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Inwardly Rectifying Whole-Cell and Single-Channel K Currents in the Murine Macrophage Cell Line J774.1

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Summary. Inward currents in the murine macrophage-like cell line J774.1 were studied using the whole-cell and cell-attached variations of the patch-clamp technique. When cells were bathed in Na Hanks' (KCl = 4.5 mM, NaCl = 145 mM), and the electrode contained Na-free K Hanks' (KCl = 145 mM) single-channel currents were observed at potentials below −40 mV which showed inward rectification, were K-selective, and were blocked by 2.5 mM Ba in the pipette. Single-channel conductance was 29 pS, and was proportional to the square root of $[K]_o$. Channels manifested complex kinetics, with multiple open and closed states. The steady-state open probability of the channel was voltage dependent, and declined from 0.9 to 0.45 between −40 and −140 mV. When hyperpolarizing voltage pulses were repetitively applied in the cell-attached patch mode, averaged single-channel currents showed inactivation. Inactivation of inwardly rectifying whole-cell current was measured in Na Hanks' and in two types of Na-free Hanks': one with a normal K concentration (4.5 mM) and the other containing 145 mM K. Inactivation was shown to have Na-dependent and Na-independent components. Properties of single-channel current were found to be sufficient to account for the behavior of the macroscopic current, except that single-channel current showed a greater degree of Na-independent inactivation than whole-cell current.

Key Words potassium · channel · macrophage · rectification · patch clamp

Introduction

Inwardly rectifying K currents have been described to date in a wide variety of cells, including frog skeletal muscle (Almers, 1972; Leech & Stanfield, 1981; DeCoursey, Dempster & Hutter, 1984), cardiac cells (Noble & Tsien, 1968; Carmeliet, 1982; Hume & Giles, 1983; Giles & Shibata, 1985), invertebrate egg cells (Miyazaki et al., 1974; Hagiwara, Miyazaki & Rosenthal, 1976; Ohmori, 1978, 1980), retinal cells (Tachibana, 1983), and recently, rat basophilic leukemia (RBL) cells (Ikeda & Weight, 1984; Lindau & Fernandez, 1986). Previously, we used the whole-cell variation of the patch-clamp technique (Hamill et al., 1981) to record inwardly rectifying K current in the macrophage-like murine cell line J774.1 and to characterize its properties (Gallin & Sheehy, 1985). The macroscopic current was shown to have the following properties: 1) activation occurred over the voltage range −50 to −120 mV in Na Hanks' and shifted to more positive potentials as $[K]_o$ was raised; 2) conductance was proportional to the square root of $[K]_o$; 3) current was completely blocked by 2 mM Ba or Cs; 4) in Na Hanks' time-dependent inactivation was present at voltages more negative than −100 mV.

In this study we used the patch-clamp technique to record single-channel and whole-cell currents in the J774.1 cell line. It is the aim of this paper to describe the properties of the single-channel current and to show that they can account for the behavior of the macroscopic inwardly rectifying current. Preliminary accounts of this research have already appeared (McKinney & Gallin, 1986, 1987).

Materials and Methods

Cell Culture

J774.1 (J774A.1) cells were obtained from American Type Tissue Culture (Rockville, Md.) and maintained in suspension at 37°C. Culture medium (RPMI 1640, GIBCO, Grand Island, N.Y.) was supplemented with 5% fetal calf serum, 5% glutamine, and 100 U/ml penicillin-streptomycin. For recording, cells were plated on glass cover slips and maintained in culture medium for varying times (hours to days). Cover slips were mounted in a Plexiglas® chamber in 300 to 400 μl of Hanks' and rinsed every 20 to 30 min. Recordings were made at room temperature (23 to 26°C) and for not more than 1 hr after cells were removed from the medium.

The diameter of adherent J774.1 cells ranged from 13 to 48 μm. Cells can appear rounded or flat and project psuedopodia that can extend tens of μm. Cells chosen for recording had a rounded appearance and had no pseudopodia.

Recording Methods

Patch electrodes of approximately 3 to 5 MΩ resistance were pulled from hematocrit glass, fire-polished and wax coated. Average seal resistance for 40 cells was 26 ± 1.7 GΩ, about 10 times larger than the input resistance of these cells. Error in zero-current potential measurements was therefore not greater than 10%.

Records were obtained in the whole-cell or cell-attached configuration, using a List (Darmstadt, FRG) EPC-7 patch clamp. Voltage pulses were generated by computer and ramp stimuli by a Krohn-Hite (Avon, Mass.) model 5200A waveform generator. Currents were digitized, displayed and analyzed using an Indec Laboratory Display System (Sunnyvale, Calif.) interfaced to a PDP 11/23 (Digital Equipment Corp., Maynard, Mass.). Data acquisition and analysis routines were written by us using Basic-23 (copyright Cheshire Data and Dan Brown). Single-channel currents were recorded on magnetic tape using a Hewlett-Packard (San Diego, Calif.) 3968A FM tape recorder (DC-5 kHz) and digitized off-line. Records were low-pass filtered at 100 to 500 Hz (−3 dB) using a 4-pole Bessel filter (Frequency Devices Inc., Haverhill, Mass.) and digitized at > two times the filtering frequency. A few records containing very small amplitude channels were filtered at 50 Hz.

In a few experiments, the patch pipette was perfused during cell-attached recording. Perfusion was accomplished by inserting tubing containing the test solution into the patch pipette to within 200 μm of the tip. A small air bubble at the tip prevented the test solution from leaking into the pipette until positive pressure was applied.

Liquid junction potentials between Na Hanks' (bath) and various pipette solutions were appropriately nulled before seal formation. The potential across the patch is given as pipette potential plus resting V_m (zero-current potential). If resting V_m was not measured directly it was assumed to be −70 mV for a cell bathed in Na Hanks' and 0 mV for a cell bathed in K Hanks'. Cells were held to within 5 mV of resting V_m.

Analytical Methods: Whole-Cell Records

The whole-cell records presented here have been corrected for leak and capacity currents. Leak current, measured either by stepping into a voltage range where no time-dependent current was present or after blocking all inward current with barium, was digitally subtracted from whole-cell records. Electrode capacitance was compensated electronically while in the cell-attached mode. Total membrane capacitance was measured in the whole-cell mode from the capacity transient compensation dial of the EPC-7, or by integrating the capacity transient, and was also compensated electronically. Experiments were halted if leak increased or if cell capacitance declined. Series resistance (R_s) was measured directly from the EPC-7 after capacity transient cancellation, or by fitting a capacitance record with a monoexponential function to find τ, and calculating R_s from the relationship $R_s = \tau/C$. Average series resistance for 28 cells was 4.8 ± 0.5 MΩ; when checked, R_s changed less than 5% during the course of the experiment ($n = 6$). In Na Hanks', inward currents were less than 1 nA, and maximum error from R_s was 5 mV. Therefore, we used R_s compensation only when cells were bathed in K Hanks', where currents were 1 to 5 nA.

Current amplitudes at the beginning and end of a voltage step were determined by eye using a cursor. In some cases, current records were fitted by a monoexponential function to obtain time constants as well as current amplitudes at time zero and at steady state. The fitting routine used the Levenberg-Marquardt algorithm (Bevington, 1969) to carry out a nonlinear least-squares fit.

