Archives of Nephrology

Volume 1, Issue 1, 2018, PP: 1-10

Effect of Nicotinamide on Phosphate Uptake by Renal Brush Border Membrane Vesicles (BBMV) from Superficial and Juxta-Medullary Cortex in Rats Fed a Normal (NPD) or Low Pi Diet (LPD)

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Abstract

The renal proximal tubule in the kidney is the major site of phosphate (Pi) conservation through controlled reabsorption and excretion. Na-dependent transport of Pi across proximal tubular brush border membrane (BBM) is an essential step that is regulated by hormones, drugs and diet. Phosphaturic hormone; parathyroid hormone and calcitonin have been shown to inhibit Pi transport in nephron segments and BBM populations under different dietary situations differently. In the present study we have investigated the effect of nicotinamide (NiAm), a non-hormone phosphaturic agent on Pi reabsorption in vivo by clearance studies and in BBM vesicles from superficial (SC) and juxta-medullary cortex (JM) in normal (NPD) and low Pi diet (LPD) fed rats. The clearance studies indicate that NiAM caused phosphaturia and decreased Pi reabsorption in vivo in rats fed NPD but had no phosphaturic effect in rats fed LPD.The effect of NiAm onuptake of Pi^{32} and L-[^{3}H]-Proline was then determined in BBMV-SC and BBMV-JM prepared from same kidneys of the same animals. Administration of NiAm caused phosphaturia and inhibited Pi transport both in BBMV-SC and BBMV-JM to the same degree in rats fed NPD. However, in rats fed LPD, NiAm failed to increase urinary excretion of Pi but inhibited Pitransport in BBMV-SC and not in BBMV-JM. The transport of L-proline, however, was not affected by NiAm in any case. Inhibition of Pi uptake in BBMV-JM rather than that in BBMV-SC seems to be associated with increased urinary excretion of Pi. NiAm also increased NAD content in BBMV-SC and BBMV-JM but to greater extent in NPD compared to LPD rats. NiAm elicited increase of NAD in BBMVs appeared to be the modulator of Pi reabsorption in vivo and in BBMV-SC and BBMV-JM inrats fed NPD and only in BBMV-JM in rats fed LPD.

Keywords: *Kidney, Proximal tubule, superficial cortex, juxta-medullary cortex, renal heterogeneity, Na^{+}-dependent Pi transport, nicotinamide, NAD, phosphate deprivation.*

Abbreviations

BBM: Brush Border Membrane

BBMV: Brush Border Membrane

BBMV-SC: BBMV-Superficial Cortex

BBMV-JM: BBMV-Juxta-medullary Cortex

BP: Blood Pressure

FE_{Pi} %: Fractional Excretion of Pi

FR_{Pi} %: Fractional Reabsorption of Pi

GFR: Glomerular Filtration Rate

LPD: Low Pi Diet

NPD: Normal Pi diet

NiAm: Nicotinamide

NAD: Nicotinamide Adenine Dinucleotide

PTH, parathyroid hormone

PCT: Proximal Convoluted Tubule

Proximal Straight Tubule: PST

$P_{Pi:}$ Plasma Pi

TPTX: Thyroparathyroidectomized

wt./ wt.: weight / weight

Introduction

Pi plays essential role in bone mineralization, intermediary metabolism, formation of high energy phosphate compounds, and chemical signalling, etc. The proximal tubule in renal cortex is the site of Pi conservation through controlled reabsorption and excretions [1].The bulk of filtered Piis reabsorbed by the proximal tubule across its luminal brush border membrane (BBM)by Na-dependent Pi transporters [2-5].However, in many but not all situations, the changes in the rate of Pi transport determined in BBMV correlated well with the changes observed in vivo [2].The rate of Na-gradient ($Na_o > Na_i$) dependent uptake of Pi by renal proximal tubule across the BBM is rate limiting step and is regulated by hormones, drugs, chemical agent and dietary Pi status [4, 6-12]. The Pi reabsorption by renal proximal tubules determined in vivo or in vitrodemonstrates both axial (intra-nephron) and inter-nephron heterogeneity that originate from different cortical regions and differ in the structure and transport functions [5, 13, and 14].

