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DIFFERENTIAL EXPRESSION OF INWARD AND OUTWARD POTASSIUM CURRENTS IN THE MACROPHAGE-LIKE CELL LINE J774.1

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SUMMARY

1. J774.1 cells, a mouse-derived macrophage-like tumour cell line, were voltage clamped using whole-cell patch-clamp techniques. Cells were maintained in suspension cultures and plated at varying times before recording.

2. The average zero-current potential of long-term adherent (> 24 h) cells was −77·6 mV. A tenfold increase in $[K]_o$ produced a 49 mV shift in zero-current potential.

3. Freshly plated cells (< 24 h) expressed two voltage-dependent currents: an outward current expressed transiently from 1 to 12 h post-plating and an inward current expressed 2–4 h post-plating which persisted in 100 % of long-term adherent cells.

4. Inward current was dependent upon voltage, time and $[K]_o^{1/2}$, similar to the anomalous rectifier of other tissues. The conductance activated at potentials negative to −50 mV and plateaued at potentials negative to −110 mV. Inactivation was evident at potentials negative to −100 mV. Both the rate and extent of inactivation increased with hyperpolarization. Inward rectification was blocked by external $BaCl_2$ or CsCl.

5. The outward current was time- and voltage-dependent. The instantaneous I/V curves derived from tail experiments reversed at the potassium equilibrium potential (E_K). A tenfold change of $[K]_o$ shifted the reversal potential 52 mV, indicating that the current was carried by potassium. This conductance activated at potentials positive to −50 mV, plateaued at potentials positive to −10 mV and inactivated completely with an exponential time course at all potentials. At voltages positive to −25 mV the rate of inactivation was independent of voltage. The outward current was blocked by 4-aminopyridine or D600.

6. During the first 10 min after attaining a whole-cell recording, the conductance/voltage relation of the outward current shifted to more negative voltages and peak conductance showed a slight increase; recordings then stabilized. The voltage

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dependence of the inward current did not shift with time but wash-out of inward current was observed in some cells.

7. The J774.1 cell line can serve as a model for the study of the role of voltage-dependent ionic conductances in macrophages.

INTRODUCTION

Leukocytes exhibit a number of voltage-dependent ionic conductances which may be related to such cellular processes as secretion, chemotaxis, and mitogenesis (Gallin & Gallin, 1975; Fukushima, Hagiwara & Saxton, 1984; DeCoursey, Chandy, Gupta & Cahalan, 1984). Three different voltage-dependent potassium currents have been described in macrophages. Long-term cultured mouse macrophages exhibit an inward rectifying potassium current activating at potentials negative to -70 mV (Gallin, 1981). An outward potassium current activating at potentials positive to -40 mV has been described in shorter-term cultures (Ypey & Clapham, 1984). In addition, a calcium-activated potassium current has been described in cultured macrophages from a variety of sources (Gallin, Weiderhold, Lipsky & Rosenthal, 1975; Olivera-Castro & Dos Reis, 1981). Recent single-channel studies have demonstrated large conductance calcium- and voltage-activated potassium channels in cultured human macrophages (Gallin, 1984) and large conductance chloride-selective channels in mouse macrophages (Schwarze & Kolb, 1984).

In this study we used whole-cell patch-clamp techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) to characterize both inward and outward voltage-dependent potassium conductances in J774.1 cells. These cells, derived from a mouse reticulum sarcoma (Ralph & Nakoinz, 1975), have been extensively studied because they provide homogeneous populations that express many properties characteristic of macrophages, including receptor-mediated endocytosis, secretion of enzymes and immunomodulatory factors, and chemotaxis (Snyderman, Pike, Fischer & Koren, 1977). In addition, the existence of variant clones defective in specific functions may make them particularly useful in relating ionic conductances to macrophage function (Unkeless, Kaplan, Plutner & Cohn, 1979; Bloom, Diamond, Muschel, Rosen, Schneck, Damiani, Rosen & Scharff, 1978; Damiani, Kiyotaki, Soeller, Sasada, Peisach & Bloom, 1980).

Macrophages express a variety of activities depending on their activation state or, *in vitro*, upon culture conditions. For example, adherent culture conditions (in contrast to suspension culture) decrease the phorbol-ester-induced release of superoxide anion (Berton & Gordon, 1983) and alter the membrane transport of certain nutrients (Pofit & Strauss, 1977). Indirect estimates of membrane potential using tetraphenylphosphonium (TPP^+) indicated that J774.1 cells in suspension had a resting membrane potential of -14 mV (Young, Unkeless, Kaback & Cohn, 1983) whereas both intracellular and patch micro-electrode studies of adherent J774.1 cells have reported values considerably more negative (Sheehy & Gallin, 1984). These and other observations suggest that the biophysical properties of the macrophage plasma membrane may depend on whether the cell is in suspension or is adherent. In this work we demonstrate the differential expression of two voltage-dependent potassium currents in J774.1 cells upon transition from suspension to an adherent form. During

the first hours after plating, an outward potassium current is evident which closely resembles that described in other leukocytes (Cahalan, Chandy, DeCoursey & Gupta, 1985; Matteson & Deutsch, 1984; Ypey & Clapham, 1984). This current disappears and is followed by an inward potassium current which is similar to the inward rectifier described in long-term cultures of primary macrophages as well as in egg and muscle cells (Gallin, 1981; Hagiwara & Takahashi, 1974; Leech & Stanfield, 1981).

METHODS

Cell culture

J774.1 cells obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) were maintained in tissue culture medium containing RPMI 1640 (Flow Labs, McLean, VA, U.S.A.) supplemented with penicillin 10 u./ml, streptomycin 10 μg/ml (Difco Labs, Detroit, MI, U.S.A.), 0·03 % glutamine (w/v) (Sigma, St. Louis, MO, U.S.A.), and 10 % fetal bovine serum (v/v) (Hyclone, Logan, UT, U.S.A.). Stock cultures were maintained as a non-adherent population in spinner flasks treated with Sigmacote (Sigma, St. Louis, MO, U.S.A.). For experiments, cells were withdrawn and plated in 35 mm tissue culture Petri dishes (Corning Glass Works, Corning, NY, U.S.A.). Cultures were kept at 37 °C in a 7 % CO_2 incubator and fed every 2–3 days. Cells were 12–14 μm in diameter.

Electrical recordings

Recordings were made using the whole-cell variation of the patch-electrode voltage-clamp technique (Hamill *et al.* 1981). Electrodes were filled with 150 mM-KCl, 10 mM-NaCl, 1·0 mM-$MgCl_2$, 1·1 mM-EGTA, 0·1 mM-$CaCl_2$ (pCa = 7·7) and 10 mM-HEPES, and brought to pH 7·3. The bath solution contained 155 mM-NaCl, 4·6 mM-KCl, 1·6 mM-$CaCl_2$, 0·6 mM-$MgCl_2$, and 10 mM-HEPES (pH = 7·30). The liquid junction potential between the electrode and bath solutions (typically 4·5–5·5 mV, electrode negative) was subtracted from the voltage record. Ionic concentrations were used to calculate reversal potentials because at these ionic strengths, internal and external activities of sodium and potassium were within 2 % of each other (Fujimoto & Kubota, 1976). Experiments were performed at room temperature (22–25 °C). In experiments where $[K]_o$ was raised, KCl was substituted for NaCl. Electrodes were fire polished and coated with beeswax to reduce electrode capacitance. Resistances ranged from 2 to 10 MΩ; seal resistances ranged from 5 to 20 GΩ. Confluent cultures were not studied to avoid the possibility of recording from electrically coupled cells.

The output of a Dagan 8900 patch-clamp amplifier with a 1 GΩ feed-back resistor (Dagan Corp, Minneapolis, MN, U.S.A.) was filtered through a low-pass filter at 1 kHz; the output was displayed on the oscilloscope and a strip-chart recorder. The output was also tape-recorded (Ampex PR2200, Ampex Corp, Redwood City, CA, U.S.A.). I/V relations generated using slow voltage ramps (dV/dt = 20 mV/s) were plotted directly onto an X–Y recorder (Hewlett Packard 7015B, Hewlett Packard, San Diego, CA, U.S.A.). No series resistance compensation was performed. Since the maximum amplitude of currents measured in this study was < 0·5 nA, the error due to series resistance was no more than 5 mV and usually much less. Unless otherwise noted, cells were maintained at the initial zero-current holding potential and voltage steps were separated by 10 s. This was subtracted from the voltage records.

Statistical analysis and curve fitting were performed on a VAX 750 computer (DEC, Boston, MA, U.S.A.) using the RS1 software package (BBN Inc., Cambridge, MA, U.S.A.).

RESULTS

Resting membrane potential

Initial studies were done on long-term adherent cultures (> 24 h) of J774.1 cells because they were judged more comparable to previous intracellular micro-electrode studies of macrophages (Gallin, 1981; Gallin & Livengood, 1981). The zero-current holding potential determined within seconds of attaining the whole-cell configuration

was used as an estimate of resting membrane potential. The zero-current potential of thirty-seven cells averaged $-77{\cdot}6$ mV $\pm 0{\cdot}78$ (S.E. of mean); values ranged from -66 to -85 mV. The observation that the zero-current potential was within 10% of the potassium equilibrium potential ($E_K = -87$ mV assuming $2{\cdot}3\ RT/F = 57$ mV where R, T and F have their usual meaning, $[K]_o = 4{\cdot}6$ and $[K]_i = 150$ mM) indicates that shunt to ground across the pipette/cell seal or conductance to ions other than K^+ was no more than 10% of the whole-cell conductance. Zero-current potentials of long-term adherent J774.1 cells were routinely stable for greater than 30 min and as long as 90 min.

