CALCIUM- AND VOLTAGE-ACTIVATED POTASSIUM CHANNELS IN HUMAN MACROPHAGES

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ABSTRACT Single calcium-activated potassium channel currents were recorded in intact and excised membrane patches from cultured human macrophages. Channel conductance was 240 pS in symmetrical 145 mM K^+ and 130 pS in 5 mM external K^+. Lower conductance current fluctuations (40% of the larger channels) with the same reversal potential as the higher conductance channels were noted in some patches. Ion substitution experiments indicated that the channel is permeable to potassium and relatively impermeable to sodium. The frequency of channel opening increased with depolarization and intracellular calcium concentration. At 10^{-7} M $(Ca^{++})_i$, channel activity was evident only at potentials of +40 mV or more depolarized, while at 10^{-5} M, channels were open at all voltages tested (−40 to +60 mV). In intact patches, channels were seen at depolarized patch potentials of +50 mV or greater, indicating that the ionized calcium concentration in the macrophage is probably $<10^{-7}$ M.

INTRODUCTION

Calcium-activated potassium conductances have been described in a wide variety of cells (1, 11). Several observations indicate that macrophages also exhibit a calcium-activated potassium conductance. First, macrophages exhibit spontaneous membrane hyperpolarizations associated with an increase in conductance, which can be blocked by the addition of EGTA, verapamil, and cobalt (2, 6). Second, injection of intracellular calcium or exposure to the calcium ionophore, A23187, produces hyperpolarizations similar to the spontaneously occurring ones (6, 10). However, because stable intracellular recordings using standard microelectrodes have been difficult to obtain in macrophages, the conductance underlying these events has not been well characterized. In this study, patch clamp techniques were used to demonstrate the presence in human macrophages of voltage- and calcium-activated potassium channels with a conductance of 130 pS in 5 mM external potassium, and 240 pS in symmetrical KCl. The frequency of channel opening increased with depolarization and increasing intracellular ionized calcium in the range of 10^{-7}–10^{-5} M. These channels are very similar to the large-conductance calcium-activated potassium channels described in other cells (1, 7, 13).

MATERIALS AND METHODS

Human peripheral blood monocytes were isolated by density centrifugation on Ficoll-Hypaque gradients (5) and cultured at 37°C in RPMI-1640 containing 5% fetal bovine serum (heat inactivated at 56°C for 30 min), 10,000 μ/ml penicillin-streptomycin, and 5% glutamine. RPMI-1640, a synthetic tissue culture medium originally designed for growing leukemia cells, was developed at Roswell Park Memorial Institute. After various periods of cultivation (up to 3 wk), cells were placed in recording solutions and patch clamp experiments were performed at room temperature (21–23°C).

Recordings were obtained using a patch clamp with 1 kHz low-pass filtering (EPC-5; List Industries, Inc. Matteson, IL). Patch electrodes had resistances of 3–6 MΩ. Seals resistances ranged from 10 to 100 GΩ. The resting membrane potential of the cells was measured following the destruction of the patch membrane by increased suction. Channel activity was recorded on a chart recorder (Gould, Inc., Instrum. Div., Santa Clara, CA) and an FM tape recorder (frequency response DC to 5 kHz). Channel data were analyzed by hand after playing back at 1/8 speed into a Gould chart recorder.

The ionic composition of the medium in the patch electrode and the bath varied in different experiments. NaCl-Hanks' contained 145 mM NaCl, 4.6 mM KCl, 1.13 mM $MgCl_2$, 1.6 mM $CaCl_2$, and 10 mM HEPES-NaOH pH 7.3. KCl-Hanks' contained 145 mM KCl, 10 mM NaCl, 1.13 mM $MgCl_2$, 10 mM HEPES-KOH, pH 7.3, and various concentrations of EGTA and calcium. K-aspartate-Hanks' contained 145 mM K-aspartate instead of KCl. For free calcium levels $<10^{-5}$ M, 1.1 mM EGTA and various amounts of calcium were added to the medium to obtain the desired levels of free calcium (1.08 mM calcium for a final concentration of 4×10^{-6} M Ca^{++}; 1.06 mM for 3×10^{-6} M Ca^{++}; 0.92 mM for 5×10^{-7} M Ca^{++}; 0.55 mM for 1×10^{-7} M Ca^{++}). The free calcium concentration was calculated using 10^{-7} M as the apparent dissociation constant for the Ca-EGTA complex (8). The free calcium concentration in each solution was checked using a calcium-sensitive electrode calibrated with calcium buffers obtained from W-P Instruments, Inc. (New Haven, CT). The calculated and measured calcium levels were in good agreement. The calcium concentration of recording medium containing no calcium or EGTA was $\sim 10^{-5}$ M, as determined by a calcium-sensitive electrode.