Current experimental evidence suggests that basal and hormonal or otherwise modulated rate of Pi reabsorption in vivo and BBM Pi transport differs in BBMV isolated fromsuperficial (BBMV-SC) and juxta-medullary (BBMV-JM) cortical regions [4, 11, 14-21]. We have reported that BBM transport of Pi was inhibited by PTH, calcitonin, fastingand renal ischemia and increased by thyroid hormone predominantly in BBMV-JM[17, 18 and 20]. In contrast, low Pi diet (LPD) and fasting/re-feedingincreasedPi transport to greater extentin BBMV-SC as compared to BBMV-JM [18, 19, 21 and 22]. Infusion of atrial natriuretic peptide, however, decreased Pi transport both in BBMV-SC and BBMV-JM to similar degree in the rats fed NPD [12].

It has been reported that administration of nicotinamide (NiAm) causes phosphaturia and inhibits proximal tubular Pi reabsorption in vivo [23] and Pi uptake in BBMV in NPD-fedrats (3). Micropuncture studies have shown that NiAm inhibit Pi reabsorption in the pars recta (PST) and not in early proximal tubule (PCT) in rat fed NPD but Pi reabsorption was markedly inhibited by NiAm in only PCT in LPD-fed rats without causing phosphaturia [23]. Nicotinamide adenine dinucleotide (NAD) also inhibits Pi reabsorption in vivo and in vitro (29 and 30). The present studies were undertaken to investigate the effect of NiAm on Pi reabsorption in vivo and in BBMV-SC and BBMV-JM in rats fed either NPD or LPD and its relationship, if any, withcellular NAD content. The results indicate that NiAm causes phosphaturia and increased NADcontent in BBMV and inhibited Pi reabsorption both in BBMV-SC and BBMV-JM to a similar degree in rats fed NPD. However, NiAm inhibited Pi uptake only in BBMV-SC but not in BBMV-JM in rats fed LPD.NiAm elicited NAD content in respective BBMV preparations appeared to be a possible modulator of Pi transport.

Material and Methods

Materials

Nicotinamide(NiAm) was purchased from Sigma Chemical Co. (St Louis, MO). Radioisotopes ^{32}Pi, and L-[^{3}H]-proline were purchased from New England Nuclear Co. (Boston, MA). Bacterial Luciferase was purchased from Worthington Biochemical (Freehold, NJ). Low-phosphorus diet containing 0.07% (wt. /wt.) phosphorus was from ICN Pharmaceuticals (Cleveland, OH). All other chemicals and bio-chemicals all of the highest were purchased either from Sigma Chemical Co, or from other standard suppliers.

Exprimental Design

Clearance Studies

As shown in Fig. 1, adult male Sprague-Dawley rats weighing 250-300 g were used in the study and were conditioned for one week on a standard rat diet and water adlibitum before the start of the experiment. All animals were kept under conditions that prevented them from experiencing unnecessary pain according to the guide lines of institutions ethical committee. The rats were then divided into four groups (8rats per group). Two groups of rats were fed a normal

phosphate (0.7% Pi) whereas the other two were fed a low (0.07% Pi) phosphate diet for four days (Fig. 1) and had free excess to water. The clearance studies were conducted as described earlier [17]. Rats were anesthetized with Inactin (100 mg/kg body weight) and were acutely thyroparathyroidectomized(TPTX) by heat cautery and tracheostomy was performed. Catheters were inserted into jugular veins for infusions and into the carotid artery for blood sampling and monitoring of blood pressure.A catheter was placed in the bladder for urine collection. Body temperature was maintained between 36° C and 38° C with a heated table. Rats were infused with 6% inulin in 0.9% NaCl which was infused at a rate of 8 ml/hr for one hour and then decreased to 2 ml/hr for the remainder of the experiment.The NiAm-treated rats were given NiAm (1g/kg, i.p.) two hours prior to anesthesia and the control rats were given the blank solution (0.9% NaCl). Clearances were started from control and nicotinamide (NiAm)-treated rats two hours after NiAm treatment; one urine collection (15 min) and an arterial blood sample were taken. Fractional excretion (FE_{Pi} %) and Fractional reabsorption (FR_{Pi} %) were determined as described elsewhere [17]. Phosphate concentrations in the plasma and urine samples were determined by the method of Chen et al. [24].

