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Electrophysiology of Phagocytic Membranes: Induction of Slow Membrane Hyperpolarizations in Macrophages and Macrophage Polykaryons by Intracellular Calcium Injection

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Summary. Some electrophysiological characteristics of macrophages and macrophage polykaryons of foreign body granuloma have been investigated. Cells were obtained from implants of small coverslips in the subcutaneous tissue or in the peritoneal cavity of rats and mice. Transmembrane potentials ranged from -5 to -40 mV. Input resistances ranged from 5 to 120 MΩ, being significantly higher in mice polykaryons. Approximately 10% of the cells exhibited spontaneous slow membrane hyperpolarizations (SH) indistinguishable from those observed in macrophages. SH responses were invariably evoked by iontophoretic injection of calcium ions into the cytoplasm of mice macrophages or macrophage polykaryons. The amplitude of these responses increased with the amount of current carried by calcium ions into the cells. The maximum amplitude of the calcium-induced SH responses is a linear function of the logarithm of $[K^+]_o$ (from 3 to 40 mM). The slope of the regression line is 43 mV for a 10-fold increase in $[K^+]_o$. Substituting sodium chloride by sodium isethionate or by choline chloride does not interfere with the occurrence of SH. The assumption that the SH is solely a consequence of an increase in the membrane conductance to K^+ was used to calculate the potassium equilibrium potential (E_K). The E_K value is also a linear function of the logarithm of $[K^+]_o$ (from 3 to 40 mM). The slope of the regression line is 46 mV for a 10-fold increase in $[K^+]_o$. These results constitute evidence of the calcium dependence of K^+ permeability during SH both in macrophages and macrophage polykaryons. Macrophage polykaryons are a more convenient model than macrophages for the study of the mechanisms underlying the SH responses and their possible physiological implications.

Key words: macrophages, macrophage polykaryons, cytoplasmic calcium, potassium permeability, slow hyperpolarization.

The macrophage membrane is the site of basic events for the host defense mechanism such as: phagocytosis, chemotaxis, secretion of enzyme granules, antigen presentation to lymphocytes, and other specific cell interactions essential to immunocompetence. The fact that these are membrane-mediated phenomena has prompted the investigation of the electrophysiologic properties of these cells.

Guinea pig, mouse, and human macrophages [5–8] have revealed slow membrane hyperpolarizations (SH) characterized by: (a) great variability in amplitude and duration, (b) spontaneous firing or triggering by mechanic, electric, or chemical stimuli and (c) simultaneous decrease of input resistance. An increase in the potassium membrane permeability has been shown to occur during these SH responses [5, 8, 21]. This mechanism appears to be calcium dependent [8, 21]. Calcium is also known to control the potassium permeability in a number of cells [14, 17]. Using alveolar macrophages it was possible to correlate an increase in cytoplasmic calcium with secretory phenomena mediated by exocytosis [34].

In the case of macrophages the small cell size and the small amplitude of the recorded electric signals are complicating factors when electrophysiologic techniques are employed. We have tried to establish criteria for the acceptance of intracellular records in these cells through the use of markers for the cytoplasmic location of microelectrodes [5]. These criteria imposed great limitations in the number of recordings. In order to overcome these limitations, we decided to study the macrophage polykaryons of foreign body granuloma [1, 16, 23].

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These large cells still exhibit some physiological properties of their cell of origin, such as phagocytosis [4, 15, 27], locomotion [24, 25], and exocytosis (26, 29). The use of macrophage polykaryons has increased the yield of successful impalements and allowed a longer intracellular recording period, minimizing cell damage by the microelectrode.

In this paper we describe the occurrence of SH responses in macrophage polykaryons. The responses are similar to those found in macrophages. With these large cells two microelectrodes can be simultaneously used. We found that iontophoretic injection of Ca^{2+} ions into the cytoplasm of macrophages and macrophage polykaryons regularly induces the firing of SH responses. The ionic dependence of the Ca^{2+}-elicited SH is studied. The data indicate that a Ca^{2+}-dependent potassium permeability change is the most important ionic event involved in membrane potential changes during the SH.

Materials and Methods

Cells

Macrophages were obtained by dextran-induced peritoneal exudate in AKR mice as previously described [5]. The exudate was plated on glass coverslips in culture medium (RPMI-1640 Gibco-Grand Island, N.Y.), with 5% fetal calf serum, 2 grams per liter of sodium bicarbonate and antibiotics (streptomycin 100 µg/ml, penicillin 100 µg/ml, fungizone 0.25 µg/ml). After a two-hour incubation at 37 °C in a 5% CO_2 humidified atmosphere the non-adherent cells were washed away. The coverslips were maintained in plastic Petri dishes for 8 days in the complete culture medium.

Macrophage polykaryons were formed on both surfaces of round glass coverslips (6 mm diameter) [28] implanted: a) in the subcutaneous tissue of outbred rats for 15 to 30 days, b) in the subcutaneous tissue of isogenic AKR or C_3H mice for 4 to 16 days, or c) in the peritoneal cavity of AKR mice for 4 to 16 days. The coverslips were removed and washed in complete culture medium buffered with 6 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). Cells were removed from one side and the coverslips placed with the other side up in the HEPES-buffered medium for electrophysiological studies. Most experiments were performed with peritoneal coverslips where an extensive and more homogeneous layer of cells were found. All data from mouse cells shown are from the AKR strain.