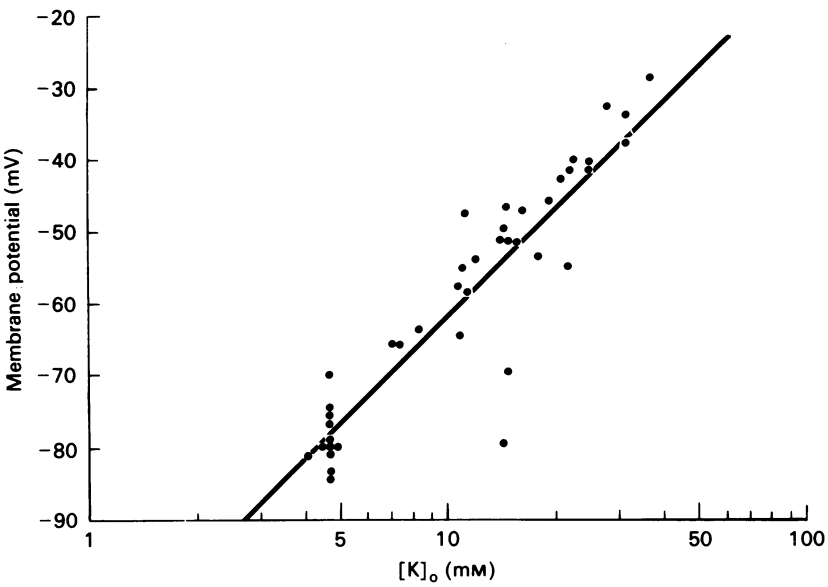

Fig. 1. Relation between membrane potential (estimated as zero-current holding potential) and $[K]_o$. Data are the summary of twelve different cells exposed to three or four increases in $[K]_o$.

To determine the relation between the resting membrane potential and the potassium equilibrium potential, studies were done in which the extracellular potassium was varied and the zero-current membrane potential was measured. The data obtained from twelve different cells each exposed to three or four different $[K]_o$ are shown in Fig. 1. The linear regression line drawn through these points has a slope of 49 mV/tenfold increase in $[K]_o$ (correlation coefficient $-0{\cdot}90$) compared to a predicted slope of 57 mV/decade change in $[K]_o$ for a potassium electrode.

I/V relations

All long-term adherent J774.1 cells showed marked inward rectification. Fig. 2*A* depicts the current responses of a cell to voltage steps taken from its zero-current potential of -80 mV. Hyperpolarizing voltage steps produced large inward currents which activated rapidly. For steps beyond -120 mV, the currents show a decrease with time. The current elicited by depolarizing steps was considerably smaller and

exhibited no time dependence. The I/V relations of this cell obtained from both voltage steps (circles) and a voltage ramp (continuous line, $dV/dT = 20$ mV/s) is shown in Fig. 2*B*. The apparent peak current, measured 10 ms after the beginning of the step (a time when the ionic current could be clearly distinguished from the capacitive transient) and the steady-state current obtained with voltage steps are

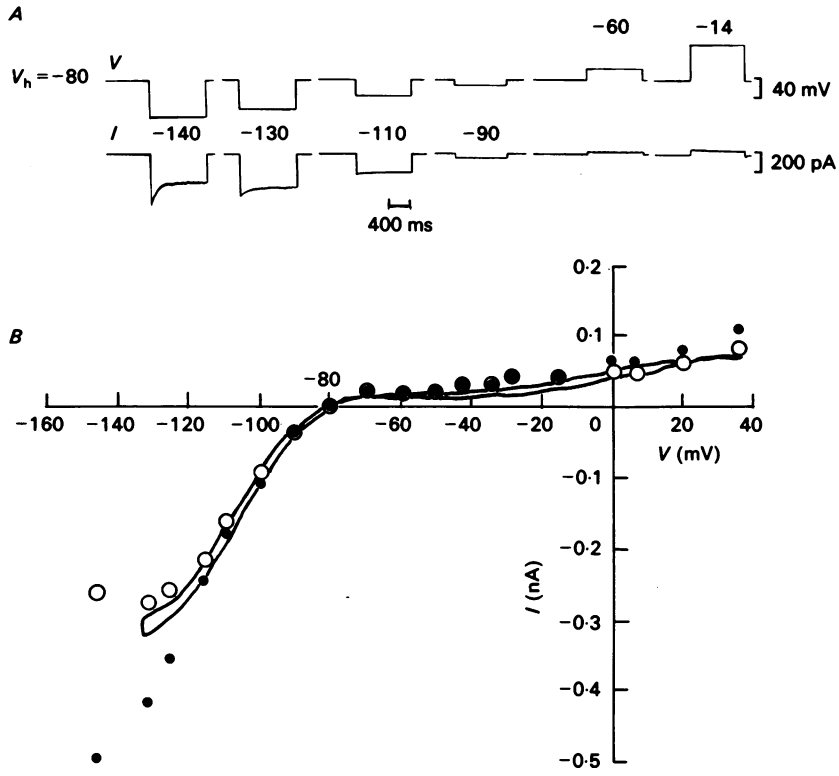

Fig. 2. *A*, current responses to a series of voltage steps from zero-current holding potential (−80 mV). Cell plated for > 24 h. *B*, I/V relation of cell shown in Fig. 2*A*. Filled circles represent current measured 10 ms after start of step. Open circles represent current measured at end of step (1 s). Voltage steps were applied every 10 s. Continuous line represents current generated by injection of a voltage ramp ($dV/dT = 20$ mV/s).

both plotted. Since there was no time dependence to current in the voltage range of +40 to −110 mV, the peak and steady-state curves as well as the ramp-derived curve are identical. However, at voltages negative to −110 mV they diverge. In other studies in which ramps to more negative potentials were done, a prominent hysteresis was evident (see Fig. 3*C* and *D*) due to time-dependent inactivation of the inward current. The cell in Fig. 2 had a maximum slope conductance of 9·6 nS for inward current calculated between −100 and −120 mV (for both the ramp- and step-derived curves) and a leak slope conductance, calculated from −50 to −40 mV, of 0·9 nS. The leak conductance of long-term adherent cells, calculated from −50 to −40 mV, was 1·01 nS ± 0·12 (mean ± S.E. of mean, $n = 22$).

Effect of adherence on zero-current potential and I/V relations

The resting membrane potential of non-adherent J774.1 cells as measured with TPP^+, an indirect probe of membrane potential, is significantly more positive than our values obtained on adherent J774.1 cells (Young *et al.* 1983). To investigate the cause of this difference, we measured the zero-current membrane potential of J774.1 cells previously grown in spinner culture and plated shortly before recordings were done. The zero-current holding potentials of cells studied at 30 min–24 h post-plating were similar to those of long-term adherent cultures (−66 mV or more negative), indicating that J774.1 cells probably hyperpolarize rapidly upon plating.

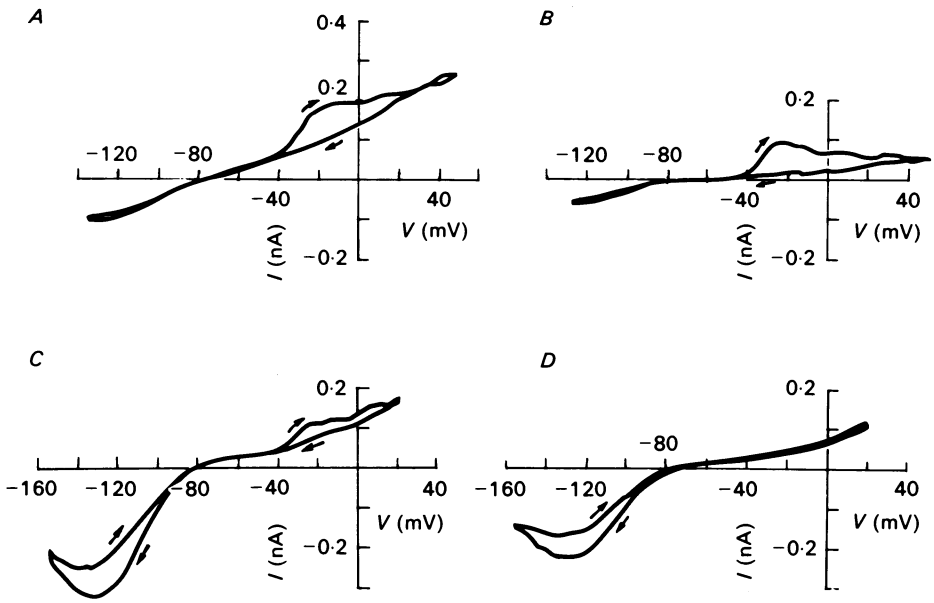

Fig. 3. *I/V* relations of four cells obtained by voltage ramp at various times after plating from suspension culture. Cells were held at zero-current potential. *A*, 1 h after plating. $V_h = -77$ mV. *B*, 3 h. $V_h = -78$ mV. *C*, 7 h. $V_h = -81$ mV. *D*, > 24 h. $V_h = -72$ mV.

The *I/V* characteristics of J774.1 cells varied considerably with time after plating. Four *I/V* curves representative of the pattern seen in the 195 cells analysed in this paper are shown in Fig. 3. Cells showed no evidence of inward rectification immediately after plating (< 1 h) although small inactivating, outward currents were sometimes noted. The increase in the slope of the *I/V* curve at voltages positive to −40 mV in the rising limb of the depolarizing ramp in Fig. 3*A* reflects activation of an outward current. The returning limb of the ramp was flat due to inactivation of this outward current. This inactivating outward current was observed up to 8 h after plating (Fig. 3*B* and *C*) but was absent in cultures more than 12 h old (Fig. 3*D*). The outward current is described in a later section of this paper. Inward rectification usually became evident about 2–4 h after plating (Fig. 3*C*) and was the characteristic feature of *I/V* curves obtained from cells plated 24 h or longer (Fig. 3*D*). Experiments with voltage steps confirmed the results obtained with ramps.

While the inward rectifier was always present in long-term cultures, expression of the outward current was variable. The fraction of cells expressing the outward current 2–4 h after plating could vary from 90 to 20 %. The consistent observation was that the inward current was not expressed upon plating and took 2 h or more to manifest. The inactivating outward current was seen between 1 and 12 h after plating and, with only one exception, never thereafter.

Analyses of the voltage dependence of the inward and outward currents which are presented later in this paper were all performed on stabilized cells. Apart from an occasional transient increase in leak current during the first 10 min of a recording, holding currents were stable throughout an experiment.

