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Mechanisms of heterogeneity of Na^+-P_i cotransport in superficial and juxtamedullary renal cortex

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To explore the mechanisms of axial heterogeneity of proximal tubular P_i transport, brush border membrane vesicles (BBMV) were prepared from superficial (BBMV-SC) and juxtamedullary (BBMV-JM) cortex of rat kidneys. Na^+ gradient-dependent P_i transport was measured after the imposition of an inside-negative electrical potential created by either a K^+ gradient ($[K^+]_i > [K^+]_o$) or a proton gradient ($[H^+]_i > [H^+]_o$) in the presence of ionophores. The initial Na^+-dependent P_i uptake was higher in BBMV-SC than in BBMV-JM, both in the presence and absence of ionophores. Na^+-dependent D-glucose uptake remained unchanged. We did not find a significant electrogenic transport component in either BBMV population when the non-specific effect of ionophores on P_i transport was taken into account. The stoichiometry of Na^+-P_i cotransport was 2:1 in BBMV-SC and BBMV-JM. Phosphonoformic acid (PFA) competitively inhibited P_i transport. The inhibitory constant (K_i) for PFA was lower in BBMV-SC ($237 \pm 1.7\ \mu M$) than in BBMV-JM ($409 \pm 53\ \mu M$) ($P < 0.05$). Arrhenius plots showed a higher rate of P_i uptake in BBMV-SC compared to BBMV-JM at all temperatures. However, the transition temperatures did not differ. We conclude that axial heterogeneity of P_i transport is not due to differences in electrogenicity or stoichiometry of transport.

Introduction

The rate-limiting step in renal P_i transport is the active, Na^+ gradient-dependent uptake of P_i across the brush border membrane (BBM) of renal proximal tubule epithelial cell. Micropuncture studies have demonstrated a heterogeneity for P_i transport along the nephron length (axial heterogeneity). Phosphate reabsorption is lower in deep nephrons compared to superficial nephrons [1–3]. Superficial cortex contains mostly the proximal convoluted tubules (PCT) and juxtamedullary cortex mostly the pars recta (PST). In dogs placed on a low P_i diet, BBM vesicles (BBMV) prepared from superficial cortex (BBMV-SC) have a greater capacity for P_i transport than BBMV prepared from juxtamedullary cortex (BBMV-JM) [4]. This difference is specific for P_i, since D-glucose transport is not different in the two BBMV populations. The administration of 3,5,3′-triiodothyronine (T_3) produces an increase in the V_{max} for P_i transport without an effect on the K_m [5]. The effect of T_3 on P_i transport is limited to BBMV prepared from the juxtamedullary cortex [7]. In primary cultured cells from rabbit PCT and PST, only cells from PST show a reduction of P_i transport after stimulation by either bPTH or 8-bromo cAMP [6]. These results are in agreement with those obtained by micropuncture studies [3].

Axial heterogeneity may be due to differences in the density of Na^+-P_i cotransporters or in the lipid composition of the BBM of the two segments resulting in differences in membrane fluidity [8]. Changes in membrane fluidity have been shown to affect P_i transport in renal BBMV [9]. Although P_i transport is considered electroneutral when total cortex is examined [10], electrogenic P_i transport may be important in late proximal tubules [11]. Recent studies suggest a dependence of P_i transport on electrical potential [12,13]. Finally, the stoichiometry of Na^+-P_i cotransport may be different in BBMV-SC and BBMV-JM, resulting in different rates of transport.

Abbreviations: P_i, inorganic phosphate (orthophosphoric acid); BBM, brush-border membrane; BBMV, brush-border membrane vesicle; PCT, proximal convoluted tubule; PST, proximal straight tubule; LPD, low-P_i diet; NPD, normal-P_i diet; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; PFA, phosphonoformic acid (foscarnet); MTH, mannitol/Tris/Hepes.

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The purpose of the present studies was to determine whether the difference in P_i transport between PCT (assessed by BBMV-SC) and PST (assessed by BBMV-JM) are secondary to differences in the electrogenic component and/or the stoichiometry of Na^+-P_i cotransport of the two proximal tubular segments. We also studied Na^+-P_i cotransport in BBMV from animals on a low-P_i diet, to determine if changes in electrogenicity or stoichiometry may contribute to the preferential increase in P_i uptake by BBMV-SC in response to P_i deprivation.

