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DISSOCIATION OF PARATHYROID HORMONE AND CYCLIC-3', 5'AMP EFFECTS ON Na-Pi UPTAKE BY CELLS ISOLATED FROM PROXIMAL STRAIGHT TUBULES OF RAT KIDNEY

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Studies in respiratory alkalotic or short-term phosphate deprived rats raised the possibility that in straight portion of proximal tubules (PST) cAMP might be not a mediator of PTH in inhibition of phosphate reabsorption. The present experiments directly compared the sensitivity of Na-dependent phosphate [^{32}P] (Na-Pi) uptake to PTH or cAMP by PCT or PST cells freshly prepared from outer cortex and outer stripe of outer medulla of rat kidney. The purity of the cells was examined by activity of enzymes specific for PST i.e. glutamine synthetase, gamma-glutamyl transpeptidase and creatine kinase, a marker enzyme for medullary thick ascending limb (MTAL) and distal convoluted tubule. Similar inhibition of Na-Pi uptake by 1—34 bPTH by PST and PCT cells was observed: −33.0 and −30.0 % (ns), respectively. In contrast, dibutyryl cAMP decreased Na-Pi uptake only by PCT but not by PST cells: −31.0 and −3.6% ($p<0.02$), respectively. The 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, resulted in slight stimulation of Na-Pi uptake by PST but strong inhibition by PCT cells: 7.8 vs −26.0% ($p<0.001$). In contrast to PCT in PST cells cAMP seems to play a minor role as a mediator of inhibition of Na-Pi uptake by PTH.

Key words: *rat proximal convoluted and straight tubule cells, sodium-dependent phosphate transport, parathyroid hormone, adenosine 3',5'-cyclic monophosphate, 3-isobutyl-1-methylxanthine*

INTRODUCTION

The resistance to phosphaturic effect of PTH observed in hamsters (1, 2) respiratory alkalosis (3, 4) and phosphate deprivation (5—7) are experimental models of human pseudohypoparathyroidism type 2 (PHP 2) (8). The results were interpreted as an induced defect in reception of cAMP signal. (1—4). In our further experiments it was shown that resistance to the phosphaturic effect of PTH observed during respiratory alkalosis (3, 4) as well as in the short term phosphate deprivation (7) was localised exclusively in the straight portion of the proximal tubule, and may be restored by the blockade of beta-adrenergic

receptors (9, 10). These studies suggest that proximal straight tubule is the segment that is responsible for the induced resistance to the phosphaturic effect of PTH. However, besides the hypothesis that there is the defect in cAMP signal reception there is another possibility i.e. namely: in PST segment there is a different than cAMP signalling system for PTH which might be defective. The last possibility is partially supported by experiments performed on cultured opossum kidney cells, which suggest the existence of Ca^{2+}-diacylglycerol-kinase C regulatory cascade in the PTH effect on phosphate transport as an alternative pathway of PTH action (11, 12). However the numerous studies on cultured kidney cells did not allow till now to conclude if PTH-cAMP signalling pathway in straight (PST) is equipotent to convoluted (PCT) portions of proximal tubule (13, 14). Such a conclusion might help to understand the role of alternative PTH signalling pathways in regulation of phosphate reabsorption within a nephron segments.

The aim of the present work was to compare the PTH-cAMP signalling system on phosphate transport in cells originating from PST and from PCT portions of rat kidney proximal tubules. We elaborated, therefore, the method of isolation of alive cells from the same rat kidney representing the PST and PCT cells, which were pure and homogenous enough to study the regulation of Na-dependent phosphate uptake. We found that PTH inhibits Na-dependent phosphate uptake in both types of cells to similar extent, but in contrast to PCT cells, the cells representing the PST segment are insensitive to inhibitory effect of dibutyryl cAMP and 3-isobutyl-1-methylxanthine (IBMX), the phosphodiesterase inhibitor.

MATERIALS AND METHODS

Solutions and chemicals

Intracellular-like solution (ICS) was prepared according to Nagineni *et al.* (15), with the following composition (mM): KCl 75, K_2HPO_4 1, $NaHCO_3$ 9, glucose 180, HEPES 5, pH 7.4 adjusted with Tris base. Uptake medium consisted of (mM): NaCl or choline chloride 142, $CaCl_2$ 1.3, Mg Cl_2 0.5, $MgSO_4$ 0.4, $NaHCO_3$ or $KHCO_3$ 4, HEPES 10, L-glutamine 2, L-β-hydroxybutyrate 1, pH 7.4 adjusted with Tris base. "Stop solution" consisted of (mM): NaCl 150, Na_2HAsO_4 10, HEPES 5, pH 7.2 adjusted with Tris base. Trypan Blue 0.2% solution in 150 mM NaCl, HEPES 10 mM, pH 7.4 was adjusted with Tris base.

