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VOLUME EXPANSION-INDUCED CHANGES IN RENAL TUBULAR MEMBRANE PROTEIN PHOSPHORYLATION

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SUMMARY The influence of volume expansion (VE) on the in vitro phosphorylation of membrane protein in the proximal brush border membrane (BBM) of the thyroparathyroidectomized (TPTX) rat was studied in the presence and absence of cyclic AMP and the results were compared to those obtained in control TPTX and intact animals.

The results indicate that the cyclic AMP-independent phosphorylation of a protein band (Mr = 72,000) was stimulated both by VE and by the presence of parathyroid hormone in the circulation, whereas the cyclic AMP-dependent phosphorylation of membrane proteins (Mr = 40,000, 52,000 and 87,000) was inhibited by the same maneuvers. These findings, taken together with data previously available, which demonstrate inhibition of BBM phosphate transport following VE, may provide a link between alterations in phosphate transport in renal BBM vesicles and the phosphorylation of membrane proteins. The results further suggest that membrane protein phosphorylation may be a common mechanism by which a number of agents and maneuvers induce an inhibition of renal tubular phosphate transport.

INTRODUCTION

Both expansion of the extracellular fluid volume (VE) and the administration of parathyroid hormone (PTH) in intact and parathyroidectomized animals have been shown to produce a phosphaturia in a variety of species (1,2). It has been proposed that the action of the hormone on the renal tubular cell may be mediated by a cyclic AMP-dependent system (3-5). However, evidence suggesting alternative mechanisms also has been documented (2,6-8).

The mechanisms by which VE diminishes phosphate reabsorption are less well understood. Some studies have indicated that VE-induced phosphaturia is

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not dependent upon PTH secretion, hemodynamic factors or the chemical composition of the serum (9). Recent studies from this laboratory utilizing the isolated tubule perfusion system or brush border membrane vesicle transport technique have indicated that phosphate uptake by the proximal straight tubules or vesicles prepared from volume expanded animals was significantly lower than that of the control group (10,11). These results strongly support the hypothesis that expansion of the extracellular fluid volume induces the release of a humoral agent or agents, which in turn alter the structure of the BBM leading to an inhibition of phosphate transport (9).

The present paper describes the effects of PTH status and extracellular fluid VE in vivo in the thyroparathyroidectomized (TPTX) rat on the in vitro phosphorylation of renal BBM proteins in the presence and absence of cyclic AMP.

METHODS AND MATERIALS

Animal Preparation TPTX was performed on female Sprague-Dawley rats (170-200 gm) at least 3 days prior to study. VE was accomplished by infusing the anesthetized animals through a jugular vein with a modified saline solution containing 126 mM NaCl, 4mM KCl, 5 mM $CaCl_2$ and 46 mM sodium acetate in an amount equivalent to 6% of body weight for 30 minutes, after which the infusion rate was adjusted to match the urine flow rate for an additional 30 minutes(maintenance period). In the control animals, the first 30 minute period urine was collected without any infusion, followed by a 30 minute period during which a maintenance infusion was given.

Preparation of Brush Border Membrane Vesicles. At the end of the maintenance period, the kidney cortex was removed to prepare BBM vesicles by a calcium precipitation procedure as described previously from this laboratory (5). Vesicles were utilized only if there was at least an 8-fold enrichment in gamma-glutamyl transpeptidase activity in the BBM fraction compared to the cortical homogenate.

Phosphorylation of Brush Border Membranes Phosphorylation of BBM by intrinsic protein kinase was carried out by a modification of the methods of Hammerman and Hruska (13). The final incubation mixture (in 100 µl) contained 200-300 µg protein; 5 mM Mes/Tris-HCl, pH 6.5; 10 mM KF; 10 µM ATP containing approximately 2.5 µCi of [gamma - ^{32}P] - ATP. Studies were performed in the presence and absence of 10 µM cyclic AMP. The mixture was preincubated in a 30 °C water bath for one minute in the absence of ATP. Incubation was continued for another minute after the addition of the nucleotide. The phosphorylation reaction was then terminated by the addition of 100 µl of an ice-cold 125 mM Tris-HCl buffer, pH 6.8, containing 4% SDS (w/v), 20% glycerol (w/v), 10% 2-mercaptoethanol (v/v) and 0.004% bromophenol blue (w/v), followed by boiling for 3 minutes.

