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PHORBOL ESTERS STIMULATE PHOSPHATE ACCUMULATION SYNERGISTICALLY WITH A23187 IN CULTURED RENAL TUBULAR CELLS

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Summary: The effects of phorbol esters and diacylglycerol on phosphate accumulation in the cultured mouse kidney cells were investigated to assess the possible role of Ca^{2+}-activated, phospholipid dependent protein kinase (protein kinase C) on the renal phosphate handling. 12-O-tetradecanoyl phorbol-13-acetate (TPA) stimulated phosphate accumulation dose-dependently. TPA-induced phosphate accumulation was synergistically enhanced with A23187. 4α-phorbol 12,13-didecanoate did not stimulate the phosphate accumulation, while 4β-phorbol 12,13-didecanoate stimulated it. Additionally, 1-oleoyl-2-acetyl-glycerol exhibited a stimulatory effect on phosphate accumulation. These data indicated that protein kinase C is one of possible regulators of phosphate transport at the renal tubules.

Protein kinase C, widely distributed in various tissues (1), has been reported to be involved in signal transduction for a variety of biologically active substances (2).

Recently, Barrett and co-workers demonstrated the presence of protein kinase C activity in renal microvillus membrane and suggested a possible contribution of this enzyme for the regulation of transmembrane transport process (3). However, no direct evidence of the involvement of protein kinase C on the phosphate handling in the kidney has been demonstrated so far.

Recent studies have shown that several phorbol esters, known tumor promotors, directly activate protein kinase C (4,5) which has been estimated to be a TPA receptor itself (6).

In the present study, the effects of phorbol esters and synthetic diacylglycerol, OAG, on the accumulation of phosphate in the cultured renal

Abbreviations used are: protein kinase C, Ca^{2+}-activated, phospholipid dependent protein kinase; TPA, 12-O-tetradecanoyl phorbol-13-acetate; OAG, 1-oleoyl-2-acetyl-glycerol; PTH, parathyroid hormone.

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tubular cells were investigated to clarify a possible role of protein kinase C on the phosphate transport at the renal tubules.

Materials and Methods

Assay of Phosphate Accumulated in the Cells

The cells from C57BL/6J male mouse kidneys were cultured according to the method described recently (7). The phosphate accumulation in the cells was studied according to the modified method of Biber et al (8) except that the incubation with ^{32}P was usually undertaken for 60 min. In brief, after reaching confluence, the cells were incubated in the experimental medium (modified Ham's F12, Ca 1.2 mM, P 0.3 mM, ^{32}P 1 μCi/ml) containing test agents or vehicle alone at 37°C. After an appropriate incubation period, the medium was discarded and the cells were washed with ice-cooled normal saline. ^{32}P accumulated in the cells was counted by a liquid scintillation counter.

Chemicals

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

Statistical Analysis

The results were expressed as the mean ± S.E. Significance of difference between groups was determined by Duncan's new multiple range test (9).

Results

The phosphate accumulation in the cells increased linearly up to 60 min at 37°C, while at 4°C, no increase of phosphate accumulation was observed. 2,4-Dinitrophenol at 10^{-4} M, or ouabain at 10^{-4} M, inhibited the phosphate accumulation remarkably (44% and 55% of the control respectively). In the sodium-free medium (NaCl (130 mM) in the experimental medium was replaced with choline chloride on an equimolar basis), the accumulation was inhibited almost completely (8.5% of the control). These data clearly indicate that the phosphate accumulation in the cells is energy-dependent and sodium-dependent. In addition, parathyroid hormone (PTH), $N^6,O^{2'}$-dibutyryl cyclic adenosine 3':5'-monophosphate, and forskolin inhibited the phosphate accumulation dose-dependently (data not shown).

