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Stimulation of Na^+/phosphate cotransport in LLC-PK_1 cells by 12-*O*-tetradecanoylphorbol 13-acetate (TPA)

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We have tested for the effect of the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) on Na^+/phosphate cotransport in an established epithelial cell line of renal origine (LLC-PK_1). Incubation of LLC-PK_1 cells with TPA produced an increase in Na^+/phosphate (P_i) cotransport. The maximal response was reached at a TPA concentration of 10 ng/ml. Other phorbol esters which have no potency or a smaller one to activate protein kinase C had no effect on Na^+/P_i cotransport. Incubation of LLC-PK_1 cells with 10 ng/ml TPA for 8 h led to a 300% increase in Na^+/P_i cotransport; in the presence of cycloheximide the increase amounted only to a 100% and was reached within 2 h. Kinetic analysis of Na^+/P_i cotransport indicated an increase in the apparent V_{max} without an effect on the apparent K_m. The increased P_i transport was retained in isolated apical vesicles. Na^+-dependent alanine transport into LLC-PK_1 monolayers was affected by TPA administration in a similar manner. TPA had under the chosen experimental conditions no effect on [^{3}H]thymidine incorporation into DNA excluding a general proliferative effect. We conclude that TPA via activation of protein kinase C regulates the number of operating transport systems. As also other Na^+-coupled transport systems are influenced, the TPA effect appears to be related to the expression of a general 'adaptive' alteration of membrane transport in LLC-PK_1 cells.

Introduction

LLC-PK_1, an established epithelial cell line of renal origin [1] contains Na^+-H^+ exchange [2], Na^+/P_i [3–5], Na^+/glucose [6,7] and also Na^+/amino acid cotransport [8,9]. Na^+-H^+ exchange [2], Na^+/glucose [10] and Na^+/P_i cotransport [5] have been characterized in detail in studies with apical vesicles isolated from LLC-PK_1 cells.

The LLC-PK_1 cells express 'adaptive' regulation of P_i transport, i.e., P_i transport is increased at the membrane level after P_i deprivation [11,12]. This transport is regulated by protein synthesis-dependent as well as protein synthesis-independent mechanisms [11,12]. Thus, similar to proximal tubular epithelial cells, LLC-PK_1 cells contain in the apical membrane an Na^+/P_i cotransport system that is capable of 'adaptive' changes. It is important to note, that LLC-PK_1 cells have no or very few receptors for parathyroid hormone and in contrast to a cell line derived from an American Opossum kidney, P_i transport is not inhibited by addition of parathyroid hormone to LLC-PK_1 cells [13].

We and others have recently shown protein kinase C activity in brush border membranes iso-

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Abbreviations: TPA, 12-*O*-tetradecanoylphorbol 13-acetate; PoDBu, 4β-phorbol 20-oxo-20-deoxy-12,13-dibutyrate; DMSO, dimethylsulfoxide; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; P_i, inorganic phosphate.

lated from rat kidney cortex [14,15]; also polypeptide substrates for protein kinase C-dependent phosphorylation could be identified in isolated brush border membranes [14]. It was therefore of interest to us to analyze the effect of phorbol esters – known activators of the protein kinase C [16] – on Na^+/P_i cotransport. The cell line which was selected expresses the adaptive response to P_i deprivation [11,12], but not parathyroid hormone-dependent inhibition of Na^+/P_i cotransport [13]. Thus, the present experiments would allow to test for an involvement of protein kinase C in the 'adaptive' regulation of P_i transport.

We show that LLC-PK_1 cells following the administration of TPA exhibit an increase in Na^+/P_i cotransport. This effect is independent from cell proliferation and can in part be blocked by inhibitors of protein synthesis. Na^+-dependent L-alanine transport is also altered.

Materials and Methods

Cell culture. LLC-PK_1 cells were cultured in Dulbecco's modification of Eagle's minimum essential medium (DMEM) containing 25 mM D-glucose, 44 mM sodium bicarbonate, 5% fetal bovine serum, 5% equine donor serum, 1% non-essential amino acids and penicillin/streptomycin (100 I.U./100 μg per ml). Cells were grown in an atmosphere of 10% CO_2/90% air and subcultured by trypsinization (0.25% trypsin). For serial passages (between passage 160 and 180), cells were maintained in plastic culture flasks (75 cm^2, Corning).

