Biochimica et Biophysica Acta 856 (1986) 362–372
Elsevier

BBA 73021

Electrophysiology of phagocytic membranes. Role of divalent cations in membrane hyperpolarizations of macrophage polykaryons *

Elizabeth G. Araujo **, Pedro M. Persechini and Gilberto M. Oliveira-Castro ***

Instituto de Biofisica, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, RJ 21.941 (Brazil)

(Received August 20th, 1985)

Key words: Macrophage polykaryon; Electrophysiology; Divalent cation; K^+ transport; Membrane potential; Hyperpolarization

The electrophysiological properties of the membrane of mouse peritoneal macrophage polykaryons are studied. Slow hyperpolarizations can be elicited by iontophoretic injections of either Ca^{2+} or Sr^{2+} into the cytoplasm. The effect of both cations is identical, since: (1) it is invariably triggered by the cation injection, (2) the amplitude is dependent on the K^+ gradient, (3) quinine blocks reversibly the response to both cation injections. Mg^{2+}, Ba^{2+} and Mn^{2+} did not elicit responses when injected into the cytoplasm. Ca^{2+} induced slow hyperpolarizations were reversibly blocked by the addition of Ba^{2+} to the external saline, but were not affected by the presence of external tetraethylammonium chloride. Cells maintained in saline containing high concentrations of Ca^{2+}, Sr^{2+} or Mn^{2+} exhibited sustained hyperpolarizations. Quinine blocked the hyperpolarization induced by high Ca^{2+} or Sr^{2+}, but was ineffective for the case of Mn^{2+}. Cells hyperpolarized by external Mn^{2+} frequently exhibited nonlinear, voltage-current characteristics. Similar patterns could also be observed in a small fraction (less than 10%) of the cells in control conditions. Current-induced shifts between two stable membrane potentials were seen either in high Ca^{2+} or normal medium. The great variability of the responses described for this phagocytic membrane is discussed. The evidence supports the assumption that Ca^{2+} and Sr^{2+} can induce transient or persistent hyperpolarized states by activating a potassium permeability. External Mn^{2+} may act in part by reducing impalement-related current leakage from the phagocytic membrane.

Introduction

The macrophage membrane is the interface involved in several mechanisms of host defense and immune recognition. Evidence for a variety of ionic channels and transport system has been accumulated, although the precise physiologic correlates of the ionic movements and macrophage functions are far from being established.

The macrophage polykaryons are multinucleated cells derived from fusion of macrophages in experimental foreign-body reactions [1–3], and they retain several of the physiological capabilities of the mononucleated cells, such as phagocytosis, locomotion and exocytosis [4–8]. Polykaryons are easier to handle than macrophages, less sensitive to electrode damage and constitute a good model for the study of phagocytic membranes [9]. Both kinds of cells display spontaneous membrane potential fluctuations characterized by transitory slow hyperpolariza-

* This paper is the fifth of a series.

** Present address: Departamento de Neurobiologia, Instituto de Biologia, UFF, Niteroi, RJ, Brazil.

*** To whom correspondence should be addressed.

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

tions with concomitant increase in membrane conductance. These fluctuations vary greatly in amplitude, are due to a transient increase in a Ca^{2+}-dependent K^+ permeability [10–12] and can be induced by intracellular Ca^{2+} injection [9].

Similar oscillations of membrane potential have been found in a variety of cells [13]. The red blood cells, where the Ca^{2+}-dependent K^+ permeability was first described [14], and the fibroblastic L cells [15,16] are cases where the triggering features, channel specificity and blocking agents have been studied. In the previous paper of this series, we presented evidence in favor of the assumption that the slow hyperpolarization is mainly a consequence of a Ca^{2+}-dependent membrane conductance to K^+ in macrophage polykaryons and their mononucleated precursors [9]. Nothing is known about the ability of other divalent cations to substitute for Ca^{2+} in the intracellular medium, nor about their effects in the extracellular medium. In this paper, we compare the ability of several divalent cations to induce membrane potential and conductance changes in macrophage polykaryons when present in the extracellular medium or when iontophoretically injected into the cytoplasm. It is shown that increased Ca^{2+} and Sr^{2+}, either inside or outside the cells, can cause an increase in K^+ conductance, whereas Ba^{2+} and Mg^{2+} cannot. Mn^{2+} induces membrane hyperpolarization only when added to the extracellular medium. This effect differs from that of Ca^{2+} and Sr^{2+}, because it is quinine-insensitive and is usually accompanied by the emergence of nonlinear, excitable-like, voltage-current characteristics. The mechanisms that may be involved in the Mn^{2+}-induced nonlinearity and depolarizing excitability are discussed, together with similar behavior that is sometimes observed in the absence of Mn^{2+} in cells exposed to normal culture medium.

Preliminary reports of this work have already been published (Ann. Acad. Bras. Cien. (1983) 55, 456; Braz. J. Med. Biol. Res. (1983) 16, 426 and Braz. J. Med. Biol. Res. (1983) 16, 427.

Materials and Methods

Cells. Macrophage polykaryons were formed on the surface of round glass coverslips (6 mm diameter) implanted for 4–60 days in the peritoneal cavity of AKR or C_3H strains or outbred albino mice [1,9]. After variable periods, the coverslips were removed and washed in culture medium (RPMI-1640, Gibco, Grand Island, NY) containing 5% fetal calf serum and buffered with 6 mM Hepes at pH 7.2–7.4. Cells were then kept is this medium at 37°C for at least 30 min prior to any electrophysiological experiment.

Solutions and reagents. The recordings were performed either in culture medium or in saline solutions. Normal medium (described above) had a potassium concentration of 5.3 mM. In high-potassium medium, $[K^+]$ was increased to 30.3 mM by the addition of concentrated KCl to the normal medium. The normal saline comprised 140 mM NaCl/5 mM KCl/1 mM $CaCl_2$/0.5 mM $MgCl_2$/6 mM Hepes. High divalent-cation salt solutions were obtained by addition of the appropriate chloride. Quinine sulfate was dissolved in normal saline at a concentration of 1.5 mM and kept frozen until dilution. Tetraethylammonium chloride was dissolved in culture medium immediately before use. Tetraethylammonium chloride, Hepes and quinine sulfate were obtained from Sigma (St. Louis, MO).

