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Ion Channels in Human Macrophages Compared with the U-937 Cell Line

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Summary. The human cell line U-937 has been used extensively to model many macrophage functions. We have examined the cell membranes of human monocyte-derived macrophages (HMDM) and U-937 cells to compare membrane properties as expressed by single ion channel currents. The patch-clamp technique was applied to isolated, nonactivated, inside-out patches of cell membranes obtained from HMDM and from the U-937 cell line. Voltage-gated potassium channels of similar conductance but different kinetics are present in both types of cells, and a calcium-activated potassium channel is present only in the HMDM. These differences in ion channel properties suggest fundamentally different behavior between these two cell types at the level of the cell membrane.

Key Words ion channels · human macrophages · U-937 cell line

Introduction

U-937, a human cell line established from a histiocytic lymphoma by Sundstrom and Nilsson (1976) has been used to model a number of macrophage characteristics (Koren, Anderson & Larrick, 1979; Larrick et al., 1980; Guyre, Morganelli & Miller, 1983; Shen, Guyre & Fanger, 1983). Uncertainties regarding the extent to which U-937 cells express certain structural and functional properties of macrophages, however, have been considered by Gourdin et al. (1985). These authors conclude that U-937 cells resemble monoblasts and promonocytes and when differentiated by phorbol esters are transformed into monocyte-like rather than macrophage-like cells. In previous studies with intracellular microelectrodes (*unpublished*) we compared electrical activity in U-937 with that in HMDM. We found that U-937 cells have low (−30 mV) resting potentials and appeared electrically inexcitable when stimulated by injection of constant current pulses. Human monocyte-derived macrophages, on the other hand, have large (−70 mV) resting potentials (Woehlck & McCann, 1986) and generate repetitive, overshooting action potentials in response to injected depolarizing current (McCann et al., 1983). These action potentials are calcium spikes (Woehlck & McCann, 1986) according to the criteria set forth by Hagiwara and Byerly (1981). Since the U-937 cells are very small, injury induced by microelectrode penetration could well reduce resting potential values and inactivate regenerative electrical responses. To circumvent microelectrode injury and to improve resolution of membrane electrical properties, we applied the patch electrode technique (Neher & Sakmann, 1976; Hamill et al., 1981) to measure single ion channels.

The question posed in this study was whether there are fundamental differences in the electrical behavior of U-937 cells compared to HMDM. Such differences could relate to functional adjustments between mononuclear phagocytes that relate to varying stages of development. We present data that demonstrate both similarities and differences in the electrical properties of U-937 cells and HMDM and suggest that maturation levels may well be a factor in the expression of membrane ion channels.

Materials and Methods

Cell cultures: The method for separating mononuclear cells (MNC) from the blood of normal human donors was modified from that described by Boyum (1966). MNC were obtained by centrifugation (350 × *g*, 30 min) of a mixture of 15 ml heparinized blood plus 10 ml normal saline over a 15-ml cushion of Ficoll-Hypaque (density 1.077). MNC were washed 3× in RPMI-1640 (KC Biological, Lenexa, Kans.) plus 50 μg/ml gentamicin (Shering, Kenliworth, N.J.) and resuspended at 2 × 10^6 cells/ml in medium plus 10% pooled human serum, 50 μM 2-mercaptoethanol, and cultured in 120 ml Teflon vials (Savillex, Minnetonka, Minn.), 5–20 ml/vial for 5 to 30 days. On the day of analysis, 1 ml was removed from the vial and added to the inverted lid of a Falcon #3001 petri dish and incubated for 30 min to 4 hr at 37°C, 5% CO_2. Just prior to analysis, nonadherent cells and proteins were removed by three washes with medium. New