Single-Channel Records

Amplitude histograms showing open and closed current levels were constructed from single-channel current records and displayed on the Indec unit. Currents were binned in intervals of 0.05 to 0.2 pA. Midpoints of the bins are connected by lines, creating the appearance of a continuous distribution of points. Peaks of the open and closed current levels were determined by eye, using a cursor. The fraction of time a channel was in the closed or open state was determined by measuring the area under the amplitude histogram for that state and dividing by the total time of the record.

Distributions of open and closed times were obtained from steady-state records containing only one channel (the maximum number of channels in any given patch was taken to be the same as the maximum number of integral current levels). For each 164-sec record, the open/closed current levels were determined using a line cursor; the threshold for counting an opening or closing even was 1/2 the channel amplitude. Records were filtered at 200 Hz; filter τ was 5 times faster than the fastest channel τ. Sample rate was 1 kHz. Open/shut intervals were measured by an automated routine with no minimum dwell time criteria. Idealized current records were reconstructed using the measured open/closed intervals and compared to the original data. Histograms of the number of events versus event duration were binned in intervals of 5 msec; values less than 5 msec were excluded from the analysis. Distributions of mean open and closed times were fitted by exponential functions; F-tests were used to evaluate whether significant improvements of the fits were obtained with two versus one exponential. The accuracy of our routines was checked by analyzing artificial single-channel data with known mean open and closed times generated by a stochastic channel simulator (Model QS-1; Instrutech Corp. Westbury, N.Y.).

Other fits of linear or sigmoidal functions to single-channel data were carried out on a VAX 11/750 using the RSE statistical package (BBN Software Products Corp., Cambridge, Mass.).

Solutions

Cells were bathed in either a Na Hanks' solution consisting of (in mM) 145 NaCl, 4.5 KCl, 1.6 $CaCl_2$, 1.1 $MgCl_2$, 10 HEPES buffer pH 7.3, or K Hanks' in which NaCl was replaced by KCl. For Na substitution experiments, Na was replaced with an equimolar amount of N-methylglucamine (NMG). For both cell-attached and whole-cell recordings, the pipette contained (in mM) 145 KCl, 1 $MgCl_2$, 1.1 EGTA, 0.1 $CaCl_2$, and 10 HEPES, pH 7.3. Free Ca in this solution was less than 10^{-8} M. In some early experiments, this solution also contained 10 mM NaCl. For conductance versus $[K]_o$ measurements, K concentration was varied by replacing KCl with an equimolar concentration of NaCl. In one case, KCl was raised to 300 mM, creating a hypertonic solution. For anion substitution experiments, KCl was replaced on an equimolar basis with K isethionate. Na and K concentrations were checked by flame photometry.

Results

Passive Membrane Properties

Resting V_m was taken to be the zero-current potential obtained immediately after obtaining the whole-cell conformation. The average value for 13 cells bathed in Na Hanks' (145 Na/4.5 K) was -70 ± 2 mV; in Na-free NMG Hanks' (4.5 K/0 Na), $V_m = -76$ mV $\pm$ 2.4 ($n = 6$). Cells bathed in K Hanks' (145 K/0 Na) were always within a few mV of zero. Average input resistance for cells bathed in Na Hanks' was 2.5 $\pm$ 0.4 GΩ ($n = 9$) and for those bathed in K Hanks' was 0.8 $\pm$ 0.1 GΩ ($n = 14$). Total membrane capacitance (C_m) was 24 $\pm$ 1.6 pF ($n = 36$). Membrane surface area was estimated from cell diameter, assuming a spherical cell shape, and was used to calculate a value for specific C_m of 1.5 $\pm$ 0.1 μF/cm^2, ($n = 16$), which is higher than the specific capacitance of most biological membranes. We interpret this value to be an overestimate, resulting from an inaccurate estimation of the membrane surface areas of J774.1 cells, which are adherent and can be irregularly shaped. We therefore assume that the true specific C_m is 1 μF/cm^2, and for purposes of estimating channel density, we calculate membrane area from measurements of total membrane capacitance.

Voltage Range of Activation

Whole-Cell Currents

Figure 1 illustrates the main properties of the inwardly rectifying K conductance. Shown in Fig. 1(*A*) are whole-cell records from a cell bathed in Na Hanks' and held at -80 mV. Inward currents were elicited by hyperpolarizing steps and showed inactivation for steps below -100 mV. When cells were bathed in K Hanks' (Fig. 1*B*) inward currents increased in size and the voltage range of activation was shifted to more positive potentials. Inactivation was reduced (*see* section on Inactivation). Peak conductance in K Hanks' was 24 $\pm$ 2 nS ($n = 14$), and was proportional to the square root of $[K]_o$ as described by Gallin and Sheehy (1985). Inward-rectifying K current was present in all J774.1 cells plated for longer than 1 to 2 hr, and usually declined slightly during the 30- to 40-min time course of an experiment.

Single-Channel Currents

Figure 2(*A*) shows an example of single-channel fluctuations recorded in the cell-attached patch con-

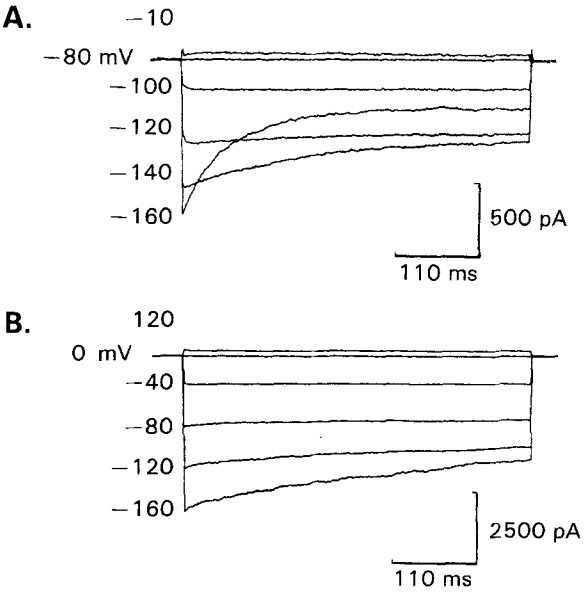

Fig. 1. Whole-cell currents from 2 cells bathed in (*A*) Na Hanks' or (*B*) K Hanks'. Records were not leak subtracted to show total current. 440-msec test pulses were given every 8 sec to potentials shown

figuration at various holding potentials. Both pipette and bath contained 145 mM K Hanks' (as in Fig. 1*B*) so that both resting V_m and E_K were near zero. Current fluctuations increased in amplitude as holding potential became more negative, and did not reverse at positive potentials, indicating rectification at the single-channel level. In some cells, very small outward fluctuations were observed, which we could not distinguish from the activity of a small conductance (~5 to 7 pS) channel that occasionally contaminated our records. Inward single-channel currents were observed in almost all cells plated for longer than 1 to 2 hr; when both single-channel and whole-cell records were obtained from the same cell, inward currents were always present when single-channel fluctuations were present. Patches contained from 1 to 6 channels, which usually persisted for as long as the patch remained intact (up to 20 min).

