtain the time constant of recovery, 20.5 sec in this experiment.

Coursey, 1990; Demo & Yellen, 1991). The apparent voltage dependence of inactivation of I_{DR} was examined using 3–4 sec prepulses to various voltages followed by a test pulse to $+40$ or $+60$ mV. Figure 2A shows the test current amplitude in one cell, fitted with a Boltzmann function (Materials and Methods). The average midpoint was -35.7 ± 4.7 mV (mean \pm SD, $n = 4$). In experiments in which we wanted to eliminate I_{DR} to observe other currents, we set V_{hold} at -20 mV.

Inactivation accumulated during rapidly repeated depolarizing pulses or voltage ramps, suggesting that the rate of recovery from inactivation was slow. The superimposed ramp records in Fig. 2B illustrate the accumu-

lation of inactivation of I_{DR} . This behavior was one criterion used to identify I_{DR} . The time course of I_{DR} inactivation during depolarizing pulses in Ringer's solution (Fig. 1A) was fit by a single exponential with time constant, τ_i . Inactivation was faster at more positive voltages and τ_i was virtually independent of voltage at potentials above -10 mV (Fig. 2C). The average τ_i at $+20$ mV in 4.5 mM $[K^+]_o$ was 334 ± 139 msec (mean \pm SD, $n = 4$), with $KMeSO_3$ in the pipette and 579 ± 148 msec ($n = 9$) with KCl. Effects of intracellular anions on τ_i of I_{DR} in human T lymphocytes have been described previously (Cahalan et al., 1985). To examine the time course of recovery from inactivation, we used a

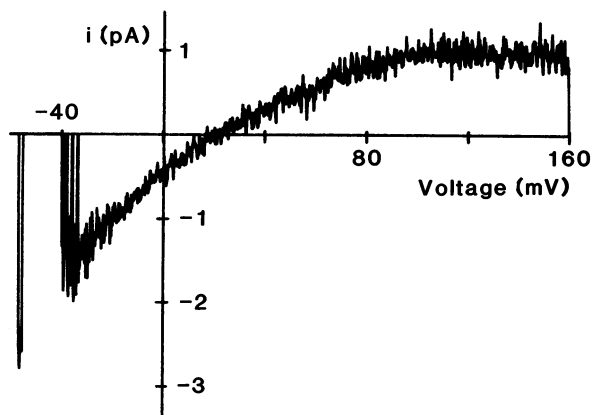


Fig. 3. Single-channel I_{DR} current-voltage relationship in a cell-attached patch. The pipette contained Mg^{2+} -free $KMeSO_3$. Currents elicited by voltage ramps were sorted according to whether a channel was open, and the average closed-channel current was subtracted from the average open-channel current. Channels like this one were identified as I_{DR} by their voltage-dependent gating, inactivation (periods of many seconds in which no channels opened), rectification, and conductance consistent with I_{DR} channels in other cells. In addition, cells in which I_{DR} channels were identified in cell-attached patch configuration had macroscopic I_{DR} in whole-cell configuration. The patch was held at RP -60 mV, and the voltage was ramped from RP -60 to RP $+160$ mV at 0.44 V/sec. The bath contained Ringer's solution, filter 1 kHz.

pulse protocol consisting of a pair of identical 4-sec voltage steps to 0 mV from a holding potential of -80 mV with variable intervals between pulses. The normalized amplitude of the second pulse is plotted in Fig. 2D as a function of the interval between pulses. The time constant of recovery from inactivation fit by first-order kinetics was $\tau = 21.2 \pm 3.2$ sec (mean \pm SD, $n = 3$). This much slower recovery than onset of inactivation is the cause of the characteristic accumulation of inactivation in cells with I_{DR} .

Single delayed rectifier channel currents were often observed in cell-attached patches of THP-1 monocytes. The net (leak-subtracted) single channel current-voltage relationship is plotted in Fig. 3. The unitary current rectifies inwardly, and actually decreases at large positive potentials, possibly due to block by an intracellular cation. The slope conductance at V_{rev} was roughly 20 pS in experiments with high $[K^+]$ pipette solutions.

SMALL CONDUCTANCE Ca -ACTIVATED K^+ CURRENT (I_{SK})

Figure 4A illustrates whole-cell ramp currents in a THP-1 monocyte studied with a pipette solution in which Ca^{2+} was weakly buffered, with 0.1 mM EGTA and no added Ca^{2+} . The only current evident in Ringer's solution was I_{DR} . Addition of ionomycin to the bath increased the conductance at all potentials. This calcium-activated conductance was evidently K^+ selective be-

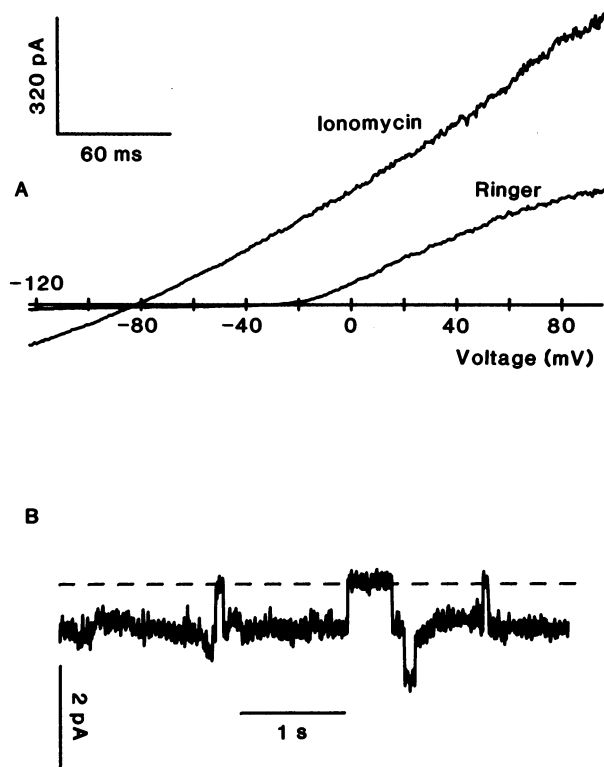


Fig. 4. (A) Ionomycin elicits I_{SK} . The whole-cell current during a voltage ramp in Ringer's solution in a cell containing KCl with no added Ca^{2+} and 0.1 mM EGTA shows only prominent I_{DR} (which inactivated progressively during subsequent ramps). After addition of 2 μ M ionomycin to the bath, a large conductance appeared which reversed near E_K and exhibited little time dependence during voltage pulses, I_{SK} . (B) Single-channel I_{SK} currents in a cell-attached patch. The pipette contained $KMeSO_3$, the bath K^+ Ringer's solution, with 10 μ M A23187 added. The resting potential of the cell was presumably "clamped" near 0 mV by this solution. The holding potential was -40 mV (relative to the resting potential), the broken line indicates the current level when no channels were open. No channel openings were detected before adding A23187 to the bath.

