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Whole-Cell Currents in Macrophages: II. Alveolar Macrophages

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Summary. Although an outwardly rectifying K^+ conductance has been described in murine peritoneal macrophages and a murine macrophage cell line, the expression of this conductance in human monocyte-derived macrophages (HMDMs) is rare. Whole-cell current recordings in this study were obtained from HMDMs differentiated in adherent culture for varying periods of time following isolation and compared to currents obtained in human alveolar macrophages (HAMs) obtained from bronchoalveolar lavage. These studies were undertaken to compare ionic current expression in the in vitro differentiated macrophage to that of a human tissue macrophage. HAMs are the major population of immune and inflammatory cells in the normal lung and are the most readily available source of human tissue macrophages. Of the 974 HMDMs in the study obtained from a total of 36 donors, we were able to observe the presence of the inactivating outward current (I_A) which exhibited voltage-dependent availability in only 49 (or 5%) of the cells. In contrast, whole-cell current recordings from HAMs, revealed a significantly higher frequency of I_A expression (50% in a total of 160 cells from 26 donors). In the alveolar cell, there was no correlation observed between cell size and peak I_A amplitude, nor was there a relationship between peak I_A amplitude and time in culture. The current in both cell types was K^+ selective and 4-aminopyridine (4-AP) sensitive. I_A in both cell types inactivated with a time course which was weakly voltage-dependent and which exhibited a time constant of recovery from inactivation of approximately 30 sec. The time course of current inactivation was dependent upon the external K^+ concentration. An increase in the time constant describing current decay was observed in elevated K^+. Current activation was half-maximal at approximately −18 mV in normal bathing solution. Steady-state inactivation was half-maximal at approximately −44 mV. The presence of the outwardly rectifying K^+ conductance may alter the potential of the mononuclear phagocyte to respond to extracellular signals mediating chemotaxis, phagocytosis, and tumoricidal functions.

Key Words K^+ channels · patch clamp · macrophage activation · inflammatory cells

Introduction

Previous studies on macrophages have described the presence of voltage-gated K^+ channels which resemble delayed rectifier K^+ channels of nerve and muscle in their selectivity, pharmacological sensitivity to 4-aminopyridine (4-AP), and kinetics of activation and inactivation. Ypey and Clapham (1984) observed that the transient outward K^+ current (I_A) in murine peritoneal macrophages appeared after one day in culture following cell isolation and suggested that I_A could be linked to functional activation. Both Gallin and Sheehy (1985) and Randriamampita and Trautmann (1987) were also able to record the inactivating outward current in the murine macrophage-like cell line J774.1; however, in contrast to the peritoneal macrophages, the current appeared only in the first 1–8 hr following plating and disappeared at later culture times.

We have used the human monocyte-derived macrophage (HMDM) as a model system in which to follow the morphologic, metabolic, and functional changes that occur when monocytes are allowed to differentiate in vitro (Zeller et al., 1988). Studies of HMDM current activation as a function of time in culture yielded the finding that the inactivating outward current, which had been previously described in the murine cells, was absent in the differentiating monocyte as described in the preceding article (Nelson, Jow & Jow, 1990). Gallin and McKinney (1988) were similarly able to observe inactivating outward currents in only 3% of the HMDMs they examined. Based on these findings, we sought to compare the electrophysiology of the in vitro differentiated cell with that of a readily available human tissue macrophage, the human alveolar macrophage (HAM). In contrast to data obtained in the in vitro differentiated HMDM, current recordings from HAMs described in this study revealed the presence of the inactivating outward current in 50% of the cells throughout time in culture. The voltage dependence, kinetics of current activation and inactivation, and selectivity of I_A in the alveolar cells were similar to that previously described for the murine cells (Ypey & Clapham, 1984; Gallin & Sheehy, 1985; Randriamampita & Trautmann, 1987).

Placed at the interface between the external and internal environments in the normal lung, the HAM is exposed to a variety of airborne as well as possibly blood-borne stimuli that can selectively activate the cells and to which the cells may respond metabolically (Brown, Monick & Hunninghake, 1988). In that the HAMs used in these studies were obtained from patients undergoing diagnostic bronchoscopy, they may well represent a population of cells exhibiting a spectrum of activated states. The alterations in macrophage metabolism and biochemistry that accompany activation are now thought to encompass extensive but selective changes in proteins of the macrophage plasma membrane (Adams & Hamilton, 1984; Hamilton & Adams, 1987). The inactivating outward K^+ current ion channel may well be a class of plasma membrane proteins whose expression is enhanced in the activated state.

Materials and Methods

Specimen Collection, Cell Culture, and Electrophysiology

The project was approved by the human experimentation committee of the University of Chicago and informed consent obtained. The subjects included men and women adult patients—smokers and nonsmokers—scheduled for bronchoscopy. The procedures used for the collection, isolation, and culture of human alveolar macrophages have been previously described (Nelson et al., 1985). The procedure for isolating the human monocyte-derived macrophages and the voltage-clamp techniques employed are described in the preceding article (Nelson et al., 1990). Current decays were analyzed as a sum of exponentials by a Fourier method (Provencher, 1976) that determined the number, amplitudes, and time constants of the components. Summary data are expressed as means ± standard error of the mean with the number of experiments in parentheses.

Results

Alveolar macrophages isolated from alveolar lavage exhibited an outwardly rectifying current which inactivated during sustained depolarization in 50% of the 160 cells examined. We have referred to this current as I_A in the preceding paper. Monocyte-derived macrophages (HMDMs) differentiated in either suspension or adherent culture exhibited this current at a significantly lower frequency (5%). This paper will describe the voltage dependence, kinetics of activation and inactivation, and selectivity of I_A. A complete characterization of the transient outward current in the HMDMs was difficult due to its low frequency of occurrence. Where possible, however, comparative data are given for the in vitro differentiated HMDM and the alveolar cell. While I_A is a prominent component of the outward current in the alveolar cell, the calcium-activated K^+ current (I_C, as seen in Fig. 4*B*, was present in 70% of the cells examined), as well as the cation nonselective current (I_D, as seen in Fig. 4*C*, was present in 30% of the cells examined) described for the HMDM in the preceding paper, were also present in the alveolar cell. The presence of I_A was confirmed in experiments performed at both a hyperpolarizing and depolarizing holding potential. In that I_A shows steady-state inactivation at depolarized potentials (*vide infra*), current amplitude was significantly decreased at the more depolarized holding potential.