Recording Arrangement

Intracellular recordings were performed on a heated stage of a special contrast Leitz microscope (Heineke condenser, UMK 50/0.60 objective) using a standard electrophysiological recording system. Glass microelectrodes filled with 2.5 M KCl had resistances from 30 to 60 MΩ (equivalent to tip diameters below 200 nm in our conditions [22]. The experimental set-up is shown in Fig. 1. Recording microelectrodes were connected to high input impedance preamplifier with a bridge circuit for simultaneous voltage recording and current injection (WP Instruments Model M4A Electrometer, Ramden, Conn.). Current measurements were made through the voltage drop across a 1-MΩ resistor inserted between the bath and the ground. Impalements were considered acceptable only when good bridge balance (within 10% of the input resistance of the cell) and return to the same base line (within 2 mV) were observed upon withdrawal of the microelectrode.

Using two microelectrodes in the same cell it was possible to inject current (I_1) through one and record the corresponding voltage drop simultaneously from both (V_1, V_2). The input resistance measurements obtained by the current-injecting microelectrode (V_1/I_1) were in good agreement with those from the other (V_2/I_1). This control validates the use of a single microelectrode for the measurement of input resistance and also gave assurance of the cytoplasmic location of our electrode tips.

Calcium Injection

Glass microelectrodes were filled with 0.5 M $CaCl_2$ solution. Current was provided by a low-impedance source connected between the Ca^{2+} microelectrode and ground and monitored by the RI drop across the same 1 MΩ resistance inserted between bath and ground mentioned above. The Ca^{2+} electrode had a d-c resistance of around 100 MΩ and the shunting of membrane voltage caused by it seemed to be negligible. The KCl recording electrode was impaled before and the $CaCl_2$ afterwards. Hyperpolarizing pulses (0.5 nA; 120 msec; 1 pulse/sec) were applied through the

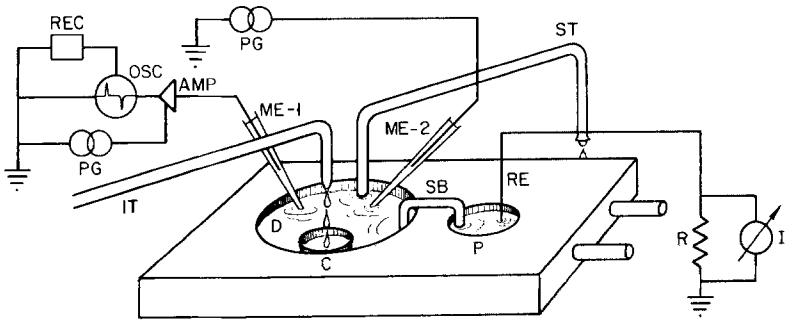

Fig. 1. Experimental set-up. Solutions were injected into two inlet tubes (*IT*) (second tube not shown). The collector (*C*) was placed inside the culture dish (*D*) to minimize vibrations. Total volume was kept constant (about 1.5 ml) by means of a siphon used as a suction tube (*ST*). Microelectrodes shown contained KCl (*ME*−1) and $CaCl_2$ (*ME*−2). A saline-agar bridge (*SB*) connected the culture dish with a 2.5 M KCl pool (*P*) containing the reference electrode (*RE*). *AMP*-Preamplifier, *OSC*-double beam oscilloscope, *REC*-strip chart recorder, *PG*-pulse generator, *I*-current monitor by means of the voltage drop across the 1 MΩ resistor (*R*). Hot water was circulated to maintain constant temperature in the culture dish stage

$CaCl_2$ electrode during the impalement. This procedure allowed control of the electrode penetration and avoided electrode blockage.

Solutions

Unless otherwise specified all recordings were performed in complete culture medium buffered with 6 mM HEPES, pH from 7.2 to 7.4, at temperatures from 34 to 37 °C. The medium was replaced every 15 to 20 min.

The normal salt solution used contained NaCl 138 mM, KCl 3 mM, $CaCl_2$ 1 mM, HEPES 6 mM. The effect of external potassium concentration ($[K^+]_0$) was tested using equimolar substitution of NaCl by KCl. In some experiments NaCl was completely replaced by sodium isethionate or choline chloride. Cells were exposed to saline solution for at least two periods of 15 to 20 min. Each period was preceded by 30 min in complete culture medium. For high potassium concentrations (above 30 mM) the exposure to the saline solution was reduced to less than 10 min.

Determination of the Potassium Equilibrium Potential (E_K)

The potassium equilibrium potential was calculated from cells where the resting input resistance returned within 10% to the initial value after the SH and the resting potential remained within 2 mV of the initial value.

Perfusion System

To obtain continuous recording from the same cell while exchanging the bathing solution a hand driven screw system was used for perfusion (Fig. 1). The flow was adjusted to perfuse up to four times the chamber volume (~1.5 ml) in 90 sec. The solution flow into the chamber and the suction system included a discontinuous liquid column allowing electrical isolation of the bathing solution from the external reservoirs.

Results

Basic Electrophysiologic Characteristics of Macrophage Polykaryons

Cells on the surface of coverslips contain from 1 up to 100 nuclei and have diameters from 15 to 150 μm. Most of the studied cells had diameters of 30 to 80 μm. Fig. 2 shows the appearance of cells.

Fig. 3 shows the distributions of the transmembrane potentials and the input resistances in different animals and sources. The transmembrane potential (V_0) ranged from −5 to −40 mV. The input resistances (R_0) ranged from 14 to 120 MΩ for mouse cells and from 5 to 45 MΩ for rat cells. Values of V_0 and R_0 may change during an impalement but the observed variations fall within the range of the histograms of Fig. 3. We did not find any correlation between the V_0 and R_0 values in the same cell population. Time constant for the majority of cells was below 100 msec.

