Decrease in equilibrative uridine transport during monocytic differentiation of HL-60 leukaemia: involvement of protein kinase C

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The dose–response curves for the inhibition of equilibrative uridine transport by dilazep, dipyrindamole and nitrobenzylthioinosine (NBMPR) in undifferentiated HL-60 cells were biphasic. Some 70% of the transport activity was inhibited with IC_{50} values of 0.7, 1 and 7 nM respectively. No inhibition of the remaining 30% of transport activity was observed until the dilazep, dipyrindamole and NBMPR concentrations exceeded 1, 0.1 and 3 μ M respectively. Exposure to phorbol 12-myristate 13-acetate (PMA) for 48 h, to induce monocytic differentiation, caused a 20-fold decrease in V_{max} of both NBMPR-sensitive and NBMPR-insensitive equilibrative uridine transport. The decrease in NBMPR-sensitive uridine transport induced by PMA corresponded to a decrease in NBMPR binding sites. A 30%

decrease in specific NBMPR binding sites occurred within 6 h of PMA exposure, and could be prevented by uridine and thymidine at concentrations as low as 100 μ M, and by staurosporine at 40 nM. However, the protective effects of these compounds diminished with prolonged PMA exposure. No protection was observed with uracil. Exogenous protein kinase C (PKC) in the presence of ATP and PMA decreased the number of specific NBMPR-binding sites in purified HL-60 cell plasma membranes. These results suggest that a PKC-induced conformational change in substrate-binding/transporting site may be responsible for the decrease in NBMPR-sensitive nucleoside transport during PMA-induced monocytic differentiation of HL-60 cells.

INTRODUCTION

The uptake of uridine by dimethyl sulphoxide-induced and phorbol 12-myristate 13-acetate (PMA)-induced differentiated human HL-60 promyelocytic leukaemia cells appears to be mediated by two different transport systems (Lee et al., 1990, 1991; Sokoloski et al., 1991; Goh et al., 1993). The first transport system is a broad-specificity transporter which operates bidirectionally in a non-concentrative and energy-independent manner. The second transport system is a concentrative Na^+ -dependent transport system insensitive to equilibrative nucleoside-transport inhibitors such as nitrobenzylthioinosine (NBMPR) and dipyrindamole and only subjected to inhibition by physiological nucleosides. Unlike murine splenocytes (Darnowski and Handschumacher, 1986; Darnowski et al., 1987), however, the Na^+ -dependent nucleoside transport system in undifferentiated HL-60 cells is unable to concentrate intracellular uridine to a level above that present in the medium. Failure to concentrate nucleosides inside the cells despite the presence of the Na^+ -dependent nucleoside transporter was also demonstrated in L1210 murine leukaemia cells (Belt and Noel, 1988; Crawford et al., 1990).

When HL-60 cells are induced to differentiate along either the granulocytic or the monocytic pathway, a marked increase in the Na^+ -dependent transport affinity for uridine occurs (K_m values decrease from 44 μ M to less than 2 μ M) (Lee et al., 1990, 1991; Sokoloski et al., 1991). Simultaneously, a decrease in the rate of equilibrative transport and metabolism for uridine and other nucleosides is observed in mature cells (Gordon and Rubin, 1982; Chen et al., 1986; Stoeckler and Li, 1987; Takimoto et al., 1988, 1989; Lee et al., 1990, 1991). The balance of these changes results in formation of intracellular uridine pools. Thus the failure to concentrate nucleosides in undifferentiated HL-60 cells may be due to both rapid metabolism and high equilibrative transport rates of these cells.

In this study, an attempt was made to characterize the transport properties of the equilibrative nucleoside transporter(s) in HL-60 cells. Both uridine transport and NBMPR-binding activities were evaluated during the induction of monocytic differentiation of these cells by PMA. Two distinct populations of equilibrative transport systems were observed. They are distinguished on the basis of their sensitivity to inhibition by equilibrative nucleoside transport inhibitors such as NBMPR, dilazep and dipyrindamole.

