Effect of Parathyrin on the Transport Properties of Isolated Renal Brush-Border Vesicles

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The transport properties of brush-border membrane vesicles isolated by a calcium-precipitation method from the renal cortex of normal and parathyrin (parathyroid hormone)-treated rats were studied by a rapid-filtration technique. Parathyrin elicited a dose-dependent decrease in the Na^+ -dependent phosphate uptake by the brush-border membrane vesicles, but the uptake of D-glucose, Na^+ and mannitol was not affected. A maximum inhibition of 30% was observed after the application of 30 U.S.P. units intramuscularly 1 h before the animals were killed. Intravenous infusion of dibutyryl cyclic AMP (0.5–1.5 mg) also decreased the phosphate uptake by the brush-border vesicles. Both dibutyryl cyclic AMP and parathyrin were ineffective when added *in vitro* to brush-border membrane vesicles isolated from normal rats. These data suggest that parathyrin exerts its action on the phosphate reabsorption in the renal proximal tubule by affecting the Na^+ /phosphate co-transport system in the brush-border membrane. The effects of parathyrin on Na^+ and glucose transport, however, seem to be due to alterations to the driving forces for transport and not to the brush-border transport systems.

Parathyrin administration causes phosphaturia by inhibiting phosphate transport in the proximal as well as in the distal tubule (Agus *et al.*, 1971, 1973; Amiel *et al.*, 1970; Brunette *et al.*, 1973; Gekle, 1971; Knox *et al.*, 1976).

With respect to the biochemical events, it seems that the action of parathyrin is initiated at the contraluminal cell side (Shlatz *et al.*, 1975) by activation of an adenylate cyclase (Nelson *et al.*, 1970), and that the appearance of cyclic AMP in the tubular fluid precedes the effect of parathyrin on the phosphate reabsorption (Chase & Aurbach, 1967; Nelson *et al.*, 1970). Butlen & Jard (1972) provided further evidence that the presence of cyclic AMP in the tubular lumen might be essential for the action of parathyrin on the phosphate reabsorption. The further reactions which in turn are influenced by cyclic AMP are, however, unknown.

From a biophysical viewpoint the alterations of phosphate transport by parathyrin can be brought about either by changes in the properties of the transport system, leaving the driving forces unaltered, or by changes in the driving forces without an alteration of the transport system itself, or by a combination of both processes. The following studies were undertaken to distinguish between these possibilities. For this purpose, the transport properties of renal brush-border membrane vesicles isolated from

proximal tubules of normal and hormone-treated animals were compared. The results demonstrate that parathyrin administration specifically decreases the maximum velocity of the Na^+ /phosphate co-transport system in the brush-border membrane. This finding indicates that parathyrin exerts its action on the phosphate transport by an alteration to the membrane-bound transport system rather than by a change in the driving forces.

Materials and Methods

Isolation of brush-border membranes

Male Wistar rats (160–180 g body wt.; ten for each experiment) were killed by a blow to the neck and their kidneys were placed as quickly as possible into ice-cold mannitol buffer (10 mM-mannitol/2 mM-Tris/HCl, pH 7.1). They were decapsulated and thin slices of the renal cortex (approx. 1–2 mm thick) were prepared. Brush-border membrane vesicles were isolated by the calcium-precipitation method originally described by Booth & Kenny (1974) as modified by Evers *et al.* (1978). In short, the renal cortex is homogenized in mannitol buffer (the most critical period for the maintenance of the altered state of the membrane seems to be the time between the death of the animal and the homogenization of the tissue in mannitol buffer; after that the parathyrin-induced decrease in phosphate transport is quite stable), then CaCl_2 is added to the suspension to a final concentra-

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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tion of 10mm. The aggregated mitochondria, lysosomes, endoplasmic reticulum and basolateral plasma membranes are removed by a low-speed (12min at 500g) centrifugation. The brush-border membranes remain in the supernatant and are then sedimented by centrifugation for 12min at 15000g. The calcium-precipitation step is repeated once and the final sediment is suspended in buffer (100mm-mannitol/20mm-Hepes/Tris, pH7.4) and used for transport studies. The brush-border membrane vesicles obtained by this procedure are predominantly oriented right-side out as judged from freeze-fracture electron-microscopy and by immunological techniques (Haase *et al.*, 1978).