Our results expand on the recent report by Levi [8] suggesting that differences in P_i transport between BBMV-SC and BBMV-JM are due to differences in the density of the cotransporters as well as the fluidity of BBM. Differences in the electrochemical behavior of BBM do not seem to play a major role in the heterogeneity of P_i uptake in BBMV-SC and BBMV-JM.

Methods

1. Preparation of brush border membrane vesicles

Sprague-Dawley rats weighing 250–300 g were used for all experiments. In some experiments, animals were placed on a low-P_i diet for 5 days. The diet contained 0.07% phosphorus, 1% Ca^{2+}. Control diets contained 0.9% phosphorus, 1% Ca^{2+}. Brush border membrane vesicles (BBMV) were prepared by a divalent cation precipitation method [14]. For preparation of BBMV from superficial and juxtamedullary cortex, the kidneys were sliced horizontally in 3 mm sections. The outer 3 mm portion of the cortex was used for preparation of BBMV-SC, and the inner cortex including the outermost portion of red medulla was used for preparation of BBMV-JM. Cortices were then suspended in a buffer containing 50 mM mannitol, 2 mM Tris-Hepes (pH 7.5), homogenized with a glass-teflon homogenizer, further diluted in the same buffer and rehomogenized with a Polytron homogenizer. $CaCl_2$ was added at a final concentration of 10 mM, and the mixture was agitated for 20 min. After centrifugation at $2000 \times g$ for 10 min, the supernatant was centrifuged at $35\,000 \times g$ for 30 min. The pellet was resuspended in a buffer containing 300 mM mannitol, 5 mM Tris-Hepes (pH 7.5) (MTH buffer), and homogenized using a tight-fitting Dounce homogenizer then recentrifuged at $35\,000 \times g$ for 20 min. The final pellet was resuspended in MTH buffer at a protein concentration of approx. 6 mg/ml and used for transport measurements.

2. Transport studies

Transport measurements were carried out at 20°C on freshly prepared BBMV, using a rapid filtration method as previously described [15]. Transport was interrupted by the rapid addition of an ice-cold stop solution containing 135 mM NaCl, 10 mM $NaHAsO_4$, 5 mM Tris-Hepes (pH 7.5). The tubes were then washed three more times over Millipore filters (0.65 μm pore size), using a suction apparatus. Media for transport contained in final concentrations: 100 mM NaCl or KCl, 5 mM Tris-Hepes (pH 7.5) and either 0.1 mM $K_2H^{32}PO_4$, or 0.05 mM D-[^{3}H]glucose. All experiments were carried out in quadruplicate, and the results were expressed relative to BBMV protein content determined by the Lowry method [16].

The effect of an inside-negative electrical potential on transport was determined after the imposition of a K^+ gradient ($[K^+]_{in} > [K^+]_{out}$) in the presence of valinomycin. BBMV prepared as above were resuspended in a medium containing 100 mM K_2SO_4, 100 mM mannitol, 5 mM Tris-Hepes (pH 7.5) (medium A), incubated for 60 min, centrifuged at $35\,000 \times g$ for 20 min. The pellet was resuspended in medium A and recentrifuged at $35\,000 \times g$ for 20 min. The final pellet was resuspended in medium A and used for transport studies. The transport media contained 100 mM Na_2SO_4, 100 mM mannitol, 5 mM Tris-Hepes (pH 7.5), and 0.1 mM $K_2H^{32}PO_4$ or 0.05 mM D-[^{3}H]glucose. Valinomycin, dissolved in ethanol, was added to a final concentration of 8 μg/mg protein. Control media contained an equal volume of ethanol (final concentration: 0.5%). In control experiments we showed that valinomycin has no effect on P_i transport in the absence of a K^+ gradient ($[K^+]_{in} = [K^+]_{out} = 100$ mM).

An inside-negative electrical potential was also created by imposing a proton gradient ($[H^+]_{in} > [H^+]_{out}$) in the presence of carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), a proton ionophore. BBMV were preloaded with a medium (medium B) containing 400 mM mannitol, 50 mM Tris-Mes (pH 5.5), by incubation at 25°C for 60 min, followed by washing and centrifugation at $35\,000 \times g$ for 20 min. Following a second wash in the same medium, the vesicles were suspended in medium B and used for transport studies. In some experiments designed to test a non-specific effect of FCCP, BBMV were preloaded with a medium containing 400 mM mannitol, 50 mM Tris-Hepes (pH 7.5) to eliminate the H^+ gradient. The transport media contained 100 mM Na_2SO_4, 100 mM mannitol, 50 mM Tris/Hepes, (pH 7.5), 0.1 mM $K_2H^{32}PO_4$ or 0.05 mM D-[^{3}H]glucose. FCCP was added to a final concentration of 20 μM. Control media contained ethanol (0.4% final concentration).

