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Electrophysiology of phagocytic membranes: intracellular K^+ activity and K^+ equilibrium potential in macrophage polykaryons *

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The role of K^+ as current carrier during the slow membrane hyperpolarizations (SH) elicited by iontophoretic Ca^{2+} injections into macrophage polykaryons is studied. The intracellular K^+ activity (a_K) and the K^+ equilibrium potential (E_K) are measured using ion-sensitive microelectrodes. The mean value of a_K is 84 ± 5 mM in a culture medium containing 5.3 mM K^+, but increases to 100 ± 8 mM when the extracellular K^+ concentration is raised to 30.3 mM. Under the same conditions the values of E_K obtained from the Nernst equation are -81 ± 2 mV and -40 ± 2 mV, respectively. The reversal potentials (E_R) of the SH are calculated from changes observed in transmembrane potential and input resistance, according to an equivalent model based only on passive ionic fluxes. The mean E_R values obtained are -74 ± 8 mV in the presence of low K^+ concentration and -37 ± 3 mV for the high K^+ medium. These values are significantly smaller than the estimated E_K for the corresponding situations. Evidence for the existence of an electogenic ($Na^+ + K^+$)-ATPase activity is also presented. The evidence indicates that an increase in the membrane potassium permeability can account for about 90% of the total permeability change occurring during the SH.

Introduction

The study of the electrophysiological properties of macrophages has demonstrated potential and conductance changes due to both voltage dependence and to Ca^{2+}-dependent permeabilities [1–5]. The recent development of the patch-clamp technique [6] has made it possible to describe a high-conductance (105–130 pS) Ca^{2+} and voltage-dependent channel and a low-conductance (16–26 pS) voltage-dependent K^+ channel [5,7,8]. These channels are probably involved in the slow hyperpolarizing oscillations (SH) and other membrane properties that have been described using classical intracellular recording techniques [1–3,9–11].

Macrophage polykaryons of the foreign body granuloma also display SH due to a Ca^{2+}-dependent K^+-conductance increase [11]. The SH can be induced by intracellular Ca^{2+} or Sr^{2+} injection and are reversibly blocked by quinine [12]. Although the Ca^{2+}-dependent K^+-conductance change has been well characterized for macrophages and macrophage polykaryons, little is known about the selectivity of the channel and the intracellular activity of K^+ (a_K). In a previous study a model was proposed which made it possible to correlate the K^+ equilibrium potential (E_K) with conductance and potential changes during a SH [11]. At that time it was not possible to determine whether deviations from the Nernst

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equation prediction reflected non ideal K^+ selectivity of the channel or high potassium-induced increase of the intracellular K^+ activity.

In this paper we present direct measurements of intracellular K^+ activities that allow us to estimate the true K^+ equilibrium potential (E_K) under various experimental conditions. When this information is applied to an analysis of the reversal potential (E_R) (see Appendix), it becomes necessary to take into account other charge carriers in addition to K^+ that are involved in the SH response. The existence of an electrogenic ($Na^+ + K^+$)-ATPase system in the membrane of macrophage polykaryons is also inferred. The data allow improvements in the previously proposed electrical equivalent circuit model for SH responses [11].

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Materials and Methods

Cells. Macrophage polykaryons were formed on the surface of round glass coverslips (6 mm diameter) kept from 4 to 60 days in the peritoneal cavity of outbred albino or AKR and C_3H mice strains [11]. The coverslips were removed and washed in culture medium (RPMI-1640, Gibco, Grand Island, NY), containing 5% (v/v) fetal calf serum and buffered with 6 mM Hepes (4-2-hydroxyethyl)-1-piperazine-ethanesulfonic acid). Cells were maintained in this medium at 37 °C for at least 30 min before electrophysiological measurements.

Solutions. Normal medium had a potassium concentration ($[K^+]$) of 5.3 mM. High-K^+ medium (30.3 mM) was obtained by the addition of concentrated KCl solution. The K^+-free saline contained 145 mM NaCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$ and 6 mM Hepes, and the high-K^+ (30 mM) saline was obtained by isosmotic substitution of KCl for NaCl. All measurements were made either in culture medium or in saline solutions at pH 7.2–7.4 and at temperatures ranging from 35 to 37 °C. Ouabain (Sigma) was dissolved immediately before use.