Measurement of Single-Channel Conductance and E_{rev}

Single-channel conductance (γ) was determined by measuring the slope of the linear portion of the single-channel *I-V* relationship (Fig. 2*B*). Data from the cell in Fig. 2(*A*) are shown as well as data from a cell bathed in Na Hanks' (pipette contained 145 K Hanks' in each case). Single-channel conductance for each cell was 30 pS; the average value for 18

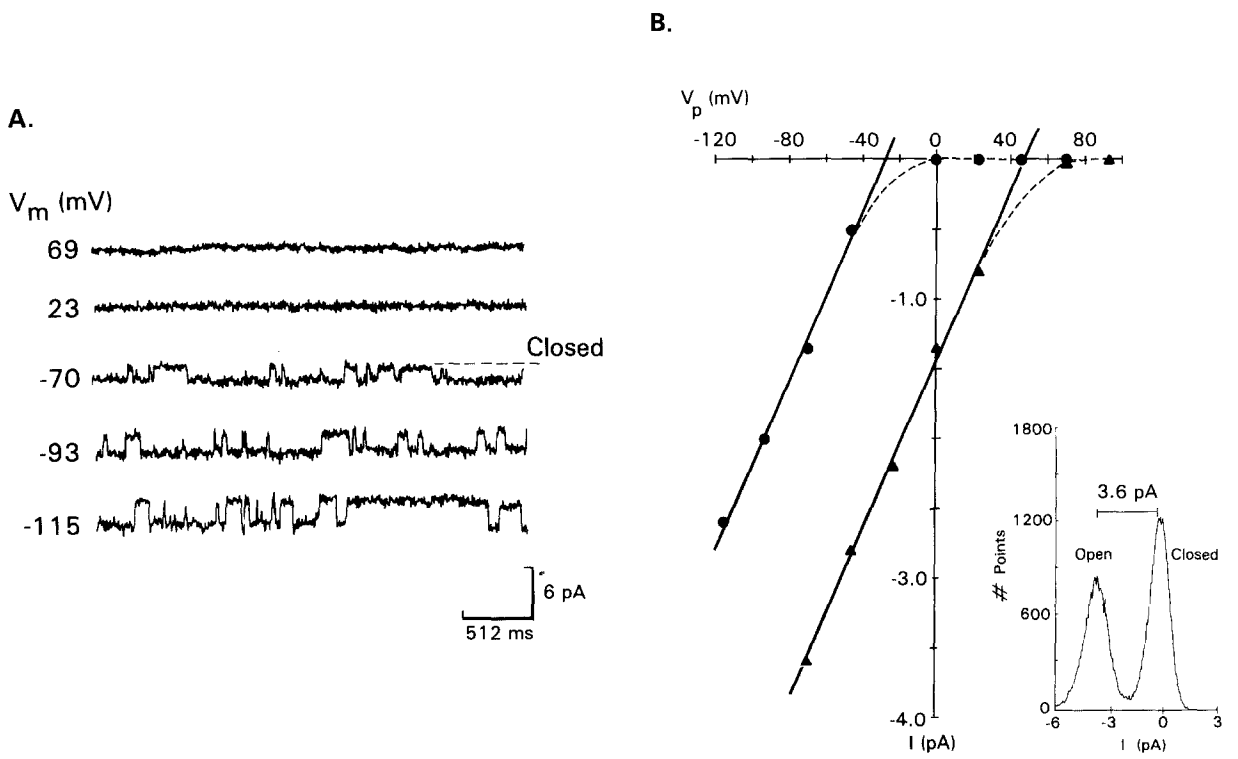

Fig. 2. (*A*) Cell-attached patch recording of single-channel currents at various holding potentials from a cell plated for approximately 3 hr and bathed in K Hanks'. Unless otherwise stated, V_m across the patch is given as pipette potential (V_p) + resting V_m. Downward current deflections represent inward current. Records were filtered at 100 Hz. (*B*) Single-channel *I-V* relationship for the cell shown in Fig. 2(*A*) (●) and for a different cell bathed in Na Hanks' (▲). Note that the X-axis is V_p not V_m. Solid lines are least-squares fits of the linear portion of the data. Dashed lines were drawn by eye to pass through E_K, which was near 0 mV for each patch. Inset shows an amplitude histogram of 33 sec of data recorded at −70 mV from the cell bathed in Na Hanks'

cells was 29 ± 1 pS. The reversal potential (E_{rev}) of the channel was estimated by extrapolating the *I-V* curve to the *X*-axis. If the channel was selective for potassium, as would be expected for the inward-rectifying channel (Gallin & Sheehy, 1985), then E_{rev} should equal E_K, which is −2 mV for each patch (assuming $[K]_i$ = 157 mM; Melmed, Karanian & Berlin, 1981; Sung et al., 1985). Since V_m across the patch = $V_{pipette}$ + resting V_m, we expect that when the cell is bathed in K Hanks', E_{rev} will occur when V_p = 0 mV ($V_m = V_p$). When the cell is in Na Hanks', it should be displaced by 70 mV, because the resting V_m of the cell bathed in Na Hanks' is near −70 mV. For the two cells in Fig. 2, the extrapolated E_{rev} values were −28 and 48 mV. Thus, while the reversal potentials showed the expected displacement along the *X*-axis (76 mV), they did not correspond to the values for E_K. A likely explanation for this discrepancy is that estimating E_{rev} by extrapolation of the *I-V* curve is inaccurate for a current that shows rectification near the reversal potential. An alternative and more accurate way to measure E_{rev} is to apply a continuous voltage ramp (in the cell-attached mode) and determine the voltage at which the open and closed current levels intersect. When E_{rev} was measured in this way, it was always found to be very close to E_K (n = 6). An example of single-channel currents measured in response to a ramp stimuli is given in Fig. 9 of the accompanying paper, and illustrates this method of estimating E_{rev}. We conclude that the extrapolated E_{rev} determined from an *I-V* curve is an underestimate of the true reversal potential of the inward-rectifying channel.

Single-Channel Conductance and E_{rev} are Proportional to $[K]_o$

One of the distinguishing features of the whole-cell inward-rectifying K conductance is that it is proportional to the square root of $[K]_o$ (Hagiwara & Takahashi, 1974; Ohmori, 1978; Leech & Stanfield, 1981; Fukushima, 1982). We therefore measured single-channel conductance using different K concentrations in the pipette to see of this relationship would hold true. Figure 3(*A*) shows examples of current fluctuations recorded from two cells at the same