The 30% and 49% Percoll solutions were prepared for each experiment by mixing 3 ml and 4 ml of 100% Percoll with 7 ml and 6 ml ICS solution respectively. 100 % Percoll solution was the mixture of 9 ml of Percoll and 1ml of 10 times concentrated ICS solution (KCl 750, K_2HPO_4 10, $NaHCO_3$ 90, glucose 1800, HEPES 50 (mM), pH 7.4 adjusted with Tris base), respectively.

Percoll was purchased from LKB Pharmacia, MSO (methionine sulfoximine), octyl-β-D-glucopyranoside from Calbiochem, IBMX (3-isobutyl-1-methylxanthine) from Aldrich, ^{32}P from Amersham, 1—34 PTH, db c-AMP, DTT (dithiothreitol), Hepes, Tris, Choline Chloride from Sigma. All other chemicals were purchased in Polskie Odczynniki Chemiczne.

Isolation of cells originating from C (outer cortex) and OS (outer stripe of outer medulla).

Male Wistar rats, 200—250 g of body weight, fed *ad libitum* on standard rat chow, with tap water as the drinking water, were used. Animals were anaesthetised with peritoneal injection of sodium pentobarbital, 40 mg/kg b.w. The abdominal aorta was then exposed and cannulated below the renal arteries to allow *in situ* perfusion (30 ml/20 s) of both kidneys with ice-cold and oxygen saturated ICS solution. Pale kidneys were rapidly excised, decapsulated and stored in ice-cold ICS solution.

The kidneys were placed on the glass plate precooled with ice and a slice from each pole of kidney was removed with sharp razor. The remaining tissue was then sliced perpendicularly to its polar axis into 2 mm slabs. The isolation of cells originating from C was initiated by dissection of about 1 mm layer of the outermost renal cortex from each slab situated on the enlighten table. To isolate the cells originating from OS of the same kidney, the outer stripe of outer medulla was dissected from the surrounding tissue. The border between cortex and outer stripe of outer medulla was preliminary identified in rats injected with trypan blue (0.1 mg/g) i.p. 24 hours before the kidneys were isolated (16) and compared to perfused kidneys of uninjected animals. This procedure allowed us to learn an easy identification of OS in perfused kidney alone. Each group of such isolated parts of renal tissue was then minced with a razor blade and transferred onto nylon mesh, 250 μm-pore-size, in order to be rubbed through with a plastic pestle. Than the rubbed through tissue was suspended in 20 ml of ICS solution and sieved through 80 and 40 μm-pore-size meshes, consecutively, to separate the single cells from tubular fragments and glomeruli. The cells were suspended with the ice cold ICS solution to the final volume of 30 ml. The suspensions were centrifuged at 1090 g (Beckman J2-21) for 4 min., and the cells pellets originated from C or OS were re-suspended in 10 ml of isotonic 40% or 30% Percoll solution, respectively, to obtain the pure cells fraction as found experimentally. Each medium was than supplemented with DTT (1μmol/ml) and the separate flasks, containing C or OS cells, were placed in ice-cold bath and gently mixed for 20 min. Than the suspensions were filtered again through the 80 μm mesh to remove a "gelly-like" coat. Afterwards filtrates were centrifuged in plastic tubes at 25.000 g for 20 min. and a few bands were separated. Each band was carefully collected and washed two times with fresh ICS medium and than the suspensions were examined microscopically for efficacy of the procedure. The upper fractions containing dead cells, fragments of the cells and other debris were thrown away. The bottom fractions contained the whole cells. About 30 min. elapsed between final suspension of cells in the uptake medium and the subsequent use for experimental studies. According to the above procedure the cells originating from dissected inner stripe (IS), possibly containing a medullary thick ascending limb (MTAL) and thin descending limb (TDL) cells were also isolated.

Protein concentration was measured according to Lowry (17) after solubilisation of the cells in 1% octyl-β-D-glucopyranoside. The concentration of protein in the final suspension was around 4 mg/ml. The concentration of the cells was counted microscopically in Bürker chamber.

Morphological and enzymatic characterisation of isolated cells

The purity and quality of isolated cells from C, OS and IS were evaluated by: a/ the light microscope and b/ Trypan Blue exclusion viability test by counting stained and unstained cells in the presence of dye (18). The enrichment of cells isolated from C, OS and IS in PCT, PST and MTAL cells, respectively was assessed by measurements of enzymes activities specific for those nephron segments.

Activity of glutamine synthetase as a PST marker (19, 20) was measured in the presence and absence of MSO (methionine sulfoximine), an inhibitor of glutamine synthetase (21). Gamma-glutamyl transpeptidase activity as another PST marker (22) and creatine kinase activity as a MTAL and DCT marker (16) were measured according to (23) and (24), respectively.