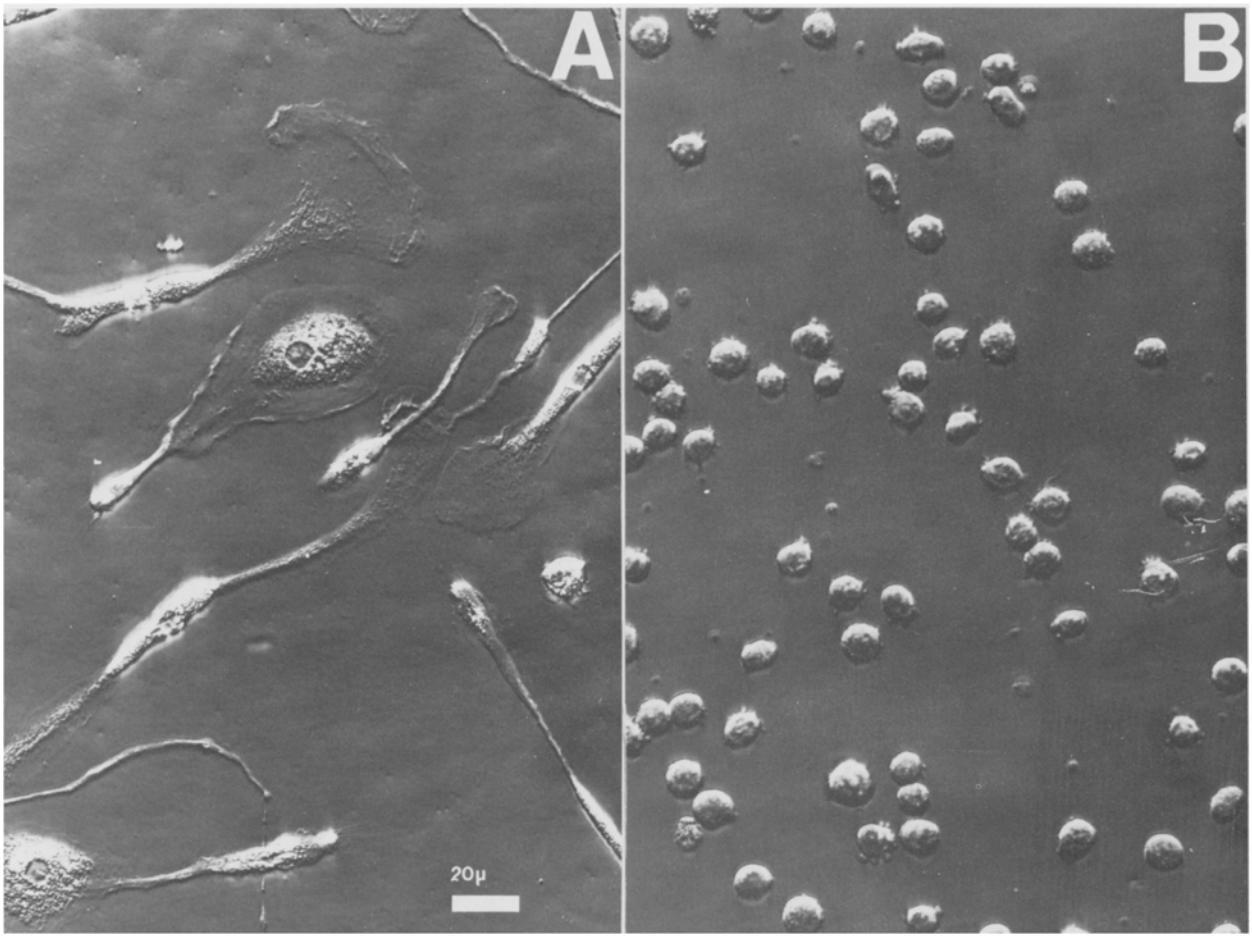

Fig. 1. (*A*) Phase contrast photo of human monocyte-derived macrophages obtained from a normal donor and maintained in culture for seven days. (*B*) Phase contrast photo of U-937 cells maintained in culture for seven days. Calibration is same for both *A* and *B*

medium was then added and cells with the characteristic "fried egg" morphology of mononuclear phagocytes were patched (*cf.* Fig. 1*A*) within 4 hr of plating.

U-937 cells (*cf.* Fig. 1*B*), obtained from the American Type Culture Collection (Rockville, Md.), were maintained at a cell density of 3 to 8 × 10^5/ml in 75 cm^2 flasks (Costar, Cambridge, Mass.) in a medium supplemented with 10% fetal bovine serum (Sterile Systems, Logan, Ut.). U937 cells were washed, plated and patched as described for monocytes.

