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Renal brush border glutamine transport: comparison between in situ and isolate membrane vesicle uptake

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Glutamine uptake by renal cortical brush-border vesicles was compared to transport expressed by the functioning isolated kidney. Comparisons were made with regard to sodium dependency and the adaptive increase induced by chronic metabolic acidosis in the rat. The results show an absolute dependency upon a sodium gradient; sodium-independent glutamine uptake has no counterpart in situ. In addition, acidosis-induced adaptive increase in vesicle glutamine uptake has no counterpart in situ. Rather, the apparent adaptation reflects extravesicular γ-glutamyltransferase-mediated conversion to glutamate and subsequent accumulation; acidosis-induced adaptation of this enzyme largely explains the apparent adaptation in glutamine uptake. Consequently the role of membrane transport in glutamine flux regulation can be assessed providing metabolic conversion is controlled.

Introduction

Glutamine is the most prevalent plasma amino acid [1] representing 60 percent or more of the total available amino and amide nitrogen pool Its central role as a nitrogen carrier and high rate of filtration coupled to near complete reabsorption [2,3] have lead investigators to the study of glutamine uptake into isolated brush-border vesicles [4] These studies concluded that uptake could be driven by either a sodium or choline gradient, although the former was more effective [4] and that uptake appeared to be independent of its metabolic conversion [4] Vesicles isolated from chronically acidotic rats exhibited an enhanced glutamine uptake [4–7], an observation that was difficult to reconcile with the efficient reabsorption of filtered glutamine already existent [2] On the other hand, the report of an adaptive increase in membrane glutamine transport holds the promise that a more generalized membrane transport system may play a role in directing interorgan glutamine flow in a variety of physiological states [8]

Because of the potential importance of such a mechanism, our concern was the comparison between vesicle glutamine uptake and that exhibited in situ Specifically, we were interested in the degree of dependency upon a sodium versus a choline gradient and the significance of the reported adaptive increase in glutamine transport during chronic metabolic acidosis The results to

Abbreviations Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid, AT-125, α-amino-4-chloro-4,5-dihydro-5-isoxazoleacetic acid

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follow confirm the applicability of isolated membrane vesicles to the study of glutamine uptake, especially when supported by parallel in situ studies

Materials and Methods

Experiments were carried out on brush-border vesicles and kidneys isolated from control and metabolically acidotic male Sprague-Dawley rats weighing between 350 and 450 g Chronic metabolic acidosis was induced by maintaining rats on 1 2% NH_4Cl in 5% Dextrose for 4 days At this time blood pH, plasma HCO_3^- concentration and urinary ammonium excretion were 7 32 ± 0 02 vs control, 7 42 ± 0 02, 18 8 ± 1 2 vs 26 2 ± 1 4 mM and 1690 ± 177 vs 106 ± 8 μmol/100 g per 24 h, $n = 8$, $P < 0$ 05 for all three parameters

Kidneys were isolated and perfused as previously described [9] The perfusate was composed of a modified Krebs-Henseleit solution containing in mM L-glutamine, 1, $NaHCO_3$, 24, glucose, 5, and albumin, 6 g% After 30 min of perfusion, the perfusate was switched [10] to one containing choline chloride and choline bicarbonate in place of sodium for a second 30-min period, finally, the perfusate was switched back to a new sodium containing media The filtered load of glutamine was determined from the glomerular filtration rate, estimated by the clearance of [^{3}H]inulin, and the average perfusate glutamine concentration over a 15 min clearance period Glutamine net transport (t-Gln) was calculated as the difference between that filtered and excreted after subtracting the excreted glutamate, fractional excretion of glutamine (FE-Gln) was determined as the percent of the filtered glutamine excreted Glutamine and glutamate in perfusate and urine were determined by microfluorimetric enzymatic assay [11]