Microscopic examination of the quality of isolated cells from cortex (C), outer stripe of medulla (OS) and inner stripe of medulla (IS) showed integral single cells with little amount of debris. No tubule fragments, glomeruli or cell clusters were present. The results of Trypan Blue exclusion test (*Tab. 1*) were similar in cells originating from C, OS and IS indicating that about 90% of cells preserved the ability to exclude a dye. These results were similar to that of Nagineni at al. (15) for cells obtained from rabbit renal tubules. Also the protein content was not different in cells isolated from C, OS and IS of rat kidney zones.

Table 1. Quality and purity of the cells isolated from the rat kidney.

	OS	C	IS
Protein [μg/10^6 cells]	59.1 ± 5.6	60.3 ± 4.7	67.7 ± 14.2
Trypan Blue Exlusion [%]	11.5 ± 1.1	9.6 ± 1.2	12.1 ± 1.5
Glutamine synthetase [μgPi/min/mg protein]	48 ± 3	13 ± 3 [d]	31 ± 5 [c]
Gamma-glutamyl transpeptidase (U/mg protein]	33 5 ± 0.1	7.5 ± 1.2 [d]	17.6 ± 7.3 [a]
Creatine kinase [mU/mg protein]	152 ± 10 [b]	199 ± 52 [b]	745 ± 225

Values are means ± SE for 6 to 10 individuall cell preparations. C, cortex; OS, outer stripe of medulla; IS, inner stripe of medulla. Significance calculated using Student's *t* test: [a]$p < 0.05$; [c]$p < 0.01$; [d]$p < 0.001$ vs. OS; [b]$p < 0.02$ vs. IS.

The marker enzymes activities for PST or MTAL and distal convoluted (DCT) segments were measured to estimate the purity of cells isolated from C, OS and IS of the rat kidney (*Tab. 1*). The activities of glutamine synthetase, an enzyme very specific for rat PST segment (19, 20), was 3.6 and 2.3 times higher in cells isolated from OS or IS, than in cells from C, respectively. The absolute activities of glutamine synthetase in cells derived from all examined zones of rat kidney were statistically different from each other (*Tab. 1*). Similarly, the absolute activities of gamma-glutamyl transpeptidase, an enzyme with the highest activity in PST segment (22), were significantly different between each examined cells. Again, the highest activity of enzyme was expressed in cells isolated from OS zone: 4.4 and 1.9 times higher than in cells prepared from C and IS zones, respectively. In contrast, the creatine kinase activity, the marker enzyme for MTAL and DCT of rat nephron segments (16), on average was 4.3 times lower in the cells isolated from C and OS than in cells originating from IS. The creatine kinase activity statistically did not differ between cells from C and IS stripes. In further experiments we examined the ^{32}P uptake in the presence of 142 mM sodium or choline (sodium-free) medium by the cells isolated from OS and C stripes, further denoted as a „PST" or „PCT" cells respectively.

Uptake studies

50 μl of the uptake medium and 25 μl of the cells suspension containing approximately 1×10^6 cells were preincubated in the shaking water bath at 37°C for 30 min. in the Eppendorf test tubes. Transport was initiated by adding 50 μl of the above medium containing 0.25 mM of Pi. The final concentration of Pi in the uptake medium was 0.1 mM (^{32}Pi 0.5 μCi/125 μl). After 5 min. (or in the time indicated in Results) uptake was terminated by adding of 1 ml of ice-cold „stop-solution" and than the tubes were immediately centrifuged for 15 sec in Eppendorf centrifuge. Supernatants were removed and the pellets were washed twice with stop solution. Each sample was done in triplicates. Radioactivity was counted in the liquid scintillation counter (Beckman LS 5801).

Statistical analysis

All results are expressed as means ±SE. Statistical comparisons were made using Student's t test for paired or unpaired data as appropriate.

RESULTS

The time course of ^{32}P uptake by PST and PCT cells in sodium and sodium-free medium is presented in the *Figure 1*. The ^{32}P uptake in the presence of sodium increased gradually with incubation time both in PST and PCT cells reaching the maximum around 10 min. of incubation. In contrast, the ^{32}P uptake in sodium-free medium by PST or PCT cells was constant beginning from 5 minutes of incubation and did not exceed 20% of that observed in Na-medium at maximum. Summing up, the time-course of ^{32}P uptake by PST and PCT cells in sodium containing media was similar as well as a sodium-dependent uptake (sodium-choline ^{32}P uptake), data not shown. In further experiments the ^{32}P uptake was measured at 5 minutes of incubation in 142 mM sodium or choline medium, concomitantly, by PST and PCT cells isolated simultaneously from the same kidney.

