Membrane Potential in Human Myeloid Leukemia Cell Line ML-1: Responsiveness of Granulocytic and Monocytic Differentiated Cells

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Abstract. The membrane potential responsiveness of human myeloid leukemia cells (ML-1 line) was studied with the voltage sensitive fluorescent dye diS-C_3-(5). The experimental procedure used in this study enabled us to assess the magnitude of the membrane potential change in cells treated with ouabain, 12-0-tetradecanoylphorbol-13-acetate (TPA) and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), relative to the membrane potential in the untreated control. Inhibition of the Na, K-ATPase by ouabain was followed by a (20 ± 4) mV depolarization. In undifferentiated homogeneous cell population TPA caused a (19.4 ± 4.4) mV depolarization while FMLP had virtually no effect. Cells in which granulocytic or monocytic differentiation was induced by retinoic acid or 1,25-dihydroxyvitamin D_3 exhibited under the effect of TPA a (57.8 ± 7.1) mV and (34.8 ± 10.9) mV depolarization, respectively. A very small transient depolarization was also observed up on treating of the cells with FMLP. The changes in the membrane potential responsiveness in the induced cells are obviously connected with the cell differentiation.

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Introduction

Membrane potential plays an important role in many cellular activities such as the transport of charged substance, stimulation of cell proliferation, etc. Changes in membrane potential occur after binding of various ligands to membrane receptors. In neutrophil granulocytes, for instance, these changes can be induced by phorbol esters or chemotactic peptides (Whitin et al. 1980; Seligmann and Gallin 1980).

We studied the membrane potential response to phorbol ester and chemotactic peptides in the human myeloid leukemia cell line ML-1 in which either granulocytic or monocytic differentiation was induced by retinoic acid (RA) or 1,25-dihydroxyvitamin D_3 (vitD), respectively. The membrane potential monitoring in ML-1 cells was based on the indirect fluorescence method using the cationic cyanine dye diS-C_3-(5) (e.g. Sims et al. 1974; Bashford 1981). In general, this method of assessment of membrane potential is not free of problems, which concern mainly the toxicity of diS-C_3-(5) and the contribution of mitochondrial potential to the probe fluorescence signal (e.g. Johnstone et al. 1982; Chused et al. 1986 and references therein) as well as certain difficulties inherent in quantitative calibration of the voltage-sensitive probe fluorescence response (e.g. Hoffman and Laris 1974; Laris et al. 1976; Hladky and Rink 1976). A modified theory of the diS-C_3-(5) fluorescence response to membrane potential will be presented elsewhere (Plášek and Hrouda 1990), according to this a linear relationship exists between the logarithmic increment of the dye fluorescence intensity in cell supernatant, $\ln(I/I^\circ)$, and the underlying change in plasma membrane potential, $\Delta\psi_p = \psi_p - \psi_p^\circ$. This theory was tested in human myeloid leukemia cells (line ML-1) in which membrane potential changes were induced by valinomycin clamping in various K^+ gradients (see also Methods).

Materials and Methods

Reagents. The cyanine dye 3,3'-dipropylthiodicarbocyanine [diS-C_3-(5)] was obtained from Molecular Probes (USA), valinomycin from Calbiochem (Switzerland), N-formyl-methionyl-leucyl-phenylalanine [FMLP], 12-0-tetradecanoylphorbol-31-acetate [TPA], all-trans retinoic acid [RA] and nitroblue tetrazolium [NBT] came from Sigma (USA). 1,25-Dihydroxyvitamin D_3 [vitD] was from Hoffman – La Roche (France), ouabain was purchased by Serva (FRG). The antibiotics were from Spofa (Czechoslovakia), other chemicals were from Lachema (Czechoslovakia).

