Effect of acidosis on glutamine transport by isolated rat renal brush-border and basolateral-membrane vesicles

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(Received 22 September 1982/Accepted 7 March 1983)

Glutamine uptake was examined in isolated renal brush-border and basolateral-membrane vesicles from control and acidotic rats. In brush-border vesicles from acidotic animals, there was a significant increase in the initial rate of glutamine uptake compared with that in controls. Lowering the pH of the medium increased the initial rate of glutamine uptake in brush-border vesicles from acidotic, but not from control, rats. In brush-border vesicles from both groups of animals, two saturable transport systems mediated glutamine uptake. There was a 2-fold increase in the $V_{\text{max}}$ of the low-affinity high-capacity system in the brush-border vesicles from the acidotic animals compared with that from control animals, with no alteration in the other kinetic parameters. There was no difference in glutamine uptake by the two saturable transport systems in basolateral vesicles from control and acidotic animals. Lowering the incubation-medium pH increased the uptake of glutamine by basolateral vesicles from both control and acidotic rats to a similar extent. The data indicate that during acidosis there are alterations in glutamine transport by both the basolateral and brush-border membrane which could enhance its uptake by the renal-tubule cell for use in ammoniagenesis.

The marked increase in ammonia production from glutamine during chronic acidosis is a critical function of the kidney (Pitts, 1973). The regulatory site controlling this change in ammoniagenesis is not certain and remains an area of intense research. It is clear that glutamine extraction by the kidney during chronic acidosis increases markedly in man (Owen & Robinson, 1963), the dog (Shalhoub et al., 1963; Pilkington et al., 1970) and the rat (Squires et al., 1976). This increased extraction occurs in part from an increase in net flux into the renal-tubule cell (Pilkington et al., 1970), which is due to one of two mechanisms. First, there could be a primary change in the cell membrane glutamine-transport systems, which would lead to more efficient glutamine uptake, especially at the basolateral aspect of the renal-tubule cell. Secondly, the increased flux could be related to increased tissue utilization allowing more glutamine to enter the cell and less to be available for efflux without a primary change in membrane transport characteristics. Previous methods of studying glutamine in vitro by using intact tubule cells noted increased uptake with acidosis (Alleyne, 1970; George & Solomon, 1981), but these studies could not determine which mechanism led to this increased uptake. To address this question, we have taken advantage of the isolated-membrane-vesicle methodology, which allows the assessment of glutamine uptake in the absence of mitochondrial and cell metabolism. In addition, the ability to separate brush-border from basolateral membranes has permitted the study of the glutamine-transport characteristics of the membrane from each cell surface independently. This paper presents the results of such an examination of glutamine uptake into brush-border and basolateral-membrane vesicles from control and acidotic rats.

Methods

Materials

Adult male Sprague–Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). L-[U-14C]Glutamine (220 Ci/mol) and L-[U-3H]glucose (17.46 Ci/mmol) were obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). Unlabelled glutamine was obtained from Sigma Chemical Corp. (St. Louis, MO, U.S.A.). Reagents for protein–dye-binding determination were obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.).
Membrane vesicle preparation

Adult male rats fed ad libitum on Purina rat chow were divided into control and experimental groups. Control rats received tap water ad libitum and experimental rats received tap water containing 15 g of NH₄Cl/litre. After 10 days, both groups of animals were killed by decapitation. A mixed arterial–venous blood sample was obtained for total CO₂ analysis by the Clinical Chemistry Laboratory, by using a Beckman Astral 8 automated analyser. The kidneys were quickly removed from the decapitated animals, decapsulated, and placed in iced saline (0.9% NaCl). Isolated basolateral-membrane vesicles were prepared by free-flow electrophoresis as described by Reynolds et al. (1980). The supernatant after the first centrifugation in the basolateral-membrane preparation, which is normally discarded, was the starting material for the isolation of brush-border vesicles. The brush-border membrane vesicles were prepared by a modification of the method described by Booth & Kenny (1974), in which 0.25 m-sucrose in 0.01 m-triethanolamine hydrochloride buffer (pH 7.4) was used initially and MgCl₂ was added to the preparation only once instead of twice. The brush-border and basolateral-membrane vesicles were both suspended in a 100 mm-mannitol plus 1 mm-Tris/Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.4) buffer for transport studies, except for studies at acid pH, where the pH of the Tris/Hepes buffer was 6.5. The final protein concentration for brush-border vesicles was 0.2–0.4 mg/ml and for basolateral vesicles was 0.15–0.25 mg/ml, as determined by protein–dye binding (Bradford, 1976).