Treatment of animals before death

Normally a crude parathyrin preparation (P-20; Lilly, Indianapolis, IN, U.S.A.) was injected (30 U.S.P. units/animal) intramuscularly 1h before the animals were killed. For dose-response experiments, a highly purified hormone preparation (Inolex, Park Forest, South Illinois, U.S.A.) was used. In some experiments [arginine]vasopressin (0.1nmol/animal; Ferring, Malmö, Sweden) instead of parathyrin was injected intramuscularly 1h before the animals were killed. The hormone preparations were always dissolved in water containing 0.2% phenol. Animals used for control experiments received only an equal amount (0.3ml/animal) of phenol solution in water. Dibutyl cyclic AMP (0.5 or 1mg) was injected into the jugular vein of animals kept in Inactin (thiobutabarbital sodium; Byk-Gulden, Konstanz, Germany) narcosis 20min before removal of the kidneys. The cyclic nucleotide was dissolved in 0.2ml of 150mm-mannitol/100mm-NaCl/2.6mm-KCl/3mm-CaCl₂. Control animals kept under anaesthesia received 0.2ml of the same solution without the cyclic nucleotide.

Determination of protein and enzymes

Protein was determined, after precipitation of the membranes with ice-cold 10% (w/v) trichloroacetic acid, by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard. Alkaline phosphatase (EC 3.1.3.1) and Na⁺ + K⁺-stimulated adenosine triphosphatase (EC 3.6.1.3) were determined as described by Berner & Kinne (1976); acid phosphatase (EC 3.1.3.2) was measured with a test kit (Merck, Darmstadt, Germany, no. 3378). These enzyme activities were measured semi-automatically with the LKB reaction-rate analyser 8600 at 37°C. Succinate dehydrogenase (EC 1.3.99.1) was determined by the method of Gibbs & Reimers (1965). Glucose 6-phosphatase (EC 3.1.3.9) was measured as described by Bode *et al.* (1974). Maltase (EC 3.2.1.20) was measured by a modification of the method of Sacktor (1968).

Transport studies

Uptake of labelled substrates by the isolated membrane vesicles was measured by a Millipore-filtration technique as described previously (Hoffmann *et al.*, 1976; Hopfer *et al.*, 1973; Kinne *et al.*, 1975a). The exact compositions of the incubation media are given in the legends to the Figures.

Materials

All chemical reagents were of the highest chemical purity available. Radioactive isotopes were purchased from New England Nuclear (Boston, MA, U.S.A.).

Results

Biochemical characteristics of the brush-border membrane vesicles isolated from normal and parathyrin-treated rats

As shown in Table 1 the administration of parathyrin to normal rats does not influence the specific activities, enrichment or recovery of brush-border marker enzymes alkaline phosphatase and maltase in the final membrane preparation. Similarly the contamination with basolateral plasma membranes and with other intracellular organelles is not altered. Therefore it can be concluded that the hormone does not alter the behaviour of the brush-border vesicles during isolation and that membrane fractions of identical purity are obtained from control and hormone-treated animals.

Transport properties of brush-border membrane vesicles isolated from normal and hormone-treated rats

Fig. 1 gives an example of the phosphate uptake by renal brush-border vesicles isolated from the proximal tubules of normal and parathyrin-treated rats. Both vesicle preparations show a very rapid initial uptake phase which leads to an intravesicular accumulation of phosphate inside the vesicles (overshoot). However, as detailed quantitatively in Table 2, in brush-border vesicles isolated from parathyrin-treated rats the phosphate uptake during the first 20s and the overshoot are decreased by about 30%.

During the present study it was observed that the phosphate uptake varied considerably from preparation to preparation. As shown in Fig. 2, after 20s of incubation uptake as low as 120% and as high as 250% of the equilibrium value could be observed. The high variability of the phosphate uptake (compared with the glucose uptake) might reflect the fact that phosphate transport in the proximal tubule is subjected to regulation by a variety of factors (hormonal and nutritional) that are difficult to control. In a given population of animals, however, the

Table 1. Specific activities, enrichment and overall recoveries in rat renal cortex and brush-border vesicles isolated from normal and parathyrin-treated rats

Specific activities are given in munits/mg of protein (1 unit corresponds to 1 μmol of substrate converted/min). The values represent mean values ± s.d., where appropriate, for the numbers of experiments in parentheses.

