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ELECTROPHYSIOLOGY OF PHAGOCYTIC MEMBRANES

I. POTASSIUM-DEPENDENT SLOW MEMBRANE HYPERPOLARIZATIONS IN MICE MACROPHAGES

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Summary

Electrophysiological properties of activated mouse macrophages cultured in vitro were studied using microelectrode techniques. In a high percentage of the individual cells analysed a slow hyperpolarization (SH) was observed with a concomitant decrease (2—4 times) of the input resistance.

Increasing doses of tetraethyl ammonium progressively reduce the amplitude of the SH and at a concentration of 15 mM complete blockade of the phenomena is observed. Valinomycin, at a concentration of 10^{-7} M produces rapid and permanent hyperpolarization, with a shift in the membrane potential to about —50 mV.

These data strongly support the previously proposed hypothesis that the development of SH is due to an increase in the membrane permeability to potassium ions.

Introduction

Mononuclear phagocytic leukocytes display a number of specialized activities in the mammalian host defense mechanism. These include cell and particle ingestion, chemotaxis and secretion [1,2]. The study of the electrophysiological properties of the macrophage membrane is thus relevant since all those phenomena are largely mediated by cell membrane events. Slow membrane

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Abbreviations: SH, slow hyperpolarization; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid; ET_4Am, tetraethyl ammonium; EGTA, ethylene glycol bis(α-aminoethyl ether)-*N,N'*-tetraacetic acid.

hyperpolarizations with simultaneous decrease in the cell input resistance [3], have been described in guinea pig macrophages. Similar events have been reported in L cells where responses can be spontaneous or induced by electrical, mechanical and chemical stimulation [4]. Although the exact mechanism of slow hyperpolarizations is unknown, a transient increase in membrane permeability to potassium ions has been proposed as the cause of the response. In addition, there is evidence that calcium ions are involved in the control of potassium conductance changes during the hyperpolarizations, for the calcium chelator EGTA, blocks the responses and the calcium ionophore A23187 augments their amplitude and duration [3].

The studies reported here on mouse activated macrophages are strong and direct evidence to support the hypothesis that potassium permeability changes are indeed the major cause of the slow membrane hyperpolarizations produced in the activated mononuclear phagocytes. The effects of substances directed specifically to the control of potassium permeability such as the K^+-selective ionophore valinomycin and an inhibitor of potassium currents in excitable cells-tetraethylammonium (ET_4Am) are described along with the macrophage current-voltage characterists.

Materials and Methods

Preparation. Dextran (35 mg/ml) or oil-induced peritoneal-exudate macrophages from different inbred strains of mice were used. Cells were collected, washed and resuspended in Roswell Park Memorial Institute medium (RPMI-1640-GIBCO, Grand Island N.Y.) with 10% fetal bovine serum and plated in plastic Petri dishes (Falcon Platics, 35 mm) at 37° C in a 5% CO_2 humidified atmosphere. After 2—3 hours of incubation the non-adherent cells were discarded. The adherent cells consisted of an uniform macrophage monolayer exhibiting viability of more than 95% (Trypan Blue exclusion test). These monolayers were kept in culture up to 72—96 hours.

Recording arrangement. An inverted phase-contrast microscope with a heated stage (35—38° C) was used for electrophysiological recordings. Immediately before the experiments HEPES-buffered fresh medium was added to the cells and changed with 15—20 min intervals. Glass microelectrodes filled with 2.5 M KCl (resistances from 30 to 70 MΩ) were connected to a standard electrophysiologic recording system, as previously described [5]. This set-up provided simultaneous current injection and voltage recording through a single intracellular electrode. The reference electrode was a Ag/AgCl/KCl half-cell, similar to the one connected to the recording electrode, in contact with the culture dish by means of an agar salt bridge. Substances were gently added to the dish by means of a micropipette during continuous monitoring of transmembrane potential of the same cell.

