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Intracellular K^+, Na^+ and Cl^- concentrations and membrane potential in human monocytes

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The relationship between the resting membrane potential and the intracellular ionic concentrations in human monocytes was investigated. Cell volume, cell water content, and amount of intracellular K^+, Na^+, and Cl^- were measured to determine the intracellular concentrations of K^+ (K_i), Na^+ (Na_i) and Cl^- (Cl_i) of monocytes, and of lymphocytes and neutrophils. Values found for monocytes were similar to those for neutrophils, i.e., cell volumes were 346 and 345 μm^3, respectively, cell water content 78%, and K_i, 128 and 125, Na_i, 24 and 26, and Cl_i, 102 and 103 mmol/l cell water, respectively. Lymphocytes, however, had different values: 181 μm^3 cell volume, 77% cell water content, and for K_i, Na_i, and Cl_i, 165, 37, and 91 mmol/l cell water, respectively. The resting membrane potential of cultured human monocytes (range −30 to −40 mV), determined by measurement of the peak potential occurring within the first milliseconds after microelectrode entry, was most dependent on extracellular K^+, followed by Cl^-, and Na^+. The membrane permeability ratio of Cl^- to K^+ was estimated by use of the constant field equation to be 0.23 (range 0.22 to 0.30).

Introduction

Reports that in mononuclear phagocytes, chemotaxis [1] and phagocytosis [2,3] are accompanied by membrane potential changes and that IgG/Fc-receptor ligation [4,5] and phagocytosis [6] induce ionic channel activity, indicate that membrane electrophysiological processes are involved in the function of these cells. The finding of ionic channel activity [7,8] and action potentials [9] in cultured human monocytes suggests a similarity between the electrical properties of monocytes and those of nerve and muscle cells. However, the resting membrane potential (rmp) of cultured human monocytes lies around −30 mV [10], which is less negative than that of nerve and muscle cells, and this suggests a difference in the ionic conditions determining the resting membrane potential.

Intracellular ion concentrations of human leucocytes have been reported for lymphocytes [11,12] and neutrophils [13,14] but not for monocytes. Intracellular potassium (K_i) and sodium (Na_i) concentrations in lymphocytes and neutrophils are comparable to those of other cells, whereas mononuclear phagocytes, i.e. alveolar macrophages, of the rat [15] and rabbit [16] have been reported to have unusually low K_i and high

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Na_i levels compared with other cell types. Thus, there is some uncertainty about the intracellular ionic concentration of mononuclear phagocytes of human origin, such as monocytes.

In this study values of K_i, Na_i and Cl_i (intracellular chloride concentration) were determined for human monocytes, lymphocytes and neutrophils. Furthermore, to determine the ionic basis of the resting membrane potential in human monocytes, microelectrode measurements were applied to cultured monocytes in the presence of solutions of different ionic composition. The constant field equation [13,14,17–20] was used to calculate the ratio between the permeability of the membrane to Cl^- and K^+.

Materials and Methods

Isolation of neutrophils, monocytes and lymphocytes

Blood was obtained from healthy donors by venipuncture. Mononuclear cells were separated from polymorphonuclear neutrophils by Ficoll-Hypaque centrifugation according to Boyum [21] as described elsewhere [22]. The pellet containing erythrocytes and neutrophils was resuspended in phosphate-buffered saline (PBS), the erythrocytes were separated from the neutrophils by dextran sedimentation [23], and the neutrophil-rich supernatant was washed twice with phosphate-buffered saline containing 0.5 U/ml heparin. Next, the remaining erythrocytes were lysed by resuspension of the pellet in 10 ml 0.8% (w/v) NH_4Cl and incubation of the suspension for 10 min at 37 °C. After centrifugation at room temperature for 10 min at $120 \times g$, the cells were resuspended in Hank's balanced salt solution (HBSS) to a concentration of about $4 \cdot 10^7$ neutrophils/ml. The purity of the neutrophil preparation obtained in this manner amounted to 97%.