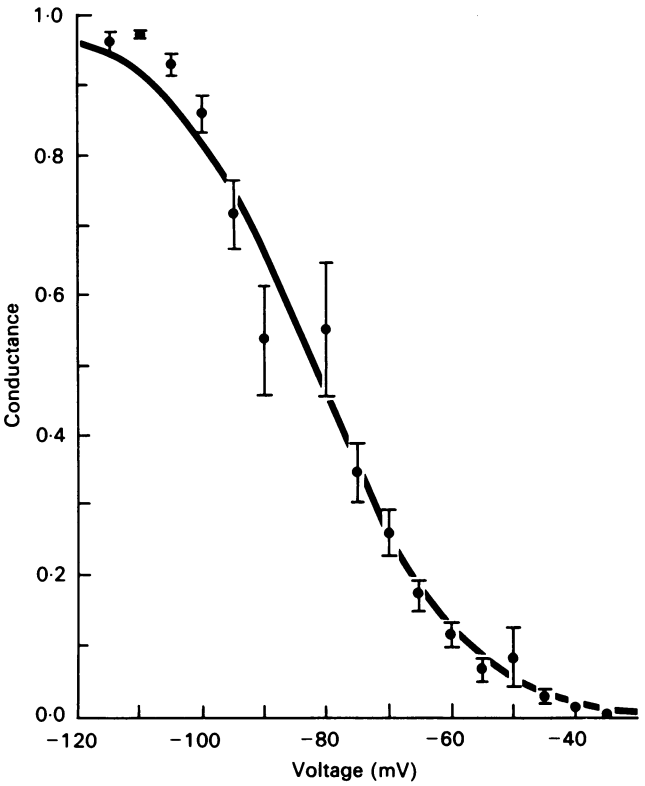

Fig. 4. Chord conductance *versus* voltage of twelve long-term adherent J774.1 cells. Leak conductance (calculated at −30 mV) was subtracted; each cell's curve was normalized by its peak conductance. Reversal potential was assumed to be −87 mV. Each point is the mean ± S.E. of mean. Continuous line conforms to eqn. (1).

Inward rectification

The conductance/voltage relation. Comparison of I/V curves obtained with voltage steps to those obtained with voltage ramps demonstrated that ramp-derived curves provided faithful representations of the peak instantaneous I/V relation up to −110 mV (Fig. 2*B*). Therefore the chord conductance of the inward current was calculated from I/V curves generated from ramps. Leak conductance, determined at −30 mV, was subtracted. The reversal potential (E_R) of the inward rectifying current

was assumed to be E_K (−87 mV). The conductance/voltage relations of twelve long-term adherent cells obtained in $[K]_o = 4{\cdot}6$ mM are presented in Fig. 4. The conductance values of each cell were normalized to 1·0 by its maximum (G_{max}) so that they could be pooled. The voltage at which the peak was observed varied from −110 to −120 mV; consequently, the pooled conductances at these potentials are slightly less than 1·0. The conductance is substantially activated at rest (about 39 % at −77 mV) and thus contributes to the resting membrane potential of the cell. At potentials beyond −110 mV the data indicate a drop in the inward rectifying conductance (this is more evident in Fig. 5*B*). This is an artifact resulting from time-dependent inactivation of the conductance during the ramp, since experiments with steps demonstrated that the inward conductance peaked by −110 mV and remained constant at more negative voltages. With the exception of the drop in conductance at potentials negative to −110 mV, the conductance/voltage parameters determined from peak currents in response to voltage steps agreed closely with those determined from current responses to voltage ramps. This indicates that inactivation positive to −110 mV during the course of the voltage ramp was insignificant.

The continuous line drawn through the points in Fig. 4 represents an empirical relation reported by Hagiwara for the anomalous rectifier in egg cells (Hagiwara & Takahashi, 1974). This relation is described by the equation:

$$G/G_{max} = 1/[1+\exp((V-V_h)/v)], \quad (1)$$

where v is a constant that characterizes the slope of the relation and V_h represents a constant that locates the curve along the voltage axis; at $V = V_h$ the conductance is half-maximal. $V = (V_m - E_K)$, the driving force of the current where V_m is the membrane potential. The data shown in Fig. 4 were fitted by non-linear least-squares regression. Values of $2{\cdot}85 \pm 0{\cdot}08$ and $11{\cdot}57 \pm 0{\cdot}25$ were obtained for V_h and v, respectively (coefficient of multiple determinations = 0·993). The relation is steep near rest; fractional activation drops from 66 % at −90 mV to 26 % at −70 mV. The average G_{max} of the cells in Fig. 4 was $6{\cdot}57$ nS $\pm 1{\cdot}06$ (mean ± S.E. of mean). Condutance/voltage characteristics of long-term adherent cells were routinely stable for greater than 30 min.

Effects of $[K]_o$. The inward rectifying potassium conductance in egg and muscle cells is a function of both the membrane potential and the $[K]_o$ (Hagiwara & Takahashi, 1974; Leech & Stanfield, 1981). To determine if this is also the case for macrophages, the effect of varying external potassium was investigated in J774.1 cells. Four I/V curves of a single cell taken after equilibration in sequentially increasing $[K]_o$ are shown in Fig. 5*A*. In addition to depolarizing the cell, raising $[K]_o$ increased the slope conductance for inward currents and shifted the voltage dependence of inward rectification to the right. In Fig. 5*B* the conductance/voltage relations of the cell are displayed for each of the four potassium concentrations shown in Fig. 5*A*. The continuous lines in Fig. 5*B* represent fits of each set of conductance data (not normalized by G_{max}) to eqn. (1); V_h and v determined at each $[K]_o$ were not substantially different from the values at $[K]_o = 4{\cdot}6$ mM when the appropriate values of E_K and G_{max} were substituted. A log–log plot of G_{max} *versus* $[K]_o$ is shown in Fig. 5*C*; linear least-squares regression gives a slope of 0·56 ($r = 0{\cdot}97$). Similar data from six different cells were normalized by dividing G_{max} determined at each $[K]_o$ by

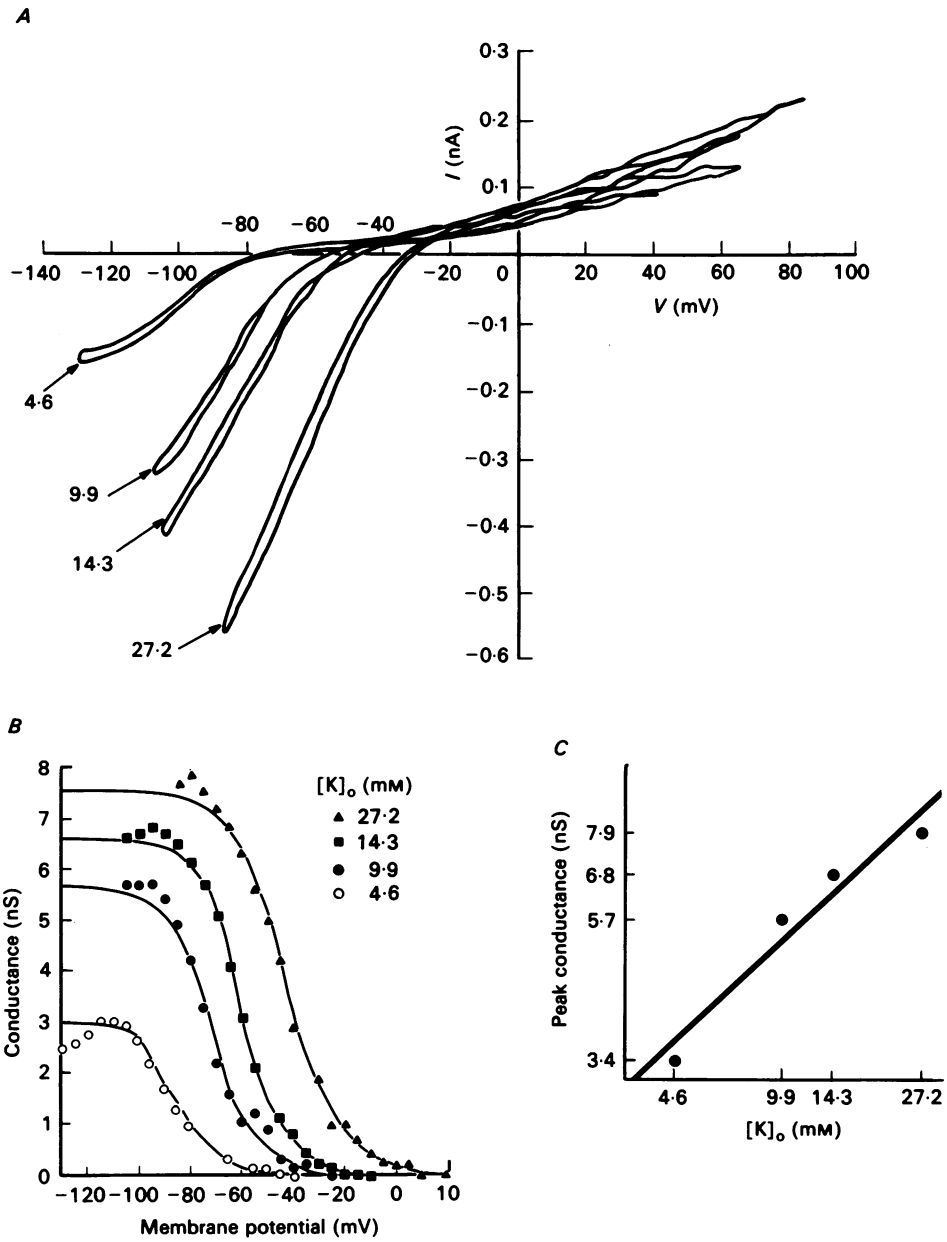

Fig. 5. *A*, *I/V* relations of the same cell taken sequentially in $[K]_o = 4{\cdot}6$, 9·9, 14·3 and 27·2 mM. Holding potentials were zero-current potentials: $V_h = -75$, -56, -50 and -33 mV, respectively. *B*, chord conductance *versus* voltage for each $[K]_o$ calculated as in Fig. 4; conductance 40 mV positive to rest was used as estimate of leak. *C*, log peak conductance *versus* log $[K]_o$. Data in panels *B* and *C* were fitted by least-squares regression ($r > 0{\cdot}97$).

G_{max} determined in $[K]_o$ 4·6 mM. The slope of a log–log plot of the normalized G_{max} *versus* $[K]_o$ for the six cells was 0·49. Thus, there is a square-root relation between the peak inward rectifying conductance and $[K]_o$, similar to that observed in egg and muscle cells (Hagiwara & Takahashi, 1974; Leech & Stanfield, 1981).