Electrodes

Patch pipettes were pulled in two steps from methanol-chloroform cleaned capillary glass (Corning, #7052) on a mechanical puller. Sylgard coating was applied and the electrode tips were fire-polished. Resistances averaged 6 to 8 MΩ.

Solutions

Test solutions had the following compositions (in mM): [A] (normal extracellular saline), 140 NaCl, 4.5 KCl, 2.0 $CaCl_2$, 1 $MgCl_2$; [B] (10^{-8} Ca^{2+}), 140 KCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 1.1 EGTA; [C] (10^{-7} Ca^{2+}) 140 KCl, 1 $MgCl_2$, 0.55 $CaCl_2$, 1.1 EGTA; [D] (10^{-6} Ca^{2+}) 140 KCl, 1 $CaCl^{2+}$, 1.1 EGTA, 1 $MgCl_2$. Solutions were buffered with 10 mM HEPES and 5 mM NaOH or 5 mM KOH as needed (pH 7.4).

Recording and Analysis

The chamber containing the cells was placed on the stage of a Zeiss inverted phase-contrast microscope equipped with a water immersion objective (×640). Experiments were carried out at room temperature (22–24°C). An IBM AT computer generated holding and command voltages that were applied to the pipette via a Dagan Patch Clamp circuit. Prior to each command pulse, the membrane was clamped at a preselected holding voltage that approximated the resting transmembrane voltage and from that value, the membrane was stepped to and clamped at various voltages. Each step consisted of a 20-sec (command) pulse followed by an interval of 20 sec at the holding voltage. Patch currents were measured during each command pulse, digitized as 12-bit (4096) numbers and stored on a floppy disk for later analysis. Computer sampling interval was 1 msec. The data acquisi-