Dose-dependency of TPA on the phosphate accumulation in the cells during 60 min incubation was shown in Fig 1. TPA at the concentration of 10 ng/ml stimulated the phosphate accumulation significantly, and raised it to 134% of the control at 50 ng/ml ($P < 0.01$, $n=6$). Fig 2 showed the effect of 4β-phorbol 12,13-didecanoate, an activator of protein kinase C, and 4α-phorbol 12,13-didecanoate, a non-activator of protein kinase C , on the phosphate

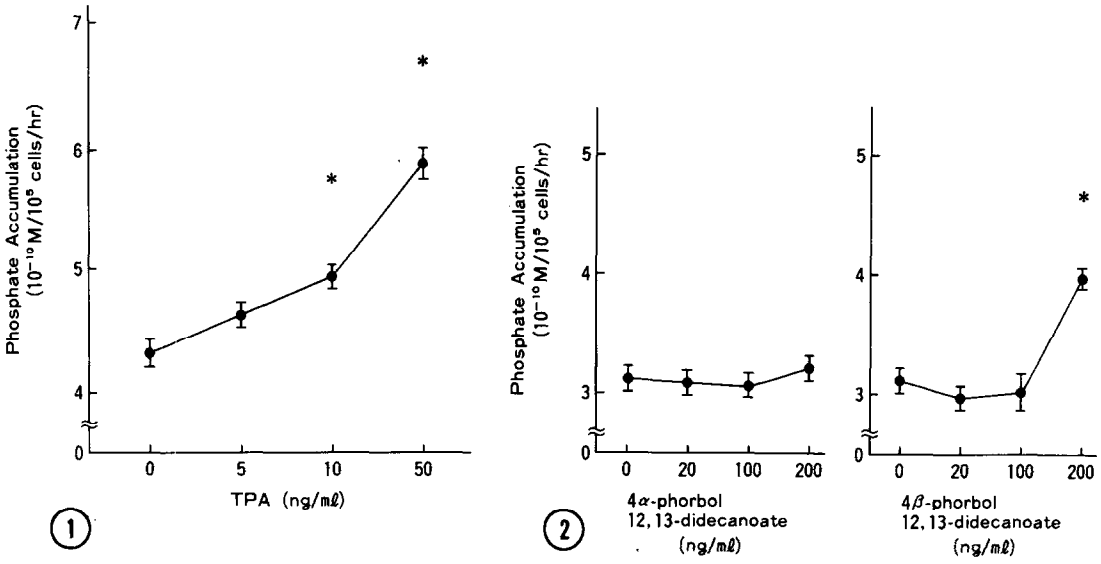
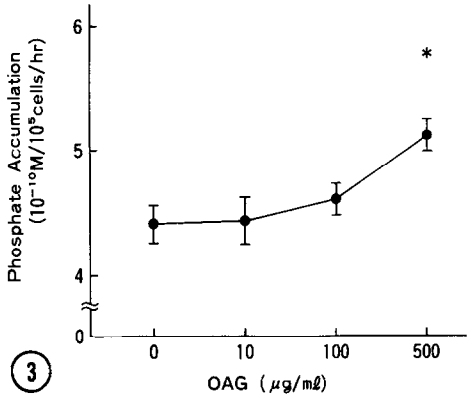


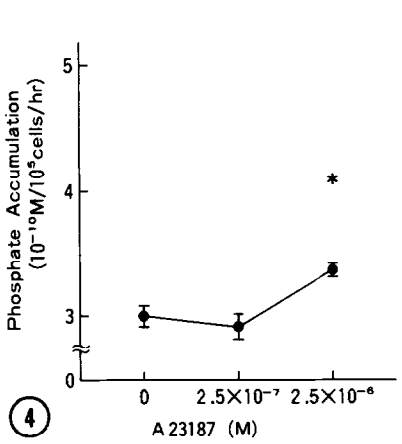
Fig. 1. Dose-dependent effect of TPA on the phosphate accumulation in the cultured renal epithelial cells. During 60 min incubation, the phosphate accumulation was significantly increased by TPA. Vertical lines represent mean ± S.E. of 6 different cultures. * $P < 0.01$ significantly different from the control.

Fig. 2. Dose-dependent effect of 4α-phorbol 12,13-didecanoate and 4β-phorbol 12,13-didecanoate on the phosphate accumulation in the cultured renal cells. 4β-phorbol 12,13-didecanoate increased the accumulation significantly, while 4α-phorbol 12,13-didecanoate failed to increase it. Vertical lines represent mean ± S.E. of 6 different cultures. * $P < 0.01$ significantly different from the control.

accumulation (4). 4α-phorbol 12,13-didecanoate failed to stimulate the phosphate accumulation even at the concentration of 200 ng/ml, while 4β-phorbol 12,13-didecanoate at the same concentration increased the accumulation significantly (125% of control, $P < 0.01$, n=6). Synthetic diacylglycerol, OAG, also stimulated the phosphate accumulation dose-dependently as shown in Fig 3. In the presence of OAG at the concentration of 500 μg/ml, the accumulation increased significantly up to 116% of the control ($P < 0.05$, n=4). Calcium ionophore, A23187, at 2.5×10^{-6} M, induced a slight but significant increase of the phosphate accumulation (110% of control, $P < 0.05$, n=6), while at lower concentration (2.5×10^{-7} M) no increase of the accumulation was observed (Fig 4).