Monocytes and lymphocytes were isolated from the Ficoll-Hypaque interphase [22]. After four washes of the interphase with phosphate-buffered saline containing 0.5 U/ml heparin the cells were resuspended in Hanks' balanced salt solution containing 0.1% (w/v) gelatin to a concentration of about $1 \cdot 10^8$ cells/ml. Monocyte- and lymphocyte-enriched suspensions were obtained by elutriation centrifugation with a Beckmann J2-21 centrifuge (Beckmann Instruments, Mijdrecht, The Netherlands) equipped with an elutriation rotor, under a constant flow rate of 18 ml/min and a stepwise reduction of the rotor speed after each 50 ml fraction starting at 3000 rpm [22]. The lymphocyte fraction was obtained at rotor speeds between 2970 and 2840 rpm, the monocyte fraction between 2300 and 2050 rpm. After centrifugation, the cells were washed twice with phosphate-buffered saline containing 0.5 U/ml heparin and resuspended in Hanks' balanced salt solution to a concentration of $3 \cdot 10^7$ cells/ml for monocytes and $6 \cdot 10^7$ cells/ml for lymphocytes. The purity of the suspensions was determined in cytocentrifuge preparations according to the criteria of Van Furth et al. [24]. The monocyte suspension contained on average 83% monocytes, 14% lymphocytes, and 3% neutrophils. In the lymphocyte suspension more than 97% of the cells were lymphocytes.

Preparation of cells for determinations of intracellular ion concentrations

Aliquots (1 ml) of the various cell suspensions were added to 1.5 ml polypropylene tubes (Merck, Darmstadt, F.R.G.) over a 0.4 ml cushion (density: 1.025 g per ml) of dibutyl phthalate (C16H2204; Merck) doped with a light mineral oil (Klearol, Sonneborn Div., Witco Chemical, New York, NY, U.S.A.), and the Hanks' balanced salt solution was removed by centrifugation for 45 s at $6500 \times g$ at room temperature (B12 Microfuge, Beckman). These cell pellets were used for determinations of the cell volume and percentage water content as well as the intracellular K^+, Na^+, and Cl^- concentrations.

Measurement of cell volume, trapped extracellular space and cell viability

Cells were counted microscopically with a Burker hemacytometer and by a Coulter Counter (Model ZF, Coulter Electronics Ltd., Luton, Beds., U.K.). Cell volumes were calculated from the mean of the Gaussian volume distribution generated by a channel analyzer (Size Distribution Analyzer Model P64, Coulter Electronics, U.K.) attached to the counter. To obtain absolute values of cell volumes polystyrene latex beads (6.5, 7.8 and 8.7 μm diameter, Poly Sciences Inc., Warrington, PA, U.S.A.) were used as standards. Cell viability was determined by Trypan-blue exclusion.

The volume of extracellular fluid trapped in the cell pellet after centrifugation through the dibutyl phthalate- klearol cushion was determined with [^{14}C]sucrose (Amersham International Limited, Amersham, Buckinghamshire, U.K.) as volume marker.

Determination of percentage cell water

To determine cell water content, the tip of the polypropylene tube containing the cell pellet was weighed in an aluminium planchet (wet weight, W_w), dried at 80 °C for 72 h in a porcelain crucible covered with a lid and then reweighed (dry weight, W_d). These values were used to calculate the weight of cell water ($W_{cell\ water} = W_w - W_d$). Control experiments showed that 72 h was sufficient for complete dehydration and that the weight of the tubes and planchets remained constant during this period. The dry weight of the pellet (W_{dp}) was measured after carefully removing it from the tip of the tube. The percentage cell water content was then calculated from the equation:

$$W_{cell\ water}/(W_{cell\ water} + W_{dp}) = \%\ \text{cell water content}/100$$

Measurement of K_i, Na_i and Cl_i

After centrifugation of the cells through the dibutyl phthalate-klearol cushion, the aqueous phase was carefully collected, the inner surface of the polypropylene tube rinsed three times with de-ionized water (Milli-Q de-ionized water; Millipore Corp, Bedford, MA, U.S.A.) and the oil phase gently aspirated and discarded. Next, the cell pellet was resuspended in 11 ml de-ionized water and mixed vigorously on a vortex mixer for 5 min. Complete cell lysis was obtained by incubation at room temperature for 60 min followed by centrifugation at $1500 \times g$ for 10 min to remove debris. The K^+ and Na^+ contents of the supernatant were determined by emission flame photometry (Perkin-Elmer X- 460 Atomic Absorption Spectrophotometer, Perkin-Elmer Ltd, Beaconsfield, Buckinghamshire, U.K.). Standard curves were based on KCl and NaCl solutions.