Cell culturing and induction of differentiation. Cells of the human myeloid leukemia ML-1 cell line were cultured in Iscove's medium supplemented with 10% (v/v) heat inactivated foetal calf serum, 50 IU/ml penicillin, 50 μg/ml streptomycin and 540 μg/ml glutamine in 5% humidified CO_2 atmosphere at 37 °C. The cells were transferred three times a week and kept in the logarithmic phase of growth. To induce differentiation, stock ethanolic solutions of RA or vitD were diluted into the cell medium to the final concentration of 1 μmol/l. The cells were then cultured in these media for 4 days. Their differentiation was monitored by the modified nitroblue tetrezolium (NBT) test. Briefly: the cell sample was incubated for 25 min at 37 °C in a freshly mixed solution of TPA (0.1 μg/ml) with NBT (0.1 mg/ml). The proportion of NBT-positive, dark cells was then scored in a total of 200 cells (Haškovec et al. 1990).

Fluorescence measurements. Cell samples were washed three times (120g × 10 min) in a buffer containing: NaCl (137 mmol/l), KCl (5.4 mmol/l), $CaCl_2 . 2H_2O$ (1.43 mmol/l), $MgSO_4 . 7H_2O$ (0.8 mmol/l), $Na_2HPO_4 . 12H_2O$ (1 mmol/l), HEPES (10 mmol/l), glucose (5 mmol/l), pH adjusted to 7.35. Afterwards the cell suspension, concentrated to the density of $2-5 \times 10^6$ cells/ml, was maintained at 4 °C. Before fluorescence measurement the cells were shaken for 10 min at 37 °C. The cell concentration was then adjusted to $1-2 \times 10^6$ cells/ml and the diS-C_3-(5) stock solution in ethanol (1 mmol/l) was added to the sample to give a final concentration of 0.5—2 μmol/l. Then the labeled suspension was left to equilibrate for 5 min at 37 °C. The equilibrium fluorescence intensity in a set of identical samples was reproducible within the range ±15%, relative to its mean value.

Changes in the dye fluorescence intensity were measured with a filter fluorimeter Spekol 10/FK (Carl Zeiss Jena, GDR) equipped with a thermostatted cuvette chamber. A photomultiplier 65 PK 414 (Tesla, Czechoslovakia) was used for the detection of fluorescence. The fluorescence signal was recorded by an X-Y recorder BME, model 79 812 (Hungary). The excitation light (tungsten lamp) was set by the instrument monochromator at the wavelength of 620 nm. The emission wavelength range was selected by interference filter at (650 ± 10) nm. Fluorescence measurements were performed in glass cuvettes at 37 °C. All the procedural hints recommended by Hladky and Rink (1976) and Tsien and Hladky (1978) were adhered to.

Calibration of the dye fluorescence response with K^+-equilibrium potential. The quantitative calibration of voltage-sensitive response of diS-C_3-(5) fluorescence in ML-1 cells was performed against a set of K^+-equilibrium potentials. The method is based on the valinomycin clamping of cells in the presence of different gradients of potassium ions (Hoffman and Laris 1974; Hladky and Rink 1976; Rink et al. 1980). We adopted the zero-point technique according to Rink et al. (1980). Valinomycin was added to cells as a 1 mmol/l stock solution in ethanol to a final concentration of 1 μmol/l. Afterwards the K^+ concentration in external medium was raised in fixed steps: usually from 5.4 mmol/l (the concentration of potassium in the standard buffer) to 25.4; 45.4; or 105.4 mol/l. After any manipulation the system was allowed to equilibrate for 4 min at 37 °C. The K^+-diffusion potential in ML-1 cells was calculated according to the Nernst equation

$$\psi_k = (RT/F) \ln([K^+]_{out}/[K^+]_{in}) \quad (1)$$

with the intracellular potassium concentration approximated by the value $[K^+]_{in} = 145$ mmol/l, which was found earlier in a very similar leukemic cell line HL-60 (Gargus et al. 1984). Other symbols have their usual meanings. As demonstrated elsewhere (Plášek and Hrouda, 1990) the logarithmic increment of diS-C_3-(5) fluorescence intensity in ML-1 cell samples can be used as a linear scale for measuring the underlying changes in the plasma membrane potential, provided that the dye-to-cell concentration of the sample is close to the range used in this study. In particular, it was found that the $\ln(I/I^\circ)$ value per 1 mV change in the plasma membrane potential equals 0.0086.

