NONLINEAR CURRENT-VOLTAGE RELATIONSHIPS IN CULTURED MACROPHAGES

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ABSTRACT

Intracellular recordings of cultured mouse thioglycolate-induced peritoneal exudate macrophages reveal that these cells can exhibit two different types of electrophysiological properties characterized by differences in their current-voltage relationships and their resting membrane potentials. The majority of cells had low resting membrane potentials (−20 to −40 mV) and displayed current-voltage relationships that were linear for inward-going current pulses and rectifying for outward-going pulses. Small depolarizing transients, occurring either spontaneously or induced by current pulses, were seen in some cells with low resting membrane potentials. A second smaller group of cells exhibited more hyperpolarized resting membrane potentials (−60 to −90 mV) and S-shaped current-voltage relationships associated with a high-resistance transitional region. Cells with S-shaped current-voltage relationships sometimes exhibited two stable states of membrane potential on either side of the high-resistance transitional region. These data indicate that macrophages exhibit complex electrophysiological properties often associated with excitable cells.

Mononuclear phagocytic cells perform a variety of specialized membrane-related functions important in host defense mechanisms including phagocytosis, secretion, and chemotaxis. It is evident that these functions must involve a coupling between events occurring at the plasma membrane and intracellular events involving contractile proteins and metabolic changes. Studying the electrophysiological properties of the macrophage membrane may provide information about the nature of the signal involved in the coupling. In addition, determining the resting membrane potential (RMP), input resistance and current-voltage (I-V) relationships of these cells yields information about both the normal membrane permeabilities of macrophages and the possible effects of small changes in membrane permeability on the membrane potential and ionic currents of these cells.

In previous studies, we have shown that a percentage of cultured macrophages exhibit both spontaneous (10) and chemoattractant-induced membrane hyperpolarizations (7). These studies indicated that macrophages within a single culture may be heterogeneous with respect to their electrophysiological properties. The studies presented here demonstrate that mouse thioglycolate-induced peritoneal macrophages from the same culture exhibit differences in both their RMP and their responses to injected current pulses. These cells exhibit nonlinear I-V relationships (rectifying, nonohmic responses to applied current pulses), indicating that macrophage membranes have voltage-dependent conductances normally associated with excitable cells. Preliminary reports of this work have been published (8, 9).

MATERIALS AND METHODS

All mouse cells used in these studies were obtained from strain B6D2F1 Cumberland Br mice (Cumberland View Farms, Clinton, Tenn.). Peritoneal exudate cells were harvested 4 d after

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were studied, because in older cultures a small number of fibroblasts usually resulted in 90% of the cells staining positive for esterase. A nonspecific esterase stain (15) was used and no difference in recorded membrane potentials was seen, except that when using two microelectrodes we could inject larger current pulses, enabling us to obtain I-V relationships over a larger range.

Intracellular recordings were done at 34-37°C in Hanks' balanced salt solution (NaCl, 128 mM; KCl, 4.2 mM; Na2HPO4, 0.912 mM; KH2PO4, 0.4 mM; Na2HPO3, 3.65 mM; MgSO4, 0.2 mM; MgCl2, 6H2O, 0.1 mM; CaCl2, 2H2O, 1.6 mM) buffered to pH 7.3 with HEPES (10 mM) and supplemented with 5% heat-inactivated fetal bovine serum or 0.5% bovine serum albumin (fraction V; Sigma Chemical Co.). A thin layer of mineral oil covering the surface of the dish prevented evaporation of the bathing solution during the experiment.

**Electrical Measurements**

Electrophysiological techniques used in these studies were similar to those previously described (7, 10). Fiber-filled potassium acetate (3 M) glass microelectrodes with resistances of 60-150 MΩ were used and connected to an active bridge amplifier through WPI half-cells (W-P Instruments, Inc., New Haven, Conn.). In a few experiments, 3 M KCl-filled electrodes were used and no difference in recorded membrane potentials was seen. I-V relationships were obtained by applying small current pulses ±0.1 nA through the recording electrode, using an active bridge amplifier. Current pulses were long enough in duration (>300 ms) to allow for the capacitative charging of the membrane. Applied current was monitored by use of a virtual ground current meter connected to the bath through a second 3 M KCl agar bridge. Both the outputs of the current monitor and the active bridge amplifier were displayed on both an oscilloscope screen and a strip chart recorder. In three experiments, recordings were made with two microelectrodes in a single cell, one for injecting current and one for recording voltage, and the I-V relationships obtained with this approach were compared with those obtained using a single microelectrode and a bridge circuit.