The amount of chloride in the supernatant was determined by the use of a chloride selective electrode (Solid State Chloride Electrode, PHI 91200, NY, U.S.A.) monitored by an Ionalyzer (Orion Research Inc, Cambridge, MA, USA). Standard KCl solutions were used for calibration. K_i, Na_i, and Cl_i were calculated after correction for the percentage cell water under the assumption that all K^+, Na^+ and Cl^- ions are free in the cytosol. Values of K_i, Na_i, and Cl_i are expressed as mmol/liter cell water.

Electrophysiological measurements

For the electrophysiological experiments, human monocytes were cultured on flying coverslips in plastic petri dishes for one to three weeks as described elsewhere [25]. Coverslips were attached to a teflon culture dish which permitted measurement under high magnification optics [25]. Membrane potentials were measured with fine-tipped open-ended glass micropipettes filled with 4 M potassium acetate. Microelectrodes were capacitively compensated, had resistances ranging from 50 to 200 Mohm. Cells were impaled by use of a piezo-stepper device as described elsewhere [26]. Due to the relative small size of the cells, the peak value of the potential transient seen in the first milliseconds after electrode entry (E_p; see inset Fig. 1) was used to measure the resting membrane potential of the cells [10,26–28]. That the value of E_p provides a reliable measure of the resting membrane potential was shown by us in recent studies in which whole cell patch clamp measurements [29] in current clamp were made in combination with microelectrode impalements [28]. Measurements were carried out at room temperature unless specified otherwise. For measurements at 37 °C, a micro-CO_2 incubator mounted on a microscope [30] was used. Communicated values are expressed as mean ± S.E. with n = number of cells.

Composition of solutions

Hanks' balanced salt solution (HBSS) was composed of 137 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 0.1 mM $MgSO_4$, 0.4 mM Na_3PO_4, 0.4 mM KH_2PO_4, and 5.6 mM dextrose. The culture medium for monocytes consisted of medium 199 (Microbiological Associates, Bethesda, MD, U.S.A.), supplemented with 10% heat-inactivated newborn calf serum (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY, U.S.A.), 2000 U/ml penicillin G (Mycofarm, Delft, The Netherlands) and 50 μg/ml streptomycin (Myco-

farm). The standard saline solution used for the membrane potential measurements was composed of 150 mM NaCl, 3 mM KCl, 4 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes-NaOH (pH 7.2).

Results

Concentrations of intracellular monovalent ions

Mean values of cell water content, cell volume, and intracellular monovalent ion concentrations in human monocytes, neutrophils, and lymphocytes are given in Table I. Almost the same cell volumes were found for neutrophils and monocytes, but a smaller value was obtained for lymphocytes. All cell types had a similar percentage of cell water. The concentrations of intracellular K^+, Na^+, and Cl^- were similar in neutrophils and monocytes, whereas lymphocytes had a higher concentration of K^+ and Na^+ and a slightly lower Cl^- concentration. Incubation of the various types of cell at 37 °C had little effect on the intracellular ion concentration as compared with the cells incubated at room temperature (data not shown). Determination of the trapped extracellular space in cell pellets gave similar low values for lymphocytes ($2.4 \pm 0.1\%$, $n = 3$), neutrophils, ($1.7 \pm 0.2\%$, $n = 3$), and monocytes ($1.7 \pm 0.1\%$, $n = 3$).

Measurement of the resting membrane potential

The average resting membrane potential of cultured human monocytes measured in culture medium at 37 °C amounted to −32.2 mV, and did not differ from the average value at room temperature (Table II). To permit correlations between concentrations of intracellular ions and resting membrane potential values, the resting membrane potential was determined in the presence of Hanks' balanced salt solution (the solution used as the final incubation medium prior to cell lysis for the determinations of K_i, Na_i and Cl_i) and also in saline in which the KCl content was increased to 5 mM so that the ionic composition of this solution would resemble that of Hanks' balanced salt solution. No difference was found between the resting membrane potential determined in these two solutions (Table II), whereas the resting membrane potential measured in standard saline (containing 3 mM KCl) was more negative than that measured either in Hanks' balanced salt solution or saline with 5 mM KCl (Table II; Fig. 1).