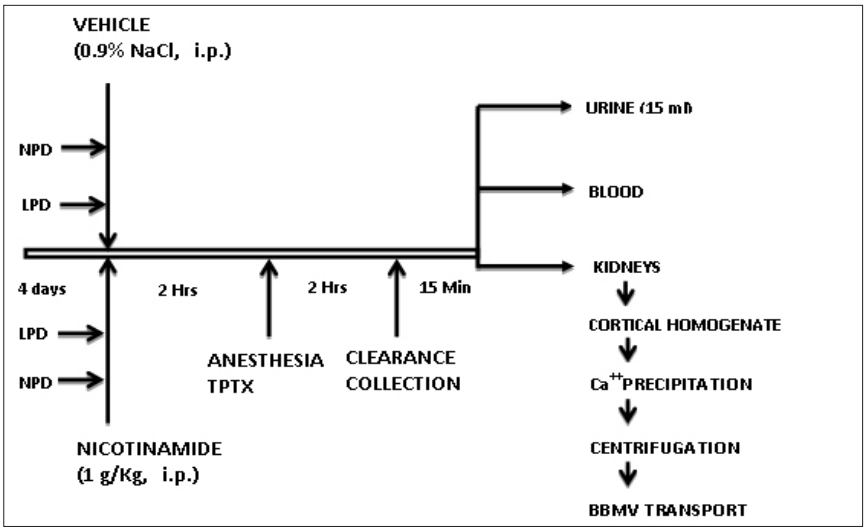

Fig 1. *Experimental Design (NPD: Normal Phosphate Diet; LPD: Low Phosphate Diet; TPTX: Thyroparathyroidectomized)*

Methods

Preparation of Brush Border Membrane Vesicles (BBMV) from Superficial Cortex (BBMV-SC) and Juxta-Medullary Cortex (BBMV-JM)

After the clearance measurements, the kidney were removed from the animal and immediately chilled and kept in ice-cold saline solution. Dissections and all other preparative procedures were conducted strictly at 0-4° C. BBM vesicles were prepared from homogenates of renal tissues using Ca-precipitation method identical to that employed and described in our previous and recent publications [17 and 19]. After decapsulation, traverse sectional slices of the kidney were cut with a razor blade in the middle of the cortical thickness between the kidney surface and cortical-medullary junction. The superficial cortex (SC) was carefully separated from the juxta-medullary (JM) cortex as described earlier [14 and 17]. Since the straight portion of proximal tubule dip beyond the cortical-medullary junction, the outer most layer of adjacent medulla (outer-stripe, i.e., "red" medulla was included with the juxta-medullary cortical tissue. BBMV from superficial and juxta-medullary cortex were prepared simultaneously from the same kidney with identical technique to avoid any experimental variation. In order to obtain sufficient tissue from both the SC- and JM- cortical region, BBMV were prepared by pooling the tissue from two rats.Briefly, freshly minced tissues were homogenized in 50 mM mannitol and 5 mM Tris-HEPES buffer pH 7.0 (20 ml/g), in a glass Teflon homogenizer with 4 complete strokes. The homogenate was then subjected to high speed homogenization in an Ultra Turex homogenizer (Type T-25, Janke & Kunkel GMBH & Co. KG. Staufen)

for three strokes of 15 s each with an interval of 15 s. $MgCl_2$ was added to the homogenate to a final concentration of 10 mM and the mixture stirred for 20 min on ice. The homogenate was centrifuged at 2000g for 10 min in a Beckman centrifuge (J2 MI, Beckman instruments Inc, Palo Alto, C.A. USA) using JA-17 rotor and the supernatant was then recentrifuged at 35,000g for 30 min. The pellet was resuspended in 300 mM mannitol and 5mM Tris-HEPES, pH 7.4, with four passes by a loose fitting Dounce homogenizer (Wheaton IL, USA) and centrifuged at 35,000g for 20 min in a 15 ml corex tube. The outer white fluffy pellet of BBMV was resuspended in small volume of buffered 300 mM mannitol. As in previous studies, the qualities of the BBMV were evaluated by enrichment of BBM marker enzymes. The protein content in the BBMV and respective homogenates was measured using the method of Lowry et al [25] as modified by Yusufi et al [14].