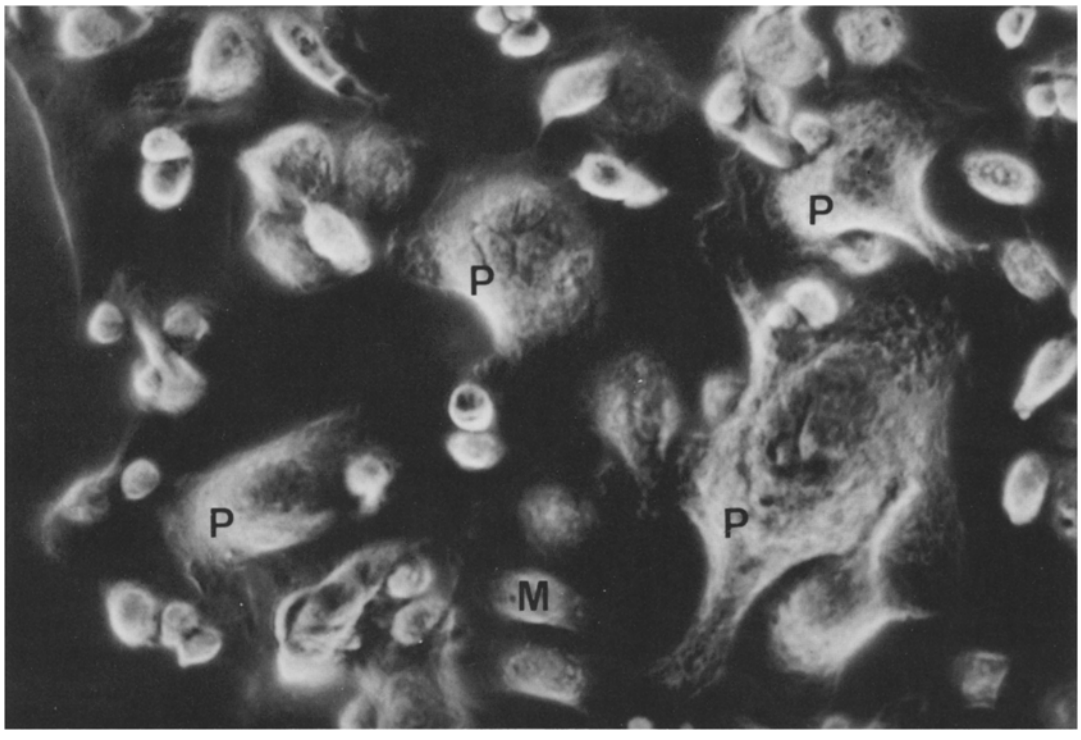

Fig. 2. Macrophage polykaryons. Cells from an intraperitoneal coverslip after five days of implant, fixed for 15 min in phosphate buffer solution with 2.5% glutaraldehyde. The picture was taken in the phosphate buffer solution using a special contrast Leitz microscope (*see* Recording arrangement). Calibration bar: 50 μm; *P* macrophage polykaryons; *M* macrophage

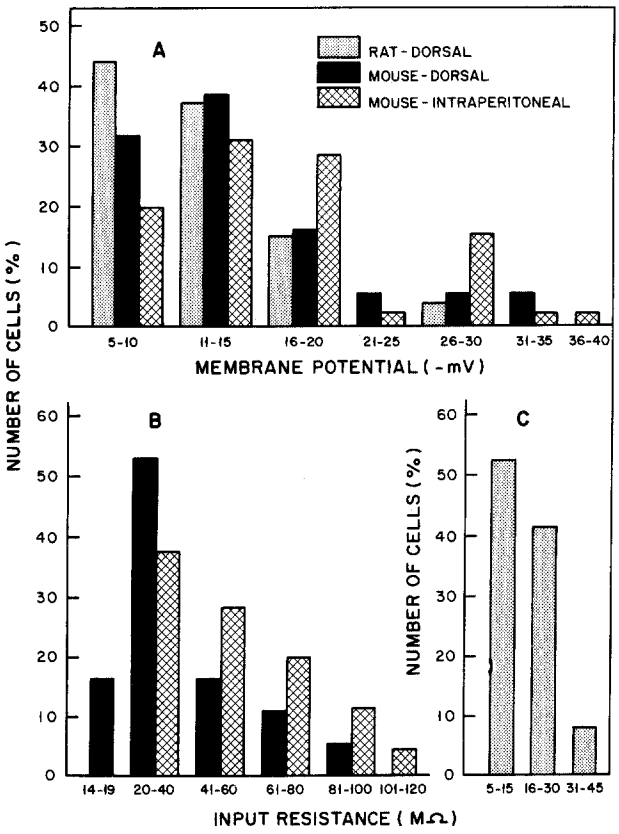

Fig. 3. Membrane potentials and input resistances of macrophage polykaryons. (*A*) Membrane potentials. (*B* and *C*) Total input resistances. The same cells were used for both measurements. Rat dorsal cells were obtained after 15 to 30 days of implant (27 cells) and mouse from 4 to 9 days after the implant (19 cells from dorsal and 27 cells from intraperitoneal coverslips)

Approximately 10% of the cells examined exhibited slow membrane hyperpolarizations concomitant with a transient decrease in input resistance. These responses are very similar to those described for macrophages [8].