Electrophysiological measurements. A standard electrophysiological recording system was used for monitoring membrane potential and input resistance simultaneously [11]. Glass recording microelectrodes (20–60 MΩ) containing a glass fiber were back-filled with 2.5 M KCl solution and connected to a high-input- impedance preamplifier (M4A Electrometer, WP Instruments, Hamden, Co.). For Ca^{2+} injections, a second microelectrode filled with 0.5 M $CaCl_2$ and connected through a 200 MΩ resistor to a voltage source was used [11]. In the single-electrode experiments, current was recorded through the current monitor of the electrometer. When a Ca^{2+}-injecting microelectrode was present, a virtual ground circuit interposed between the bath and ground allowed current measurements. Transmembrane potentials (E_o) ranged from −10 mV to −76 mV, but most of the data presented here are from cells with E_o smaller than −40 mV, which corresponds to 90% of the impalements.

Electrical recordings included in this study met the following criteria: (a) a positive pre-potential of less than 3 mV (obtained with the microelectrode at the cell surface, corresponding to tip potentials below 10 mV (13,14]; (b) the total baseline change during the impalement did not exceed 2 mV; (c) the microelectrode resistance change after withdrawl from the cell was less than 5% of the smallest cell input resistance recorded during the measurement.

Determination of E_R. The reversal potential of the SH was calculated from Eqn. 3 (Results). In order to minimize cumulative errors, potential and resistance values were accepted only when: (a) the membrane potential during the current pulses used to measure input resistance was in the linear range of the I–V relationship (−80 to 10 mV); (b) resting membrane potential measurements performed immediately before and after the SH did not exceed 1 mV; (c) changes in the resting membrane resistance (ΔR_o) measured as in (b) were below 5%; (d) the resolution limit for data from the strip-chart recorder corresponded to 5 mV or 10 MΩ.

Determination of a_K. The intracellular K^+ activity was calculated from the difference between the potential detected by an ion-sensitive microelectrode (E_S) and the transmembrane potential measured simultaneously by a conventional microelectrode (E_M) [15,16]. The liquid ion exchanger Corning No. 477317 (a kind gift of Dr. E. Garcia Filho) was used as the ion-sensitive element.

The variables were estimated from Eqns. 1 and 2 [15–18]:

$$V_K = S \log(a_K) + S_o \quad (1)$$

where $V_K = E_S - E_M$ and S and S_o were adjusted to fit the calibration curve (see below). Micropipettes were pulled in the conventional way and heated for 2 h at 100 °C after exposing their interior to dimethyldichlorosilane vapour. Tips were then back-filled with the liquid ion exchanger and connected to an amplifier of 10^{15} Ω impedance through a 1 M KCl solution and by means of an Ag/AgCl connection. Calibrations were performed in solutions containing 100 mM NaCl and KCl concentrtions ranging from 50 to 200 mM. The values of S and S_o in Eqn. 1 were then determined by linear correlation. The activity coefficient for potassium (γ_K) was obtained from the following relation [19–21]:

$$\log \gamma_K = \frac{-0.509\sqrt{I}}{1 + B\sqrt{I}} + CI \quad (2)$$

where I is the ionic strength in mol per liter, $B = 1.2638$ and $C = 0.0078$. The value of γ_K was 0.75 for the normal medium and 0.74 for the high-K^+ medium. All ion-sensitive microelectrodes selected for use had an S value ranging from −55 to −65 mV and a selectivity greater than 30 for K^+ over Na^+. They were calibrated before and after a series of 1 to 15 impalements. Measurements that satisfied the following criteria were accepted: (a) the V_K values recorded from cells in culture medium did not vary by more than 5% throughout a series of measurements; (b) calibration curves were linear and correlation coefficients were 0.995 or better; (c) the responses to K^+ activity changes occurred with $t_{1/2}$ smaller than 1 s; (d) localization of both microelectrodes within the same intracellular compartment was ascertained by the invariability of V_K values during current-induced membrane potential changes (see Fig. 1).