For transport experiments, cells were grown in 35-mm (diameter) petri dishes (NUNC) in 2 ml of complete DMEM medium (seeding density, approx. 10^4 cells/cm^2). After 3 days the cell monolayer had reached approx. 90% confluency. For prolonged cultures the medium was changed every second day thereafter. Apical membranes were prepared from cells grown for 10 days in glass roller bottles (840 cm^2).

Membrane isolation. Apical membranes were isolated by a divalent cation (Mg^{2+}) precipitation method as described previously [5]. Compared to the homogenate, alkaline phosphatase, a marker enzyme for the apical membrane, was enriched 6–8-fold in the purified membrane fraction, whereas ($Na^+ + K^+$)-ATPase, a marker enzyme for basolateral membranes, and marker enzymes for intracellular membrane systems were not enriched (data not shown). There was no difference in enzyme content, enrichment factor and membrane recovery in membrane preparations obtained from either control cells or TPA-treated cells (data not shown).

Transport experiments. The uptake rates for inorganic phosphate (P_i) and L-alanine by intact cells were determined as described earlier [4,11,12]. Transport experiments were performed at room temperature under the following extracellular conditions (mM): NaCl, 138; KCl, 5,4; $CaCl_2$, 2,8; $MgSO_4$, 1,2 and Tris-HCl, 14 (pH 7.4). The substrates $K_2H^{32}PO_4$ (1 μC_i/ml) and L-[^{3}H]alanine (2,5 μC_i/ml) were routinely used at 0.1 mM final concentration. To obtain sodium-free conditions, sodium was replaced isoosmotically by *N*-methylglucamine. Transport was stopped by washing the cell monolayers three times with 5 ml cold buffer (138 mM NaCl/14 mM Tris-HCl, pH 7.4). The monolayers were solubilized in 1 ml 0.5% Triton X-100, and aliquots were used for determining the accumulated radioactivity.

Under the described experimental conditions the Na-dependent uptake rates of P_i and L-alanine were linear for 20 and 3 min, respectively (data not shown). Net sodium-dependent uptake rates were determined as the differences between sodium and *N*-methylglucamine conditions.

Transport of P_i into the isolated apical membranes vesicles was performed in a buffer of 300 mM mannitol, 20 mM Hepes-Tris (pH 7.4). The rates of uptake were determined under the condition of inwardly directed gradients of 100 mM NaCl and 0.1 mM $K_2H^{32}PO_4$. For sodium-free conditions, sodium was replaced by choline chloride. The uptake was stopped by rapid filtration [17,18].

Other methods. Enzymatic assays were performed according to Berner and Kinne [19]. Protein was determined by a modification of the method of Lowry [20] for cell monolayers and by the method of Bradford [21] for isolated membranes. Incorporation of [^{3}H]thymidine was determined according to Moran et al. [22].

Presentation of data. All uptake data were corrected for a blank obtained by immediately stop-

ping the uptake ('zero-time' uptake). Data are expressed as means ± S.D. of quadruplicate (monolayers) or triplicate determinations (vesicles). Typical experiments are presented, which have been repeated with similar results at least twice. For kinetic analysis, regression lines of the Hanes–Woolf plot were calculated by a least-squares fit. The correlation coefficients (r^2) were larger than 0.95. For statistical comparisons we have calculated the P values using the Student's t-test for unpaired data. P values smaller than 0.01 were taken as evidence for a significant difference.

Materials. Cell culture reagents were obtained from Amimed (Basel, Switzerland). All other reagents were of normal analytical grade. Radio-isotopes were purchased from New England Nuclear Corporation (Boston, MA). Phorbol esters (Sigma, St. Louis, MO) were prepared as stock solutions in DMSO (1 mg/ml) and kept at −20°C. For experimental purposes, stock solutions were diluted with DMEM. Amounts of these dilutions were added directly to the culture medium. At 10 ng/ml ($1.6 \cdot 10^{-8}$ M) of TPA the final concentration of DMSO in the medium was 0.01‰.