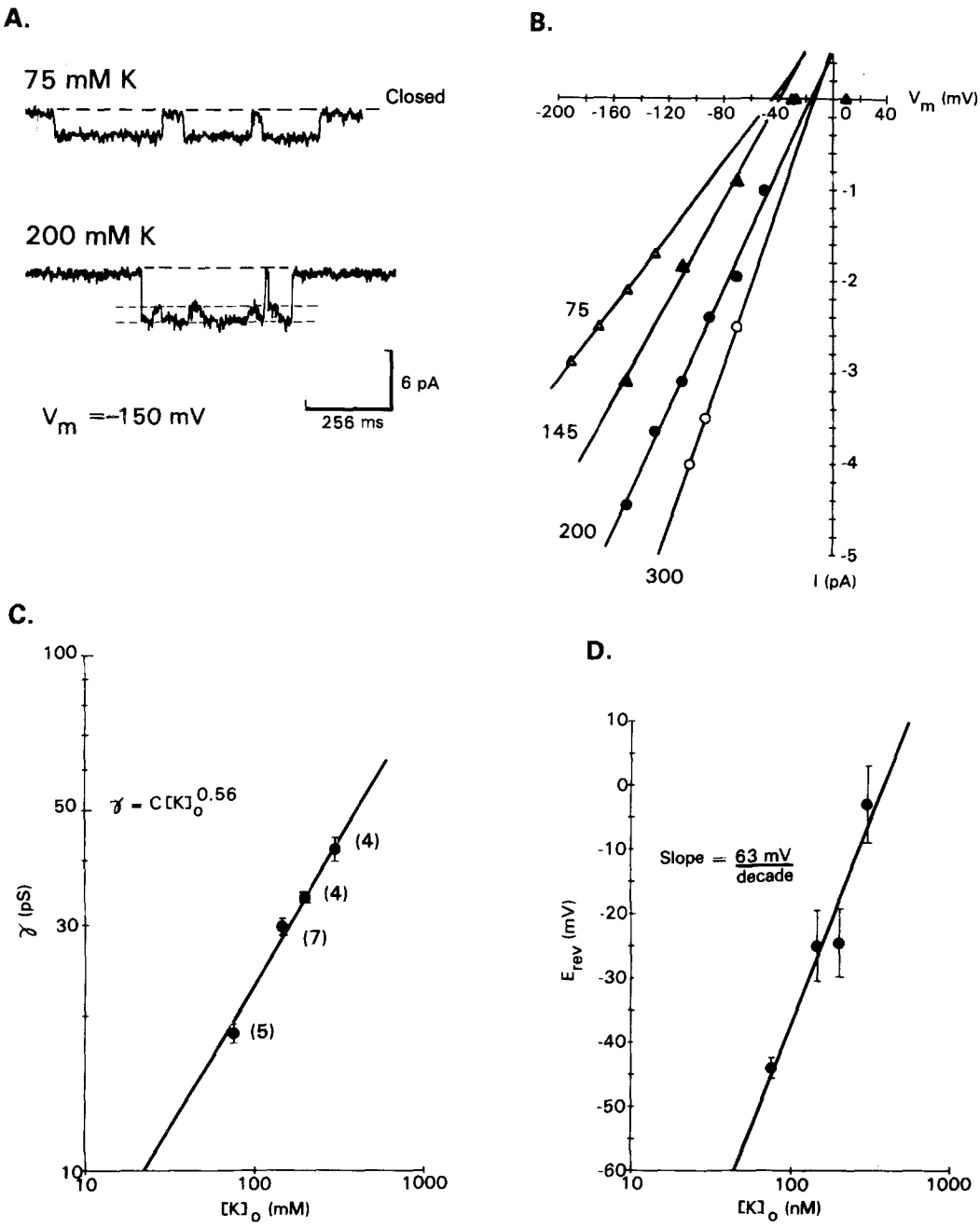

Fig. 3. (*A*) Single-channel records from two cells bathed in Na Hanks' with different K concentrations in the pipette, 75 and 200 mM. V_m = −150 mV. Note fluctuations to a smaller current level in the lower record. (*B*) *I-V* relationships of four cells, each bathed in Na Hanks', but with different [K] in the pipette: 75 (△), 145 (▲), 200 (●), and 300 (○) mM. [Na] was 75, 10, 10, and 10 mM, respectively. [Cl] was 157, 157, 216, and 316 mM. The number of measurements for each point are indicated in parentheses. Note that the *X*-axis is V_m across the patch, not V_p. (*C*) Log-log plot of γ *vs.* $[K]_o$, fitted by a straight line with slope 0.56. Values are given as mean ± SEM. (*D*) Extrapolated E_{rev} *vs.* log $[K]_o$, fitted by a straight line

holding potential, but with 75 or 200 mM K in the pipette. Current amplitude increased as $[K]_o$ increased. Figure 3(*B*) shows the *I-V* relationship for these two cells and for two others, which had 145 and 300 mM K in the pipette. Current amplitudes for $[K]_o$ = 75 mM were small and difficult to resolve unless membrane potential was large. For $[K]_o$ = 300 mM, patches were unstable, and values at only three potentials could be obtained. Single-channel conductance values of 19 ± 0.8, 29 ± 1.1, 34 ± 0.7, and 42 ± 2.2 pS, and extrapolated E_{rev} values of −44 ± 1.6, −25 ± 5.5, −24 ± 5.3, and −3 ± 6 mV

were obtained for 75, 145, 200, and 300 mM K_o, respectively. A log-log plot of single-channel conductance versus $[K]_o$ is shown in Fig. 3(*C*). The data were fit by the equation $\gamma = C[K]_o^x$ with a coefficient of 0.56, which is in excellent agreement with the value of 0.57 obtained for the whole-cell inward-rectifying conductance in J774.1 cells by Gallin and Sheehy (1985). The conductance of the channel in 4.5 mM K was calculated to be 4.1 pS. Figure 3(*D*) shows a plot of the extrapolated E_{rev} values versus log $[K]_o$. The data were fit by the Nernst equation for potassium with a slope of 63 mV per tenfold change in $[K]_o$. We ascribe the large variability in E_{rev} values to unknown variability in resting V_m (−70 mV was assumed for all cells) and to the sensitivity of the extrapolated E_{rev} to small differences in the slope of the *I-V* curve.

Ion Substitution Experiments

The permeability of the channel to sodium was not directly measured. However, we can infer that permeability to Na must be low because when Na in the pipette was elevated from 10 to 75 mM (for measurements with 75 K), γ and E_{rev} still followed $[K]_o$. In addition, in two other experiments, single-channel activity was recorded with 30 K/120 Na or 30 K/120 N-methylglucamine in the pipette, and current amplitudes were comparable. Channel conductance was in the range expected for 30 mM K_o (9 to 10 pS). Likewise, we infer a low permeability to anions, since E_{rev} values did not follow E_{Cl} as [Cl] in the pipette changed. In addition, several anion substitution experiments were carried out in which all but 12 mM chloride was replaced by the impermeant anion isethionate in the pipette solution. Average single-channel conductance for four cells was 30 ± 1.3 pS. Extrapolated E_{rev} was −40 ± 4.8 mV (corrected for a −70 mV resting potential), more negative than control, but shifted in the wrong direction to indicate chloride permeability. We assume the shift was due to variability in our E_{rev} measurements. Whole-cell inward currents recorded using the various pipette solutions described above were normal.

Block by Barium

The divalent cation barium is a characteristic blocker of inward-rectifying K current (Hagiwara et al., 1978; Standen & Stanfield, 1978; DeCoursey & Hutter, 1984; DiFrancesco, Ferroni & Visentin, 1984; Lindau & Fernandez, 1986). In J774.1 cells, externally applied barium (100 or 200 μM) reduced macroscopic inward K current in a concentration- and voltage-dependent manner (Fig. 4). As voltage steps became more negative, peak and steady-state currents were reduced and the time course of inactivation became faster. If the single-channel fluctuations described here do indeed comprise the inward-rectifying current, they should also be blocked by barium. Figure 5(*A*) shows single-channel activity obtained with K Hanks' in the pipette; when the pipette was subsequently perfused with K Hanks' + 2.5 mM $BaCl_2$, channel activity disappeared. Since channels are usually quite stable in the cell-attached mode, we can assume that they were blocked by Ba, even though we did not demonstrate the reversibility of the effect. The next set of traces (Fig. 5*B*) shows data from cell-attached patches from two cells bathed in Na Hanks' and held at −150 mV. Only one channel was visible in each patch. In the presence of 100 μM barium, the number of channel openings was reduced (% closed time of the channel was 87% compared to 59% ± 6 (n = 6) for control cells at this potential) while single-channel conductance was comparable (γ = 26 pS). Similar results were obtained in three other