To find out whether a Na^+/K^+ pump is present in human monocytes, 0.5 mM ouabain, which is known to inhibit Na^+/K^+ pump activity, was added to the saline. This led to an immediate depolarization (within 30 s) of the resting membrane potential to -24.6 ± 1.8 mV ($n = 30$) as compared with -35.8 ± 1.7 mV ($n = 30$) without ouabain. From these results it may be concluded that monocytes have a Na^+/K^+ pump [31]. In subsequent experiments ouabain was not added to block this pump, because blockade of this pump was found to alter intracellular ionic concentrations in monocytes [32].

The dependence of the resting membrane potential on K_e, Na_e, and Cl_e

To study the dependence of the resting membrane potential on extracellular concentrations of K^+, Na^+, and Cl^- ions (K_e, Na_e and Cl_e, respectively) the concentrations of these ions were varied

TABLE I

PERCENTAGE CELL WATER, MEAN CELL VOLUME (MCV) AND INTRACELLULAR CONCENTRATIONS OF K^+, Na^+ AND Cl^- OF HUMAN LYMPHOCYTES, NEUTROPHILS AND MONOCYTES

K_i, Na_i and Cl_i are expressed in mmol/l cell water.

Cell type	% cell water	MCV (μm^3)	K_i	Na_i	Cl_i
Lymphocytes	77.9	181.4	165.0	37.0	91.5
(S.E., n)	(0.6, 4)	(2.5, 5)	(2.5, 4)	(0.8, 4)	(2.3, 4)
Neutrophils	75.6	345.4	125.2	25.5	103.1
(S.E., n)	(0.7, 8)	(2.9, 5)	(1.9, 7)	(1.4, 5)	(2.1, 4)
Monocytes	78.3	346.3	136.1	23.5	102.0
(S.E., n)	(0.7, 5)	(2.3, 5)	(1.5, 5)	(0.5, 5)	(2.5, 4)

TABLE II

RESTING MEMBRANE POTENTIALS OF CULTURED MONOCYTES IN DIFFERENT SOLUTIONS

Unless otherwise stated all measurements were carried out at room temperature; n = number of cells; rmp, resting membrane potential; HBBS, Hanks' balanced salt solution.

Solution	rmp	S.E.	n
Culture medium at 37 °C	−32.2	1.1	95
Culture medium	−30.4	1.0	36
HBBS	−32.0	1.3	30
Saline (5 mM KCl)	−31.3	0.3	147
Saline (3 mM KCl)	−36.6	0.5	338

in saline. Measurements were completed within 30 min after substitution of the culture medium with the test solution. The effect of Na_e on the resting membrane potential was studied by equiosmolar substitution of choline for Na^+ in the saline. The results of these experiments show that variation of Na_e from 150 mM to 3 mM has almost no effect on the resting membrane potential (Fig. 2). Variation of K_e was achieved by replacing Na^+ by K^+. The results showed a strong dependence of the

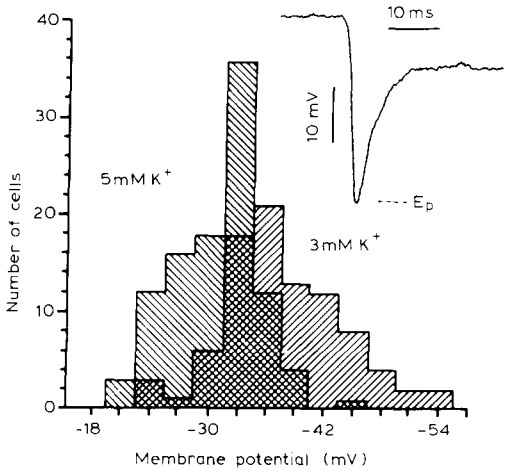

Fig. 1. Amplitude distribution of the resting membrane potentials of cultured human monocytes measured in saline containing 3 mM (▨) and 5 mM (▧) showing a dependence of the resting membrane potential on K_e. The inset shows a representation of the potential transient seen during the initial milliseconds after microelectrode entry into a cultured monocyte. The peak potential (E_p) is taken as the resting membrane potential of the cell prior to impalement.