Activation

Current activation in a HAM in normal bathing solution in response to a series of depolarizing pulses from −50 to +20 mV is seen in Fig. 1*A* and *B*. The corresponding peak current-voltage relationship is given in Fig. 1*C*. The time course of current activation determined in 12 cells was well described by first order kinetics with a time constant of 17.5 ± 2.3 msec at −20 mV which decreased to a value of 4.5 ± 0.6 msec at +10 mV. In the experiment shown in Fig. 1*A*, the time constant of current inactivation at the most depolarized potential of +20 mV was 1180 msec.

Changes in peak current amplitude as well as the time constants describing current activation and inactivation were observed as a function of time following whole-cell formation. After an initial 3-min period following whole-cell formation to allow for current stabilization, peak current amplitude appeared to remain stable over time; however, large changes in the time course of current activation and inactivation were observed to take place over the same time period. Peak current amplitude at −20 mV after 20 min was 103 ± 5% (4) of the initial value. The time constant describing current inactivation decreased by 38 ± 14% (4). The time constant describing current activation at −20 mV decreased by 26.5 ± 9% (4) over the same time period. Similar shifts in the voltage dependence of K^+ channel gating have been observed over time following whole-cell recording for the inactivating delayed rectifier current in T lymphocytes (Fukushima, Hagiwara & Henkart, 1984; Cahalan et al., 1985; Deutsch, Krause & Lee, 1986).

Figure 1*D* shows the peak conductance *versus* voltage relationship for the currents in normal bath solution illustrated in Fig. 1*A*. The continuous line through the data points represents the best fit of the

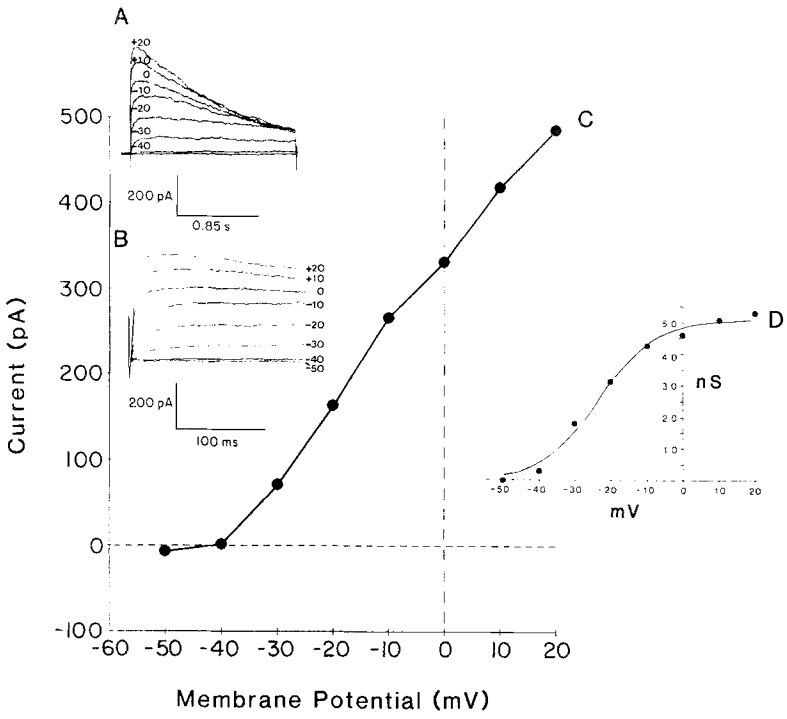


Fig. 1. Time course and voltage dependence of transient outward current (I_A) activation in a HAM. Whole-cell current recordings were made after three days in culture following isolation. (*A*) Current recording made from a holding potential of −60 mV in response to step depolarizations between −50 and +20 mV in 10-mV increments obtained in standard saline. Pulse potentials are given above each current trace. The interval between pulses was 40 sec to allow for complete recovery from inactivation. Pulse length was 1.7 sec. Currents are leak and capacity transient subtracted. (*B*) Current traces in *A* displayed in an expanded time scale showing voltage-dependent activation phase. (*C*) Peak current-voltage relationship measured approximately 15 min after establishing the whole-cell clamp. (*D*) Relationship between the chord conductance at the peak of the outward current and the membrane potential. The reversal potential in this experiment was −78 mV as determined from tail currents. Cell capacitance 23 pF

peak conductance to a Boltzmann distribution of the form:

$$g(V) = g_{max}/(1 + e^{(V-V_{1/2})/k}) \quad (1)$$

where $V_{1/2}$ is the voltage at the midpoint of the curve and k gives the steepness of the voltage dependence. Maximal I_A conductance in three experiments on HAMs, as determined from Boltzmann fits to the data, was achieved at potentials greater than 0 mV with an average maximal conductance of 10.1 ± 4.6 nS. Half-maximal current activation, $V_{1/2}$, was −17.7 ± 6.0 mV with a k of −8.8 ± 1.2 mV (n = 3).

There was no clear relation between peak current amplitude and cell capacitance in the alveolar cells as can be seen in Fig. 2. This is in contrast to data obtained from murine T lymphocytes where a close correlation was found between T cell enlargement and the characteristic expression in activated cells of large numbers of type n K^+ channels (DeCoursey et al., 1987). Peak current amplitude was determined at +10 mV in all cells expressing the inactivating outward current. There is minimal activation of other contaminating outward currents in this voltage range. I_A frequency and cell capacitance for HAMs throughout time in culture are given in the Table.