Transport of ^{32}Pi and L-[^{3}H]-Proline in BBMV-SC and BBMV-JM

The uptake of Pi and L-proline were measured by using aliquots from the freshly prepared BBMV preparations, at 20 ˚C by rapid filtration techniqueeither in the presence or absence of Na-gradientas described in detail in our previous studies (14 and 17).Uptake was initiated by addition of 30 µl of incubation medium containing 100 mM mannitol, NaCl/KCl 100 mM, 5mM Tris–HEPES, pH 7.5, 0.1 mM $K_2H^{32}PO_4$ to 15 µl BBM suspension (50–100µg protein) and incubated for the desired time intervals (see Results). The uptake was stopped by the addition of 3 ml ice-cold stop solution (containing 135 mM NaCl, 5 mM Tris–HEPES and 10mM sodium arsenate, pH 7.5) and filtered immediately through 0.45 µm DAWP Millipore (USA) filter and washed three times with the stop solution using a Cornwall-type syringe (Wheaton, IL). Correction for non-specific binding to filters was made by subtracting from all data the value of corresponding blank obtained by filtration of the incubation buffer without vesicles. The uptake of L-proline (0.025 mM) was also determined by rapid filtration technique using aliquots from same BBMV preparations as described earlier [17]. The radioactivity of dried filters was measured in a Beckman liquid scintillation counter with 10 ml scintillation fluid.All transport parameters were measured, as a rule, in quadruplicate for each BBMV preparation.

Determination of NAD

The NAD content in the BBMV was determined by a bioluminescence micro assay by the method [26] described in detail in our previous studies [27]. Briefly, acidic extraction of NAD from BBMV was performed by mixing 0.01 ml of the BBMV suspension with 0.02 ml of a solution containing 0.1 M HCl and 4 mM cysteine-HCl. After incubation at room temperature for 15 min, the samples were neutralized by the addition of 0.02 ml of 0.1 M NaOH, and an aliquot (0.005 ml) of the final mixture was added to 0.044 ml of light yielding solution containing bacterial luciferase. After a delay of 6 s, the bioluminescence was measured 3 times at intervals of 12 s in a Photomultiplier (Packard Pico-lite Model 6100, Packard Instrument Co., and Downers Grove, Ill, USA). Standards and blanks were prepared in the same way; the luminescence was proportional to NAD concentration the range 10^{-12} – 10^{-6}M.

Statistical Analyses

All data are expressed as Mean ± SEM for at least 4-5 different preparations. Statistical evaluation was conducted by one-way ANOVA and by paired/ group student's t test using SPSS 7.5 software. A probability level of $p<0.05$ was selected as indicating statistical significance. The changes between various groups were compared with control values for better understanding and clarity.

Results

Effect of Nicotinamide (NiAm) on Pi Reabsorption in Vivo Determined by Clearance Studies in Normal (NPD) And Low Pi (LPD) Diet Fed Rats by Clearance Studies

The clearance data, summarized in Table-1, showed that NiAm treatment produced phosphaturia in rats fed NPD which is evidenced by an increase in fractional excretion of Pi (FE_{Pi} %) or a reciprocal decrease in fractional reabsorption of Pi (FR_{Pi} %). However, NiAm had no phosphaturic effect in rats fed LPD. There was no significant difference in glomerular filtration rate (GFR) and plasma phosphate (P_{Pi}) between control and NiAm treated rats in both NPD and LPD fed rats. However, a slight decrease was observed in blood pressure (BP) after NiAm treatment irrespective of the feeding of NPD or LPD.

