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Changes in the kinetics of inositol transport during TPA-induced differentiation of HL60 cells towards monocytes

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When exposed to the phorbol ester TPA, HL60 cells undergo growth arrest and differentiate towards monocytes. During TPA-induced differentiation there was a 2.6-fold increase in the rate of inositol transport (V_{max}), a 2.1-fold increase in intracellular inositol and a 1.5-fold increase in inositol lipid. An increase in the V_{max} of inositol transport did not occur when the variant cell line HL60Ast3 was exposed to TPA, which has been shown in this cell line to induce growth arrest but not differentiation. This observation suggests that the change in inositol transport during HL60 monocyte differentiation is specifically associated with the process of cell differentiation as opposed to growth arrest.

Inositol transport; Monocyte differentiation; HL60 cell; Phorbol ester

1. INTRODUCTION

The HL60 cell lines is a well established model for studying the morphological and biochemical changes, including those in inositol metabolism, which occur during myeloid differentiation [1–4]. Exposure of HL60 cells to DMSO induces differentiation towards neutrophil-like cells [1] and it has been shown that this is accompanied by a 3.5-fold increase in the rate of inositol transport and an 11-fold increase in the intracellular concentration of inositol [4]. In HL60 cells it has been shown that the intracellular concentration of inositol is controlled, at least in part, by the rate of transmembrane inositol transport [4]. These observations have led to the suggestion that regulation of the rate of inositol transport, so as to effect changes in the intracellular inositol concentration, may have a role in the process of cell differentiation.

In this study we have investigated the role of inositol transport in the TPA-induced differentiation of HL60 cells towards monocytes [2]. We have also studied the changes in inositol transport when the variant cell line HL60Ast3 was treated with TPA. When exposed to TPA HL60Ast3 cells undergo growth arrest but do not differentiate [5] and therefore these cells allow the processes related to growth arrest to be clearly delineated from those associated with cell differentiation.

Abbreviations: DMSO, dimethylsulphoxide; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

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2. MATERIALS AND METHODS

2.1. *Cell culture and treatment of cells with TPA*

HL60 and HL60Ast3 cells were cultured as described previously [5]. To induce differentiation 10 nM TPA was added to cells at 5×10^5 cells/ml and differentiation was assessed by the cells' ability to phagocytose complement coated yeast [6] and their expression of A-naphthyl-acetate-esterase activity (ANAE) [7].

2.2. *Inositol transport assay*

This assay has been described and validated previously [4]. In this study the cells were harvested by centrifugation through an oil mixture composed of 70% (v/v) diphenylmethane and 30% (v/v) dibutylphthalate oil.

2.3. *Extraction of inositol and inositol derivatives*

The extraction of inositol and inositol phosphates from HL60 cells has been described previously [4]. Extracts were applied to a Dowex anion-exchange column (formate form) and the inositol-containing fraction eluted with distilled water. The inositol phosphates were eluted as a single fraction using 2 M ammonium formate/0.1 M formic acid. Inositol lipids were extracted from the cell pellet as described previously [4].

2.4. *Measurement of inositol concentration in media*

Inositol was measured using the chemiluminescent assay described by Guderman and Cooper [8].

3. RESULTS

Exposure of HL60 cells to TPA induced a rapid arrest of their growth and the induction of monocyte morphology (Fig. 1a). After 72 h of TPA treatment up to 35% of cells phagocytosed yeasts and 77% stained positive for ANAE. HL60Ast3 cells also underwent growth arrest when exposed to TPA, however, these cells failed to differentiate towards monocytes (Fig. 1b). After 48 h exposure to TPA only 6% of HL60 Ast3 cells phagocytosed yeast and 6% were ANAE-positive.