Transport measurements into membrane vesicles

The measurement of glutamine uptake by membrane vesicles by using filtration on Millipore HAWP filters (0.45 μm) was performed by the technique described by McNamara et al. (1976). All experiments were performed at 22°C. Trapped and diffused spaces were measured with L-[3H]glucose as previously described (Reynolds et al., 1980), and results are expressed as uptake in excess of diffusion.

Enzyme determinations

Enzyme activities were determined on samples of the initial cortical homogenate, brush-border membrane vesicles and pooled basolateral membrane fractions after electrophoresis. These determinations were performed by standard procedures: alkaline phosphatase (EC 3.1.3.1) (Sigma Chemical Corp., 1977); (Na⁺ + K⁺)-dependent ATPase (EC 3.6.1.3) (Kinsolving et al., 1963); and γ-glutamyltransferase (EC 2.3.2.2) component activities of γ-glutamyl transeptidase and γ-glutamyl hydrolase (Glossman & Neville, 1972).

Metabolism

Samples of isolated brush-border membranes were incubated with either 0.02 mm- or 2.1 mm-[14C]glutamine for 0.25 and 20 min to determine the extent of intravesicular metabolism. After the incubation the membranes were filtered on HAWP filters (0.45 μm) with a Millipore filtration apparatus. Immediately after filtration, the filters were pooled in 4 ml of hot water in a 15 ml test tube and the labelled intravesicular contents were extracted by placing this tube in a boiling-water bath for 5 min. The water extract was freeze-dried overnight and then redissolved in 0.5 ml of water. This was then chromatographed on Whatman 3MM paper by descending chromatography, with acetic acid/butan-1-ol/water (3:12:5, by vol.) as the solvent. The chromatograms were cut into 0.5 cm segments and assayed for radioactivity by liquid-scintillation techniques. The radioactivity peaks were compared with simultaneously chromatographed standards stained with ninhydrin for localization. Each radioactivity peak was expressed as a percentage of the total radioactivity.

Data analysis

In concentration-dependence studies, observed transport-kinetic parameters were determined from an Eadie–Hofstee plot drawn with a Monroe model 1775 programmable calculator to obtain the best fit of the data by the least-squares methods. Comparisons of data for significance were performed by Student’s t test.

Results

Animals

The method of adding NH₄Cl to the drinking water to induce chronic acidosis in rats has been well established (Parry & Brosnan, 1978; McFarlane-Anderson & Alleyne, 1979). Acidosis was induced in the experimental group of rats, as demonstrated by a serum total [CO₂] of 17 ± 2 mm (mean ± s.e.m., n = 8) compared with a serum total [CO₂] of 26 ± 1 mm (n = 3, P < 0.01) in the control group. In spite of the decrease in serum total [CO₂], the animals appeared healthy otherwise.

Marker-enzyme determination

The purity of the brush-border membrane preparation was assessed by the marker enzymes alkaline phosphatase and γ-glutamyl transpeptidase. As shown in Table 1, there was a 10-fold enrichment in the specific activity of alkaline phosphatase and a 9-fold enrichment of γ-glutamyl transpeptidase activity in the brush-border preparation from control rats compared with the homogenate. An 11-fold enrichment of alkaline phosphatase and a 17-fold increase in γ-glutamyl transpeptidase activity...
Table 1. Marker enzyme activities