Electrophysiological measurements. A standard electrophysiological recording system was used for simultaneously monitoring membrane potential and input resistance [9], Glass recording microelectrodes (20–60 MΩ) were filled with 2.5 M KCl solution and connected to a high input-impedance preamplifier with an active bridge circuit (M4A Electrometer, WP Instruments, Hamdem, CT). For divalent cation injections, a second microelectrode pulled in the same conditions and filled with a 0.5 M solution of the appropriate chloride was connected through a 200 MΩ resistor to a voltage source, as previously described [9]. Injected current was recorded through the current monitor of the electrometer, when a single recording electrode was used. When an ion-injecting microelectrode was used, a virtual ground circuit was interpolated between the bath and ground, allowing current measurements.

In experiments where the saline was changed during an impalement, a peristaltic perfusion pump (Minipuls 2, Gilson, Middleton, WI) was employed at a flow-rate of 5 or 1.4 ml/min. Complete renewal of the solution occurred from

1 min. In some experiments, the increase of divalent cation concentration was achieved by direct addition of 0.1 ml of 0.5 M chloride solution during an impalement (final concentration, 10 mM).

Impalements were considered acceptable only when good bridge balance (within 10% of the input resistance of the cell) and return to base line (within 10% of the resting potential) were observed upon withdrawal of the electrode.

Results

Intracellular injection of divalent cation

We have previously demonstrated that intracellular Ca^{2+} injection evokes a slow membrane hyperpolarization in macrophages and macrophage polykaryons [9]. In Fig. 1 we show that iontophoretic injection of Sr^{2+} into the cytoplasm of macrophage polykaryons also produces slow hyperpolarization. Similar results were seen in 51 cells (100%). The amplitude of the slow hyperpolarization increases with the intensity and duration of the stimulus. Injections of similar currents through the KCl microelectrode, on the other hand, never evoked a slow hyperpolarization response. Membrane input resistance reaches a minimum at the peak of the slow hyperpolarization and is smaller for larger hyperpolarizations (Fig. 1B). No significant changes in membrane potential or input resistance are observed after Ba^{2+}, Mg^{2+} or Mn^{2+} injection (Fig. 1C–E).

Both Sr^{2+}- and Ca^{2+}-induced slow hyperpolarizations are similar in their sensitivity to external K^+. The effect of the extracellular K^+ concentration is better observed when a maximal reduction in the input resistance follows stimulation (Fig. 2). In this case, the value of the membrane potential at the peak of the slow hyperpolarization is maximum [9]. It can reach -70 mV in normal K^+ (5.3 mM) and only about -36 mV when K^+ is raised to 30.3 mM (compare Fig. 1A and B with Fig. 2).

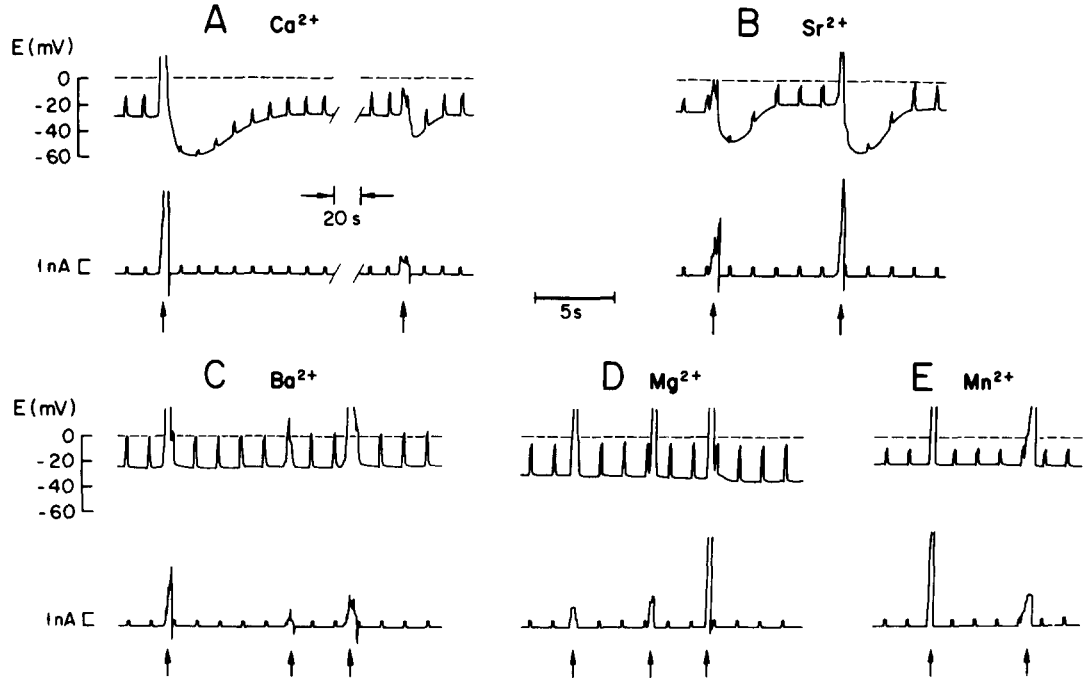


Fig. 1. Iontophoretic injection of divalent cations. Upper trace: voltage recording; lower trace: current monitor. Arrows indicate ion injection through a second microelectrode containing the chloride of the divalent cation. (A) Ca^{2+} and (B) Sr^{2+} injections induce slow hyperpolarization of different amplitudes. (C), (D) and (E) Ba^{2+}, Mg^{2+} and Mn^{2+} injections, respectively.

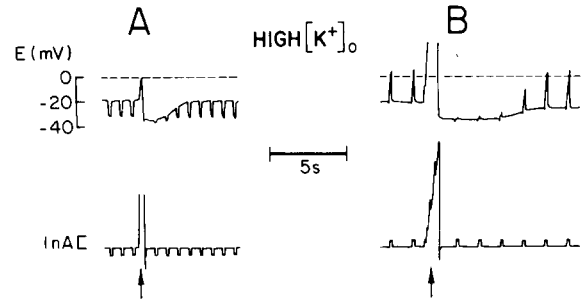


Fig. 2. Potassium dependence of the slow hyperpolarization amplitude. Representative recordings of two different cells in culture medium containing 30.3 mM K^+. Arrows indicate injections of Ca^{2+} (A) or Sr^{2+} (B). Sr^{2+} injection to the same cell in (B) in normal K^+ medium is shown in Fig. 1B.