In contrast to earlier data reported for the HAM (Nelson et al., 1985) using pipette solutions buffered

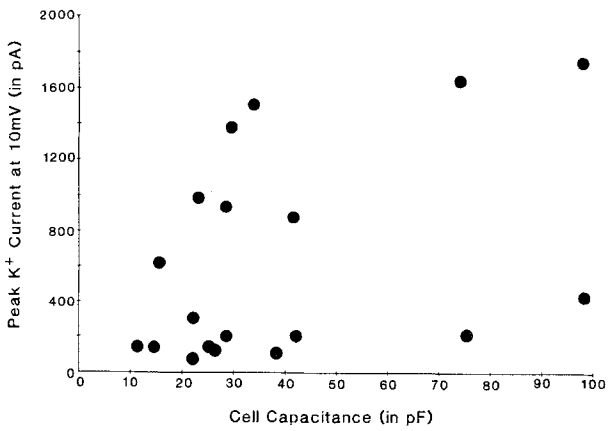


Fig. 2. Peak K^+ (I_A) current in HAMs as a function of cell capacitance. Peak current was determined for a 10-mV depolarization where contamination by the Ca^{2+}-activated K^+ current (I_C) is minimized. Capacitance was measured by integrating the current during a 5- to 10-mV voltage step and subtracting a baseline established about 20 msec after the step as determined from a nonlinear fit to the data. Capacitance measurements were made throughout the culture period, capacitance appeared to remain relatively constant throughout the culture period (*see also* the Table)

to a higher internal free-Ca^{2+} concentration (8 μM Ca^{2+}, using 1 mM Ca^{2+} with 1 mM EGTA), we were unable to observe any significant shift in the HAM resting potential (measured as the zero current po-

Table. Frequency of the transient inactivating outward K^+ current (I_A) and capacitance in human alveolar macrophages with time in culture

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 6	Day 7
I_A frequency (%)	60 (n = 37)	59 (n = 34)	67 (n = 18)	53 (n = 17)	25 (n = 8)	88 (n = 8)	67 (n = 6)
Capacitance (pF)	60 ± 15 (n = 5)	41 ± 14 (n = 4)	36 (n = 1)	30 ± 3 (n = 3)	—	53 ± 9 (n = 4)	100 (n = 1)

Cell capacitance was measuured by integrating the current during a 5- to 10-mV voltage step and subtracting a baseline established about 20 msec after the step as determined from a nonlinear fit to the data. The presence of I_A was confirmed in experiments performed at both hyperpolarizing and depolarizing holding potentials.

tential) with time in culture using internal solutions with a free-Ca^{2+} concentration of approximately 38 nM. Zero current potentials in the present studies ranged from -44 ± 6 mV ($n = 8$) on day of isolation compared to -46 ± 2 mV ($n = 5$) on day six following isolation. The fact that a shift in the zero current potential to more negative potentials with time in culture is observed only in the presence of high internal free calcium, may indicate that a Ca^{2+}-activated K^+ conductance is being expressed with time in culture.

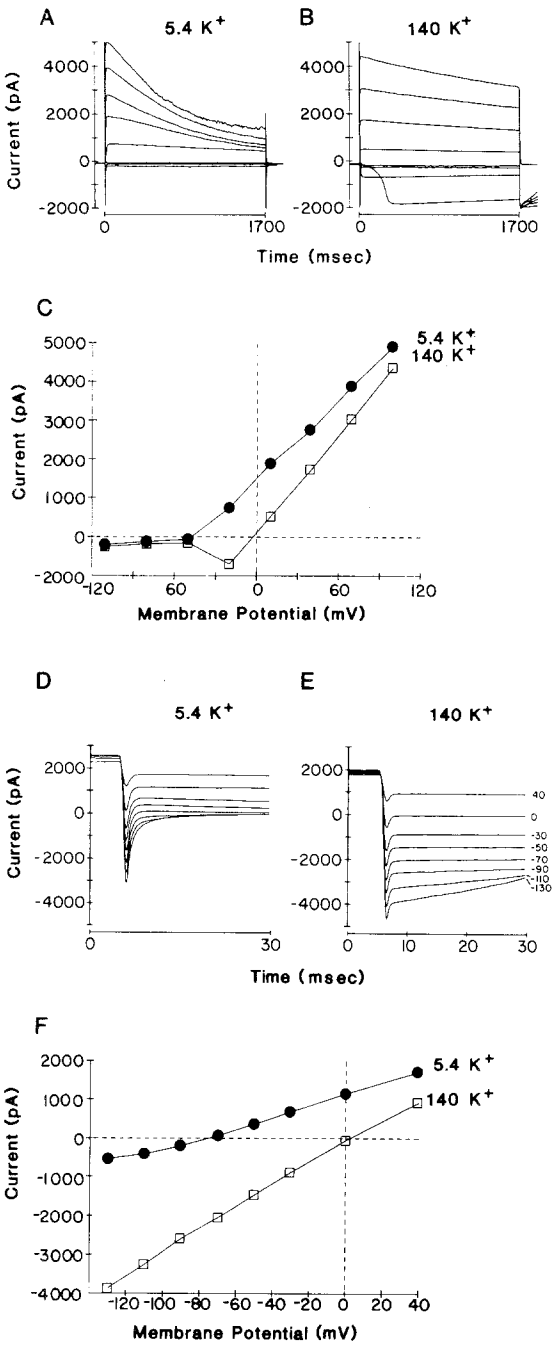
Selectivity

The selectivity of I_A was determined from the reversal potential of currents made in solutions containing 5.4 K^+ and solutions containing 140 K^+ (Fig. 3*A* and *B*). Inward currents under these ionic conditions were small and therefore the reversal potential is an estimate and was taken as the potential at which outward currents could not be detected. Exchange of the low K^+ bathing solution with one containing high K^+ shifted the reversal potential in the depolarizing direction by 44.7 mV in the experiment illustrated in Fig. 3*A* and *B*. The peak current-voltage relation for the currents in both solutions (Fig. 3*C*) shows a larger slope conductance in the high K^+ solution. Note also, the increase in the time course of current decay, as well as complex current activation kinetics in the hyperpolarized range in the presence of the high K^+ solution. This complex kinetic pattern was observed in all cells in high K^+ solutions, and may well be due to the activation of an inwardly rectifying K^+ current. The time constant describing current decay at +100 mV was 640 msec in the standard bath solution containing 5.4 K^+ and 2832 msec in the presence of 140 mM extracellular K^+ for the currents in Fig. 3*A* and *B*.