Brush-border vesicles were obtained by differential precipitation using $MgCl_2$ [12] Briefly, 1 g of diced cortex was homogenized in 9 vol of 250 mM sucrose, 10 mM Hepes buffer (pH 7 4) using 10 strokes of a motor driven Potter-Elvejhem homogenizer, 800 rpm The resulting homogenate was then spun down at 10 000 × g for 10 min, the pellet discarded and the supernatant recentrifuged at 24 000 × g for 20 min The fluffy layer was transferred into 4 ml of buffered sucrose and $MgCl_2$ was added to 15 mM After allowing the suspension to stand for 20 min on an ice bath with occasional stirring, it was successively centrifuged at 8000 × g and at 21 000 × g for 15 min The final pellet was washed and resuspended in 100 mM mannitol, 10 mM Hepes (pH 7 2) All studies were performed on freshly prepared membrane fractions Marker enzymes, γ-glutamyltransferase [13] and alkaline phosphatase [14] for the brush border and ($Na^+ + K^+$)-ATPase [15] for basolateral membranes were run on each preparation The media for both transport and metabolism studies contained 100 mM mannitol, 10 mM Hepes (pH 7 2) and either 100 mM NaCl or 100 mM choline chloride Glutamine (Sigma) containing tracer amounts of L-[U-^{14}C]glutamine, spec act 285 mCi/mmol (New England Nuclear) were added to a final concentration of 100 μM just prior to the study Radioactive purity of the glutamine was assessed in every run and found to be 98 3 ± 1 2% pure, chemical glutamine, was checked by microfluorimetric assay and found to be 98 5 ± 1 6% glutamine Vesicle uptake of ^{14}C label was studied at 23°C employing the rapid-filtration technique [16], uptake was initiated by the addition and prompt mixing of 100 to 150 μg of membrane protein to 300 μl media Protein concentration was determined with a dye binding assay kit (Bio-Rad) using bovine serum albumin as the standard At timed intervals the reaction was terminated by the addition of a 10-fold volume of ice-cold stop solution containing media minus glutamine, this solution was then promptly applied to a Millipore filter, HAWP 0 45 μm, under light suction The filter was washed using an additional 3 ml of stop solution, placed in a scintillation vial and monitored for radioactivity by liquid scintillation spectrometry, quenching was monitored by using the channel ratio method Conversion of glutamine to glutamate was determined in the stock media, in the media plus vesicles and in the filtered vesicles For the latter, washed filters containing the vesicles were trimmed, placed in a small volume of water and boiled for exactly 3 min, samples of the media and stock were handled the same way Aliquots of each were then applied to cellulose thin-layer chromatograms (Kodak) and developed using n-butanol/ pyridine/ water/ acetic acid (30 30 20 20, v/v), areas corre-

TABLE I

QUANTITATIVE RELATIONSHIP BETWEEN FILTERED AND EXCRETED GLUTAMINE

Kidneys isolated and perfused with 1 mM L-glutamine (Gln) Gln In and Gln Out are filtered and excreted glutamine, respectively, calculated as described in Methods t-Gln represents the net transport of glutamine, see Methods FE-Gln, fractional excretion of glutamine Results are mean ± S E in nmol/min per kidney for five control rat kidneys

	Gln In	Gln Out	Glu Out	t-Gln	FE-Gln
Control	728 ± 193	62 ± 16	17 ± 4	649 ± 182	8 5 ± 2 6
$-Na^+$	390 ± 65 [a]	303 ± 75 [a]	89 ± 33 [a]	−2 ± 68 [a]	77 7 ± 15 [a]

[a] Significantly different from Na^+ media, $P < 0.05$

sponding to authentic glutamine and glutamate standards were transferred to counting vials and monitored as above

Results

The quantitative relationship between filtered glutamine and that excreted in the presence and absence of sodium is presented in Table I In control rat kidneys glutamine was filtered at the rate of 728 ± 193 nmol/min of which 62 ± 16 nmol/min, 8 5 ± 2 6 percent, was excreted as glutamine, glutamine net transport was 649 ± 182 nmol/min In the absence of sodium 78% of the filtered glutamine was excreted, the remaining 22% could be accounted for as excreted glutamate The rate of glutamine transport fell from 649 to −2 nmol/min reflecting virtual elimination of net transport, intraluminal conversion of glutamine to glutamate and its excretion, on the other hand, dramatically increased Returning to 140 mM sodium results in decreased glutamine excretion, 73 ± 60 and glutamate excretion 31 ± 12 nmol/min, the fractional excretion of glutamine returns to 17 ± 8% Thus the effect of choline is largely, but not completely, reversible Glomerular filtration rate was only slightly diminished by exposure to choline falling from 698 ± 110 to 490 ± 96 μl/min, $P < 0.05$ with a return toward control, 560 ± 112 μl/min, during reperfusion with sodium

In acidotic rat kidneys, Table II excreted glutamine was reduced, but this can not be attributed to either increased glutamine net transport or a reduced fractional excretion, rather, it was associated with a significantly increased glutamate excretion, 56 ± 17 vs 17 ± 4 nmol/min In the absence of sodium ($-Na^+$, Table II) glutamine net transport fell to a value not significantly different from zero with a huge increase in glutamate excretion, 216 ± 42 vs 56 ± 17 nmol/min In fact, glutamate excretion exceeded that of the control rat kidney perfused in the absence of sodium, 216 ± 42 vs 89 ± 33 nmol/min That glutamate excretion reflects intraluminal conversion of glutamine to glutamate catalyzed by γ-glutamyltransferase can be shown by employing the potent inhibitor of this enzyme, AT-125 Adding AT-125 to the perfusion media at the concentration of 0 23 mM reduced γ-glutamyltransferase activity from 1208 ± 176 to 52 ± 12 nmol/min per mg protein; our previous studies have shown that this residual activity is associated with the luminal brush border [17] With greater than 95% inhibition of the enzyme the fractional excretion of glutamine increased from 5 ± 2 to 22 ± 9% but