EXPERIMENTAL

Materials

[5- 3H]Uridine (20 Ci/mmol), [3H]NBMPR (25 Ci/mmol) and [^{14}C]sucrose (475 mCi/mmol) were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.). 3H_2O (18 μ Ci/mmol) was purchased from Amersham (Arlington Heights, IL, U.S.A.). PMA, staurosporine, NBMPR, nitrobenzylthioguanosine (NBTGR), dilazep and dipyrindamole were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Protein kinase C (PKC) α (1.5 μ mol/min per mg of protein) was obtained from Gibco (Gaithersburg, MD, U.S.A.). All other reagents were of analytical grade.

Cell culture

Human HL-60 promyelocytic leukaemia cells were cultured and induced into monocytic differentiation as described previously (Lee et al., 1991), with slight modification. Briefly, the cells were maintained in continuous culture in RPMI 1640 medium (Gibco), supplemented with 10% heat-inactivated (50 $^{\circ}C$ for 30 min) fetal-bovine serum (Gibco) plus 50 units/ml penicillin and 50 μ g/ml streptomycin (Gibco) until passage 65. Cell differentiation was induced by incubating HL-60 cells at an initial density of 2×10^6 cells/ml with 200 ng/ml PMA for various time intervals.

[³H]Uridine transport

Cells were harvested and prepared as described previously (Lee et al., 1991). The uptake of [³H]uridine (final concn. 50 μ M, at 22–24 °C) was initiated by simultaneously injecting 20 μ l of cell suspension [(8–9) $\times$ 10⁵ cells] and 40 μ l of [³H]uridine, by using a Biohit Proline electronic pipettor (Biohit OY, Helsinki, Finland), into an 'oil-stop' tube (Lee et al., 1991). After 5 s, the cell mixture was separated from the transport medium by centrifugation in a Beckman model E microcentrifuge. This time point was chosen as it was within the initial linearity of the transport curve, and no metabolites other than uridine were detected as analysed by h.p.l.c. (results not shown). In inhibition studies, test compounds and [³H]uridine were added simultaneously, except for NBMPR, dipyridamole and dilazep, which were preincubated for 15 min with the cells. Studies using [³H]NBMPR were performed in parallel to determine the free NBMPR concentration. The detailed composition of the final incubation medium is given in each Figure legend. 'Time zero' values for transport, attributed to radioactivity trapped in the cell pellet, were determined by centrifuging a cell suspension (20 μ l) through a layer of [³H]uridine (40 μ l) layered over the oil in an 'oil-stop' tube. These values were subtracted from measurements of uptake by HL-60 cells. The intracellular volume of HL-60 cells was calculated in all experiments by using ³H₂O to determine total water space, and extracellular volume was computed by using [¹⁴C]sucrose as a measurement of extracellular space. For experimental purposes, equilibrative transport of uridine is defined as the difference between the intracellular concentration of [³H]uridine in cells suspended in Na⁺-free medium (choline replacement) treated with or without 20 μ M dipyridamole.

[³H]NBMPR binding to HL-60 cells

Equilibrium [³H]NBMPR-binding assays were initiated by incubating 20 μ l of cell suspensions [(8–9) $\times$ 10⁵ cells] with graded concentrations of [³H]NBMPR (0.1–14 nM) in a total volume of 0.2 ml at 22–24 °C in the absence or presence of 20 μ M NBTGR as a competing non-radioactive ligand. To ensure that the studies were performed under true equilibrium conditions, a long incubation time of 60 min was used. We had found that an incubation time of 30 min is insufficient, as this resulted in a curvilinear relationship in Scatchard analysis of the data (results not shown). The reactions were stopped by the 'oil-stop' method. Specific binding is defined as the difference in membrane content of [³H]NBMPR in the presence and absence of 20 μ M NBTGR.