Chemicals. Tetraethylammonium chloride (Eastman Kodak Co.) was used for final concentrations ranging from 7.5 to 20 mM. Valinomycin (Calbiochem) was dissolved in 1% dimethyl sulfoxide for final concentrations from 10^{-7} to 10^{-8} M. After these treatments the cells remained morphologically normal and viable by dye exclusion tests.

Results and Discussion

Slow membrane hyperpolarizations in mice macrophages. Dextran-induced peritoneal exudate gave promptly penetrable large macrophages exhibiting an average transmembrane potential of −26 mV with total input resistances averaging from 100 to 220 MΩ.

Almost all the impaled cells exhibited slow hyperpolarizations (SH) either spontaneously or by electrical stimulation with a large (10^{-8} *A*) current pulse. These responses consisted of simultaneous membrane hyperpolarizations and considerable decrease in the total input resistance of the cells. Fig. 1 shows a typical record of SH during which depolarizing pulses were used to measure input resistance. The time course of the hyperpolarization (Fig. 1A) is the same of that observed in the decrease of resistance (Fig. 1B). These findings are in good agreement with the previous studies that described the SH responses in guinea pig macrophages [3].

Effects of ET_4Am. Since in excitable cells of different origins ET_4AM has been shown to block specifically potassium conductance increments during

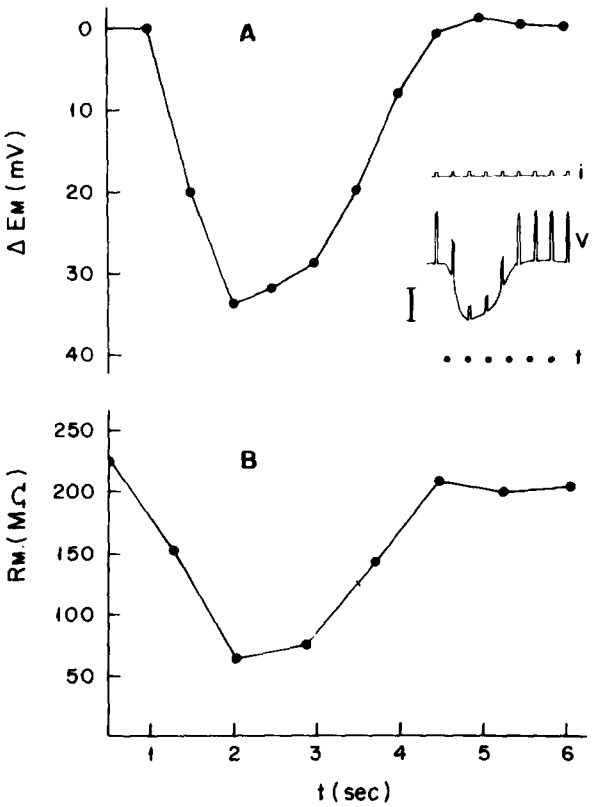

Fig. 1. Time course of a typical slow hyperpolarization from a mouse macrophage. (A), change of transmembrane potential (E_m) and (B) simultaneous fall of input resistance (R_m). Inset, strip-chart recording of the SH plotted in this figure. Note that hyperpolarizing phase has a higher rate of change than the repolarizing portion of the SH. Calibration bar, 20 mV and 10^{-9} *A*. Outward current test pulses (i) have a duration of 100 ms. (ABY dextran induced macrophage, 72 h in culture).

impulse generation [6,7] we tested this substance in our system. A clear blocking effect on the SH was observed. Fig. 2 shows three superimposed SH taken from the same cell before and after ET_4Am treatment. The cell reached an almost complete blocking of the responses upon the application of 15 mM Et_4Am in the bathing medium, without significant membrane potential changes (−12 mV). In some instances when ET_4Am was locally applied cells underwent depolarization and decrease in the input resistance indicating cell damage. Homogeneous distribution of ET_4Am in the dish resulted in blockade of SH without cell lesion. After Et_4Am treatment other macrophages were either quiescent or displayed barely detectable deflections of the membrane potential (as seen in Fig. 2).