cause it reversed near the K^+ equilibrium potential, E_K . To study the properties of this conductance more systematically, we used pipette solutions with $[Ca^{2+}]$ buffered to various levels. We use the term I_{SK} advisedly, recognizing that small conductance Ca^{2+} -activated K^+ channels vary widely in their properties (Latorre et al., 1989); the term "intermediate" or IK has also been applied to ChTX-sensitive, apamin-insensitive channels (Varnai et al., 1993). In some cells, delayed rectifier currents could be seen in the presence of the I_{SK} , but in others I_{SK} was so large that it swamped out other currents which might have been present.

Single-channel currents attributable to I_{SK} channels are illustrated in Fig. 4B. In cell-attached patch configuration, there was initially no channel activity. When A23187 was added to the bathing solution, two channels appeared. The unitary conductance was roughly 20 – 25

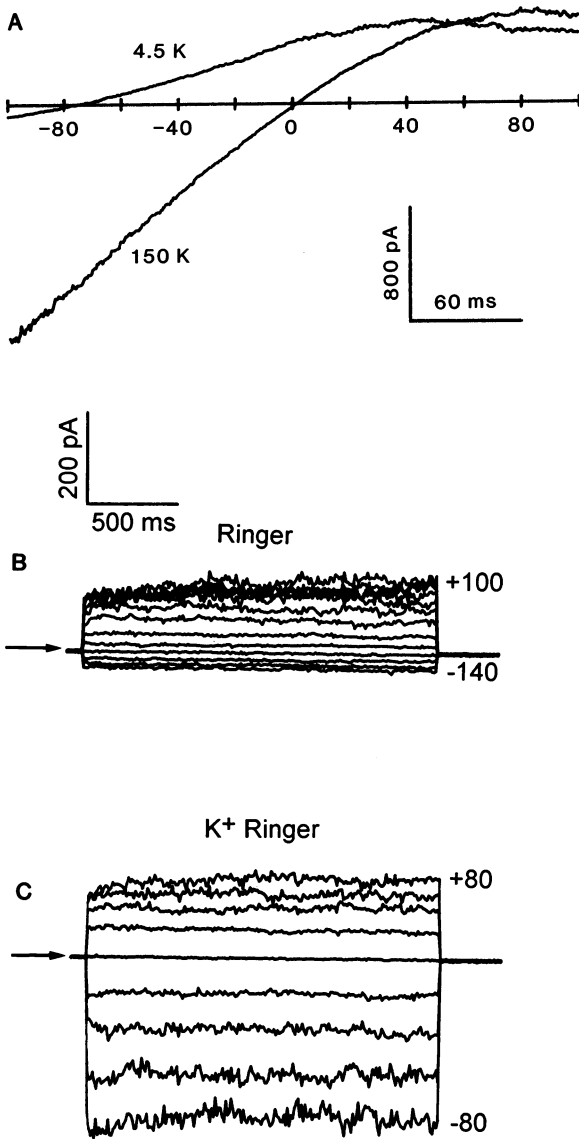


Fig. 5. (A) I_{SK} during voltage ramps in the same cell in Ringer's solution (4.5 K⁺) and K⁺ Ringer's (150 K⁺), with 10 μ M [Ca²⁺]_i KCl in the pipette. Note the pronounced inward rectification at depolarized potentials. The time-independence of I_{SK} can be seen in families of currents during voltage pulses in Ringer's solution (B) and in K⁺ Ringer's solution (C) in another cell studied with the same pipette solution. Voltages were incremented in 20-mV steps between the indicated extremes. V_{hold} was -80 mV in Ringer's and 0 mV in K⁺ Ringer's solution; arrows indicate zero current.

pS. Similar currents were seen in inside-out patches exposed to elevated [Ca²⁺]_i.

I_{SK} at Different [K⁺]_o

In the whole-cell experiment illustrated in Fig. 5A, the pipette contained 10 μ M free Ca²⁺. In Ringer's solution (4.5 mM K⁺) the membrane conductance was high at all

potentials, but V_{rev} was near -80 mV, suggesting that a K⁺ selective conductance was active, rather than a simple leak. When the bath was changed to 150 mM K⁺ Ringer's solution, V_{rev} was 0 mV, confirming the K⁺ selectivity of this conductance. In other experiments with 3–10 μ M Ca²⁺ in the pipette solution, whole-cell currents usually reversed between -70 and -80 mV in Ringer's solution and near 0 mV in K⁺ Ringer's, both close to E_K . Any nonselective leak conductance will bring V_{rev} in Ringer's solution toward more positive values, and in a given cell a shift of V_{rev} towards 0 mV in Ringer's solution was taken as an indication that the membrane was becoming damaged and nonselectively leaky. We did not pursue the alternative possibility that high [Ca²⁺]_i also activates nonselective channels.

Figure 5B and C illustrate I_{SK} currents during voltage pulses. In general, there was no obvious or consistent time dependence. In the cell illustrated, there was a suggestion of turn-on during pulses to positive potentials, but in other cells this was not observed and sometimes partial decay occurred. Because it was not possible to be sure in any given cell that the entire conductance was due to I_{SK} alone, we are reluctant to ascribe much significance to the weak and variable evidence of time dependence, and therefore consider I_{SK} to be practically time independent. A consistent feature of I_{SK} however, was the reduced conductance at large positive potentials, apparent both during ramps (Fig. 5A) and during pulses in both Ringer's and K⁺ Ringer's solution (Fig. 5B and C, respectively). This behavior is strongly reminiscent of voltage-dependent ionic blockade by intracellular cations. The only cation other than K⁺ in the pipette solution was 2 mM Mg²⁺, which produces similarly weak block of outward current through a number of K⁺ channels. Whether Mg²⁺ block causes the observed rectification was not explored.