SDS - Polyacrylamide Gel Electrophoresis Samples containing up to 100 µg of BBM protein were added to SDS-polyacrylamide slab gels and

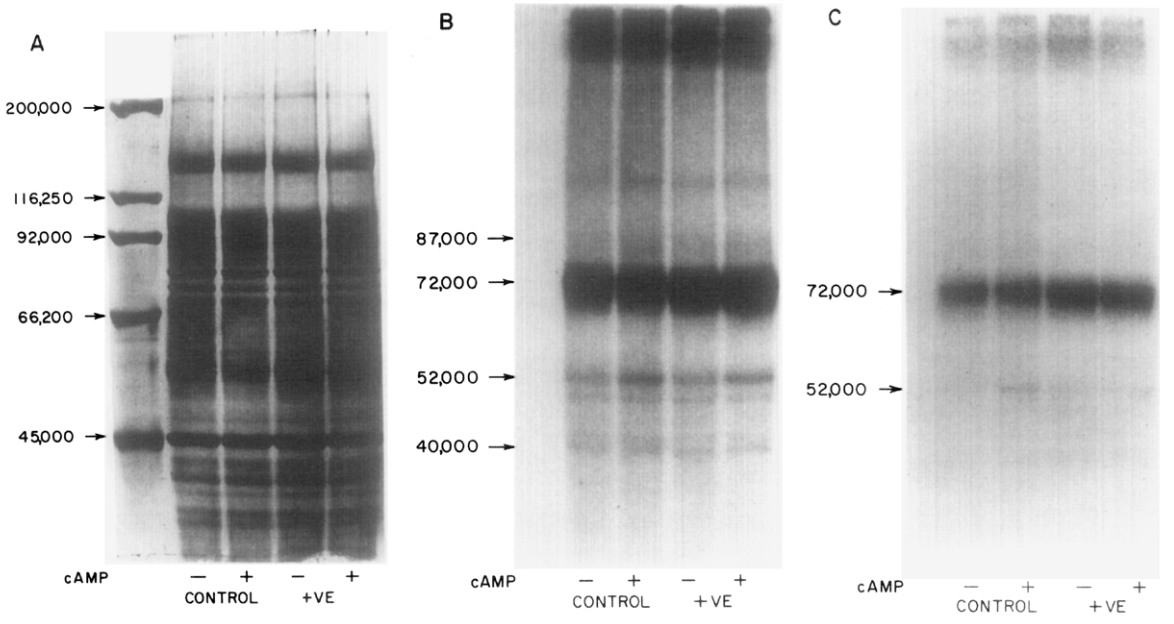

Figure 1. SDS - polyacrylamide gel electrophoresis profile (A) and autoradiograms (B and C) of phosphorylated brush border membrane (BBM) proteins prepared from control and volume expanded (VE) thyroparathyroid-ectomized (TPTX) rats. A. The column on the left is an electrophoresis profile of five standard proteins with molecular weights shown on their left. Columns 2-5 represent a typical electrophoresis profile of BBM proteins that were ^{32}p-phosphorylated in the presence and absence of cyclic AMP. The BBM were prepared either from control (-VE) or volume expanded (+VE) TPTX rats. B. An autoradiogram obtained by exposing the SDS-gel shown in A to a radiographic film for 72 hours. The estimated molecular weights of the ^{32}P-phosphorylated protein bands are shown on the left. C. An autoradiogram obtained from a shorter exposure period (18 hours) than that shown in B.

electrophoresis was performed according to the method of Laemmli (13). The final concentrations in the resolving gel were as follows: 7.5% acrylamide, 0.375M Tris-HCl (pH 8.8), 0.1% SDS, 0.05% (by volume) tetramethylethylenediamine (TEMED) and 0.075% ammonium persulfate. The running buffer contained 0.025 M Tris-HCl, 0.192M glycine, pH 8.6, and 0.1% SDS. SDS-polyacrylamide gels were calibrated for molecular weights using known standard proteins: ovalbumin (Mr = 45,000), bovine serum albumin (Mr = 66,200), phosphorylase B (Mr = 92,500), β-galactosidases (Mr = 116,250) and myosin (Mr = 200,000). Protein concentration was determined by the method of Bradford (14) using crystalline bovine serum albumin as a standard.

Materials [Gamma-^{32}P] - ATP (>4,000 Ci/mmole) was obtained from ICN Radiochemicals Inc. (Irvine, California). ATP and cyclic AMP were obtained from Sigma Chemical Co. (St. Louis,Mo). All other chemicals were reagent grade and were obtained from commercial sources.

