Transport of Glutamine by Rat Kidney Brush-Border Membrane Vesicles

By Norma McFARLANE-ANDERSON and George A. O. ALLEYNE
Department of Medicine, University of the West Indies, Kingston 7, Jamaica

(Received 16 October 1978)

Transport of glutamine by brush-border vesicles prepared from the renal cortex was studied. The transport system had both Na⁺-dependent and Na⁺-independent components. The presence of Na⁺ in the incubation resulted in an 'overshoot' at 30s at which time the rates of transport were approx. 8 times the values obtained in the absence of Na⁺. Variation of the glutamine concentration showed that the system obeyed Michaelis-Menten kinetics with $K_m$ and $V_{max}$ values for the Na⁺-dependent system of 0.86 mM and 9.6 nmol/min per mg of protein respectively. Vesicles obtained from chronically acidic rats showed similar kinetic characteristics. The $K_m$ and $V_{max}$ values for the Na⁺-dependent system were 0.76 mM and 9.6 nmol/min per mg of protein respectively. There was increased uptake of glutamine by vesicles from acidic rats and this increase was associated with increased activity of γ-glutamyltransferase in these preparations. Vesicles from acidic rats, however, showed no increase in glucose transport and no increase in the activity of maltase, another brush-border enzyme.

The luminal or brush-border membrane and the contraluminal or basolateral membrane of the kidney are two sites at which substrates may be transported into the cell. Vesicles prepared from the brush border of the kidney have been used extensively in the study of sugar and amino acid transport. The uptake of alanine, proline (Hammerman & Sacktor, 1977; Fass et al., 1977) and phenylalanine (Evers et al., 1976) by kidney brush-border vesicles has been shown to be a rapid active process which is stimulated by Na⁺. It is remarkable that, although glutamine is the amino acid present in the highest concentration in the plasma from man, rat and dog, and is a major substrate for ammoniagenesis, there are no reports on the transport characteristics of this amino acid by the kidney.

Since glutamine is filtered and none appears in the urine, there must be active transport at some site in the nephron. Recently, we have found that utilization of glutamine by renal cortical slices of the rat has both Na⁺-dependent and -independent components and have suggested that the Na⁺-independent process may be mediated in part by γ-glutamyltransferase, an enzyme that is present in the luminal brush border and is thought to play a role in amino acid transport in general (Meister, 1975), and we have shown that its activity in the kidney is increased in metabolic acidosis (McFarlane-Anderson & Alleyne, 1977).

An additional response to metabolic acidosis is increased utilization of glutamine by the kidney (Kamm & Strope, 1972; Alleyne & Roobol, 1974). It is not clear, however, whether this increase is controlled by a primary acceleration of transport of glutamine into the cell with subsequent adaptation of various enzymic steps or whether it is the transport into and metabolism in mitochondria that represent the rate-controlling steps.

The present paper describes experiments in which the uptake of [¹⁴C]glutamine by a luminal brush-border-vesicle preparation was measured to determine the characteristics of this transport, and to discover whether this process was affected by acidosis and if there was any relationship between glutamine uptake and γ-glutamyltransferase activity.

Materials and Methods

Experiments were with adult Sprague-Dawley rats, which were locally bred and fed routinely with Purina Laboratory Chow. Chronic metabolic acidosis was induced by giving rats 280 mM NH₄Cl to drink for 7–10 days. Controls received 280 mM NaCl. In experiments in which the degree of acidosis was varied, rats were (a) tube-fed with 200 mM NH₄Cl (0.5 mmol/100 g body wt.) at 12h intervals for 48 h, with 100 mM NH₄Cl as drinking fluid, (b) tube-fed with 400 mM NH₄Cl (1 mmol/100 g body wt.) at 12h intervals for 48 h, with 200 mM NH₄Cl as drinking fluid. Controls were tube-fed with 400 mM NaCl (1 mmol/g body wt.). All rats were starved overnight before they were killed which was done routinely by cervical dislocation. For determination of pH and
pCO₂ rats were anaesthetized with sodium pentobarbital (60 mg/kg) intraperitoneally. Blood was taken from the abdominal aorta into heparinized syringes and determinations were carried out on a blood micro-system acid-base analyser (Radiometer, Copenhagen, Denmark).