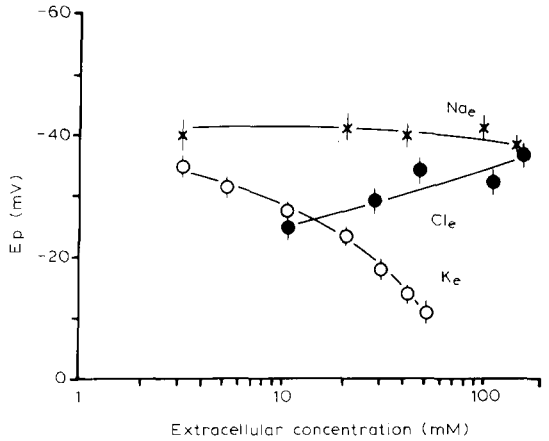

Fig. 2. Graph showing the dependency of the mean resting potential (the peak of the impalement transient (E_p)) on K_e, Na_e and Cl_e. The horizontal axis represents the concentration of the extracellular ion indicated next to each curve. Na_e was varied by equimolar replacement in saline of NaCl by choline chloride (×———×). K_e was varied by equimolar substitution of KCl for NaCl (○———○). This experiment is described by Eqn. 2 (E_{ml}; see text). Sodium glutamate was substituted for NaCl to vary Cl_e (●———●). Each point represents the mean of 30 measurements. The bars represent the S.E. of these values.

resting membrane potential on K_e (Fig. 2). The dependence of the resting membrane potential on Cl_e was investigated by equiosmolar substitution of sodium glutamate for the NaCl in saline. Decreasing Cl_e from 160 to 10 mM resulted in a depolarization of the resting membrane potential (Fig. 2). To exclude the possibility that this depolarization was due to presence of glutamate and not to the absence of Cl^-, the resting membrane potential was measured in a 10 mM Cl_e solution in which SO_4^{2-} was used instead of glutamate to replace the Cl^- in the saline. Mannitol was added to maintain osmolarity. Under these conditions the resting membrane potential amounted to -27.1 ± 0.9 mV ($n = 72$), which is similar to the value obtained in the 10 mM Cl_e solution with glutamate substituting for Cl^- (-25.0 ± 0.5 mV, $n = 31$). Furthermore, a similar depolarization (-31.6 ± 0.45 mV, $n = 34$) was obtained in a solution consisting only of 3 mM KCl supplemented with sucrose to maintain osmolarity.

To find out whether the intracellular ionic composition of cells alters during ion substitution experiments, the intracellular ionic concentration

of monocytes was determined after incubation of the cells in saline solutions of various ionic concentrations. Since the resting membrane potential depended most strongly on K_e (Fig. 2), K_i was determined in monocytes incubated in saline (K_e = 3 mM) for 30 min at room temperature. The results gave a value for K_i of 113.7 ± 0.9 mmol/l cell water, $n = 2$. Incubation of cells for 30 min in saline in which Na^+ had been replaced by K^+ to obtain a K_e of 50 mM, resulted in a K_i value of 155.1 ± 5.7 mmol/l cell water $n = 2$. Since the resting membrane potential also depends to some extent on Cl_e, the effect of a low Cl_e on Cl_i was investigated by incubation of cells for 30 min in a 10 mM Cl_e solution where glutamate was used as a substitute for Cl^-. The results showed a decrease of Cl_i from 99.4 (control) to 77.2 mmol/l cell water.

