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POSSIBLE ROLE OF CALCIUM MEDIATORS IN PARATHYROID HORMONE ACTION ON PHOSPHATE TRANSPORT IN RABBIT RENAL BRUSH BORDER MEMBRANE

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The possibility of the involvement of intracellular calcium in the action of parathyroid hormone on phosphate transport in renal brush border membrane was examined. Preincubation of rabbit renal proximal tubules with parathyroid hormone or 8-bromo-cAMP induced a significant inhibition on phosphate uptake by the brush border membrane vesicles isolated therefrom. The addition of intracellular Ca antagonists, trifluoperazine or W-7, to the preincubation medium, alone was without effect on phosphate uptake by the brush border membrane vesicles, but abolished the inhibitory effects of parathyroid hormone and 8-bromo-cAMP.

In renal proximal tubules (PT), parathyroid hormone (PTH) stimulates cAMP formation and inhibits phosphate (P) reabsorption. It has become widely accepted that cAMP is the intracellular mediator of PTH action because its administration reproduces the effect of the hormone (1). The exact mechanism whereby cAMP inhibits P reabsorption in PT remains unclear, however. Previous studies have demonstrated that treatment with PTH, either *in vivo* (2,3) or *in vitro* (4,5), suppresses the P uptake by the subsequently isolated brush border membrane (BBM) vesicles of PT. In addition, it has been shown that cAMP-dependent protein kinase is present on BBM (6) and that cAMP-induced protein phosphorylation in BBM is associated with a decrease in P uptake (7,8). These findings led to the proposition that protein phosphorylation by cAMP-dependent protein kinase on BBM represents the actual final step of the inhibitory effect of PTH on P reabsorption in PT.

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On the other hand, with the increasing evidence for the role of intracellular calcium (Ca) as an universal intracellular mediator in various hormonal systems (9,10), it is possible that Ca may be involved in the PTH actions in PT. Ca has been shown to play a pivotal role in the stimulatory effect of PTH on gluconeogenesis in PT (11). It is less clear whether or not Ca is also involved in the inhibitory effect of PTH on P reabsorption in PT. Such possibility has been suggested from previous observations made under different diseased and experimental conditions (12-16). Our recent observations have also suggested such possibility in the isolated PT of the rabbit kidney (17, 18). It is not known, however, whether or not the interaction with Ca involves the PTH effect at the level of BBM.

The present studies examine the in vitro effects of two structurally different intracellular Ca antagonists, trifluoperazine (TFP) (19) and N-(6-aminohexyl)-5-chloro-1-naphthlenesulfonamide (W-7) (20), on the inhibitory effects of PTH and cAMP on P uptake by BBM vesicles isolated from the PT of the rabbit kidney.

METHODS

Experimental animals. Male New Zealand white rabbits, weighing 1.5 to 2 kg, were maintained on an ad lib diet of standard rabbit chow with free access to tap water for drinking. The rabbits were sacrificed by cervical dislocation.

PT preincubation. In order to improve exposure to various testing agents, PT was first isolated from renal cortex and preincubated with these agents prior to the preparation of BBM. PT was prepared from renal cortex by homogenization and sieving processes similar to those described previously (21,22). The step of kidney perfusion was omitted in these studies to avoid BBM damage from this procedure. The solution used during PT preparation was Krebs-Ringer bicarbonate buffer preequilibrated with 95% O_2/5% CO_2 (v/v) at 37°C (pH 7.4) containing (mM): NaCl 125, $NaHCO_3$ 25, KCl 5.0, $CaCl_2$ 1.0, $MgSO_4$ 1.2, and NaH_2PO_4/Na_2HPO_4 1.2. PT was then suspended in the preincubation medium containing (mM): NaCl 125, $NaHCO_3$ 25, KCl 5, $MgSO_4$ 1.2, NaH_2PO_4/Na_2HPO_4 2, $CaCl_2$ 3, Na-acetate 1, L-alanine 5, D-glucose 7, a-ketoglutarate 3, and 6% fatty acid-poor bovine serum albumin, preequilibrated with 95% O_2/5% CO_2 (v/v) at 37°C (pH 7.4). The final ionized Ca concentration in this preincubation medium,