Results

The effect of TPA (10 ng/ml) on Na^+/P_i cotransport in LLC-PK_1 was analyzed in subconfluent (approx. 90% confluency) cell monolayers (Fig. 1). After 1 h of incubation, TPA had no effect on Na^+/P_i cotransport. However, after prolonged incubation with TPA, Na^+/P_i cotransport was increased; 8 h of incubation with TPA resulted in a 3-fold increase as compared to control cells (incubation in DMSO only). Na^+-independent P_i uptake was not affected by TPA (Fig. 1).

To test for an involvement of de novo protein synthesis in the observed activation of Na^+/P_i cotransport activity (Fig. 1), parallel experiments were performed in the presence of an inhibitor of protein synthesis. Cycloheximide was added to the cells at a concentration of 20 μM together with TPA. This concentration of cycloheximide was shown previously [12] to block the incorporation of L-[^{35}S]methionine into total protein within minutes. As also shown in Fig. 1, cycloheximide blocked to a great extent the effect of TPA on

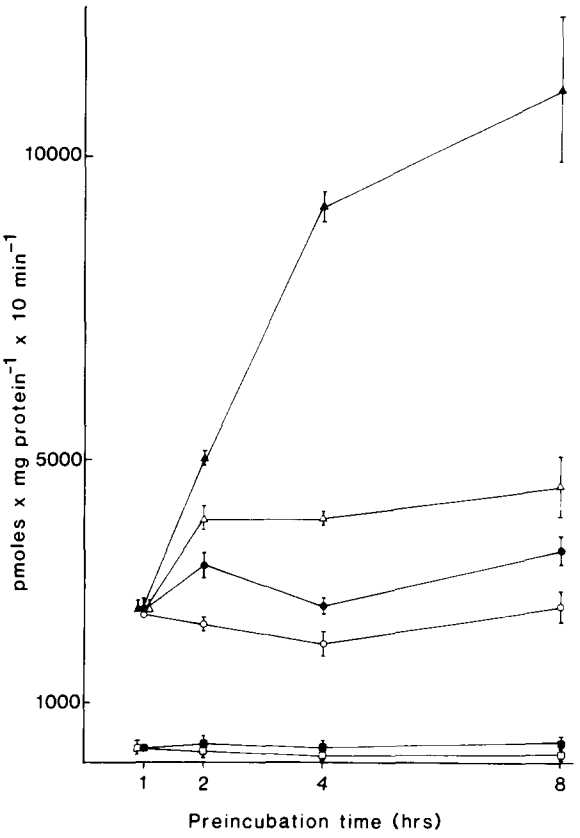

Fig. 1. Effct of TPA on Na^+/P_i cotransport activity in subconfluent LLC-PK_1 cells. Cells were incubated in serum containing DMEM at 37°C either in the presence of 10 ng/ml of TPA or 0.01‰ DMSO (as a control). Where indicated cycloheximide was present at a concentration of 20 μM. P_i uptake was measured either in the presence or absence of NaCl (see methods). ▲———▲, TPA in the presence of NaCl; △———△, TPA and cycloheximide in the presence of NaCl; ●———●, DMSO in the presence of NaCl; ○———○, DMSO and cycloheximide in the presence of NaCl; ■———■, DMSO in the absence of NaCl; □———□, TPA in the absence of NaCl. Each point represents the mean ± S.D. of four determinations.

Na^+/P_i cotransport. However, in the presence of cycloheximide TPA still caused a 2-fold increased Na^+/P_i cotransport as compared to control cells (cycloheximide plus DMSO). Interestingly, also in the presence of cycloheximide, the TPA effect appeared after a lag period of 1 h. In contrast to the increases observed in absence of cycloheximide, the increase in presence of cycloheximide was maximal after 2 h and remained constant over the following 6 h.

It should be noted here that similar findings were obtained with highly confluent cell monolayers (10 days in culture, data not shown). As reported earlier [12], highly confluent LLC-PK_1 cells exhibit a much lower Na^+/P_i-cotransport activity. Similar reduction in transport has also been reported for other transport systems (e.g., Na^+-dependent amino acid uptake, see Ref. 8).