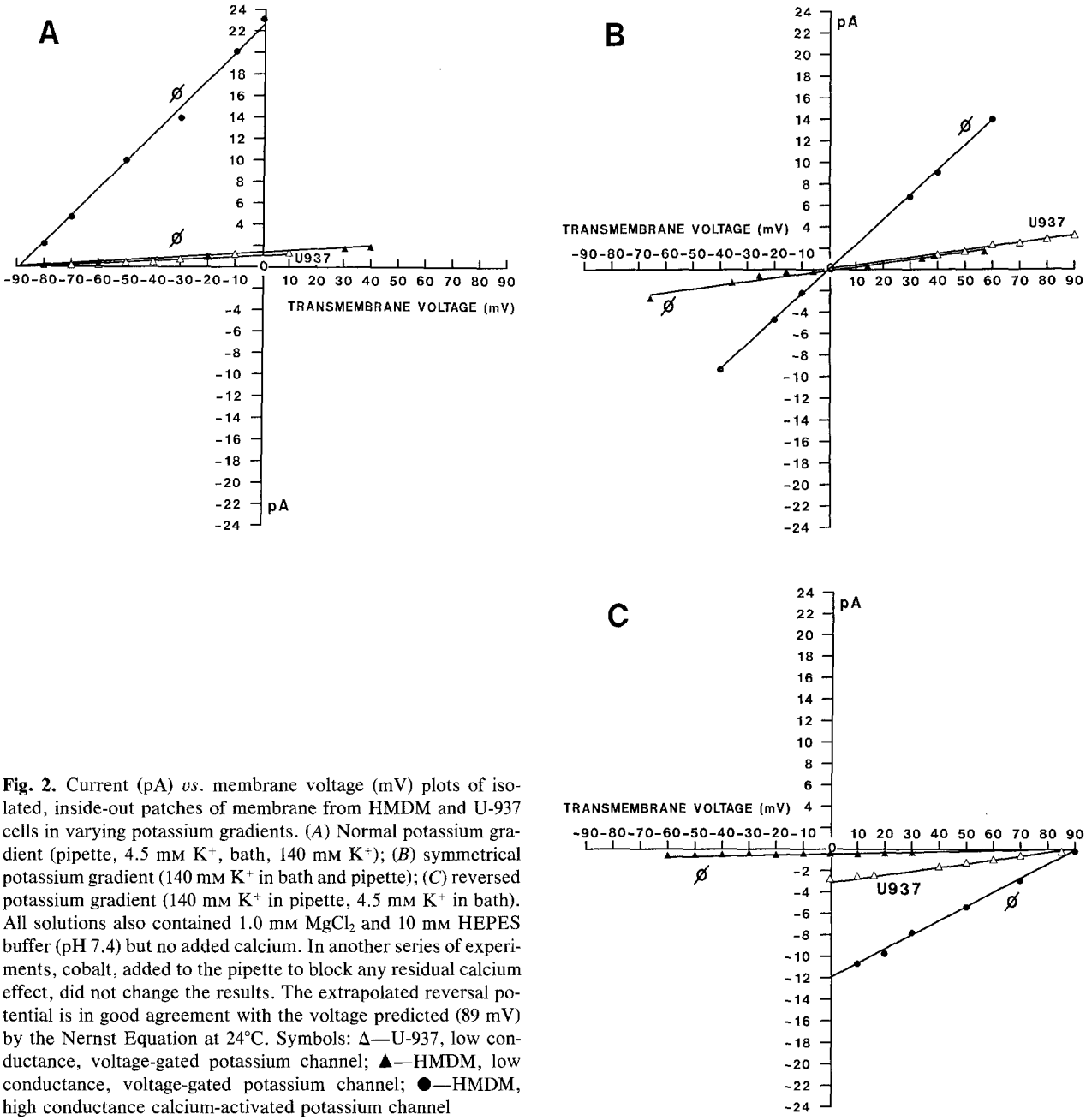

Fig. 2. Current (pA) *vs.* membrane voltage (mV) plots of isolated, inside-out patches of membrane from HMDM and U-937 cells in varying potassium gradients. (*A*) Normal potassium gradient (pipette, 4.5 mM K^+, bath, 140 mM K^+); (*B*) symmetrical potassium gradient (140 mM K^+ in bath and pipette); (*C*) reversed potassium gradient (140 mM K^+ in pipette, 4.5 mM K^+ in bath). All solutions also contained 1.0 mM $MgCl_2$ and 10 mM HEPES buffer (pH 7.4) but no added calcium. In another series of experiments, cobalt, added to the pipette to block any residual calcium effect, did not change the results. The extrapolated reversal potential is in good agreement with the voltage predicted (89 mV) by the Nernst Equation at 24°C. Symbols: Δ—U-937, low conductance, voltage-gated potassium channel; ▲—HMDM, low conductance, voltage-gated potassium channel; ●—HMDM, high conductance calcium-activated potassium channel

tion and analysis programs are described in detail in another publication (McCann, Stibitz & Keller, 1987).

Results

Morphology

The two types of cells used in this study are shown in Fig. 1. The cells in *A* are HMDM that have been maintained in culture for 14 days. U-937 cells (*B*) are about five times smaller in diameter than HMDM and appear to have multiple protrusions on their surface.

Potassium Channels

Both cell-attached and inside-out configurations were tested on HMDM and U937 cells with essen-