TABLE II

EVIDENCE FOR γ-GLUTAMYLTRANSFERASE HYDROLYSIS OF INTRALUMINAL GLUTAMINE

Results are mean ± S E in nmol/min per kidney for five acidotic rat kidneys and five acidotic rat kidneys perfused with 0 23 mM AT-125 in addition to 1 mM L-glutamine (Gln) For abbreviations, see Table I

	Gln In	Gln Out	Glu Out	FE-Gln (%)	FE-Gln + Glu (%)
Acidotic	481 ± 150	22 ± 4	56 ± 17	4 6 ± 2	19 ± 4
$-Na^+$	509 ± 153	248 ± 56	216 ± 42	48 7 ± 7	101 ± 15
+AT-125	378 ± 56	87 ± 39 [a]	17 ± 8 [a]	22 ± 9 [a]	26 ± 9
$-Na^+$ +AT-125	430 ± 72	276 ± 11	35 ± 3 [b]	67 ± 8 [b]	75 ± 9

[a] Significantly different from acidotic, $P < 0.05$

[b] Significantly different from acidotic $-Na^+$ media, $P < 0.05$

TABLE III

MARKER ENZYME DISTRIBUTION AND EFFECT OF ACIDOSIS

Results are mean ± S E for the number of kidneys shown in parentheses Values are nmol/min per mg protein for γ-glutamyltransferase, GGT, alkaline phosphatase, AP and $(Na^+ + K^+)$-ATPase are expressed in μmol/min per mg

Preparation	GGT	AP	$(Na^+ + K^+)$-ATPase
Homogenate			
Control (5)	716 ± 64	77 ± 6	45 ± 9
Acidotic (6)	1080 ± 31	70 ± 5	46 ± 7
Brush border			
Control (4)	5754 ± 443	626 ± 42	108 ± 15
Acidotic (5)	7810 ± 118	544 ± 36	80 ± 12

[a] Significantly different from control, $P < 0.05$

the fractional excretion of glutamine plus glutamate remained unchanged, 19 ± 4 vs 26 ± 9% In the absence of sodium the full potential of the luminal glutaminase to hydrolyze glutamine is unveiled, now elimination of γ-glutamyltransferase reduces glutamate excretion from 216 ± 42 to only 35 ± 3 nmol/min These in situ results clearly demonstrate the existence of both a Na^+-dependent glutamine transport and γ-glutamyltransferase-dependent glutamine hydrolysis

Chronic metabolic acidosis induces an apparent adaptation in brush-border Na^+-dependent-glutamine uptake, Fig 1, and, as well as adaptation in brush-border γ-glutamyltransferase activity (Table III) Furthermore this adaptation was not paralleled by an increase in neither alkaline phosphatase (Table III) nor maltase activity [5] The question therefore becomes the role of metabolic conversion of glutamine to glutamate in the

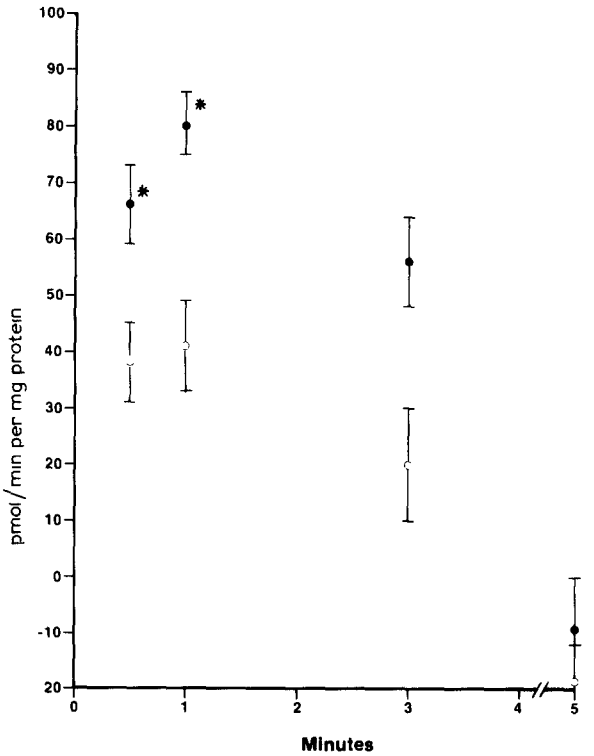

Fig 1 Sodium-dependent uptake of label ^{14}C from media containing [^{14}C]glutamine, 100 μM, by brush-border vesicles prepared from control (○) and acidotic (●) rat kidneys Points are mean ± S E from duplicate runs from techniques from five control and five acidotic rats Asterisks indicate statistically significant difference, $P < 0.05$

apparent adaption Table IV shows the Na^+-dependent uptake of label as either glutamine or glutamate in relation to the media glutamine hydrolysis At 1 min, 14% of the media glutamine had been converted to glutamate while 50% of the label within the vesicles appears as glutamate, uptake of label is therefore significantly contributed to by the high-affinity glutamate carrier