[Ca²⁺]_i Dependence of I_{SK}

I_{SK} was obvious in cells studied at 10 μ M [Ca²⁺]_i, and was also seen in many cells at lower nominal [Ca²⁺]_i. The average slope conductance in Ringer's solution at V_{rev} was 2.3 ± 1.8 nS (mean \pm SD, $n = 21$) at 10 μ M [Ca²⁺]_i, 1.8 ± 1.3 nS ($n = 11$) at 3 μ M [Ca²⁺]_i, and 0.5 ± 0.5 nS ($n = 3$) at 1 μ M [Ca²⁺]_i. With nominally 38 nM [Ca²⁺]_i (Table, KCl pipette solution) small inward currents resembling I_{SK} were observed in only 7 of 37 cells and had an average amplitude of 0.24 nS in those cells. With pipette solutions of 1 or 3 μ M [Ca²⁺]_i, the amplitude of I_{SK} appeared to be somewhat variable with time in some cells, presumably related to changes in the access resistance, i.e., the patency of the pipette tip. Accurate determination of the [Ca²⁺]_i dependence of I_{SK} would require a more effective Ca²⁺ buffer than EGTA or an independent estimate of [Ca²⁺]_i. Nevertheless, it is ap-

Table. Composition of solutions

Name	K ⁺	Ca ²⁺	Mg ²⁺	Cl ⁻	MeSO ₃ ⁻	EGTA	pH	HEPES
Pipette solutions (mM)								
KCl	167	1	2	146	0	11	7.2	10
KCl, 10 μM Ca ²⁺	162	9.65	2	143.3	0	10	7.2	10
KMeSO ₃	161	1	2	6	133	11	7.2	10
KMeSO ₃ , Mg ²⁺ free	167	2	0	4	120	10 ^a	7.2	10
External solutions (mM)								
Name	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Cl ⁻	MeSO ₃ ⁻	pH	buffer
Ringer's	4.5	149	2	1	150.5	0	7.4	5 ^b
K ⁺ Ringer's	150	0	2	1	151	0	7.4	10 ^b
KMeSO ₃	150	0	2	0	4	150	7.0	20 ^c
^d X ⁺ Ringer's	164	0	2	1	166	0	7.4	10 ^b

^a Includes also 10 mM EDTA. The final K⁺ concentration after titrating the pH with KOH is given. CsCl, NaCl, and TMACl pipette solutions were identical to KCl except all of the K⁺ was replaced with the appropriate cation.

^b HEPES, ^c BES, ^d X⁺ means any cation (Rb⁺, TMA⁺, Na⁺, Li⁺, Cs⁺, NH₄⁺, TEA⁺). See Materials and Methods for a description of the Tl⁺ Ringer's solution.

parent that I_{SK} is activated by elevated $[Ca^{2+}]_i$. The data are generally consistent with $[Ca^{2+}]_i$ sensitivity of I_{SK} channels in human T lymphocytes or HL-60 cells, which are maximally activated at $\sim 1 \mu M$ $[Ca^{2+}]_i$ (Grissmer, Nguyen & Cahalan, 1993; Varnai et al., 1993).

Selectivity of I_{SK}

The selectivity of I_{SK} was explored by applying voltage ramps and changing the cation in the bath solution, with a $10 \mu M$ $[Ca^{2+}]_i$ K⁺ solution in the pipette (*data not shown*). We assumed that the main conductance under these conditions was I_{SK} . For K⁺, Rb⁺, and Cs⁺ measurements V_{hold} was set at 0 mV to inactivate I_{DR} , and for less permeant ions with quite negative V_{rev} , I_{DR} would not be active near V_{rev} anyway. The permeability to a specific cation, relative to K⁺, P_X/P_K , was calculated for these bi-ionic conditions from the Goldman-Hodgkin-Katz voltage equation (Hille, 1992). Best estimates for the relative permeabilities are:

$$Tl^+ > K^+ > Rb^+ > NH_4^+ > Cs^+ > Li^+ \geq Na^+ > TMA^+, TEA^+ \\ 1.77 \quad 1.0 \quad 0.89 \quad 0.22 \quad 0.094 \leq 0.005 \leq 0.004 < 0.001 < 0.001$$

The measured value for V_{rev} , particularly for the weakly permeant ions, may be erroneously positive due to any leak current which is present. The P_X values given are based on 1 to 6 measurements, but each represents the average of the two values with the lowest P_X , because we did not correct for leak current. Leak current would lead to an overestimate of P_X . The sequence should be correct, unless the leak itself is selective, because it is based on comparisons within individual cells. For example,

studied in the same cell V_{rev} was distinctly more negative in TMA⁺ or TEA⁺ solutions than in Na⁺ or Li⁺.

Pharmacological Sensitivity of I_{SK}

The traditional K⁺ channel blocker tetraethylammonium, TEA⁺, was moderately effective for I_{SK} . Fig. 6A illustrates a family of I_{SK} currents in Ringer's solution. Addition of 30 mM TEA⁺ (Fig. 6B) appeared to scale down the current uniformly at all potentials. In the presence of 30 mM TEA⁺ I_{SK} was evidently not completely blocked, because V_{rev} was still near E_K . This is more apparent in Fig. 6C, which shows the dose-response relationship for TEA⁺ using voltage ramps. Without correcting for leak current, K_i was estimated to be 10–25 mM in Ringer's solution in two cells. Figure 6D and E illustrate that ChTX inhibits I_{SK} , like TEA⁺, without obvious voltage dependence. The dose-response relationship for ChTX using voltage ramps (Fig. 6F) indicates that this toxin is a potent inhibitor of I_{SK} , although it is a more potent blocker of I_{DR} (Fig. 1F). The K_i estimated for ChTX block of I_{SK} was 4–12 nM in Ringer's solution ($n = 2$) and 10–30 nM in K⁺ Ringer's solution ($n = 2$).