A dose–response relationship for the TPA effect on Na^+/P_i cotransport was obtained after 2 h of treatment with TPA either in the absence or in the presence of cycloheximide (Fig. 2). In agreement with the observations given in Fig. 1 the TPA-dependent increase in Na^+/P_i cotransport is blocked to about 50% at concentrations of 5 ng/ml ($0.8 \cdot 10^{-8}$ M) TPA and higher in the presence of cycloheximide. However, at low TPA concentrations (below 3 ng/ml), the protein synthesis-dependent component of transport activation is minimal. Thus, an apparent difference in sensitivity to TPA of the protein synthesis-dependent and -independent activation of Na^+/P_i cotransport exists.

Fig. 3 demonstrates the effects of different phorbol esters with respect to an increased Na^+/P_i cotransport activity. All phorbol esters were tested at a concentration of 10 ng/ml after 4 h of incubation in the absence or presence of cycloheximide. Among the various phorbol esters tested only TPA showed a specific effect (compared to DMSO-treated cells); equimolar concentrations of 4β-phorbol 12,13-dibutyrate and phorbol 12,13-didecanoate (both having lower tumor promoter activity than TPA, Ref. 23) or 4β-phorbol 20-oxo-2--deoxy-12,13-dibutyrate (showing no tumor activity, Ref. 24) did not stimulate Na^+/P_i cotransport.

Reversibility of the TPA induced increase of Na^+/P_i cotransport in LLC-PK_1 cells was investigated as described in Fig. 4. Cell monolayers were incubated with 10 ng/ml of TPA for 2 h in serum-containing DMEM (in the presence or absence of 20 μM cycloheximide). The cell monolayers were then rinsed three times with serum-containing DMEM and incubated for an ad-

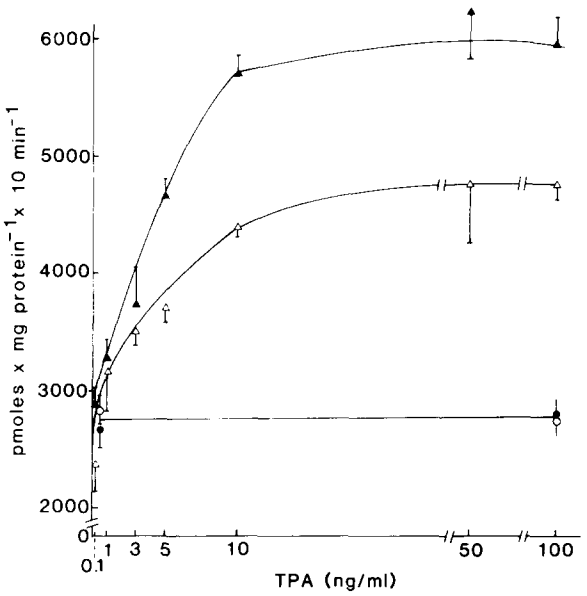

Fig. 2. Effect of different concentrations of TPA on net sodium-dependent P_i transport in LLC-PK_1 cells. Cell monolayers were incubated for 2 h with TPA ranging from 0.1 to 100 ng/ml or as controls with the corresponding amounts of DMSO either in the absence or presence of 20 μM cycloheximide. ▲——▲, TPA; △——△, TPA and cycloheximide; ○——○, DMSO; ●——●, DMSO and cycloheximide. Each point represents the mean ± S.D. of four determinations.

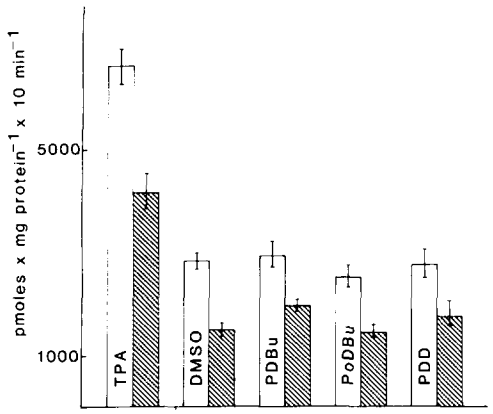

Fig. 3. Effect of different phorbol ester derivatives on Na^+/P_i cotransport. Cell monolayers were exposed to the different phorbol esters for 4 h at 10 ng/ml each in the absence (open bars) or presence (hatched bars) or 20 μM cycloheximide. The results represent the mean ± S.D. of four determinations of net sodium-dependent P_i uptake. PDBu, 4β-phorbol 12,13-dibutyrate; PoDBu, 4β-phorbol 20-oxo-20-deoxy-12,13-dibutyrate; PDD, phorbol didecanoate.