Aspects of SH responses from mononuclear, binuclear and multinucleated cells from a single coverslip observed immediately after impalement are shown in Fig. 4*A*, 4*B* and 4*C*. A pattern of spontaneous SH in mice peritoneal polykaryon is shown in Fig. 4*D*. The SH characteristics (amplitude, duration and frequency) are variable from cell to cell. It was possible to elicit SH responses by electrical (Fig. 4*E*) and mechanical stimulation of polykaryons. The current intensity required to elicit SH responses was generally higher than that required for macrophages [6]. Mechanical stimulation generally caused a decrease in the input resistance of the polykaryons.

Effects of Iontophoretic Calcium Injection

SH responses are invariably elicited when calcium ions are injected into the cytoplasm of peritoneal exudate macrophages. The amplitude of the SH increases with the total outward current carried by calcium ions (Fig. 5*A*). Similar results were obtained in macrophage polykaryons (Fig. 5*B*). To exclude the effects of the current flow which can itself induce SH as shown, we injected equivalent outward or inward currents alternatively through both the $CaCl_2$ and

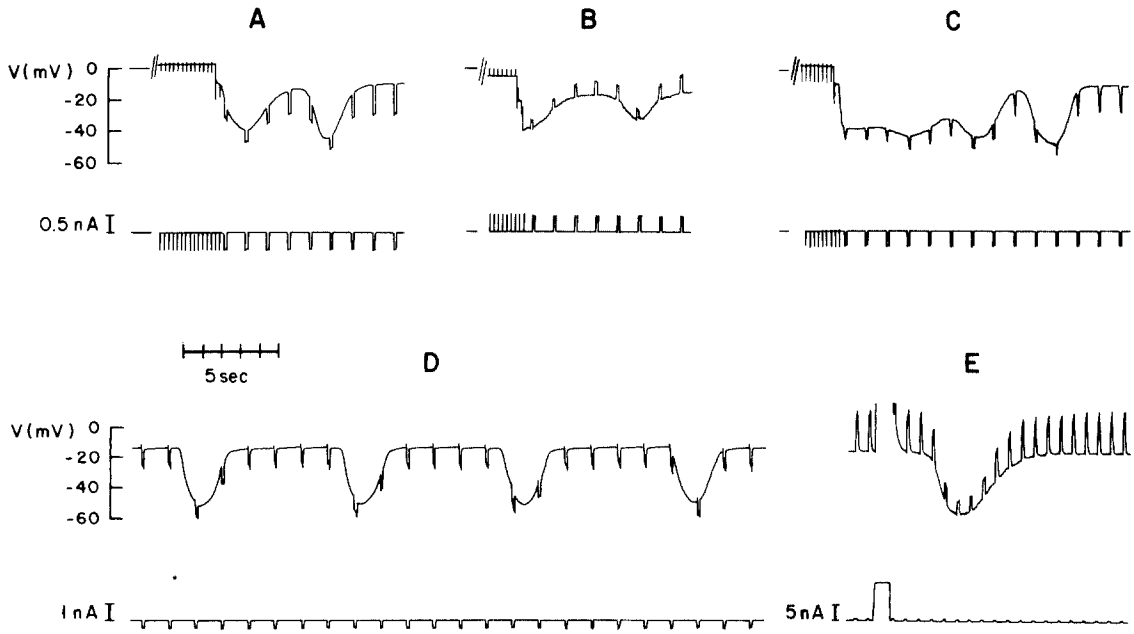

Fig. 4. Different patterns of SH. Records from the same dorsal coverslip from a rat are shown to compare spontaneous SH immediately after impalement of a mononuclear cell (*A*), a binuclear cell (*B*) and a macrophage polykaryon (*C*). (*D*) Repetitive spontaneous SH of intraperitoneal macrophage polykaryons (mouse). (*E*) Electrically elicited SH by the large current pulse shown in lower trace (mouse intraperitoneal polykaryon). Upper trace is voltage monitored by the microelectrode and lower trace is the injected current

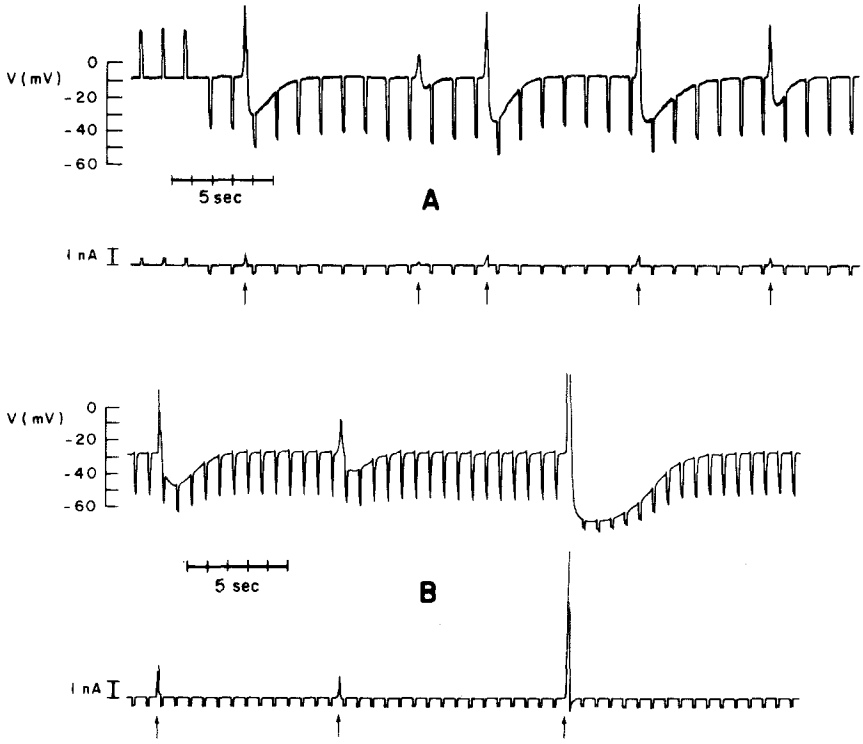

Fig. 5. Ionophoretic calcium injection. Arrows indicate outward current pulses through the $CaCl_2$ microelectrode. Other pulses were injected by the KCl microelectrode. (*A*) Mouse peritoneal exudate macrophage. (*B*) Mouse macrophage polykaryon from intraperitoneal coverslip