Figure 7 illustrates block of I_{SK} in K⁺ Ringer's solution by TEA⁺ (A), Cs⁺ (B), and Ba²⁺ (C). No time-dependence was detected during voltage pulses for any blockers of I_{SK} , so the voltage dependence seen in ramps is a reasonable indication of steady-state behavior. As in Ringer's solution, TEA⁺ added to K⁺ Ringer's solution produced practically voltage-independent block (Fig. 7A). Half block of I_{SK} in K⁺ Ringer's solution by TEA⁺ was estimated in two cells at several potentials to be 15–25 mM. Onset and removal of block occurred as rap-

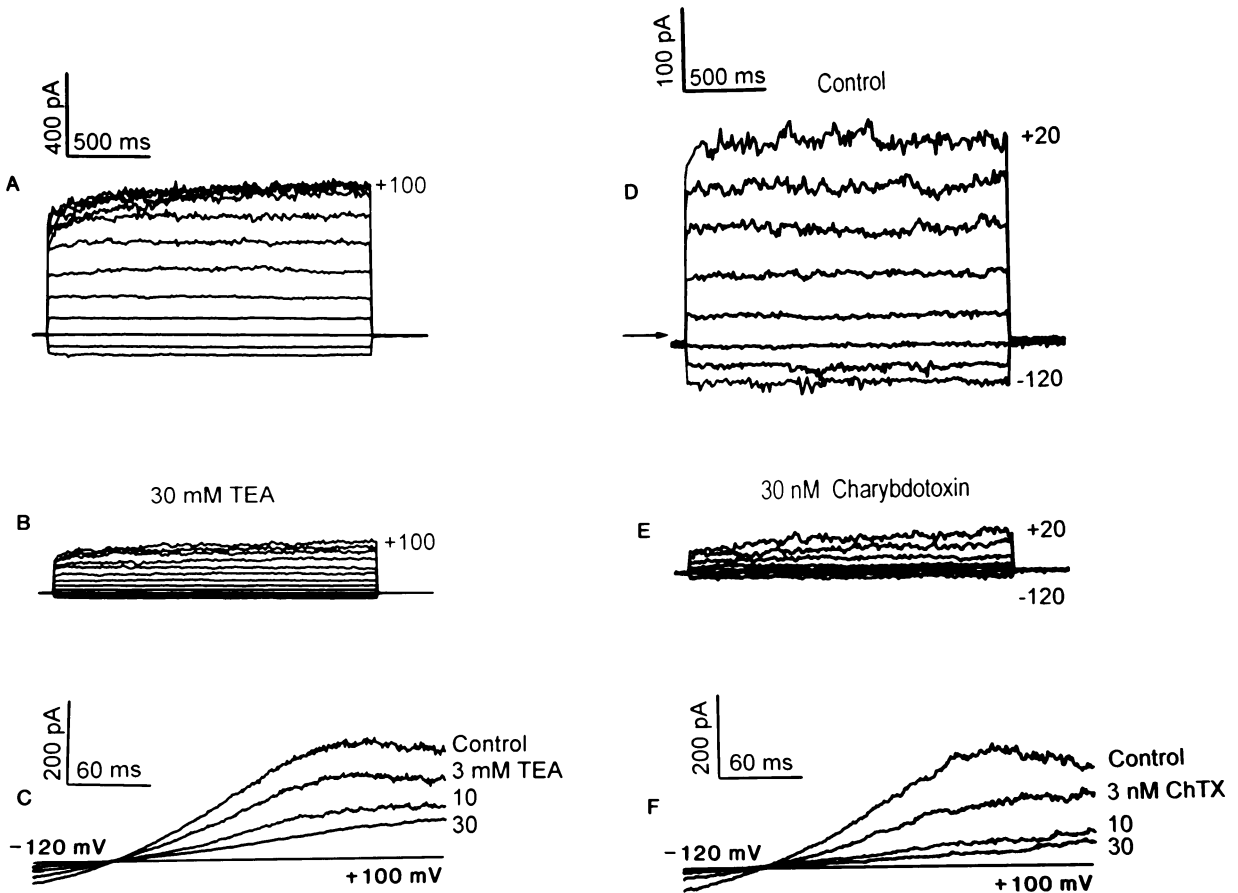


Fig. 6. Block of I_{SK} by TEA^+ and ChTX in Ringer's solution. (A) I_{SK} elicited by voltage pulses, in 20 mV increments from -120 to $+100$ mV from $V_{hold} = -80$ mV. The zero current potential was near V_{hold} here and in B. Pipette solution $10 \mu M [Ca^{2+}]_i$ KCl, $V_{hold} = -80$ mV. (B) The identical pulse family in the presence of 30 mM TEA^+ . (C) Currents during voltage ramps from -120 to $+100$ mV in the same cell in the presence of the indicated concentrations of TEA^+ , substituted for Na^+ . $V_{hold} = -80$ mV. (D) A family of currents during voltage pulses in a different cell studied with $10 \mu M [Ca^{2+}]_i$ KCl in the pipette. Pulses were applied in 20 mV increments between -120 and $+20$ mV. Arrow indicates zero current level, which was near V_{hold} in E. (E) Currents during pulses identical with D applied in the presence of 30 nM ChTX. (F) Block of I_{SK} currents by ChTX in the same cell. Ramps from -120 to $+100$ mV from $V_{hold} = -80$ mV.

idly as the solutions were changed (on the order of 10 sec). For Cs^+ and Ba^{2+} , block was much greater at more negative potentials. The outward current was reduced in an apparently voltage-independent manner, but this inhibition appeared qualitatively different from the voltage-dependent block of inward currents. Voltage-dependent block of inward current occurred immediately when Cs^+ or Ba^{2+} was added, whereas outward currents were reduced gradually and progressively over tens of seconds after the bath change was complete. As the outward current was reduced, there was also a comparable scaling down of inward currents. The illustrated ramp currents were recorded 1–2 min after each solution change. Upon washout, the voltage-dependent block was immediately removed, but the scaling down of the conductance was reversed more slowly, and not always completely. The impression received was that some

slow process modulated the overall level of activation of the I_{SK} conductance, in addition to the specific reversible voltage-dependent block by Cs^+ or Ba^{2+} of the open channel. The voltage-dependent ionic blockade of I_{SK} by Cs^+ or Ba^{2+} was rather weak, being distinctly incomplete at moderate negative voltages even at 150 mM Cs^+ or 100 mM Ba^{2+} .