Results

Intracellular activity and equilibrium potential of potassium

The value of the intracellular potassium activity (a_K) was obtained from the potential difference (V_K) between the outputs of the ion-sensitive microelectrode and the conventional KCl microelectrode (Eqn. 1). Typical examples are shown in Figs. 1A and B, where the values of a_K in normal- and high-K^+ medium were, respectively, 78 mM and 97 mM. It can also be seen from this figure that V_k is stable (except for the transient) during a DC current pulse, indicating that both microelectrodes detect the same membrane potential and are correctly located in the cytosol. An increase in a_K in response to an increase in extracellular K^+ was observed in all the cells studied. The observation that a_K values remained constant throughout the measurement indicates that although an input resistances decrease may sometimes occur, no measurable potassium leakage was found after the introduction of a second microelectrode (Fig. 1B). In fact, a marked decrease in a_K can only be observed in the case of intentional and severe lesion of cell membrane, as shown in Fig. 1C. The efflux of K^+ that occurs during SH is also not sufficient to induce a measurable decrease in a_K, as shown in Fig. 1B. The mean values of a_K and E_K in normal- and high-K^+ medium are shown in Table I.

A model for the reversal potential of the SH

The reduction of the input resistance that occurs during the SH has been ascribed to a K^+ conductance increase [1,2,11] but other charge carriers contributing to these potential oscillations can not be excluded. We therefore propose a

TABLE I

EFFECT OF EXTERNAL K^+ ON THE VALUES OF VARIABLES

Data show the mean ± S.D. (number of cells). Measurements of membrane potential and resistance for the E_R calculation (Eqn. 3) were taken near the minima of the SH. E_K values were calculated from Nernst Equation, using the a_K values estimated for each cell. E_R values given are significantly smaller than the corresponding E_K values ($P < 0.05$ according to 'Student's t-test).

	Normal medium	High-K^+ medium
a_K (mM)	84 ± 5 (29)	100 ± 8 (22)
E_K (mV)	−81 ± 2 (29)	−40 ± 2 (22)
E_R (mV)	−74 ± 8 (23)	−37 ± 3 (9)