[³H]NBMPR binding to HL-60 cell plasma membranes

Plasma membranes were separated on sucrose gradients after homogenization of the cells in 1 mM ZnCl₂ by the method described by Bardin and Johnstone (1978). To study the involvement of PKC, the purified plasma membranes, in 20 mM Hepes (pH 7.4), 10 mM MgCl₂, 0.2 mM EGTA, 0.5 mM CaCl₂, 1 mM ATP and 100 μ g/ml phosphatidylserine, were exposed to PMA (10 μ g/ml) in the absence or presence of PKC α (125 ng/ml) for 3 h at 37 °C. [³H]NBMPR binding was measured as described above by using the membrane filtration method (Lee and Jarvis, 1988a).

Data analysis

Kinetic constants of mediated transport of uridine (apparent K_m and V_{max}) and site-specific binding of NBMPR (apparent K_d and

B_{max}) were analysed with computer programs 'Ligand' (McPherson, 1985) and 'GraphPad Inplot' (GraphPad Software, 1992).

RESULTS

Effect of equilibrative nucleoside-transport inhibitors on uridine transport by HL-60 cells

The dose-response curves for inhibition of equilibrative uridine transport by NBMPR, dipyridamole and dilazep in undifferentiated HL-60 cells were compared. Figure 1 shows that the inhibition curves for these inhibitors were biphasic. About 70 % of the transport activity was inhibited by dilazep, dipyridamole and NBMPR, with IC₅₀ values of 0.7, 1 and 7 nM respectively. No further inhibition of the remaining 30 % of transport activity was observed until the dilazep, dipyridamole and NBMPR concentrations exceeded 1, 0.1 and 3 μ M respectively. The wide plateaus in these inhibition curves of uridine transport suggest that there are two mechanisms for equilibrative uridine transport by undifferentiated HL-60 cells. They can be distinguished on the basis of their sensitivity to inhibition by equilibrative nucleoside-transport inhibitors. Thus further characterization of these two transport components was performed by using cells pretreated with or without 100 nM NBMPR. Transport activity remaining in the presence of 100 nM NBMPR was defined as NBMPR-insensitive, whereas the difference between transport rates determined in the presence and absence of 100 nM NBMPR represents NBMPR-sensitive transport.

Effect of nucleosides on NBMPR-sensitive and NBMPR-insensitive uridine transport

The ability of other nucleosides to interact with the uridine-transport mechanisms in undifferentiated HL-60 cells was studied by investigating the effect of adenosine, inosine, guanosine, thymidine and cytidine on uridine influx by NBMPR-treated and untreated cells. Table 1 shows that adenosine was the most effective inhibitor of NBMPR-sensitive transport of uridine, with an IC₅₀ value of 170 $\pm$ 35 μ M. Adenosine, inosine and thymidine, however, are equally effective in inhibiting NBMPR-insensitive uridine transport, with IC₅₀ values of 130 $\pm$ 20, 110 $\pm$ 5 and 110 $\pm$ 40 μ M respectively.

Figure 1 Effect of NBMPR, dilazep and dipyridamole on equilibrative uridine transport by undifferentiated HL-60 cells

HL-60 cells were preincubated with various concentrations of NBMPR (■), dipyridamole (○) or dilazep (●) for 15 min before the addition of 50 μ M [³H]uridine. The results are expressed as a percentage of the control influx rate (measured at 5 s uptake intervals) versus the inhibitor concentration. Data are means $\pm$ S.E.M. of three separate experiments each performed in triplicate.

Table 1 Effect of nucleosides on NBMPR-sensitive and NBMPR-insensitive transport of uridine by undifferentiated HL-60 cells

[³H]Uridine (final concn. 50 μ M) and nucleosides were added simultaneously to HL-60 cells treated with or without NBMPR (100 nM). After 5 s at 22–24 °C, uridine transport was terminated as described in the Experimental section. NBMPR-sensitive transport was defined as the difference between the total transport rates and the transport rates determined in the presence of 100 nM NBMPR. NBMPR-insensitive transport was defined as transport in the presence of 100 nM NBMPR after subtracting the background determined in the presence of 20 μ M dipyrindamole. Values are means $\pm$ S.E.M. of at least three separate experiments, each performed in triplicate.