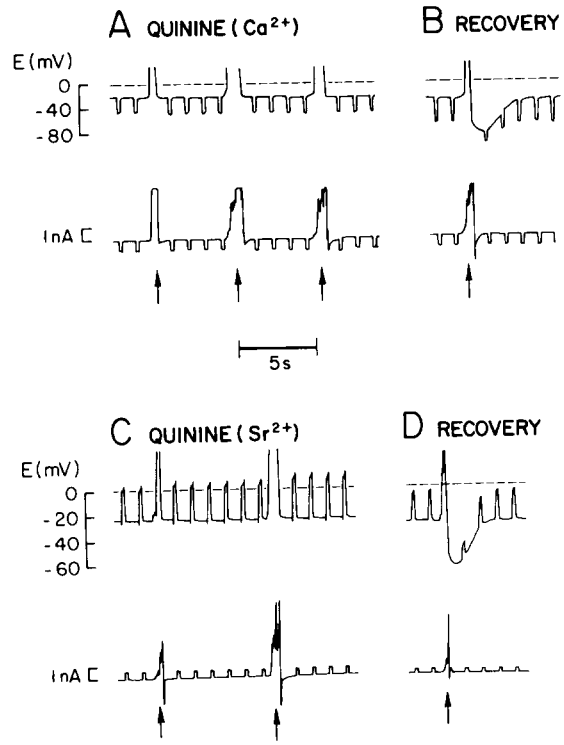


Fig. 3. Effects of channel blockers on slow hyperpolarization. (A) Record from a cell maintained for 20 min in a saline solution containing quinine sulfate (1.5 mM). (B) Record of another cell from the same coverslip, 11 min after removing the quinine (culture medium). (C) Quinine (0.15 mM) also blocks Sr^{2+}-induced slow hyperpolarization (20 min in saline solution). (D) Removal of quinine restores response to injected Sr^{2+} (18 min in culture medium, another cell). Arrows indicate Ca^{2+} injection in (A) and (B), and Sr^{2+} injection in (C) and (D).

Effect of channel blockers on slow hyperpolarization

Fig. 3 illustrates the inhibitory effect of quinine on slow hyperpolarization elicited both by Ca^{2+} and by Sr^{2+} injection. This potent blocker of Ca^{2+}-sensitive K^+ permeability [13,17] is active at concentrations ranging from 1.5 μM to 1.5 mM. Throughout this concentration range, the effect is noted in less than 10 min and is completely reversible after a few minutes of washing.

The application of external Ba^{2+} has a marked and reversible inhibitory effect on the Ca^{2+}-induced slow hyperpolarization (Fig. 4A). When a dose-response analysis is performed, it is found

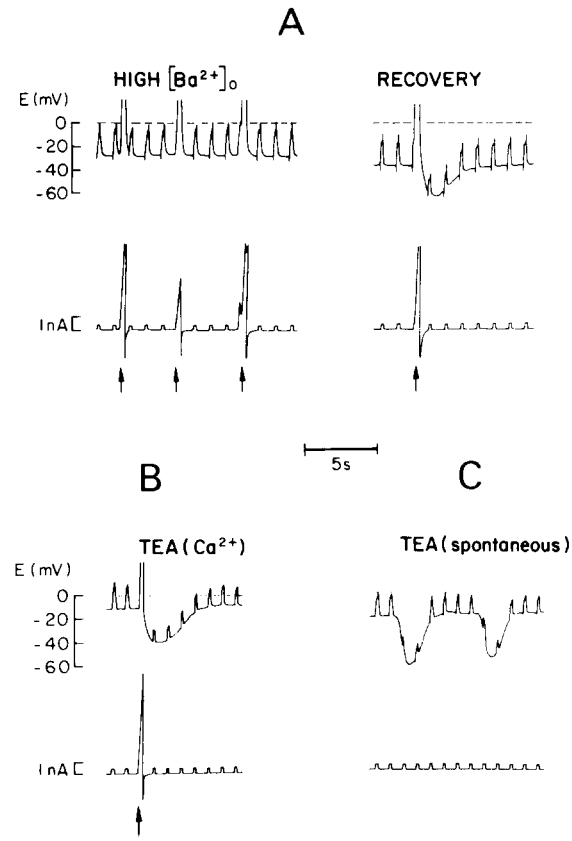


Fig. 4. Effect of external Ba^{2+} and tetraethylammonium chloride (TEA) on the slow hyperpolarization. (A) Left: Ca^{2+}-induced slow hyperpolarization is blocked after 10 min in 20 mM Ba^{2+}. Right: slow hyperpolarization response in the same cell recovers after 15 min in normal medium. (B) tetraethylammonium chloride (50 mM, 10 min in culture medium) does not affect Ca^{2+}-induced slow hyperpolarization. (C) Recording of spontaneous slow hyperpolarization, after 23 min in 50 mM tetraethylammonium chloride (culture medium). Arrows indicate Ca^{2+} injections.

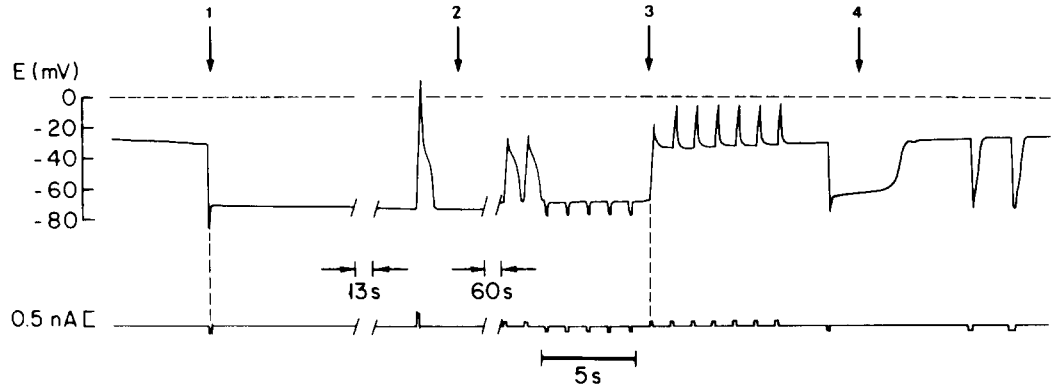
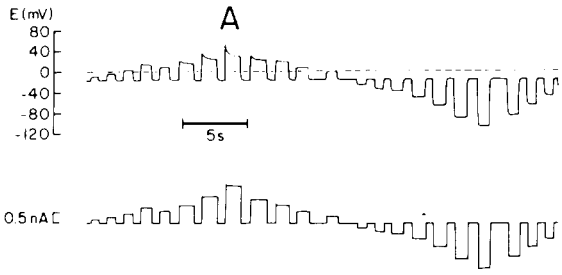