RESULTS

Cell-attached patches from cells bathed in KCl-Hanks' exhibited large, brief (30 ms or less) outward currents at

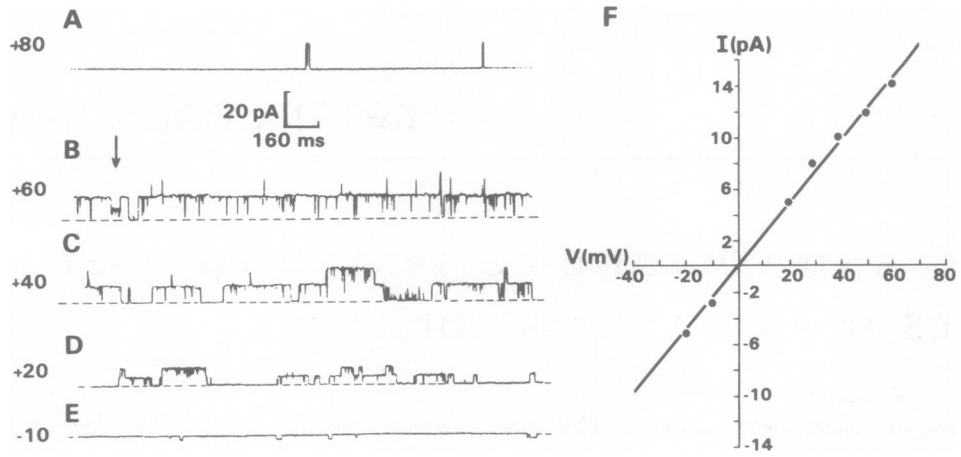

FIGURE 1 (*A–E*) Single-channel current traces. Both electrode and bath contained KCl-Hanks' (4×10^{-6} M Ca^{++}). (*A*) Current trace obtained in the cell-attached mode at a holding potential of +80 mV. (*B–E*) Current traces from the same patch as in *A* following excision. Patch is inside-out. Potentials for each tracing and zero current level (dashed line) are indicated. Traces are typical exerpts of longer records. Upward deflections represent outward current in this and other figures. (*F*) Current-voltage relationship of the same patch. Channel amplitudes were read from the chart records.

depolarized patch potentials of +50 mV or greater. Channel activity increased with increasing depolarization. These channels were rarely seen in cells bathed in NaCl-Hanks' at voltages ranging from −80 to +80 mV. However, channels were often recorded at potentials of +100 mV or more depolarized. Fig. 1 *A* shows an in situ current-tracing at a patch holding potential of +80 mV in a cell bathed in KCl-Hanks' (4×10^{-6} M Ca^{++}). No channel activity was noted at less depolarized holding potentials. Upon detachment of the patch in the inside-out configuration, channels were absent at voltages negative to −20 mV. Fig. 1, *B–E*, depicts channel activity recorded at various voltages. The frequency of opening and open-time increased as the patch was depolarized. As shown in Fig. 1 *D*, channel conductance (225 pS) was constant in the voltage range tested, and reversed at 0 mV. The channel conductance of 13 patches in symmetrical potassium solutions ranged from 200 to 275 pS and averaged 240 pS.

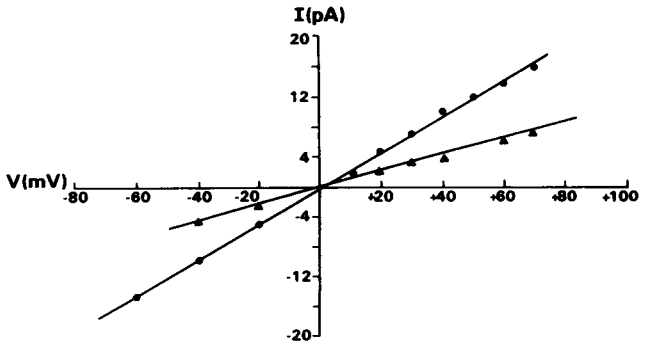

FIGURE 2 Single-channel current amplitude for large (●) and small (▲) conductance fluctuations as a function of voltage. Excised inside-out patch. Electrode contained K-aspartate-Hanks' and bath contained KCl-Hanks' (10^{-5} M Ca^{++}).