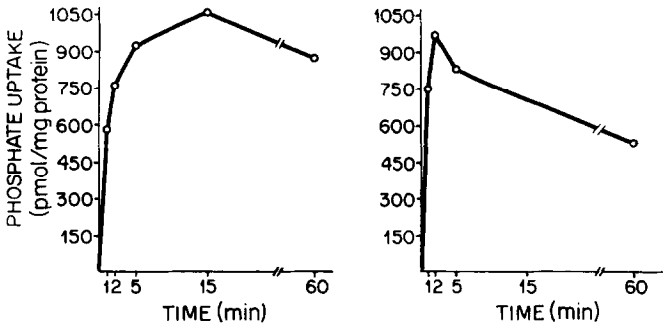

Figure 1. Improvement in P uptake function of BBM vesicles prepared with modified method (details see text). BBM vesicles isolated from PT suspension with modified method (right) had a clearer overshoot uptake within shorter time period and a lesser uptake at equilibrium as compared to BBM vesicles prepared with the conventional method (left).

measured by Orion flow-through Ca electrode, was approximately 1 mM. The preincubation was carried out in a shaker bath at 37°C with 95% O_2/5% CO_2 for 20 minutes. The effects of different testing agents including 1,34-bPTH (1 U/ml), 8-bromo-cAMP (0.1 mM), TFP (0.01 mM), and W-7 (0.01 mM) were examined by adding to the preincubation medium. All the chemicals were obtained from Sigma Chemical, St. Louis, MO.

BBM vesivles preparation. After the preincubation, BBM vesicles were isolated from PT by the conventional Ca-precipitation method (23,24). Since more vigorous homogenization was required in isolating BBM from PT suspension than from the conventional renal cortex, which appeared to impair the uptake function of BBM vesicles, modifications in the homogenization step were made. PT was repeatedly homogenized with six strokes of a Potter-Elvehjem Teflon pestle at 1000 rpm for three times. At each time, the homogenate was separated from the remaining PT by centrifugation so that it would not be damaged by rehomogenization. It was found that such modification improved the uptake function of the BBM vesicles and allowed for the inhibitory effect of PTH or cAMP to become apparent (Fig. 1,2). The preparation of BBM vesicles, after PT preincubation, was carried out at 4°C. The enrichment of BBM enzyme marker, alkaline phosphatase, averaged 13.8 ± 0.87 (n=12) folds from the crude renal cortex or 8.8 ± 0.43 (n=24) folds from the PT suspension. Protein concentration was assayed with Lowry's method (25).

P uptake measurements. Transport studies were performed at room temperature using a Millipore rapid-filtration procedure under an inward Na gradient of 100 mM. Uptakes were calculated from the accumulated [^{32}P]-phosphoric acid (New England Nuclear, Boston, MA.) and expressed in pmol/mg protein. All measurements were carried out in triplicate with freshly prepared membranes.

Statistics. Data are expressed as means ± SEM and analyzed with Student t test for paired data.

RESULTS

Preincubation of PT for up to thirty minutes in the preincubation medium without testing agents did not affect the transport

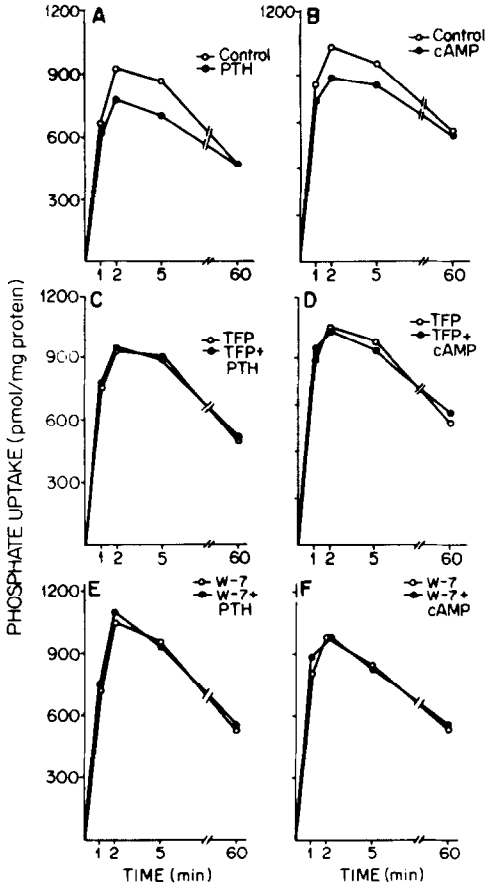

Figure 2. Effects of PTH, 8-bromo-cAMP, TFP and W-7 on phosphate uptake by BBM vesicles. Preincubation of PT suspension with PTH (1 U/ml) (left upper panel) or 8-bromo-cAMP (0.1 mM) (right upper panel) for 20 minutes induced a significant inhibition on the P uptake by the BBM vesicles isolated therefrom. The presence of either TFP (0,01 mM) (middle panels) or W-7 (0.01 mM) (lower panels) in the preincubation medium, however, abolished the inhibitory effects of both PTH and cAMP. Representative studies are shown in the figure.