Inhibitor	IC ₅₀ (μ M)	
	NBMPR-sensitive	NBMPR-insensitive
Adenosine	170 $\pm$ 35	130 $\pm$ 20
Inosine	700 $\pm$ 130	110 $\pm$ 5
Guanosine	430 $\pm$ 85	590 $\pm$ 60
Thymidine	1430 $\pm$ 310	110 $\pm$ 40
Cytidine	510 $\pm$ 40	1150 $\pm$ 110

Figure 2 Effect of PMA on the NBMPR-sensitive and NBMPR-insensitive uridine transport kinetics

HL-60 cells were cultured in the presence of 200 ng/ml PMA for 0–48 h before starting the transport experiments. Apparent K_m (a) and V_{max} (b) values for NBMPR-sensitive (●) and NBMPR-insensitive (○) transport were determined by using an uptake interval of 5 s with [³H]uridine concentrations ranging from 50 to 600 μ M. NBMPR-insensitive transport was defined as transport in the presence of 100 nM NBMPR after subtracting the linear component determined in the presence of 20 μ M dipyrindamole. NBMPR-sensitive transport was defined as the difference between transport determined in the presence of 100 nM NBMPR and the total uptake rate. Values are means $\pm$ S.E.M. of at least three separate experiments each performed in triplicate.

Effect of PMA on the kinetics of NBMPR-sensitive and NBMPR-insensitive uridine transport

The concentration-dependence of uridine influx in the presence and absence of 100 nM NBMPR was determined in HL-60 cells treated with PMA (200 ng/ml) for 0–48 h. Figures 2(a) and 2(b) show the K_m and V_{max} of both NBMPR-sensitive and NBMPR-

Figure 3 Effect of PMA on the NBMPR equilibrium-binding kinetics

HL-60 cells were cultured in the presence of 200 ng/ml PMA for 0–48 h before starting the [³H]NBMPR (0.1–14 nM)-binding experiments. Apparent K_d (a) and B_{max} (b) values were computed from Scatchard plots by using computer programs. Values are means $\pm$ S.E.M. of three to five separate experiments each performed in triplicate.

insensitive transport components after correcting for the linear component estimated in the presence of 20 μ M dipyrindamole. Exposure to PMA for 48 h causes a 20-fold decrease in V_{max} of both components (Figure 2b), with little change in their apparent K_m values (Figure 2a). The apparent K_m values of uridine influx in undifferentiated cells were 80 $\pm$ 11 and 430 $\pm$ 160 μ M, with V_{max} values of 29 $\pm$ 1 and 23 $\pm$ 5 μ M/s, for NBMPR-sensitive and NBMPR-insensitive transport components respectively. In cells differentiated with PMA for 48 h, the apparent K_m values for NBMPR-sensitive and NBMPR-insensitive transport components were 65 $\pm$ 29 and 445 $\pm$ 145 μ M, with V_{max} values of 1.2 $\pm$ 0.1 and 2.2 $\pm$ 0.4 μ M/s respectively.

Effect of PMA on the kinetics of NBMPR equilibrium binding

The effect of PMA-induced differentiation on the number of NBMPR-sensitive nucleoside-transporter molecules in HL-60 cells was studied by investigating the availability of NBMPR-binding sites on the cell membrane. Figure 3(a) shows that the K_d values for NBMPR binding were not changed significantly in response to PMA. After 48 h of PMA exposure, the apparent K_d value was 0.41 $\pm$ 0.16 nM, compared with 0.52 $\pm$ 0.07 nM at 0 h of PMA exposure. However, a rapid decrease in B_{max} values for NBMPR binding was observed in the presence of PMA (Figure 3b). After 48 h of PMA exposure, the B_{max} value was 12000 $\pm$ 2000 sites/cell, compared with 198000 $\pm$ 12000 sites/cell at 0 h. These results suggest that the decrease in transporter capacity caused by PMA may be explained by a decrease in the number of transporters at the plasma membrane. This decrease in number of transporters could be due to a molecular modification of the transporter on treatment with PMA, which would hinder the recognition of NBMPR by the transporters, or because internalization of these transporters into subcellular membrane fractions had occurred, or a combination of both.