Structure	Enzyme	Homogenate		Brush-border membranes		Enrichment (fold)		Overall recovery (%)	
		Control	+Parathyrin	Control	+Parathyrin	Control	+Parathyrin	Control	+Parathyrin
Brush-border	Alkaline phosphatase (7)	1741 ± 295	1731 ± 205	13780 ± 3969	14053 ± 3442	7.8 ± 1.3	8.2 ± 1.9	72.2	80.8
	Maltase (2)	4.6	3.8	47.4	44.4	10.3	11.7	71.5	77.2
Basolateral plasma membranes	Na ⁺ +K ⁺ -stimulated adenosine triphosphatase (7)	230 ± 76	255 ± 61	108 ± 53	100 ± 51	0.47 ± 0.27	0.39 ± 0.22	82.7	84.0
Endoplasmic reticulum	Glucose 6-phosphatase (2)	20.5	16.7	Not detectable	Not detectable	—	—	90.2	106.7
Lysosomes	Acid phosphatase (2)	75.7	70.1	151.4	155.5	2.0	2.2	88.0	89.0
Mitochondria	Succinate dehydrogenase (2)	4.5	3.1	0.8	0.7	0.18	0.23	58.7	75.5
Protein (mg) (2)		501	579	10.0	10.7	—	—	91.2	84.1

Fig. 1. Effect of parathyrin on the phosphate uptake by isolated renal brush-border membrane vesicles

Control rats (○) received 0.3ml of 0.2% phenol intramuscularly 1 h before death; parathyrin-treated rats (●) received 0.3 ml of Parathormone (Eli Lilly, 100 U.S.P. units/ml). Phosphate uptake was studied in 100mM-mannitol / 20mM-Hepes / Tris (pH 7.4) / 0.1 mM-phosphate/100mM-NaCl medium. The results are expressed as percentage of the uptake observed after 60min (equilibrium), which amounted to 0.357nmol/mg of protein for the control rats and 0.322 for the parathyrin-treated rats. Results from one typical experiment are given.

phosphate uptake by brush-border vesicles isolated after treatment with parathyrin was always lower than uptake by the membranes isolated from the non-treated animals, and seemed to be linearly related to the transport observed in the control membranes (Fig. 2). This means that the percentage inhibition was quite constant, although the absolute decrease varied greatly. Therefore paired data obtained from the same population of animals at the same time and under identical conditions are always given. Representative experiments are shown which could be repeated at least three times with different animal populations yielding qualitatively similar results.

Table 3 shows that the decrease in phosphate uptake can be elicited by different preparations of parathyrin (highly purified hormone preparation provokes similar alterations to those with crude preparations), and that [arginine]vasopressin, another peptide hormone acting on the kidney but at different sites on the nephron, does not seem to influence the characteristics of the phosphate transport.

The effect of parathyrin on the phosphate uptake is also dependent on the concentration of parathyrin

Table 2. Uptake of phosphate, glucose, Na⁺ and mannitol by brush-border membrane vesicles isolated from control and parathyrin-treated rats. The values are expressed as percentage of the equilibrium uptake and represent mean values $\pm$ S.D. derived from *n* experiments. *P* values were calculated by using Student's *t* test for independent data.