External 4-AP at 1 mM and apamin at 1 μM had no effect on I_{SK} .

NONSELECTIVE CATION CURRENT (I_{cat})

A time-independent outward current (I_{cat}) was observed in addition to the inactivating outward K^+ current in some cells. Figure 8A shows both I_{cat} and I_{DR} elicited by depolarizing voltage steps from a holding potential of -80

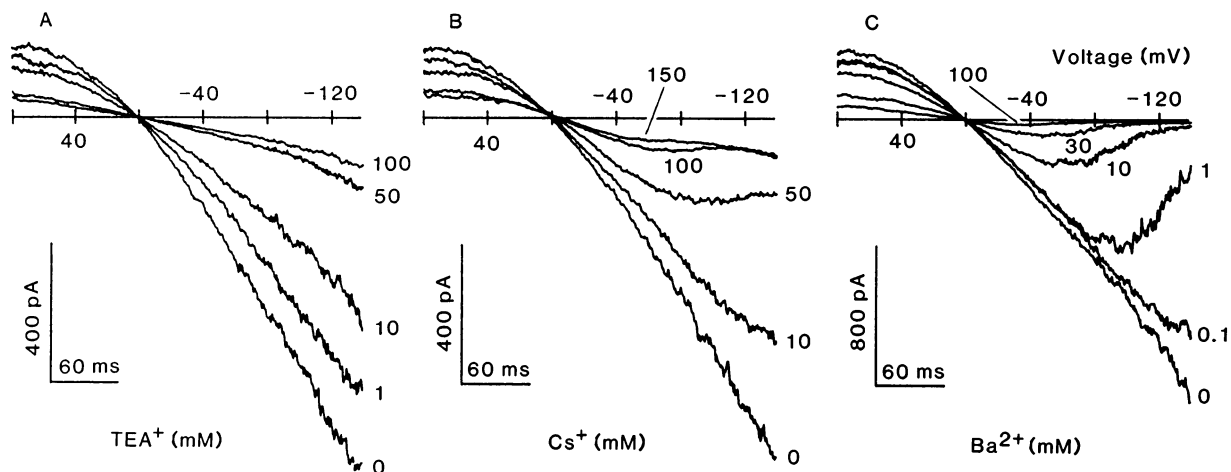


Fig. 7. Block of I_{SK} by TEA^+ , Cs^+ , and Ba^{2+} in a cell studied in K^+ Ringer's solution, with $10 \mu M$ Ca^{2+} KCl in the pipette. All blockers were added from 1 M stock solutions to K^+ Ringer's solution, resulting in some dilution of its constituents (e.g., by 10% for 100 mM blocker), as well as hypertonicity. (A) I_{SK} during voltage ramps from +80 to -140 mV in the indicated concentrations of TEACl added to K^+ Ringer's solution. Block and recovery from block of both inward and outward currents occurred rapidly during solution changes. (B) I_{SK} in the presence of the indicated concentrations of Cs^+ . Both block and unblock were rapid for the voltage-dependent component of block, but slower for the voltage-independent scaling down of both inward and outward currents. (C) I_{SK} in the indicated concentrations of Ba^{2+} . The I_{SK} current recovered only half its original amplitude after washout of high Ba^{2+} concentrations. The ramps in Ba^{2+} were done before those in TEA^+ (A) or Cs^+ (B) thus their control currents are smaller. Illustrated ramps currents were recorded 1–2 min after each solution change. Note the pronounced enhancement of block at more negative voltages.

mV. When V_{hold} was -30 mV (Fig. 8B) I_{DR} was inactivated and only the time-independent I_{cat} remained. I_{cat} was evidently responsible for most of the current at the end of long depolarizing pulses. In some cells (e.g., Fig. 1A) little current remained after I_{DR} had inactivated, but in others (e.g., Fig. 8A and B) I_{cat} was prominent.

I_{cat} was small immediately after establishing whole-cell configuration, becoming larger over several minutes. Figure 8C illustrates the development of I_{cat} in a THP-1 monocyte studied with NaCl in the pipette. Whole-cell currents during voltage ramps are superimposed, measured at several times after establishing whole-cell configuration. At 2 min there was only a small linear leak current. By 10 min there was pronounced I_{cat} , with its characteristic steep voltage dependence, activating above +40 mV. The amplitude increased further over the next 8 min. When voltage pulses were applied in this cell (*not shown*), the currents exhibited little time-dependence and the same voltage dependence as in the ramps.

Large I_{cat} was observed in cells studied with K^+ (Fig. 8A and B), Cs^+ (Fig. 9A-C), or Na^+ (Fig. 9D-F) in the pipette solution, but not TMA^+ or TEA^+ (*not shown*). The identity of the extracellular cation (Na^+ , K^+ , Cs^+ , TEA^+ , TMA^+) had no obvious effect on these currents. Replacing external Cl^- with $MeSO_3^-$ did not reduce I_{cat} , which thus appears to be selective for small cations. The following ion channel inhibitors, applied externally, did not inhibit I_{cat} : 1 mM Zn^{2+} , >100 mM TEA^+ , 1 mM 4-AP, 100 nM ChTX, 5 mM Ba^{2+} , 1 mM Gd^{2+} , 1 mM SITS, 100 μM amiloride. No inhibitor of I_{cat} has been found.