Table 2 Effect of nucleosides and nucleobase on decrease by PMA of NBMPR-binding sites

HL-60 cells were cultured in the presence of 200 ng/ml PMA and various concentrations of uridine, thymidine or uracil in the range 0–10 mM for 6 h. [3 H]NBMPR binding was measured at a concentration of 8 nM. Bound [3 H]NBMPR represents the difference between membrane-associated [3 H]NBMPR in the absence and presence of NBTGR (20 μ M). The results are expressed as a percentage of site-specific NBMPR binding activity in the absence of PMA and substrate. Values are means $\pm$ S.E.M. of four separate experiments each performed in triplicate: *significantly different from the corresponding value determined in the absence of the corresponding substrate (analysis of variance, $P \leq 0.05$).

Substrate concn. (mM)	[3 H]NBMPR bound (% control)		
	Uridine	Thymidine	Uracil
0	67 $\pm$ 13	70 $\pm$ 4	70 $\pm$ 3
0.1	81 $\pm$ 2*	83 $\pm$ 7*	64 $\pm$ 2
1	83 $\pm$ 1*	86 $\pm$ 1*	68 $\pm$ 3
5	96 $\pm$ 9*	91 $\pm$ 8*	72 $\pm$ 8
10	108 $\pm$ 7*	94 $\pm$ 7*	69 $\pm$ 7

Figure 4 Effect of staurosporine on decrease by PMA of NBMPR-binding sites

HL-60 cells were cultured in the presence (●, ○, ▲) or absence (■, □) of 200 ng/ml PMA together with either 5 mM uridine (Urd: ○, □) or 40 nM staurosporine (STSP: ●) for 6 h (a) and 24 h (b) before starting the binding experiments. The data are representative of three to five separate experiments each performed in triplicate. Free NBMPR is expressed in units of nM.

Effect of uridine, thymidine and uracil on decrease by PMA of NBMPR-binding sites

To investigate whether the decrease in NBMPR binding sites is due to the molecular modification of the substrate-recognition sites by PMA, HL-60 cells were cultured in the presence of 200 ng/ml PMA and various concentrations of uridine, thymidine and uracil (0–10 mM) for 6 h. Table 2 shows that the decrease in total specific NBMPR binding sites, at a [3 H]NBMPR concentration of 8 nM, was effectively prevented by both uridine and thymidine. At initial extracellular nucleoside concentrations of 5 mM, no decrease in the number of NBMPR-binding sites

Figure 5 Effect of exogenous PKC on NBMPR binding in purified plasma membranes

Purified HL-60 plasma membranes were treated with PMA (10 μ g/ml), ATP (1 mM) and Ca^{2+} (0.5 mM) in the presence (●) or absence (○) of PKC α (125 ng/ml) for 3 h at 37 $^{\circ}\text{C}$, after which the [3 H]NBMPR binding was measured by the membrane filtration method. Data are representative of three separate experiments each performed in triplicate. Free NBMPR is expressed in units of nM.

was observed in the presence of PMA. Some protection was also observed with initial nucleoside concentrations of 100 μ M, even though little of these nucleosides were left in the culture medium at the end of 6 h incubation (h.p.l.c. results not shown). No prevention of the decrease in NBMPR-binding sites by PMA was observed with uracil at concentrations as high as 10 mM.

Protective effect of uridine and staurosporine on NBMPR-binding sites on prolonged exposure to PMA

HL-60 cells treated with or without 5 mM uridine or 40 nM staurosporine (a PKC inhibitor) were exposed to 200 ng/ml PMA for 6 or 24 h. Figure 4(a) shows Scatchard plots of the data at 6 h of PMA exposure. Uridine and staurosporine effectively prevented the decrease in NBMPR-binding sites in cells treated with PMA. However, both uridine and staurosporine only partially prevented the decrease in NBMPR-binding sites on prolonged exposure (24 h) to PMA (Figure 4b). Uridine (5 mM) alone caused no down-regulation of NBMPR-binding sites.