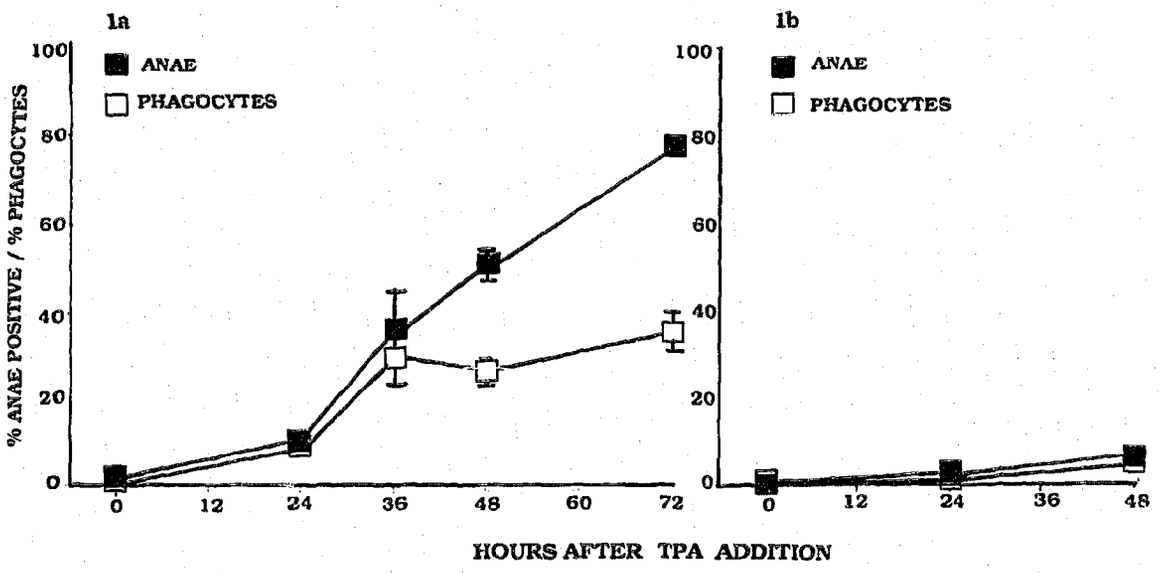

Fig. 1. The development of phagocytic ability and acquisition of ANAE activity in both HL60 (a) and HL60Ast3 (b) cells is shown plotted against time (h) of exposure to 10 nM TPA. Data are the mean ± SE of 5–8 experiments.

After 24 h exposure of HL60 cells to TPA, there was a small but significant increase ($0.01 < P < 0.05$) in the rate of inositol transport (V_{max} which reached a maximum value of 12.0 ± 1.4 pmol/min/10^6 cells at 48 h ($P < 0.01$: 2.6-fold increase, Fig. 2a). The development of a mature phenotype was associated with a small but significant decline in inositol transport V_{max} ($0.01 < P < 0.05$ from the 48 h point: Fig. 2a).

TPA-induced differentiation of HL60 cells towards monocytes was associated with a small but significant rise in the K_m for inositol transport from 77 ± 7 μM ($n = 6$) to 154 ± 18 μM ($n = 6$) ($0.01 < P < 0.05$). In the experiments reported in this study the concentration of inositol in the culture medium was 1.0 mM which was derived principally from fetal calf serum which has a high inositol content (10.5 mM) and which is present in