ditional 13 h (15 h total). After removal of TPA no further increase of Na^+/P_i cotransport was observed (Fig. 4A). The 2-fold increase of net sodium-dependent P_i uptake observed after 2 h of TPA incubation remained constant during the following 13 h. In the experiment presented in Fig. 4b, cycloheximide was added together with TPA. In agreement with our initial observation (Fig. 1) cycloheximide largely prevented the further increase in Na^+/P_i cotransport usually observed after 2 h of TPA incubation and reduced minimally the TPA-dependent activation during the initial 2 h. Similar to the observation given in Fig. 4a, removal of TPA did not diminish during the next 13 h the initial (2 h) stimulation of Na^+/P_i cotransport by TPA. These observations suggest that although the TPA can be washed off (no further increase after 2 h in Fig. 4A), the fast and largely protein synthesis-independent increase (see also Fig. 1) is not reversed after TPA removal (Fig. 4A and B).

Table I summarizes a kinetic analysis of Na^+/P_i cotransport into intact LLC-PK_1 cell monolayers after a 2 or 4 h treatment with TPA (10 ng/ml) or DMSO (0.01‰; as control) in the presence and absence of cycloheximide. Saturation kinetics of net sodium dependent P_i uptake was determined using P_i at 25–1000 μM. The data show that after 2 h of treatment with TPA the V_{max} of the uptake increased by a factor of 2, whereas the apparent K_m for P_i did not change. In agreement with our initial observation (Fig. 1), the addition of cycloheximide during the 2 h of incubation with TPA did not reduce sigificantly the V_{max}. The effect of TPA incubation on V_{max} was more pronounced after 4 h of treatment with TPA. Incubation of the cells with TPA for 4 h in the presence of cycloheximide also leads to an increase in the V_{max}. However, there was a significant reduction in V_{max} by the presence of cycloheximide during the 4 h of incubation with TPA. In fact, in the presence of cycloheximide the TPA-dependent increase (4 h of incubation) in V_{max} was similar to that observed after 2 h of incubation with TPA in the presence or absence of cycloheximide.

Na^+/P_i cotransport experiments were also performed with isolated apical membrane vesicles as shown in Fig. 5. Apical membranes were isolated from cells treated for 4 h with TPA (10 ng/ml) or

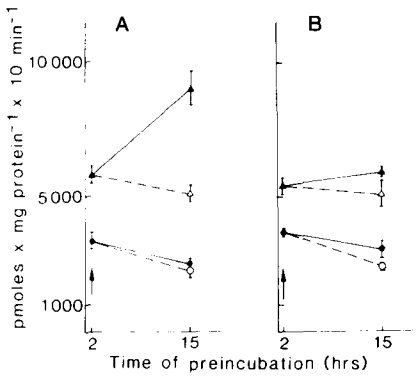

Fig. 4. Reversibility of the TPA-mediated increase of Na^+/P_i cotransport. Panel A: after an incubation with 10 ng/ml TPA (▲, △) or 0.01‰ DMSO (●, ○) for 2 h, half of the monolayers were rinsed three times (↑) with serum-containing DMEM-medium (△, ○). The other half was kept in TPA-, respectively, DMSO-containing medium (▲, ●). At the indicated time points, net sodium-dependnet P_i transport was determined. Panel B: same experiments as in panel A, but in the presence of 20 μM cycloheximide during the first 2 h of incubation. Each point represents the mean ± S.D. of four determinations.

TABLE I

EFFECT OF TPA ON KINETIC PARAMETERS OF PHOSPHATE TRANSPORT IN LLC-PK_1 CELLS

Subconfluent monolayers were treated with TPA (10 ng/ml) or DMSO (0.01‰) for 2 or 4 h. V_{max} and apparent K_m were calculated on the basis of Hanes–Woolf plots of phosphate uptake measured at 7 concentrations between 25 and 1000 μM. The values represent the mean ± S.D. of four deteminations. The values labelled with an asterisk are statistically different from their control values (± TPA).