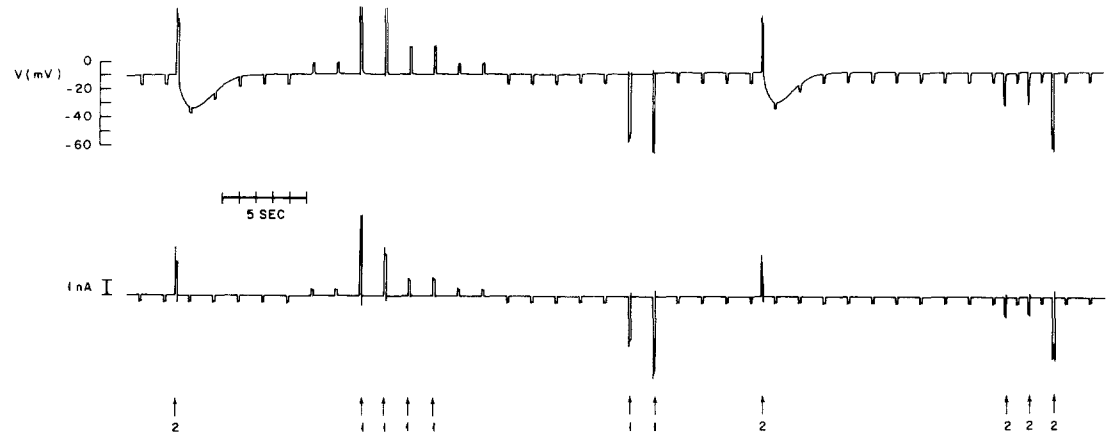

Fig. 6. Requirement of calcium as a charge carrier to elicit SH with small current pulses. Arrows indicate when current was injected through the KCl (1) or $CaCl_2$ (2) microelectrodes. Note that Ca^{2+} injection (depolarizing current 2) invariably triggers SH, while Cl^- injection by any of the electrodes (hyperpolarizing currents 1 and 2) or K^+ injection (depolarizing current 1) fails to elicit the response. Mouse macrophage polykaryon from a dorsal coverslip

the KCl microelectrodes. Only the outward current through the $CaCl_2$ microelectrode triggered SH responses (Fig. 6). In some cells smaller pulses of outward current through the $CaCl_2$ microelectrode (below the standard 0.5 nA during 120 msec pulses used to measure input resistance) were also capable of inducing SH responses. Increasing the stimulus intensity caused the SH amplitude to increase up to a maximum (Fig. 7). In this situation a maximum decrease of the input resistance at the peak of the SH was observed. The inset on Fig. 7 shows that a maximal amplitude is also reached after repetitive stimulation during an SH response.

Effects of External Potassium Concentration on SH

The previous analysis of SH responses in macrophages produced evidence for a calcium-dependent increase in potassium permeability [8, 9, 21]. To characterize the potassium dependence of spontaneous and calcium-induced SH, we used salt solutions with different potassium concentrations.

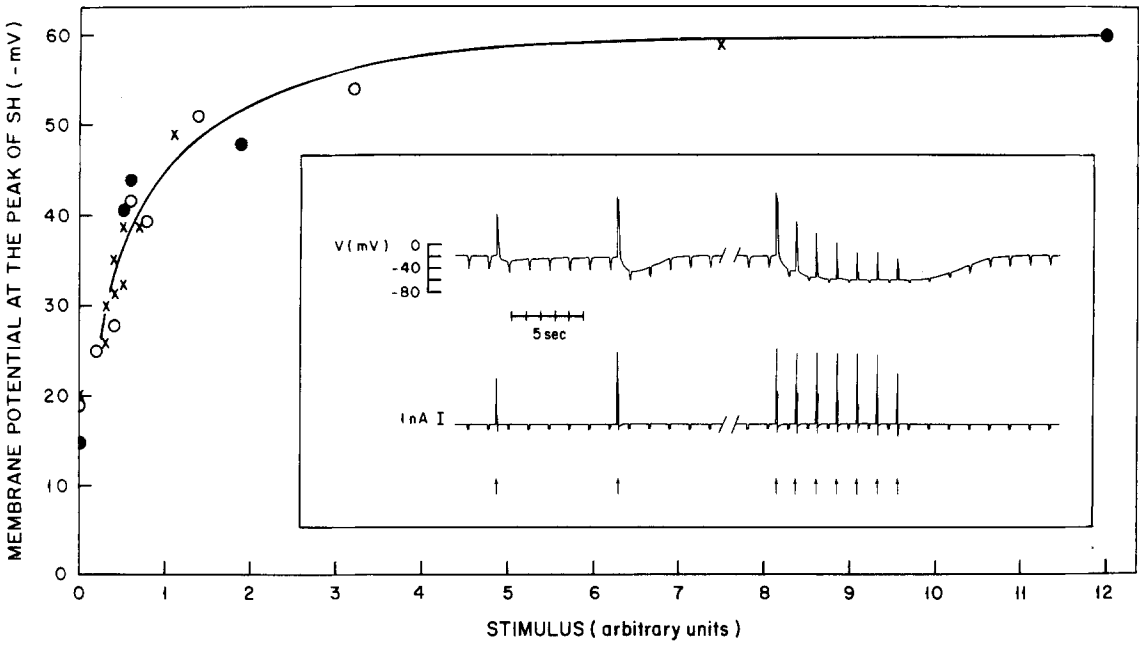

Fig. 7. Effect of different calcium stimuli in murine macrophage polykaryons. Value of transmembrane potential at the peak of the SH as a function of the stimulation intensity. Stimuli (total charge injected) were measured by the area under the current versus time records. Symbols indicate three different intraperitoneal cells. *Inset:* repetitive stimulation by calcium injections causes saturation of the response (dorsal cell). Arrows indicate calcium injections