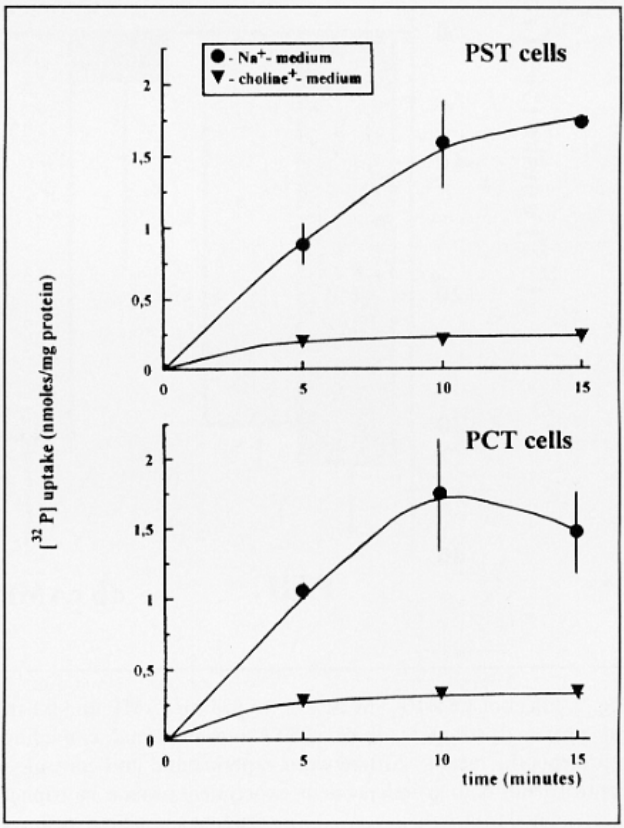

Fig. 1. Time dependence of ^{32}P uptake in Na^+ or $choline^+$ (142 mM) medium by cells originated from PST (proximal straight tubules) and PCT (proximal convoluted tubules) of the rat kidney. Uptake was determined in 0.1 mM of phosphate in the medium as described in MATERIALS AND METHODS. Each point represents the mean±SE of 3 independent experiments made in triplicates and was significantly different from baseline ($p<0.05$). Each point in sodium medium was significantly different from respective point in choline medium. Significance calculated using Student's *t* paired test.

The effect of 1—34 b PTH, db cAMP and IBMX is shown in *Table 2*. In the presence of 1.6×10^{-8} M PTH the ^{32}P uptake by PST as well as by PCT cells was markedly decreased to similar extent only in Na-medium without any effect on uptake in choline medium when compared to respective own control values. The net result was a significant inhibition of Na dependent ^{32}P uptake by PST and PCT cells (*Fig. 2*).