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**RESULTS**

**Distribution of Membrane Potentials**

Only data obtained from recordings that were stable for at least 10 min were used to determine the average RMP of mouse macrophages. In some cases, single cell penetrations were maintained for several hours. The RMP of the 47 cells studied showed a bimodal distribution (Fig. 1), with the majority of the cells (31/47) exhibiting an RMP of −40 mV or less (referred to as low RMP cells; average = 24.7 ± 1.8 SE). A smaller number (16/47) of cells with RMP ≥−60 mV had an average membrane potential of −70 ± 1.8 SE (referred to as high RMP cells). Macrophages with low RMP frequently exhibited small spontaneous membrane hyperpolarizations 10–20 mV in amplitude and several seconds in duration. Spontaneous membrane hyperpolarizations associated with an increase in permeability, primarily to potassium ions, have been reported previously in both guinea pig and mouse oil-induced exudate macrophages as well as in cultured human peripheral blood monocytes (7, 10). Cells with high RMP frequently exhibited low membrane potentials (~10 to −40 mV) immediately after penetration; these cells then hyperpolarized to more negative potentials. In only one instance did a high RMP cell exhibit spontaneous membrane hyperpolarizations. Some cells changed from a low RMP state to a high RMP state and back again several times during the course of the recording, indicating that these two groups of cells were in a dynamic state.

**Cells with Low Membrane Potentials**

To determine whether macrophages from either of these two groups (high and low RMP cells) exhibited voltage-dependent conductances normally associated with excitable cells, I-V relationships were determined for cells in both groups. Fig. 2a and b shows two I-V relationships from cells with low RMP along with insets showing two of the voltage responses to current pulses of similar amplitude but different polarity used to determine the I-V relationships. Most low RMP cells had I-V relationships that were slightly rectifying for
FIGURE 2  (a and b) Current-voltage (I-V) relationships of two macrophages. The voltage shift measured after the charging of the membrane capacitance is plotted on the ordinate and the current pulses on the abscissa. The lines are arbitrarily drawn through the data points. Insets are photographs, taken directly from the oscilloscope screen, of consecutive sweeps showing two voltage responses of each cell to current pulses of the same amplitude but different polarity. Top tracing shows injected current pulses and bottom tracing voltage responses of the cell. (c) "Active response" induced by depolarizing current pulses. Same cell as in Fig. 2b. Top tracing monitors both current pulses and changes in DC current at different potential levels. Bottom tracings show the voltage response to depolarizing current pulse at different DC potentials. Top voltage tracing is the response of the cell at RMP = -30 mV. The cell was then hyperpolarized to -50 mV and slowly repolarized back to its RMP. An active response (arrows) can be seen at DC potentials of -43 and -46 mV.

outward (depolarizing) current pulses (delayed rectification) but linear for inward (hyperpolarizing) current pulses of ≤0.6 nA, similar to that shown in Fig. 2a. The delayed rectification is evident in the inset of Fig. 2a depicting two voltage responses of a cell to 0.3-nA current pulses of opposite polarity. In this cell, the depolarizing current pulse (top tracing, upward deflection) produced an 18-mV voltage response (bottom tracing, downward deflection), whereas the hyperpolarizing current pulse (top tracing, downward deflection) produced a 32-mV voltage response (bottom tracing, downward deflection). In two cells, one of which is shown in Fig. 2b, a more marked rectification was noted. In this cell, an active response at the beginning of the depolarizing current pulse as well as a small off response to the hyperpolarizing current pulse can be seen in the inset. In addition, the active response to depolarizing current pulses could be brought out by a conditioning hyperpolarization. This is shown in Fig. 2c in which little or no active response was produced.

162  RAPID COMMUNICATIONS
when a 0.1-nA depolarizing current pulse was applied to the cell at its RMP of -30 mV (top voltage and current tracings). When the cell was hyperpolarized to -53 mV (bottom voltage and current tracings) and then brought back in stepwise fashion toward the resting level, a small active response appeared at DC potentials of -43 and -46 mV, as indicated by the arrows. The effects of the conditioning hyperpolarization are compatible with the existence of an inactivation of the active response at low (depolarized) membrane potentials.

In addition to the small active responses induced by current pulses in two cells with low RMP, small spontaneous depolarizing transients (≤10 mV), occurring for brief periods, were seen in several mouse cells as well as in cultured human macrophages. A chart recorder tracing of a human cell showing spontaneous "spikelike" transients is shown in Fig. 3. The amplitude of the spontaneous spikelike transients in this cell increased when the cell was hyperpolarized to -60 mV from the RMP of -44 mV by applying DC current and decreased when the cell was depolarized to -30 mV. The variation in size of the depolarizing transients with the DC potential of the cell indicates that the transients are associated with an increase in permeability to a particular ion or ions with an equilibrium potential more depolarized than the RMP. This is consistent with the hypothesis that these transients result from an increase in permeability to either calcium or sodium or perhaps both ions.