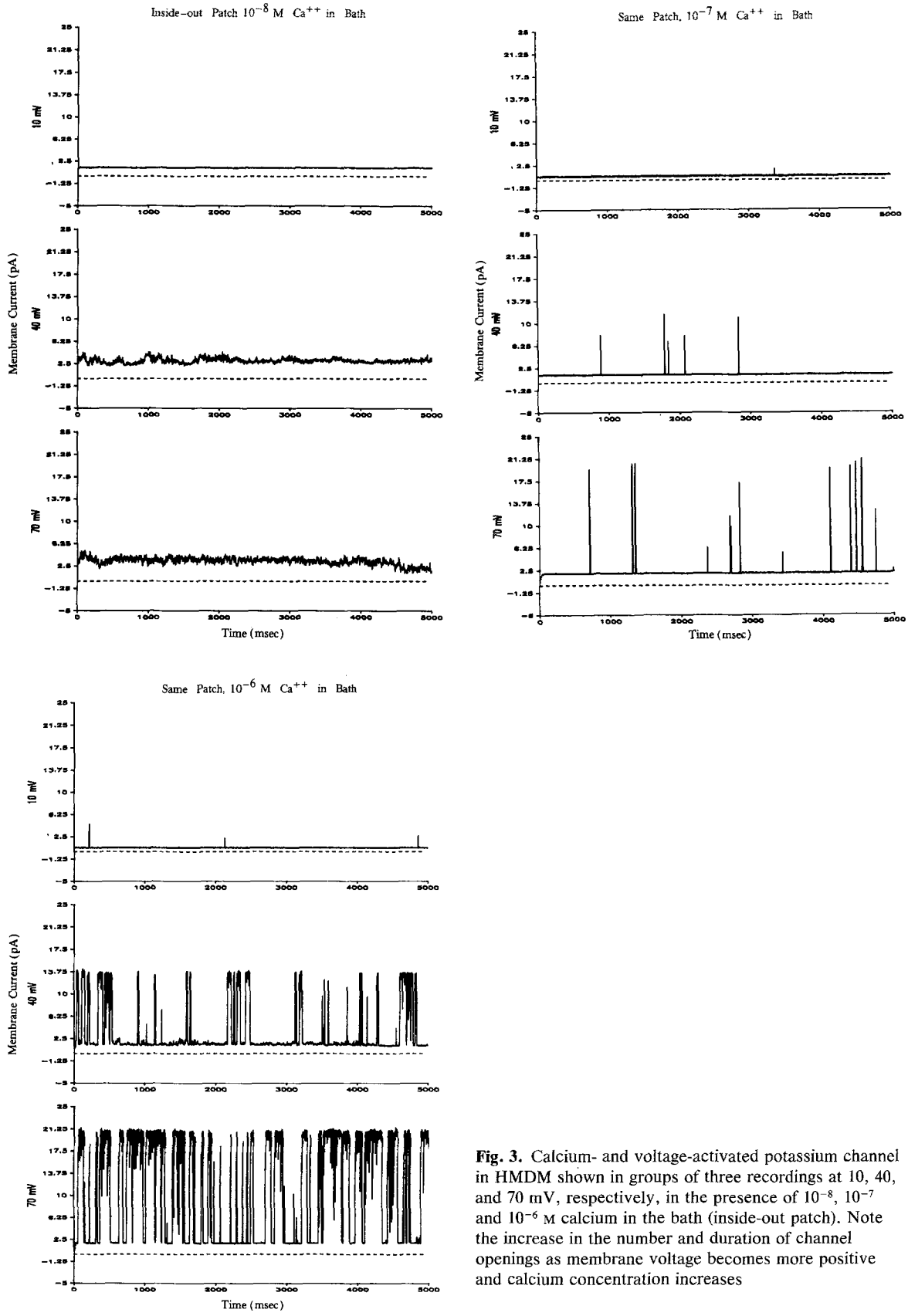

Fig. 3. Calcium- and voltage-activated potassium channel in HMDM shown in groups of three recordings at 10, 40, and 70 mV, respectively, in the presence of 10^{-8}, 10^{-7} and 10^{-6} M calcium in the bath (inside-out patch). Note the increase in the number and duration of channel openings as membrane voltage becomes more positive and calcium concentration increases

