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An increase in intracellular pH is a general response of promonocytic cells to differentiating agents

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Intracellular pH (pHi) was measured in HL60 and U937 cells before and after differentiation into monocyte-macrophage like cells. 12-*O*-Tetradecanoyl phorbol-13-acetate (PMA), butyrate, interferon, retinoic acid and 1,25-dihydroxyvitamin D_3 all increased pHi. The increases elicited were rapid with PMA, much slower with retinoic acid and interferon and still slower with 1,25-dihydroxyvitamin D_3. Increases in pHi are due to an activation of the Na^+/H^+ exchange system. High pHi values are unlikely to serve as an early intracellular signal for initiating monocytic differentiation.

intracellular pH; Na^+/H^+ exchange; Interferon; Phorbol ester; Vitamin D_3; (Monocyte)

1. INTRODUCTION

Maintenance of a defined pHi value seems to be important for a number of cellular functions. pHi is controlled in eukaryotic cells by membrane ion transporting systems, the most important being that of Na^+/H^+ exchange and the different bicarbonate transporting systems [1–3]. The Na^+/H^+ exchange system is a target for a large variety of agents, including hormones and growth factors [3–5]. We previously showed that in the human myeloid cell lines HL60 and U937, retinoic acid activates about 2-fold the Na^+/H^+ exchange system, and produces a cellular alkalinization [6,7]. Retinoic acid has no action on the activity of a second pHi regulating mechanism, the Na^+-dependent HCO_3^-/Cl^- exchange system [6,7]. Retinoic acid induces HL60 and U937 cells to differentiate along the granulocytic and monocytic differentiation pathways respectively [8–10]. In this paper we investigate the action of other agents that promote the monocytic differentiation in HL60 and U937 cells. These are phorbol esters [11,12], rIFN [13], butyrate [14] and 1,25-$(OH)_2D_3$ [15,16].

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Abbreviations: 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; PMA, 12-*O*-tetradecanoylphorbol-13-acetate; rIFN, recombinant γ human interferon; pHi, intracellular pH; EIPA, ethylisopropylamiloride

2. MATERIALS AND METHODS

[^{14}C]Benzoic acid (19.3 mCi/mmol) was from New England Nuclear. 2′,7′-Biscarboxyethyl-5(6)-carboxyfluoresceinacetoxymethylester was purchased from Calbiochem. RPMI 1640 culture medium was from Gibco. Fetal bovine serum was from Boehringer. Nigericin and PMA were from Sigma. EIPA was synthesized as described [17]. 1,25-$(OH)_2D_3$ and recombinant human γ interferon (RU 42369) were from Roussel Uclaf.

HL60 cells and U937 cells were grown in RPMI 1640 medium supplemented with 20% (HL60 cells) or 15% (U937 cells) heat inactivated fetal bovine serum, 2 mM glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Cells were seeded twice weekly at 3×10^5 cells/ml.

pHi values were determined from the distribution of

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[^{14}C]benzoic acid or from the fluorescence of 2',7'-biscarboxyethyl-5(6)-carboxyfluorescein as described [6]. The two techniques gave identical values of the pHi. In all cases, measurements were performed after equilibration of the cells for 30 min in a bicarbonate free Hepes buffered RPMI 1640 medium. Calibration was achieved using the nigericin/KCl method.

For immunofluorescent detection of HLA-DR, 10^6 HL60 cells, incubated in RPMI 1640 medium supplemented with 2% fetal bovine serum were stained with OKla-1 (Ortho Diagnostic System) for 1 h at 4°C, washed three times and incubated with goat anti-mouse IgG fluorescein isothiocyanate conjugate for an additional hour. Cells were then washed three times at 4°C and analyzed on a fluorescence activated cell sorter (Ortho-50H) [18]. FACS analyses were performed in the 'Laboratoire de Cytométrie en Flux', Hôpital Saint Louis, Paris. Radioimmunoassay of HLA-DR was carried out as described [19] using D1-12 anti-human HLA-DR antibodies.