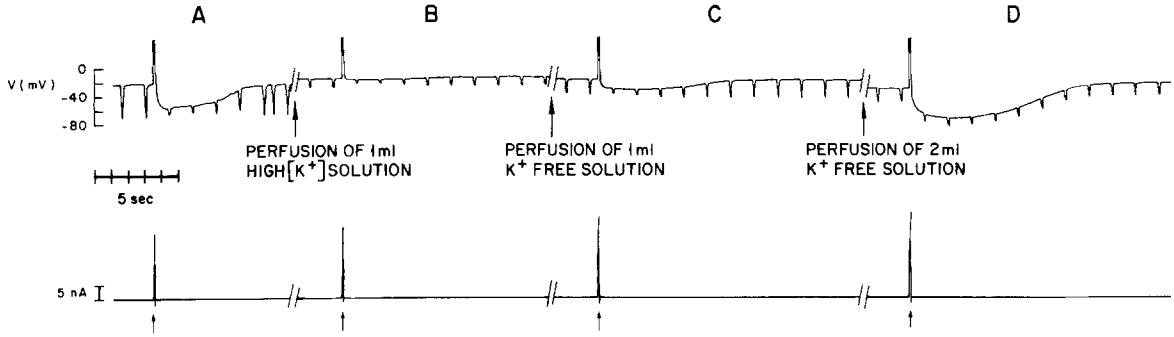

Fig. 8. Effect of $[K^+]_0$ on the amplitude of calcium-induced SH. Continuous records from the same mouse macrophage polykaryon (intraperitoneal coverslips) during increase and reduction of external potassium concentration. The records were made: (*A*) in the culture medium; (*B*) after perfusion of 1 ml of salt solution containing 108 mM of KCl; (*C*) after perfusion of 1 ml of the K^+-free solution; (*D*) after another perfusion of 2 ml of the K^+-free solution. Arrows indicate calcium injections. Current pulses from KCl microelectrode (not shown) are of 0.3 nA. Intervals between the records are: *A–B*: 35 sec, *B–C*: 40 sec and *C–D*: 44 sec. Note: estimated $[K^+]_0$ are: *A*, 5.4 mM; *B*, 45 mM; *C*, 18 mM and *D*, 3 mM

We compared the transmembrane potential, the input resistance and the SH (spontaneous and elicited by current or Ca^{2+} injection) in culture medium and in salt solutions. The values at V_0 and R_0 in salt solutions with $[K^+]_0$ ranging from zero to 30 mM were similar to those of the complete culture medium. In salt solutions with a $[K^+]_0$ greater than 30 mM the cells generally depolarized and had lower input resistances. This effect disappeared upon return to normal $[K^+]_0$. Spontaneous SH were observed at $[K^+]_0$ as high as 40 mM. With bathing solutions containing increasing $[K^+]_0$ the maximum amplitude of the Ca^{2+}-induced SH decreased reversibly as illustrated in Fig. 8. This record was obtained by continuous monitoring from the same cell. Note in Fig. 8*B* a decrease in the input resistance without change in the membrane potential. The maximal amplitude of the SH-induced by calcium injection is a linear function of the logarithm of $[K^+]_0$ as shown in Fig. 9*A*. The values shown here are from the maximum responses among all of the cells studied. The slope of the regression line is 43 mV for a 10-fold increase in $[K^+]_0$. The results obtained with cells in the culture medium are also shown.