Table 1. *Effect of Nicotinamide (NiAm) on Fraction excretion (FE_{Pi}%)and reabsorption (FR_{Pi}%) in Normal and Low Phosphate Diet fed rats.*

Groups (ml/min)	GFR (mM)	FE_{Pi}% (mm Hg)	FR_{Pi}%	P_{Pi}	BP
NPD Control	3.00±0.40	8.00±3.00	92.00±3.00	2.10±0.10	127.00±7.00
NiAm	2.30±0.20	40.00±10[b]	53.00±11[b]	2.00±0.20	87.00±8.00[b]
LPD Control	2.40±0.20	1.00±0.50	99.70±0.30	1.50±0.20	122.00±8.00
NiAm	2.70±0.20	11.00±7.00	89.00±7.00	1.50±0.20	88.00±4.00[b]

[a]The results are expressed as mean ± SEM for four separate experiments (2 rats in each experiment).

FE_{Pi}%, fractional excretion of phosphate; FR_{Pi}%, fractional reabsorption of phosphate; P_{Pi}, Plasma phosphate; BP, Blood Pressure, GFR, glomerular filtration rate

[b]Significantly difference from corresponding control values.

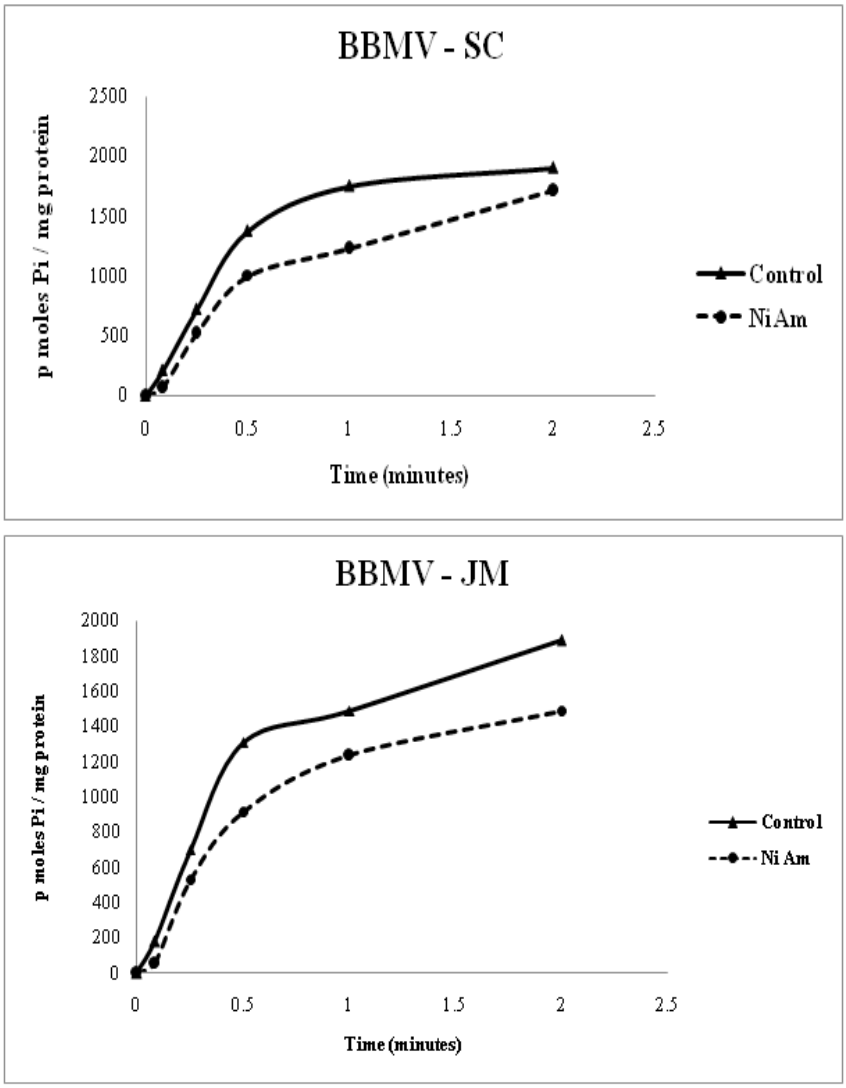

Fig 2. *Time course of Na+ gradient – dependent uptake of phosphate by BBMV – SC and BBMV – JM in NPD fed rats*