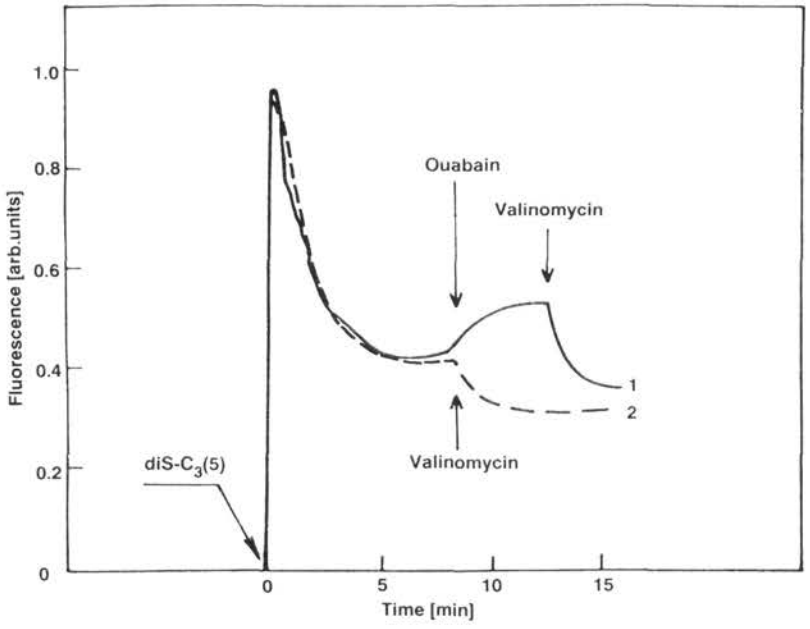

Fig. 1. The typical response of diS-C_3-(5) fluorescence in ML-1 cells to ouabain depolarization: 1 — Sequential effects of ouabain and valinomycin; 2 — Effect of valinomycin alone. Measured at 37 °C in the standard medium ($[K^+] = 5.4$ mmol/l) in samples containing 2 μmol/l diS-C_3-(5) and 1×10^6 cells/ml. The additions of valinomycin (1 μmol/l) and ouabain (15 mmol/l) are indicated by arrows.

Results and Discussion

Effect of ouabain. The addition of ouabain to ML-1 cells was followed by a considerable increase of the diS-C_3-(5) fluorescence intensity which has obviously indicated partial cell depolarization (Fig. 1). This can be attributed to the elimination of the Na,K-ATPase contribution to the membrane potential (Bashford and Pasternak 1984, 1985).

The effect of depolarization consequent upon blocking the Na^+-pump with ouabain was compared to the hyperpolarization induced by valinomycin clamping of ML-1 cells at a low external potassium concentration. This assay was carried out in the standard buffer, $[K^+]_{out} = 5.4$ mmol/l, for which the expected value of K^+-equilibrium potential is $\psi_k = -87$ mV, see Eq(1). When valinomycin was applied after the partial ouabain-induced depolarization, the fluorescence intensity reached a higher value., i.e. the membrane potential was less negative than with valinomycin alone.