The instantaneous current-voltage relationship for I_A was determined from tail current experiments in normal (5.4 K^+) and high K^+ solutions (*see* Fig. 3*D* and *E*). Instantaneous current reversal potentials were determined by applying a +80-mV depolarizing prepulse from a holding potential of −60 mV for 40 msec and estimating the potential at which the tail current appeared to reverse during successive repolarizing steps to various potentials. Inward tail currents were, in general, difficult to detect in normal bath solutions (*see* Fig. 3*D* and also Fig. 5*A*) and, therefore, in most cases were taken as the potential at which outward tail currents could no longer be detected. The appearance of outward rectification under these experimental conditions could be due to (*i*) the ionic gradient for the permeant ionic species, (*ii*) rapid voltage-dependent channel deactivation in the inward current range, and/or (*iii*) the presence of a blocking ion in the external solution inhibiting inward current movement. The fact that the current-voltage relationship is linear in the high K^+ solution indicates that it is indeed the magnitude of the ionic gradient for K^+ which accounts for the outward rectification. In two experiments, the mean apparent shift in the reversal potential of the instantaneous current-voltage relationship was 64 mV. The predicted shift in reversal potential in this case would have been 82 mV.

Voltage-Dependent Current Availability

The inactivating outward K^+ current was completely blocked when the internal K^+ was replaced with Na^+ and showed steady-state inactivation at depolarized holding potentials. The currents in Fig. 4*A* were obtained at a holding potential of −60 mV and the currents in Fig. 4*B* were obtained at the depolarized holding potential of −30 mV. The cell was held at −30 mV for 1 min before the currents illustrated in Fig. 4*B* were obtained. The amplitude of the transient component of the outward current was significantly decreased at the depolarized holding potential leaving only the time-independent



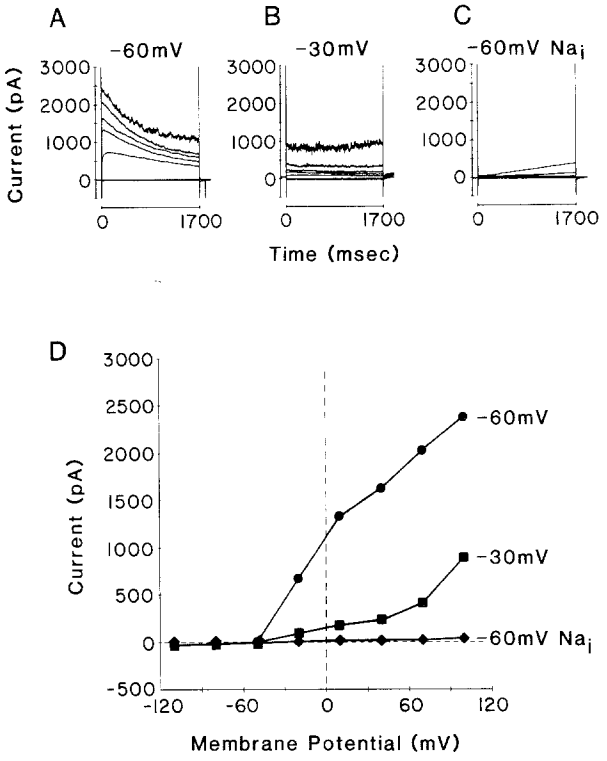


Fig. 4. Effect of holding potential and intracellular Na^+ on I_A activation in a HAM. (*A*) Current recording from a holding potential of −60 mV. Hyperpolarizing and depolarizing steps were made from −110 to +100 mV in 30-mV increments. (*B*) Current recording from the same cell from a depolarized holding potential of −30 mV. Note the inactivation of the transient component of the outward current (I_A) at the depolarized holding potential. (*C*) Current recording from the same cell at the hyperpolarized holding potential after exchange of the internal (intrapipette) solution to one in which all the K^+ was isosmotically replaced with Na^+. (*D*) Corresponding current-voltage plot; currents were measured approximately 40 msec after the voltage step

Fig. 3. Whole-cell current recordings from a HAM in normal saline solution containing 5.4 K^+ and in a solution in which all the extracellular Na^+ has been replaced with K^+. (*A*) Current recording in normal saline, voltage steps were applied every 40 sec using the voltage protocol described in Fig. 1. Currents were recorded approximately 60 min after the transition to the whole-cell configuration. Zero current potential was −48 mV. Leak and capacity currents have not been subtracted. (*B*) Whole-cell currents recorded after replacement of the external solution with one containing a K^+ concentration of 140 mM. Note the complex pattern of current activation in the inward current range for currents measured in the presence of elevated external K^+. Zero current potential in the presence of the high K^+ solution was −3 mV. The holding potential in both high and low K^+ solutions was −60 mV. (*C*) Corresponding peak current-voltage relationship in 5.4 K^+ and 140 mM K^+. Cell capacitance was 95 pF. (*D*) Tail currents in the presence of normal bathing solution containing 5.4 K^+. Currents were measured after the capacity transient about 4 msec after the test voltage step. Test voltage pulses followed brief (40 msec) conditioning pulses to +80 mV to maximally activate the K^+ conductance. Holding potential, −60 mV; sampling interval, 200 μsec; filter, 1 kHz. Leak current was not subtracted. (*E*) Tail currents in solutions containing elevated K^+ (140 mM). Same pulse protocol as in *D*. (*F*) Instantaneous current-voltage relationship in normal and high K^+ solutions. The instantaneous current reversal potential in standard bath solution (5.4 K^+) was −75.6 mV. In the 140 K^+ bathing solution, the reversal potential shifted to +2.1 mV