RESULTS AND DISCUSSION Figure 1A illustrates SDS-polyacrylamide gel electrophoresis profiles and autoradiograms of phosphorylated brush border membrane proteins prepared from control and volume expanded TPTX rats. More

Table 1

Effects of volume expansion and thyroparathyroidectomy on the phosphorylation of the 52,000 and 72,000 molecular weight proteins*

Phosphorylated Protein	VE/Control† (TPTX)	Intact/TPTXΔ
cAMP-dependent,‡ 52,000 MW	0.36 ± 0.09 (n=6, p<0.005)	0.39 ± 0.18 (n=4, p<0.025)
cAMP-independent, 72,000 MW	1.69 ± 0.03 (n=8, p<0.005)	1.71 ± 0.43 (n=7, p<0.005)

* Results are means ± SD. Number of experiments and P value are in parentheses. The data presented represent the ratios of arbitrary units obtained by determining the area under the peak of the densitometry tracings of autoradiograms for the two proteins.

†Ratio of the values obtained in volume expanded (VE) animals to those from control rats.

ΔRatio of the values obtained in intact to those from thyroparathyroidectomized (TPTX) animals.

‡The data for cAMP-dependent protein phosphorylation were determined by subtracting the value for the observations obtained in the absence of cAMP from those obtained in the presence of the cyclic nucleotide.

than 20 bands were resolved by the electrophoresis. When radiographic films were exposed to gels for 48 to 72 hours, 8 to 10 bands were regularly phosphorylated (Figure 1B). The addition of cyclic AMP increased phosphorylation of two protein bands with apparent Mr of 52,000 and 87,000. However, a 3-fold attenuation in the cyclic AMP-dependent phosphorylation of the 52,000 Mr protein was shown in vesicles isolated from kidneys of VE animals relative to the control group (Table 1). A shorter period of film exposure time (18-24 hours) revealed a significant difference between control and VE groups in ^{32}P incorporation into a protein band with an apparent Mr of 72,000 (Figure 1C). Tracings of the autoradiograms from 8 preparations indicated a 69% stimulation of the 72,000 Mr protein in vesicles isolated from the VE rats as compared to the controls (Table 1). Phosphorylation of this protein was also demonstrated to be cyclic AMP-independent.

Figure 2A shows a representative SDS-polyacrylamide gel of a BBM suspension taken from the kidneys of normal and TPTX rats. Although no