3. Enzymatic assays

The purity of the BBMV preparations was assessed by the enrichment of the specific activity of the BBM-associated enzymes and the reduction of the activity of the basolateral and mitochondrial enzymes. The BBM enzymes routinely measured, included alkaline phosphatase, gammaglutamyl transferase, leucine amino-

peptidase, and maltase [17–19]. BBMV-associated enzymes were enriched 11–14-times. There were no differences in enzyme enrichments of BBMV-SC and BBMV-JM. To assay basolateral membrane (BLM) contamination, the activity of Na^+/K^+-ATPase was measured, using the method of Kinsolving et al. [20]. The activity of this enzyme was enriched 0.80 ± 0.12-times ($n = 8$). Succinate dehydrogenase was determined as indicative of mitochondrial contamination using the method of Pennington [21]. Its activity was markedly reduced (0.04 ± 0.01-times, $n = 13$). All enzymatic assays were performed on BBMV and cortical homogenates snap frozen and stored at −80°C.

Results are expressed as means ± S.E. of several experiments or of replicate samples. Data comparisons were made by Student's *t*-test for group or paired comparisons. Where appropriate, one way ANOVA was used for multiple comparisons. Values of $P > 0.05$ were considered non-significant.

Results

In preliminary studies we showed that P_i uptake remains linear up to 15 s incubation in both BBMV-SC and BBMV-JM. Beyond 15 s the linearity was lost (results not shown). The initial Na^+ gradient-dependent P_i uptake was higher in BBMV-SC than in BBMV-JM at all incubation times (835 ± 132 and 688 ± 104 pmol/mg at 5 s; 1351 ± 202 and 1070 ± 157 pmol/mg at 10 s for BBMV-SC and BBMV-JM, respectively, $n = 3$).

Electrogenicity of P_i transport

In the first series of experiments, the electrogenicity of P_i transport was tested by creating an inside-negative electrical potential with the imposition of 100 mM K^+ gradient ($[K^+]_i > [K^+]_o$), in the presence or absence of the K^+ ionophore, valinomycin. Under these conditions, the initial Na^+ gradient-dependent P_i uptake was higher in BBMV-SC than in BBMV-JM, both in the presence and absence of valinomycin (Table I). Furthermore, the addition of valinomycin did not produce a significant change in P_i uptake in BBMV-SC or BBMV-JM, indicating that P_i uptake is an electroneutral process in both BBMV preparations. In contrast, the initial Na^+-dependent D-glucose uptake was somewhat lower in BBMV-SC than in BBMV-JM. The addition of valinomycin resulted in a significant increase in D-glucose uptake in both BBMV preparations, indicating an electrogenic transport. The equilibrium uptake of P_i and of D-glucose, measured in the same BBMV preparations was higher in BBMV-SC than in BBMV-JM ($P < 0.05$) but remained unchanged in the presence or absence of valinomycin. To rule out a non-specific effect of valinomycin on P_i transport, transport was determined in the absence of a K^+ gradient ($[K^+]_{in} = [K^+]_{out} = 100$ mM). Under these conditions, P_i transport was significantly reduced, representing < 4% of that in the presence of Na^+ on the extravesicular side. Furthermore, valinomycin had no effect on P_i transport in the absence of an inside-negative electrical potential: 24 ± 7 versus 27 ± 8 pmol/mg per 15 s in BBMV-SC and 22 ± 4 versus 16 ± 3 pmol/mg per 15 s in BBMV-JM, in the absence and presence of valinomycin, respectively.

TABLE I

Electrogenicity of transport in BBMV-SC and BBMV-JM

BBMV were prepared from superficial (BBMV-SC) and juxtamedullary (BBMV-JM) cortex, then preincubated with a medium containing 100 mM mannitol, 100 mM K_2SO_4, 5 mM Tris-Hepes (pH 7.5) for 1 h, followed by centrifugation and suspension in the same medium. Transport media contained 100 mM Na_2SO_4, 100 mM mannitol, 5 mM Tris-Hepes (pH 7.5), and either 0.1 mM $K_2H^{32}PO_4$, or 0.05 mM D-[^{3}H]glucose, with (+Val) or without (−Val) 8 μg valinomycin/mg protein. Results are means ± S.E. of four or five experiments. * $P < 0.05$, BBMV-SC versus BBMV-JM; ** $P < 0.01$, +Val versus −Val (paired *t*-test).