Estimation of the relative membrane permeability of monocytes for Cl^- as compared with K^+

As was shown in the preceding section the resting membrane potential of monocytes is strongly dependent on K_e, to a lesser extent on Cl_e, and hardly at all on Na_e. Since the Cl_i level in human monocytes is relatively high and their resting membrane potential depends on Cl_e, the generally applied simplification of the constant field equation [16,19] cannot be used to determine the relative permeability P_n of monocytes membranes to Na^+ compared with K^+. However, the relative permeability of monocytes membranes to Cl^- compared with K^+ ($P_c = P_{Cl}/P_K$) can be estimated under constant field assumptions and this is needed to gain insight into the ionic basis of the value of the resting membrane potential in human monocytes. For this purpose the constant field equation was rewritten by the addition of a term E_c to include constant contributions to E_p not affected by K_e, Na_e, or Cl_e, such as Na^+/K^+ pump contributions and E_p underestimation of E_m. This leads to the following equation:

$$E_m = E_c + \frac{RT}{F} \ln \frac{K_e + P_n Na_e + P_c Cl_i}{K_i + P_n Na_i + P_c Cl_e} \quad (1)$$

in which E_m is the resting membrane potential, R is the gas constant, T is the temperature in K, F is the constant of Faraday and K_e, Na_e and Cl_e are the extracellular and K_i, Na_i, and Cl_i the intracellular ion concentrations. For the calculation of P_c, the following assumptions were made: (1) membrane permeabilities are not altered by any of the experimental conditions applied, (2) the values of K_i, Na_i and Cl_i remain constant under our experimental conditions, (3) E_c is constant for solutions with similar K_e, and (4) the ionic concentrations measured in suspensions do not substantially differ from those of cultured cells.

Applications of the constant field equation to the series of experiments in which Na_e was replaced by K_e such that the sum of their concentrations remained constant (i.e., $K_e + Na_e$ = 155 mM) and with Cl_e equal to 165 mM (see Fig. 2), results in the following equation:

$$E_{m1} = E_c + \frac{RT}{F} \ln \frac{K_e(1 - P_n) + P_n 155 + P_c Cl_i}{K_i + P_n Na_i + P_c 165} \quad (2)$$

In the next series of experiments, Na_e was exchanged by K_e such that the sum of their concentrations remained constant (i.e. $K_e + Na_e$ = 155 mM), while Cl_e was kept low (10 mM) by the use of K_2SO_4 and Na_2SO_4 instead of KCl and NaCl, respectively. Osmolarity was kept constant by addition of mannitol. The results of these experiments are shown in Fig. 3 The constant field

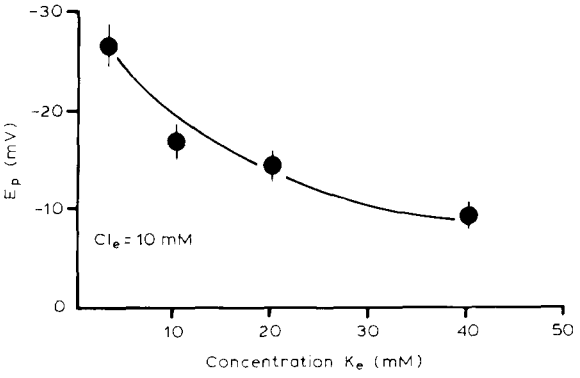

Fig. 3. Dependence of the resting membrane potential on K_e under conditions of low Cl_e (E_{m2}; see Eqn. 3 in text). Cl_e was kept at 10 mM by substitution of K_2SO_4 for KCl and Na_2SO_4 for NaCl. Osmolarity was maintained by addition of mannitol. K_e was then varied by substituting K^+ for Na^+ (●——●). Each point represents the average value of 30 measurements, and the bars represent the S.E. of these values.

equation for such experiments becomes:

$$E_{m2} = E_c + \frac{RT}{F} \ln \frac{K_e + P_n(155 - K_e) + P_c Cl_i}{K_i + P_n Na_i + P_c 10} \quad (3)$$