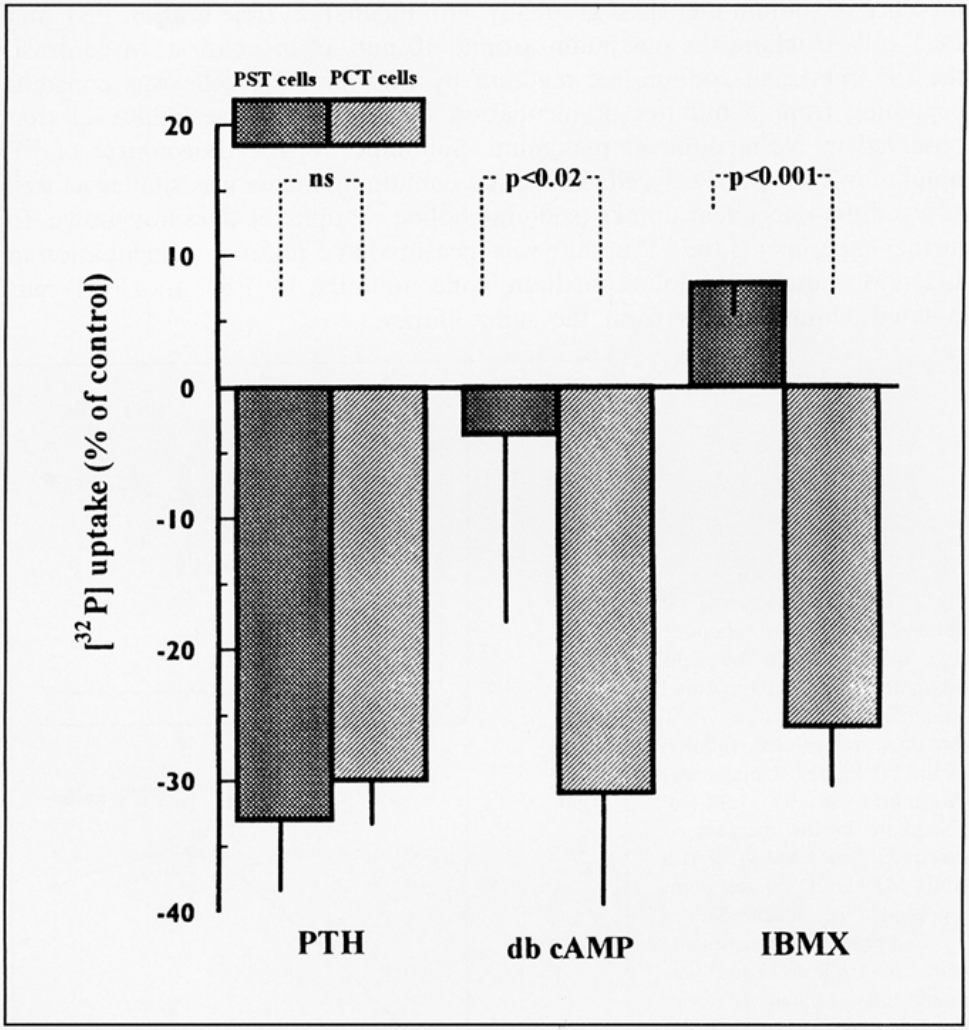

Fig. 2. Effect of 1.6×10^{-8} M bPTH, 15 μM db cAMP and 0.1 mM IBMX on phosphate uptake by rat proximal straight tubule (PST) and proximal convoluted tubule (PCT) cells. Each bar represents the mean($\pm$SE) between experimental and control values expressed as % of respective control from 5 to 6 independent experiments made in triplicates. Significance calculated using Student's *t* paired test.

In contrast, addition of 15 μM db cAMP to Na-medium elicited a marked decrease of the ^{32}P uptake only by PCT cells with no significant inhibition of uptake by PST cells. Again, no inhibition of uptake either by PST nor by PCT cells in choline medium was observed. The result was significant, over 30% decrease ($p<0.02$ vs 0), of Na dependent ^{32}P uptake by PCT cells with no statistical effect on uptake by a PST cells (NS vs 0), as shown in *Figure 1*.

Finally, the effect of IBMX, an cAMP phosphodiesterase inhibitor, on ^{32}P uptake was examined. The presence of IBMX in Na medium resulted in pronounced inhibition of ^{32}P uptake only by PCT cells whereas in PST cells, if anything, a slight increase in uptake was observed. No effect on ^{32}P uptake by PST or PCT cells in choline medium was noted (*Tab. 2*). As a result, the Na dependent uptake by PCT cells was also markedly decreased (-26%, $p<0.005$ vs 0). In contrary, a small but significant increase in sodium-dependent ^{32}P uptake by PST cells was observed ($+7.8\%$, $p<0.03$ vs 0, *Fig. 2*). In summary both PST and PCT cells were sensitive to inhibition of Na dependent ^{32}P uptake by PTH. However, the inhibitory effect of db cAMP or IBMX was observed only in PCT cells but no in PST cells.

Table 2. Effect of PTH, db cAMP and IBMX on sodium-dependent phosphate uptake by proximal tubule cells.

	Control	+PTH	Control	+db cAMP	Control	+IBMX
	$n=9$		$n=5$		$n=6$	
	nmoles/mg protein/5 min					
	PST cells					
A) Na-medium	0.98±0.13	0.66±0.08	0.80±0.16	0.77±0.11	0.96±0.13	1.01±0.12
B) Choline-medium	0.15±0.01	0.15±0.03	0.15±0.02	0.19±0.02	0.19±0.04	0.17±0.02
Na-dependent ^{32}P uptake (A—B)	0.83±0.13	0.51±0.06[b]	0.64±0.15	0.58±0.13	0.77±0.12	0.83±0.12[a]
	PCT cells					
A) Na medium	1.03±0.11	0.70±0.12	0.93±0.14	0.75±0.05	0.96±0.06	0.72±0.06
B) Choline-medium	0.15±0.03	0.11±0.02	0.18±0.03	0.26±0.07	0.12±0.03	0.11±0.01
Na-dependent ^{32}P uptake (A—B)	0.87±0.12	0.61±0.09[d]	0.75±0.14	0.49±0.08[a]	0.83±0.06	0.61±0.05[c]

Each value represents the mean±SE. Significant differences [a] $p<0.04$, [b] $p<0.03$, [c] $p<0.004$, [d] $p<0.001$ vs. Control calculated using Student's t paired test. PST, proximal straight tubule cells; PCT, proximal convoluted tubule cells. Phosphate transport was studied for 5 min. at 37°C. Concentration of 1—34 bPTH was 1.6×10^{-8} M; db cAMP 15 μM and IBMX 0.1 mM.