	[^{32}P]P_i transport			
	−Val		+Val	
	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM
15 s	1799 ± 227	1410 ± 179	1937 ± 279	1552 ± 223
120 min	337 ± 46	274 ± 39 *	344 ± 52	272 ± 30 *
	D-[^{3}H]Glucose transport			
	−Val		+Val	
	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM
15 s	124 ± 13	194 ± 33	195 ± 28 **	287 ± 49 **
90 min	73 ± 14	62 ± 11 *	71 ± 15	60 ± 12 *

The electrogenicity of transport was also tested by creating an inside-negative electrical potential with the imposition of a proton gradient ($[H^+]_i > [H^+]_o$), in the presence or absence of FCCP. In the presence of an outwardly directed H^+ gradient, the Na^+-dependent P_i uptake was higher in BBMV-SC than in BBMV-JM, both in the presence and absence of FCCP. Furthermore, the addition of FCCP produced a significant increase in P_i uptake in both BBMV-SC and BBMV-JM, suggesting a significant electrogenic transport component in these BBMV preparations (Table II). To test if the increased P_i uptake observed in these experiments may be secondary to a non-specific effect of FCCP on P_i transport, the latter was determined in the absence of a H^+ gradient ($pH_{in} = pH_{out} = 7.5$) with and without FCCP. Under these conditions, P_i transport was higher in the presence of FCCP in both BBMV populations, indicating that the increased P_i uptake observed after the addition of FCCP is secondary to a non-specific effect of this ionophore on P_i transport itself rather than due to the presence of an electrogenic transport component for P_i. When a H^+

gradient was present, the percent increase in the initial (5 s) P_i uptake after the addition of FCCP was 52 ± 12 and 46 ± 2% for BBMV-SC and BBMV-JM, respectively. In the absence of a H^+ gradient, uptake increased 56 ± 13 and 46 ± 6% in BBMV-SC and BBMV-JM, respectively. In both BBMV populations, the initial Na^+-dependent D-glucose uptake was markedly and significantly increased in the presence of FCCP, consistent with an electrogenic transport. The equilibrium uptake of P_i and D-glucose, measured in the same BBMV preparations, remained unchanged both in the presence and absence of FCCP.

Effect of LPD on the electrogenicity of P_i transport

BBMV were prepared from the total cortex of rats placed on a normal or low-P_i diet. The Na^+ gradient-dependent transport of P_i and D-glucose was determined as described above, in the presence and absence of valinomycin. The initial Na^+-dependent P_i uptake was significantly increased in BBMV prepared from animals on LPD compared to BBMV from animals on NPD (+54.5% and +57.4%, respectively, without and with valinomycin) (Table III). The equilibrium uptake (120 min) was not significantly different in the two groups. P_i uptake remained unchanged after the addition of valinomycin. The initial D-glucose uptake was not different in BBMV prepared from animals on NPD or LPD. In contrast to P_i uptake, the initial D-glucose uptake was significantly higher after the addition of valinomycin (+51.2% and +41.0% for BBMV from NPD and LPD animals, respectively). The equilibrium uptake remained unchanged.

TABLE II

Electrogenicity of transport in BBMV-SC and BBMV-JM

BBMV were prepared from superficial (BBMV-SC) and juxtamedullary (BBMV-JM) cortex, then preloaded with a medium containing 400 mM mannitol, 50 mM Tris-Mes (pH 5.5) or 400 mM mannitol, 50 mM Tris-Hepes (pH 7.5) for 1 h, followed by two washing and centrifugation cycles, and resuspension in the same medium. Transport media contained 100 mM Na_2SO_4, 100 mM mannitol, 50 mM Tris-Hepes (pH 7.5), and either 0.1 mM $K_2H^{32}PO_4$ or 0.05 mM D-[^{3}H]glucose, with (+FCCP) or without (−FCCP) 20 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone. Results are means ± S.E. of three or four experiments. * $P < 0.05$, ** $P < 0.005$; +FCCP versus −FCCP (paired *t*-test).