CHLORIDE CURRENTS (I_{Cl})

Chloride currents, I_{Cl} , were often present in THP-1 cells shortly after whole-cell configuration was established, but ran down progressively over a few minutes or tens of minutes. The typical appearance of I_{Cl} is illustrated in Fig. 10A. Whole-cell currents during voltage ramps are plotted in normal Ringer's solution, and in low Cl^- Ringer's solution. Replacing the external Cl^- with $MeSO_3^-$ greatly reduced the outward current and shifted the zero current potential to more positive potentials. These changes were reversible. Some outward rectification persisted in symmetrical $MeSO_3^-$ solutions, suggesting that $MeSO_3^-$ carries some current through Cl^- channels. No time- or voltage-dependent gating was evident at moderate potentials in some cells, but in other cells the outward current decayed at large positive potentials.

Block

Figure 10B illustrates block of I_{Cl} by 1 mM SITS (4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid). In this experiment the pipette contained TMACl, thus the outward rectification cannot be attributed to asymmetrical Cl^- concentrations and must reflect a property of the g_{Cl} . Addition of SITS decreased both inward and outward currents. Block by SITS was not reversible. Because of the temporal changes in g_{Cl} and the difficulty of distinguishing it from leak current, we did not attempt to quantify the amplitude of I_{Cl} .

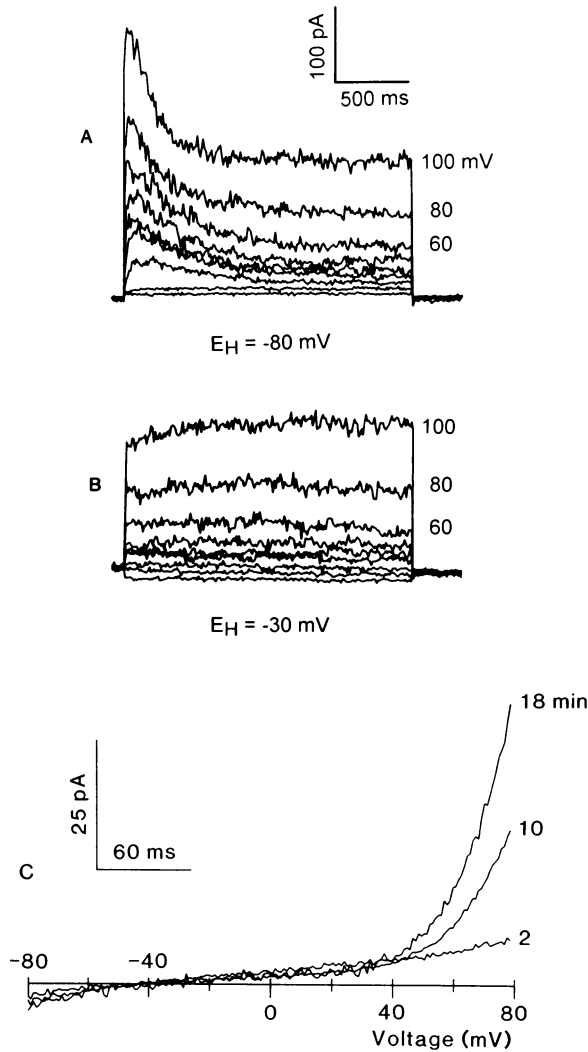


Fig. 8. Family of currents in a cell containing KCl pipette solution and bathed in Ringer's solution, exhibiting both I_{DR} and nonselective cation current, I_{cat} . In A the holding potential was -80 mV and both currents are seen, I_{DR} inactivating and I_{cat} time-independent. When V_{hold} was set at -30 mV (B) the same pulses elicited only I_{cat} . In both the pulses were in 20 mV increments from -60 to $+100$ mV. (C) Development of I_{cat} with time in a different cell. Currents during voltage ramps are superimposed for the indicated times (min) after establishing whole-cell configuration. Several consecutive ramp currents were averaged for each record. The bath contained Ringer's solution, and the pipette NaCl.

Large Anion Channels

In rare instances we observed large unitary currents which behaved differently than I_{BK} , the high-conductance voltage- and $[Ca^{2+}]_i$ -activated K^+ channels described in the third paper in this series (DeCoursey et al., 1996). These channels were observed too infrequently to allow complete characterization. Their open probability

was not obviously voltage dependent at moderate potentials. In one inside-out patch, outward single-channel currents persisted when Na^+ replaced K^+ in the bath, but substituting $MeSO_3^-$ for Cl^- reduced the conductance from 350 pS to 210 pS.

PROTON CURRENT (I_H)

Voltage-activated H^+ selective currents were observed in THP-1 cells before and after differentiation. The properties and expression of I_H are described in the next paper (DeCoursey & Cherny, 1996).

Discussion

DELAYED RECTIFIER K^+ CURRENT (I_{DR})

The delayed rectifier in THP-1 monocytes, I_{DR} , activated with depolarization above -50 mV, inactivated during sustained depolarizations with a τ_i ~ 300 – 600 msec (faster with $MeSO_3^-$ than Cl^- pipette solutions), recovered slowly from inactivation with τ ~ 21 sec, and was inhibited by external TEA $^+$ (K_i 12.5 mM) and ChTX (K_i 1.7 nM). Single I_{DR} channels had a conductance of ~ 20 pS at V_{rev} in symmetrical high $[K^+]$. I_{DR} in THP-1 monocytes thus resembles that in other human and mouse macrophages (Ypey & Clapham, 1984; Gallin & Sheehy, 1985; Randriamampita & Trautmann, 1987; Nelson, Jow & Popovich, 1990b), although to our knowledge ChTX block of I_{DR} has not been previously demonstrated in macrophages, and only recently in microglia (Eder et al., 1995). The properties, especially the ChTX sensitivity and inactivation kinetics, of the delayed rectifier K^+ channel in THP-1 cells are virtually identical with those of type 'n' K^+ channels in rat alveolar epithelial cells (DeCoursey, Jacobs & Silver, 1988; Jacobs & DeCoursey, 1990; DeCoursey, 1990) and in human and murine T lymphocytes (Cahalan et al., 1985; Deutsch, Krause & Lee, 1986; DeCoursey et al., 1987; Sands, Lewis & Cahalan, 1989). In a companion study we use the polymerase chain reaction (PCR) to show that Kv1.3 mRNA is present in THP-1 cells and suggest that it codes for I_{DR} (DeCoursey et al., 1996).