Fig. 5. Transition between two levels of stable potential in normal medium. Arrows 1 and 3 indicate stable transitions induced by current injections. Note the slow repolarization that follows each of the first three inward-going pulses at arrow 2, and the slow repolarization after an outward-going pulse at arrow 4.



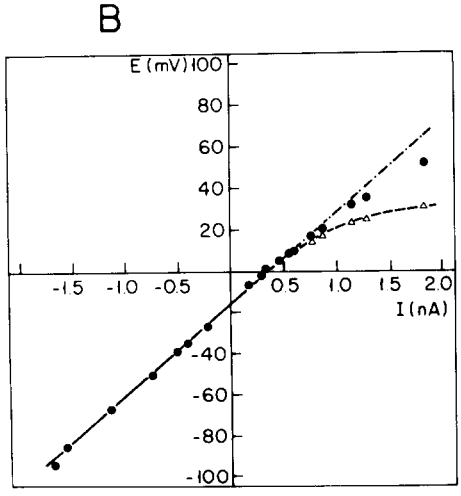
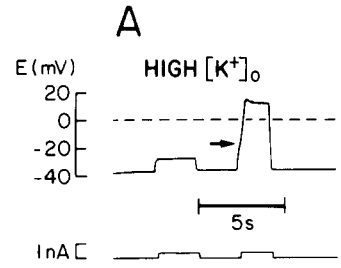


Fig. 6. Current-voltage relationship of macrophage polykaryons in normal medium. (A). Potential changes (upper trace) induced by different current injections (lower trace). Note conductance changes during the inward current pulses. (B) Current-voltage relationship of the cell in (A). Measurements were made at the onset of the pulses (●) and just before (△) the cessation of current injection. The straight line was obtained by linear regression on the negative potentials.



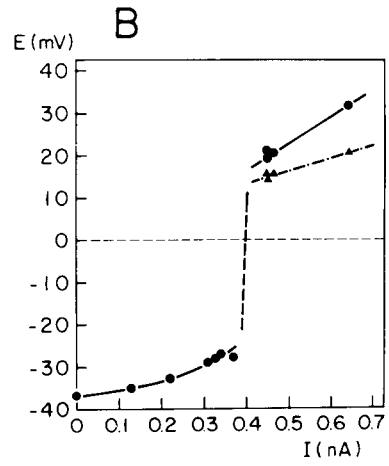


Fig. 7. Current-voltage relationship in high-potassium medium . (A) Record from a cell in culture medium. An increase of 19% in the amplitude of the injected current causes a 440% increase in the peak depolarization. Arrow indicates an inflection point in the upstroke of depolarization. (B) Nonlinear current-voltage relationship of the cell in (A), revealing a discontinuity (dashed line). Voltage measurements were taken at the onset (●) and the cessation (▲) of current pulses.

that at 20 mM Ba^{2+}, 100% of the cells fail to respond to the Ca^{2+} injection and at 4 mM Ba^{2+}, only some cases of partial inhibition are seen. Tetraethylammonium chloride, another K^+ permeability blocker, was ineffective on either Ca^{2+}-induced or spontaneous slow hyperpolarization in concentrations up to 100 mM (Fig. 4B and C). Maximum exposure time to tetraethylammonium chloride was 60 min.

Two levels of membrane potential

Fig. 5 illustrates the shifts between two stable levels of membrane potential in a cell maintained in normal medium. In this cell, both hyperpolarizing and depolarizing current pulses (arrows) caused a 40 mV shift in membrane potential, and slow conductance activations were induced by pulses of different amplitudes and durations applied in both directions. This record shows also a spontaneous shift from the high to the low level of membrane potential (after arrow 4). Hyperpolarized cells with excitable-like characteristics may occur either in normal medium or in the presence of a high divalent cation concentration (as will be shown later). Similar findings have been described for mononuclear macrophages in culture medium containing normal Ca^{2+} concentration [18,19]. In the case of macrophage polykaryons, 11% of the cells studied in normal culture medium had membrane potentials between -40 and -75 mV. In the high-K^+ medium, values greater than -40 mV have never been found.

Current-voltage characteristics

Current-voltage relationships of the macrophage polykaryon membrane are shown in the next two figures. Fig. 6 represents a cell with low membrane potential and a linear response to current injection from -100 to $+5$ mV. Fig. 7 illustrates the occurrence of nonlinear voltage-current characteristics in high-K^+ medium. In these cases, the cells are not hyperpolarized, but it is still possible to observe nonlinear curves with a sharp discontinuity.

Effects of externally added divalent cations

Addition of Ca^{2+}, Sr^{2+} or Mn^{2+} to normal saline induces a rapid and sustained increase in membrane potential, whereas Mg^{2+} and Ba^{2+} have no effect (Fig. 8). Membrane potential is strongly dependent on extracellular divalent cation concentration, as shown in Table I. In normal saline ($[Ca^{2+}] = 1$ mM), the mean membrane potential is -22 mV. If this mean plus two standard deviations is taken as the criterion for a hyperpolarized cell, we find that 5% of the cells in control saline are hyperpolarized (membrane potential greater than -40 mV). The mean membrane potential increases to -55 mV in high-Ca^{2+} and high-Sr^{2+} salines. In these situations, 75% of the cells are hyperpolarized, according to our criteria. In high-Mn^{2+} saline, the mean membrane potential is -79 mV and again 75% of the cells have membrane potentials greater than -40 mV. It is noteworthy that in the presence of Mn^{2+} this parameter may reach -132 mV. As already shown for the slow hyperpolarization responses, the action of external Ca^{2+} or Sr^{2+} can also be blocked by quinine

TABLE I

EFFECT OF DIVALENT CATIONS AND QUININE ON MEMBRANE POTENTIAL

Membrane potentials were measured in normal saline (control) and in salines containing the indicated divalent cation concentrations, in the presence or absence of 1.5 mM quinine sulfate. Values are expressed as means ± S.D. (number of cells). Figures in square brackets indicate minimum and maximum values.

