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PHORBOL ESTERS INHIBIT PHOSPHATE UPTAKE IN OPOSSUM KIDNEY CELLS: A MODEL OF PROXIMAL RENAL TUBULAR CELLS

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Summary: The effects of phorbol esters and diacylglycerol on phosphate uptake in opossum kidney (OK) cells were investigated to assess the possible role of Ca^{2+}- activated, phospholipid dependent protein kinase (protein kinase C) on renal phosphate handling. OK cells are widely used as a model of proximal renal tubular cells and are reported to possess a Na^{+}-dependent phosphate transport system. Phorbol-12,13-dibutyrate (PDBu) inhibited phosphate uptake. This inhibitory effect was synergistically enhanced with A23187. 4ß-phorbol 12,13-didecanoate inhibited phosphate uptake, while 4α-phorbol 12,13-didecanoate did not. 1-oleoyl-2-acetyl-glycerol (OAG), a synthetic diacylglycerol, also exhibited an inhibitory effect on phosphate uptake. These data suggest the possible involvement of protein kinase C in proximal renal tubular phosphate transport.

We recently reported that phorbol esters and OAG affect the phosphate transport in cultured renal tubular cells and suggested the involvement of protein kinase C in the regulation of renal tubular handling of phosphate (1). It was difficult, however, to investigate the role of protein kinase C in the individual segment of the renal tubules, due to the heterogeneous composition of these cells (a mixture of the cells originating from both proximal distal tubules). The OK cell line, recently developed using a normal kidney from the american opossum (2), is reported to have

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Abbreviations used are: OK cell: opossum kidney cell; PTH: parathyroid hormone; PDBu: phorbol-12,13-dibutyrate; OAG: 1-oleoyl-2-acetyl-glycerol; DMEM: Dulbecco's modified Eagle's medium; IP_3: inositol 1,4,5-triphosphate.

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characteristics corresponding to proximal tubular cell function. Therefore this cell line has become a model for studying the physiologic role of proximal tubules (3,4,5). In the present study, we employed these cell line to investigate the possible involvement of protein kinase C in the phosphate transport of the proximal renal tubules.

Materials and Methods

Cell culture. OK cells were kindly provided by Dr. L. R. Forte, Columbia, MO. The cells were cultured according to the method described recently(4) using DMEM containing 10% fetal calf serum and 100U/ml penicillin. All studies were performed between passages 86 and 92.

Chemicals. 4α- and 4ß-phorbol 12,13-didecanoate, PDBu, and A23187 were purchased from Sigma Chemicals Co. (St. Louis, MO), and the OAG from Avanti Polar-Lipids, INC. (Birmingham, AL). Other reagents were obtained from standard suppliers and were of the highest quality available.

Phosphate uptake study. The phosphate uptake in the cell was measured according to the method of our previous report (6). In brief, after reaching confluence, the cells were incubated at 37°C for 60 min in either the experimental medium (modified DMEM, P 0.3 mM) containing test agents or vehicle alone as a control. The medium was then discarded and the uptake medium containing ^{32}P (1μCi/ml) was added and incubated for 5 min followed by 3 separate washes with normal cool saline solution. The cells were dispersed and ^{32}P was counted by a liquid scintillation counter.

Statistical analysis. The results were expressed as the mean ± S. E. Significant of differences among the groups were determined by Duncan's new multiple range test (7).

Results

Fig. 1 shows the effect on phosphate uptake of 4α-phorbol 12,13-didecanoate, a non-activator of protein kinase C, and 4ß-phorbol 12,13-didecanoate, an activator of protein kinase C (8). While 4α-phorbol 12,13-didecanoate failed to inhibit phosphate uptake at concentrations as high as 500nM, 4ß-phorbol 12,13-didecanoate inhibited phosphate uptake in a dose-dependent fashion, up to 67.0% of control at 500 nM ($P<0.01$, n=8).

As indicated in Fig 2A, PDBu, one of the phorbol esters which activate protein kinase C at 100 ng/ml, also inhibited the phosphate uptake significantly (18.2% inhibition of control, $P<0.01$, n=8). On the other hand,

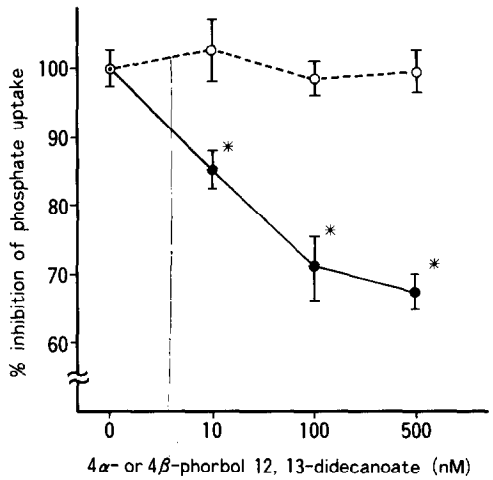

Figure 1. Dose-dependent effect of 4α-phorbol 12,13-didecanoate and 4β-phorbol 12,13-didecanoate on the phosphate uptake in OK cells. 4β-phorbol 12,13-didecanoate inhibited the uptake significantly, while 4α-phorbol 12,13-didecanoate failed to inhibit it. Phosphate uptake without 4α- or 4β-phorbol 12,13-didecanoate was 119.2 ± 1.7 x 10^{-12} moles/5min/10^5cells. Vertical lines represent the mean ± S.E. of 8 different cultures. * P<0.01 significantly different from the control.
(●——●) : 4β-phorbol 12,13-didecanoate.
(○---○) : 4α-phorbol 12,13-didecanoate.