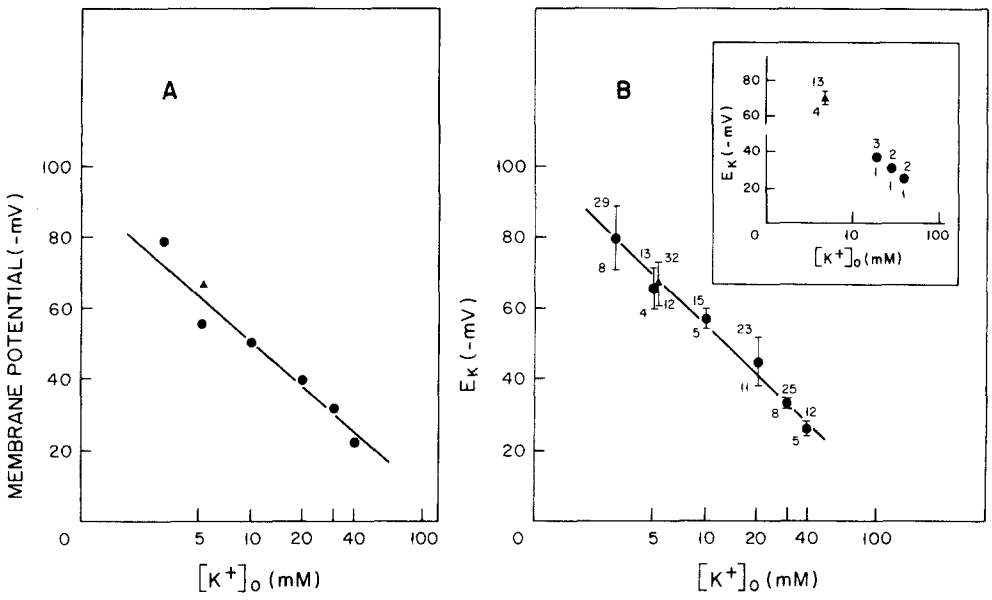

Fig. 9. Maximal amplitude of calcium-induced SH and potassium equilibrium potential. (*A*) Effect of $[K^+]_o$ in the maximum value of membrane potential at the peak of the calcium-induced SH. (*B*) Calculated values of $E_K \pm$ SD (*see* Appendix) for calcium-induced SH. *Inset:* E_K for spontaneous SH. Data from mouse macrophage polykaryons (intraperitoneal, 4 to 9 days of implant). Circles indicate measurements from salt solutions. Triangles indicate measurements in culture medium (5.4 mM of K^+). Regression lines were calculated using only data in salt solutions. Figures indicate the total number of cells (lower) and the total SH included (upper)

When the sodium chloride was entirely substituted by sodium isethionate or choline chloride in the 3 mM KCl salt solution no differences in SH responses could be observed (results not shown). These observations suggested that the absence of Na^+ or Cl^- ions in the external medium does not interfere with the occurrence of the SH. We have also observed similar SH responses (spontaneous and elicited by Ca^{2+} injection) in salt solution without $CaCl_2$ (data not shown).

Determination of Potassium Equilibrium Potential

In some cells the maximal amplitude of SH was obtained only after injection of current pulses of about 1 sec duration and more than 2 nA amplitude through the calcium microelectrode. This procedure can cause irreversible reduction of the input resistance. This was not accompanied by depolarization or any morphological indication of cell damage.

In order to avoid damage resulting from high current injection a different approach was devised based on measurements of submaximal SH. The method consisted of determination of the potassium equilibrium potential (E_K) and was based on the evidences of the preceding section. The calculation assumes that the membrane potential change at the peak of the SH is exclusively dependent on a potassium conductance increase. As shown in the Appendix the potassium equilibrium potential can be calculated by the following equation:

$$E_K = E_0 + \Delta E \left(1 - \frac{R}{R_0}\right)^{-1} \quad (1)$$

where E_0 is the membrane resting potential, ΔE is the change in the membrane potential at the peak of the SH, R_0 is the resting input resistance of the cell

Table 1. E_K values in different conditions

Cell[a]	External solution	$[K^+]_o$ (mM)	Type of SH	E_K (mV)[b]
Polykaryon	culture medium	5.4	Ca^{2+}-induced	-67 ± 6 [12,32]
Polykaryon	culture medium	5.4	Spontaneous	-71 ± 3 [4,13]
Polykaryon	normal saline	3.0	Ca^{2+}-induced	-80 ± 9 [8,29]
Polykaryon	choline saline	3.0	Ca^{2+}-induced	-71 ± 11 [4, 14]
Polykaryon	isethionate saline	3.0	Ca^{2+}-induced	-72 ± 11 [7, 18]
Macrophage	culture medium	5.4	Ca^{2+}-induced	-69 ± 2 [2, 5]

[a] Mouse macrophage polykaryons from intraperitoneal coverslips with 4 to 9 days of implant. Peritoneal exudate macrophages were kept 8 days in culture.

[b] $E_K \pm$ SD [number of cells, number of SH].

immediately before the SH and R is the value of the input resistance at the peak of the SH. The values of E_K were obtained applying Eq. (1) to SH of various amplitudes in the same cell. The mean of these values was considered as the E_K for that cell.

Fig. 9*B* represents the plot of the calculated values of E_K as a function of the logarithm of $[K^+]_0$, for calcium-induced SH. The slope of the regression line is 46 mV for a 10-fold increase in $[K^+]_0$. The inset of Fig. 9*B* is a plot of calculated E_K from spontaneous SH.

Table 1 shows values of E_K obtained under various experimental conditions. The values obtained in culture medium for spontaneous SH or SH triggered by calcium injection are not significantly different (Student's t test $p<0.05$). Substitution of sodium by isethionate or by chloride choline in normal potassium did not change significantly the values of E_K ($p<0.05$). Also shown are values of E_K for peritoneal exudate macrophages.

Discussion

The observations described here show that SH activity previously studied in macrophages can also be observed in macrophage polykaryons.

Calcium is involved in the control of the K^+ permeability in a number of cells [2, 10, 12–14, 17, 20, 30–33], including macrophages [8, 21]. The results obtained by iontophoretic calcium injection are a more direct indication for a calcium control of G_K both in macrophages and macrophage polykaryons.

The ionic dependence of the membrane potential and input resistance during the SH was analyzed. The maximum amplitude of the calcium-induced SH responses is a linear function of the logarithm of $[K^+]_0$. This result is similar to that observed for spontaneous SH in guinea pig macrophages [8, 21].

The calculated value of E_K is also a linear function of the logarithm of the $[K^+]_0$. There is a good agreement between these calculated values and the maximum amplitude of the calcium-induced SH.

The calculated values of E_K gave a regression line with a slope smaller than the value expected by the Nernst Equation (46 mV for a 10-fold increase in the $[K^+]_0$ as compared to 61 mV). This discrepancy may be due either to an increase in the free cytoplasmic $[K^+]_0$ in the high K^+ saline or to the small influence of other ions.