P_c can be calculated by subtraction of E_{m2} (Eqn. 3) from E_{m1} (Eqn. 2). Such a subtraction also cancels out systematic errors present in both expressions and gives:

$$\exp\left(\frac{F\Delta E}{RT}\right) = \frac{K_i + P_c 10 + P_n Na_i}{K_i + P_c 165 + P_n Na_i} \quad (4)$$

in which

$$\Delta E = E_{m1} - E_{m2} \quad (5)$$

Eqn. 4 predicts that $\exp(F\Delta E)/(RT)$ is independent of the value of K_e. That this is the case is confirmed by subtraction of the experimentally obtained values for E_{m2} from E_{m1} and plotting $\exp(F\Delta E)/(RT)$ as a function of K_e (Fig. 4). Neglecting the contribution of the $P_n Na_i$ term in Eqn. 4 (due to the small value of both factors) and substituting the value of K_i determined in the intracellular ion concentration measurements (i.e., 122.3 mmol/l cell water at $K_e = 5$ mM), together with the experimentally determined value for $\exp(F\Delta E)/(RT)$ of 0.77, gave a P_c of 0.23. Substitution of the K_i values of 113.7 and 155.1 mmol/l cell water, measured at K_e values of 3 and 50 mM, respectively, gave values for P_c of 0.22 and 0.30, respectively. Thus, variation of the K_i within the range found in the present study has no dramatic effect on the value of P_c.

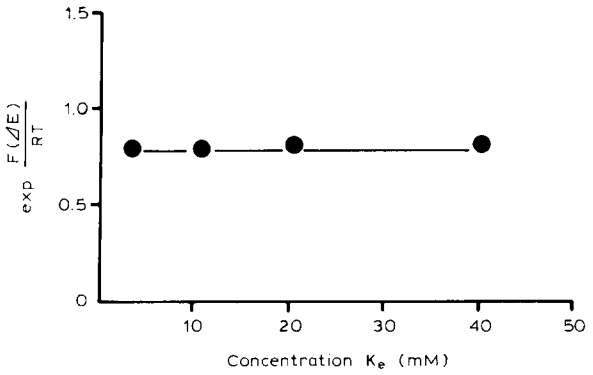

Fig. 4. Determination of P_c under constant field assumptions. Subtraction of the mean resting membrane potential for each corresponding K_e of the E_{m2} measurements (Fig. 3) from that of the E_{m1} values for the same K_e (Fig. 2, open circles) gives ΔE. $\exp(F\Delta E/RT)$ is then plotted as a function of K_e (●———●). The horizontal relationship between $\exp(F\Delta E/RT)$ and K_e, predicted by the constant field equation (see text) has a value of 0.77. S.E. values lay below 0.01.

Discussion

The present study was undertaken to gain a better understanding of the ionic basis of the resting membrane potential of cultured human monocytes and to obtain values for the intracellular ionic concentrations needed for patch clamp studies on human leucocytes [7,8,33–35]. To this end, the concentrations of the intracellular monovalent ions (K_i, Na_i, and Cl_i) were determined. To our knowledge this is the first report of the intracellular concentrations of K^+, Na^+ and Cl^- in human monocytes. Since isolation and handling of the cells are known to affect these concentrations [36], the ionic composition of human lymphocytes and neutrophils was determined for comparison with values reported for these cells by others (Tabel III). Because the monocyte suspensions contained on average 14% lymphocytes, whereas the neutrophil and lymphocyte suspensions were more than 97% pure, the values for monocytes in Table I were corrected for the K_i, Na_i and Cl_i contributed by the lymphocytes. These corrected values are listed in Table III. After correction, the values of K_i and Na_i were almost identical for monocytes and neutrophils, and higher for lymphocytes (Table III). The Cl_i values of monocytes and neutrophils were also similar whereas in lymphocytes the Cl_i level was slightly lower. As Table III shows, our values for lymphocytes and neutrophils agree well with those reported by others. Comparison of the ionic composition of rabbit [16] and rat [15] alveolar macrophages with that of human monocytes revealed a considerable difference despite the similarity of the resting membrane potential (-37 mV for rat alveolar macrophages, as determined with membrane potential sensitive dyes) [15]. Differences in isolation techniques may have played a role in this discrepancy.

The peak potential upon microelectrode impalement, taken as a direct measure of the resting

TABLE III

CONCENTRATIONS OF MONOVALENT IONS IN HUMAN LEUCOCYTES AND IN MONONUCLEAR PHAGOCYTES

Intracellular concentrations are expressed as mmol/l cell water.