TABLE IV

BRUSH-BORDER VESICLE UPTAKE OF GLUTAMINE (Gln) AND GLUTAMATE (Glu)

Vesicles were incubated in 100 mM mannitol, 100 mM NaCl containing 10 mM Hepes buffer (pH 7 2) and 100 μM L-glutamine Measurements were made after 1 min of incubation at 23° C Results are mean ± S E from the number of rats shown in parentheses

	Media (μM)		Vesicle (%)		Uptake (pmol/min per mg)	
	Gln	Glu	Gln	Glu	Gln	Glu
Control (5)	86 ± 3	14 ± 3	50 ± 5	50 ± 6	22 ± 4	21 ± 5
Acidosis (5)	78 ± 3	22 ± 5 [a]	30 ± 7 [a]	70 ± 8 [a]	25 ± 6	55 ± 8 [a]

[a] Significantly different from control, $P < 0.05$

[4,16] Vesicles from chronically acidotic rat kidneys hydrolyze more of the glutamine to glutamate, 22 ± 5%, consistent with the enzymatic adaptation shown in Table III, glutamine uptake was not different from control whereas glutamate uptake increased from 21 ± 5 to 55 ± 6 pmol/min per mg This difference in glutamate uptake adequately explains the apparent adaptation in glutamine uptake shown in Table IV

Discussion

Although brush border glutamine uptake has been studied previously, no attempt was made to relate uptake to that occurring in situ The importance of comparing transport characteristics at these two levels has recently been emphasized [12,18] Accordingly, our purpose was to apply this approach to luminal glutamine transport, specifically regarding Na^+ dependency and the apparent acidosis-induced adaptation The present study revealed an absolute dependency of in situ glutamine uptake upon a sodium gradient as previously predicted [19,20], these finding, therefore, rule out the existence of the putative γ-glutamyl cycle mediated [21] and choline gradient driven processes [4]

Our second concern was the apparent adaptive increase in glutamine uptake exhibited by brush-border vesicles from chronically acidotic rat kidneys [5–7] As noted by those reporting enhanced transport, an adaptation is paradoxical since all filtered glutamine is normally reabsorbed Consequently an apparent 30–50% increase [5,7] conveys no obvious physiological advantage Unlike the in vivo kidney [2], the isolated kidney exhibits less than complete glutamine reabsorption, this, of course, is advantageous since it leaves room for an adaptation to be expressed However, no adaptation was observed, neither in absolute absorption rate nor in expressed efficiency at a given load What was evident was an increase in intraluminal glutamine hydrolysis catalyzed by γ-glutamyltransferase McFarlane and Alleyne [5] previously correlated the adaptive uptake of glutamine exhibited by brush-border vesicles with a parallel adaptation in γ-glutamyltransferase, an observation confirmed in the present study Unlike the previous study, our results show no increase in glutamine uptake, on the other hand, uptake of label in the form of glutamate was clearly increased suggesting that the association of γ-glutamyltransferase and uptake is dependent upon the metabolic conversions of glutamine Noteworthy brush border glutamate uptake exhibits a lower V_{max} but higher affinity than does the glutamine transporter [4,16,22] Our results suggest vesicle uptake of glutamate derived from glutamine may be dependent upon extravesicular hydrolysis, it may also be that both the luminal glutamine and the glutamate transporter adapt in harmony

The physiological significance of intraluminal glutamine hydrolysis has been carefully studied by Silbernagl [23] His in vivo studies clearly show that little of the filtered glutamine undergoes intraluminal hydrolysis because the afferent tubule contains the glutamine transporter and relatively little of the hydrolytic enzyme [24], accordingly, the higher concentration of γ-glutamyltransferase downstream should be deprived of filtered glutamine However, Silbernagl [23] has estimated a peritubular capillary to lumen flux of glutamine which allows γ-glutamyltransferase to contribute 5 to 10% of the excreted ammonium Although agreeing in the main with this analysis, we suspect there may be more to the role of γ-glutamyltransferase in glutamine hydrolysis in vivo, particularly in metabolic acidosis [9,25,26] In this regard the acidosis-induced adaptation is largely limited to the outer cortex [21] and expressed on both the luminal and antiluminal regions of proximal tubules [25], the adaptation reflects both an increased V_{max} and decreased K_m [26] Finally the enzyme's adaptation is specific for glutamine [26] consistent with significant extracellular glutamine hydrolysis and subsequent enhanced glutamate uptake by tubule cells in chronic metabolic acidosis

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