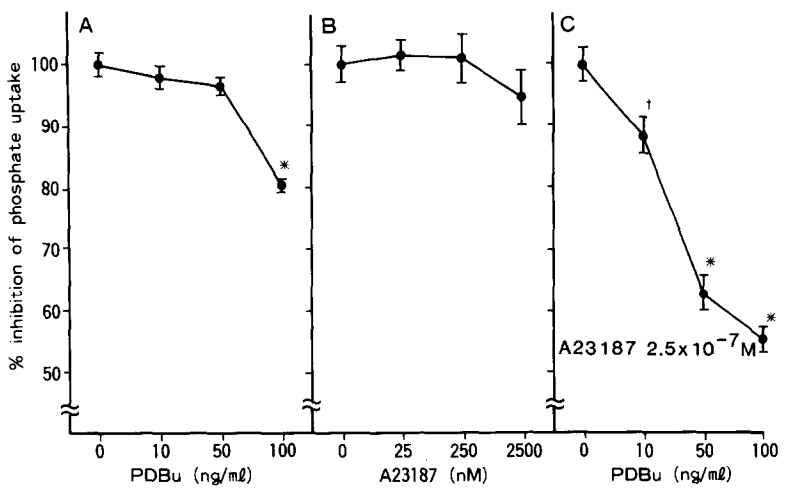

Figure 2. Individual and combined effect of PDBu and A23187 on the phosphate uptake. The effect of PDBu was synergistically enhanced by 2.5 x 10^{-7}M A23187. Phosphate uptake at the basal state was 122.2 ± 2.5 x 10^{-12} moles /5min/10^5cells. Vertical lines represent the mean ± S.E. of 8 different cultures. + P<0.05 , * P<0.01 significantly different from control.

A: effect of PDBu.
B: effect of A23187.
C: combined effect of PDBu and 2.5x10^{-7}M A23187.

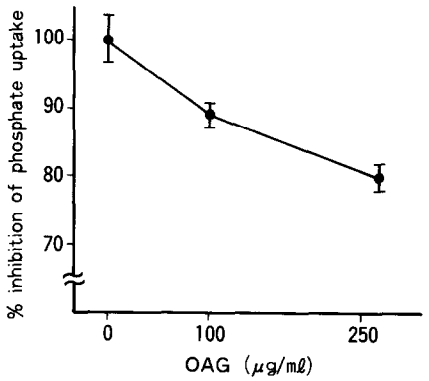

Figure 3. Dose-dependent effect of OAG on the phosphate uptake in OK cells. The phosphate uptake was significantly inhibited by OAG. Phosphate uptake at the basal level was 86.1 ± 3.1 x 10^{-12} moles/5min/10^5cells. Vertical lines represent the mean ± S.E. of 8 different cultures.
+ P<0.05, * P<0.01 significantly different from control.

A23187, calcium ionophore, failed to inhibit phosphate uptake even at a concentration of 2.5x10^{-6}M (Fig. 2B). When PDBu were used in combination with A23187, the inhibitory effect was synergistic (44.4% inhibition of control, P<0.01, n=8) as shown in Fig. 2C.

Furthermore, OAG, synthetic diacylglycerol, inhibited the phosphate uptake dose-dependently up to 80.1% of control at 250 μg/ml (P<0.01, n=8) (Fig.3).

Discussion

In the present study, we first demonstrated that phosphate transport in OK cells was inhibited by the phorbol esters- and OAG-induced activation of protein kinase C.

OK cells possess several important functional properties of proximal tubules (2,3,4,5), particularly, a sodium dependent phosphate transport system and high affinity PTH receptors. These characteristics make the cell line a good model for studying phosphate transport in proximal renal tubules. In addition, several lines of investigation reveal that PTH inhibits phosphate transport in OK cells (5,9), and that the action is similar to that observed in the proximal renal tubules (10,11,12). Hruska and co-workers recently described PTH-stimulated phosphatidylinositol breakdown, and the resulting

production of diacylglycerol and IP_3 in both cultured proximal renal tubular cells and OK cells (13).

In the physiologic state, protein kinase C, involved in signal transduction for various hormones (14), may regulate cellular functions after being activated by diacylglycerol. Synthetic diacylglycerol, OAG, and tumor promoting phorbol esters are widely used to directly stimulate protein kinase C and to study possible involvements of protein kinase C in cellular functions. In the present investigation, we found that when protein kinase C was stimulated by these compounds, phosphate uptake in OK cells was reduced. This suggests the possible involvement of protein kinase C in phosphate transport in the proximal renal tubular cells. Therefore, in addition to the adenylate cyclase system, the activation of protein kinase C by phosphatidylinositol breakdown might be another second messenger of PTH on the renal tubular phosphate transport. Further studies are required to fully elucidate this mechanism.

In the present study, phosphate uptake in the proximal tubular cells was inhibited via the stimulation of protein kinase C. However, previously, in mixed renal tubular cells phorbol esters and OAG stimulated phosphate uptake (1). This discrepancy may be due to a stimulatory effect on the distal tubular cells of the mixed culture, by the phorbol esters and OAG. Indeed, phorbol esters and OAG remarkably stimulate the phosphate uptake in LLC-PK_1 cells, which share many characteristics of distal tubular cells such as calcitonin and vasopressin responsiveness (15).

This study, therefore, provides new evidence that phorbol esters and OAG inhibit phosphate transport in OK cells and also suggests that protein kinase C is involved in phosphate handling in proximal renal tubular cells.

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