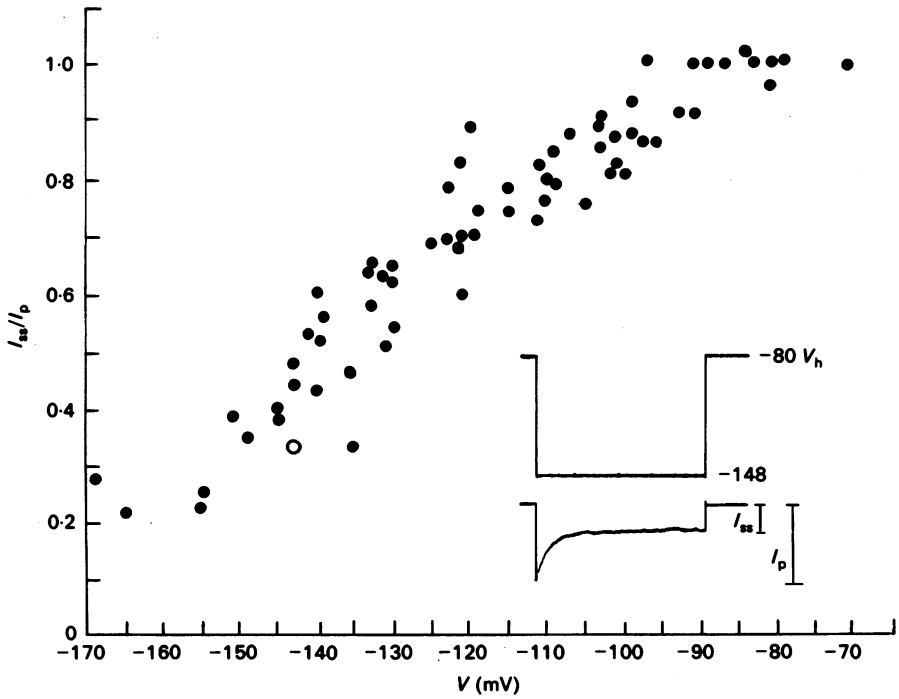

Fig. 6. Steady-state current/peak current (I_{ss}/I_p) *versus* voltage. Data from eight cells. I_p determined 10 ms after start of step, I_{ss} determined at end of 1 s step. Inset represented by open circle at −148 mV.

Inactivation. The *I/V* relations in Fig. 2*A* and *B* show that inward current was time independent for voltage steps up to −110 mV but decreased with time at more negative voltages and suggest that the inactivating component of total inward current increases with hyperpolarization. The ratio of the steady-state current (I_{ss}) to the apparent peak current (I_p) is plotted *versus* voltage for eight cells in Fig. 6. The peak was measured 10 ms after the initiation of the voltage step when the capacitive transient was clearly over. The data in Fig. 6 indicate that inactivation occurs at voltages negative to −100 mV and that the inactivating component of inward current increases with hyperpolarization. At the most negative voltages studied (−150 to −175 mV), the steady-state inward current was still greater than leak. It was not possible to determine whether the ratio of I_{ss}/I_p plateaued at very negative voltages since it was difficult to apply voltage steps to −150 mV or greater.

Fig. 7*A* shows the time-dependent current ($I_p - I_{ss}$) measured from current records as a function of time for four different voltage steps. The current relaxations were fit by single exponentials ($P < 0·01$). The time constants determined for voltage steps

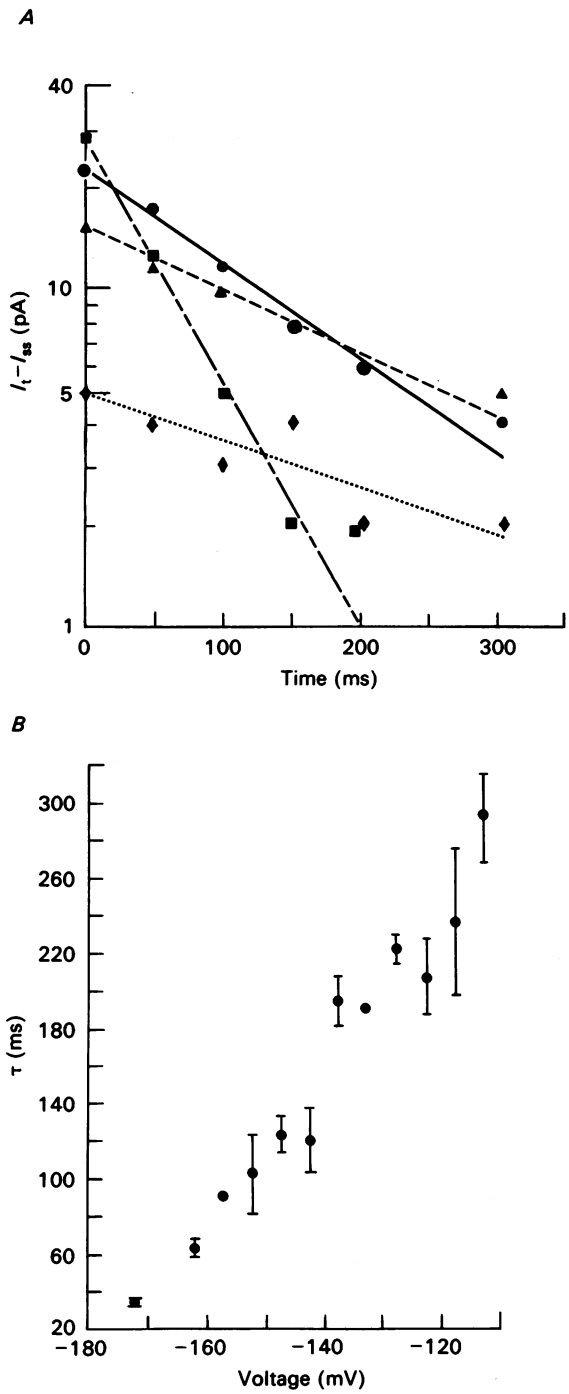

Fig. 7. *A*, semilog plot of inactivating inward current as function of time for voltage steps to −154 (square), −145 (filled circle), −136 (triangle) and −114 (diamond) mV. *B*, time constant of decay (τ) *versus* voltage for cells of Fig. 6. Individual points represent average time constant from a number of cells (one to five) ± S.E. of mean. The two points with no error bar represent single cells; eight different cells were analysed in all.

to −154, −145, −136, and −114 mV were 58, 148, 218, and 298 ms, respectively. Fig. 7*B* shows the time constants of decay as a function of voltage for the same eight cells analysed in Fig. 6. Time constants decreased with increasing hyperpolarization.

Blockers. Addition of barium (2–3 mM) to the bathing medium blocked inward rectification in J774.1 cells. The *I/V* curves from a cell before and after the application of barium are shown in Fig. 8*A*. The *I/V* curve of the cell following addition of barium became linear with a slope resistance of 3·7 GΩ, a value equal to its resistance in the voltage range −60 to −40 mV before the addition of barium. The zero-current holding potential of this cell shifted from −81 to −51 mV. Barium completely eliminated inward rectification in all cells tested. The average zero-current holding potential after barium was −49 mV ± 11·3 ($n = 18$). Block was not voltage dependent at the barium concentrations and voltages studied. Note that the control and barium-tested *I/V* curves intersect at −84 mV, indicating that barium-sensitive current was carried by K^+ ($E_K = -87$ mV).

Caesium (1–3 mM) reduced the inward rectifying conductance in J774.1 cells ($n = 5$). Block was voltage dependent, as shown in the *I/V* curves of Fig. 8*B*. Block was incomplete at rest and increased with hyperpolarization, producing a region of negative slope resistance.

Outward rectification

I/V relation. As noted earlier, outward currents were obtained in cells that had been plated for 1–8 h. Fig. 9*A* shows current responses of a cell, plated for 3 h, to voltage steps from a holding voltage of −80 mV. Cells in these studies were held at −80 mV regardless of their actual zero-current potential and a 40 s interpulse interval was used. A 10 min interval after attaining the whole-cell confirmation was instituted before initiating voltage steps to allow the conductance to stabilize (see section on *Stability of currents*, p. 493). Fig. 9*A* shows that voltage steps positive to −40 mV activated an outward current that declined with time. The outward current produced by the step to −32 mV peaked at 30 ms. With increasing depolarization, the time to peak was reduced (Fig. 9*B*). The time course of the rise of the outward current was not systematically investigated because the capacitative transients obscured very early events. Fig. 9*C* shows the *I/V* curve plotting the steady-state and peak currents from the cell in Fig. 9*A* and *B*. The peak outward currents were measured at least 10 ms after the voltage step (at a time when the capacitative transient was over). The measured values for the peak currents for large depolarizing steps (where the current activates rapidly) therefore underestimate the actual peak current. It is evident from the steady-state *I/V* curve that the current completely inactivates.