	$[^{32}P]P_i$ transport ($[H^+]_{in} > [H^+]_{out}$)			
	−FCCP		+FCCP	
	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM
5 s	678 ± 31	440 ± 74	1033 ± 113 *	647 ± 116 *
15 s	1638 ± 256	996 ± 50	1832 ± 69 *	1230 ± 81 *
120 min	662 ± 10	511 ± 7	669 ± 10	526 ± 7
	$[^{32}P]P_i$ transport ($[H^+]_{in} = [H^+]_{out}$)			
	−FCCPs		+FCCP	
	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM
5 s	583 ± 71	± 73	912 ± 161	531 ± 125
15 s	1241 ± 117	± 61	1561 ± 238	854 ± 129
	D-[^{3}H]Glucose		nsport	
	−FCCP		+FCCP	
	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM
5 s	81 ± 10	90 ± 10	330 ± 21 **	367 ± 51 **
15 s	158 ± 9	225 ± 26	602 ± 65 **	745 ± 101 **
90 min	67 ± 13	68 ± 10	66 ± 10	56 ± 7

TABLE III

Effect of low-P_i diet on electrogenicity of P_i transport

BBMV prepared from total cortex were preloaded for 1 h with a medium containing 100 mM mannitol, 100 mM K_2SO_4, 5 mM Tris-Hepes (pH 7.5), followed by centrifugation at 37000 × *g* and suspension in the same medium. Transport media contained 100 mM Na_2SO_4, 100 mM mannitol, 5 mM Tris-Hepes (pH 7.5), and either 0.1 mM $K_2H^{32}PO_4$ or 0.05 mM D-[^{3}H]glucose without (−Val) or with (+Val) 8 μg valinomycin/mg protein. Results are means ± S.E. of four or five experiments. * $P < 0.05$ +Val versus −Val (paired *t*-test).

	$[^{32}P]P_i$ transport	
	−Val	+Val
NPD: 15 s	1138 ± 123	1140 ± 126
120 min	216 ± 32	210 ± 36
LPD: 15 s	1758 ± 156	1795 ± 228
120 min	215 ± 41	214 ± 37
	D-[^{3}H]Glucose transport	
	−Val	+Val
NPD: 15 s	119 ± 16	180 ± 25 *
90 min	70 ± 15	66 ± 18
LPD: 15 s	95 ± 16	134 ± 22 *
90 min	60 ± 15	65 ± 17

Stoichiometry of $[^{32}P]P_i$ transport

The initial P_i uptake was measured in the presence of increasing extravesicular Na^+ concentrations, in BBMV-SC and BBMV-JM prepared from animals placed on a low- or normal-P_i diet. In animals on a normal-P_i diet, the plots of P_i uptake versus $[Na^+]$ had a sigmoidal appearance in both BBMV populations, indicating cooperative binding of Na^+ to the cotransporter (Fig. 1). When the data were plotted in a double-reciprocal form as $1/V$ versus $1/[Na^+]$, $1/V$ versus $1/[Na^+]^2$, or $1/V$ versus $1/[Na^+]^3$, only the plots of $1/V$ versus $1/[Na^+]^2$ gave a linear relationship (Fig. 2A), suggesting a stoichiometry of 2 Na^+: 1 P_i for either BBMV-SC or BBMV-JM. When the same data were plotted in the form of V versus $V/[Na^+]$, V versus $V/[Na^+]^2$, or V versus $V/[Na^+]^3$, again only the plot of V versus $V/[Na^+]^2$ gave a linear relationship (Fig. 2B), suggesting a stoichiometry of 2 Na^+: 1 P_i. Except for higher uptake rates, identical results

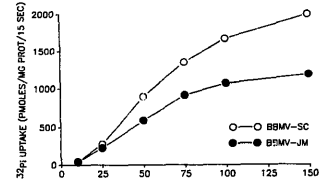

Fig. 1. Dependency of BBMV P_i transport on Na^+ concentration. P_i uptake was measured in BBMV-SC (○) and BBMV-JM (●) prepared from animals on a normal-P_i diet, in the presence of increasing Na^+ concentrations. Osmolarity was maintained by replacing Na^+ with equimolar concentrations of choline. Transport media contained in addition, 100 mM mannitol, 5 mM Tris-Hepes (pH 7.5), and 0.1 mM $K_2H^{32}PO_4$. Results shown are of a representative experiment performed in quadruplicate and repeated three times.

were obtained in BBMV-SC and BBMV-JM from animals placed on a low-P_i diet (results not shown).