Preincubation time	Experiment	Apparent K_m for phosphate (μM)	V_{max} (nmol/mg protein per 10 min)
2 h	DMSO	242 ± 46	12.1 ± 1.6
	DMSO + cycloheximide	337 ± 58	11.0 ± 1.5
	TPA	318 ± 40	25.2 ± 2.5 *
	TPA + cycloheximide	332 ± 76	21.3 ± 3.9 *
4 h	DMSO	323 ± 40	15.7 ± 1.4
	DMSO + cycloheximide	267 ± 30	11.3 ± 0.6
	TPA	256 ± 24	36.1 ± 1.7 *
	TPA + cycloheximide	259 ± 22	27.7 ± 1.1 *

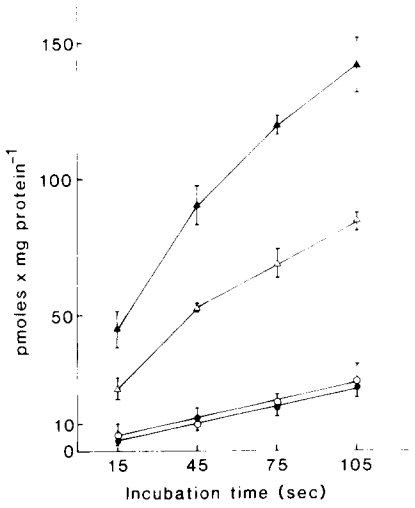

Fig. 5. Effect of TPA treatment of the cells on Na^+/P_i cotransport in apical membrane vesicles isolated thereafter. Cells were treated with 10 ng/ml TPA (▲, ○) or 0.01‰ DMSO (△, ●) for 4 h. Uptake of P_i was determined either in the presence of an inwardly gradient of 100 mM NaCl (▲, △) or 100 mM choline chloride (●, ○). Each point represents the mean ±S.D. of three determinations.

DMSO (0.01‰; as control) in the absence of cycloheximide. Uptake data during the first 105 s are shown, since the equilibrium values were reached only after 18 h of incubation (data not shown). After 4 h of treatment with 10 ng/ml TPA, the Na^+-dependent uptake of P_i into the apical membrane vesicles was found to be increased by a factor of two. TPA treatment of the cells did not lead to a change in the Na^+-independent uptake of P_i in the apical membrane vesicles.

TABLE II

EFFECT OF TPA ON [^{3}H]THYMIDINE INCORPORATION IN LLC-PK_1 CELLS

Subconfluent or confluent monolayers were treated with TPA (10 ng/ml) for 2 (T_2) to 4 (T_4) h. After preincubation, thymidine incorporation into DNA during 30 min was measured. The given values represent the man±S.D. of six determinations. There were no significant differences between DMSO and T_2 or T_4.

	Thymidine incorporation (pmol/mg protein per 30 min)	
	Cells, 3 days in culture	Cells, 10 days in culture
Control (DMSO)	7.8±1.8	4.1±0.9
TPA, 2 h preincubation	6.3±1.1	3.0±0.4
TPA, 4 h preincubation	7.2±2.4	4.3±1.2

We measured also sodium-dependent L-alanine transport in order to test whether other transport mechanisms were affected by TPA administration. Fig. 6 shows that similar to sodium-dependent P_i uptake, sodium-dependent L-alanine transport is also stimulated by TPA incubation. Again, part of the TPA-dependent stimulation is prevented by cycloheximide.

The effect of TPA administration on Na^+-dependent transport of P_i and L-alanine may be an expression of a general stimulation of cell proliferation [8] with a concomitant increase in amino-acid and P_i transport during accelerated cell growth.