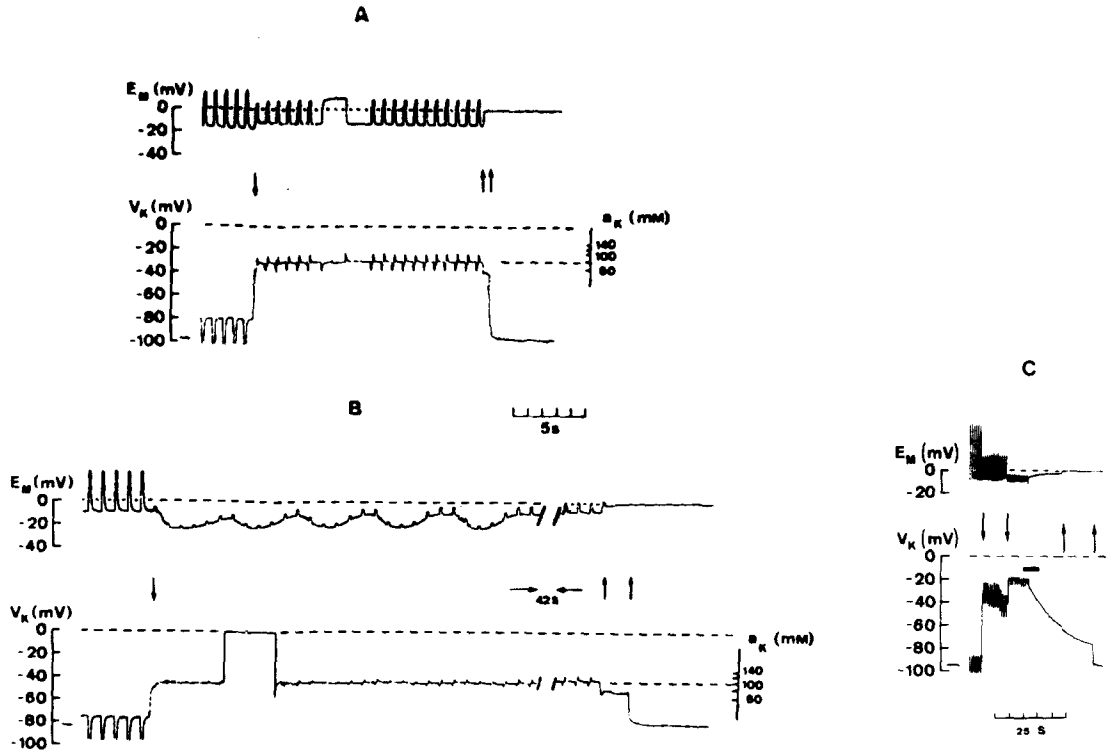

Fig. 1. Determination of intracellular K^+ activity. Upper traces: membrane potential. Lower traces: intracellular activity of K^+. Downward arrows (↓) indicate penetration by the ion-sensitive microelectrode and upward arrows (↑) indicate withdrawal of the potential and of the ion-sensitive microelectrodes. Current pulses (0.5 nA) are not shown. (A) Normal medium; $a_K = 78$ mM. (B) High-K^+ medium; $a_K = 97$ mM. Note the stability of the a_K recording during a spontaneous SH. (C) Membrane lesion induced by mechanical vibration in normal medium (horizontal bar indicates the vibration). The $V_K = 0$ interval in (B) marks the electrical ground.

simple model for the calculation of the reversal potential of the SH (E_R), which will enable us to compare E_K and E_R, obtaining information about the K^+ selectivity of the conductance change. This model is an extension of the one we have previously proposed [11] and its derivation is in the Appendix. In brief, we suppose that typical SH (as shown in Fig. 2A) are due mainly to an incrase in an ohmic K^+ conductance but that the conductance to an ion X may also change. The model cannot distinguish whether the ion X flows through the K^+ channel or through a different Ca^{2+}-activated channel. Uner these conditions, the reversal potential of SH can be obtained from experimental values by the following relations:

$$E_R = E_o - \Delta E \frac{R_o}{\Delta R} \tag{3}$$

$$\Delta E = E - E_o \tag{4}$$

$$\Delta R = R - R_o \tag{5}$$

where E_o and R_o are membrane potential and input resistance measured either before or after the SH, E and R are the potential and resistance values measured during the SH. The value of E_R is related to the equilibrium potential of K^+ (E_K) and to that of the ion X (E_X) by the following equation:

$$E_R = \frac{E_K + \alpha E_X}{\alpha + 1} \tag{6}$$

where $\alpha = \Delta G_X / \Delta G_K$ represents the ratio between the changes in K^+ conductance (ΔG_K) and in the conductance to the ion X (ΔG_X). This parameter will be called 'selectivity coefficient'. Eqn. 3 is also valid if we include conductance changes for other ions, but in this case Eqn. 6 should be modified to include contributions for their equilibrium poten-

tials (see Eqn. A-10, Appendix). On the other hand, if we consider K^+ as the only charge carrier during SH, Eqn. 6 leads to the conclusion that $E_R = E_K$. As we will show in the next section, this seems not to be the case.

Determination of E_R

Before applying Eqn. 3 to calculate E_R, its ability to predict some specific situations should be tested. Eqn. 3 may be rewritten as follows:

$$\Delta E = \frac{(E_o - E_R)\Delta R}{R_o} \tag{7}$$

This expression indicates that a proportionality exists between ΔE and ΔR during the SH response. This result is not dependent on the E_R value and its validity can be experimentally observed as shown in Fig. 2. We can now determine E_R by applying Eqn. 3 to a Ca^{2+}-induced SH of any amplitude. The results,shown in Table I, indicate values of -74 mV in the normal medium and -37 mV in the high K^+ medium.