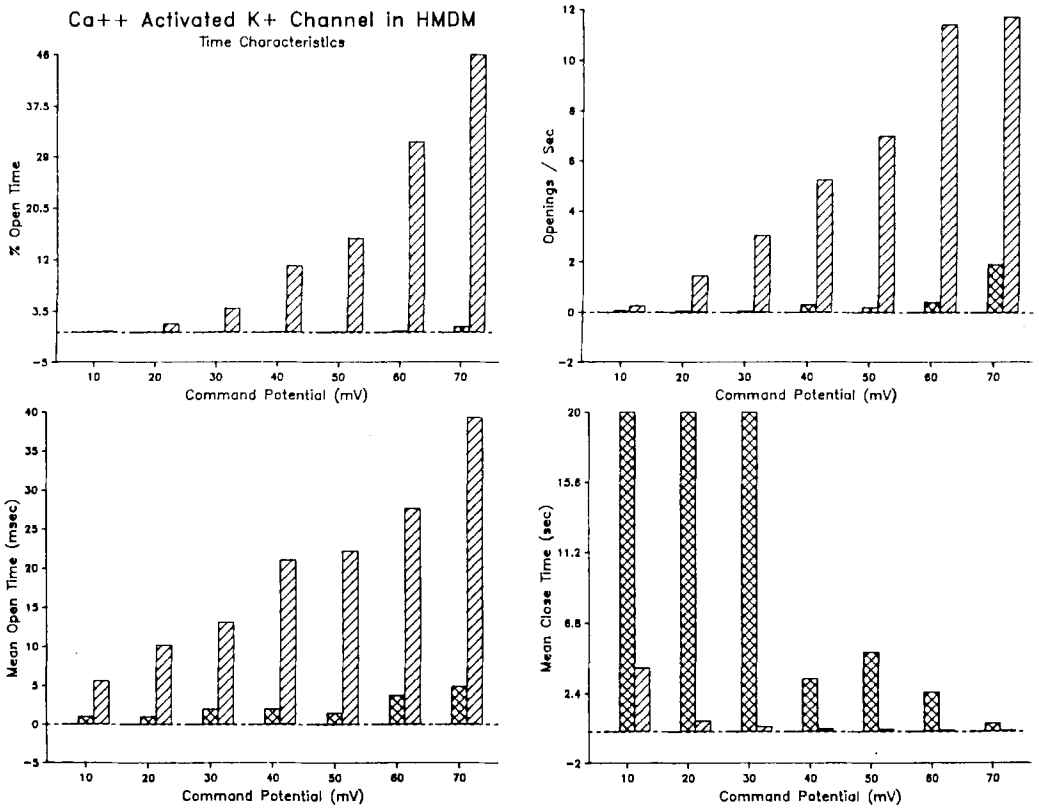

Fig. 4. Time characteristics of the calcium- and voltage-activated potassium channel in HMDM. Data from a single excised patch in symmetrical K^+ with the indicated Ca^{2+} concentration in the bath. *Percent open time* is defined as the length of time the channel spends in the open state (dwell time) divided by the total recording time. *Openings per second* is the number of transitions from the baseline, and the *mean open time* is the total open dwell time divided by the number of openings in the record. Similarly, the *mean closed time* is the total closed dwell time divided by the number of closings in the record. ▨, Inside-out patch, 10^{-6} M Ca^{2+} in bath. ▩, Same patch, 10^{-7} M Ca^{2+} in bath. ▧, Same patch, 10^{-8} M Ca^{2+} in bath

tially similar results. Inside-out patches were much more difficult to achieve in U937 than in HMDM. Current-voltage plots for HMDM and U937 cells in three different potassium gradients, normal (*A*), symmetrical (*B*) and reversed (*C*) are summarized in Fig. 2. It can be seen that two different channels, one with a low conductance and one with a higher conductance, are present in HMDM. In symmetrical potassium these conductances were 37 ± 15.8 pS (5 of 9 patches) and 255 ± 43 pS (25 of 40 patches), respectively. Only the low conductance (51 ± 23.1 pS, 10 of 21 patches) channel is present in the U-937. The errors are reported as 2 standard deviations from the mean. The *I vs. E* curves were linear over the voltage range tested. Reversal potentials, in normal, symmetrical and reversed potassium gradients were −90, 0 and +90 mV, respectively. Aspartate substitution for chloride did not alter the reversal potentials; thus, this channel is not chloride selective.

Calcium and Voltage-Activated Potassium Channels

The large conductance potassium channel identified in HMDM is sensitive to the level of intracellular calcium as well as transmembrane voltage. A sample recording of the effects of increasing levels of calcium and increased positive voltages on this channel are shown in Fig. 3. In a total of 25 successful patches, the conductance averaged 255 (±43) pS. A kinetic analysis of this channel, shown in Fig. 4, demonstrates that the percent open time, openings/second and mean open time increase as the membrane voltage becomes more positive and as the calcium concentration increases.