Basolateral and brush-border membranes were isolated and enzyme assays were performed as described in the Methods section. Values for the enzyme activities represent means ± S.E.M. for n determinations. Values in parentheses are the ratio of the enzyme specific activity in the membrane preparation to that in the cortical homogenate before the isolation of the membranes.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Membrane Type</th>
<th>Alkaline phosphatase (nmol of p-nitrophenol/min per mg of protein)</th>
<th>γ-Glutamyl transpeptidase (nmol of p-nitroaniline/min per mg of protein)</th>
<th>(Na⁺ + K⁺)-dependent ATPase (mmol of P₄/h per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control n</td>
<td>Acidotic n</td>
<td>Control n</td>
</tr>
<tr>
<td>Homogenate</td>
<td>Kidneys</td>
<td>124 ± 30</td>
<td>99 ± 25</td>
<td>264 ± 23</td>
</tr>
<tr>
<td>Brush-border membranes</td>
<td>Basolateral</td>
<td>1269 ± 371</td>
<td>1145 ± 317</td>
<td>2445 ± 547</td>
</tr>
<tr>
<td>membranes</td>
<td></td>
<td>(10.2)</td>
<td>(11.6)</td>
<td>(9.3)</td>
</tr>
<tr>
<td></td>
<td>Basolateral</td>
<td>97 ± 11</td>
<td>66 ± 1</td>
<td>141 ± 29</td>
</tr>
<tr>
<td>membranes</td>
<td></td>
<td>(0.8)</td>
<td>(0.7)</td>
<td>(0.5)</td>
</tr>
</tbody>
</table>

Table 2. Effect of incubation pH on glutamine uptake

Isolated membrane vesicles from control and acidic rats were incubated with 0.02 mM L-[¹⁴C]glutamine under an inwardly directed 100 mM-NaCl gradient, and uptake in a 2 mM-Tris/Hepes buffer at pH 6.5 was compared with that at 7.4. Uptake was assayed after 0.25 min of incubation with brush-border membranes and after 1.0 min of incubation with basolateral membranes. Values (nmol/mg of protein) are given as the means ± S.E.M.; the numbers of determinations are shown in parentheses. *P < 0.05 when comparing uptake in pH 6.5 buffer with that in pH 7.4 for control or acidic membranes.

<table>
<thead>
<tr>
<th>Membranes</th>
<th>Glutamine uptake (nmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basolateral</td>
<td>pH 7.4</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.0265 ± 0.003 (6)</td>
</tr>
<tr>
<td>Basolateral</td>
<td>0.0282 ± 0.006 (7)</td>
</tr>
<tr>
<td>Brush border</td>
<td>0.0598 ± 0.008 (12)</td>
</tr>
<tr>
<td>Acidotic</td>
<td>0.0900 ± 0.0114 (16)</td>
</tr>
</tbody>
</table>

In the brush-border preparation from acidic rats compared with the homogenate was observed. The increase in the activity of γ-glutamyl transpeptidase in the brush-border membrane preparation from the acidic rats compared with that of the control rats is consistent with the increase previously reported (McFarlane-Anderson & Alleyne, 1979; Dass & Welbourne, 1980), and is as yet an unexplained consequence of acidosis unrelated to a difference in purity of the membranes. There was a small increase in the activity of (Na⁺ + K⁺)-dependent ATPase in the brush-border vesicle preparations from both groups of animals compared with the homogenate, suggesting some contamination of the brush-border preparation with basolateral membranes.

There was a 16-fold enrichment of (Na⁺ + K⁺)-dependent ATPase in the basolateral-membrane preparation compared with the homogenate from control animals and a 14-fold enrichment in the basolateral-membrane preparation from acidic animals, demonstrating the purity of this preparation, as reported previously (Reynolds et al., 1980). Further, the specific activities of the brush-border membrane markers, alkaline phosphatase and γ-glutamyl transpeptidase, in the basolateral-membrane preparation from both groups of animals were not significantly increased compared with the homogenate.

Glutamine uptake by brush-border membranes.

The time course of [¹⁴C]glutamine uptake by brush-border membrane vesicles prepared from control and acidic rat kidneys is plotted in Fig. 1. The uptake of 0.02 mM-glutamine by brush-border membrane vesicles from control animals under an inwardly directed Na⁺ gradient had only a small 'overshoot' of Na⁺-stimulated transport. The uptake of 0.02 mM-glutamine with brush-border membrane vesicles from acidic animals demonstrated a more marked 'overshoot' under an inwardly directed Na⁺ gradient. The uptake at 1 min of incubation was 23% higher in vesicles from acidic animals than in controls. At steady state after 20 min of incubation, both groups of animals had similar uptake values.