Under conditions of maximal stimulation, the conductance increase and the change in the membrane potential are also maximal and we can expect from Eqn. 7 that the value of E at the point of maximal amplitude approaches the E_R value. In fact this value was never greater than -80 mV in the normal medium and -40 mV in the high-K^+ medium, in agreement with the calculated values of E_R. Fig. 3 provides further evidence that Eqn. 3 yields correct E_R values. In this case the amplitude of the negative current pulses used for input resistance measurements was adjusted so that the membrane potential during a pulse was driven to -78 mV. This should be equal to the E_R value for this cell (see Appendix). This value is within the limites of the mean E_R obtained from Eqn. 3 (Table I), indicating again the consistency of the model.

Electrogenic potassium pumping

In most cells the intracellular K^+ activity is maintained at a high level by a $(Na^+ + K^+)$-

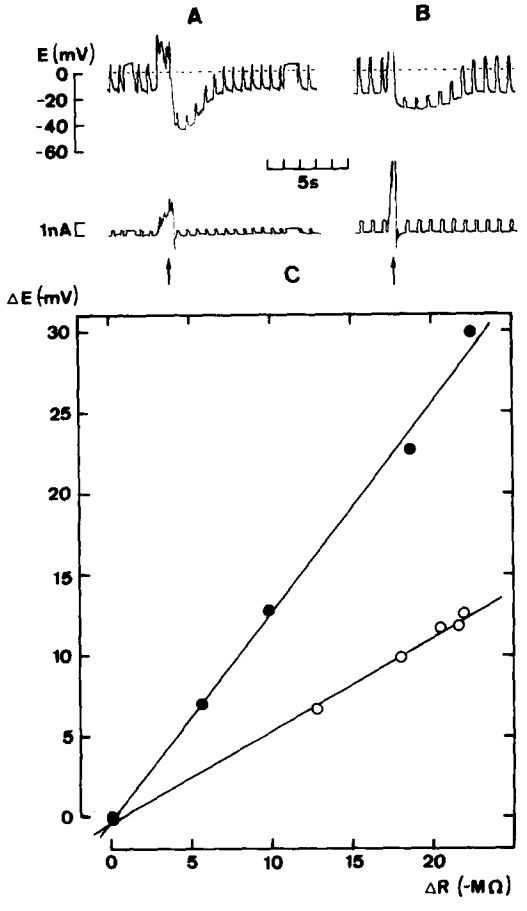

Fig. 2. Linear relationship between ΔE and ΔR supports the model. (A) and (B) represent two Ca^{2+}-induced SH in normal- and high-K^+ medium, respectively. Arrows under current traces indicate the Ca^{2+}-injecting currents. (C) Relation between ΔE and ΔR for the records of SH shown in (A) ● and (B) ○. In both cases the correlation coefficient is better than 0.995.

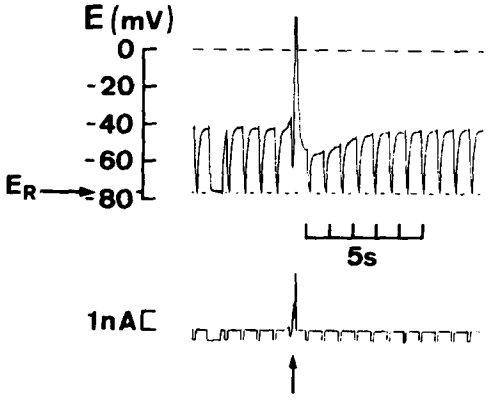

Fig. 3. Reversal potential of the SH. Slow hyperpolarization in normal medium. Arrow indicates the Ca^{2+}-injecting current. Pulses injected through the recording microelectrode were previously adjusted to maintain a constant level of membrane potential during this SH. The dotted line below the pulses indicates the calculated E_R value (-78 mV).