Absence of Calcium-Activated Potassium Channels in U937

No large conductance channel of any type was observed in 50 excised patches from U937 cells with

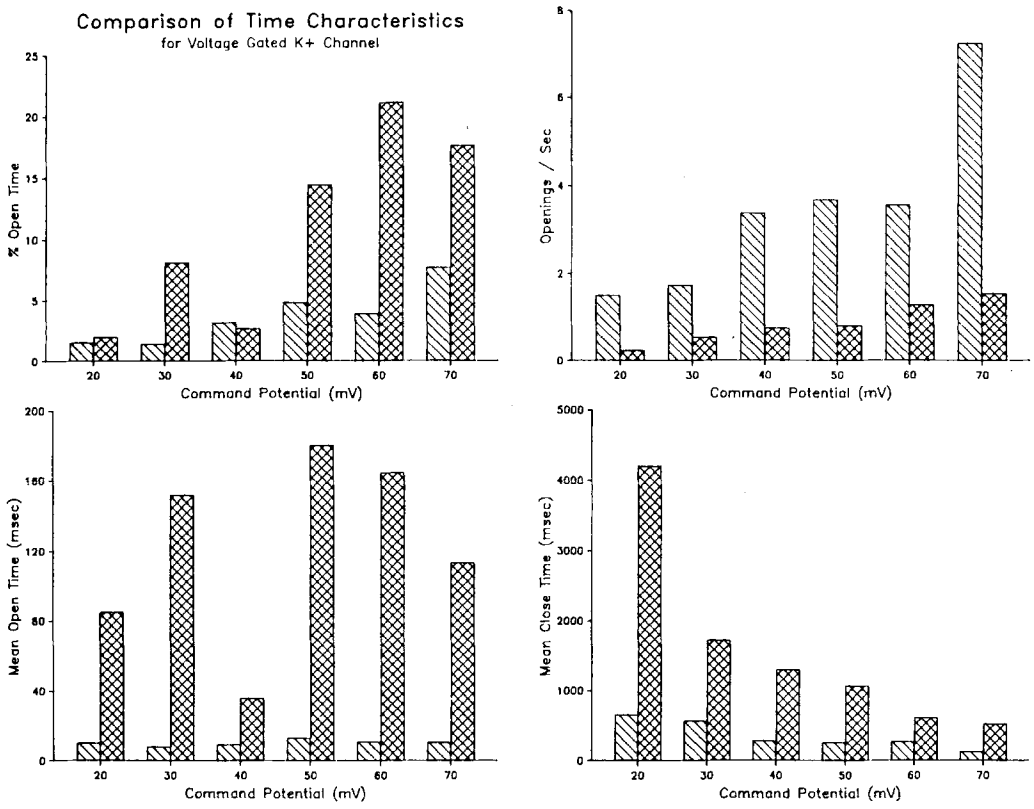

Fig. 5. A comparison of kinetic behavior of the voltage-gated potassium channel in the HMDM and in the U-937 cell. Data are from excised patches in symmetrical K^+ and with 10^{-7} M Ca^{2+} in the bath. The number of openings per second is greater in the HMDM, but the time spent in the open state is much longer in U-937 than in HMDM. Dwell times do not appear voltage sensitive. ⧹⧹, Seven day cultured HMDM. ⧸⧸, U937 cell line

10^{-6} M Ca^{2+} in the bath. In HMDM the Ca^{2+}-activated K^+ channel was observed in 25 of 40 excised patches with 10^{-6} M Ca^{2+} in the bath. In those patches in which this latter type of channel was present, there was an average of two channels per patch. Assuming that the patches where no activity was observed possessed no channels, that the distribution of channels is uniformly random, and that the average patch had a surface area of 5 μm^2, then the channel density would be about 0.4 channels/μm^2. The probability of finding one of these channels in any given patch of HMDM is 0.65. If the channel were expressed at a similar level in the U937 cell line then the probability of no observations in 50 trials is vanishingly small.

Voltage-Gated Potassium Channels

A comparison of the time characteristics of the small conductance channel in HMDM and U-937 cells is presented in Fig. 5. These data demonstrate that the voltage-gated potassium channel of low conductance, present in both HMDM and the U-937 cell line, exhibits different kinetic behavior. The number of openings/sec is markedly greater in HMDM, but the percent and mean open times of the U-937 channel is far greater than that in the HMDM. In both cell types it is clear that the mean open dwell time appears to be insensitive to the membrane potential; however, the mean closed dwell time is clearly voltage dependent, decreasing as the membrane is depolarized.