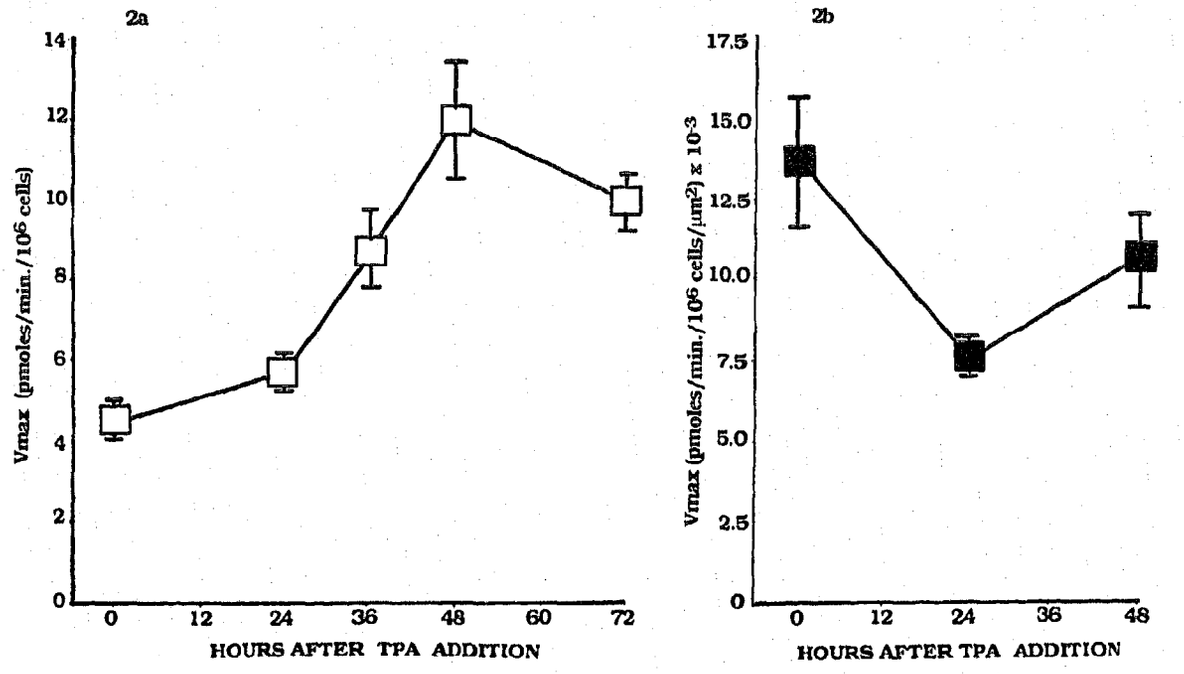

Fig. 2. The V_{max} for inositol transport in HL60 (a) and HL60Ast3 (b) is shown following exposure of the cells to 10 nM TPA. The V_{max} and K_m values for inositol transport were determined from a Hanes plot (S/V vs S). S represents the concentration of inositol in the range 0–100 μM and V equals the rate of inositol transport in pmol/min/10^6 cells. The assay of inositol transport was as described in section 2 and the R value for all Hanes plots was 0.96–1.00. K_m values appear in the text. Data are the mean ± SE of 6–8 experiments and statistical significance was calculated using a paired Student's t-test.

the cell culture medium at 10%, v/v. The concentration of inositol in the culture medium (1.0 mM) would clearly fully saturate the inositol transporter which has a K_m for inositol in the 0.07–0.15 mM range. Therefore the increase in the K_m value for inositol seen after TPA treatment would not, under the conditions of the experiments reported in this study, influence the overall rate of inositol transport. However, such a change in the transporter's affinity for inositol may be more significant in a physiological situation where the serum concentration of inositol in adults is reported to be 30–50 μM [9]. In contrast, the 3-fold increase in the V_{max} of inositol transport, at saturating concentrations of inositol, would result in a significant increase in the overall rate of inositol transport (3-fold).

It may be predicted from the above observations that TPA-induced differentiation of HL60 cells towards monocytes would be accompanied by an increase in the intracellular content of inositol, inositol lipids and/or inositol phosphates. Data presented in Fig. 3 show the absolute changes in the levels of these compounds in HL60 cells following exposure to TPA. After 48 h exposure of HL60 cells to TPA there was a 2.1-fold increase in the intracellular inositol content (Fig. 3a). Throughout HL60 differentiation towards monocytes there was a small but consistent rise in inositol lipids which reached statistical significance ($P < 0.01$) at the 48 h time point (1.5-fold increase: Fig. 3b). In contrast to the observations with inositol and inositol lipids, the total level of inositol phosphates in HL60 cells was unaffected by exposure to TPA (Fig. 3c).

The V_{max} and K_m values for inositol transport were determined for HL60Ast3 cells treated with TPA. HL60Ast3 cells are considerably larger than HL60 cells and during their growth arrest they undergo a significant reduction in size [5]. This is in contrast to HL60 cells which during monocyte differentiation undergo a relatively small increase in their volume (50% or less). Therefore, the data obtained for V_{max} values for TPA-treated HL60Ast3 cells are expressed in relation to the cell surface area. In contrast to the observations with HL60 cells, when HL60Ast3 cells stopped growing after treatment with TPA there was no change in either the V_{max} (Fig. 2b) or the K_m of inositol transport (0 h = 83.7 ± 6.9 μM (6): 48 h = 86.2 ± 6.9 μM (8)).

4. DISCUSSION

It has been shown that during DMSO-induced differentiation of HL60 cells towards neutrophils there is a 3-fold increase in inositol transport and an 11-fold increase in intracellular inositol [4]. This study demonstrates that there is a similar rise (2.6-fold) in the V_{max} for inositol transport during TPA-induced differentiation of HL60 cells towards monocytes. During both neutrophil and monocyte differentiation of HL60 cells the rise in V_{max} precedes the appearance of cells with a mature phenotype and terminal differentiation