DISCUSSION

The present study demonstrates that Na-dependent ^{32}P uptake by cells isolated from rat kidney cortex enriched in Proximal Convoluted Tubule (PCT) cells or Proximal Straight Tubule (PST) cells, if harvested from outer stripe of outer medulla, is susceptible to PTH inhibition in both types of cells. However, only in PCT cells the Na dependent ^{32}P uptake decreases in response to db cAMP or IBMX but not in PST cells.

We applied the method of cells isolation from rabbit renal proximal tubules originally described by Nagineni *et al.* (15) to rat kidney. The principle is to use the intracellular-like solution to perfuse the kidney in order to preserve the morphological and functional integrity of the cells. The outer cortex in the rat kidney contains predominantly the proximal convoluted tubule (PCT) while proximal straight tubule (PST) occupies 68% of the outer stripe of outer medulla space (25). In contrast, the inner stripe of outer medulla is predominantly (70%) occupied by medullary thick ascending limb segments (26, 27). Careful dissection of outer cortex and outer stripe of outer medulla from their neighbourhood was the crucial step to harvest a material containing predominantly PCT or PST segments, respectively. The further isolation and purification of viable single cells with no glomeruli, debris, tubule fragments or cell clusters was achieved by combination of sieving and centrifugation in Ficoll/Percoll gradient (see Methods).

The ratio of gamma-glutamyl transpeptidase activity in OS to C isolated cells in present work is similar to the ratio of activities in PST to PCT microdissected segments (22): 4.5 and 5, respectively. These results strongly indicate that isolated cells from OS are mainly PST but not PCT. This conclusion is further supported by inspection of glutamine synthetase activity results (*Tab. 1*). The glutamine synthetase activity is exclusively present in PST but not in other fragments of rat nephron as found by microdissection studies (19, 20). In our studies the highest acitivity was found in cells originating from OS stripe indicating again the predominantly PST origin. The cells isolated from C zone were slightly contaminated by PST cells judging from glutamine synthetase activity (*Tab. 1*). Finally, the PST and PCT cells are similarly slightly contaminated with cells from a more distal parts of nephron as indicated by creatine kinase activity. This enzyme is almost absent in microdissected rat proximal (PCT and PST) but present in more distal (thick ascending loop of Henle and distal convoluted) segments of nephron (16).

PST and PCT cells isolated with a described method preserved the ability of Na-dependent phosphate uptake (*Fig. 1*) which was reproducible and lasting at least 4 hours (data not shown). The accumulation of ^{32}P by PST and PCT cells was similar (*Fig. 1*), it increased with time in Na-medium during 10 minutes of experiment and was about 5 times higher than in Na-free

medium. The direct comparison of the results of uptake by PST and PCT cells was possible because cells were isolated from the same kidney and the transport studies were carried out parallel. We chose the 5-th minute of incubation for further experiments as a time in which measurable and reproducible uptake was reflecting the initial velocity as closely as possible. The 1—34 b PTH at concentration 1.6×10^{-8}M inhibited markedly Na-dependent ^{32}P uptake in PST as well as in PCT cells ($-33.0 +/-6.2$ and $-30.0+/-3.0\%$, respectively, $p<0.001$ vs 0%) (*Tab. 2, Fig. 2*). The extent of inhibition was similar to that observed by Quamme *et al.* (29) in OK cells. There was no inhibitory effect in choline (Na-free) medium, which indicates that PTH specifically affects only the Na-dependent process. The similarity between PTH effects on PST and PCT was lost when the cells were exposed to 15 μM db cAMP (*Tab. 2, Fig. 2*). There was over 30% decrease of Na-dependent ^{32}P uptake in db cAMP treated PCT cells while it was a little (if any) inhibition observed in PST cells (3,6%, NS vs 0) when compared to their own control values. Again, no inhibitory effect on ^{32}P uptake in choline medium was observed in both types of cells. The results suggest (in spite of higher variation of results) that Na-dependent ^{32}P uptake by PST cells is refractory to the db cAMP, while in PCT cells it mimics the well known (28) inhibitory effect of PTH. In order to examine whether or not the endogenous cAMP plays the role as a messenger of PTH inhibitory effect on phosphate transport in PST cells, IBMX was applied as an inhibitor of cAMP phosphodiesterase. The increase of the intracellular cAMP level with concomitant inhibition of Na-dependent ^{32}P uptake in OK cells incubated with IBMX was shown (12, 29). In the present work the incubation of PCT cells with 0.1 mM IBMX was inhibiting markedly and significantly Na-dependent ^{32}P uptake by $26+/-5.4\%$ ($p<0.001$ vs 0%), while in PST cells, if anything, a small stimulation but not inhibition was observed (*Tab. 2, Fig. 2*). No effect on this uptake in Na-free media was observed both in PST and PCT cells.