Effect of Nicotinamide (NiAm) on Pi Reabsorption in Vitro Determined in Brush Border Membrane (BBM) Vesicles Isolated from Superficial Cortex (BBMV-SC) and Juxta-Medullary Cortex (BBMV-JM) in Normal (NPD) and Low Pi (LPD) Diet Fed Rats

Figure-2 describes the times course for Na-gradient dependent transport of Pi in BBMV-SC and BBMV-JM of control and NiAm –treated rats fed NPD. As can be seen in Fig.2, NiAm treatment decreased Na-gradient transport of Pi both in BBMV-SC and BBMV-JM to a similar extent at all-time points measured in the concentrative "uphill" phase (5 s to 2 min) However, NiAm had no effect on phosphate uptake in the equilibrium phase at 120 min (data not shown). Therefore, in all subsequent determinations, the uptake of Pi in the concentrative "uphill" phase was measured at 30 sec and for the equilibrium at 120 min. The uptake of Pi or L-proline (for comparison) by BBMV was expressed both as a net rate, and in relative terms as a percentage of the uptake in the uphill phase relative to uptake at 120 min as percent "overshoot" (%Δ) as in previous studies [14 and 17].

The results summarized in Table 2 show that NiAm treatment caused a marked decrease of the Na-dependent uptake of Pi at 30 s in both BBMV-SC and BBMV-JM of rats fed NPD to a similar degree. However, the equilibrium uptake (120 min) of Pi was not changed significantly by NiAm. In the same aliquots of the BBMV, Na dependent transport of L-[^{3}H]-Proline was not affectedneither at early phase of 15 s nor at 120 min (Table 2). The effect of NiAm on Pi and L-Proline transport was also determined in rats fed LPD and the results are summarized in Table 3. In contrast to the effect of NiAm in rats fed NPD. NiAm administration markedly decreased the Pi transport only in BBMV-SC and no significant decrease was observed in BBMV-JM. NiAm also had no effect on L-[^{3}H]-Proline transport in BBMV-SC and BBMV-JM in rats fed LPD (Table 3).

Table 2. *Effect of Nicotinamide on Na^{+} gradient Uptake of ^{32}Pi, and L-[^{3}H]-Proline by BBMV-SC and BBMV-JM of NPD-fed rats*

	[^{32}Pi]-phosphate intake, (pmole/mg protein)			L-[^{3}H]-Proline uptake, (pmole/mg protein)		
	30s	120min	+Δ%	15 sec	120 min	+Δ%
BBMV-SC Control	2227±188	959±66	132±7	230±37	42±3	445±51
NiAm	1580±126** (-29±3%)	840±95	90±10** (-32±6%)	258±64 (NS)	45±7 (NS)	492±156 (NS)
BBMV-JM Control	1290±104	451±39	191±22	278±39	22±3	1175±161
NiAm	913±59** (-29±6%)§	436±27	110±13 (-42±2%)	319±41 (NS)	27±4 (NS)	1114±210 (NS)

*Values are mean ± SEM of four separate experiments (2 rats in each experiment) experiments.

+The ratio of initial uptake (30sec for Pi, 15 sec for L-proline) expressed relative to the equilibrium (120 min) point, taken as 100%, i.e., Δ% overshoot.

§Values in parentheses are percent inhibition with respect to corresponding control values.

**Significantly different from corresponding controls (t-test/ ANOVA)

Table 3. *Effect of Nicotinamide on Na^{+} gradient Uptake of ^{32}Pi, and L-[^{3}H]-Proline by BBMV-SC and BBMV-JM of LPD-fed rats*

	[^{32}Pi]-phosphate intake, (pmole/mg protein)			L-[^{3}H]-Proline uptake, (pmole/mg protein)		
	30s	120min	+Δ%	15 sec	120 min	+Δ%
BBMV-SC Control	3539±450	1086±93	224±22	274±7	41±8	604±115
NiAm	2439±348 (-31±7%)	975±100	151±24** (-32±10%)	244±25 (NS)	43±8 (NS)	485±49 (NS)
BBMV-JM Control	1740±131	585±65	202±16	333±8	21±2	1489±173
NiAm	1502±73 (-12±7%)	525±33	188±15 (-6±7%)	287±26 (NS)	23±3 (NS)	1157±125 (NS)

*Values are mean ± SEM of four separate experiments (2 rats in each experiment) experiments.