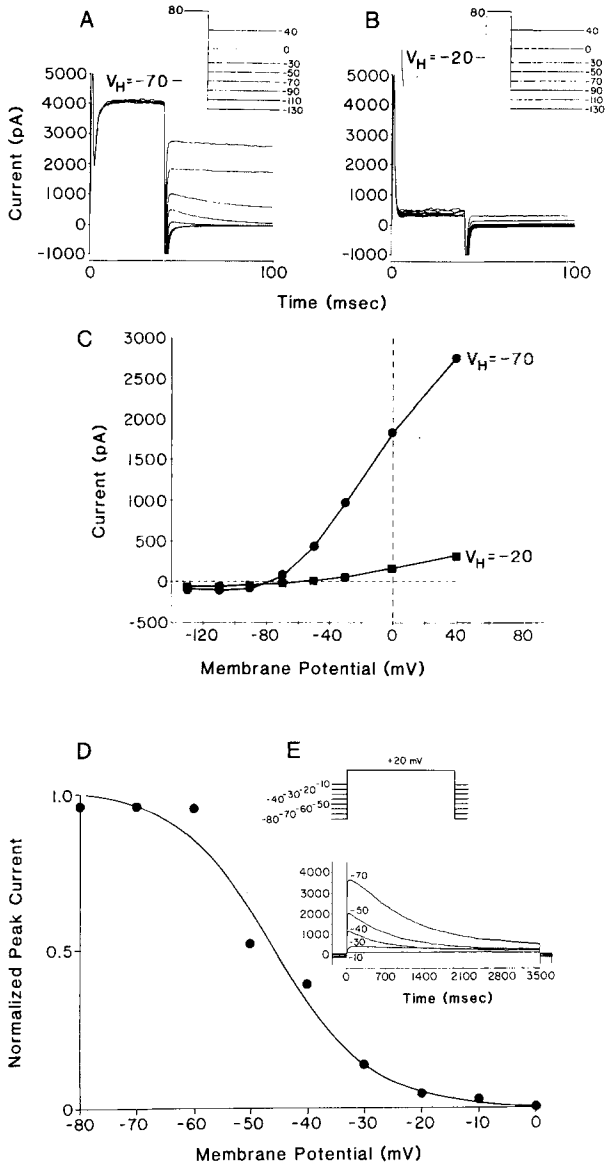


Fig. 5. Deactivation of I_A in a HAM at different holding potentials. Tail current recordings were made from a holding potential of −70 mV (*A*) and −20 mV (*B*). Outward current was maximally activated during 40-msec voltage steps to +80 mV. Deactivation was brought about with steps back to increasingly hyperpolarized potentials. The cell was held at −20 mV prior to the initiation of the tail current protocol to allow for full steady-state inactivation of the current at the depolarized holding potential. Sampling interval 200 μsec; filter 1 kHz. Leak current was not subtracted. (*C*) Peak current-voltage relationship of currents measured at $V_H = -70$ and $V_H = -20$. "Instantaneous" currents were measured after the capacity transient, about 8 msec after the test pulse. Tail currents in *A* and *B* reversed at −78 and −53 mV, respectively. (*D*) Voltage dependence of steady-state outward current inactivation in a HAM. Steady-state inactivation was determined by varying the holding potential and recording the peak outward current at a constant potential of +20 mV following several minutes which ensured the establishment of equilibrium. The data points were fitted with a Boltzmann function of the form $I/I_{max} = 1/(1 + e^{(V-V_{1/2})/k})$ allowing the mid-point, $V_{1/2}$, the slope factor, k, and the maximum normalized peak K^+ current, I_{max}, to vary. (*E*) Experimental data and pulse protocol used to construct the curve in *D*. In the experiment which is illustrated, $V_{1/2}$, the midpoint was −46 mV and the slope factor k was 8.57 mV

component, I_C, and the slowly activating component, I_D. The corresponding current-voltage relationship is given in Fig. 4*D*. Following recovery of the current at the hyperpolarized holding potential, internal pipette perfusion (*see* preceding article, Nelson et al., 1990) was used to exchange the internal solution for one in which K^+ was replaced isosmotically with Na^+ (Fig. 4*C*). In the presence of high internal Na^+, both the time-dependent and time-independent components of the outward K^+ current were inhibited (*see* current-voltage relationship in Fig. 4*D*) leaving only the slowly activating, nonselective outward current, I_D.

The instantaneous current-voltage relationship at both holding potentials was determined (Fig. 5). Current recordings were made from either a holding potential of −70 mV (Fig. 5*A*) or −20 mV (Fig. 5*B*). Outward current was activated by 40-msec duration voltage steps to +80 mV. Current deactivation, which reflects the kinetics of channel closure, was brought about with voltage steps back to decreasingly negative potentials. Instantaneous current-voltage plots for the tail currents in a HAM at the two holding potentials are illustrated in Fig. 5*C*. As in the ion substitution studies, inward current rectification was seen in the instantaneous current-voltage relationship at the hyperpolarized holding potential. The shift in the instantaneous current reversal potential obtained at the depolarized holding potential was approximately 48.5 ± 11.9 mV (n = 3), less than that obtained in the ion substitution studies indicating the presence of a significant amount of current which remained available at the depolarized holding potential.

Steady-State Inactivation

Steady-state inactivation as a function of membrane potential was determined by varying the holding potential for a period of minutes prior to recording the K^+ current at a constant test potential of +20 mV. A schematic of the pulse protocol along with current obtained during the test pulse are plotted in Fig. 5*E*, with the conditioning holding potential indicated above each pulse. In Fig. 5*D*, peak currents were normalized, plotted as a function of the holding potential, and fit with a Boltzmann function of the form:

$$I/I_{max} = 1/(1 + e^{(V-V_{1/2})/k}) \quad (2)$$

where $V_{1/2}$ is the voltage at which the current is half inactivated and k is the slope factor. In two HAMs, the average midpoint $V_{1/2}$ obtained from similar fits to the data was −44.2 mV with a slope factor of 6.7 mV. In one experiment, steady-state inactivation was determined approximately 5 min following whole-cell formation and again 20 min later. $V_{1/2}$ and k remained relatively constant over this period, shifting only from −36.9 to −40.7 mV and 4.2 to 4.1 mV, respectively.