The individual and combined effects of TPA and A23187 on the phosphate accumulation were demonstrated in Fig 5. A23187 alone at 2.5×10^{-7} M failed to increase the accumulation, while in combination with 10 ng/ml and 50 ng/ml





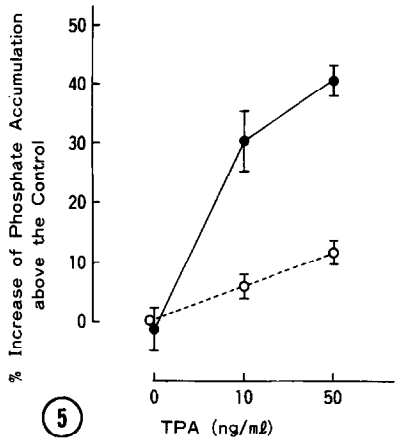


Fig. 3. Dose-dependent effect of OAG on the phosphate accumulation in the cells. The phosphate accumulation was significantly increased by OAG. Vertical lines represent mean ± S.E. of 4 different cultures. * $P < 0.05$ significantly different from the control.

Fig. 4. Dose-dependent effect of A23187 on the phosphate accumulation. Vertical lines represent mean ± S.E. of 6 different cultures. * $P < 0.05$ significantly different from the control.

Fig. 5. Combined effect of TPA and A23187 on the phosphate accumulation in the cultured renal cells. The effect of TPA was synergistically enhanced by 2.5×10^{-7} M A23187. Vertical lines represent mean ± S.E. of 6 different cultures.
(●—●): with 2.5×10^{-7} M A23187.
(o----o): without A23187.

TPA, the phosphate accumulation was synergistically increased up to 30.6 and 40.4% above the control respectively.

Discussion

The possible involvement of protein kinase C in the regulation of phosphate handling in the kidney was first demonstrated using a phosphate accumultion system.

The phosphate accumulation in our cells was an energy-dependent and sodium-dependent and compatible with the report of Biber et al (8). In addition, inhibitory effect of PTH on phosphate accumulation was also maintained in our cells.

The tubular transport of phosphate is under the control of two major factors. One is the serum PTH and the other is an intrinsic adaptation mechanism to phosphate depletion that operates independently of PTH (10,11). It is generally accepted that the effects of PTH on renal phosphate transport are mediated by adenosine 3':5'-cyclic monophosphate. On the other hand, the intracellular mechanism of renal tubular adaptation is still unclear.

Protein kinase C has been reported to be involved in the regulation of various cellular functions (2). Recently, Barrett and co-workers demonstrated the presence of this enzyme in the renal tubular microvillous membrane and speculated the involvement of protein kinase C in the transmembrane transport. However, no evidence that this enzyme alters the renal ion transport has been shown untill now. Our results clearly demonstrated that TPA, 4β-phorbol 12,13-didecanoate and OAG, activators of protein kinase C, stimulated the phosphate accumulation in the cells, while 4α-phorbol 12,13-didecanoate, a non-activator of protein kinase C, failed to stimulate it. These data suggested the involvement of protein kinase C in the regulation of renal tubular phosphate handling. The synergistic role of protein kinase C activation and Ca^{2+} mobilization in the cytosol has been reported in many systems such as serotonin release from platelets (12,13) and calcitonin secretion from a rat C-cell line (14). These observations are also compatible with our result that the effect of TPA was synergistically enhanced with A23187 (Fig 5). Although the mechanism of the action of protein kinase C on renal phosphate handling remains unclear, our present study disclosed that besides A-kinase system, protein kinase C may participate in the regulation of phosphate handling in the kidney.

In summary, the present data suggest that protein kinase C could become a new candidate for a regulator of renal tubular phosphate transport.

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