The PST and PCT cells were exposed to high (1.6×10^{-8}M) concentration of PTH, which resulted in maximal cAMP concentrations in OK cells (27). Also, the db cAMP (15 μM) as well as IBMX (0.1 mM) were shown to cause maximal or submaximal inhibition of Pi uptake in OK cells (29). The effect of all three compounds resulted in similar inhibition of Pi uptake in PCT as reported for OK cells, while in PST cells only PTH affected Pi uptake but not cAMP or IBMX. These observations suggest the basic difference in mechanism of PTH inhibitory effect between PCT and PST. The existence of such the difference was implicated by the micropuncture studies examining the sensitivity of Pi transport to inhibitory effect of PTH in respiratory alkalotic or short term phosphate deprived rats (9, 10, 30). In these models of resistance to phosphaturic effect of PTH the decreased sensitivity to PTH was found only in PST but not in PCT segment (9, 10). Despite the resistance to phosphaturic

effect of PTH in both models the increase in urinary cAMP excretion in response to PTH was preserved as in control rats (3, 9). Moreover, in both models the resistance to PTH induced phosphaturia, the sensitivity of PST to inhibitory effect of PTH on Pi reabsorption was restored by beta adrenergic-receptors blockade, while no change in sensitivity to PTH in PCT segment was observed. All those findings indicated the existence of different mechanism(s) of PTH signal transduction in PST when compared to PCT segment. It corresponds with present finding that in contrast to PCT the PST cells are resistant to exogenous or endogenous cAMP inhibitory effect on Na-dependent Pi uptake with the same sensitivity to PTH. The present results lead us to the hypothesis that in respiratory alkalosis or in short term phosphate deprivation (3, 9, 10) the resistance to PTH induced phosphaturia is the defect in other than cAMP pathway in PST segment but not the erroneous reception of cAMP signal. This hypothesis might explain why urinary cAMP excretion is not decreased in both models of resistance to PTH: in contrast to PST in PCT segment, the phosphate reabsorption is not affected (9, 10) and Na-dependent phosphate uptake is sensitive to extra- and intracellular cAMP (present work). This implies that probably the source of PTH induced increase in urinary cAMP excretion is mainly a convoluted but not straight portion of proximal tubule in certain conditions (3, 8, 9, 10). This in turn might explain the observed dissociation between inhibition of phosphate transport by PTH and cAMP formation. It is possible that in proximal straight tubule the alternative to cAMP pathway, involving Ca^{2+}-diacylglycerol-kinase C cascade, may predominantly mediate the PTH induced inhibition of phosphate uptake as it is reported in literature (11, 12).

In summary, the present results as well as *in vivo* clearence and micropuncture studies (3, 4, 9, 10) strongly suggest that in PST (a "fine tuning segment" in renal handling of phosphate) some other than cAMP signalling system is also present. This system transmitting the information from PTH to phosphate reabsorbing site(s) seems to be blocked by respiratory alkalosis, short term phosphate deprivation or pseudohypoparathyroidism type II (3, 8, 9) resulting in phosphate conservation. The lack of sensitivity of PST cells to dbcAMP and IBMX might explain why the PTH-induced increase in urinary cAMP excretion may be not accompanied with phosphaturia.