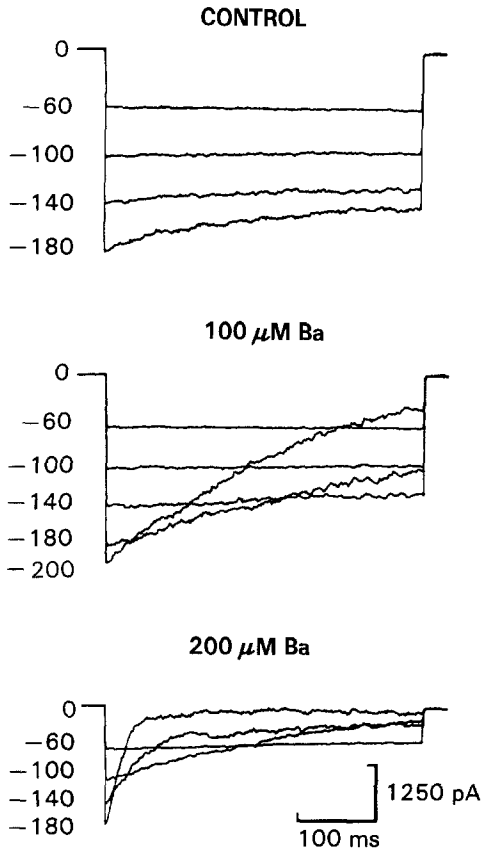

Fig. 4. Whole-cell records from a cell bathed in K Hanks', before and after addition of 100 or 200 μM Ba^{2+} to the bath. 440-msec pulses were applied every 8 sec to the potentials shown

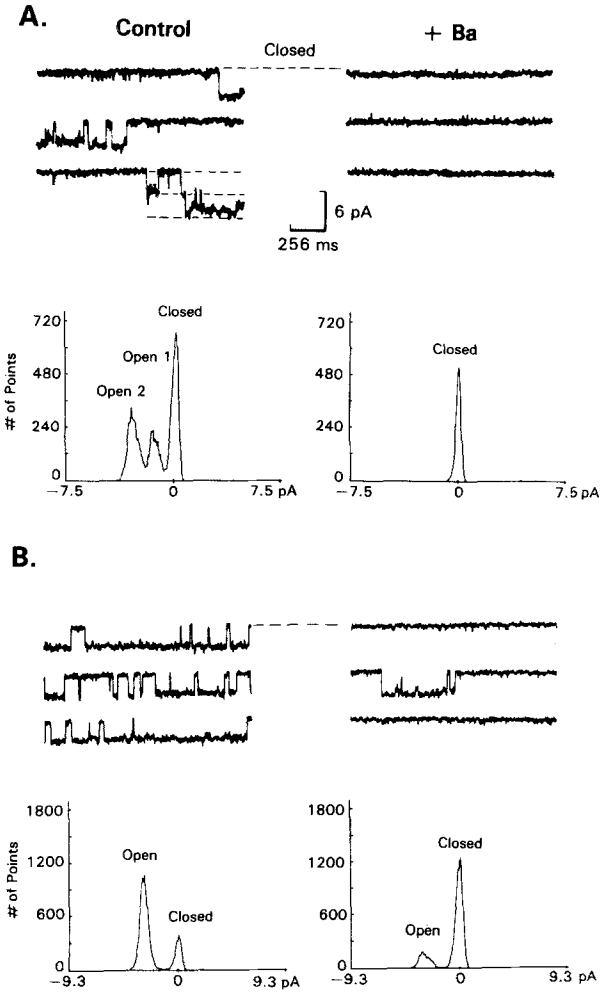

Fig. 5. (*A*) Cell-attached patch records showing single-channel currents and corresponding amplitude histograms from a single cell before and after perfusion of the pipette with K Hanks' + 2.5 mM Ba^{2+}. $V_h = -150$ mV. Records filtered at 200 Hz. (*B*) Single-channel records and amplitude histograms from two cells, with and without 100 μM Ba^{2+} in the pipette. $V_h = -150$ mV. Records filtered at 200 and 100 Hz. External solution was Na Hanks'

experiments in which cells were bathed in K Hanks'. Percent closed time was increased at all potentials tested (−40 to −120 mV). Macroscopic inward currents recorded with 100 to 200 μM barium in the cell were of normal magnitude as were resting membrane potentials, indicating little if any block by barium from the inside.

Inactivation of Inwardly Rectifying Current

Whole-Cell Currents

Inward-rectifying whole-cell currents recorded from cells bathed in Na Hanks' ($[K]_o$ + 4.5 mM) showed time-dependent inactivation below −100 mV that increased as voltage steps became more negative (Fig. 6*A*). Removing Na from the medium greatly reduced, but did not completely eliminate, inactivation, which was already shown for cells bathed in Na-free 145 mM K Hanks' (Figs. 1*B*, and 4*A*). Similar results were obtained for cells bathed in Na-free 4.5 mM K Hanks', where all sodium was replaced by NMG (Fig. 7*B*). We estimated the amount of inactivation by calculating the ratio of current (I_2) measured at the end of a 440- or 880-msec pulse to peak current (I_1) as a function of voltage (Fig. 7). Currents obtained in the presence of Na reproducibly inactivated to 25% of their peak value at −160 mV (*see also* Gallin & Sheehy, 1985). In the absence of Na, currents inactivated to only 75 to 80% of their peak value at this voltage. The percent inactivation in low-K and high-K Na-free Hanks' is the same, even though current magnitude differs by about fivefold. Inactivation did not depend on the order in which pulses were given. The time course of inactivation, determined by fitting a monoexponential function to the current trace, was very slow in Na-free solution compared to that in Na Hanks' (τ = 403 msec ± 72 (n = 8) versus 48 msec ± 7 (n = 5) at −160 mV). Thus, currents recorded in Na-free solution did not always reach steady state by the end of the current pulse, and the amount of inactivation was underestimated (*see* Discussion). Attempts to apply longer duration pulses to very negative voltages resulted in loss of the seal.

Single-Channel Currents

To determine whether inactivation occurs at the single-channel level, we recorded currents in response to voltage steps in the cell-attached mode (Fig. 8). Cells were bathed in Na Hanks' and held at −30 mV, where no channel activity was visible. Upon stepping to a more negative potential, single-channel fluctuations appeared, which often declined in number during the pulse. When many such sweeps were averaged, the resultant current trace showed inactivation. The three groups of sweeps shown in Fig. 8 illustrate this point. The top five sweeps of each group are single-channel currents recorded in response to a fixed voltage step. The bottom sweep is the average of 40 to 48 such sweeps. Inactivation was present at each voltage, although it was less apparent at voltages where single-channel currents were smaller. The ratio of current measured at the end of the voltage pulse (I_2) to