Effects of valinomycin. Addition of this potassium-selective ionophore at concentrations from 10^{-8} to 10^{-7} M produced a rapid and sustained membrane hyperpolarization followed by simultaneous reduction of the input resistance. Cells were electrically quiescent within 4 s of the ionophore treatment. Fig. 3 shows a typical example of this effect, where the time course of membrane potential change is parallel to that observed in the total cell resistance.

Most cells exhibited a linear current-voltage relationship. Fig. 4 was taken from an electrically quiescent cell and shows ohmic responses up to $1.5 \cdot 10^{-9}$ *A* of outward current pulses. For higher currents a rectification was observed in a few cells. Valinomycin produces a shift of the membrane resistance, which in the case of Fig. 4 is a 2-fold reduction. Using higher concentrations of the ionophore (10^{-7} M) it was possible to reduce the input resistance by a factor of 4.

These results provide strong evidence for the assumption that a slow transient increase in potassium permeability is involved in the slow responses observed. Since it has been demonstrated that valinomycin molecules act by

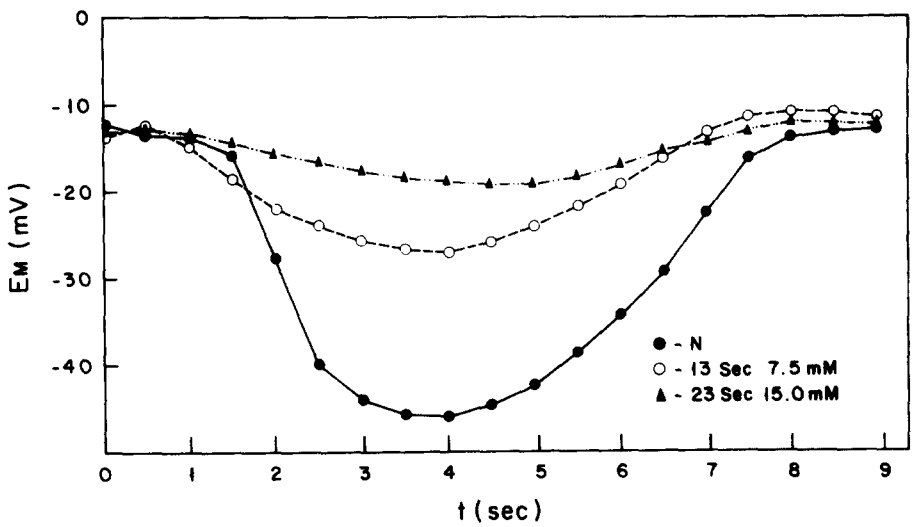

Fig. 2. Effect of ET_4Am treatment in the amplitude of continuously monitored SH responses of the same cell. ●, control; ○, after the addition of 7.5 mM of ET_4Am and ▲, after a second application of ET_4Am to a final concentration of 15 mM (ABY dextran induced macrophage, 48 h in culture).

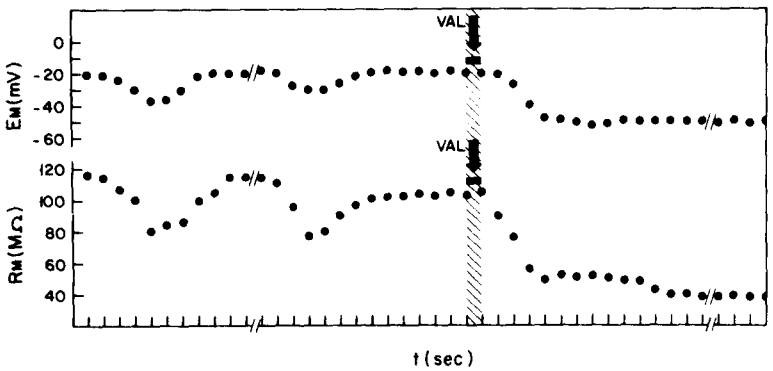

Fig. 3. Effect of valinomycin in membrane potential (E_m) and input resistance (R_m) of a spontaneously active macrophage. Note the two SH responses shown before addition of $0.5 \cdot 10^{-7}$ M valinomycin (VAL). Within 4 s of the ionophore addition a sustained hyperpolarization is observed along with a 2.5 times reduction of the input resistance. This cell as well as all the others tested in the dish were quiescent and hyperpolarized, but remained viable before impalement (B10.D2, oil induced macrophage, 48 h in culture).