3. RESULTS AND DISCUSSION

Undifferentiated HL60 cells have a mean pHi of 7.00 ± 0.03 [6]. After 4 days of exposure to 0.5 mM butyrate or to 0.1 μM 1,25-$(OH)_2D_3$, pHi increased to 7.16 ± 0.02 (n = 5) and to 7.20 ± 0.02 (n = 5) respectively. After 5 days of treatment with 100 IU/ml rIFN, pHi increased to 7.13 ± 0.01 (n = 5). PMA induced HL60 and U937 cells to attach to plastic surfaces [12]. The pHi of adherent cells, measured after 2 days of exposure to 5 nM PMA was 7.23 ± 0.02 (n = 6). Similarly exposure of U937 cells for 4 days to 1 μM retinoic acid induced a cellular alkalinization from 7.03 ± 0.02 to 7.23 ± 0.03 [7]. After treatment with these agents, blocking Na^+/H^+ exchange activity with EIPA [6,7,20,21] decreased the steady state pHi value to 6.95 ± 0.02 irrespective of the agent used. These experiments indicated (i) that all inducers of the monocytic-macrophage differentiation pathway produced an increase in pHi and (ii) that this increase resulted from an increased activity of the Na^+/H^+ exchange system. The increase in pHi was lowest with rIFN (0.14 pH unit) and highest with retinoic acid (in U937 cells) or PMA (0.23 pH unit). It seems therefore that pHi changes are linked in some way to the acquisition of monocytic properties. An increase to a pHi value of 7.14–7.23 characterizes the monocytic lineage. A larger increase to pHi values of 7.30–7.37 has previously been shown to occur during the granulocytic differentiation of HL60 cells [6].

We next compared the time courses of pHi changes after treatment of the cells with different inducers. Addition of PMA (0.1 μM) to HL60 cells (fig.1A) or to U937 cells (fig.1B) produced a rapid and monophasic increase in the pHi of 0.3 pH units which was not observed in the presence of EIPA (fig.1). The rapid effect of PMA on pHi is typical of an action mediated by protein kinase C [22,23]. The dose-response curves for PMA action on the pHi of HL60 and U937 cells are presented in fig.2. ED_{50} values were observed at 5 nM for the two cell types. The ED_{50} value for PMA action on the cell ability to adhere to plastic surfaces was 0.5 nM in the two cell lines (fig.2), i.e. a value 10 times lower than the ED_{50} value for PMA action on pHi. As a consequence, PMA at a concentra-

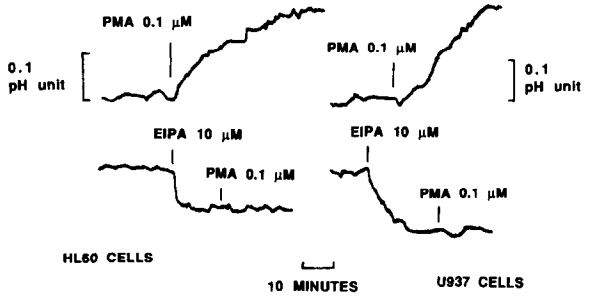

Fig.1. Effect of PMA on the pHi of HL60 and U937 cells. 2',7'-Biscarboxyethyl-5(6)-carboxyfluorescein loaded HL60 cells (left) and U937 cells (right) were suspended in 140 mM Na^+ Earle's salt solution. When indicated, PMA (0.1 μM) or EIPA (10 μM) was added to the cells.

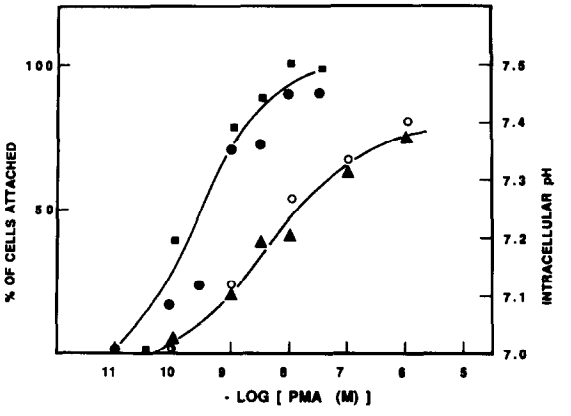

Fig.2. Dose-response curves for PMA action on the pHi and the differentiation of leukemic cells. Cells used were HL60 cells (○,●) and U937 cells (▲, ■). pHi was measured 30 min after the addition of PMA to the cells using [^{14}C]benzoic acid (○, ▲). Each point is the mean of 5 to 6 determinations. Differentiation (■,●) was measured as the percent of cells adhering to plastic surfaces after 2 days of exposure to PMA.