With 2.1 mM-glutamine as the substrate, an obvious 'overshoot' of uptake was observed with brush-border membrane vesicles from both groups of animals. The values for uptake by the brush-
border vesicles from the acidotic rats were significantly higher compared with controls at 0.25, 1 and 2 min. At steady state after 20 min of incubation, again both groups of animals had reached similar values. This increased uptake by brush-border vesicles from acidotic animals appeared to be specific for glutamine, since there was no difference in the uptake of 0.06 mM-L-proline, 0.06 mM-glycine or 1 mM-D-glucose between brush-border vesicles from control and acidotic rats.

Because George & Solomon (1981) previously noted that glutamine uptake was increased when the pH of the medium was lowered in renal-cortical slices from acidotic rats, uptake studies of 0.02 mM-glutamine in pH 6.5 buffer were compared with similar ones in pH 7.4 buffer (Table 2). Lowering the buffer pH appeared to increase the early entry of glutamine, as measured by 15 s of incubation, in brush-border vesicles from control animals, but this effect was not statistically significant. However, there was a statistically significant enhancement of early uptake by lowering the pH of the medium in vesicles from acidotic animals. Table 2 also shows the marked increase in brush-border membrane uptake in acidotic kidneys at both pH values.

Metabolism

Since the enzyme γ-glutamyl transpeptidase resides on the brush-border membrane and is known to catalyse glutamine hydrolysis, analysis of the incubation media and intravesicular contents was performed. After 15 s of incubation, only 13% of the intravesicular label was found as glutamate and 87% was unchanged in brush-border membrane vesicles from control rats. The same ratio of labelled glutamine to glutamate was also found in the intravesicular contents of the brush-border membrane vesicles from the acidotic rats. With 2.1 mM-glutamine as the substrate, 95% of the intravesicular label was unchanged and 5% was glutamate in the control brush-border vesicles after 0.25 min of incubation, and 91% of the intravesicular label was glutamine and 9% was glutamate in the brush-border vesicles from acidotic animals. After 20 min of incubation, 61% of the intravesicular label was associated with glutamate and 39% remained as glutamine with 0.02 mM-glutamine as the substrate. With 2.1 mM-glutamine as the substrate, on 35% of the intravesicular label was associated with glutamate and 65% with glutamine after 20 min of incubation. Glutamine was the only labelled species found in the media after 15 s of incubation in both groups of vesicles. Previous work from our laboratory (Weiss et al., 1978) has shown that the brush-border membrane can metabolize 0.06 mM-[14C]glutamine such that by 30 min of incubation 32% of the label in the medium has been converted into glutamate. Because of this extensive metabolism, the value after 20 min of incubation in brush-border vesicles does not represent equilibration of glutamine across the vesicles, but only of radioactivity.
Glutamine transport by renal membrane vesicles in acidosis

Concentration-dependence

Since a difference in the initial rate of uptake of glutamine was found in the two groups of animals, the concentration-dependence of the initial rate of uptake was examined over the substrate concentration range 0.009–3.9 mM. A Hofstee plot of this is shown in Fig. 2. The two-limbed nature of this plot for uptake by the vesicles from both groups of animals indicated that multiple transport systems mediated the entry of glutamine into the brush-border membrane vesicles. These findings were similar to those previously reported by our laboratory for glutamine transport by brush-border membrane vesicles (Weiss et al., 1978; Hsu et al., 1980). The observed kinetic parameters for glutamine uptake into the control vesicles over the substrate range 0.009–0.207 mM were $K_m = 0.263 \pm 0.025$ mM, $V_{max_1} = 1.72 \pm 0.26$ nmol/15 s per mg of protein, and the observed kinetic parameters over the substrate range 0.207–3.9 mM were $K_m = 1.05 \pm 0.11$ mM, $V_{max_2} = 4.77 \pm 0.36$ nmol/15 s per mg of protein. The observed kinetic parameters for glutamine uptake over the same substrate concentration ranges into brush-border membrane vesicles from acidotic rats were $K_m = 0.296 \pm 0.079$ mM, $V_{max_1} = 2.17 \pm 0.43$ nmol/15 s per mg of protein and $K_m = 1.72 \pm 0.28$ mM, $V_{max_2} = 9.08 \pm 1.05$ nmol/15 s per mg of protein. Comparison of these observed kinetic parameters from the two groups of animals revealed no statistically significant difference between $K_m$, $K_m$, and $V_{max_2}$. There was, however, a significant difference in the observed $V_{max_2}$ from the two groups of animals: that in vesicles from the acidotic rats was 1.9 times that from the control animals ($P < 0.02, n = 6$).