function of the BBM vesicles isolated therefrom (data not shown). Preincubation of PT with PTH (1 U/ml) or 8-bromo-cAMP (0.1 mM) significantly suppressed the P uptake by BBM vesicles (Figure 2 and Table 1). BBM vesicles P uptake was not affected when PT was preincubated with either TFP (0.01 mM) (1172.0 $\pm$ 132.2 vs. 1187.9 $\pm$ 137.9 pmol/mg protein/2 min., n=3), or W-7 (0.01 mM) (926.0 $\pm$ 116.9 vs. 910.3 $\pm$ 111.1 pmol/mg protein/2 min., n=5). In the presence of either TFP (0.01 mM) or W-7 (0.01 mM), however, the addition of PTH (1 U/ml) or 8-bromo-cAMP (0.1 mM) to the preincu-

Table 1. Effects of PT preincubation with PTH and cAMP, in the absence and presence of TFP or W-7, on phosphate uptake by BBM

(n=5)	Control	PTH(1U/ml)	Control	8-bromo-cAMP(0.1mM)
	1126.2 ± 89.7	985.2* ± 82.2	1003.1 ± 131.6	874.9* ± 127.0
+ TFP (0.01 mM)	1250.6 ± 150.2	1223.7 ± 141.6	1371.3 ± 35.8	1337.8 ± 57.2
+ W-7 (0.01 mM)	1001.8 ± 37.8	986.2 ± 31.1	909.0 ± 45.2	879.7 ± 60.4

Results of 2 minute-uptake (pmol/mg/2 min.) from five experiments in each group are shown. (Mean ± S.E.)

* $p < 0.01$

bation medium failed to suppress the BBM vesicles P uptake (Figure 2 and Table 1).

DISCUSSION

Isolation of BBM vesicles from PT dispersed from renal cortex provides a convenient in vitro experimental system for studying the regulatory mechanisms of the transport function of BBM vesicle. The data found in these studies are consistent with those suggested in previous reports (12-18), regarding the involvement of intracellular Ca mediators (ICM) in the PTH or cAMP effects on P reabsorption in renal PT. The fact that TFP or W-7 abolishes the cAMP effect suggests that ICM may, at least in part, be involved in the action of PTH at steps distal to the generation of cAMP. If indeed cAMP-induced protein phosphorylation of BBM fully accounts for the inhibitory effect of PTH on phosphate uptake by BBM vesicles, it is possible that ICM may in some way interact with this pathway so that in the presence of ICM antagonists the effect does not occur. More definitive studies on the effects of these agents on the cAMP-dependent protein phosphorylation of BBM may clarify this possibility.

On the other hand, the contention that cAMP dependent protein phosphorylation fully accounts for the inhibitory effect of PTH on

P uptake by BBM has been challenged recently. Biber et al. have shown that BBM protein phosphorylation, induced by cAMP in vitro, is not associated with a decreased P uptake (26). It is therefore possible that mechanisms other than cAMP-dependent BBM protein phosphorylation may, either in part or in total, account for the final PTH action, and that ICM may participate in such mechanism so that in the presence of ICM antagonists the effect does not occur. At present, however, there is no evidence in support of this possibility.

Finally, reservation must be maintained in interpreting the data of the present studies. The specificity of the actions of various calmodulin antagonists, including both TFP and W-7, have been questioned recently (27,28). In addition to the possibility that ICM other than calmodulin can be affected (29,30), it has also been suggested that these antagonists may affect membrane properties due to their lipophilic structures. Although the fact that pretreatment of PT with TFP or W-7 in these studies did not affect the BBM vesicles P uptake may provide a dispute against such nonspecific membrane effects of these agents at the concentration used, the possibility that these antagonists may interfere with PTH or cAMP action through ICM-independent mechanisms cannot be excluded. The definitive conclusion on the involvement of ICM, and the identification of the specific ICM involved in the PTH effect on P uptake by BBM vesicles, therefore, awaits for the availability of more specific findings related to ICM inhibitors and a further understanding of the roles of various ICM.

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