Solute	Salt gradient	Uptake after 20 s		Uptake after 1 min		Uptake after 2 min	
		Control	+Parathyrin	Control	+Parathyrin	Control	+Parathyrin
KH ₂ ³² PO ₄ (0.1 mM)	100 mM-NaCl	193.5 $\pm$ 45.2 (<i>n</i> = 11)	132.7 $\pm$ 34.8 (<i>n</i> = 11)	275.4 $\pm$ 72 (<i>n</i> = 7)	195.3 $\pm$ 46 (<i>n</i> = 7)	253.6 $\pm$ 57 (<i>n</i> = 5)	191.2 $\pm$ 64 (<i>n</i> = 5)
		0.01 > <i>P</i> > 0.005		0.05 > <i>P</i> > 0.02		0.2 > <i>P</i> > 0.1	
KH ₂ ³² PO ₄ (0.1 mM)	100 mM-Choline chloride	3.8 $\pm$ 1.4 (<i>n</i> = 4)	3.6 $\pm$ 3.1 (<i>n</i> = 4)	4.8 $\pm$ 2.3 (<i>n</i> = 4)	5.9 $\pm$ 3.7 (<i>n</i> = 4)	6.5 $\pm$ 1.4 (<i>n</i> = 4)	7.4 $\pm$ 1.3 (<i>n</i> = 4)
D-[³ H]Glucose (1 mM)	100 mM-NaSCN	263.4 $\pm$ 34.3 (<i>n</i> = 4)	276.2 $\pm$ 18.2 (<i>n</i> = 4)	200.1 $\pm$ 35.5 (<i>n</i> = 4)	216.8 $\pm$ 37.8 (<i>n</i> = 4)	154.5 $\pm$ 16.7 (<i>n</i> = 4)	158.6 $\pm$ 14.4 (<i>n</i> = 4)
²² NaSCN (100 mM)	100 mM-NaSCN	36.9 $\pm$ 2.7 (<i>n</i> = 4)	36.3 $\pm$ 2.8 (<i>n</i> = 4)	45.2 $\pm$ 3.7 (<i>n</i> = 4)	40.8 $\pm$ 4.4 (<i>n</i> = 4)	53.8 $\pm$ 2.5 (<i>n</i> = 4)	56.6 $\pm$ 8.7 (<i>n</i> = 4)
D-[³ H]Mannitol	100 mM-NaCl	32.0 $\pm$ 1.5 (<i>n</i> = 3)	39.4 $\pm$ 8.2 (<i>n</i> = 3)	43.9 $\pm$ 0.4 (<i>n</i> = 3)	42.9 $\pm$ 8.1 (<i>n</i> = 3)	48.3 (<i>n</i> = 2)	36.7 (<i>n</i> = 2)

Fig. 2. Correlation between initial uptake rates of P_i in renal brush-border vesicles isolated from control animals and from parathyrin-treated animals

The experimental conditions were identical with those given in Fig. 1. The solid line represents the regression line calculated from all experimental points by the least-squares method (*r* = regression coefficient). The dashed lines indicate 0, 25 and 50% inhibition of uptake.

administered to the animals (see Fig. 3a); however, addition of parathyrin to the vesicles after isolation of membranes from normal animals does not change the transport properties (Fig. 3b).

Table 2 shows in addition that parathyrin under the conditions of these experiments specifically decreases the Na⁺-dependent phosphate uptake by the brush-border vesicles. The uptake of phosphate in the presence of choline instead of Na⁺ is not affected by the hormone. Similarly parathyrin seems not to affect significantly the uptake of D-glucose and ²²Na by the membrane vesicles, or the amount of phosphate, glucose and Na⁺ found in the vesicles after 2 h of incubation in the Na⁺-containing medium. Therefore it can be concluded that both preparations contain the same intravesicular volume of 3.3 μ l per mg of protein. Since the rate of mannitol entry (which monitors unspecific permeability properties of the membrane) into the two vesicle preparations is also identical, one can further assume that the surface/volume ratio and therefore the size of the transporting vesicles is not different (Evers *et al.*, 1978).

Table 4 shows the effect of parathyrin on the kinetic parameters of the phosphate-transport system in the brush-border membrane. Both at 100 mM- and 40 mM-NaCl the hormone mainly seems to affect the

Table 3. *Effects of parathyroid gland extract (Lilly), highly purified parathyrin (Inolex) and [arginine]vasopressin (Ferring) injection on phosphate uptake by isolated brush-border vesicles*

The experiments were carried out as indicated in the legend to Fig. 1. The values are expressed as percentage of equilibrium uptake. For the experiments with parathyroid extract the mean values $\pm$ s.d. of 11 experiments are indicated. For purified parathyrin three independent experiments are given. For [arginine]vasopressin two independent experiments are given.

		Uptake after 20s (% of equilibrium value)		
Hormone preparation				
Parathyroid extract (Lilly, P-20)	Control (n = 11)		193.5 $\pm$ 45.2	
			0.01 > P > 0.005	
	Hormone injection (n = 11) (30 U.S.P. units/animal)		132.7 $\pm$ 34.8	
Parathyrin (Inolex)	Control	170	187	276
	Hormone injection (10 U.S.P. units/animal)	135	136	178
[Arginine]vasopressin	Control		310	290
	Hormone injection (0.1 nmol/animal)		300	280

Fig. 3. *Effect of parathyrin on phosphate uptake by isolated brush-border membrane vesicles; comparison of application of parathyrin in vivo (a) versus the effect of parathyrin on isolated membrane vesicles in vitro (b)*

The experiments *in vivo* (a) were carried out as indicated in the legend to Fig. 1 and the results are expressed as percentage of phosphate uptake by brush-border vesicles isolated from control rats. Results from one typical experiment are given. For the experiments *in vitro* (b), brush-border membrane vesicles were isolated from normal rats and parathyrin at doses indicated in the Figure was added to the incubation media. The results are expressed as percentage of phosphate uptake after 20s (○) and after 1 min (●) in the incubation media containing no parathyrin. For experiments *in vivo* and *in vitro* highly purified hormone (Inolex) was used. The incubation media were as indicated in the legend to Fig. 1. $-\log [M]$ is the negative logarithm of the concentration of parathyrin in the incubation medium.