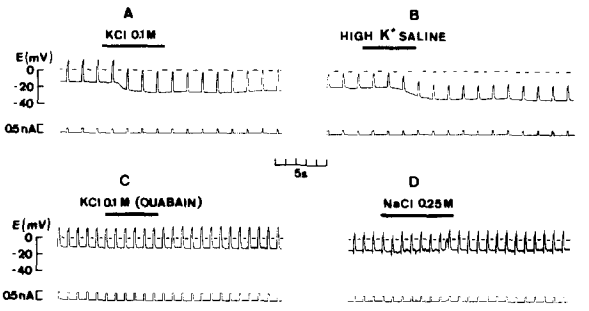

Fig. 4. Evidence for an electrogenic $(Na^+ + K^+)$-ATPase. Cells were incubated in K^+-free saline and 0.1 ml of the indicated solutions were added during the impalement (horizontal bars). Final volume was 10 ml. (A) and (B) show membrane hyperpolarizations induced by K^+ addition. (C) In the K^+-free saline containing 1 mM ouabain this response did not occur. (D) NaCl addition is unable to induce membrane potential change.

ATPase. The possible contribution of this pumping system to electrogenesis of the macrophage polykaryon membrane was assessed by experiments in which K^+ was added to a K^+-free extracellular solution during impalement. In this situation it is expected that if the $(Na^+ + K^+)$-ATPase was inactivated by the absence of external K^+, K^+ addition should re-activate it [22,23]. Fig. 4 shows that addition of a KCl solution or a K^+-containing saline induces a sustained hyperpolarization of the cell membrane without significant change in the input resistance. Ouabain blocks this effect and Na^+ is unable to substitute for K^+. These findings indicate the existence of an electrogenic $(Na^+ + K^+)$-ATPase in the macrophage polykaryon membrane.

Discussion

We have measured the intracellular K^+ activity in macrophage polykaryons and compared the values of the K^+ equilibrium potential with those of the reversal potential for the slow hyperpolarizing oscillations triggered by intracellular Ca^{2+} injection. The intracellular K^+ activity found (84 mM in cells exposed to the normal medium) corresponds to an intracellular ion concentration of 112 mM. This value is greater than the 50–75 mM found in rat and rabbit alveolar macrophages [24,25] and is smaller than the 135 mmol per liter of cell water found for human mononuclear cells [26]. The ion-sensitive microelectrode gives direct measurements of the ionic activity in the cytosol, which is the more convenient parameter for the electrophysiological studies. Another relevant finding is that a_K increases to 100 mM when the extracellular K^+ concentration is raised to 30 mM. Although our studies are not directed towards determining the precise mechanisms by which these changes occur, the data are of importance when a model for the resting membrane potential and its oscillations is proposed.

Potassium equilibrium potentials calculated from the above data are, respectively, -81 mV and -40 mV for cells in normal- and high-K^+ medium. The comparison of these values with the calculated reversal potentials for the SH (-74 mV and -37 mV, respectively) lead to the conclusion that the E_R values are less negative than the corresponding E_K. This indicates that K^+ is not the only charge carrier during the SH response. According to the proposed model we can suppose that an ion X also contributes to the membrane potential oscillation. Eqn. 6 then enables us to determine E_X and α. Thus, using the data from Table I we have:

$$E_R(N) = \frac{E_K(N) + \alpha E_X(N)}{\alpha + 1} \tag{8}$$

$$E_R(H) = \frac{E_K(H) + \alpha EX(H)}{\alpha + 1} \tag{9}$$

where (N) and (H) represent, respectively, the media containing normal and high concentration of KCl. Two distinct situations have to be considered: (a) if ion X is not Cl^-, $E_X(N) = E_X(H)$ and we conclude that $\alpha = 0.11$ and $E_X(N) = -10$ mV; (b) if ion X is Cl^- (a hypothesis we cannot discard), and we suppose that its intracellular concentration is constant in both situations, $E_{Cl}(H) = E_{Cl}(N) + (RT/F)\ln[Cl(N)]/[Cl(H)]$ where R, T and F have their usual meanings and [Cl(N)] and [CL(H)] are the extracellular chloride concentrations in normal and high KCl medium, respectively. In this situation we can calculate $\alpha = 0.08$ and $E_{Cl}(N) = 14$ mV. This results should be considered as approximations, since accumulation of errors makes Eqns. 8 and 9 sensitive to the standard deviations of E_R and E_X. In conclusion,

we can say that the model presented here leads to the prediction that the SH of macrophage polykaryons is due to a increase in the conductance to K^+ with an ion X participating in a proportion of about 10 : 1. It is not possible at this moment to distinguish whether the current carried by the ion X flows either through the Ca^{2+}-activated K^+ channel or through another channel. Patch-clamp studies performed with the Ca^{2+}-activated K^+ channel of macrophage membranes [7,8] indicate that it is highly selective for K^+, but more precise measurements have to be done to clarify this point. A voltage-dependent anionic channel of large unit conductance has also been described in macrophages [27] and must be considered as a possible current carrier.