Tail Current Kinetics

Single exponential fits to the tail currents similar to those depicted in Figs. 3*D* and 5*A* (tail currents obtained under conditions of normal bath K^+ concentrations) revealed the voltage dependence of channel deactivation. The time constant describing current deactivation at −30 mV was 44.0 ± 5.8 msec (3); whereas, current deactivation at −90 mV was complete in 7.9 ± 1.7 msec (3). The complex kinetics of channel deactivation and possible contamination of inward rectifier K^+ current which is present in the HAM precluded a comparison of tail current kinetics in the high K^+ experiments.

Inactivation

The currents shown in Fig. 1*A* inactivated with a time course that could be adequately fitted with a single exponential, suggesting that the kinetics of inactivation were describable as a first order process. This was the case for both the HMDM and HAM. A summary of the voltage dependence of the time constants of current inactivation for HMDMs (n = 11) and HAMs (n = 11) is given in Fig. 6. The time constant for the decline of the current decreased as the membrane potential became more positive; however, the time constant describing inactivation was observed to be relatively independent of voltage at potentials at which the inactivating outward K^+ current was maximally activated, i.e., at potentials greater than 0 mV. Current inactivation occurred over a period of seconds at the more hyperpolarized potentials where the conductance was only partially activated. Figure 6*B* summarizes the voltage dependence of I_A current kinetics, i.e., current activation, inactivation, and deactivation in a single HAM. The activation time constant decreases over the entire range at which I_A current can be activated. The deactivation time constant is largest at the threshold for current activation and decreases as the potential becomes more negative.

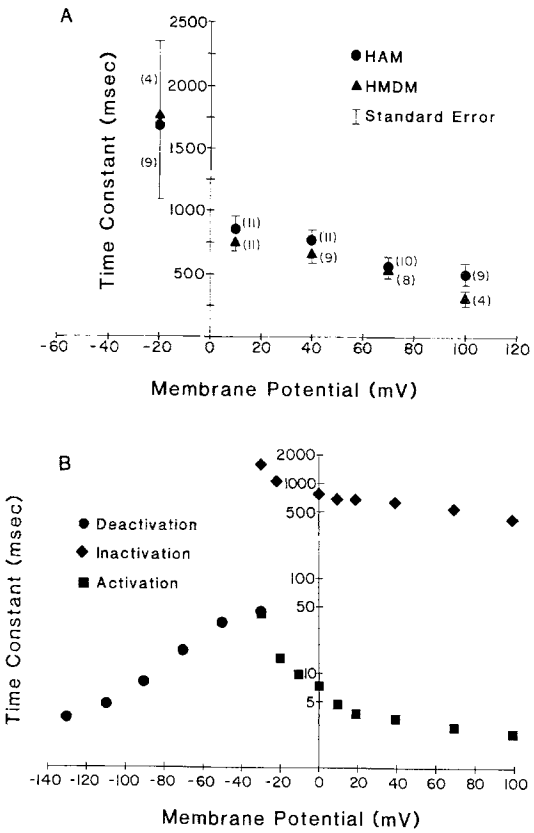


Fig. 6. Kinetics of I_A activation, inactivation, and deactivation. (*A*) Voltage dependence of I_A inactivation. Whole-cell voltage-clamp current records were obtained from both HAMs and HMDMs in standard bathing solution according to the pulse protocol described in Fig. 1. Theoretical fits to current decays were analyzed as a sum of exponentials by a Fourier method (Provencher, 1976). Mean time constants of inactivation derived from exponential fits to outward transient currents were obtained from a total of 22 cells. The number of fits at each potential are given in parentheses with the SEM indicated by the error bars. (*B*) Summary of the voltage dependence of I_A kinetics in a single HAM. Time constants obtained for current activation, inactivation, and deactivation (obtained from fits to the tail currents), as described in the text, are plotted

Recovery from Inactivation

Figure 7 illustrates the time course of current recovery from inactivation. Pairs of identical pulses to +80 mV separated by varying intervals were applied to the voltage-clamped cell from a holding potential of −70 mV. Figure 7*A* shows the second current obtained from each one of the pair of pulses with the interval between the two pulses shown to the left of each trace. With 60 sec separating the two pulses, the currents obtained were identical. The ratio of the peak current during the second pulse (I) to that during the first (I_{max}) is a measure of the degree of recovery from inactivation during the in-