SMALL CONDUCTANCE Ca-ACTIVATED K^+ CURRENT (I_{SK})

Selectivity

The selectivity sequence found for I_{SK} is similar to that of many types of K^+ channels (Hille, 1992), and is consistent with the relative permeability of I_{SK} channels in human erythrocytes (Christoffersen, 1991) and in hu-

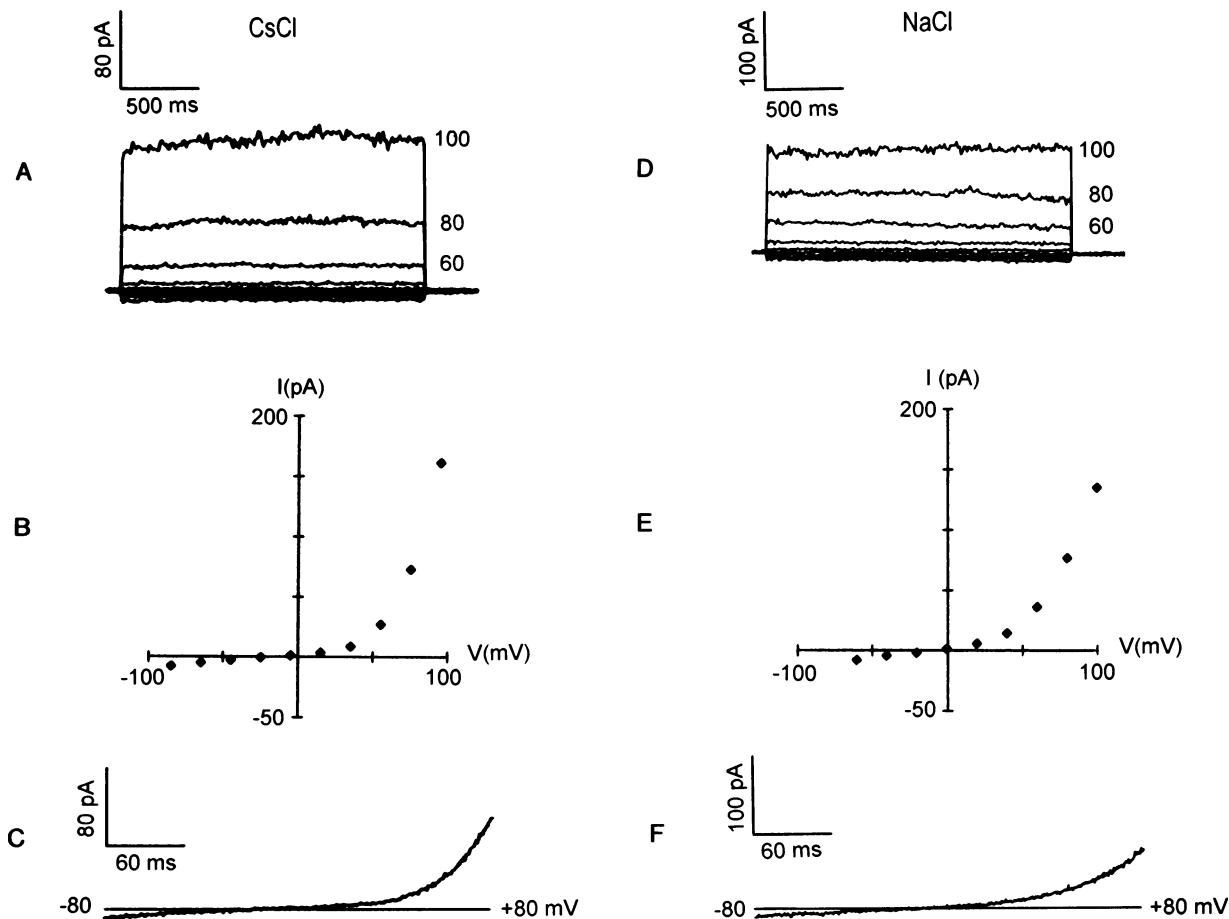


Fig. 9. Nonspecific cation currents in cells perfused with CsCl (A–C) or NaCl (D–F) pipette solutions, all in Ringer's solution. Illustrated for each cell is a family of currents during voltage pulses between -80 and $+100$ mV (A and D), the corresponding current-voltage relationship (B and E), and the current during a voltage ramp (C and F). V_{hold} was 0 mV in both experiments; in A and D the holding current was near zero.

man T lymphocytes (Grissmer et al., 1993). Cs^+ was clearly permeant through I_{SK} in THP-1 cells. The bionic reversal potentials in both Li^+ and Na^+ were clearly positive to those in TMA^+ or TEA^+ when measured in the same cell, thus Li^+ and Na^+ are detectably permeant as well.

Block

I_{SK} was blocked by ChTX and by TEA^+ , in both cases somewhat less potently than was I_{DR} . Block of I_{SK} by both drugs appeared to be voltage independent. The slightly more potent block of I_{DR} than I_{SK} by both TEA^+ and ChTX is quite similar to the pattern observed in activated human B lymphocytes (Partiseti et al., 1992); in activated T lymphocytes I_{DR} was more sensitive than I_{SK} to TEA^+ but was equipotently blocked by ChTX (Grissmer et al., 1993). Both Cs^+ and Ba^{2+} produced distinctly voltage-dependent block of I_{SK} , but were rela-

tively weak in comparison with their potency for blocking inward rectifier K^+ channels in THP-1 cells (DeCoursey et al., 1996). Thus, block of I_{SK} was not quite complete at 100 mM Ba^{2+} , and was clearly incomplete at 30 mM Ba^{2+} . In 150 mM Cs^+ there was still substantial inward and outward current. Similarly, in human macrophages I_{SK} was blocked in a voltage-dependent manner by Ba^{2+} and more weakly than was I_{IR} (Gallin, 1989).