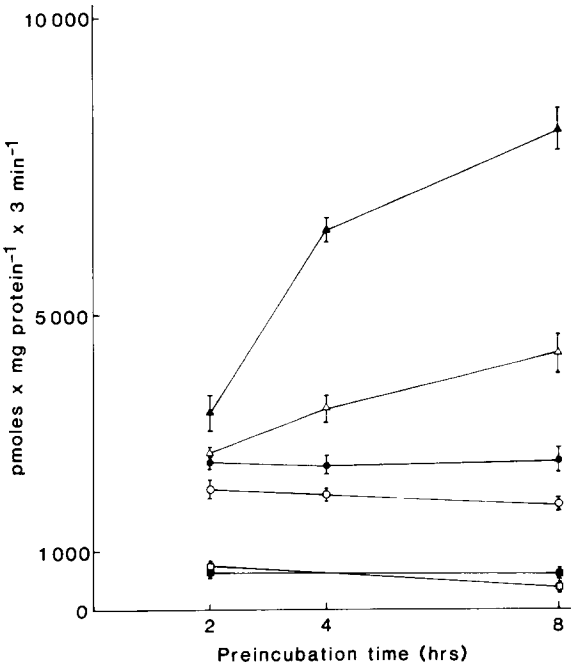

Fig. 6. Stimulation of Na^+-dependent L-alanine uptake into LLC-PK_1 cells by TPA. Three-days-old cell monolayers were treated as described in Fig. 1. L-Alanine transport (0.1 mM) was measured either in the presence of sodium (○, ●, △, ▲) or in the presence of *N*-methylglucamine (□, ■). Cells were treated with 10 ng/ml TPA (▲, □), TPA in the presence of 20 μM cycloheximide (△), DMSO (0.01‰) (●) or DMSO in the presence of cycloheximide (○). Each point represents the mean ± S.D. of four determinations.

This, however, seems unlikely under our conditions, while the TPA effects on P_i transport was also observed in highly confluent cells, i.e., in growth arrested cells (data not shown). Furthermore, [^{3}H]thymidine incorporation was not affected in subconfluent cells (3 days of culture) and highly confluent cells (10 days of culture) ruling out a major proliferative effect of TPA under our experimental conditions (Table II).

Discussion

Two lines of evidences suggest that the TPA-dependent increase of Na^+/P_i cotransport activities of LLC-PK_1 cells is mediated by an activation of protein kinase C. First the concentration of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) required for stimulation of Na^+/P_i cotransport is similar to the reported concentration to activate the protein kinase C [25]. Secondly, TPA, the phorbol ester with a high potency to accelerate protein kinase C activity [23,24], was the only one to affect Na^+/P_i cotransport in LLC-PK_1 cells at concentrations of 10 ng/ml ($1.6 \cdot 10^{-8}$) which were used as the arbitrary concentrations.

We have analyzed Na^+/P_i cotransport activity under initial linear flux conditions, i.e., under constant driving force conditions. However, the difference between transport rate in control cells and that in TPA-treated cells could be related to an increased force, e.g., due to a reduced cellular Na^+ concentration (increased Na^+ gradient) after TPA incubation. This driving force effect could also explain the increase in Na^+-dependent L-alanine influx (Fig. 6). In order to decide whether increased transport would be the result of changes in the P_i transport systems themselves or the consequence of altered driving forces we have performed an experiment with isolated apical vesicles. As shown in Fig. 5, an increased P_i uptake was observed in vesicles isolated from TPA-treated cells. Since this experiment has been performed under conditions of an inwardly directed Na^+ gradient (zero-*trans*) and under conditions approaching initial linear uptake of P_i (15 s), the observed increased transport rate is most likely not the consequence of alterations in the decay rate of the Na^+ gradient (driving force), but rather the result of a change in the P_i-transport characteristics of the membrane. Therefore, we have good evidence that the observed alterations in P_i transport in the experiments with monolayers are – at least in part – related to altered transport properties of the apical membrane, i.e., to an increase in Na^+/P_i cotransport activity. It will be the task of a future study, to analyze the effects of TPA incubation of cell monolayers on all the different properties of apical membrane transport as analyzed in vesicles. It will be certainly most interesting to extend our studies on vesicular Na^+-H^+ exchange, Na^+/alanine cotransport and Na^+/glucose cotransport.