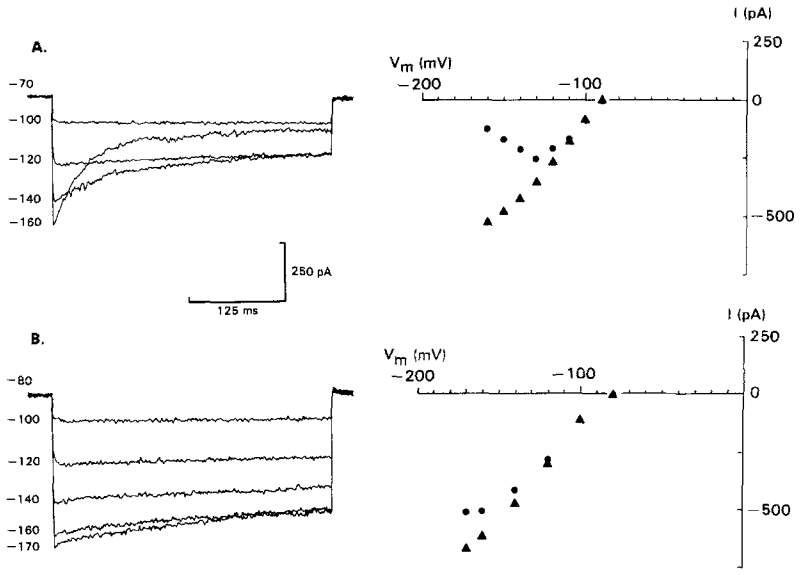

Fig. 6. (*A*) Whole-cell records from a cell bathed in Na Hanks'. $V_h = -70$ mV. 440-msec steps were applied every 8 sec to voltages shown. (*B*) Whole-cell records from a cell bathed in Na-free (NMG) Hanks'. $V_h = -80$ mV. Records in (*B*) were not leak subtracted; leak conductance was 10% of total. Current amplitudes measured at the beginning (▲) and end (●) of the current pulse are plotted *vs.* voltage in adjacent panels

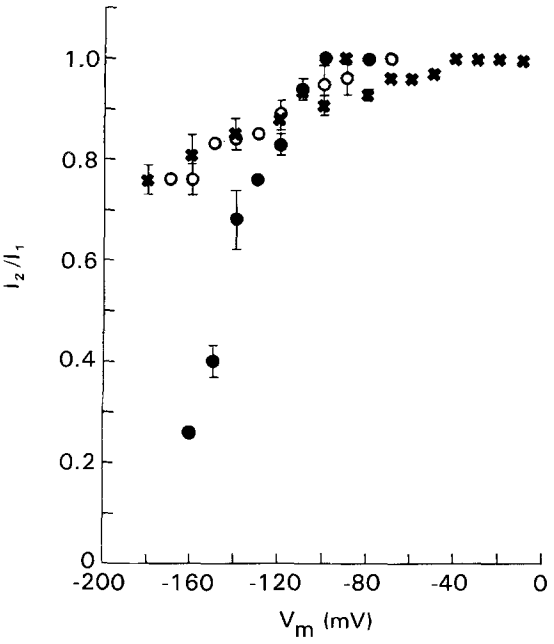

Fig. 7. Graph of the ratio of current measured at the beginning (I_1) and end (I_2 of the test pulse as a function of voltage for cells bathed in (●) Na Hanks' (4.5 K/145 Na), $n = 3$; (○) NMG Hanks' (4.5 K/0 Na), $n = 6$; and (X) K Hanks' (145 K/0 Na), $n = 8$. No cell spanned the entire voltage range shown. Pulse durations were 440 msec for data in Na and NMG Hanks' and 880 msec for K Hanks'. Some SEM values were within the points

peak current (I_1) for steps to −190, −150, and −110 mV were 48% ± 4 ($n = 3$), 47% ± 7 ($n = 4$), and 57% ± 5 ($n = 3$), respectively. Thus, single-channel currents appear to inactivate to a greater extent than whole-cell currents at comparable voltages.

Single-Channel Steady-State Open Probability

Since inward currents inactivate, the steady-state open probability of the inward-rectifying channel should decline as potential becomes more negative. Shown in Fig. 9(*A*) are records from three different cell-attached patches, each at a different holding potential and each containing only one channel. As potential became more negative, channel closures became longer and more frequent. Both short (msec) and long (sec) duration closures were evident at all voltages; the longest closures lasted tens of seconds. The dependence of open probability (defined as % open time/100) on voltage is shown in Fig. 9(*B*). All data are from cells that were bathed in K Hanks' to remove uncertainty regarding resting potential. Data were pooled from patches held at only one potential for very long times and from those held at various potentials for shorter times; in two cases, the patch contained two channels. Single-channel open probability (p) was calculated according to the equation

$$p = \sum_{n=0}^{N} \frac{n \cdot P_n}{N} \tag{1}$$

where P_n = probability that n channels are open in the patch and N = total number of channels in the patch. Open probability declined by about 45% between −45 and −120 mV. Data were fitted by the sigmoid equation

$$p(V) = A + \frac{B}{1 + \exp\frac{-(V-C)}{D}} \tag{2}$$

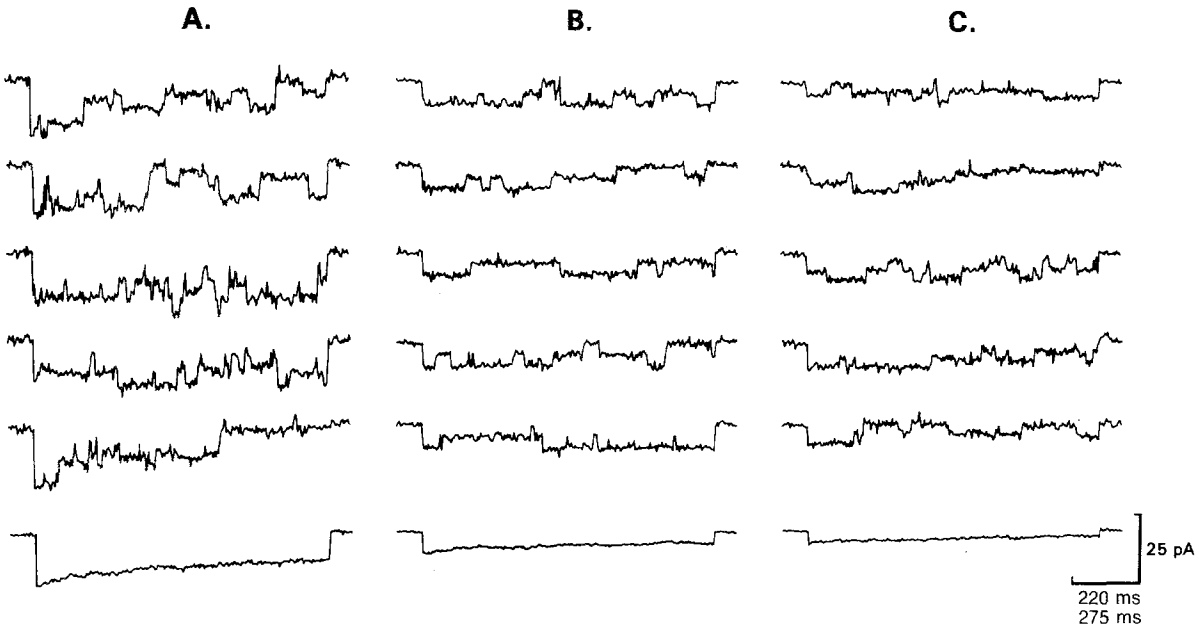

Fig. 8. Cell-attached patch records of currents elicited in response to different sized voltage steps. Patches were held at −30 mV and stepped to −190 (*A*), −150 (*B*), and −110 mV (*C*). At the bottom of each group of sweeps is the averaged current record for that voltage, comprised of 40 or 48 sweeps applied at 5-sec intervals. Records at −150 and −190 mV are from the same cell and are of longer duration (1100 msec) than those to −110 mV (880 msec). Leak and capacitive currents recorded in response to eight equal and opposite sized steps were averaged and subtracted from each record. Bathing solution was Na Hanks'

with four free parameters. Maximum ($A + B$) and minimum (A) open probabilities were 87 and 43.5%, respectively; the inflection point (C) occurred at −88 mV; (D) was 10.5 mV. Data obtained from cells bathed in Na Hanks', or from experiments where nonstandard solutions (high-K or Cl-free) were used in the pipette showed the same sigmoidal dependence on voltage, and in particular showed the same maximum and minimum values.