Ionic basis of outward rectification. The ionic basis of the outward current was investigated using a two-step pulse protocol. Fig. 10*A* presents oscilloscope records of tail currents obtained in $[K]_o = 4{\cdot}6$, 9·7, 14·8 and 20 mM. The first pulse was to −10 mV which fully activated the outward conductance. The instantaneous *I/V* curves (Fig. 10*B*) were generated by plotting the amplitude of the tail current (measured 10 ms after the second step minus steady-state current) *versus* the voltage of the second step. The tail currents measured in this way underestimate the actual tail currents, particularly for large currents and therefore were not used to calculate conductance. However, they can be used to determine the reversal potential of the

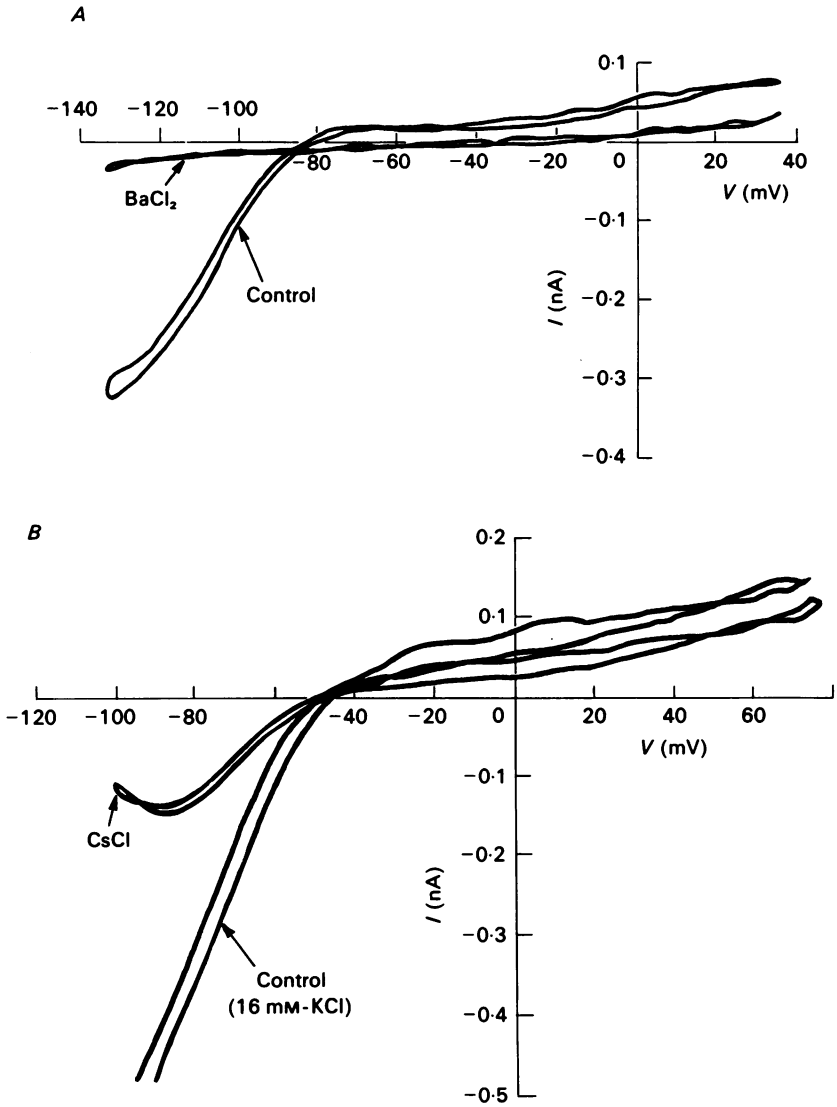

Fig. 8. *A*, *I*/*V* relations before and after addition of 2·5 mM-$BaCl_2$ to bath. $V_h = -80$ mV. *B*, *I*/*V* relations before and after addition of 1 mM-CsCl to bath. $[K]_o = 16$ mM. $V_h = -46$ mV.

current. It is evident from Fig. 10*B* that the reversal potential of the outward current shifted to the right as $[K]_o$ increased. A plot of the reversal potentials *versus* log $[K]_o$ for these data is shown in Fig. 10*C*. The points were fit by linear-regression analysis, and they have slope of -52 mV per tenfold increase in potassium ($r = 0{\cdot}96$) indicating that these outward currents are carried by potassium.

Conductance/voltage relations. The chord conductance of the outward rectifying current was calculated assuming potassium as the predominant charge carrier and E_K as -87 mV. Fig. 11 presents results from three cells. The rectifying current

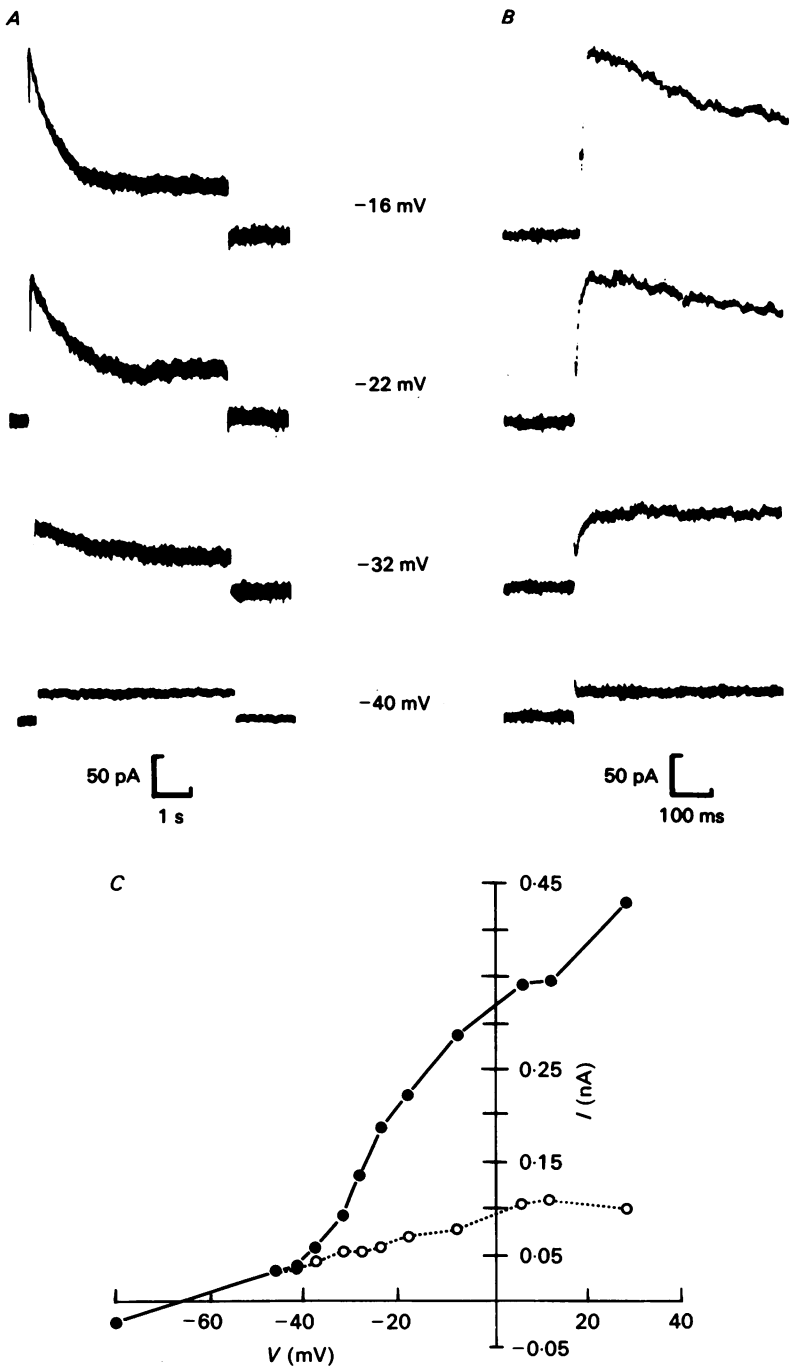

Fig. 9. *A*, current responses to series of voltage steps taken from −80 mV. Cell plated for 3 h. *B*, same currents as in Fig. 9*A* but at 10 times sweep speed. *C*, *I*/*V* relation of cell in Fig. 9*A*. Each point is mean of three steps. Filled circles represent current measured 10 ms after start of step, open circles represent the current at end of 5 s step. Voltage steps were applied every 40 s.

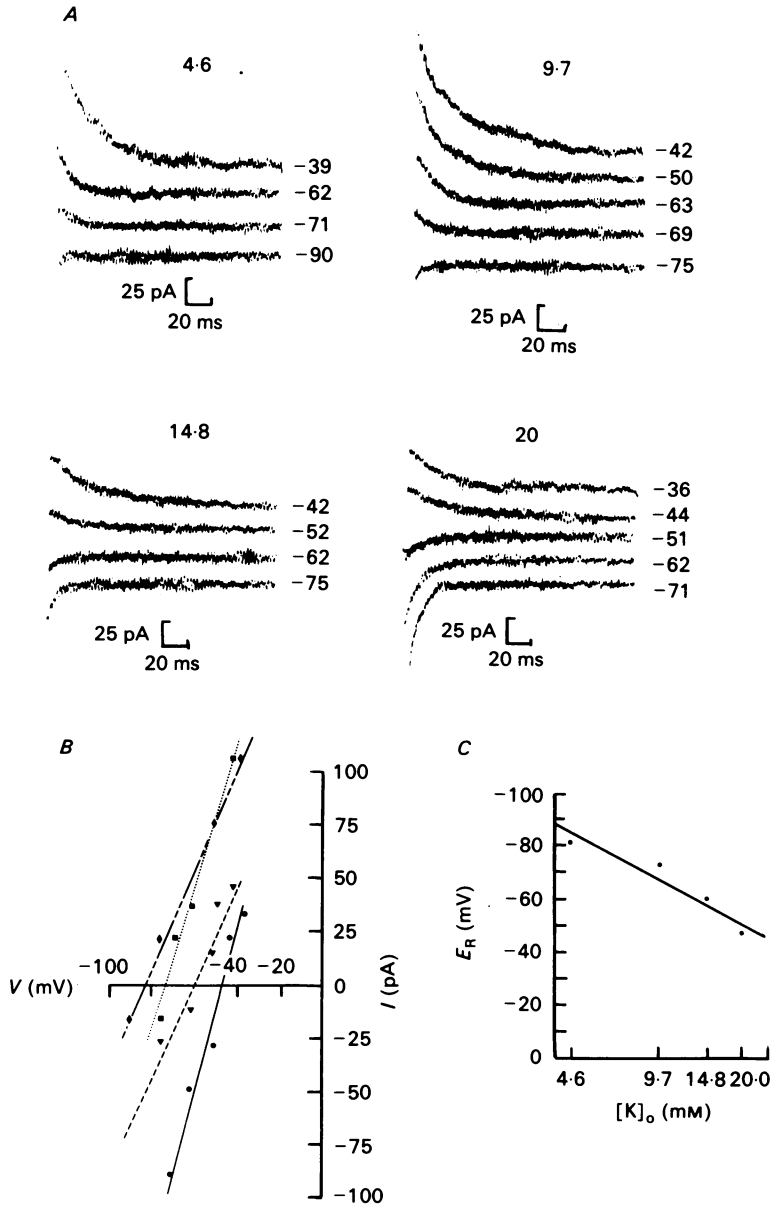

Fig. 10. Tail current analysis of ionic basis of outward rectification. Cell was held at −80 mV and outward conductance fully activated by a step to −10 mV. *A*, current responses of same cell obtained in $[K]_o$ = 4·6, 9·7, 14·8 and 20 mM. *B*, instantaneous *I*/*V* relations from data of Fig. 10*A*. *C*, reversal potential determined from Fig. 11*B* plotted *versus* log $[K]_o$. Lines in both Fig. 10*B* and *C* were fitted by least-squares linear regression ($r > 0{\cdot}98$).

activated positive to −50 mV and plateaued around −10 mV. The dashed line corresponds to a best fit of the data to the Hodgkin–Huxley equation for the delayed rectifier:

$$G/G_{max} = 1/(1+\exp[-(V_m-V_h)/v])^x, \quad (2)$$

where V_m and V_h have the same meanings as in eqn. (1) and x indicates an unknown degree of exponentiation. The values of V_h and v determined from these data using a first-power relationship are −31 and 5·7 mV, respectively (non-linear least-squares fit, coefficient of multiple determinations = 0·98). Statistically significant fit could be obtained for both first- or fourth-power relations.