**Preparation of brush-border membranes**

Luminal brush-border membranes were prepared by a modification of the method of Turner & Silverman (1977). A 10% (w/v) homogenate of renal cortex was made in a 10 mm-triethanolamine/HCl buffer, pH 7.6, which contained 250 mm-sucrose (buffer A). The homogenate was filtered through glass wool and a double layer of gauze. The filtrate was then centrifuged twice for 10 min at 190 g and the pellets were discarded. The supernatant was centrifuged at 16000 g for 20 min. The pellet was resuspended in buffer A (approx. 2 ml/g of cortex). A portion (about 2 ml) of this crude membrane fraction was centrifuged for 30 min at 36000 g. The pellet was resuspended in 20 ml of 1 mm-Tris/Hepes buffer containing 100 mm mannitol, pH 7.4 (buffer B) and centrifuged for 30 min at 36000 g. The pellet was suspended in approx. 2 ml of buffer B, passed once through a 25-gauge needle, twice through a 30-gauge needle and the volume made up to 20 ml with buffer B. MgCl₂ was added to give a final concentration of 10 mm was added to the suspension, which was placed on ice and stirred occasionally for 10 min. The suspension was then centrifuged at 2000 g for 10 min. The supernatant was centrifuged for 30 min at 36000 g. The pellet was resuspended in 1 ml of 100 mm-Tris/Hepes, pH 7.4, containing 300 mm mannitol (buffer C) (except where stated otherwise), passed twice through a 25-gauge needle and centrifuged at 30000 g for 20 min. The pellet was resuspended in 500-750 μl of buffer C (except where stated otherwise), passed twice through a 30-gauge needle, incubated at 37°C for 15 min, and stored on ice until used.

Maltase and γ-glutamyltransferase were used as marker enzymes for the preparation, and (Na⁺+K⁺)-stimulated ATPase was used as a marker for the basolateral membranes. The enrichment of the three enzymes is shown in Table 1 and indicates that the preparation was predominantly one of luminal brush-border membranes. Electron microscopy of samples fixed in glutaraldehyde/OsO₄ and stained with uranyl acetate was carried out to assess the purity of the preparation (Plate 1).

**Uptake method**

In most experiments 25 μl of brush-border suspension (50-100 μg of protein) was preincubated for 45 s at 25°C. Incubation was started by the addition of 125 μl of the appropriate buffer containing 50 μM-L-[U-¹⁴C]glutamine (appro. 0.5 μCi) or 50 μM-D-[¹⁴C]glucose (appro. 0.25 μCi). After incubation for the appropriate time the reaction was stopped by the rapid addition of 5 ml of an ice-cold buffer containing 1 mm-Tris/Hepes, pH 7.4, and 154 mm-NaCl, and the mixture poured on to Millipore filters (HAWP 0.45 μm) prewetted with buffer. The incubation tube was rinsed twice and the filter washed with 10 ml of the buffer. In the experiments to determine the time course of uptake, 250 μl of the brush-border suspension (0.5–1 mg of protein) was preincubated at 25°C for 1 min. At t = 0 min, 750 μl of incubation buffer was added; 150 μl samples were removed at the times indicated, pipetted on to a prewetted filter and the filter was rapidly washed with 20 ml of buffer.

Filters were placed in 10 ml of Tritosol scintillation fluid (Fricke, 1975) and radioactivity was counted in a Beckman LS150 liquid-scintillation counter. Non-specific retention by the filters was determined by filtering 150 μl of vesicle-free medium, the filter was counted for radioactivity and this radioactivity was subtracted from that obtained with brush-border samples. The radioactivity in the medium was determined by the application of 50 μl of incubation medium to a dry filter, which was then counted. Uptake of [¹⁴C]glucose was carried out as described for glutamine.