Added cation	Concn. (mM)	Membrane potential ($-$mV)	
		no quinine	quinine (1.5 mM)
Ca^{2+}	1	22 ± 9 (49) [6, 62]	16 ± 4 (13) [10, 20]
	5	49 ± 14 (14) * [26, 70]	17 ± 7 (6) [10, 30]
	20	55 ± 12 (14) * [28, 70]	23 ± 7 (6) [16, 36]
Sr^{2+}	5	44 ± 17 (17) * [20, 80]	17 ± 2 (15) [14, 20]
	20	55 ± 15 (8) * [32, 72]	–
Mn^{2+}	5	49 ± 23 (47) * [22, 116]	48 ± 32 (18) * [16, 100]
	20	79 ± 35 (29) * [24, 132]	–

* Significantly different from control ($P < 0.05$), according to Student's t-test.

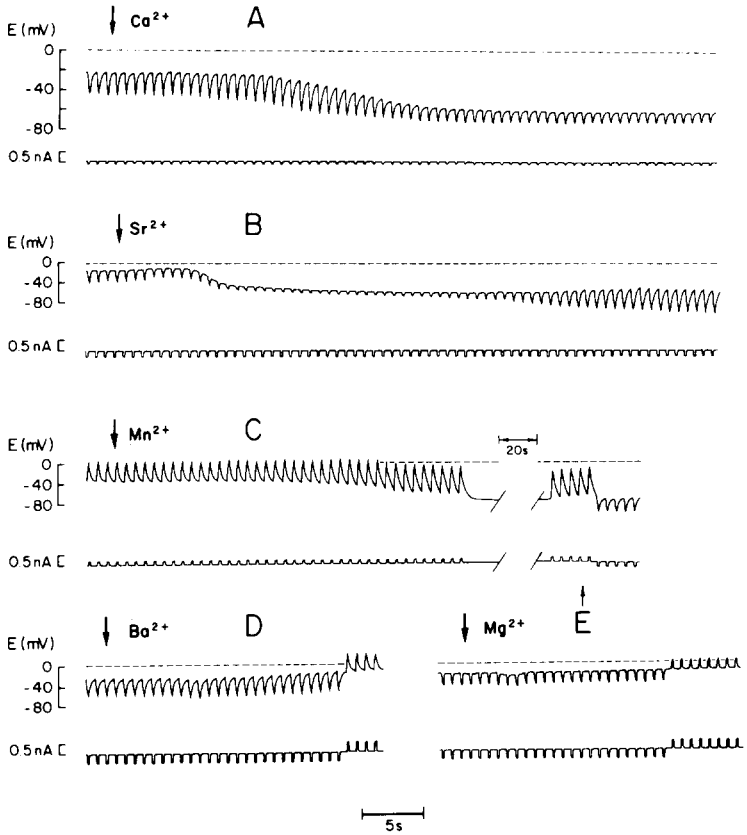


Fig. 8. Effects of divalent cations in the bathing medium. Records from cells perfused with salines containing 20 mM divalent cation. Arrows indicate the arrival of this solution in the chamber. Hyperpolarization is seen in the presence of Ca^{2+} (A, from -24 to -60 mV), Sr^{2+} (B, from -20 to -60 mV) and Mn^{2+} (C, from -36 to -76 mV). In the case of Ba^{2+} (D) and Mg^{2+} (E), no hyperpolarization is observed.

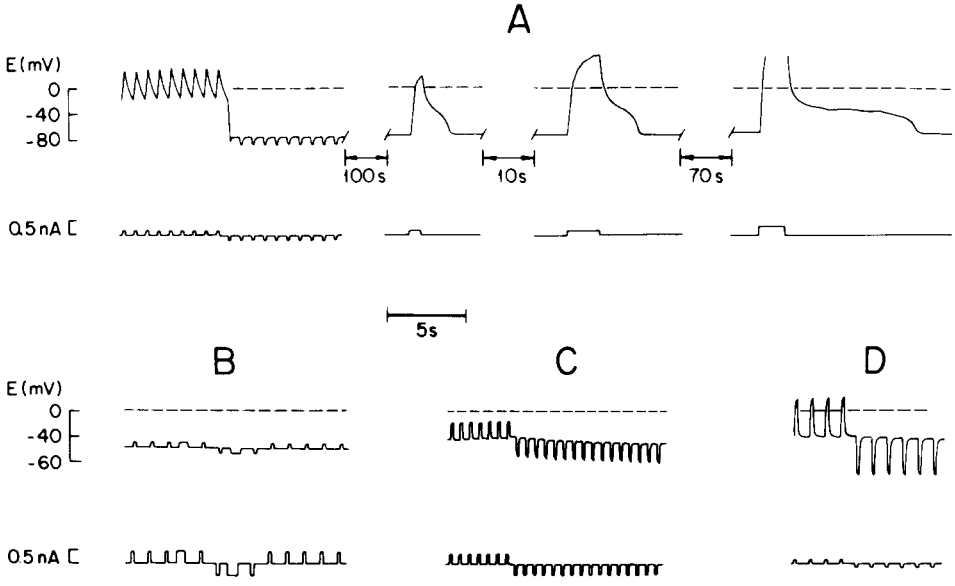


Fig. 9. Current-induced responses in the presence of Ca^{2+} and Sr^{2+}. (A and C) Patterns of response to current injection in high-Ca^{2+} saline (20 mM). Note in (A) the shift of transmembrane potential from -16 to -76 mV and a rectifying behavior. (B) Hyperpolarized cell with ohmic responses in 20 mM Sr^{2+}. (D) Another cell in 5 mM Sr^{2+}.