+The ratio of initial uptake (30sec for Pi, 15 sec for L-proline) expressed relative to the equilibrium (120 min) point, taken as 100%, i.e., Δ% overshoot.

§Values in parentheses are percent inhibition with respect to corresponding control values.

**Significantly different from corresponding controls (t-test/ ANOVA).

Effect of Nicotinamide (NiAm) on NAD Content in BBMV-SC and BBMV-JM of Normal (NPD) and Low Pi (LPD) Diet Fed Rats

The effect of NiAm was determined on NAD content in BBMV-SC and BBMV-JM isolated from rats fed either NPD or LPD by bioluminescence technique as described earlier [26 and 27]. The data summarized in Table 4 show that the content of NAD in general, was slightly higher in BBMV-SC compared to BBMV-JM in control rats fed either NPD or LPD. Administration of NiAm significantly increased NAD content in both BBMV-SC and BBMV-JM. In NPD fed rats, NiAM markedly increased the NAD content in BBMV-SC (+311%) as compared to BBMV-JM (+226%). NiAm also increased the NAD content to much greater extent in BBMV-SC (+246%) than in BBMV-JM (+178%) in rats fed LPD. However, the increase of NAD in both BBMV preparations was significantly higher in rats fed NPD compared to LPD fed rats (Table 4).

Table 4. *Effect of Nicotinamide (NiAm) on NAD content in BBMV-SC and BBMV-JM of Normal Phosphate Diet (NPD) and Low Phosphate Diet (LPD) fed rats*

Groups	BBMV-SC	BBMV-JM
Normal Phosphate Diet Control	259±19	227±14
NiAm	1049±207* (+311%)	739±159* (+226%)
Low Phosphate Diet Control	236±17	216±27
NiAm	817±134* (+246%)	601±82* (+178%)

The results are mean ± SEM (pmole/mg protein) of four different experiments. Each BBMV preparation included tissue pooled from two rats.

Values in parentheses represent percentage change from respective control.

* Significantly different from control at P< 0.05 by t-test and/or by one ANOVA.

Discussion

The renal handling of Pi is of paramount importance in the maintenance of Pi in the body. Pi transport across the renal proximal tubule BBM occurs by Na-Pi co-transport system [4 and 5]. Evidenceindicates that BBMV-SC and BBMV-JM arederived from different population of nephrons located in superficial and juxta-medullary cortical regions and contain BBM predominantly from proximal convoluted (PCT) and proximal straight tubule, (pars recta, PST), respectively [14].BBMV-SC and BBMV-JM also exhibit diverse Na-Pi transport properties and BBM marker enzyme distributions [14]and respond differentially to pharmacological stimuli and dietary contents [16-22].

We have reported that phosphaturic hormones, such as PTH and calcitonin inhibit Pi reabsorption in vivo and in BBMV-SC and BBMV-JM alike in rats fed NPD or LPD (17). However, the effect on Pi transport wasalways greater in the BBMV-JM than in BBMV-SC in both rats fed NPD or LPD. Furthermore, the effect of PTH and calcitonin seems greater in BBMV-JM in LPD fed compared to NPD-rats [17].The present studies were performed to understand whether NiAm, a non-hormonal phosphaturic agent would inhibit the Pi reabsorption in vivoand in BBMV-SC and BBMV JM of rats fed NPD/LPD similar to PTH and calcitonin or not. The results obtained indicate that administration of NiAm in rats fed NPD caused phosphaturia (FE_{pi} %) and inhibits Pi reabsorption (FR_{pi} %) in vivo(Table 1) and inhibits Pi transport both in BBMV-SC and BBMV-JM to similar extents (Table 2). However, NiAm administered to rats fed LPD increased fractional excretion (FE_{Pi} %) but did not inhibit Pi reabsorption (FR_{Pi} %, Table 1). NiAm inhibited Pi transport in the BBMV also selectively. NiAm inhibited BBM uptake of Pi in BBMV-SC but BBM uptake of Pi in BBMV-JM was not significantly lowered (Table 3). These effects of NiAm on BBM transport of Pi clearly different from those of PTH and calcitonin as observed in rats fed NPD or LPD [17]. The hormonal effects on Pi transport were greater in the BBMV-JM than in BBMV-SC both in rats fed NPD or LPD [16 and 17]. The effect of NiAm was similar both in BBMV-SC and BBMV-JM in NPD fed rats but NiAm inhibited BBM Pi only in BBMV-SC in LPD rats. The observed differences on the effect of PTH, calcitonin and NiAm can be attributed to the differences in their mode and site of actions. While the