Glutamine uptake in basolateral-membrane vesicles

In Fig. 3, the uptake of 0.02 mM-glutamine by basolateral membrane vesicles is plotted. In this preparation, 50% of the vesicles are right-side out and 50% inside out (Reynolds et al., 1980). As previously noted (Reynolds et al., 1980), an 'overshoot' of Na$^+$-stimulated transport was not observed. The uptake values represent glutamine uptake, since basolateral vesicles do not hydrolyse glutamine to glutamate (Reynolds et al., 1980). There was no difference in the glutamine-uptake values by the basolateral-membrane vesicles from the control and acidotic rats throughout the time course. Glutamine uptake by the basolateral membranes was only one-fifth of that by the brush-border membrane vesicles after 1 min incubation.

Performing the transport studies in a pH 6.5 medium increased glutamine uptake by 193% as measured over 1.0 min incubation in basolateral-membrane vesicles from controls and by 158% in those from acidotic animals (Table 2). There was no significant difference in glutamine uptake between basolateral-membrane vesicles from control and acidotic rats incubated in pH 6.5 buffer, suggesting that enhanced glutamine transport with lowered pH of the medium is an intrinsic property of the basolateral membrane and not a response to chronic acidosis.

Fig. 2. Concentration-dependence of initial uptake of glutamine by brush-border vesicles from control (●) and acidotic (▲) rats

Vesicles were incubated with various concentrations of L-[14C]glutamine for 15 s at 22°C under Na$^+$-gradient conditions as described in the Methods section. Uptake in excess of diffusion, as measured by the L-[3H]glucose space, is shown as a plot of $v$ versus $v/s$, where $v$ is in nmol/15 s per mg of protein and $s$ is in mM. Values shown are the means of at least 12 determinations.
The time course of uptake of 0.02 mM L-[14C]glutamine by isolated rat renal basolateral-membrane vesicles was measured under Na⁺-gradient conditions at 22°C by rapid filtration as described in the Methods section. Each symbol represents at least 12 determinations of uptake in excess of diffusion as assessed by the simultaneous determination of the L-[3H]glucose space. The S.E.M. is contained within each symbol used to represent the mean.

The concentration-dependence of uptake over the concentration range 0.009–1.76 mM is plotted in Fig. 4. There was a two-limbed curve, suggesting multiple transport systems, and the observed kinetic parameters obtained from this plot were $K_m = 0.04$ mM, $V_{max} = 0.09$ nmol/min per mg of protein, and $K_m = 3.18$ mM, $V_{max} = 2.22$ nmol/min per mg of protein. There was no difference between these parameters for basolateral-membrane vesicles from control and acidotic rats.

Discussion

We have shown that the rate of glutamine uptake is increased in brush-border membrane vesicles from chronically acidotic rats, and amplified the findings of McFarlane-Anderson & Alleyne (1979). The increase is related to a doubling of the $V_{max}$ of the high-$K_m$ system with chronic acidosis. This change in vesicles from acidotic animals was not related to an increase in glutamine hydrolysis, as demonstrated by little conversion into glutamate in 15 s despite the increase in y-glutamyl transpeptidase activity (Table 2), which catalyses the deamination of glutamine (Meister, 1973). The independence of glutamine uptake by brush-border vesicles from the y-glutamyl cycle has been suggested by the work of Hsu et al. (1980).