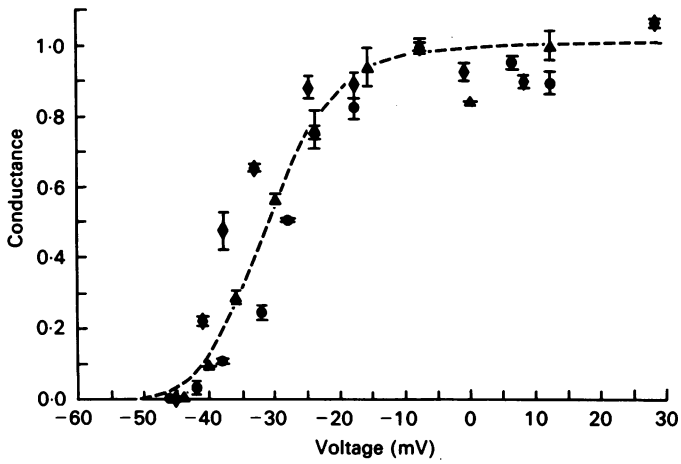

Fig. 11. Chord conductance *versus* voltage of three freshly plated J774.1 cells ($V_h = -80$ mV), each pulsed to same voltage three times (each point is mean ± S.E. of mean). Conductances were leak subtracted (calculated at −60 mV) and normalized by peak conductance for each cell. Reversal potential was estimated as −85 mV. Dashed line conforms to eqn. (2), $x = 1$.

Inactivation. As shown in Fig. 9, the outward currents completely inactivated with time during the voltage step. The time course of the inactivation process was described by a single exponential (Fig. 12*A*). Fig. 12*B* plots the time constant of inactivation as a function of voltage for three different cells. Positive to −25 mV the time constant was insensitive to voltage, averaging 538 ± 25 ms (mean ± S.D.); negative to this potential, inactivation was consistently slower and appeared to show some voltage dependence. However, since the amount of inactivating current negative to −25 mV was very small, reliable estimates of the time constants at these voltages could not be made.

The outward rectifying current recovered relatively slowly from inactivation. Recovery from inactivation was examined using paired 250 ms pulses to −10 mV separated by a variable interval. The summary of three experiments is shown in Fig. 13 in which the ratio of the peak current elicited by the second pulse to the first pulse is plotted *versus* the interpulse interval. The time course of recovery was described by a single exponential with a time constant of 13·7 s.

Blockers. The effects of 4-aminopyridine (4-AP) and D600 on the outward currents

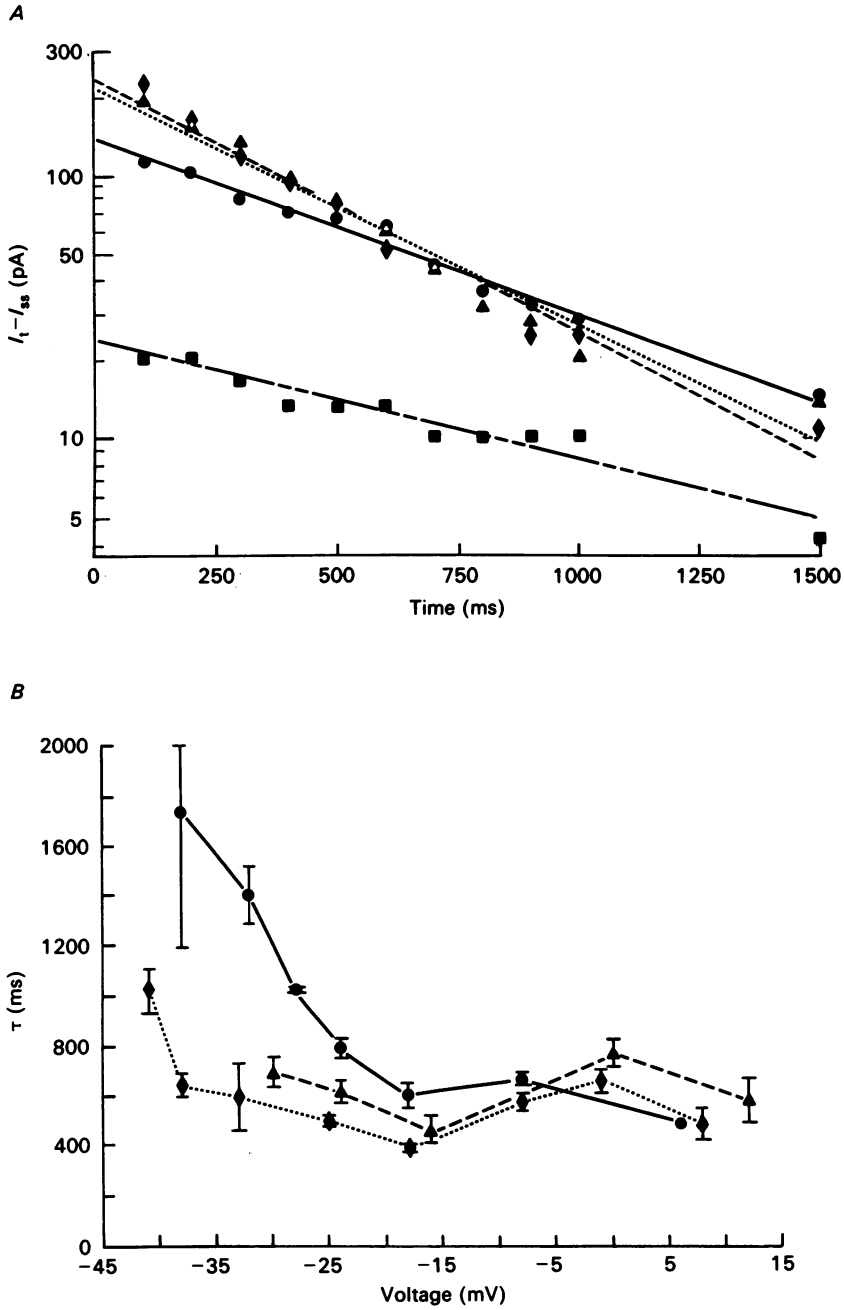

Fig. 12. Time course of inactivation of outward rectification. *A*, semilog plot of inactivating outward current as a function of time for steps to −32 mV (squares), −24 mV (circles), −8 mV (triangles) and +12 mV (diamonds). *B*, time constant of outward current decay *versus* voltage for three different cells.

were investigated because it has been shown that 4-AP (DeCoursey *et al.* 1984; Ypey & Clapham, 1984), and verapamil (Chandry, DeCoursey, Cahalan, McLaughlin & Gupta, 1984) block outward potassium currents in other leukocytes. Addition of 10 mM-4-AP (introduced at pH 7·4) or 1 mM-D600 (methoxyverapamil) to the bath during recordings from cells exhibiting inactivating outward currents produced a block of the current within 3–5 min. Lower concentrations of these agents (1 mM-4-AP or 500 μM-D600) had only partial blocking action.

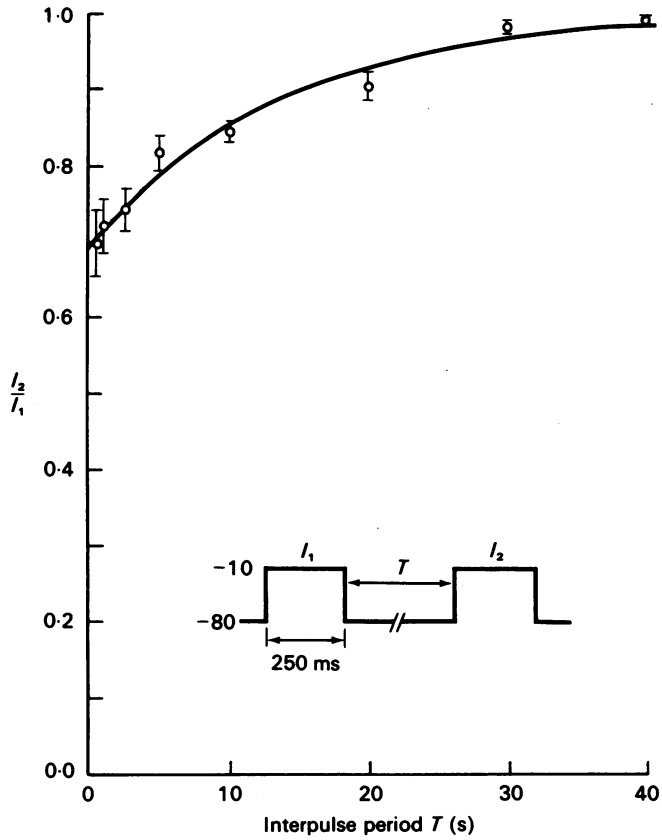

Fig. 13. Time course of recovery from inactivation of outward rectification. The ratio of the peak current during the second pulse (I_2) to that during the first pulse is plotted as a function of the interpulse interval. The continuous line conforms to $I_2/I_1 = 1-0{\cdot}30 \exp(t/-13{\cdot}7)$ where t is seconds ($P < 0{\cdot}01$).