We can use the values for open probability to estimate steady-state conductance from the equation $G(V) = \gamma \cdot p(V) \cdot N$ where $G(V)$ = whole-cell chord conductance, and N = number of channels. Assuming that N is constant, the ratio of steady-state conductance at −40 versus −160 mV should be about 0.5. The observed ratio of steady-state whole-cell conductance values between the two voltages was, at most, 0.8 (data obtained from four cells bathed in K Hanks'). These data support our previous finding that inactivation of whole-cell currents is less than that of single-channel currents.

Steady-State Single-Channel Kinetics

Because patches containing only one channel were relatively rare, we have not yet obtained enough data to permit an extensive analysis of channel kinetics. A preliminary estimate of mean open and closed times was obtained by pooling data from three cells bathed in K Hanks' and held at −80 mV. Open/shut intervals were measured as described in Materials and Methods. Shown in Fig. 10(*A*) are samples of the digitized current record along with idealized traces that were reconstructed from the measured open/shut intervals. Open and closed time histograms are shown in Fig. 10(*B*), (*C*), and (*D*). The distribution of open durations was fitted by a biexponential function with τ values of 6 and 99 msec. Closed times were also fitted by a biexponential function; τ values were 4 and 37 msec. To estimate the mean closed time of the infrequent, long duration (>0.5 sec) closures, the durations of 34 such closures were measured directly from chart paper to the nearest 0.1 sec. Values were binned in intervals of 0.5 sec, and fitted by a monoexponential function; τ was 0.6 sec (Fig. 10*D*).

Discussion

Properties of Inwardly Rectifying Single-Channel and Whole-Cell Currents are Similar

In this paper we described a channel found in J774.1 cells that was active at negative voltages, showed

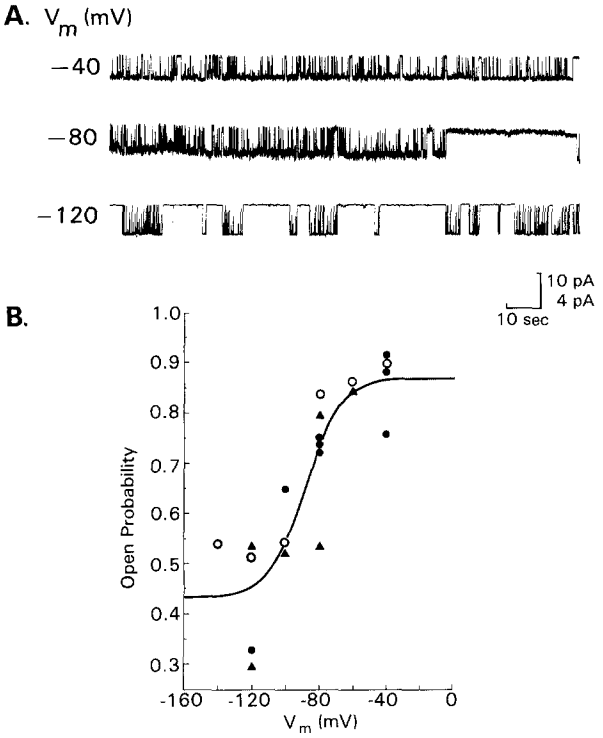

Fig. 9. (*A*) Cell-attached patch records from three cells bathed in K Hanks'. Currents were photographed from chart paper in order to show long (130 sec) segments of continuous channel activity. Current scale for upper two traces is 4 pA, and for lower trace is 10 pA. (*B*) Graph of open probability *vs.* voltage. (●) Values from eight cells (including those shown in panel *A*) where records were obtained at only one voltage; records were 164 to 459 sec in duration. (○) Values from a single cell where records were obtained at six different voltages in random sequence; records were 40 to 120 sec long. (▲) Values from patches containing two channels; records were 33 to 147 sec long

inward rectification, and was blocked by barium. The conductance of this channel was 29 pS in 145 mM K_o and was proportional to the square root of $[K]_o$. The extrapolated reversal potential of the channel was consistently below E_K, although reversal potentials measured using ramp stimuli were not. Others who have studied inward-rectifying K channels similar to ours (Kameyama, Kiyosue & Soejima, 1983; Sakmann & Trube, 1984*a*,*b*; Kurachi, 1985) have also found that the extrapolated E_{rev} of the channel is below E_K. We suggest that the extrapolated E_{rev} is an underestimate, possibly due to rectification of the channel near its reversal potential. The fact that E_{rev} shifted appropriately as $[K]_o$ was changed indicates that the channel is primarily K selective.

To a first approximation, the properties of the single-channel current can account for the behavior of the macroscopic inwardly rectifying current previously characterized in J774.1 cells by Gallin and Sheehy (1985). The voltage dependence, selectivity, and block by barium of single-channel and whole-cell currents were similar.

Calculation of Single-Channel Density

The density of the inward-rectifying K channel in the membrane of J774.1 cells was estimated from the following relation: number of channels = $G/p\gamma$ where G = peak whole-cell conductance in K Hanks', γ = single-channel conductance, and p = maximum open-state probability of a single channel. The average value for 14 cells (normalized to

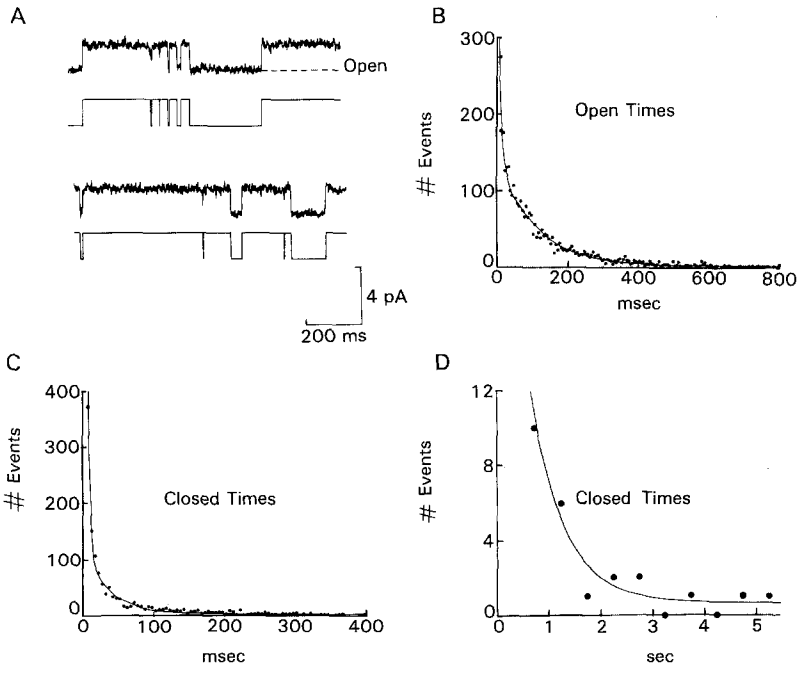

Fig. 10. (*A*) Examples of single-channel fluctuations along with idealized current traces reconstructed from measured open/closed durations. V_h = −80 mV. (*B*, *C*) Open and closed time histograms. Midpoints of the bins are represented as dots. Solid lines are nonlinear least-squares fits of the data by biexponential functions. (*D*) Histogram of very long (>0.5) closed times with monoexponential fit. Total duration of the records used was 9.3 min

cell capacitance) was 47 ± 7 channels/pF; assuming a specific capacitance of 1 μF/cm^2 membrane, this equals a channel density of 0.47 channels/μm^2, or roughly 1100 channels/cell. Others (Sakmann & Neher, 1983) have estimated that the area of free membrane comprising a patch is 2 to 15 μm^2 for electrodes of 2 to 3 MΩ resistance. Assuming this value is reasonable in our case, the number of channels per patch should range from 0 to 7, which is exactly what we observed. Lower channel densities tended to be found in cells that had been plated for short times, an observation consistent with previous work showing that the inwardly rectifying current is expressed with time after plating (Gallin & Sheehy, 1985).