The data were also analyzed by Hill plots. In animals on a normal-P_i diet, the Hill coefficients (n_H) calculated from three separate experiments were 1.73 ± 0.06 for BBMV-SC and 1.72 ± 0.13 for BBMV-JM. In animals on a low-P_i diet, n_H were 1.69 ± 0.06 and 1.55 ± 0.02 for BBMV-SC and BBMV-JM, respectively. The differences were not statistically significant. The results confirm a stoichiometry of 2 Na^+: 1 P_i, consistent with electroneutral transport of one HPO_4^{2-} with 2 Na^+ ions in both BBMV populations, under low- or normal-P_i diets.

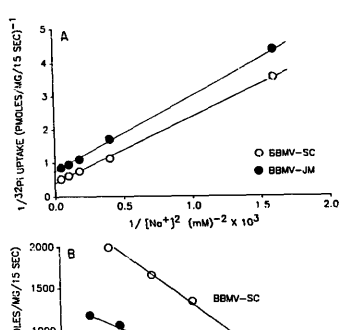

Fig. 2. Determination of stoichiometry of Na^+-P_i cotransport. Panel A: Data from Fig. 1 plotted as double-reciprocal plots of $1/V$ versus $1/[Na^+]^2$; $r \geq 0.998$. Panel B: The same data plotted as V versus $V/[Na^+]^2$; $r \geq 0.992$. ○, BBMV-SC; ●, BBMV-JM.

TABLE IV

Kinetics of P_i transport inhibition by phosphonoformic acid (PFA) in BBMV-SC and BBMV-JM

Na^+-dependent P_i uptake was measured at 5 s, using K_2HPO_4 concentrations ranging from 0.025 to 1.0 mM, without or with 1 mM PFA in the incubation media. Results are means ± S.E. of three separate experiments performed in quadruplicate. * $P < 0.05$ from control. [a] not significant.

	BBMV-SC		
	K_m (μM)	V_{max} (pmol mg protein per 5 s)	K_i (μM)
Control	90 ± 5.2	1588 ± 205	–
PFA (1 mM)	454 ± 42 *	1637 ± 211 [a]	237 ± 1.7
	BBMV-JM		
	K_m (μM)	V_{max} (pmol/mg protein per 5 s)	K_i (μM)
Control	83 ± 6.2	917 ± 124	–
PFA (1 mM)	292 ± 11 *	689 ± 101 [a]	409 ± 53

Kinetics of inhibition of P_i transport by PFA

We first compared the kinetics of P_i transport between BBMV-SC and BBMV-JM. The apparent V_{max} for P_i transport was significantly higher in BBMV-SC than in BBMV-JM (1.59 ± 0.20 vs. 0.92 ± 0.12 nmol/mg protein per 5 s; $n = 3$, $P < 0.01$). The apparent K_m was not different between the two BBMV populations (Table IV). We then determined the kinetics of P_i transport inhibition by PFA in BBMV-SC and BBMV-JM. PFA was added to transport media at 1 mM final concentration. The inhibitory effect of PFA on P_i transport was competitive in both BBMV preparations, resulting in an increase in the apparent K_m with no change in the apparent V_{max}. The increase in K_m after addition of PFA was more pronounced in BBMV-SC than in BBMV-JM ($P < 0.05$, $n = 3$). The inhibitory constants (K_i) for PFA were 237 ± 1.7 μM for BBMV-SC and 392 ± 69 μM for BBMV-JM and significantly different ($n = 3$, $P < 0.05$, paired t-test).

Effect of temperature on P_i transport

Since increasing temperature results in increased membrane fluidity, we compared P_i uptake at various temperatures in BBMV-SC and BBMV-JM. The results, plotted in the form of Arrhenius plots (Log V_{max}

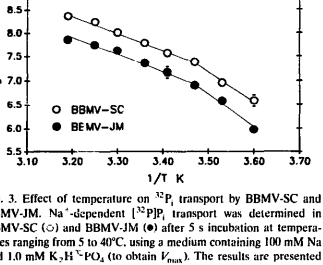

Fig. 3. Effect of temperature on $^{32}P_i$ transport by BBMV-SC and BBMV-JM. Na^+-dependent [^{32}P]P_i transport was determined in BBMV-SC (○) and BBMV-JM (●) after 5 s incubation at temperatures ranging from 5 to 40°C, using a medium containing 100 mM Na and 1.0 mM K_2HPO_4 (to obtain V_{max}). The results are presented as Arrhenius plots. Each data point is the mean ± S.E. of four experiments. S.E. bars smaller than symbols are not shown.