Effect of exogenous PKC on NBMPR binding

To study further whether molecular modification of nucleoside transporters was occurring by activation of PKC, purified HL-60 cell plasma membranes were treated with pure PKC purified from baculovirus-infected insect cells expressing rabbit brain PKC α (Goswami and Glazer, 1991). PKC significantly decreased the B_{max} values of NBMPR-binding sites (Figure 5); the decrease was approx. 15% after 3 h of incubation (B_{max} values of 2.0 ± 0.1 and 2.4 ± 0.1 pmol/mg of protein for PKC-treated and untreated membranes respectively). PKC had no effect on the dissociation constants (apparent K_d values of 1.2 ± 0.2 and 1.1 ± 0.2 nM for PKC-treated and untreated membranes respectively). The controls contained all activators of PKC, but not the enzyme itself. These experiments provided additional evidence to support the hypothesis that a chemical modification of transporters occurs through PKC.

DISCUSSION

There is increasing evidence to show the presence of at least two equilibrative nucleoside-transport systems in most mammalian cell types (Plagemann and Wohlhueter, 1985; Jarvis and Young, 1986; Marangos and Deckert, 1987; Lee and Jarvis, 1988a,b;

Gati and Paterson, 1989; Crawford et al., 1990; Meckling-Gill and Cass, 1992). These transport systems can be distinguished on the basis of their sensitivity towards equilibrative nucleoside-transport inhibitors such as NBMPR. In the literature, they are thus classified as the equilibrative NBMPR-sensitive and equilibrative NBMPR-insensitive transport systems. The NBMPR-sensitive transport is inhibited by nanomolar (1–500 nM) concentrations of NBMPR, whereas the NBMPR-insensitive transport functions in the presence of micromolar concentrations of NBMPR.

The data presented in this paper continue to demonstrate the co-existence of these two distinct equilibrative uridine-transport components. In HL-60 leukaemia cells, the NBMPR-sensitive component is inhibited by nanomolar concentrations of NBMPR, with an apparent K_i of 4.3 nM. The NBMPR-insensitive component was not inhibited by concentrations of NBMPR as high as 1 μ M. The nature of the low-affinity interaction between NBMPR and the nucleoside-transport mechanism(s) is not known. Both the dilazep and dipyrindamole concentration–effect relationships with respect to uridine transport are also biphasic. The apparent K_i values for the inhibitor-sensitive component were 0.43 and 0.62 nM for dilazep and dipyrindamole respectively. This is in agreement with all available evidence to suggest that the NBMPR-insensitive pathway is similarly insensitive to inhibition by dilazep and dipyrindamole (Paterson et al., 1980; Plagemann and Wohlhueter, 1984, 1985).

The possibility that the sensitivity of uridine transport to nucleoside-transport inhibitors was attributable to contaminating cells also needed to be considered. We have therefore isolated and analysed eight independent clones of HL-60 cells. All exhibited similar kinetics of NBMPR binding, number of binding sites and proportion of NBMPR-sensitive and NBMPR-insensitive uridine transport (results not shown). These results indicate that the variation in the expression of these two transport components in HL-60 cells is a relatively stable property.

Aside from their differences in sensitivity towards nucleoside-transport inhibitors, the NBMPR-sensitive and NBMPR-insensitive transport systems in HL-60 cells also differed in their substrate specificity. Uridine and cytidine had a significantly higher affinity for the NBMPR-sensitive system than for the NBMPR-insensitive system. In contrast, inosine and thymidine were significantly less potent as inhibitors of NBMPR-sensitive uridine transport than as inhibitors of NBMPR-insensitive transport, indicating that they may be preferentially transported by the NBMPR-insensitive system. Affinities for adenosine and guanosine for both systems, however, were indistinguishable. These results are in contrast with those obtained from Chinese hamster ovary cells (Plagemann and Wohlhueter, 1984), rat erythrocytes (Jarvis and Young, 1986), guinea-pig synaptosomes (Lee and Jarvis, 1988b), and Ehrlich ascites cells (Hammond, 1991), which showed that substrate affinities for the NBMPR-sensitive system were generally higher than the NBMPR-insensitive system. The reason for this discrepancy is unclear.