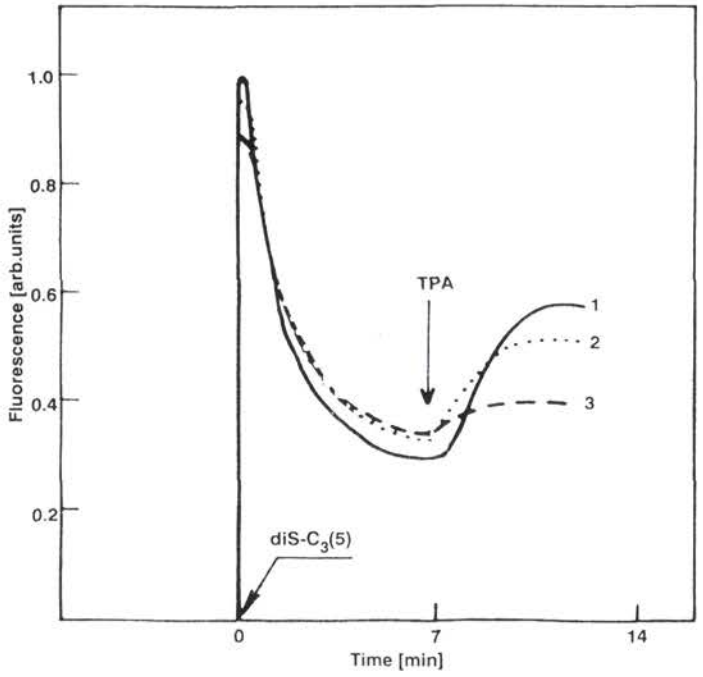

Fig. 2. The response of diS-C_3-(5) fluorescence in ML-1 cells to the action of TPA: 1 — RA-induced cells; 2 — VitD-induced cells; 3 — Undifferentiated control cells. Measured at 37 °C in samples containing 2 μmol/l diS-C_3-(5) and 1×10^6 cells/ml. The addition of TPA (0.1 μmol/l) is indicated by the arrow.

Using the linear calibration scale, (see Methods), the observed effects can be quantified. The voltage changes estimated in the case of valinomycin-induced hyperpolarization in intact and ouabain-treated cells are $-(50 \pm 13)$ mV and $-(32 \pm 14)$ mV, respectively. The mean values ot the voltage change and corresponding experimental errors were calculated from the results of six experiments.

Apparently, for valinomycin-induced hyperpolarization the voltage changes observed at two different experimental conditions do not exceed the limits of the experimental error. This result complies with the Hodgkin—Horowitz equation. In particular, according to it the plasma membrane potential equals a weighted sum of the ion diffusion potentials, $\Sigma \tau \psi_i$, and Δ^{act}, the contribution of the Na^+-pump. i.e. $\psi_p = \Sigma T_i \psi_i + \Delta^{act}$ (Kotyk et al. 1988). Thus the voltage change during the ouabain-induced depolarization can be taken as a reasonable

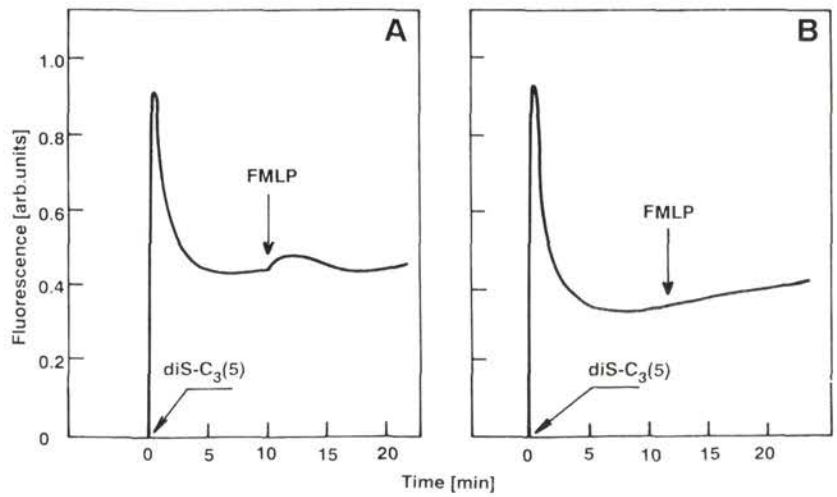

Fig. 3. The response of diS-C_3-(5) fluorescence in ML-1 cells to the action of FMLP: *A* — RA-induced cells; *B* — Undifferentiated control cells. Measured at 37 °C in samples containing 2 μmol/l diS-C_3-(5) and 1×10^6 cells/ml. The additions of FMLP (5 μmol/l) are indicated by the arrows.

estimation of the pump contribution, Δ^{act}, to the plasma membrane potential in the control ML-1 cells. We found the value of $+(20 \pm 4)$ mV.