(Table I). However, Mn^{2+}-treated cells undergo a distinctive effect, since a large hyperpolarization insensitive to blockade by quinine is observed (Table I). Persistent membrane hyperpolarization induced by high extracellular concentrations of either Ca^{2+} or Sr^{2+} is dependent on the transmembrane K^+ gradient. The amplitude of Ca^{2+}-induced membrane hyperpolarization in high-K^+ medium never exceeded -40 mV, as compared to -70 mV in normal K^+ medium.

The addition of high Ca^{2+} or Sr^{2+} salines induces a variable pattern of responses to injected current, as shown in Fig. 9. In many cases, a rectifying effect is observed, since potential changes induced by inward current are greater than those observed for similar outward currents. A shift between two levels of membrane potential, an increase of the time constant and an active depolarization may also occur (Fig. 9A). The most frequent pattern found in hyperpolarized cells exposed to high Ca^{2+} or Sr^{2+} is shown in Fig. 9B, where both inward and outward currents induced time-invariant voltage responses and the cells had relatively low input resistance (68% of the hyperpolarized cells). Non-hyperpolarized cells that exhibit ohmic behavior are also frequent (25%) in the presence of either high Ca^{2+} (Fig. 9C) or Sr^{2+} (Fig. 9D).

The presence of Mn^{2+} usually induces changes in the voltage-current characteristics. Current-induced active potential changes that depend on amplitude and duration of the stimulus may be

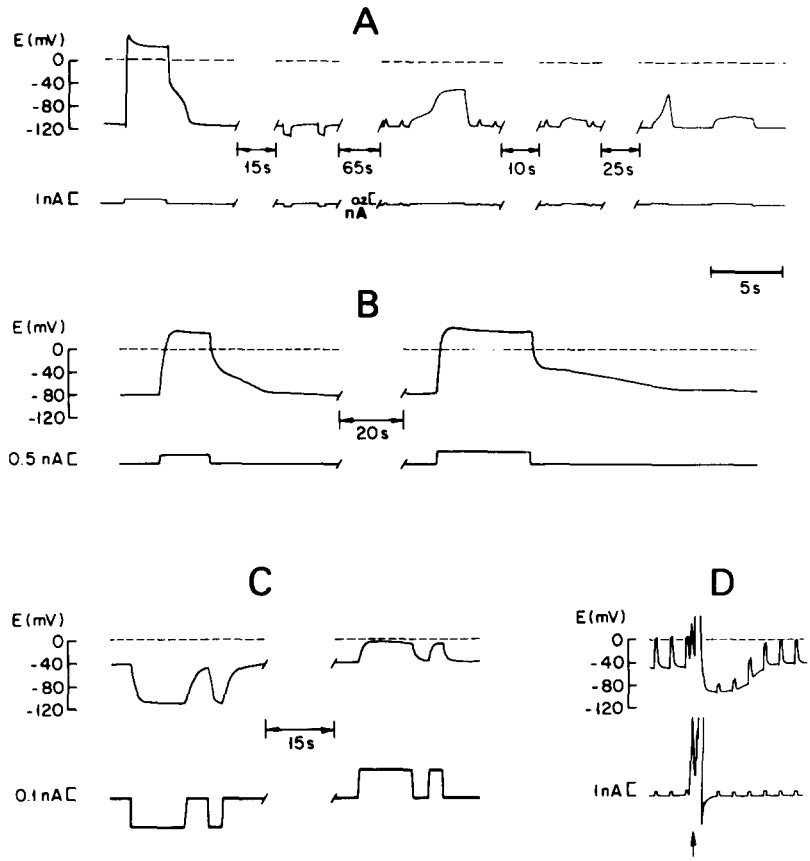


Fig. 10. Current-induced responses and Ca^{2+} injection in the presence of Mn^{2+}. (A) A hyperpolarized cell (-116 mV) exhibiting a rectifying behavior and slow conductance variations induced by inward current pulses. (B) Another hyperpolarized cell (-80 mV, 10 mM Mn^{2+}) showing slow voltage variations at the termination of current pulses. (C) A cell exhibiting rectification in 5 mM Mn^{2+} (membrane potential of -40 mV). (D) Slow hyperpolarization induced by Ca^{2+} injection after 20 min in the presence of 10 mM Mn^{2+}.

observed (Fig. 10). Rectification is seen in the first two panels of Fig. 10A and also in a less hyperpolarized Mn^{2+}-treated cell (Fig. 10C). In contrast to experiments with Sr^{2+} and Ca^{2+} (Fig. 8A, B and 9B), a decrease in the input resistance during Mn^{2+}-induced hyperpolarization was never observed.

It is interesting to note that a concentration of Mn^{2+} which is sufficient to hyperpolarize the cells has no effect on the Ca^{2+}-induced slow hyperpolarization (Fig. 10D). This observation suggests that different mechanisms are involved in the two situations.

Discussion

The similarities between the polykaryons and their mononuclear macrophage precursors are extensive: (a) macrophage polykaryons fused in vitro present Ia antigens in their membranes [20]; (b) the phagocytosis of different opsonized and non-opsonized particles [7]; (c) the formation of phagolysosomes [8]; (d) the release of lysosomal enzymes [5]; (e) the locomotory behavior [6]; and (f) the electrophysiological characteristics of macrophages previously described [9,11,12,21] and recently reviewed [22] correlate well with the findings for the larger polykaryon model [9,23].

The experiments described here provide evidence about the selectivity of the triggering mechanism of a divalent cation-dependent K^+ channel in a phagocytic membrane model. We show here that Sr^{2+} substitutes for Ca^{2+} in inducing the activation of the K^+ channel. The following evidence supports this hypothesis: (a) each cation injection invariably triggers one slow hyperpolarization; (b) slow hyperpolarization time-course is similar whether Ca^{2+} or Sr^{2+} is injected; (c) slow hyperpolarization amplitude increases with total charge injected; (d) slow hyperpolarization amplitude shows a similar K^+ dependence to that previously described in a model where K^+ conductance changes are the main events underlying the slow potential variations [9,23]; (e) the quinine sensitivity described here is very similar for the case of both alkaline-earth cations. Quinine blockade is considered a strong indication that we are dealing with a relatively ubiquitous Ca^{2+}-activated K^+ channel (for reviews see Refs. 13, 17, 24, 25, and the volume containing Ref. 22).