These conclusions are based on a model that considers only passive ionic fluxes. The validity of this hypothesis was confirmed by the correct prediction of the E_R value and the proportionality between ΔE and ΔR (Figs. 2 and 3) which makes fitting of these data into other models difficult. Nevertheless, we cannot disregard the contribution of other components such as active ion fluxes and voltage-dependent channels contributing to the Ca^{2+}-induced membrane potential changes observed in these cells. In fact, the existence of a K^+-induced, ouabain-sensitive membrane hyperpolarization indicates that the $(Na^+ + K^+)$-ATPase of macrophage polykaryons is electrogenic (Fig. 4) and may contribute to the resting membrane potential in a similar way to that proposed for mononuclear macrophages [26,28]. Voltage-dependent phenomena are also present in the multinuclear [12] and mononuclear [3–5] cells and its calcium dependence deserves further analysis.

The limitations of the measurements described here deserve discussion, since it has been recently demonstrated that mononuclear macrophages studied by means of the conventional electrophysiological technique may display reduced membrane potential and input resistance due to leakage induced by the microelectrode itself [8,29]. The spike going in the direction of hyperpolarization observed at the moment of microelectrode penetration and from whole-cell path-clamp data is indicative of a fast depolarization upon impalement. Macrophage polykaryons are significantly larger than the mononuclear cells but are also sensitive to microelectrode-induced leakage [14]. The first conclusion derived from these observations is that membrane potential and conductances values have been underestimated and that the described electrophysiological properties of these cells have to be reconsidered. On the other hand, the existence of a Ca^{2+}-dependent K^+ permeability is quite well established [1,2,11] and has in fact been cofirmed by the use of the path-clamp technique [7,8]. What is then the extension of this leakage? The finding that macrophage polykaryons sustain an intracellular K^+ activity that is 20-times greater than that of the extracellular medium, even during the impalement with two microelectrodes (Fig. 1), excludes the existence of a gross lesion and indicates that the ionic gradients are preserved. The small S.D. of the values of a_K (Table I) also point to the same conclusion, since diffierent microelectrodes and distinct cells are not expected to exhibit similar leakages. So we can now say that conventional electrophysiological technique may continue to yield relevant information about voltage and ion-dependent conductances when proper caution is taken.

The influence of the microelectrode-induced leakage on the calculated value of E_R can now be taken into consideration. The model we have proposed is compatible with the existence of a constant leakage conductance (see Appendix). The difference between E_R and E_K could be at least partially ascribed to a leakage conductance only in those cases where it exhibits a Ca^{2+} dependence similar to that of G_K. Although this hypothesis seems improbable, we cannot disregard it when based only on the data presented here. The calculation of E_R using Eqn. 3 was made possible because the Ca^{2+}-dependent K^+ channel has a unit conductance large enough to dominate total membrane conductance and to enable ΔR measurements even in the presence of leakage.

Appendix

Reversal potential of the SH

Assuming that the membrane potential change (ΔE) that occurs during SH is due to the changes in the K^+ conductance (ΔG_K) and in the conductance to another ion X (ΔG_X), we can write the

following equation:

$$G = G_o + \Delta G_K + \Delta G_X \quad \text{(A-1)}$$

where G_o and G are the membrane conductance at rest and during the SH, respectively. Now, if we propose an equivalent circuit for the cell membrane consisting of a parallel arrangement of the ohmic ionic conductances (G_j) and potential sources (E_j) we conclude that the total ionic current before and after SH is zero, and the resting membrane potential (E_o) may satisfy the following equation [30,31]:

$$\sum_j (E_o - E_j) G_j = 0 \quad \text{(A-2)}$$

The total ionic current at the minimal point of the SH (where $dE/dt = 0$) is also zero and we can write:

$$\sum_j (E_o + \Delta E - E_j) G_j + (E_o + \Delta E - E_K) \Delta G_K + (E_o + \Delta E - E_X) \Delta G_X = 0 \quad \text{(A-3)}$$

where G_j can include any ohmic ion-specific and leakage conductances that contributes to E_o and G_o.