tion of 0.1 nM, induced the adhesion of 17% to 38% of the cells without detectable increase in pHi. This suggests that an increase in pHi may not be necessary for inducing macrophage differentiation in response to PMA.

rIFN produced no change in pHi during the first hours of its exposure. Then pHi increased steadily to reach a maximum after 4 to 5 days (fig.3). The time course of pHi change induced by rIFN closely paralleled the time course of appearance of HLA-DR (fig.3) and of receptor sites for interleukin 2 (not shown). A similar time course of pHi change has been reported for U937 cells that have been treated with retinoic acid [7]. The inset of fig.3 shows the dose response curves for rIFN action on the pHi and on HLA-DR expression. Half maximum effect was observed at 10 IU/ml for both pHi changes and HLA-DR expression.

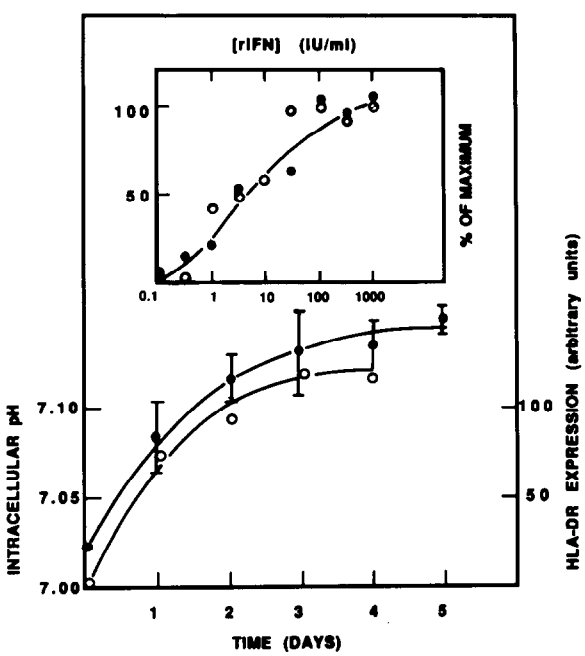

Fig.3. Effect of rIFN on the pHi of HL60 cells. Time course of pHi changes and of appearance of HLA-DR after treatment of HL60 cells with 100 IU/ml of rIFN. pHi (●) was measured using 2′,7′-biscarboxyethyl-5(6)-carboxyfluorescein. Bars indicate the mean ± SD from 3 to 5 determinations. HLA-DR (○) was assayed by flow cytometric analysis of cells labelled with fluorescent anti-HLA-DR antibodies. (Inset) Dose response curve for rIFN action on the pHi (●) and HLA-DR (○). Experiments were performed after 3 days of treatment with rIFN. 100% on the ordinate scale corresponds to 0.13 pH unit. Each point represents the mean from 3 determinations. HLA-DR was determined by radioimmunoassay.

A third situation was found for $1,25\text{-}(OH)_2D_3$. After treatment of HL60 cells with 0.1 μM $1,25\text{-}(OH)_2D_3$, pHi remained stable for 2 days. It increased to 7.20 ± 0.08 (n = 6) at day 3 and then stabilized. Thus the different agents that induce myeloid cells to acquire monocytic properties modify pHi with very different time courses. Variations in pHi are almost immediate with PMA, much slower with retinoic acid [7] and rIFN and still slower with $1,25\text{-}(OH)_2D_3$. It is not known whether the different time courses of pHi change correspond to distinct activation pathways for the Na^+/H^+ exchange system. Finally we confirmed the observations by Carlson et al. [24] showing that *N*-5 disubstituted derivatives of amiloride such as EIPA, at concentrations sufficient to block most of the activity of the Na^+/H^+ exchange system, did not prevent the appearance of the differentiated phenotype.

Taken together the results suggest that an increase in pHi is unlikely to be important as an early intracellular signal for initiating monocytic differentiation. High pHi values probably arise as a consequence of the differentiation process. They might be important for late stages of differentiation or for the expression of functional macrophage properties such as superoxide generation [25,26].

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