Ninety percent of the patches from which recordings were taken exhibited these channels, and patches often contained three or more channels, as judged by the number of conductance states. Smaller current fluctuations, with a conductance of ~40% of the higher conductance state, were noted in a number of experiments. The arrow in Fig. 1 *B* points to one of these events. These fluctuations were only seen in patches exhibiting the 240-pS channels, and they always had the same reversal potential as the larger conductance channels. The current-voltage relationship of both the high (240 pS) and low (100 pS) conductance state from an excised inside-out patch is shown in Fig. 2. In some studies, small outward currents with a much lower conductance (<20 pS) were also evident (Fig. 4 *D*). These currents have not been characterized, but they occurred in isolation and were often noted at lower calcium concentrations, conditions under which the higher conductance channels were not active.

A series of experiments to determine which ion(s) was responsible for this conductance indicated that the channel was permeable predominantly to potassium. The data from one of these studies are shown in Fig. 3 *A*, in which an excised outside-out patch with K-aspartate-Hanks' in the electrode was exposed to extracellular solutions containing different concentrations of potassium. The voltages are given with respect to the intracellular surface. Large channels with a conductance of 200 pS, that inverted at zero holding potential, were noted in 145 mM KCl outside, indicating that the channel was relatively impermeable to chloride, which had a negative equilibrium potential under these conditions. Replacing half the KCl in the bath with NaCl decreased the conductance of the channel from 200 to 160 pS, and shifted the reversal potential by 15 mV. Subsequent replacement of all the KCl with NaCl further reduced the channel conductance, produced a nonlinear