The concentration of 4-AP needed to completely block the outward currents produced rapid and extensive vacuolization of the cells and whole-cell recordings were often lost following block of the outward current. To ensure that the outward current block was not related to a general run down of the cells, the following study was done. Recordings were obtained from different cells in the same culture dish before and after a 20 min incubation in 10 mM-4-AP and the resting membrane potential, leak conductance, and outward currents measured. Both populations before and after 4-AP addition had the same average resting membrane potential and leak conductance

(−78·7 *versus* −78·5 mV and 1·1 *versus* 1·2 nS, respectively). However, the expression of outward rectification decreased from six of seven cells observed before addition of 4-AP to none of eight after. No release of the intracellular enzyme lactate dehydrogenase was detected during a 1·5 h incubation in 10 mM-4-AP (S. W. Green & E. K. Gallin, unpublished observations).

Stability of currents

Several groups of investigators have reported that some parameters of voltage-dependent sodium, potassium and calcium conductances change with time during patch-clamp recordings (Fenwick, Marty & Neher, 1982; Cahalan *et al.* 1985; Fernandez, Fox & Krasne, 1984). In J774.1 cells, the outward current increased and its activation shifted over the course of the first 10 min of a whole-cell recording. After an initial stabilization period, outward current routinely remained stable for up to an hour. I/V curves generated with voltage steps immediately after attaining the whole-cell conformation and again after stabilization indicated that peak outward conductance increased and activation shifted. The conductance/voltage curves from four different cells fitted to eqn. (2) exhibited a 8 mV shift in V_h to the left; no change was found in v. The 8 mV shift may be an underestimate since it was apparent that a significant shift occurred even as the first I/V curve was obtained. The inward potassium current showed a different pattern of changes with time under whole-cell recording conditions. The conductance/voltage relation did not shift and, in most cells, maximum inward current was stable. In a few cells, voltage-dependent inward current disappeared within 10–20 min without a detectable shift in its conductance/voltage relation. Activation of the outward current shifted in such cells but no run-down was observed.

DISCUSSION

This report describes two time- and voltage-dependent potassium currents in J774.1 cells, a macrophage-like cell line derived from a mouse reticulum sarcoma (Ralph & Nakoinz, 1975). The two currents have different voltage sensitivities: one activating at voltages negative to −60 mV, and the other activating at voltages positive to −40 mV. The former is similar to the inward rectifying potassium conductance described in long-term cultures of mouse spleen and thioglycolate-elicited peritoneal macrophages (Gallin, 1981; Gallin & Livengood, 1981). The latter is similar to the outward rectifying potassium conductance described in mouse resident peritoneal macrophages (Ypey & Clapham, 1984). These conductances are sequentially expressed after J774.1 cells are switched from suspension to adherent culture.

Resting membrane potential

The relation of the zero-current potential to $[K]_o$ indicates that approximately 90 % of the resting membrane potential of J774.1 cells is determined by the potassium electrochemical gradient. The remaining component, i.e. permeability to other ions, may be induced by the recording system (i.e. it may reflect a shunt conductance across the electrode–cell seal), or may normally be present in unperturbed J774.1 cells. Our previous studies using intracellular micro-electrodes demonstrated a broad distri-

bution of resting membrane potentials in J774.1 cells, with resting membrane potentials ranging from −18 to −86 mV (Sheehy & Gallin, 1984). A bimodal distribution of resting membrane potentials, from −20 to −40 and −50 to −90 mV, was found in primary cultures of mouse spleen and peritoneal macrophages (Gallin & Livengood, 1981; Gallin, 1981). In those studies it was suggested that the depolarized cells might represent a damaged subpopulation. The patch-clamp data in this paper, demonstrating a unimodal distribution of hyperpolarized resting membrane potentials, indicate that this is the case.

The values of resting membrane potential for adherent J774.1 cells are very different from the value of −14 mV obtained by Young *et al.* (1983) using the lipophilic cation TPP^+ to indirectly monitor membrane potential. However, TPP^+ measurements on adherent cells yield resting membrane potential values of −70 mV, which are similar to those obtained in these studies (J. Young, personal communication). Therefore, it is likely that the process of adherence produces a rapid (within 30 min) increase in the resting membrane potential. The change in resting membrane potential must involve either an increase in potassium permeability or a decrease in other permeabilities. The depolarization induced by barium indicates that the inward rectifier contributes significantly to the resting membrane potential of long-term adherent cells. On the other hand, cells soon after plating have −77 mV potentials but do not exhibit inward rectification, indicating that the inward rectifier is not essential for the establishment of negative resting membrane potential.

Conductance changes following adherence

J774.1 cells express both voltage-dependent inward and outward potassium currents at different times after plating. The outward current appears first, about an hour after plating, and is lost within 12 h post-plating. In these studies the number of cells exhibiting outward current in any given dish was variable, but the time frame of its expression remained consistent. With only a single exception (in 195 cells), outward currents were not observed more than 12 h post-plating. All long-term cultured J774.1 cells express the inward rectifying potassium current; it was only occasionally seen in cells plated for less than 3 h. As already noted, similar inward and outward rectifications have been described in primary cultures of mouse peritoneal macrophages (Gallin & Livengood, 1981; Ypey & Clapham, 1984). Ypey & Clapham (1984) reported that the outward current was not present until cells had been cultured under adherent conditions for 12 h and was expressed for at least 4 days (the duration of the studies). They did not observe inward rectification during the 4 day culture period. Previous work in this laboratory has demonstrated that 2–4 week cultured mouse peritoneal macrophages exhibit prominent inward rectification (Gallin & Livengood, 1981). Although these two conductances are expressed at later times after plating in primary macrophages than in J774.1 cells, the expression of the inward rectifying current appears to follow the expression of the outward current in both cell types.

The differential appearance of these two conductances could result from any one or a combination of the following processes: (1) the synthesis and subsequent insertion of new channels in the membrane, (2) the insertion of pre-existing channels in the membrane, and (3) the modulation of membrane channels. Since the expression

of the outward rectifier in J774.1 cells occurs rapidly, as early as 25 min following plating, it is unlikely that *de novo* protein synthesis is required. Inward rectification develops slightly later, 2–4 h after plating; therefore, its full expression may involve synthetic processes. Studies examining this possibility are in progress.

In addition to influencing the expression of potassium conductances, adherence produces other physiological changes in macrophages. For example, Lazdins, Koech & Karnovsky (1980) have shown that within 3 h after plating, the glycogen levels in mouse peritoneal macrophages triple. Furthermore, Cohen, Ryan & Root (1981) have demonstrated that 90 min after adherence, the release of reactive oxygen metabolites increases in response to phagocytosis or phorbol myristate acetate. An increase in a voltage-dependent inward calcium current which parallels the development of immunoglobulin-secreting activity has been reported in lymphocyte–myeloma hybridomas, another cell type of leukocyte origin (Fukushima *et al.* 1984). It is possible that the expression of the two potassium conductances in J774.1 cells relates to a functional change(s) occurring following adherence.

The two conductances will have different effects on a cell. In response to depolarizing stimuli the inward rectifier shuts off whereas the outward rectifier activates; thus voltage responses to depolarizing stimuli will be amplified for a cell exhibiting inward rectification but blunted for a cell exhibiting outward rectification. Future studies using pharmacological agents to block the inward and outward potassium conductances in J774.1 cells may be useful in elucidating the relation between these conductances and cell function.

Inward rectification

The dependence of the inward current in J774.1 cells on both voltage and $[K]_o^{\frac{1}{2}}$ is similar to that described for the anomalous rectifying potassium conductance in muscle and egg cells (Leech & Stanfield, 1981; Hagiwara & Takahashi, 1974). Previous work in this laboratory using intracellular micro-electrodes has demonstrated a similar inward rectification blocked by barium and enhanced by increasing $[K]_o$ in mouse spleen and thioglycollate-induced peritoneal macrophages cultured for 2–4 weeks (Gallin & Livengood, 1981). Thus, J774·1 cells can serve as a model to study the role of the inward rectifying current in macrophages.

The unique relation of the anomalous rectifier to $[K]_o$ is particularly interesting in the context of the role of the macrophage as a scavenger. Macrophages are commonly found in sites of dead and dying tissue where $[K]_o$ may be locally elevated, and the increased inward rectifying conductance could be associated with enhanced expression of some effector function. Macrophages are not unique among leukocytes in expressing this conductance. A similar inward rectifying potassium current has recently been observed in a rat-derived basophil leukaemic cell line (Ikeda & Weight, 1984).

The conductance/voltage relation of the inward current (eqn. (1)) was similar to that of egg cells (Hagiwara & Takahashi, 1974). However, in J774.1 cells, activation was faster than in skeletal muscle or starfish eggs (Leech & Stanfield, 1981; Hagiwara, Miyazaki & Rosenthal, 1976), usually fully activated before the capacitative transient was over (< 10 ms). In contrast to activation, the voltage dependence of both the rate and extent of inactivation of the inward current in J774.1 cells generally

paralleled that of muscle and egg cells. Inactivation followed first-order kinetics with a rate that increased with membrane hyperpolarization, similar to the process described in skeletal muscle (Standen & Stanfield, 1979) and tunicate egg cells (Ohmori, 1978). However, the time constant of decay was generally longer in J774.1 cells than in muscle and egg cells. As in muscle and egg cells, the amount of inactivation in J774.1 cells increased with voltage, resulting in steady-state I/V relations with regions of negative-slope resistance for potentials negative to -150 mV.

In other cells, inactivation of the inward rectifying potassium conductance has been shown to result from one or a combination of three possible mechanisms: (1) a change in driving force due to a potassium redistribution (Almers, 1971*a*, *b*), (2) block of the open channel by weakly permeant ions such as Na^+ (Standen & Stanfield, 1979), and (3) actual channel closure (Sakmann & Trube, 1984). It is unlikely that there is a significant change in K^+ gradient in J774.1 cells since they are isolated in tissue culture, and there is no evidence that they contain an extensive system of membrane invaginations. The issue of open-channel block *versus* true channel closure would best be approached by single-channel techniques rather than the whole-cell method used in this study. However, the observation that significant current decay (up to 40%) was seen at potentials where Na^+ block is not thought to predominate in other cells (Standen & Stanfield, 1979) suggests that channel closure is the predominant mechanism in J774.1 cells in the voltage range studied in these experiments.