opening specific potassium channels in the membrane [8] our results can be easily explained by a shift of the membrane potential from the original value to a value that is closer to the expected potassium equilibrium potential. The cell is thus rendered quiescent since further increases in potassium conductance can not occur. The sustained hyperpolarization observed has a similar time course to that seen in the SH. The fact that Et_4Am reduces and prevents the SH responses is also in good agreement with this hypothesis. Cells highly active in SH production became quiescent after ET_4Am treatment since K^+-conductance increase was prevented.

Potassium activation can cause hyperpolarizing responses in excitable cells [6]. Slow hyperpolarizations have been described in L cells [4] and guinea-pig macrophages [3] under the name of hyperpolarizing activations. These responses were ascribed to an increase in potassium permeability because their

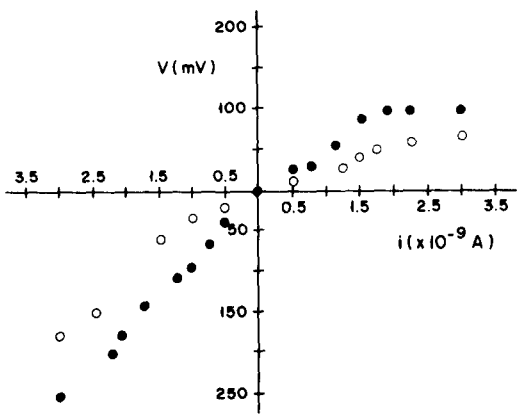

Fig. 4. Current-voltage relationship and valinomycin treatment. Measurements with 100 ms duration pulses at a rate of 75 pulses/s were done before (•) and 80 s after addition of $0.5 \cdot 10^{-7}$ M valinomycin (○). Note the rectification observed in both cases for outward pulses larger than $1.5 \cdot 10^{-9}$ A.

amplitude is dependent upon external potassium concentration. These observations, however, do not rule out the participation of other ions in the SH. It is likely that chloride can be excluded since an increase in external Cl^- should shift the chloride equilibrium potential in the hyperpolarizing direction magnifying the response. However the reverse effect has been described [3,4]. Membrane potential changes during the SH could be due to a decrease in sodium permeability, which would necessarily lead to a marked increase in membrane resistance. The pronounced fall observed in membrane resistance (Fig. 1B) seems to rule out this possibility. The data presented here with potassium specific substances are very indicative that this ion plays a major role in SH production.

Cytoplasmic calcium concentration can regulate a number of cell functions, including K^+ permeability in several excitable cells [9—11]. In guinea-pig macrophages the divalent cation ionophore A23187 induces prolonged hyperpolarizations in quiescent cells [3]. The effect was reversed by the use of Mg-EGTA. These results prompted Gallin and coworkers to propose that an increase in intracellular calcium concentration could be an early event in the triggering of K^+-conductance changes. We have tested verapamil, an extremely potent calcium antagonist [12] and found that it blocks SH production. This result brings additional support to the idea that calcium is involved in the potassium-dependent SH responses. It is difficult to ascribe a more precise role to calcium in this process since this ion is involved in a variety of cell functions ranging from cell-to-cell communication [13—15] to a possible role in conveying information from a specific region of the cytoplasm to another [16].

The physiological significance of the membrane potential changes described here remains unclear. Further studies are being conducted to attempt a correlation of the SH responses with function of the macrophage's biology.

Acknowledgments

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