All vesicle preparations were used on the day of preparation and all incubations were carried out in triplicate. Uptake values for different preparations sometimes varied over a 2–3-fold range, but were consistent and reproducible for a given preparation. In some instances therefore, the results of single experiments are presented. This is a procedure adopted by other workers and the variation seems to reflect different degrees of vesicle formation/mg of

<table>
<thead>
<tr>
<th>Enzyme Marker</th>
<th>Homogenate</th>
<th>Vesicle suspension</th>
<th>Enrichment factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltase</td>
<td>29.5 ± 1.07</td>
<td>539.4 ± 69.6</td>
<td>18.3</td>
</tr>
<tr>
<td>γ-Glutamyltransferase</td>
<td>14.6 ± 1.3</td>
<td>129.7 ± 4.9</td>
<td>8.9</td>
</tr>
<tr>
<td>(Na⁺+K⁺)-ATPase</td>
<td>0.31 ± 0.05</td>
<td>1.0 ± 0.18</td>
<td>3.2</td>
</tr>
</tbody>
</table>

1979
EXPLANATION OF PLATE I

*Electron micrograph of brush-border-membrane vesicles*

The preparation was fixed in glutaraldehyde/OsO₄ and stained with uranyl acetate. Magnification × 25000.
GLUTAMINE TRANSPORT IN KIDNEY

Vol. 182

protein for any preparation (Sigrist-Nelson et al., 1975).

Chromatography

After uptake for 30s four or five filters were placed in 1ml of water on a boiling-water bath for 15min. Samples of the eluate, glutamine, glutamate and a mixture of these amino acids were spotted on Whatman no. 1 paper and subjected to ascending chromatography in butanol/acetic acid/water (10:2:5, by vol.). Amino acid spots were detected by spraying with ninhydrin.

Determination of protein and enzymes

The protein content of the brush-border preparation was determined by the method of Lowry et al. (1951), with bovine serum albumin as standard. Malate (EC 3.2.1.20) was determined as described by Bergmeyer et al. (1974). Analysis of γ-glutamyltransferase (EC 2.3.2.2) was by the method of Tate & Meister (1974a), and the glutamate produced was assayed by reaction with glutamate dehydrogenase (Bernt & Bergmeyer, 1974). (Na⁺+K⁺)-stimulated ATPase (EC 3.6.1.3) was determined by the method of Post & Sen (1967).

Materials

All chemical reagents were analar grade, and biochemicals were purchased from Sigma Biochemicals Co. L-[U-¹⁴C]Glutamine (49mCi/mmol) and D-[U-¹⁴C]Glucose (3.0mCi/mmol) were obtained from Amersham-Searle (Arlington Hts., IL, U.S.A.).

Results

Identity of the transported molecule

Chromatography of the eluate obtained in boiling water showed that the substance taken up by the vesicles had an R_f value identical with that of glutamine. It was also possible to displace the radioactivity from the intravesicular space by the addition of unlabelled glutamine as shown in Fig. 1. This indicates that glutamine was being taken up in an unaltered form.

Transport versus binding

Fig. 2 shows that uptake represents transport rather than binding. Uptake at 45min was measured as a function of medium osmolality and found to be inversely proportional to osmolality over a mannitol concentration range of 100–500mm. Extrapolation to infinite mannitol concentration indicates intersection at zero uptake, consistent with transport into an osmotically active space.

Time course of uptake

Uptake of 50μM-glutamine at 25°C was determined over a 60min period. Steady-state values of 115pmol of glutamine/min per mg of protein were obtained in
approx. 45 min in the absence of Na+, and addition of Na+ to the incubation medium resulted in an 'overshoot'. Initial rates increased to 400 pmol of glutamine/min per mg of protein at the peak of the 'overshoot' and were approx. 4 times that of the equilibrium value.

**Effect of Na+**

Fig. 3 illustrates the effect of variation of the Na+ concentration in the medium (range 0–150 mM) on the uptake by membranes incubated for 30 s. Concentrations of mannitol were adjusted to maintain medium iso-osmolarity. At a Na+ concentration of 150 mM, the rate is approx. 5 times that obtained in the absence of Na+.

**Variation of glutamine concentration**

The effect of varying the glutamine concentration (50 µM–2 mM) on the uptake in 30 s in the absence or presence of 150 mM-Na+ is shown in Fig. 4(a). The transport in both media was non-linear, providing evidence of saturability. Na+-dependent uptake obtained by subtracting values obtained in the absence of Na+ from total uptake also showed saturability and the system obeyed Michaelis-Menten kinetics (Fig. 4b). The calculated $K_m$ and $V_{max}$ values for the Na+-dependent system were 0.86 mM and 9.6 nmol/min per mg respectively.