A likely Δ^{act}-estimation error due to the dye toxicity can be roughly assessed by evaluating the rate of spontaneous depolarization in labelled cells. This effect, if present, will be manifested by a persistent increase of the fluorescence signal (e.g. Fig. 3*B*). Its typical rate was less than 1.5 mV/min in samples containing 1×10^6 cells/ml and 2 μmol/l of diS-C_3-(5). It indicates that the uncertainty in the assessment of Δ^{act} is about 6 mV in a 4 min experiment.

Effect of phorbol ester and of a chemotactic peptide on the membrane potential of differentiated ML-1 cells. The TPA addition to ML-1 cells caused a considerable depolarization in all samples, as revealed by the increase in the diS-C_3-(5) fluorescence (Fig. 2). The effect of TPA was much higher in the RA- and vitD-induced cells than in the undifferentiated controls. Based on the results of three experiments, the magnitudes of this depolarization were estimated. Values of (57.8 ± 7.1) mV; (34.8 ± 10.9) mV; and (19.4 ± 4.4) mV were measured for RA-induced, vitD-induced and control samples, respectively.

The response to TPA apparently coresponds to the proportion of NBT-positive cells in the culture. The percentages of the NBT-positive cells in RA-

induced, vitD-induced and control cells were 85; 50; and 0 %, respectively. In RA-induced cells the most pronounced depolarization occurred after the TPA treatment. Unfortunately, the process of the dye redistribution between various cell subpopulations in heterogeneous samples is likely to occur if their respective membrane potentials are different. Hence the individual contribution of a particular subpopulation to the resultant fluorescence signal cannot be measured. Nevertheless, we may conclude that the mean value of TPA stimulated depolarization is much more pronounced in the differentiated ML-1 cells than in the undifferentiated control (at least in RA-induced cells).

In contrast to TPA, no response to the FMLP action was observed in the control undifferentiated homogeneous cell population. The diS-C_3-(5) fluorescence in these samples exhibited only a monotonous increase independent of the FMLP addition. We attributed it to an advancing cell depolarization caused by the dye toxicity (Fig. 3*B*). In both RA- and vitD-induced cells, however, the chemotactic peptide elicited a transient partial depolarization which lasted about 4 min. It was manifested by a modest enhancement of the fluorescence intensity over its persistent increase caused by the dye toxicity (Fig. 3*A*). For RA-induced cells, an apparent voltage change that can be attributed to the maximal increase of the probe fluorescence intensity is (5.3 ± 3.1) mV. For vitD-induced cells a similar change in the membrane potential was observed (data not shown).

Our results obtained on both monocytic and granulocytic differentiated ML-1 cells extend the findings on TPA-stimulated granulocytes (Whitin et al. 1980) in which the changes in membrane potential consequent upon the stimulation were found to be associated with the production of O_2^- (as reflected by the results of NBT test). The observed effects also resemble the situation found in myeloid precursor cells (Sullivan et al. 1987), which did not respond to TPA in contrast to mature granulocytes, and in HL-60 cell line in which the response of the membrane potential to TPA increased on induction of granulocytic differentiation (Whitin et al. 1980; Newburger et al. 1983; Brown et al. 1984). The type of response of the ML-1 cells to FMLP is similar to that found in mature granulocytes (Seligmann et al. 1980; Sato et al. 1986; Sullivan et al. 1987).

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