PMA-induced differentiation of HL-60 cells by activation of PKC rapidly decreased the number of high-affinity binding sites for NBMPR, with a parallel decrease in uridine uptake capacity. There are several possible explanations for the decrease in transport capacity as a result of PMA treatment. One possible way of regulation of uridine transport by PKC is the translocation of transporters between the plasma membrane and microsomal pools. This possibility has been demonstrated with the adenosine transporter in cultured chromaffin cells (Delicado et al., 1991) and the glucose transporter in erythrocytes (Kitagawa et al., 1989). Alternatively, there may be some molecular changes to the transporter, as demonstrated in chromaffin cells (Delicado and

Miras-Portugal, 1987). In the present study, the direct molecular modification of the transporter seems to play a significant role, as the inhibitory effects of PKC activation can be prevented by nucleosides such as uridine and thymidine. This possibility is further supported by the direct action of pure PKC on HL-60 cell plasma membranes. In the absence of an organized structure and the presence of its effector, PKC decreased the number of specific NBMPR-binding sites. Nevertheless, the data do not rule out a contribution from other possible factors, such as an altered rate of transporter internalization/recycling, or a decreased rate of synthesis/increased rate of breakdown, as an indirect consequence of PKC activation.

Staurosporine has been used as an effective and useful tool to demonstrate the participation of PKC in various biological processes (Tamaoki et al., 1986; Ruegg and Burgess, 1989). However, in this study, the inhibitor failed to block completely the inhibitory effects of PKC activation on prolonged exposure to PMA. According to other studies, the effectiveness of staurosporine in preventing protein kinase-mediated phosphorylation of intracellular substrates is dependent both on the nature of these substrates and on the relative participation of the PKC and the proteolysis-derived protein kinase M (PKM) in the phosphorylation process (Yamamoto et al., 1989; Junco et al., 1990). This is especially relevant in the case of phorbol-ester-stimulated cells, since these diacylglycerol analogues promote a rapid proteolysis of PKC and therefore generate an important amount of PKM (Tapley and Murray, 1985). Unlike PKC, however, PKM is not affected by staurosporine at concentrations up to 1 μ M (Junco et al., 1990). So staurosporine does not block the effects of PMA, but it does significantly inhibit them, indicating that the effects of PMA are at least in part due to PKC activation.

In conclusion, although the equilibrative transport systems have differential sensitivity towards uptake inhibition by NBMPR, the usefulness of NBMPR as a binding probe for nucleoside-transport sites in HL-60 cells may still be valid. It is a property that differentiates multiple forms of nucleoside transport. In addition to PKC, other kinase(s) such as PKM may also be involved in molecular modification of these transporters during PMA-induced differentiation. Whether this is caused by a direct phosphorylation of the transporter remains a subject for further investigation.