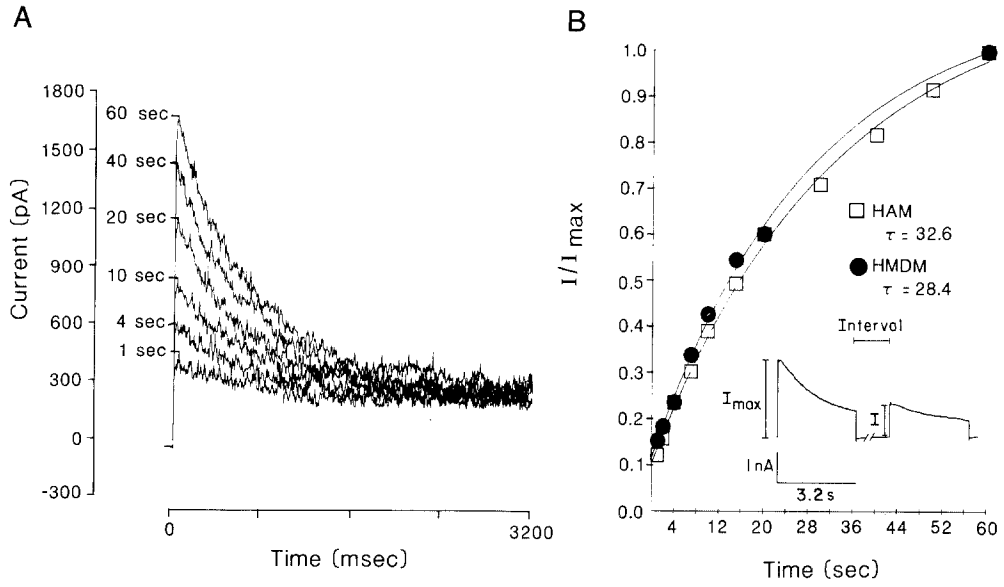


Fig. 7. Comparison of the time course of recovery from inactivation in HAMs *versus* HMDMs. Pairs of identical pulses to +80 mV separated by increasing intervals of time were applied to the cell from a holding potential of −70 mV. The pulse duration was 3.2 sec to allow for full current inactivation. Each pair of pulses were separated from the next by an interval of 60 sec. (*A*) Current recordings from a typical experiment in a HAM. (*B*) Recovery from inactivation was expressed as the ratio of the peak amplitude of the time-dependent current during the second pulse (I) to that of the current during the first pulse (I_{max}). This ratio, the inactivated fraction of the peak amplitude is plotted as a fraction of the interval between the pulses. Recovery time constants were derived from nonlinear least-squares fits to the data points. These results demonstrate that recovery follows a single-exponential time course with a time constant of recovery of 32.6 sec in the HAM and 28.4 sec in the HMDM

terval between the two pulses. The continuous curve through the data points in Fig. 7*B* shows that the time course of recovery from inactivation was well fitted by a single exponential for both the HAM and HMDM with a time constant of 32.6 sec for the HAM and 28.4 sec for the HMDM.

Current Inhibition

The transient component of the outward K^+ current was pharmacologically inhibited in the presence of low concentrations of bath applied 4-AP, as has been previously observed for the murine peritoneal macrophage (Ypey & Clapham, 1984). Low concentrations of 4-AP (0.4 to 4 mM) significantly inhibited I_A current in both HAMs (two experiments, 97% inhibition at −20 mV) and HMDMs (three experiments, 98 ± 1.4% at 0 mV) throughout the voltage range. Figure 8 illustrates currents obtained prior to and following bath application of 4-AP to a HAM which had been maintained in culture for six days. Note that 4-AP was ineffective in inhibiting the time-dependent component of the outward current, I_C.

Discussion

Inactivating Outward Current: Development and Expression

Human alveolar macrophages express the transient outward K^+ current (I_A) at a significantly higher frequency than human peripheral blood monocytes cultured for varying periods of time. The transient inactivating currents which were observed in the HAMs in this study resembled those previously described in mouse peritoneal macrophages (Ypey & Clapham, 1984; Randriamampita & Trautmann, 1987) and the murine cell line J774 (Gallin & Sheehy, 1985; Randriamampita & Trautmann, 1987). Ypey and Clapham (1984) reported that I_A developed 24 hr after peritoneal macrophage isolation and that other time- and voltage-dependent conductances were not observed in the cells they studied. This is to be contrasted to the work of Randriamampita and Trautmann (1987) who observed that the murine peritoneal macrophages expressed I_A currents on the first day of culture which could not be detected at later times in culture. Gallin and Sheehy (1985), as well as Randriamampita and

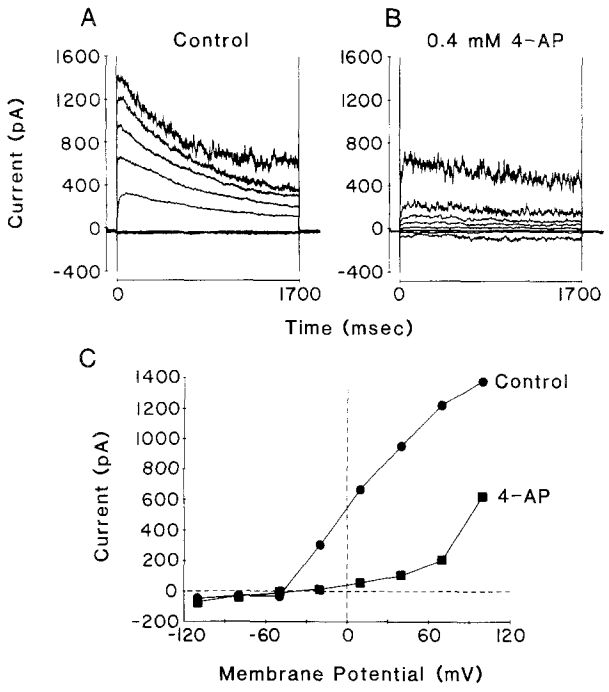


Fig. 8. The effect of external 4-AP on I_A current activation in a HAM. Current records are from a cell six days after isolation and four days after plating. Whole-cell current records under voltage clamp were made from a holding potential of −60 mV. Currents were recorded using the voltage protocol described in Fig. 1. The interval between pulses was 40 sec. (*A*) Control current record made with standard bathing solution. (*B*) Current record made with 4-AP (0.4 mM) applied via a bath perfusion system. The zero current potentials in *A* and *B* were −47 and −37 mV, respectively. (*C*) Peak current-voltage relationship of currents in presence and absence of 4-AP

Trautmann (1987), observed that I_A current expression in J774 cells declined over time in culture. In contrast to the peritoneal macrophage and the J774 cells, a significant percentage of HAMs express I_A current immediately upon isolation and introduction into adherent culture. Neither frequency of current expression nor cell capacitance was significantly altered with time in culture. In addition, there was no clear relation between cell size and current amplitude indicating that current density was not constant from cell to cell. The HAMs expressed, to a variable degree, other voltage-dependent currents which have been described for the HMDM (*see* preceding article, Nelson et al., 1990).