vs. $1/T_K$) are shown in Fig. 3. Within the wide range of temperatures studied (5–40°C), the plots could be best fitted by two linear-regression lines with breaks in linearity occurring at about 17°C for both BBMV-SC and BBMV-JM. The energies of activation calculated from the slopes of the plots were significantly different above and below the transition temperatures. The mean values for energies of activation were 3.77 ± 0.28 and 3.45 ± 0.46 kcal/mol above 17°C and 6.28 ± 0.78 and 7.40 ± 0.95 kcal/mol below 17°C, in BBMV-SC and BBMV-JM, respectively. The energies of activation were not significantly different between BBMV-SC and BBMV-JM ($n = 4$). These results are similar to those reported in BBMV from total renal cortex of rats [22] where a break in linearity was observed at about 15°C.

Discussion

Several studies have demonstrated the heterogeneity of P_i transport along the nephron length (axial heterogeneity). Micropuncture studies in phosphate-loaded rats indicate that phosphate reabsorption is lower in deep nephrons compared to superficial nephrons [1–3]. Kinetic studies of P_i transport in BBMV using a wide range of P_i concentrations, show a break in the linearity of the regression lines. This may either represent two populations of vesicles, or the presence of two transport systems on the same vesicle [22,23]. Two P_i transport systems, namely a high-capacity and a high-affinity system have been described in the dog [4] and the pig kidney [24]. Similarly, a high-capacity, low-affinity system has been described for Na^+/D-glucose cotransport in BBMV-SC [25].

The reasons for the differences in P_i transport between the superficial and juxtamedullary nephrons are not known. Apart from anatomical considerations, other factors may be involved. These may include: (1) Differences in the density of Na^+-P_i cotransporters of the two tubular segments [8]; and (2) Differences in the lipid composition of BBM of the two segments, resulting in differences in membrane fluidity. Recent studies by Levi [8] have indicated that BBMV-SC and BBMV-JM have different lipid compositions and fluidity. (3) Electrogenicity of P_i transport may also play a role. Although P_i transport is considered electroneutral when total cortex is examined [10], it has been suggested that electrogenic P_i transport may be important in late proximal tubules [11]. Recent studies have demonstrated an electrogenic phosphate transport in rat renal BBMV [12,13]. It is therefore not unreasonable to expect different electrogenic components for P_i transport in BBMV-SC and BBMV-JM. (4) The stoichiometry of Na^+-P_i cotransport may also be different in BBMV-SC compared to BBMV-JM. Such a difference has been reported for Na^+-dependent D-glucose transport which also demonstrates axial heterogeneity [26]. The stoichiometry of Na^+: D-glucose is 1:1 in BBMV-SC and 2:1 in BBMV-JM [25,26].

The early and the late proximal tubules differ significantly in their response to dietary and hormonal factors. One of the potent stimuli for P_i transport at the level of renal BBM is the feeding of a low P_i diet to the animals [4,23]. Turner and Dousa [4] prepared BBMV from superficial and juxtamedullary cortex of dogs placed on high-, normal-, or low-P_i diets. In animals on low-P_i diet, BBMV-SC had a greater capacity (higher V_{max}) for P_i transport than BBMV-JM). Thyroid hormone (T_3) administration also results in an increase in Na^+-dependent P_i transport [5,7,27]. However, in contrast to changes observed with a low-P_i diet, the effect of T_3 on P_i transport is limited to BBMV-JM [7].

In the present study, using an inside-negative electrical potential in the presence of valinomycin, we were unable to show any significant electrogenic component for P_i transport in either BBMV-SC or BBMV-JM, while D-glucose transport remained electrogenic under the same conditions. These results are similar to those described by others [10] using BBMV from total cortex, suggesting that the divalent form of phosphate (HPO_4^{2-}) is the preferred species for transport by both BBMV populations. However, these results are at variance with recent reports by Béliveau and Ibnoul-Khatib [12] and Béliveau and Strevey [13] who found a significant electrogenic component for [^{32}P]P_i transport in BBMV prepared from the total cortex of rats. The reasons for these differences are not clear but may be due to different methods of BBMV preparation (we used Ca^{2+}-precipitation whereas these authors used a Mg^{2+}-precipitation method). BBMV prepared by Ca^{2+}-precipitation are more leaky to K^+ and H^+ ions than BBMV prepared by Mg^{2+}-precipitation [29]. The

higher permeability of the membranes may lead to faster dissipation of the ionic gradients imposed when the uptake is measured.