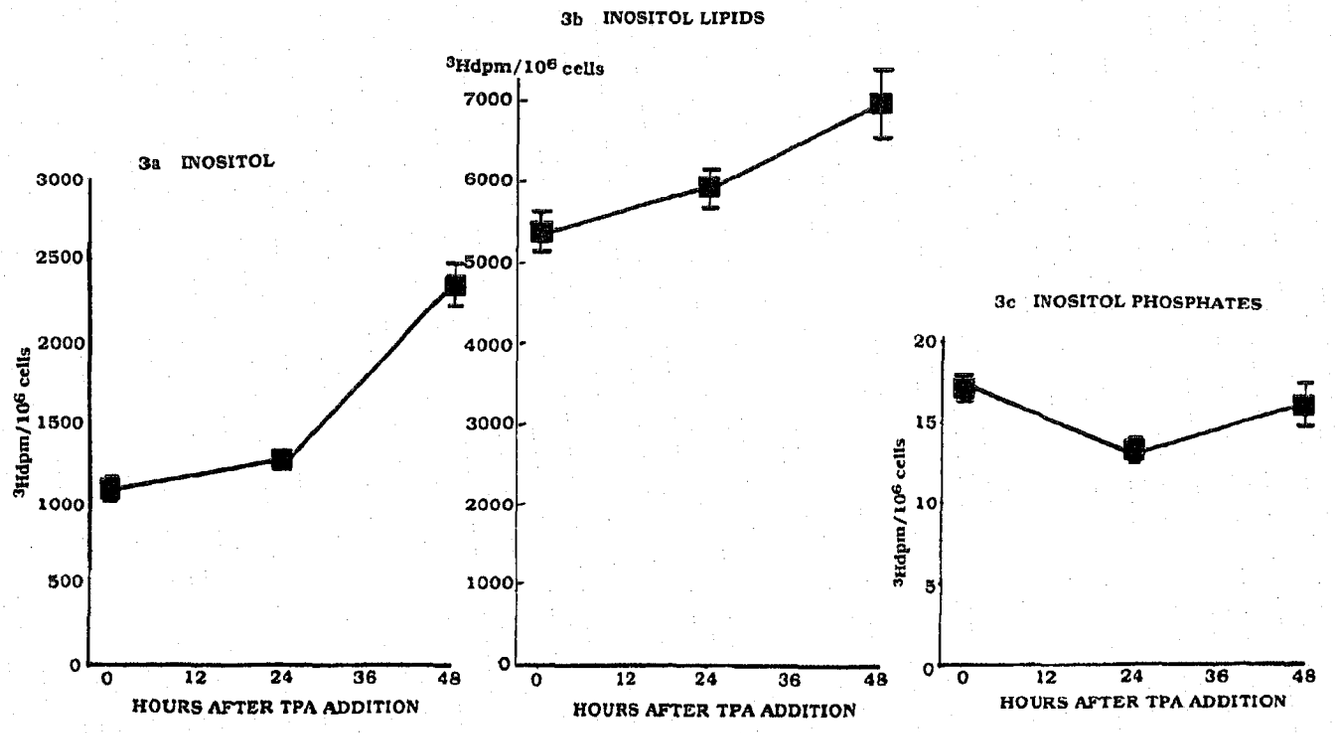

Fig. 3. The amounts of free inositol (a), inositol lipids (b) and total inositol phosphates (c) in HL60 cells are shown following exposure of the cells to TPA (0–48 h). Extraction of inositol and inositol compounds was as described in section 2. D.P.M. are derived from [^{3}H]inositol. Data are mean ± SEM of 6 experiments. Statistical significance of changes in each of the parameters measured was determined using a paired Student's *t*-test.

was associated with a decline in the inositol transport rate towards the level seen in undifferentiated cells [4].

The 2.6-fold rise in the V_{max} of inositol transport which occurs during TPA-induced differentiation of HL60 cells would be predicted to cause a significant increase in the rate of inositol transport, leading to a rise in the intracellular levels of inositol and/or inositol compounds. This assertion is supported by the observations that during TPA-induced differentiation of HL60 cells there was an overall increase in free inositol (2.1-fold) and inositol lipids (1.5-fold). Although these changes in inositol and inositol lipids are similar to those seen during DMSO induced differentiation of HL60 cells towards neutrophils [2,4] the magnitude of change is considerably smaller (11-fold increase in inositol after DMSO treatment vs 2.1-fold increase after TPA treatment). This may reflect differences in the differentiating cells' requirements for inositol which may be dependent on the pathway of differentiation.

Data obtained from studies of the variant cell line HL60Ast3 show that neither the K_m nor the V_{max} of inositol transport are affected following exposure of these cells to TPA. Since TPA induces HL60Ast3 cells to growth arrest but not differentiate [5], the changes in inositol transport observed in HL60 cells differentiating towards monocytes are related to the process of cell differentiation and are independent of cell growth arrest.

Consideration of data from studies of DMSO and TPA-induced differentiation of HL60 cells reveals that an increased capacity for inositol transport (V_{max}) occurs as HL60 cells mature along both pathways of differentiation. The increased rate of inositol transport, with subsequent increases in the intracellular amounts of inositol and inositol lipids, may be an important and general biochemical event which underlies cell differentiation. The level of activity of the inositol transporter appears to be an important regulator of the intracellular concentration of inositol. The mechanisms which regulate the activity of the inositol transporter are currently under investigation.

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