V_{max} . value of the system, but the affinity of the phosphate/ Na^+ co-transport system to phosphate seems to be unchanged. Similarly the sensitivity of the phosphate/ Na^+ co-transport to Na^+ is apparently unaffected by parathyrin treatment of the animals (Fig. 4).

Effect of cyclic AMP on the transport properties of isolated brush-border vesicles

Since cyclic AMP is thought to be the mediator of the action of parathyrin on the phosphate transport in renal cells, the effect of cyclic AMP on the transport properties of the vesicles was investigated. In a first set of experiments, dibutyl cyclic AMP was infused into the animals; the membranes were isolated after infusion and phosphate transport was studied in the absence of cyclic AMP in the incubation medium. As shown in Fig. 5, under these conditions a decrease in the phosphate transport by the brush-border vesicles (even higher in magnitude than the parathyrin effect) could be observed. The effective cyclic AMP concentration can be estimated to be approx. $3 \mu M$ at the time of removal of the kidney, if the same clearance and distribution volume as in man (Broadus *et al.*, 1970) is assumed. Addition of cyclic AMP *in vitro* to brush-border membrane vesicles isolated from control rats did not alter significantly the transport properties of the vesicles (Fig. 5).

Discussion

Nature of alteration in phosphate uptake by isolated brush-border vesicles

The brush-border membrane contains a Na^+ /phosphate co-transport system which at pH 7.4 facilitates the electroneutral transfer of secondary

Table 4. *Effect of parathyrin on kinetic parameters of phosphate uptake by brush-border membranes*

The incubation media contained 100mM-D-mannitol, 20mM-Hepes/Tris, pH 7.4, $\text{KH}_2^{32}\text{PO}_4$ and 100mM-NaCl or 40mM-NaCl and 60mM-choline chloride. The phosphate concentration of the incubation media was varied from 0.1 to 1.0mM. Apparent K_m and apparent $V_{\max}$ values were calculated from regression lines obtained by least-squares analysis in Eadie-Hofstee plots (regression coefficients = 0.96–0.99). Five different concentrations of phosphate were used and the transport experiments were performed in triplicate.

Rats	NaCl (mM)	K_m (mM)	$V_{\max}$. (nmol/20s per mg of protein)
Control	100	0.36	1.56
	40	0.32	0.65
+Parathyrin	100	0.30	0.96
	40	0.28	0.27

Fig. 4. *Effect of parathyrin on Na^+ activation of phosphate uptake*

The experiments were carried out as indicated in Fig. 1. The phosphate concentration was 0.1 mM, and the NaCl concentration was varied from 25 to 100 mM as indicated in the Figure (sodium was replaced by potassium). The results of one typical experiment are expressed as percentage of the Na^+ -stimulated uptake of phosphate at 100 mM-NaCl. Control rats: ○, uptake after 20s; ●, uptake after 1 min; parathyrin-treated animals: □, uptake after 20s; ■, uptake after 1 min.

phosphate and two Na^+ ions (Hoffmann *et al.*, 1976). In such a co-transport system, both the chemical concentration difference of phosphate and the chemical concentration difference of Na^+ across the vesicle membrane act as predominant driving forces for the phosphate uptake. Therefore a decrease in phosphate transport (measured in the presence of a Na^+ and phosphate gradient) as observed after parathyrin treatment does not necessarily imply that the phosphate-transport system itself is altered but could also be due to a decrease in the driving forces. Such a decrease could be brought about by an increase in the Na^+ permeability of the brush-border membrane.