effect of PTH and calcitonin has been shown to be mediated by a second messenger mechanism e.g., by cAMP (Dousa, 1996), NiAM might exert it effect on Pi transport via intra cellular product of NiAm [5 and 29].The differences on the effect of hormones and NiAm in NPD and LPD rats might be due to influence of LPD that increases Pi uptake predominantly in BBMV-SC compared to BBMV-JM [5 and 22]. Secondly, differences between BBMV-SC and BBMV-JM which are mainly derived from early proximal convoluted tubule (PCT) and from the late proximal straight tubule, respectively [14]. Thus renal handling of Pi appeared to be due the effect of both axial as well as inter-nephron heterogeneity.

The mechanism by which NiAm exert its effect on Pi transport is not very clear. Evidence has suggested that NAD in vitro or increased concomitantly in response to administrationof NiAm serves as an inhibitor of Pi reabsorption in vivoand/or BBM transport of Pi[29 and 30]. Micropuncture studies showed that the administration of NiAm to rats fed a normal Pi diet (NPD) inhibited Pi reabsorption only in the late proximal tubule (PST) whereas in rats fed LPD, NiAm inhibited Pi reabsorption only in PCT and not PST [23].We have reported that administration of NiAm increased the NAD content differentially in proximal convoluted tubules (PCT) and proximal straight tubules (PST) in rats fed either NPD to LPD [27]. In rats fed NPD. NAD was significantly increased both in PCT and PST, although to a greater extent in PCT. In contrast, in rats fed LPD, the NAD was increased only in PCT [27].These findings may suggest thatsignificant increased levels of intracellularNAD in both PCT and PST in rats fed NPD would lead to the inhibition of Pi transport both in BBMV-SC and BBMV-JM. On the other hand, the significant increase of NAD only in PCT and not PST in rats fed LPD would lead to the inhibition of Pi transport only in BBMV-SC. The present results show that NiAm increased NAD in both BBMV-SC and BBMV-JM to similar extent in NPD rat but in LPD rats the increase of NAD was much greater in BBMV-SC than in BBMV-JM. Secondly, the increased NAD levels were much higher in NPD as compared to LPD rats. Thus, it appears that NiAm elicited NAD might have inhibited BBM Pi transport in both BBMV-SC and BBMV-JM in NPD rats and only in BBMV-SC in LPD rats.

Taken together, the past and present results lead us to conclude that NiAm administration increases intracellular NAD content in different nephron segments namely, PCT and PST and in extension in BBMV-SC and BBMV-JM differently and inhibitthe Pi transport in BBMV-SC and BBMV-JMin NPD and LPD rats accordingly.The results further suggests that inhibition of Na-dependent Pi transport in BBMV-JM rather than that in BBMV-SC seems to be associated with increased urinary excretion of phosphate. The nephron heterogeneity also play critical role in renal handling of Pi transport.

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Citation: **Ahad N. K. Yusufi, Sheeba Khan, Faiz N. K. Yusufi, et al.** *Effect of Nicotinamide on Phosphate Uptake by Renal Brush Border Membrane Vesicles (BBMV) from Superficial and Juxta-Medullary Cortex in Rats Fed a Normal (NPD) or Low Pi Diet (LPD). Archives of Nephrology. 2018; 1(1): 1-10.*