Voltage Dependence of Current Activation and Inactivation

The voltage dependence of I_A current activation as well as kinetics of deactivation kinetics qualitatively resembled that observed for cultured murine macrophages (Ypey & Clapham, 1984) and the murine macrophage-like cell line J744 (Gallin & Sheehy, 1985). Prolonged voltage depolarizations gave rise to current inactivation, the time course of which increased in the presence of elevated external K^+ concentrations. The time constant describing current inactivation was weakly voltage dependent except for voltages near the threshold of current activation. The time course of recovery from inactivation was described by a single exponential process with a time constant of approximately 30 sec at the most depolarized potential of +80 mV. Current activation was complete within 4.5 msec at a depolarization of +10 mV. Maximal I_A conductance occurred at about 10 mV and was in general greater than 1 nS. Current activation was half-maximal at −18 mV, as determined from fits to the conductance *versus* voltage curve, with a slope factor of −8 mV. Gallin and Sheehy (1985) obtained similar values for the J774 cell with a half-maximal activation at −31 mV and a slope factor of −5.7 mV. Ypey and Clapham (1984) reported that in the peritoneal macrophage current activation reached a maximum at +40 mV within 6 msec and that current inactivation was a single exponential process which was virtually independent of voltage in the range of −30 to +40 mV. The time constant describing current decay of 460 msec which Ypey and Clapham (1984) calculated (in the voltage range of −30 to +40 mV), was somewhat faster than the time constant of 538 msec (for potentials positive to −25 mV) that Gallin and Sheehy (1985) observed in the J774 studies, and the time constant of approximately 550 msec obtained for the HAMs and HMDMs at +70 mV in this study.

Steady-state inactivation in the HAM was half-maximal at −44 mV and remained incomplete even at the most positive potentials. Recovery from inactivation was complete in 60 sec with a time constant of approximately 32 sec as compared to 12 sec for the peritoneal macrophage (Ypey & Clapham, 1984) and 13.7 sec for the J774 cell (Gallin & Sheehy, 1985). The pulse length used to study the recovery process in the HAMs was 3.2 sec to allow for full inactivation; whereas, that in the peritoneal studies was 400 msec and that in the J774 cells was 250 msec. The depolarizing test pulse in both of the murine studies was considerably less depolarized (−10 mV for the peritoneal cell and +10 mV for the J774 cell) than the +80-mV depolarizing test pulse used in our studies on the HMDMs and the HAMs. The substantial difference in pulse length and the larger depolarizing test pulse would account for the long recovery time constant we obtained in our

studies as compared to that obtained in the earlier studies in the murine cells.

Current Selectivity and Inhibition

Using ion substitution and tail current experiments, I_A was determined to be K^+ selective as in the studies of Gallin and Sheehy (1985) and Ypey and Clapham (1984). The inactivating component of the outward current was completely blocked when the internal K^+ was isotonically replaced with Na^+. An average shift of 64 mV was obtained in the instantaneous current reversal potential when the normal bath solution was replaced with one in which the extracellular Na^+ has been completely replaced with K^+. This is somewhat less than the expected shift of 82 mV, but does indicate the current is highly K^+ selective. A 48-mV shift in the instantaneous current reversal potential was obtained when I_A was inactivated at a depolarized holding potential; however, the magnitude of the shift would indicate that the current which remains at the depolarized potential is somewhat K^+ selective.

The transient inactivating current in the HAM and the HMDM was blocked by low concentrations of extracellularly applied 4-AP. Similar current inhibition effects were reported for the peritoneal cell (Ypey & Clapham, 1984) and the J774 cell (Gallin & Sheehy, 1985). Recently, it has been reported that 4-AP, acting upon voltage-gated K^+ channels, inhibits IL-1 production by adherent human macrophages (Gupta et al., 1985). This occurred in a dose-dependent manner similar to that reported to block K^+ currents and proliferation in T lymphocytes (Chandy et al., 1984; DeCoursey et al., 1984; Lee et al., 1986; Dos Reis, Nobrega & Persechini, 1988) implying that K^+ channels may play a role in a variety of activation pathways. Type *n* K^+ channels have also been implicated in the regulation of cell volume during osmotic challenge in T lymphocytes and appear to mediate K^+ efflux during the regulatory volume decrease (Cahalan & Lewis, 1988). The regulatory volume decrease can be inhibited by external application of 4-AP and TEA with a pharmacological dose response which most closely matches that of type *n* K^+ channels.

Gallin and Sheehy (1985) have also suggested that enhanced K^+ conductance expression in macrophages may well be related to the enhancement of effector function. Indeed, Jow and Nelson (1989) have recently reported that lipopolysaccharide-activated HMDMs express I_A current at a significantly higher frequency than that observed in nonlipopolysaccharide-activated cells. Macrophages are commonly found at sites of inflammation and necrotic tissue where the external environment is likely to be elevated in K^+ concentration. The profound slowing of the inactivation kinetics of I_A observed in the presence of elevated external K^+ is consistent with the suggested K^+ conductance-enhanced increase in macrophage effector function. The role of the outwardly rectifying K^+ channel in macrophages may be similar to that suggested by Schell et al. (1987) for T lymphocytes. Their data support the hypothesis that, although K^+ channels in lymphocytes probably do not play a direct role in the biochemical processes that are specific for signaling through the T cell antigen receptor, their role in the maintenance of a hyperpolarized membrane potential may be crucial in promoting cell growth and secretory function through a variety of voltage-dependent nutrient uptake pathways.

Summary

We have compared the frequency of expression of the transient inactivating current, I_A, in the in vitro differentiated HMDM to the expression in the HAM, the most readily available source of human tissue macrophages. The presence of I_A was observed in only 5% of the HMDMs studied independent of time in culture development while it was observed in 50% of the HAMs studied. The current in both cell types was K^+ selective, showed steady-state inactivation, and was 4-AP sensitive. Current activation was half-maximal at −18 mV. Steady-state inactivation was half-maximal at −44 mV. I_A inactivated with a time course which was weakly voltage dependent and which exhibited a slow time constant of recovery from inactivation of approximately 30 sec. The time constants describing current inactivation as well as deactivation were slowed by raising the external K^+ concentration. The time course of current activation and inactivation decreased during the first 20 min following whole-cell formation, while peak current amplitude remained constant over the same time period. Based on the dramatic increase in I_A frequency in the tissue macrophage over that observed in the in vitro differentiated macrophage, we postulate that current frequency might well be related to functional cellular activation. The presence of the outwardly rectifying K^+ conductance may alter the potential of the mononuclear phagocyte to respond to extracellular signals mediating chemotaxis, phagocytosis, and tumoricidal functions.

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