The similarity of the effects of Ca^{2+} and Sr^{2+} is also seen when the cations are applied to the external fluid. In this case, the main event also seems to be activation of a K^+ permeability, since: (a) persistent membrane hyperpolarization can be induced in the presence of increasing divalent ion concentrations (Table I); (b) both cations can cause a decrease in input resistance; (c) quinine blocks both effects of external cations; (d) K^+ modulation is observed in the presence of high external Ca^{2+} concentrations.

The effects of Ca^{2+} and Sr^{2+} in the induction of the K^+ channel activation are similar to those described for L-cells [26] and in red blood cells [27]. A possible mechanism for the external effects of Ca^{2+} and Sr^{2+} is the intracellular activation of the K^+ channel causing a hyperpolarization and a reduction of input resistance.

The finding that tetraethylammonium chloride does not block Ca^{2+}-induced or spontaneous slow hyperpolarization differs from our previous report in macrophages [11]. One possible explanation may be that macrophage membranes are more permeable to tetraethylammonium chloride, which may act only on the intracellular side of the membrane, as described for other cells [17,28].

Ba^{2+} is a blocker of voltage-dependent K^+ channels in axons [29], muscles [30] and macrophages [29]. In macrophage polykaryons, we have shown that 20 mM Ba^{2+} reversibly blocks the Ca^{2+}-induced slow hyperpolarization responses. However, at a concentration of 4 mM, the majority of the cells were insensitive to Ba^{2+}. This could indicate competition of Ba^{2+} for the site of Ca^{2+} action, as proposed for pancreatic β-cells [31].

Mn^{2+}-induced effects differ from those caused by Ca^{2+} or Sr^{2+}. These cations cause a hyperpolarization when present in the extracellular medium, but Mn^{2+}-induced hyperpolarization is quinine-insensitive. Unlike Ca^{2+} and Sr^{2+}, Mn^{2+} does not elicit slow hyperpolarization responses when iontophoretically injected into the cytoplasm. These findings differ from those described for L-cells [26]. We have observed that the Mn^{2+}-induced hyperpolarization is K^+-dependent, since increasing external K^+ causes a decrease in membrane potential (data not shown). In the presence of Mn^{2+}, the cells respond normally to calcium

injections, indicating that the mechanism involved in slow hyperpolarization is not influenced by Mn^{2+} itself. However, Mn^{2+} can hyperpolarize the cells to amplitudes of the order of -130 mV (Table I), posing another question. This transmembrane potential is about 50 mV more negative than the maximum value found in all other situations. One of the possible effects of Mn^{2+} may be an increase in the intracellular K^+ concentration. This seems to occur, since slow hyperpolarization peak amplitude, which can be used as an indication of the K^+ equilibrium potential [9], is also signfiicantly increased. It can reach values higher than -90 mV in the presence of Mn^{2+} (Fig. 10D), while in normal medium it never exceeds -67 mV [9].

An interesting effect of Mn^{2+} is that it induces excitability in response to currents that usually cause ohmic voltage variations. These active responses displayed by hyperpolarized cells are not confined to cells in Mn^{2+} solutions; they occur in less than 10% of the cells in normal medium and in the presence of high Ca^{2+}. In macrophages, a nonlinear voltage-current relationship has been related to the existence of a voltage-dependent, Ba^{2+}-sensitive, K^+ channel [32], and a delayed outward-rectifying K^+ conductance has been demonstrated with the use of the patch-clamp technique [33]. The occurrence of two stable levels of membrane potential has also been described in heart cells [34]. Similar mechanisms may occur in the Mn^{2+}-induced behavior of the membrane of the macrophage polykaryons.

The evidence available does not enable a description of the pathway by which Mn^{2+} can induce such membrane properties. A possible explanation is that Mn^{2+}, and to a lesser degree Ca^{2+} and Sr^{2+}, may somehow stabilize the cell membrane and reduce leakage induced by incomplete sealing at the site of microelectrode penetration. We do not have direct measurements of this sealing, but it seems that leakage is present in these cells. Macrophages depolarize as a consequence of microelectrode penetration [35,36], and we have evidence indicating that a considerable depolarization due to leakage currents may also occur in the case of polykaryons: (a) microelectrode penetration is followed by a rapidly decaying negative potential (10–100 ms) with an amplitude that may reach -84 mV [23]; (b) this peak potential is usually greater than the more stable transmembrane potential that follows and its value is K^+-dependent, reaching a maximum of only -43 mV in high K^+ [23]; (c) impalement by a second microelectrode may induce depolarization and membrane resistance decrease, which can be reversed by electrode withdrawal [23]; (d) the beginning of the record rarely reveals hyperpolarized cells immediately after electrode penetration. The usual pattern is an increase in membrane potential during the initial 5–60 s after impalement.

Gross leakage can be discarded here, because even the non-hyperpolarized cells exhibit characteristics of a healthy cell, such as: (a) impaled macrophages are viable, since they exclude dyes used to indicate damage [37]; (b) impaled cells retain their phagocytic capacity [37]; (c) fluorescein injected into macrophages is retained by the cytoplasm of previously impaled cells for about 15 min [21]; (d) stable levels of potential and input resistance can be maintained for considerable lengths of time [9,22,23,37]; (e) some cells exhibit spontaneous slow hyperpolarization for more than 2 min even when impaled by two electrodes [23]; (f) intracellular K^+ activity, measured with ion-sensitive electrodes, is maintained at constant levels in impaled cells [23]. These arguments suggest that leakage currents are small and are not accompanied by severe cell damage.

The use of Mn^{2+} in the bathing solution may be a way to circumvent the small leakage currents. However, since the Mn^{2+} induction of nonlinear voltage-current relationships is more effective than induction by Ca^{2+} or Sr^{2+}, and since Mn^{2+} induces levels of transmembrane potential which are frequently higher than the expected equilibrium potential for K^+ in normal conditions [9], it seems that besides damage prevention other effects of Mn^{2+} on the polykaryon membrane are likely to exist. Two possibilities that cannot be excluded in analyzing divalent cation effects are: (a) the contribution of electrogenic pumps such as the $(Na^+ + K^+)$-ATPase, already demonstrated for the case of macrophages [38] and macrophage polykaryons [23], and (b) modifications of membrane characteristics induced by the screening of negative surface charges [39,40].