Inactivation

The mechanism of inactivation of inward-rectifying currents was also investigated. In normal Na-containing Hanks', whole-cell currents showed time-dependent inactivation below −100 mV that increased at negative potentials. Inactivation was reduced by more than half in Na-free medium. Na-dependent inactivation of inward-rectifying currents has been well characterized in skeletal muscle (Standen & Stanfield, 1979) and tunicate egg (Ohmori, 1978; Fukushima, 1982), where it has been shown that Na acts as a low-affinity blocking particle. In addition to Na-dependent inactivation, whole-cell inward currents in J774.1 cells also showed inactivation in the absence of external sodium (Fig. 7). Inactivation was shown to be a property of single-channel currents. Ensemble averages showed inactivation in response to hyperpolarizing voltage steps (Fig. 8).

Quantitatively, however, the amount of inactivation observed for whole-cell and averaged single-channel currents was not the same. Whole-cell currents inactivated to, at most, 25% of their peak value in the absence of external Na, and required very large hyperpolarizing pulses (to −160 mV) to do so. In contrast, for steps to −110 mV or more negative, averaged single-channel currents inactivated by about one-half. There are several possible sources of error in our measurements of whole-cell current that could account for some of the discrepancy between inactivation of whole-cell and averaged single-channel currents. First, because inactivation of whole-cell currents was slow, measurements of inactivation were not made at steady state. We measured true steady-state currents by fitting traces with a monoexponential function and found we underestimated the "steady-state" (I_2)/peak (I_1) current ratio by approximately 10%. Values for peak current were underestimated by a few percent at most and did not significantly affect this ratio. A second source of error could be incompletely compensated series resistance (R_s), which would lead to an incorrect measurement of membrane voltage and thus to a misplacement of the I_2/I_1 ratio on the voltage axis. However, since both large- and small-amplitude currents show about the same amounts of inactivation, this error was probably not significant. Since experimental errors cannot fully account for the observed difference between the amount of whole-cell and single-channel inactivation, we must raise the possibility that the difference was due to perfusion of the cell with the contents of the patch pipette. Trube and Hescheler (1984) compared the properties of the cardiac inward-rectifying channel in the cell-attached versus excised patch, and specifically showed that the open probability of the channel increased by a factor of 3 when it was removed from the membrane. This observation is consistent with our finding that steady-state conductance ratios obtained from whole-cell records are greater than expected from measurements of open-channel probability. Attempts to measure the open probability of the channel in a detached patch were unsuccessful. When patches were excised in the inside-out conformation, with the inside of the patch exposed to low-Ca K Hanks', channel activity slowly subsided within 10 min.

Although we have not proven that inactivation of the inward-rectifying current is truly a function of voltage, we feel it unlikely that inactivation is due to block by the external divalent cations Ca^{2+} or Mg^{2+}, since each is present at low concentration. A few preliminary experiments indicate that whole-cell currents in the absence of external Mg^{2+} still show inactivation (*data not shown*). It should also be recalled that averaged single-channel currents showed inactivation, even when [Ca] in the pipette was very low.

Comparison to Other Inwardly Rectifying Channels

Inwardly rectifying K channels have been described in a wide variety of cell types, including skeletal muscle (Ohmori, Yoshida & Hagiwara, 1981), cardiac muscle (Bechem, Glitsch & Pott; 1983; Kameyama et al., 1983; Sakmann & Trube, 1984*a*,*b*; Hume & Uehara, 1985; Kurachi, 1985; Payet, Rousseau & Suave, 1985; Josephson & Brown, 1986), retinal cells (Shingai & Quandt, 1986), invertebrate egg cells (Fukushima, 1981, 1982), HeLa cells (Sauve, Roy & Payet, 1983), and RBL cells (Lindau & Fernandez, 1986). Not all have been fully characterized. Of those that have, the inward-rectifying K channel in J774.1 cells is very similar to one that is present in cardiac ventricular cells and is responsi-

ble for generating the so-called 'background' K current, or I_{K1} (Sakmann & Trube, 1984*a*,*b*). The conductance of the cardiac channel is 27 pS in symmetric K and increases with the square root of $[K]_o$. It has a subconductance state that is three-fourths the amplitude of the fully conducting state. We have also noted fluctuations to less than the fully open state (Fig. 4*A*). Both the cardiac channel and the J774.1 channel have complex kinetics, manifesting closures of short and long duration that indicate the presence of more than one closed state. The steady-state open probability of each channel is voltage dependent; maximum open probability for both is about 0.9 at −40 mV. Minimum open probabilities are slightly different: about 0.4 for J774.1 cells and 0.1 for cardiac cells at −120 mV (Kameyama et al., 1983; Kurachi, 1985; Payet et al., 1985).

Voltage-dependent inactivation of single-channel currents has been well described in ventricular cells from guinea pig (Sakmann & Trube, 1984*b*; Kurachi, 1985) and rabbit (Kameyama et al., 1983) but have not yet been compared to whole-cell currents from the same preparation. While inactivation rates vary, percent inactivation in either preparation is at least 50% at negative voltages, similar to J774.1 cells. Josephson and Brown (1986) described inactivating whole-cell currents in neonatal and adult rat myocytes, but found that three different inward-rectifying channels contributed to this current.

Function of the Inwardly Rectifying Channel

To date, the inwardly rectifying K conductance has been found in macrophages derived from mouse peritoneum (Gallin, 1981) and spleen (Gallin & Livengood, 1981) and in human macrophages (*see* accompanying paper), where its apparent function is to help set the membrane potential near E_K. Another function of this conductance may be to buffer external K, as has been suggested for retinal glial cells (Brew et al., 1986). Since macrophages are often found at sites of cell damage, where $[K]_o$ may be locally elevated, such a function is plausible.

The expression of inward-rectifying channels in J774.1 cells appears to be regulated; currents increase in size with time after plating (Gallin & Sheehy, 1985), which may account for part of the difference between resting potential of cells in the suspended versus adherent state (−15 *vs.* −70 mV, Sung et al., 1985). The appearance of this conductance has not been correlated with the appearance of any specific function in J774.1 cells. Its presence is not necessary for J774.1 cells to be able to carry out chemotaxis or phagocytosis or to generate an oxidative burst; these functions still occur in the presence of 2 mM barium (*unpublished results*, this laboratory). The involvement of the inward-rectifying channel in other macrophage functions, such as secretion or target cell lysis, remains to be tested.

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