Gating

I_{SK} appeared to be largely time- and voltage-independent. Sometimes there was a suggestion that the current turned on or off at certain potentials, but this behavior was neither consistent from cell-to-cell nor was it always convincingly distinguishable from poor capacity compensation or contamination from other ion channels which may have been present. However, the I_{SK} current consistently was reduced at large positive potentials. This

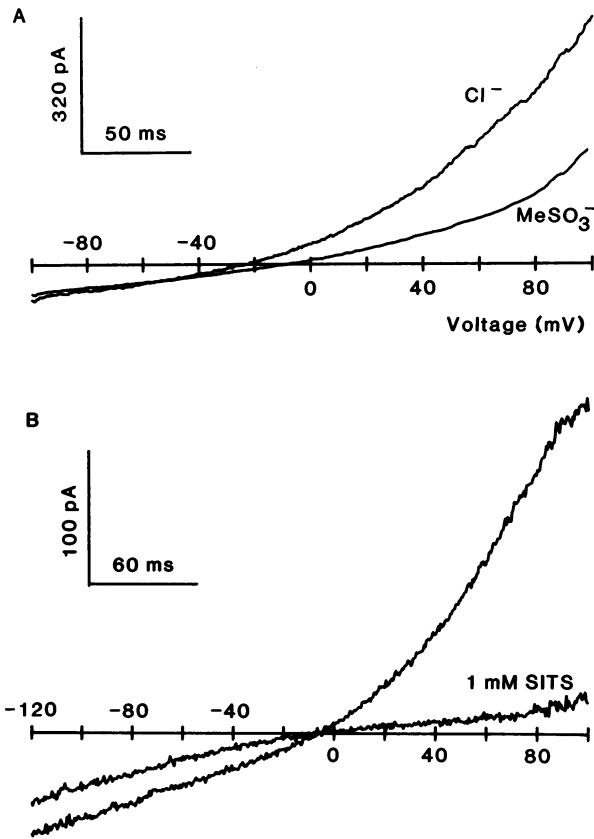


Fig. 10. (A) Whole-cell chloride currents during voltage ramps. The record labeled 'Cl⁻' was recorded in Ringer's solution, the smaller trace is the average of several currents recorded before and after in 'Cl-free' Ringer's solution, identical to Ringer's but with all but 6 mM of the Cl⁻ replaced by MeSO₃⁻. Recorded 6 min after whole-cell configuration was established. Pipette solution KMeSO₃. (B) Block of I_{Cl} by SITS. Whole-cell I_{Cl} during a voltage ramp in the absence and presence of 1 mM SITS. Pipette solution TMACl, bath Ringer's solution. Ramps were applied from -120 to +100 mV with 5 sec at V_{hold} = 0 mV between successive ramps.

reduction produced a region of negative slope conductance in some cells.

Presence in Other Monocytes

A similar I_{SK} channel has been described in human macrophages (Ince et al., 1987; Kakuta et al., 1988; Gallin, 1989), murine macrophages (Randriamampita & Trautmann, 1987; Hara et al., 1990), HL-60 cells (Wieland et al., 1992; Varnai et al., 1993), human B lymphocytes and rat thymocytes (Mahaut-Smith & Schlichter, 1989), and activated human T lymphocytes (Grissmer et al., 1993). The extent of inward rectification of the I_{SK} current-voltage relationship is somewhat variable from one study to another; however, we observed substantial cell-to-cell variability in this property. This variability may indicate that multiple types of I_{SK} channels are involved, that

other types of channels contaminate the data in some cells, or that I_{SK} is influenced by additional, unidentified factors. A voltage- and time-independent K⁺ conductance induced by G protein activators in murine macrophages and J774 cells generally resembles I_{SK} but is not blocked by ChTX (McKinney & Gallin, 1992). This conductance however, has been shown to appear spontaneously at high [Ca²⁺]_i in the absence of receptor stimulation (Fan & McCloskey, 1994) and therefore might conceivably have contaminated I_{SK} measurements in some cells in our study, because ChTX block was not tested in every cell. This G protein-related K⁺ conductance rectifies outwardly, whereas I_{SK} usually rectified inwardly. Occasional cells in which K⁺ selective currents at high [Ca²⁺]_i that rectified outwardly were observed may have expressed both types of K⁺ channels. The G protein-related K⁺ channel was described in murine macrophages and J774 cells (McKinney & Gallin, 1992; Fan & McCloskey, 1994), but to our knowledge has not been reported previously in human macrophages.

NONSELECTIVE CATION CURRENT (I_{cat})

A nonselective cation current, I_{cat}, was present in some THP-1 cells. This current was time-independent and became pronounced at potentials above +20 mV. I_{cat} was not affected by holding the cell at depolarized potentials which inactivated I_{DR}. No blockers were found. An apparently similar nonselective cation current has been described in corneal endothelial cells (Watsky, 1995). Although a variety of nonselective currents have been reported at the single-channel level (reviewed by Gallin, 1991), to our knowledge no comparable conductance has been described in macrophages.

CHLORIDE CURRENT (I_{Cl})

We observed outwardly-rectifying Cl⁻ currents early in whole-cell experiments, which disappeared over several minutes. Generally similar macroscopic Cl⁻ currents have been reported in human monocyte-derived macrophages (Nelson, Jow & Jow, 1990a). In human neutrophils, an outwardly-rectifying Cl⁻ conductance is activated by cell swelling (Stoddard, Steinbach & Simchowitz, 1993). Although we did not explore its sensitivity to osmotic stress, it is possible that I_{Cl} in THP-1 cells disappeared with time as a transient osmotic imbalance equilibrated. Large conductance voltage-dependent Cl⁻ channels have been described in murine macrophages (Schwarze & Kolb, 1984; Randriamampita & Trautmann, 1987) and in other cells. Generally similar large anion channels were observed infrequently in THP-1 cells. The macroscopic I_{Cl} described here exhibited neither voltage-dependence nor discrete current levels of comparable size, and thus must be due to a different

channel. Smaller conductance single Cl^- channels have been reported in human U937 monocytes (Kanno & Takishima, 1990). A SITS-sensitive anion conductance contributes to regulatory volume decrease in THP-1 cells challenged with hypotonic solutions (Gallin et al., 1994).

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