The great variability of the electrical signals

described in this paper derives from the variety of complex ion permeability changes occurring in phagocytic cells. One example of this heterogeneity is the occurrence of action potentials described in macrophages as a response to injected current [19]. In the case of macrophage polykaryons, we have not observed typical spike activity, even in the high-Mn^{2+} saline. The physiological meaning and ionic characteristics of the electrophysiological properties should be further investigated. Improvements in monitoring techniques could provide a higher resolution picture of the phagocytic membranes. Our data suggest that Mn^{2+} may be a useful tool in such studies.

Acknowledgements

E.G.A. is a Fellow, and P.M.P. and G.M.O.-C. are Investigators of the National Research Council (CNPq). This work was supported by grants from the CNPq, the National Found for Development of Science and Technology (FINEP-FNDCT), and the Council for Graduate Education, Federal University of Rio de janeiro (CEPG-UFRJ). We wish to thank Martha Sorenson and George A. Dos Reis for discussions and suggestions, Laura, E.P. Freitas for assistance during some experiments, and Tânia Lúcia T. Ventura for typing the manuscript.

References

1 Papadimitriou, J.M. and Spector, W.G. (1971) J. Pathol. 105, 187–203
2 Mariano, M. and Spector, W.G. (1974) J. Pathol. 113, 1–19
3 Papadimitriou, J.M. (1979) J. Pathol. 128, 93–97
4 Mariano, M., Nikitin, T. and Malucelli, B.E. (1976) J. Pathol. 120, 151–159
5 Papadimitriou, J.M. and Wee, S.H. (1976) J. Pathol. 120, 193–199
6 Papadimitriou, J.M. and Kingston, K.J. (1977) J. Pathol. 121, 27–36
7 Felipe, I. and Oliveira-Castro, G.M. (1982) Ann. Acad. Bras. Cien. 54, 262
8 Persechini, P.M., Vale, M.A.B., Machado, R.D. and Oliveira-Castro, G.M. (1983) Braz. J. Med. Biol. Res. 16, 435
9 Persechini, P.M., Araujo, E.G. and Oliveira-Castro, G.M. (1981) J. Membrane Biol. 61, 81–90
10 Gallin, E.K., Wiederhold, M.L., Lipsky, P.E. and Rosenthal, A.S. (1975) J. Cell. Physiol. 86, 653–662
11 Dos Reis, G.A. and Oliveira-Castro, G.M. (1977) Biochim. Biophys. Acta 469, 257–263
12 Oliveira-Castro, G.M. and Dos Reis, G.A. (1981) Biochim. Biophys. Acta 640, 500–511
13 Lew, V. and Ferreira, H.G. (1978) Curr. Top. Membrane Transp. 10, 217–277
14 Gardos, G. (1958) Biochim. Biophys. Acta 30, 653–654
15 Nelson, P.G., Peacock, J. and Minna, J. (1972) J. Gen. Physiol. 60, 58–71
16 Okada, Y., Doida, Y., Roy, G., Tsuchiya, W., Inouye, K. and Inouye, A. (1977) J. Membrane Biol. 35, 319–335
17 Schwarz, W. and Passow, H. (1983) Annu. Rev. Physiol. 45, 359–374
18 Gallin, E.K. and Livengood, D.R. (1980) J. Cell. Biol. 85, 160–165
19 McCann, F.V., Cole, J.J., Guyre, P.M. and Russell, J.A.G. (1983) Science 219, 991–993
20 Schlesinger, L., Musson, R.A. and Johnston, R.B., Jr. (1984) J. Exp. Med. 159, 1289–1294
21 Dos Reis, G.A., Persechini, P.M., Ribeiro, J.M.C. and Oliveira-Castro, G.M. (1979) Biochim. Biophys. Acta 552, 331–340
22 Oliveira-Castro, G.M. (1983) Cell Calcium 4, 475–492
23 Persechini, P.M. (1984) PhD Thesis, pp. 1–190, Instituto de Biofísica da Universidade Federal do Rio de Janeiro
24 Meech, R.W. (1976) in Calcium in Biological Systems (Duncan, C.J., ed.), pp. 161–191, Cambridge University Press, Cambridge
25 Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696
26 Okada, Y., Tsuchiya, W. and Inouye, A. (1979) J. Membrane Biol. 47, 357–376
27 Simons, T.J.B. (1976) J. Physiol. 256, 227–244
28 Hille, B. (1967) J. Gen. Physiol. 50, 1287–1302
29 Armstrong, C.M. and Taylor, S.R. (1980) Biophys. J. 30, 473–488
30 Sperelakis, N., Schneider, M.F. and Harris, E.S. (1967) J. Gen. Physiol. 50, 1665–1583
31 Atwater, I., Rosario, L. and Rojas, E. (1983) Cell Calcium 4, 451–461
32 Gallin, E.K. and Livengood, D.R. (1981) Am. J. Physiol. 241, C9–C17
33 Ypey, D.L. and Clapham, D.E. (1984) Proc. Natl. Acad. Sci. USA 81, 3083–3088
34 Gadsby, D.C. and Cranefield, P.F. (1977) J. Gen. Physiol. 70, 725–746
35 Ince, C., Ypey, D.L., Van Furth, R. and Verveen, A.A. (1983) J. Cell. Biol. 96, 796–801
36 Ince, C., Leijh, P.C.J., Meyer, J., Van Bavel, E. and Ypey, D.L. (1984) J. Physiol. 352, 625–636
37 Gallin, E.K. (1982) in Phagocytosis – Past and Future (Karnovsky, M. and Bolis, L., eds.), pp. 29–46, Academic Press, New York
38 Gallin, E.K. and Livengood, D.R. (1983) Am. J. Physiol. 245, C184–C188
39 Frankenhaeuser, B. and Hodgkin, A.L. (1957) J. Physiol. 137, 218–244
40 Hille, B. (1968) J. Gen. Physiol. 51, 221–236