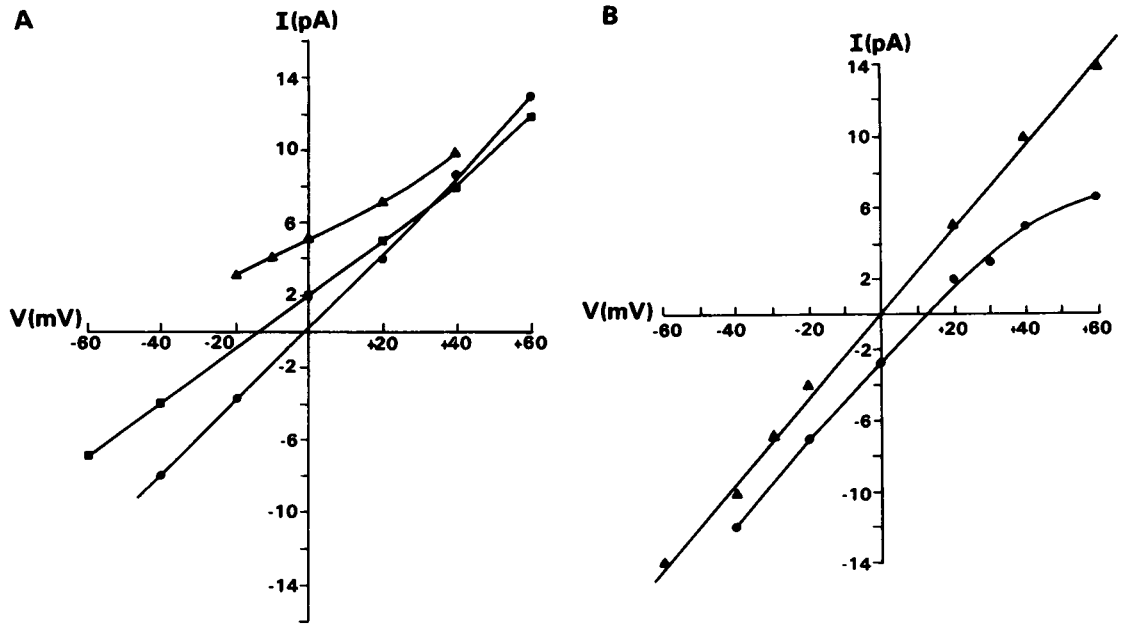

FIGURE 3 Single-channel current amplitude as a function of voltage. (*A*) Excised outside-out patch. The electrode contained K-aspartate-Hanks' (3×10^{-6} M Ca^{++}). (●) Channel current amplitudes obtained with bathing solution same as electrode solution except K-aspartate was replaced with 145 mM KCl. Bath KCl was subsequently reduced initially to 76 mM KCl by increasing NaCl to 79 mM (■), and finally to 10 mM KCl by increasing NaCl to 145 mM (▲). (*B*) Excised inside-out patch. Electrode contained the same solution as in *A*. (▲) indicates channel amplitudes with bath containing KCl-Hanks' (4×10^{-6} M Ca^{++}). (●) Bath potassium reduced to 73 and Na to 83 mM.

I-V relationship, and shifted the reversal potential to a more negative level, indicating that the channel was relatively impermeable to sodium. The null potential could not be obtained under these conditions, since channels did not open at levels more negative than −20 mV current. This experiment was repeated six times with similar results. The average conductance of seven different excised patches when NaCl bathed the outside of the membrane and KCl the inside, was 130 pS. These results indicate that (*a*) the channel behaves as if it is permeable to potassium and relatively impermeable to sodium or chloride, (*b*) increasing the sodium concentration decreases the channel's conductance, and (*c*) in the presence of 145 mM extracellular Na, the conductance is nonlinear, decreasing with hyperpolarization.

Similar experiments were performed on inside-out patches. That is, the concentration of potassium in the bath was decreased, while that of sodium was increased. The current-voltage relationship from one of these studies is shown in Fig. 3 *B*. Replacing 75 mM potassium with sodium shifted the reversal potential from 0 to +12 mV. In addition, the conductance decreased, as would be expected with the reduction of the permeant ion (potassium).

Changing the level of ionized calcium at the intracellular surface altered channel activity (the number of openings per second and the open-time) without affecting channel conductance or reversal potential. Changing the ionized calcium in the extracellular medium had no effect on the channels. Fig. 4 depicts single channel currents from an inside-out patch at a holding potential of +40 mV with various concentrations of ionized calcium in the bath. In 3×10^{-6} M Ca^{++} (Fig. 4 *A*) channels opened frequently. Decreasing the free calcium to 5×10^{-7} M reduced

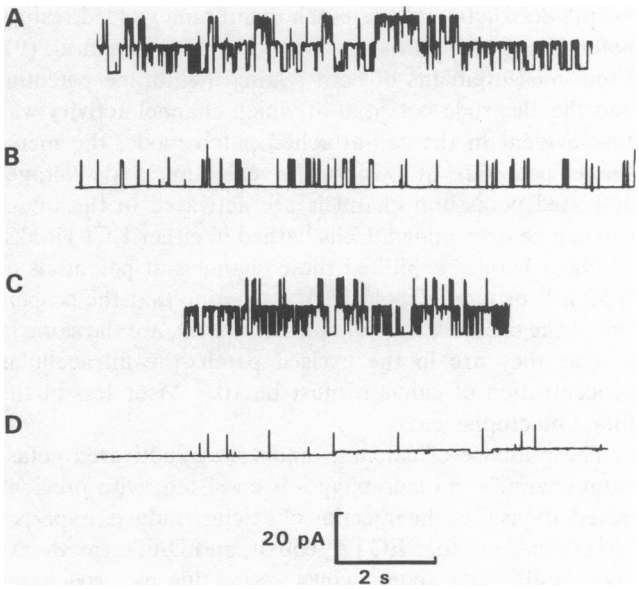

FIGURE 4 Single channel current traces from an excised inside-out patch at +40 mV holding potential. Tracings obtained in KCl-Hanks' (3×10^{-6} M Ca^{++}) in the electrode. Bath contained same medium as electrode except ionized calcium concentration was 3×10^{-6} M in *A* and *C*, 5×10^{-7} M in *B*, and 1×10^{-7} M in *D*.

channel activity (Fig. 4 *B*), which returned to the original level when the medium was changed back to one containing 3×10^{-6} M calcium (Fig. 4 *C*). Subsequent reduction of ionized calcium to 1×10^{-7} M (Fig. 4 *D*) further reduced channel activity. Similar records were obtained with eight other patches. At a calcium concentration of 10^{-5} M (calcium-free-EGTA-free Hanks'), channels were open at all holding potentials studied (+60 to −40 mV). In Fig. 5, the percent channel open-time at different holding potentials is plotted for the patch shown in Fig. 4.