Cell type	K_i	Na_i	Cl_i	Reference
Human leucocytes				
Lymphocytes	120	34		Lichtman (1969)
	165.5			Holian (1979)
	129			Negendank (1979)
	176	35.4	95	Negendank (1982)
	198	42.3		Averdunk (1980)
	165	37.0	91.5	present paper
Neutrophils	118	30		Lichtman (1969)
	130			Dunham (1974)
	120.4	24.7		Simchowitz (1982)
			78.9	Simchowitz (1986)
	125.2	25.5	103.1	present paper
Monocytes [a]	122.3	21.2	103.7	present paper
Mononuclear phagocytes of other species				
Rat alveolar macrophages	50	97	64	Castranova (1979)
Rabbit alveolar macrophages	75	83	59	Robin (1971)

[a] Values for monocytes (Table I) were corrected in this table for the contribution of the K_i, Na_i, and Cl_i from the lymphocytes present in the monocytes fraction.

membrane potential, showed that for cultured human monocytes the resting membrane potential is about −30 mV which is less negative than the resting membrane potential of around −70 mV, generally associated with nerve and muscle cells. The relatively low rmp of cultured human monocytes predicted earlier by peak potential measurements [10] was recently confirmed by whole-cell patch clamp measurements (in current [8] as well as in voltage clamp [7]). Microelectrode impalements of human monocytes during monitoring of the membrane potential by a patch electrode in the whole-cell current clamp mode have shown that on average the peak potential in human monocytes is 6 mV less negative than the pre-impalement resting membrane potential [28]. Even though the peak potential can lead to such an underestimation, this has no effect on the calculation of P_c because the method introduced in the present study to calculate P_c, allows P_c to be derived independent of the absolute value of the resting membrane potential.

The strong dependency of the resting membrane potential of cultured monocytes on K_e suggests the presence of a K^+ conductance which could be due to K^+ channel activity known to be present in these cells [7,8]. The observation that the resting membrane potential of human monocytes is, unlike that of neutrophils [13], dependent on Cl_e, implies that the value of P_c is neither extremely large or small but lies close to unity. Several observations besides the calculations presented in this study support such an intermediate value for P_c. For cells with a high P_c the equilibrium potential for Cl^- is equal to the resting membrane potential [20,27,40]. If monocytes had such a high P_c, a value of 40 mmol or less would have been found for Cl_i but this is clearly not the case (Table III). Furthermore, incubation in low-Cl^- solutions of cells with a high membrane permeability for Cl^- would result in rapid depletion of the Cl_i. This occurs in rabbit alveolar macrophages [16], where all of the intracellular Cl^- is lost within one minute of exposure to Cl^--free solutions. For cells with intermediate values of P_c a much slower Cl_i depletion would be expected. This occurs in human monocytes, where after 30 min of incubation in a low-Cl^- solution (10 mM), about a 20% loss of Cl_i was found. A similar decrease of Cl_i after incubation of cells in low-Cl^- solutions has been reported for salamander erythrocytes, which also have intermediate P_c values [14,41]. If human monocytes had a low value of P_c, according to the constant field equation, variation of Cl_e should not affect the resting membrane potential. However, the resting membrane potential found in low-Cl^- solutions containing either glutamate or SO_4^{2-} and the experiments where the bathing solutions consisted only of 3 mM KCl with sucrose added to maintain osmolarity, was dependent on Cl_e, so that these results argue against a small P_c and also favour an intermediate value for P_c.

The intermediate value of P_c found in the present study, suggests the presence of a Cl^- conductance in cultured human monocytes. In patch clamp measurements in mouse peritoneal macrophages Cl^- channels have been identified [42]. In human macrophages, such as cultured

human monocytes, however, no Cl^- channels have as yet been found [7,8], although such channels could have been below the detection threshold in these studies since Cl^- channels can have very small conductances [43].

Taken together, the present results show that the resting membrane potential of cultured monocytes is strongly dependent on K_e, less on Cl_e and almost independent on Na_e. According to the constant field equation, the relatively low value of the resting membrane potential of cultured human monocytes is based on a relatively high Cl_i combined with a membrane conductance that is greater for Cl^- than for Na^+, but lower than that for K^+.

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