**Effect of acidosis**

Vesicles obtained from acidotic rats showed increased uptake of glutamine, as shown in Table 2.

---

**Fig. 3. Effect of Na+ on glutamine uptake**

Uptake of 50 µM-glutamine was measured after 30 s in media containing 0 to 150 mM-Na+. Medium osmolarity was kept constant by the addition of mannitol. Values are given as means ± S.E.M.

**Fig. 4. Relationship between glutamine concentrations and uptake (a) and double-reciprocal plot of Na+-dependent uptake (b)**

(a) Incubations were for 30 s. The range of glutamine concentrations was 50 µM–2 mM. ○, 300 mM-Mannitol; ●, 150 mM-NaCl. ●, Na+-dependent uptake was obtained by subtracting uptake obtained in the absence of Na+ from that obtained in its presence. The values given as means ± S.E.M. are from three experiments carried out in triplicate.
Table 2. Effect of chronic acidosis on uptake of [14C]glutamine by brush-border vesicles
Uptake of 50 μM-glutamine was measured after 30 s incubation in 1 mM-Tris/Hepes medium containing various amounts of Na+. Medium osmolarity was maintained by variation of the mannitol concentration. Values are means ± S.E.M. for three observations.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Glutamine uptake (pmol/mg of protein)</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Acidotic</td>
</tr>
<tr>
<td>300 mM-Mannitol</td>
<td>68.6 ± 1.4</td>
<td>114.9 ± 10.4</td>
</tr>
<tr>
<td>50 mM-Na+</td>
<td>263.5 ± 19.9</td>
<td>345.0 ± 23.1</td>
</tr>
<tr>
<td>100 mM-Na+</td>
<td>378.2 ± 7.8</td>
<td>604.0 ± 1.8</td>
</tr>
<tr>
<td>150 mM-Na+</td>
<td>518.2 ± 31.8</td>
<td>784.6 ± 17.7</td>
</tr>
</tbody>
</table>

Table 3. Effect of the degree of acidosis on the activity of γ-glutamyltransferase and glutamine uptake
Varying degrees of acidosis were achieved as described in the Materials and Methods section. Control 1, rats were tube-fed with 400 mM-NaCl. Control 2, rats received 280 mM-NaCl for 8 days and are the controls for rats drinking NH4Cl for 8 days. Glutamine uptake by controls is designated 100%, and 50 μM-glutamine was the substrate. Uptake was measured after 30 s in medium containing 150 mM-Na+. Results are means ± S.E.M. for the numbers of determinations in parentheses.

<table>
<thead>
<tr>
<th>Plasma[HCO3⁻] (mM)</th>
<th>γ-Glutamyltransferase activity (nmol/min per mg)</th>
<th>Increase in glutamine uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>21.6 ± 0.9 (5)</td>
<td>129.7 ± 4.9 (4)</td>
</tr>
<tr>
<td>Acidosis (rats tube-fed with 200 mM-NH4Cl)</td>
<td>18.7 ± 1.1 (6)</td>
<td>162.4 ± 4.3 (4)</td>
</tr>
<tr>
<td>Acidosis (rats tube-fed with 400 mM-NH4Cl)</td>
<td>13.9 ± 1.9 (6)</td>
<td>203.0 ± 2.5 (4)</td>
</tr>
<tr>
<td>Control 2</td>
<td></td>
<td>145.3 ± 28.1 (6)</td>
</tr>
<tr>
<td>Chronic acidosis</td>
<td></td>
<td>270.2 ± 66.6 (6)</td>
</tr>
</tbody>
</table>

Glutamine uptake by vesicles from chronically acidic rats was 30–50% higher than that by vesicles from control animals. Vesicles from both groups of rats exhibited similar characteristics with respect to Na⁺-dependence. The kinetic characteristics of vesicles from acidic rats were identical with those from control rats and the $K_m$ and $V_{max}$ values for glutamine uptake were 0.76 mM and 9.6 nmol/min per mg respectively. Uptake of glucose by vesicles was not increased by chronic acidosis. Vesicles from control animals incubated in the buffer containing 150 mM-Na⁺ had a glucose uptake at 30 s of 36.2 ± 4.4 (mean ± S.E.M., n = 5) pmol/mg of protein, whereas the uptake by vesicles from acidic animals was 34.2 ± 5.9 (n = 5) pmol/mg of protein.