In a number of studies, the patch membrane was destroyed following recordings in the cell-attached patch configuration. Cells in NaCl-Hanks' (recording electrodes contained KCl-Hanks' and 10^{-6} M Ca^{++} or less) had resting membrane potentials averaging −43 mV (range −25 to −60 mV). Cells bathed in KCl-Hanks' predictably had potentials near zero (range +5 to −3 mV).

DISCUSSION

The calcium- and voltage-activated potassium channel described in this paper has a similar conductance and voltage dependence to the calcium-activated potassium channel described in a variety of cells (1, 11, 13). The calcium sensitivity of these channels in the macrophage is similar to that of skeletal muscle (1). Two other channels have been described in macrophages. The first is a large conductance (>100 pS) cation-nonselective and calcium-independent channel (7), and the second is an inward-rectifying channel with a conductance in the range of 16–30 pS (4). Both of these channels have properties different from the channels described in this paper.

Membrane potential measurements of macrophages following destruction of the patch membrane yielded resting potentials similar to those reported by McCann et al. (9). From measurements of both resting membrane potential and the electrode potential at which channel activity was first evident in the cell-attached patch mode, the membrane potential at which the calcium- and voltage-activated potassium channels are activated in the intact cell can be determined. Cells bathed in either KCl-Hanks' or NaCl-Hanks' exhibited these channels at potentials of +50 mV or more depolarized. Assuming that the properties of the channel, i.e., calcium sensitivity, are the same in situ as they are in the excised patch, the intracellular concentration of calcium must be 10^{-7} M or less in the intact macrophage.

The presence of calcium- and voltage-activated potassium channels in macrophages is consistent with previous observations that the injection of calcium induces hyperpolarizations, and that EGTA, cobalt, and D-600 (methoxyverapamil) block spontaneously occurring hyperpolarizations in macrophages (6, 10). It is likely that the channels described in this paper represent the individual events that produce these spontaneous and induced membrane hyperpolarizations.

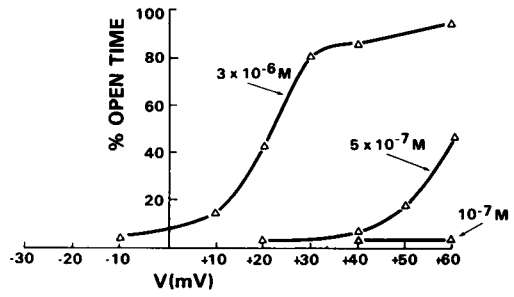

FIGURE 5 Plot of percentage of time channels were open against holding potential for the indicated $(Ca)^{++}_i$. Channel open time was determined by playing back 30–40 s of channel data at 1/8 speed into a Brush chart recorder and analyzing the data by hand. Data were obtained from same patch as in Fig. 4.

The relationship of the calcium-activated potassium conductance to macrophage function is unclear. Macrophages are well known for their secretory, phagocytic, and motile properties. Changes in membrane potential and intracellular calcium levels have been implicated in some of these functions (5, 14). Both the calcium ionophore A23187 and endotoxin-activated serum stimulate secretion in macrophages and produce membrane hyperpolarizations, presumably by activating a calcium-activated potassium conductance (5, 6). However, it is not known whether or not these changes in conductance play an important role in the secretory response to these stimuli. The calcium-activated potassium conductance, once triggered, might modulate other voltage-dependent conductances in much the same way it does in neuronal cells. In addition, potassium released from macrophages following activation of this conductance may influence cells, such as T lymphocytes, that are often found in close physical contact with the macrophage (12).

In summary, this paper demonstrates that both intracellular calcium and voltage activate a potassium channel in human macrophages that is similar to the large-conductance calcium-activated potassium channel in other cells (1, 11).

I thank S. Green for valuable technical assistance, J. Metcalf for providing human monocytes, and J. Freschi for advice on patch clamp techniques and critical review of the manuscript.

This work was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Research Work Unit MJ 00020. The views presented in this paper are those of the author. No endorsement by the Defense Nuclear Agency has been given or should be inferred.

Received for publication 3 April 1984 and in final form 6 August 1984.

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