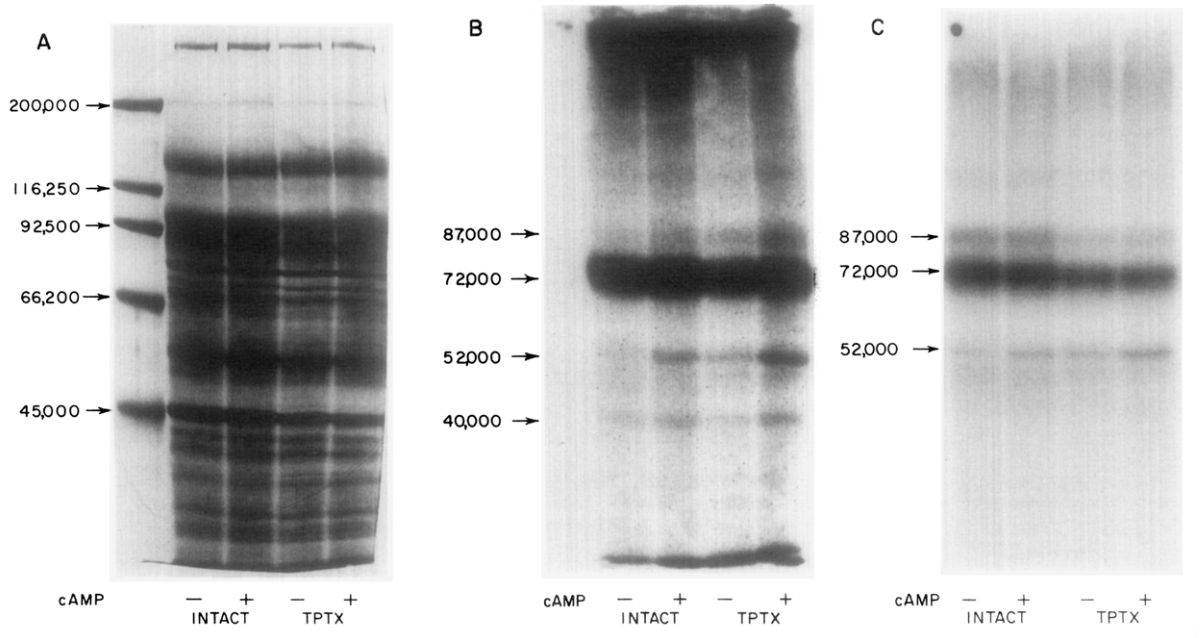

Figure 2. SDS - polyacrylamide gel electrophoresis profile (A) and autoradiograms (B and C) of phosphorylated BBM proteins prepared from intact or TPTX rats. A. The column on the left is an electrophoresis of five standard proteins with molecular weights shown on the left. Columns 2-5 represent a typical electrophoresis profile of in vitro ^{32}P-phosphorylated BBM proteins prepared from intact or TPTX rats, in the presence or absence of cyclic AMP. B. An autoradiogram obtained by exposing the SDS-gel shown in A to a radiographic film for 72 hours. The estimated molecular weights of the ^{32}p-phosphorylated protein bands are shown on the left. C. An autoradiogram obtained from a shorter exposure period (18 hours) than that shown in B.

significant difference in protein pattern can be found between these two groups of animals, the incorporation of ^{32}P from ^{32}P-ATP into membrane components is quite different. The addition of cyclic AMP to the phosphorylation reaction mixture stimulated the incorporation of ^{32}P into protein bands with apparent Mr of 87,000, 52,000, and 40,000, suggesting that a cyclic AMP-dependent protein kinase is involved in the phosphorylation of these proteins (Figure 2B). The effect is greater in the preparations derived from TPTX animals than in those of intact rats (Table 1). These findings with respect to the influence of cyclic AMP on BBM protein phosphorylation are similar to those reported by Sacktor and his associates (4,13) and Hammerman and Hruska (3). Phosphorylation of the 72,000 Mr protein was found to be dramatically reduced in membranes prepared from TPTX rats relative to control

animals (Figure 2C). In seven experiments, the phosphorylation of the 72,000 molecular weight protein was 71% higher in preparations obtained from intact rats than in those prepared from TPTX animals (Table 1). Again, cyclic AMP did not alter the phosphorylation of this protein in either intact or TPTX animals.

The experimental findings presented in this study indicate that the renal proximal tubular BBM possesses both cyclic AMP-independent and -dependent protein kinase activities which were involved in the phosphorylation of intrinsic membrane proteins. In experiments comparing intact vs TPTX animals, we have shown that the removal of the parathyroid glands, a procedure known to enhance renal phosphate reabsorption (1,2), caused a significant decrease in the cyclic AMP-independent phosphorylation of a protein with an apparent Mr of 72,000 and a small but consistent increase in the cyclic AMP-dependent phosphorylation of proteins with apparent Mr of 40,000, 52,000, and 87,000 (Figure 2 and Table 1). Contrarily, extracellular fluid VE, a phosphaturic maneuver, caused a significant increase in the cyclic AMP-independent phosphorylation of the 72,000 protein and a decrease in the cyclic AMP-dependent phosphorylation of the 52,000, and 87,000 proteins (Figure 1 and Table 1). Thus, cyclic AMP-independent phosphorylation was stimulated both by volume expansion and by the presence of PTH in the circulation, whereas cyclic AMP-dependent phosphorylation was inhibited by the same maneuvers. These findings as well as the data previously available from our laboratory (11) provide a link between the inhibition of phosphate transport in renal BBM vesicles and the phosphorylation of membrane proteins. These results suggest that membrane protein phosphorylation may be a common mechanism by which a number of agents and maneuvers induce their specific biological responses, including an inhibition of renal tubular phosphate transport.

In a series of experiments previously reported from this laboratory, we have demonstrated that not all of the nephron effects of PTH are mediated by the adenylate cyclase system (2). In their studies of the effect of PTH on the phosphorylation of phosphoinositide, Meltzer et al (7) have also found

that cyclic nucleotides and a phosphodiesterase inhibitor failed to reproduce the hormone's effects on phosphoinositide metabolism, suggesting a mechanism independent of cyclic AMP. Therefore, the phosphorylation of the 72,000 molecular weight BBM protein by a cyclic AMP-independent mechanism and its close relation to phosphate transport may provide a possible molecular mechanism for this phenomenon.

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