Discussion

Ion channels in human macrophages and in other cells of the immune system appear to be an integral link in the chain of events that activate immune responses (Gallin, 1984*a*; Chandy et al., 1985; Nelson et al., 1985). Types of ion channels described in human and murine macrophages include outwardly (Ypey & Clapham, 1984) and inwardly (Gallin & Sheehy, 1985) rectifying channels, calcium- and voltage-activated potassium channels (Gallin, 1984*b*), chloride channels of very large conductance

(Schwarze & Kolb, 1984) and ligand-activated channels (Young et al., 1983; Young, Unkeless & Cohn, 1985). While a definitive link has yet to be established between specific macrophage functions and membrane excitability, several studies suggest that ion channel behavior may correlate to transduction events such as F_c receptor activation (Young et al., 1983; Nelson et al., 1985), chemotaxis (Gallin & Gallin, 1977) and phagocytosis (*cf.* review by Gallin, 1986).

The voltage-gated potassium channels described here in the HMDM and the U-937 cell line have similar conductances (37 pS, 51 pS, respectively), but somewhat different kinetic properties. The U-937 channel exhibits fewer openings per second than HMDM, but the individual channels have open dwell times some tenfold greater. The much longer open times would allow for a higher rate of potassium flux in the U-937 and would imply that the channels may be functionally dissimilar. Further, the channel expressed in the U937 cell line is only active as the membrane is depolarized and shows no sign of inactivation with time, while that of HMDM is activated at both hyper- and depolarizing potentials and also shows no time inactivation. The mean open time appears voltage independent in both cell types.

This channel in the HMDM is most similar to the outward rectifier described in 4 day or younger macrophages by Ypey and Clapham (1984) although its conductance is somewhat larger. From the single channel recordings we have made there is no apparent time inactivation of the channel. In symmetrical K^+ the channel was active at all potentials tested (−90 to 90) mV; steps to more negative potentials were not tried. This channel clearly is not identical in behavior to that described by Ypey and Clapham and may therefore represent a separate type of activity. However, the cells used in this study averaged 10 days in culture and were selected for patching on the basis of their morphology as mature macrophages; thus, the differences observed in behavior could be due to differences in the degree of maturation.

The presence of a calcium-activated potassium conductance was suggested by earlier studies with microelectrodes (Gallin et al., 1975; Persechini, Araiyo & Oliviero-Castro, 1981; Gallin, 1981; Oliviera-Castro & Dos Reis, 1981). The calcium- and voltage-activated potassium channel we describe here appears identical to that reported by Gallin (1984). This channel is not observed in immature macrophages and develops only after 2 to 4 days in culture (Gallin, 1985). This channel is not found in the U-937 cell line, and it may be that either this channel is not synthesized or that it has not been "turned-on" by activating factors at early stages of mononuclear phagocyte maturation.

U-937 cells exhibit some morphological and functional characteristics of activated and/or differentiated macrophages following treatment with a number of mediators. These include lymphokines such as gamma interferon (Koren et al., 1979; Larrick et al., 1980; Guyre et al., 1983; Ralph et al., 1982; Shen et al., 1983), retinoic acid (Olsson & Breitman, 1982), 1,25 dihydroxy-vitamin D_3 (Amento et al., 1984; Rigby et al., 1984; Gullberg et al., 1985), phorbol esters (Nilsson et al., 1981; Ralph et al., 1982; Radzun et al., 1983; Gourdin et al., 1985).

Gourdin et al. (1985) concluded, based on peroxidase cytochemistry, that U-937 cells correspond developmentally to monoblasts and promonocytes, and that stimulation by phorbol esters caricatures transformation into monocytes but not macrophages. Our results demonstrate two significant differences in the ion channels observed in the two cell types, the presence of a voltage- and calcium-activated potassium channel in HMDM but not in U-937 cells and a voltage-gated potassium channel which displays different kinetics. The absence of the calcium-activated channel in the U-937 may be further evidence of its immaturity, for Gallin (1985) has determined that this channel is not present in immature macrophages. The voltage-gated potassium channel may simply display different kinetics or it may be a different type of channel with different functions. It will now be important to compare the ion channels of normal monocytes with those of macrophages and to ascertain the effects of various mediators on ion channel development in U-937 cells. In particular, the voltage- and calcium-activated potassium channel may be a unique marker of mature macrophages and may not be present in less mature cells of the mononuclear phagocyte lineage.

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