**Cells with Hyperpolarized Membrane Potentials**

The I-V relationships of cells with RMP ≥-60 mV were S-shaped, displaying both delayed and anomalous rectification and a very high-resistance transitional region in between. An S-shaped I-V relationship from a cell with an RMP of -80 mV is shown in Fig. 4a. The dotted portion of the curve represents the transitional region in which the response of the cell to constant current pulses is unstable. The rectifying properties of the cell are evident in the inset showing oscilloscope tracings of two voltage responses to current pulses of the same amplitude but different polarities. The arrows in the inset in Fig. 4a point to the two inflexion points present in the voltage response to the depolarizing current pulse. These inflexion points indicate the limits of the high-resistance transitional region present in the I-V curve of this cell. This type of I-V relationship is compatible with the existence of two stable states of membrane potential (11, 18), and in a few cells the RMP did fluctuate between two levels in a nongraded manner on either side of the high-resistance transitional region. These fluctuations were variable, lasting from several seconds to minutes. Current and voltage pulses from a cell that fluctuated between two potentials four times during a period of 8 min are shown in Fig. 4b. A spontaneous shift in the RMP from -68 to -46 mV resulted in the high-resistance region being evident during hyperpolarizing current pulses rather than during the depolarizing current pulses. In the right-hand portion of Fig. 4b, a number of complex potential responses to five hyperpolarizing current pulses of two different amplitudes (three of smaller amplitude and two of larger amplitude) are shown. The off response for current pulses in the high-resistance region had a distinct notch, suggesting a complex activation and/or inactivation process (11). In some cases, cells could be flipped between the two membrane potential states on either side of the high-resistance region by applying small current pulses of the appropriate polarity. However, applying depolarizing current pulses never resulted in producing a more linear I-V relationship even though the membrane potential was shifted to a more depolarized level. These cells did not appear to be morphologically different from cells exhibiting low RMP and spontaneous hyperpolarizations.

**DISCUSSION**

These results indicate that cultured macrophages can exhibit nonlinear I-V relationships and depo-
noninactivating sodium and/or calcium conductances are thought to produce similar rectifying properties in *Aplysia* neurons (5, 23). Ion substitution experiments are in progress to determine which ions contribute to the nonlinearities seen in macrophages. The small depolarizing transients (Fig. 3), which appear to be abortive spikes, have an equilibrium potential more positive than the RMP, implicating sodium and/or calcium conductances in these events. Both sodium and calcium spikes have been reported in a number of secretory cells including pancreatic cells, chromaffin cells, and pituitary cells (2, 4, 22, 16), and recently, active responses have been demonstrated in megakaryocytes (17). The voltage-dependent conductances reported here could produce the oscillations in membrane potential previously described in macrophages (9). Cyclic variations in inward voltage-sensitive calcium and/or sodium currents and outward voltage-sensitive potassium currents have been shown to produce oscillatory behavior in a number of different cells (1).

Two possibilities exist to explain the finding that cells within a single culture can exhibit different electrophysiological characteristics. Variation in leak current produced by microelectrode impedance may shift normally hyperpolarized cells to depolarized levels resulting in low RMP cells. We consider this unlikely because low RMP cells frequently had high input resistances (>100 MΩ) indicating that they were not very leaky. In addition, recordings could be maintained for long periods of time (>1 h) during which the cells appeared healthy and ingested IgG-coated bovine erythrocytes (E. Gallin, unpublished observations). Moreover, some cells switched from a high RMP state (S-shaped I-V relationship state) to a lower RMP state (more linear I-V relationship state) and back again, indicating that macrophages within a culture dish are in a dynamic situation in which cells can change their electrophysiological properties.

The rectifying properties of egg cells that are similar to those of macrophages change during maturation (17) and can be influenced by variations in the intracellular sodium concentration (12). Similar rectifying properties were also reported by Tasaki and Kamiya (25) in amoeba. The physiological significance of these rectifying properties in egg cells, amoeba, and macrophages is unclear, although similarities exist among all of these cells. For example, all three cells are rich in contractile proteins that are probably modulated by calcium (14, 24), and Klebanoff et al. (13)
recently demonstrated biochemical similarities between phagocytosis by leukocytes and fertilization of sea urchin eggs. It is likely that the rectifying properties of these cells reflect the ionic makeup of the internal milieu, i.e., the calcium, hydrogen, and/or sodium concentrations. Changes in cation levels within the cell might also be controlling factors for membrane-related events such as chemotaxis, secretion, and phagocytosis.

The membrane potential of cells with S-shaped I-V curves would be expected to be very sensitive to small changes in membrane permeability (i.e., small membrane perturbations could produce large membrane potential changes). In fact, the data shown in Fig. 4b indicate that these cells can exist at two different potential states >20 mV apart and spontaneously flip back and forth between them. From experiments in which the diffusion rates of cell-surface antigens in mouse-human heterokaryons were measured, Edidin and Wei (6) postulated that membrane potential changes can influence lateral diffusion within the surface membrane. If this is true, then a macrophage flipping between two potential levels may be changing its membrane fluidity as well. It is well known that macrophages can exist in different states of activation, ranging from cells with low rates of membrane turnover as well as decreased phagocytic and secretory abilities to cells showing increased rates of enzyme secretion, phagocytosis, and membrane turnover (3). The possibility that these functional states of activation relate to the varied electrophysiological properties that macrophages can exhibit is intriguing and opens up new possibilities in the study of macrophage activation.

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