The physiological significance of the increased ability of the brush-border membrane to transport glutamine is not clear. Even during normal acid–base conditions, approx. 99% of the filtered load of glutamine is re-absorbed (Pitts, 1973), leaving little for the more efficient brush-border membrane from acidotic rats to re-absorb further. In addition, the filtered load falls slightly during acidosis because of a slight decrease in plasma concentration (Squires...
et al., 1976; Schrock & Goldstein, 1981). Therefore the large increase in glutamine extraction by the kidney during acidosis must in part be due to net uptake across the basolateral membrane. This has been pointed out by Pilkington et al. (1970). Data from these investigators indicate that at least one-third of total glutamine extracted by the kidney in acidic dogs has to come from basolateral influx. Silverman et al. (1981), using the pulse multiple-indicator-dilution technique in dogs, found that influx across the basolateral membrane was nearly twice that across the luminal membrane. In chronic acidosis, the basolateral influx of glutamine was nearly halved, with little change in the luminal influx. The markedly increased renal extraction of glutamine by the acidic dog kidney was due to increased cellular metabolism of glutamine, which was reflected in decreased basolateral efflux. Silverman et al. (1981) concluded that the major site of cellular uptake of glutamine under both normal and chronically acidic conditions is across the basolateral membrane. They did not find adaptation of either the basolateral or luminal membrane to acidosis.

Our data indicate that there is no difference in glutamine transport by basolateral-membrane vesicles from acidic rats and controls. Glutamine uptake across basolateral membranes was shown to be enhanced by a low pH. This effect is not a response by the basolateral membrane to chronic acidosis, but rather an intrinsic characteristic of glutamine transport across this membrane, since no difference could be shown in this response between basolateral membranes from acidic and control animals. This property of glutamine transport by the basolateral membrane would allow it to react quickly to a fall in blood pH with increased glutamine uptake.

The pH of the medium does appear to be important for glutamine entry into the renal-tubule cell, as pointed out by George & Solomon (1981). They found that glutamine uptake was increased by lowering the pH of the medium to 6.8 in renal-cortical slices from acidic animals, but not in those from controls. The changes that we have observed in isolated membrane vesicles with decrease in pH, especially glutamine uptake is significantly increased in brush-border membrane vesicles from acidic rats and not from controls, may underlie the observations in cortical slices. In the intact cell, however, increased glutamine uptake could also be a response to altered glutamine utilization. Lowry & Ross (1980) noted increased ammoniagenesis and gluconeogenesis from glutamine with increased activity of 2-oxoglutarate dehydrogenase soon after lowering the pH of the incubation medium.

Comparison of events in vitro with the situation in vivo is difficult. In the intact kidney, plasma continuously flows past the basolateral membrane and glomerular filtrate past the luminal membranes, which does not occur in our isolated-membrane studies in vitro. Further, ionic and electrical gradients are constantly maintained across these membranes in situ, but such gradients are dissipated while glutamine transport is studied in membrane vesicles. However, comparison of glutamine entry rates, as measured after 1 min of incubation, does not indicate that basolateral-membrane influx would be twice that of brush border, as observed by Silverman et al. (1981). Of course the renal plasma flow is 3-5 times that of the glomerular filtration rate, so that the basolateral membrane would be exposed to more glutamine per unit time than the luminal membrane. Compartmentalization of glutamine after entry into the intact cell could also play a role in the differences in the magnitude of the flux across each membrane, which would not be reflected in transport by isolated membrane vesicles. In this regard, the mitochondria, where the majority of glutamine catabolism occurs, are in close proximity to the basolateral membrane, and this may contribute to the flux differences across the two membranes. Finally, a species difference could account for the variance between our data obtained in the rat and those of Silverman et al. (1981) obtained in the dog.

This work was supported by Grant AM10894 from the National Institute of Health, Bethesda, MD. J. W. F. is a recipient of the Daland Fellowship from the American Philosophical Society. We acknowledge the excellent technical assistance provided by Louise M. Pepe.

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