In further experiments, we determined P_i transport in the presence or absence of a proton gradient with and without FCCP. Under these conditions, the initial Na^+-dependent P_i uptake increased significantly after the addition of FCCP (+51.6% in BBMV-SC and +46.3% in BBMV-JM). The extent of P_i transport stimulation by FCCP was similar in the presence or absence of a H^+ gradient, indicating a non-specific effect of the ionophore on Na^+-P_i cotranasporter, rather than an electrogenically driven transprt. FCCP is therefore not a suitable ionophore for studies of the electrogenicity of P_i transport. D-Glucose uptake, on the other hand, was exquisitely sensitive to an inside negative electrical potential, in agreement with other reports [10]. Our results also show a somewhat higher vesicle volume, as measured by the equilibrium uptake of P_i, in BBMV-SC than in BBMV-JM. This is in agreement with other reports [4,8]. However, the volume differences were modest and not statistically significant. Differences in methodology may be responsible for these differences.

The stoichiometry of Na^+-P_i cotransport was found to be 2:1, in agreement with other reports using BBMV prepared from total cortex [11]. Furthermore, there was no difference in the stoichiometry of Na^+:P_i between BBMV-SC and BBMV-JM, with the stoichiometry remaining at 2:1. These results are in complete agreement with a recent report by Levi [8]. Furthermore our results expand on those reported by Levi in that, phosphate deprivation did not result in a change in the stoichiometry of Na^+-P_i cotransport in either BBMV preparation. The differences in the rate of Na^+-dependent P_i transport between BBMV-SC and BBMV-JM are therefore not due to the transport of different species of phosphate (HPO_4^{2-} versus $H_2PO_4^-$) in these two BBMV populations.

Taken together, our results suggest that differences in electrogenicity and stoichiometry of Na^+-P_i cotransport do not play a major role in the heterogeneity of P_i transport in PCT and PST or in the heterogeneous response of these segments to regulatory factors such as P_i-deprivation.

As it has been recently suggested by Yusufi et al. [27] and by Levi [8], the differences in the V_{max} of Na^+-P_i cotransport in BBMV-SC and BBMV-JM may be secondary to both a difference in the number of P_i carriers (as determined by [^{14}C]-PFA binding) and to differences in membrane fluidity between BBMV-SC and BBMV-JM. Kinetic experiments confirmed a higher V_{max} for P_i transport in BBMV-SC compared to BBMV-JM, suggesting a higher number of Na^+-P_i cotransporters in BBMV-SC. These results are in agreement with other reports [4,8]. However, contrary to the studies by Yusufi et al. [7] and by Levi [8], who found a higher affinity (lower K_m) for P_i in BBMV-SC compared to BBMV-JM, our results show no difference in the K_m for P_i in these two BBMV populations. The reasons for these discrepancies are not clear but may be related to different methods of BBMV preparation (Ca^{2+} versus Mg^{2+} precipitation) and dissection techniques for separation of the two cortical layers. When the inhibitory effect of PFA was measured over a wide range of P_i concentrations, allowing for calculation of the inhibitory constants (K_i), there was a difference between the two BBMV populations. The K_i for PFA was significantly lower in BBMV-SC than in BBMV-JM, suggesting that there are proportionately more Na^+-P_i cotransporters in BBMV-SC to which PFA can bind, than in BBMV-JM. Differences in the affinity of the Na^+-P_i cotransporters to PFA may also explain these results. The P_i-protectable [^{14}C]PFA binding has been used to quantify the density of Na^+-P_i cotransporters in renal BBMV [30]. Levi [8] has conducted detailed and elegant studies of [^{14}C]PFA binding in BBMV-SC and BBMV-JM, demonstrating a higher density of cotransporters in the latter. We therefore did not repeat these experiments.

The temperature dependency experiments extend the results of previous studies using the total renal cortex [22,31]. We found a break in the linearity of the Arrhenius plots of P_i transport in both BBMV-SC and BBMV-JM at about 17°C with no differences in the energies of activation above and below the transition temperature. This indicated that, although membrane fluidity may be different in BBMV-SC and BBMV-JM, as suggested by a higher P_i transport rate at all temperatures in the former than in the latter, the physical interactions of the Na^+-P_i cotransporters with the membrane appear to be similar in early and late proximal tubules.

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