Kinetic analysis showed that increased Na^+/P_i cotransport is characterized by an increase of the V_{max} value with no change in the apparent K_m for P_i (Table I). An increase in the V_{max} of the transport system could be explained either by an activation of preexisting transport systems or by an increase of the number of transport systems in the apical membrane due to a (specific or non-specific) stimulation of protein de novo synthesis. Experiments performed in the presence of cycloheximide have shown that part of the TPA effect on Na^+/P_i cotransport is via a protein synthesis-independent pathway. In the presence of cycloheximide, TPA still enhanced Na^+/P_i cotransport by a factor of two. TPA action showed the same lag-period of 1 h in the presence or absence of cycloheximide. However, the maximal effect of TPA in the presence of cycloheximide was already reached after 2 h of incubation and remained stable thereafter. Therefore, TPA seems to act via two different pathways with respect to a stimulation of Na^+/P_i cotransport in LLC-PK_1 cells. (i) A protein synthesis-independent stimulation of Na^+/P_i cotransport by TPA occurs. This effect was fully developed after 2 h and was related to an increase in the V_{max}, suggesting an activation of preexisting Na^+/P_i cotransport systems. (ii) Prolonged (more than 2 h) incubation of the cells with TPA leads to a further stimulation of transport by increased protein de novo synthesis. This increased protein synthesis, however, is not parallelled by an increased DNA synthesis rate (proliferation) as measured by [^{3}H]thymidine incorporation (see Table II). Interesting observations with respect to this 'biphasic' action of TPA are given in Figs. 2 and 4. Upon removal of TPA

(Fig. 4) a further increase was prevented and the initial increase (2 h) remained during further incubation (13 h) in the presence or absence of cycloheximide. This observation could indicate that it was possible to wash out the TPA (see Fig. 4A). However, it is puzzling that Na^+/P_i cotransport does not return to its control level. As it is rather unlikely that 'fast' and protein synthesis-independent activation is 'irreversible', another explanation seems to be more plausible. We would like to speculate that the TPA sensitivity of the fast and protein synthesis-independent activation requires very small concentrations of TPA, whereas the protein synthesis-dependent portion only occurs at the higher concentrations. This speculation seems to be supported by the dose-response curve given in Fig. 2, where at, e.g., 3 ng/ml of TPA only a minimal protein synthesis-dependent activation occurs, whereas the protein synthesis-dependent stimulation is large at, e.g., 10 ng TPA/ml. Thus, by an incomplete 'removal' of TPA we would abolish only the mechanism with 'low' sensitivity to TPA, i.e., the protein synthesis-dependent portion. Residual amounts of TPA remaining in the system, e.g., in the apical membrane, may guarantee a maintenance of protein synthesis-independent activation. Certainly this speculation requires further experimentation.

The effect of TPA on Na^+/P_i cotransport cannot be regarded as a specific one, since similar effects of TPA were observed on the Na^+-dependent L-alanine transport (Fig. 6). After 2 h of incubation with TPA Na^+-dependent L-alanine, uptake in whole cells was stimulated. This stimulation could also be prevented in part by cycloheximide. In contrast to our observation Amsler et al. [8] reported complete inhibition by cycloheximide for TPA-stimulated aminoisobutyric acid transport in LLC-PK_1 cells. No explanation can be given at present for this apparent discrepancy.

Recently, our laboratory has shown an 'adaptive' response of P_i transport to P_i deprivation in LLC-PK_1 cells. Similar to the TPA effect, we observed a protein synthesis-dependent and a protein synthesis-independent component, the latter being expressed after short periods of P_i deprivation [11,12]. Thus, there might be some similarities between the TPA-dependent and deprivation-dependent activation of phosphate transport. However, in the contrast of P_i deprivation both the suggested pathways of the TPA action seem not to be specific for the Na^+/P_i cotransport system, since Na^+-dependent L-alanine transport was also stimulated. Thus, one might speculate that activation of protein kinase C initiates an 'adaptive' regulation (changes in the number of operating units) of various Na^+-dependent transport systems via protein synthesis-dependent and protein synthesis-independent pathways. In this respect it is certainly of interest to note that a treatment with phorbol esters activates also transport systems in other cell membranes, e.g., Na^+-H^+ exchange [23] and glucose uptake in lymphocytes [26]. However, the present data are not in favor of a major role of protein kinase C activation in specific adaptation of phosphate transport by phosphate deprivation, while phosphate deprivation leads to increase in phosphate transport but not in alanine transport [11,12].

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