Thereby the Na^+ gradient across the vesicle membrane which is present at the beginning of the transport experiment would be dissipated faster, and consequently a lower initial rate of phosphate uptake would be observed. This seems unlikely, however, since the uptake of ^{22}Na by the brush-border vesicles is not altered by parathyrin and another Na^+ -driven co-transport system, the D-glucose/ Na^+ co-transport, is also not changed by the hormone. Therefore we are inclined to conclude that the phosphate/ Na^+ co-transport system itself is affected by the hormone. This conclusion is supported by the results of the experiments performed to study the properties of the phosphate-transport system. The hormone-induced decrease of the apparent $V_{\max}$ might be explained by a decrease in the number of transport sites or by a lower mobility of a hypothetical carrier system for P_i .

The biochemical nature of the parathyrin- and cyclic AMP-elicited change of the phosphate-transport system has yet to be clarified. Our data suggest that the mere presence of cyclic AMP at the luminal membrane is not sufficient to provoke a change in the phosphate-transport system, but that additional cellular processes are required. Some of our own preliminary experiments (results not shown) indicate that cyclic AMP even in the presence of ATP is unable to decrease the phosphate transport by the vesicles, although cyclic AMP-dependent protein kinases have been found to be concentrated in renal brush-border membranes (Kinne *et al.*, 1975b). This might simply be due to the fact that the brush-border vesicles used in this study are oriented right-side out (Haase *et al.*, 1978), i.e. the former cytoplasmic side of the membrane is at the inside of the vesicles, and thus the catalytic centre of protein kinase might not be accessible for ATP.

There is another noteworthy aspect of the studies presented above concerning a possible relationship between the phosphate-transport system and the alkaline phosphatase present in the brush-border membrane. After the application of parathyrin the alkaline phosphatase activity in the homogenate and

Fig. 5. Effect of cyclic AMP on phosphate uptake by isolated brush-border vesicles; comparison of application of cyclic AMP *in vivo* (a) versus the effect of cyclic AMP on isolated membrane vesicles *in vitro* (b)

Dibutyl cyclic AMP was injected *in vivo* (a) as described in the Materials and Methods section and uptake experiments were carried out as indicated in the legend to Fig. 1. The results are expressed as percentage of uptake of P_i after 20 s (○) and after 1 min (●) observed in brush-border vesicles isolated from animals that received no cyclic AMP. For the experiments *in vitro* (b) brush-border membrane vesicles were isolated from normal rats (no injection) and dibutyl cyclic AMP in amounts indicated in the Figure was added to the incubation media. The results are expressed as percentage of uptake of P_i in the incubation media containing no cyclic AMP. $-\log [M]$ is the negative logarithm of the cyclic AMP concentration (M) in the incubation medium.

in the isolated brush-border membranes remained constant, although the phosphate transport was decreased by about 30%. Thus it seems very unlikely that alkaline phosphatase and the phosphate-transport system are functionally inter-related.

Mechanism of action of parathyrin on transport processes in the proximal tubule

Since it has been demonstrated that the active phosphate reabsorption in rat proximal tubule is Na^+ -dependent (Baumann *et al.*, 1975b), it could be concluded that this transport involves a coupling of phosphate and Na^+ flux across the brush border via a Na^+ /phosphate co-transport system. This system is inhibited after parathyrin administration to the rats. The observed change of 30% *in vitro* in the rate of phosphate uptake of the vesicles agrees quite well with the degree of inhibition of phosphate transport observed in micropuncture experiments *in vivo* (Agus *et al.*, 1971; K. J. Ullrich, G. Rumrich, S. Klöss, unpublished work). This indicates that in these experiments *in vivo* and in ours *in vitro*, the same rate-limiting factors existed; other factors such as decreased phosphate permeability of the peritubular cell membrane, or changes in the driving forces for the Na^+ -phosphate co-transport (which include the electrochemical potential difference of Na^+ and phosphate across the luminal membrane) are apparently not crucial for the parathyrin-cyclic AMP action on phosphate transport.

The latter factors and perhaps even effects on the paracellular pathways of transport might be involved in the inhibitory effect of parathyrin and cyclic AMP

on iso-osmotic volume reabsorption and glucose transport in the proximal tubule (Baumann *et al.*, 1975a), since our data provide no evidence for an inhibition of transport systems mediating the trans-cellular transport of Na^+ and D-glucose. The permeability of the luminal membrane for ^{22}Na , and the activity of the Na^+ + K^+ -stimulated adenosine triphosphatase in the homogenate of the renal cortex, apparently remain unchanged. Similarly no alteration of the glucose/ Na^+ co-transport system in the brush-border membrane as studied under the conditions *in vitro* could be detected.

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