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REFERENCES

- Bardin, C. and Johnstone, R. M. (1978) *J. Biol. Chem.* **253**, 1725–1732
- Belt, J. A. and Noel, D. L. (1988) *J. Biol. Chem.* **263**, 13819–13822
- Chen, S. F., Cleveland, J. S., Hollmann, A. B., Wiemann, M. C., Parks, R. E., Jr. and Stoeckler, J. D. (1986) *Cancer Res.* **46**, 3449–3455
- Crawford, C., Ng, C. Y. C., Noel, D. L. and Belt, J. A. (1990) *J. Biol. Chem.* **265**, 9732–9736
- Darnowski, J. W. and Handschumacher, R. E. (1986) *Cancer Res.* **46**, 3490–3494
- Darnowski, J. W., Holdridge, C. and Handschumacher, R. E. (1987) *Cancer Res.* **47**, 2614–2619
- Delicado, E. G. and Miras-Portugal, M. T. (1987) *Biochem. J.* **243**, 541–547
- Delicado, E. G., Sen, R. P. and Miras-Portugal, M. T. (1991) *Biochem. J.* **279**, 651–655
- Gati, W. P. and Paterson, A. R. P. (1989) in *Red Blood Cell Membranes* (Agre, P. and Parker, J. C., eds.), pp. 635–661, Marcel Dekker, New York
- Goh, L. B., Sokoloski, J. A., Sartorelli, A. C. and Lee, C. W. (1993) *Biochem. J.* **294**, 693–697
- Gordon, P. B. and Rubin, M. S. (1982) *J. Membr. Biol.* **64**, 11–21
- Goswami, B. B. and Glazer, R. I. (1991) *Biotechniques* **10**, 626–630
- GraphPad InPlot (1992) GraphPad Software, San Diego
- Hammond, J. R. (1991) *J. Pharmacol. Exp. Ther.* **259**, 799–807
- Jarvis, S. M. and Young, J. D. (1986) *J. Membr. Biol.* **93**, 1–10

- Junco, M. Diaz-Guerra, M. J. M. and Bosca, L. (1990) *FEBS Lett.* **263**, 169–171
- Kitagawa, T., Tanaka, M. and Akamatsu, Y. (1989) *Biochim. Biophys. Acta* **980**, 100–108
- Lee, C. W. and Jarvis, S. M. (1988a) *Biochem. J.* **249**, 557–564
- Lee, C. W. and Jarvis, S. M. (1988b) *Neurochem. Int.* **12**, 483–492
- Lee, C. W., Sokoloski, A. J., Sartorelli, A. C. and Handschumacher, R. E. (1990) *Proc. Am. Assoc. Cancer Res.* **31**, 10
- Lee, C. W., Sokoloski, J. A., Sartorelli, A. C. and Handschumacher, R. E. (1991) *Biochem. J.* **274**, 85–90
- Marangos, P. J. and Deckert, J. (1987) *J. Neurochem.* **48**, 1231–1236
- McPherson, G. A. (1985) *Kinetic, EBDA, Ligand*, Lowry, Biosoft, Cambridge
- Meckling-Gill, K. A. and Cass, C. E. (1992) *Biochem. J.* **282**, 147–154
- Paterson, A. R. P., Lau, E. Y., Dahlig, E. and Cass, C. E. (1980) *Mol. Pharmacol.* **18**, 40–44
- Plagemann, P. G. W. and Wohlhueter, R. M. (1984) *Biochim. Biophys. Acta* **773**, 39–52
- Plagemann, P. G. W. and Wohlhueter, R. M. (1985) *Biochim. Biophys. Acta* **816**, 387–395
- Ruegg, U. T. and Burgess, G. M. (1989) *Trends Pharmacol. Sci.* **10**, 218–220
- Sokoloski, J. A., Sartorelli, A. C., Handschumacher, R. E. and Lee, C. W. (1991) *Biochem. J.* **280**, 515–519
- Stoeckler, J. D. and Li, S.-Y. (1987) *J. Biol. Chem.* **262**, 9542–9546
- Takimoto, T., Kubota, M., Tsuruta, S., Kitoh, T., Tanizawa, A., Akiyama, Y., Kiriya, Y. and Mikawa, H. (1988) *Leukemia* **2**, 443–446
- Takimoto, T., Kubota, M., Tsuruta, S., Kitoh, T., Tanizawa, A., Akiyama, Y. and Mikawa, H. (1989) *FEBS Lett.* **247**, 173–176
- Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F. (1986) *Biochem. Biophys. Res. Commun.* **135**, 397–402
- Tapley, P. M. and Murray, A. W. (1985) *Eur. J. Biochem.* **151**, 419–423
- Yamamoto, S., Kiyoto, I., Aizu, E., Nakadate, T., Hosoda, Y. and Kato, R. (1989) *Carcinogenesis* **10**, 1315–1322

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