Outward rectification

The inactivating outward potassium current of J774.1 cells is very similar to that of peritoneal macrophages and T lymphocytes (Ypey & Clapham, 1984; Cahalan *et al.* 1985; Fukushima, Hagiwara & Henkart, 1984). The voltage dependence of activation of the outward conductance in J774.1 cells could be described by Hodgkin–Huxley-type kinetics. The values of V_h and v determined in this study (-31 and 5·7 mV, respectively) are quite close to those reported in T lymphocytes (-37 and 4·2 mV) by Cahalan *et al.* (1985).

The time course of inactivation of the outward current followed a single exponential at all voltages studied, and showed no systematic voltage dependence at voltages positive to -25 mV. In two cells studied at potentials negative to -25, the rate of decay clearly decreased, but the measurement error was too large to make accurate estimates for the whole population. These results are similar to the findings of Cahalan *et al.* (1985) in T lymphocytes.

The inactivating outward current in J774.1 cells was completely blocked by 10 mM-4-AP. This concentration, although high, is similar to the blocking concentration reported in mouse peritoneal macrophages (5 mM-4-AP; Yepey & Clapham, 1984) and human T lymphocytes (10 mM; DeCoursey *et al.* 1984). The sensitivity of the outward current to D600, an agent which blocks calcium channels suggests that the conductance might be activated by the influx of calcium. Studies of excised patches from human macrophages have demonstrated large conductance calcium- and voltage-dependent potassium channels (Gallin, 1984). If similar channels exist in J774.1 cells (and were to play a role in these outward currents), the internal calcium

would have to be in the order of 10^{-5} M to produce the voltage dependence reported here. This is unlikely since the electrode contains 1·1 mM-EGTA and inward (calcium) currents were not seen in these cells. Furthermore, the time course of the activation and inactivation are inconsistent with a calcium-activated potassium conductance. Thus, this conductance differs from the calcium-dependent potassium conductance previously described in human macrophages. In T lymphocytes, the outward potassium conductance also is blocked by high concentrations of calcium antagonists but shows no apparent calcium dependence (DeCoursey *et al.* 1984).

Stability of currents

The zero-current holding potential recorded immediately after obtaining a whole-cell patch recording usually remained stable for an hour or longer. However, I/V curves frequently changed during the first 10 min of recording. After stabilization, all cells showed a decrease in steady-state current at voltages positive to −40 mV. Activation of the inactivating outward current shifted to more negative voltages in cells exhibiting this current. Our estimate of a 8 mV shift in the conductance/voltage relation is most likely an underestimate because of the time (3–4 min) required to obtain the initial I/V relation. The time course and magnitude of the shift in these macrophage-like cells is similar to that seen in lymphocytes by Fukushima *et al.* (1984). Two general mechanisms have been proposed to account for the conductance changes with time seen using patch electrodes: (1) dilution/loss of intracellular modulatory substances, and (2) alterations of membrane surface charge (Fernandez *et al.* 1984). The observation that activation of the inward current did not shift under whole-cell recording conditions suggests that the shift of the activation of the outward current was not due to a generalized phenomenon such as a change in surface charge.

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REFERENCES

ALMERS, W. (1972*a*). Potassium conductance changes in skeletal muscle and the potassium concentration in the transverse tubules. *Journal of Physiology* **225**, 33–56.

ALMERS, W. (1972*b*). The decline of potassium permeability during extreme hyperpolarization in frog skeletal muscle. *Journal of Physiology* **225**, 57–83.

BERTON, G. & GORDON, S. (1983). Superoxide release by peritoneal and bone marrow derived mouse macrophages. Modulation by adherence and cell activation. *Immunology* **49**, 693–704.

BLOOM, B. R., DIAMOND, B., MUSCHEL, R., ROSEN, N., SCHNECK, J., DAMIANI, G., ROSEN, O. & SCHARFF, M. (1978). Genetic approaches to the mechanism of macrophage functions. *Federation Proceedings* **37**, 2765–2771.

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.

CHANDRY, K. G., DECOURSEY, T. E., CAHALAN, M. D., MCLAUGHLIN, C. & GUPTA, S. (1984). Voltage gated potassium channels are required for human T lymphocyte activation. *Journal of Experimental Medicine* **160**, 369–385.

COHEN, M. S., RYAN, J. L. & ROOT, R. K. (1981). The oxidative metabolism of thioglycollate-elicited mouse peritoneal macrophages: The relationship between oxygen, superoxide and hydrogen peroxide and the effect of monolayer formation. *Journal of Immunology* **127**, 1007–1011.

DAMAINI, G., KIYOTAKI, C., SOELLER, W., SASADA, M., PEISACH, J. & BLOOM, B. R. (1980). Macrophage variants in oxygen metabolism. *Journal of Experimental Medicine* **152**, 808–822.

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.

FENWICK, E. M., MARTY, A. & NEHER, E. (1982). Sodium and calcium channels in bovine chromaffin cells. *Journal of Physiology* **331**, 599–635.

FERNANDEZ, J. M., FOX, A. P. & KRASNE, S. (1984). Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary (GH3) cells. *Journal of Physiology* **356**, 565–585.

FUJIMOTO, M. & KUBOTA, T. (1976). Physicochemical properties of a liquid ion exchanger microelectrode and its application to biological fluids. *Japanese Journal of Physiology* **26**, 631–650.

FUKUSHIMA, Y., HAGIWARA, S. & HENKART, M. (1984). Potassium current in clonal cytoxic lymphocytes from the mouse. *Journal of Physiology* **351**, 645–656.

FUKUSHIMA, Y., HAGIWARA, S. & SAXTON, R. E. (1984). Variation of calcium current during the cell growth cycle in mouse hybridoma lines secreting immunoglobulins. *Journal of Physiology* **355**, 313–321.

GALLIN, E. K. (1981). Voltage clamp studies on macrophages from mouse spleen cultures. *Science* **21**, 458–460.

GALLIN, E. K. (1984). Calcium- and voltage-activated potassium channels in human macrophages. *Biophysical Journal* **46**, 821–825.

GALLIN, E. K. & GALLIN, J. I. (1977). Interaction of chemotactic factors with human macrophages. *Journal of Cell Biology* **75**, 277–289.

GALLIN, E. K. & LIVENGOOD, D. R. (1981). Inward rectification in mouse macrophages: Evidence for a negative resistance region. *American Journal of Physiology* **241**, C9–17.

GALLIN, E. K., WEIDERHOLD, M. L., LIPSKY, P. E. & ROSENTHAL, A. S. (1975). Spontaneous and induced membrane hyperpolarizations in macrophages. *Journal of Cellular Physiology* **86**, 653–662.

HAGIWARA, S., MIYAZAKI, S. & ROSENTHAL, N. P. (1967). Potassium current and the effect of caesium on this current during the anomalous rectification of the egg cell membrane of a starfish. *Journal of Physiology* **67**, 621–638.

HAGIWARA, S. & TAKAHASHI, K. (1974). The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *Journal of Membrane Biology* **18**, 61–80.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

IKEDA, S. R. & WEIGHT, F. F. (1984). Inward rectifying potassium currents recorded from rat basophilic leukemia cells by whole cell patch clamp. *Society for Neuroscience Abstracts* **10**, 870.

LAZDINS, J. K., KOECH, D. K. & KARNOVSKY, M. L. (1980). Oxidation of glucose by mouse peritoneal macrophages: A comparison of suspensions and monolayers. *Journal of Cellular Physiology* **195**, 191–196.

LEECH, C. A. & STANFIELD, P. R. (1981). Inward rectification in frog skeletal muscle and its dependence on membrane potential and external potassium. *Journal of Physiology* **319**, 295–309.

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

OHMORI, H. (1978). Inactivation kinetics and steady-state current noise in the anomalous rectifier of tunicate egg cell membranes. *Journal of Physiology* **281**, 77–90.

OLIVERA-CASTRO, G. M. & DOS REIS, G. A. (1981). Electrophysiology of phagocytic membranes: III. Evidence for a calcium-dependent permeability change during slow hyperpolarizations of activated membranes. *Biochimica et biophysica acta* **640**, 500–511.

POFIT, J. F. & STRAUSS, P. R. (1977). Membrane transport by macrophages in suspension and adherent to glass. *Journal of Cell Physiology* **92**, 249–256.

RALPH, P. & NAKOINZ, I. (1975). Phagocytosis and cytolysis by a macrophage tumor and its cloned cell line. *Nature* **257**, 393–394.

SAKMANN, B. & TRUBE, G. (1984). Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *Journal of Physiology* **347**, 641–657.

SCHWARZE, W. & KOLB, H. A. (1984). Voltage-dependent kinetics of an ionic channel of large unit conductance in macrophages and myotube membranes. *Pflügers Archiv* **402**, 281–291.

SHEEHY, P. A. & GALLIN, E. K. (1984). Electrophysiological properties of the macrophage-like cell line J774.1. *Biophysical Journal* **45**, 58a.

SNYDERMAN, R., PIKE, M., FISCHER, D. & KOREN, H. (1977). Biologic and biochemical activities of macrophage cell lines P388D1 and J774.1. *Journal of Immunology* **119**, 2060–2066.

STANDEN, N. B. & STANFIELD, P. R. (1979). Potassium depletion and sodium block of potassium currents under hyperpolarization in frog skeletal muscle. *Journal of Physiology* **294**, 497–520.

UNKELESS, J. C., KAPLAN, G., PLUTNER, H. & COHN, Z. A. (1979). Fc-Receptor variants of a mouse macrophage cell line. *Proceedings of the National Academy of Sciences of the U.S.A.* **761**, 1400–1404.

YOUNG, J., UNKELESS, J. C., KABACK, H. R. & COHN, Z. A. (1983). Macrophage membrane potential changes associated with γ_2b/γ_1Fc receptor-ligand binding. *Proceedings of the National Academy of Sciences of the U.S.A.* **80**, 1357–1361.

YPEY, D. L. & CLAPHAM, D. E. (1984). Development of a delayed outward-rectifying K^+ conductance in cultured mouse peritoneal macrophages. *Proceedings of the National Academy of Sciences of the U.S.A.* **81**, 3083–3087.