Eqn. A-3 can be rewritten in the following form:

$$\sum_j (E_o + \Delta E - E_j) G_j + (E_o + \Delta E - E_R) \Delta G = 0 \quad \text{(A-4)}$$

where we have defined E_R, ΔG and the selectively coefficient α as:

$$E_R = \frac{E_K + \alpha E_X}{\alpha + 1} \quad \text{(A-5)}$$

$$\Delta G = \Delta G_K + \Delta G_X \quad \text{(A-6)}$$

$$\alpha = \frac{\Delta G_X}{\Delta G_K} \quad \text{(A-7)}$$

We can now simplify Eqn. A-4 using Eqn. A-2 and Eqn. A-1 and considering that $G_o = \sum_j G_j$:

$$\Delta E = (E_R - E_o)\left(1 - \frac{R}{R_o}\right) \quad \text{(A-8)}$$

which is the same as Eqn. 3.

The meaning of E_R becomes clear from Eqn. A-8. If we consider the theoretical situation in which the resistance R is equal to zero, we conclude that:

$$E_R = E_o + \Delta E \quad \text{(A-9)}$$

indicating that E_R is the value of the potential when the Ca^{2+}-activated conductances short-circuits the membrane. So we can say that E_R is the equilibrium or reversal potential of the slow hyperpolarizations. The value of E_R would be equal to E_K only if $\alpha = 0$. If $\alpha \neq 0$, E_R should have a contribution of E_X.

Eqn. A-9 was deduced considering that $dE/dt = 0$. In fact we can extend its validity to all situations where the time constant of the SH is much greater than that of the membrane, which means for any point during a SH. In other words, we can say that as the SH are so slow (3 s or more) the capacitive current can be disregarded.

Eqn. A-9 is also valid when several ions are involved in the membrane potential change. To substantiate this we have only to include the ionic contributions in Eqns. A-1 and A-3 and define E_R as follows:

$$E_R = \frac{E_K + \alpha_X E_X + \alpha_Y E_Y + \ldots}{1 + \alpha_X + \alpha_Y + \cdots\cdots} \quad \text{(A-10)}$$

where $\alpha_i = \Delta G_i / \Delta G_K$ represents the selectivity for each ion.

We can now study the situation in which DC current pulses of intensity I are injected through the microelectrode. In this case the new membrane potential values during the resting state (E'_o) and during the SH (E') are given by:

$$E'_o = E_o + R_o I \quad \text{(A-11)}$$

$$E' = E + RI \quad \text{(A-12)}$$

The sum of ionic currents should now be equal to I and Eqns. A-2 and A-3 assume the following forms:

$$\sum_j (E'_o - E_j) G_j = I \quad \text{(A-13)}$$

$$\sum_j (E'_o + \Delta E' - E_j) \Delta G_j + (E'_o + \Delta E' - E_K) \Delta G_K + (E'_o + \Delta E' - E_X) \Delta G_X = I \quad \text{(A-14)}$$

where $\Delta E' = E' - E'_o$ is given by:

$$\Delta E' = \Delta E + \Delta RI \quad \text{(A-15)}$$

Eqns. A-13 and A-14 can be handled in a similar way to that used for Eqns. A-3 and A-4, giving the following relation for E_R:

$$E_R = E'_o - \Delta E' \left(\frac{R_o}{\Delta R} \right) \quad \text{(A-16)}$$

where E_R and α are defined by Eqns. A-5 and A-6. Eqn. A-16 is analogous to Eqn. A-9 and gives another method for the calculation of E_R. This is achieved by injecting a current I of such an amplitude that makes $E'_o = E'$. In this case $\Delta E' = 0$ and $E_R = E'_o$, which can then be measured directly (as illustrated in Fig. 3).

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