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REFERENCES

1. Hoppe A, Destro M, Knox FG. Effect of NH_4Cl on phosphaturic response to PTH in the hamster: dissociation from acidemia. *Am J Physiol* 1980; 239: E328-E332.
2. Knox FG, Preiss J, Kim JK, Dousa TP. Mechanism of resistance to the phosphaturic effect of the parathyroid hormone in the hamster. *J Clin Invest* 1977; 59: 675—683.
3. Hoppe A, Metler M, Berndt TJ, Knox FG, Angielski S. Effect of respiratory alkalosis on renal phosphate excretion. *Am J Physiol* 1982; 243: F471—F475.
4. Hoppe A, Rybczyńska A, Knox FG, Angielski S. β-Receptors in resistance to phosphaturic effect of PTH to phosphaturic effect of PTH in respiratory alkalosis. *Am J Physiol* 1988; 255: R557—R562.
5. Knox FG, Berndt TJ, Haas JA. Resistance to the phosphaturic effect of parathyroid hormone during phosphate conservation is due to enhanced phosphate reabsorption by the pars recta. Massry SG, Olmer M, Ritz E in.: *Phosphate and Mineral Homeostasis* 1986; 208: 101—108.
6. Pastoriza-Munoz E, Mishler DR, Lechene C. Effect of phosphate deprivation on phosphate reabsorption in rat nephron. *Am J Physiol* 1983; 244: F140—F149.
7. Rybczyńska A, Hoppe A, Knox FG. Propranolol restores phosphaturic effect of PTH in short-term of phosphate deprivation. *Am J Physiol* 1990; 258: R120—R123.
8. Drezner M, Nelson FA, Lebovitz HE. Pseudohypoparathyroidism type II: a possible defect in the reception of the cAMP signal. *N Engl J Med.* 1972; 289: 1056—1060.
9. Rybczyńska A, Hoppe A, Knox FG. Effect of propranolol on phosphate reabsorption by superfiicial nephron segments in response to Parathyroid Hormone in phosphate-deprived rats. *J Am Soc Nephrol* 1990; 1: 200—204.
10. Rybczyńska A, Berndt TJ, Hoppe A, Knox FG. Site of restoration of the effect of PTH by propranolol in respiratory alkalosis. *Kidney Int* 1990; 38: 258—262.
11. Cole JA, Eber SL, Poelling RE, Thorne PK, Forte LR. A dual mechanism for regulation of kidney phosphate transport by parathyroid hormone. *Am J Physiol* 1987; 253: E221—E227.
12. Malmstrom K, Stange G, Murer H. Intracellular cascades in the parathyroid-hormone-dependent regulation of Na^+/phosphate cotransport in OK cells. *Biochem J* 1988; 251: 207—213.
13. Amiel C, Escoubet B, Silve C *et al.* Hypo-hyperphosphataemia. In Clinical Nephrology, AM Davidson, JS Cameron (eds). *Oxford University Press*, 1998, pp. 249—269.
14. Murer H, Forster I, Hilfiker H *et al.* Cellular/molecular control of renal Na/Pi-cotransport. *Kidney Int.* 1998; 53: S2—S10.
15. Nagineni N., Leveille PJ, Lee DBN, Yanagawa N. Isolation of cells from rabit renal proximal tubules by using a hyperosmolar intracellular-like solution. *Biochem J* 1984; 223: 353—358.
16. Bastin J, Cambon N, Thompson M, Lowry OH, Burch HB. Change in energy reserves in different segments of the nephron during brief ischemia. *Kidney Int* 1987; 31: 1239—1247.
17. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurements with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265—275.
18. Patterson MK Jr. Measurement of growth and viability of cells in culture.In: Methods Enzymology, Ed.: Jacoby WB, and Pastan IH, Academic Press Inc., *New York* 1979; 58: 141—152.
19. Burch HB, Choi S, McCarthy WZ, Wong PY, Lowry OH. The location of glutamine syntheatse within the rat and rabbit nephron. *Biochem Biophys Res Commun* 1978; 82: 498—505.
20. Guder WG, Ross BD. Enzyme distribution along the nephron. *Kidney Int* 1984; 26: 101—111.
21. Griffith OW, Meister A. Differential inhibition of glutamine and γ-glutamylcysteine synthetases by α-alkyl analogs of methionine sulfoxymine that induce convulsions. *J Biol Chem* 1978; 253: 2333—2338.

22. Yusufi AN, Murayama N, Gapstur SM, Szczepanska-Konkel M, Dousa TP. Differential propertiesa of brush-border membrane vesicles from early and late proximal tubules of rat kidney. *Biochi. Biophys Acta* 1994; 1: 117—132.
23. Gerhardt W, Wulf K. Creatin kinase. In: Methods of Enzymatic Analysis. 3rd ed. H.U. Bergmeyer, J. Bergmeyer, M. Grassl, Eds. Weiheim, Verlag-Chemie, 1983; 3: 508—539.
24. FCC Expert Panel on Enzymes: IFCC methods for the measurement of the catalytic concentrations of enzymes. Part 4. IFCC method for γ-glutamyl transferase. *J Clin Chem Clin Biochem* 1983; 21: 633—646.
25. Kriz W, Kaissling B. Structured organization of the mammalian kidney. The Kidney: *Physiology and Pathophysiology. Raven Press, New York* 1985; 14: 265—306.
26. Knepper MA, Danielson RA, Saidel GM, Post RS. Quantitative analysis of renal medullary anatomy in rats and rabits. *Kidney Int* 1977; 12: 313—323.
27. Kriz W. Structural organization of the renal medulla: comparative and functional aspects. *Am J Physiol* 1981; 241: R3-R16.
28. Gmaj P, and Murer H. Cellular mechanisms of inorganic phosphate transport in kidney. *Physiol Rev* 1986; 66: 36—70.
29. Quamme G, Pfeilschifter J, Murer H. Parathyroid hormone inhibition of Na^+/phosphate cotransport in OK cells. Generation of second messengers in the regulatory cascades. *Biophys Res Comm* 1989; 158: 951—957.
30. Berndt TJ, Knox FG. Nephron site of resistance to phosphaturic effect of PTH during respiratory alkalosis. *Am J Physiol* 1985; 249: F919—F922.

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