The basic assumption used for the calculation of E_K (that K^+ permeability changes are the main event responsible for the peak value of the SH) appears to be correct, since the E_K values are not significantly dependent of the Na^+ or Cl^- concentration (*see* Table 1).

Previous findings from our laboratory have shown that the SH-reversal potential for guinea-pig macrophages varies in accordance with Nernst prediction when external potassium is increased. Alternative explanation for the deviation from Nernst prediction is the inaccuracy of the recording technique, since the microelectrode tip potentials alter significantly the measured E_0 values. In macrophages we have been able to show a positive correlation between tip potentials and E_0 measurements [6]. In the case of macrophage polykaryons a similar study could not be performed because of the greater variability of E_0 (Fig. 3*A*). Other possibilities that we cannot yet discard are, for example, contribution of an active transport system during the SH, possible nonlinear current-voltage relationship for the membrane resistance to K^+ or other ions and possible resistance-voltage dependence.

The E_K values for spontaneous SH are in agreement with those obtained for calcium-induced SH (Table 1 and Fig. 9). This suggests that both types of SH are due to the same mechanism.

The values for maximum SH amplitude and E_K in culture medium correlate well with those in salt solutions (triangles in Fig. 9). Thus it is reasonable to suppose that the use of simple salt solutions does not interfere with the basic mechanisms of SH.

Using the Nernst Equation and the estimated E_K values for Ca^{2+}-induced SH (Table 1) we obtain an internal $[K^+]$ of 65 mM for the macrophage polykaryon and of 70 mM for the mouse macrophage in the culture medium. In rat macrophage polykaryons the calculated E_K was -55 ± 6 mV (3 cells, 17 SH) for spontaneous SH in the culture medium. This value yields an internal potassium of 42 mM. In a recent report a value of 50 mM for the internal $[K^+]$ of rat alveolar macrophages was obtained [3].

Transformed giant L cells [18, 19] have membrane potential oscillations similar to the SH of macrophages and macrophage polykaryons. In L cells an increase in the cytoplasmic calcium also causes the increase of the potassium conductance [10, 20]. Okada et al. [20] using a similar reasoning to that presented here (Appendix), derived a formula correlating ΔE with ΔG_K at the peak of the SH response of L cells. The expression presented in their paper differs from Eq. (6) (Appendix). Using this equation when ΔG_K is very high, we obtain the value $E_K = E_0 + \Delta E$. This means that the maximum SH amplitude possible is equal to E_K as predicted if this equilibrium potential is the driving force for the SH. From Okada's formula we would arrive at the inconsistent value $E_K = E_0 + 2\Delta E$.

The source of calcium in spontaneous SH of macrophages and of L cells is not definitively estab-

lished [10, 20, 21]. The fact that we have observed spontaneous and Ca^{2+}-induced SH in solution without $CaCl_2$ does not exclude the possibility that membrane-bound or residual Ca^{2+} may play a role in triggering spontaneous SH responses.

Iontophoretic injection of Ca^{2+} always triggered SH responses. This procedure enabled us to study the ionic events of these responses in more detail, since spontaneous SH occurred erratically and electrical stimulation sometimes failed to elicit SH.

We conclude that the macrophage polykaryon is a valuable model for analysis of the basic events of the macrophage membrane for the following reasons: a) The ionic mechanisms of SH responses are identical to those of macrophages; b) the large cell dimensions minimize cell damage, yielding a greater number of successful impalements and increasing the recording period; c) cells are easily obtained; d) some basic macrophage functions such as phagocytosis and exocytosis persist in the polykaryons.

In view of these advantages it seems worthwhile to study polykaryon membranes in even greater detail especially with regard to the physiological function of the slow hyperpolarization.

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Appendix

Calculation of the Potassium Equilibrium Potential (E_K)

Assuming that the reduction of the input resistance of a cell during the SH is a consequence only of an increase in potassium conductance (ΔG_K), the total membrane conductance (G) at the peak of the SH is given by:

$$G = G_0 + \Delta G_K \tag{2}$$

where G_0 is the resting conductance.

If the resting potential (E_0) is determined by the specific ionic conductances (G_j), and their respective equilibrium potentials E_j [11, 35] we have:

$$\sum_j (E_0 - E_j) G_j = 0. \tag{3}$$

The value of the potential at the peak of the SH ($E_0 + \Delta E$) will then be determined by the analogous expression:

$$\{\sum_j (E_0 + \Delta E - E_j) G_j\} + (E_0 + \Delta E - E_K) \Delta G_K = 0. \tag{4}$$

This expression is valid only if we assume that at the peak of the SH the membrane potential is at equilibrium.

Assuming that leak conductances remain constant during the SH it is not necessary to include them explicitly in Eqs. (3) and (4).

The total resting conductance (G_0) is:

$$G_0 = \sum_j G_j. \tag{5}$$

Using Eqs. (3), (4) and (5) we obtain:

$$-\Delta E = \frac{(E_0 - E_K)\,\Delta G_K}{G_0 + \Delta G_K}. \tag{6}$$

According to Eq. (2): $\Delta G_K = G - G_0$. Substituting this expression in Eq. (6) we than obtain:

$$E_K = E_0 + \Delta E \left(1 - \frac{G_0}{G}\right)^{-1}. \tag{7}$$

This relation can be used to calculate E_K substituting $1/R_0$ for G_0 and $1/R$ for G, where R_0 and R are the measured input resistances at rest and at the peak of the SH, respectively. Eq. (1) used in the last section of the Results, follows directly.

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