γ-Glutamyltransferase and glutamine uptake
Measurement of γ-glutamyltransferase activity and glutamine uptake were carried out in vesicles from rats subjected to varying degrees of acidosis (Table 3). Increased uptake of glutamine was associated with increased γ-glutamyltransferase activity. The activity of another brush-border enzyme, maltase, was not increased as a result of acidosis. Specific activities of maltase were 539.4 ± 69.6 nmol of glucose/min per mg of protein (n = 4) and 559.2 ± 23.5 nmol of glucose/min per mg of protein (n = 4) in vesicles from control and acidic animals respectively.

Discussion
The results presented in this study describe the active uptake of L-glutamine into isolated renal brush-border vesicles from normal and acidic rats.

In the presence of Na⁺ there was increased uptake with an 'overshoot' at 30 s, the rates of uptake increasing with increasing Na⁺ concentration. Other workers have obtained similar evidence of active Na⁺-dependent transport of other amino acids and glucose into brush-border vesicles prepared from the gut and the kidney of the rat (Aronson & Sacktor, 1975; Fass et al., 1977; Sigrist-Nelson et al., 1975; Evers et al., 1976). Vesicles prepared from chronically acidic rats exhibit the same kinetic characteristics as those from control rats, but the most significant finding in this study is the observation that the vesicles from acidic rats show a consistent significant increase in uptake over those from normal rats. Teleologically the reason for an increase in glutamine transport across the proximal tubule may be questioned. It is known that glutamine is completely reabsorbed in the kidney but the principal
site of reabsorption is not known. The elegant micro-dissection studies of Curthoys & Lowry (1973) show that, although phosphate-dependent glutaminase (EC 3.5.1.2) is present in highest concentration in the distal tubule, it is in the proximal convoluted tubule that the increase mediated by acidosis is noted. Unfortunately, there are no results for glutamine concentrations at the end of the proximal tubule. It is quite feasible that in acidosis the increased transport of glutamine into the proximal tubule is a precursor to the increase in activity of the phosphate-dependent glutaminase, which is known to occur at that site.

It is possible that the increase in α-glutamyltransferase that we have demonstrated here in vesicles and previously in whole kidney (McFarlane-Anderson & Alleyne, 1977) is related to the increase in glutamine transport, and our data show that with increasing acidosis there is an increase in both transport and enzyme activity. Novogrodsky et al. (1977) have also shown that in lymphoid cells γ-glutamyltransferase activity was highest in those that had the highest rate of glutamine uptake. There is still controversy as to whether the primary role of γ-glutamyltransferase is in the transport of amino acids, in the degradation of glutathione (Elce & Broxmeyer, 1976) or as a glutaminase (Welbourne, 1975). Pruisner et al. (1976) have proposed a simple scheme which gives a role for γ-glutamyltransferase in translocation of amino acids across membranes and which could be a system for the transport of glutamine. This process is more economical in that it requires only to be primed by the donor substrate glutathione, after which the transported molecules, which are rapidly converted into their original form, can act as donor substrate.

From kinetic data, Tate & Meister (1974b) have suggested that glutamine could serve both as donor and acceptor substrates and indeed it seems likely that glutamine could prime the cycle in a scheme such as that proposed by Pruisner et al. (1976). Whereas glutamine is present in plasma at concentrations of about 0.5 mM, the concentration of glutathione is about 5 μM (Tietze, 1969) and is probably largely in the form of the disulphide, which is a poor substrate (Tate & Meister, 1974b).

The specificity of the increased transport of glutamine is shown by the fact that in our studies there is no increase in the activity of maltase and no increase in glucose transport. We would therefore propose that increased transport of glutamine across the proximal-luminal membrane may be one of the primary mechanisms in the adaptive increases in the utilization of glutamine and ammoniagenesis that occur in response to chronic metabolic acidosis in the rat.

N. McF.-A. was supported by the Wellcome Trust. We also acknowledge the support of the Medical Research Council of Great Britain.

References