Regulation of expression of the SN1 transporter during renal adaptation to chronic metabolic acidosis in rats

ANNE M. KARINCH, CHENG-MAO LIN, CHRISTOPHER L. WOLFGANG, MING PAN, AND WILEY W. SOUBA
Department of Surgery, Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Received 19 March 2002; accepted in final form 13 July 2002

Regulation of expression of the SN1 transporter during renal adaptation to chronic metabolic acidosis in rats. Am J Physiol Renal Physiol 283: F1011–F1019, 2002; 10.1152/ajprenal.00106.2002.—During chronic metabolic acidosis, renal glutamine utilization increases markedly. We studied the expression of the system N1 (SN1) amino acid transporter in the kidney during chronic ammonium chloride acidosis in rats. Acidosis caused a 10-fold increase in whole kidney SN1 mRNA level and a 100-fold increase in the cortex. Acidosis increased Na\(^+\)-dependent glutamine uptake into basolateral and brush-border membrane vesicles (BLMV and BBMV, respectively) isolated from rat cortex (BLMV, 219 ± 66 control vs. 651 ± 180 pmol·mg\(^{-1}\)·min\(^{-1}\) acidosis; BBMV, 1,112 ± 189 control vs. 1,652 ± 148 pmol·mg\(^{-1}\)·min\(^{-1}\) acidosis, both \(P < 0.05\)). Na\(^+\)-independent uptake was unchanged by acidosis in BLMV and BBMV. The acidosis-induced increase in Na\(^+\)-dependent glutamine uptake was eliminated by histidine, confirming transport by system N. SN1 protein was detected only in BLMV and BBMV from acidic rats. After recovery from acidosis, SN1 mRNA and protein and Na\(^+\)-dependent glutamine uptake activity rapidly returned to control levels. These data provide evidence that regulation of expression of the SN1 amino acid transporter is part of the renal homeostatic response to acid-base imbalance.

renal acid-base homeostasis; basolateral membrane vesicle transport; brush-border membrane vesicle transport; ammonium chloride acidosis; system N1

AN ESSENTIAL FUNCTION OF THE kidney is maintenance of acid-base homeostasis, a role that assumes even greater importance under conditions of chronic metabolic acidosis. During acidosis, ammoniagenesis is greatly increased in the kidney, allowing excretion of protons in the form of ammonium ions in the urine. Glutamine, the most abundant free amino acid in the blood, is the precursor of 80–90% of the ammonia produced during acidosis (37). Under normal conditions, renal uptake of glutamine from the blood is minimal. During chronic metabolic acidosis, however, renal extraction of glutamine increases markedly while extraction of other amino acids is virtually unchanged (34, 36). Enhanced glutamine uptake and metabolism by the kidney are the result of a coordinated series of events triggered by the disruption of normal acid-base balance. The homeostatic response involves alterations in interorgan glutamine flux, such that glutamine release from skeletal muscle is doubled, the splanchnic bed shifts from net glutamine uptake to glutamine release, and the kidney becomes the major site of glutamine consumption (33). Intrarenal alterations in glutamine utilization also occur. Ammoniagenesis and HCO\(_3^-\) production are greatly increased by augmented flux through mitochondrial glutaminase and glutamate dehydrogenase (GDH) (23) and subsequent \(\alpha\)-ketoglutarate metabolism (4). Asymmetric secretion in the proximal tubule of NH\(_4^+\) ions into the tubular lumen and HCO\(_3^-\) ions into the venous blood is critical for maintenance of acid-base balance.

Almost all the glutamine filtered at the glomerulus is reclaimed in the convoluted proximal tubule (35), leaving little margin for increased glutamine uptake from the tubular lumen during acidosis. The major portion of the increased renal glutamine taken up must therefore enter the tubular epithelium across the basolateral membrane from the blood (28, 34). Although it has been appreciated that glutamine transport must increase during acidosis (38), the carriers responsible for glutamine transport across the basolateral and brush-border membranes of kidney tubules have not been identified. System N1 (SN1) is an Na\(^+\)-dependent amino acid transporter that transports glutamine, histidine, and asparagine in various tissues. Its activity was first characterized in rat liver, in which it is the principal transporter of glutamine (19), and subsequently system N-like activities were described in brain (10) and skeletal muscle (17). The cDNA for SN1 has recently been cloned (6, 11, 14), and the protein has been expressed in cells and Xenopus laevis oocytes, allowing functional study of the transporter (6, 11, 14). In addition to its expression in liver, brain, and skeletal muscle, SN1 mRNA is expressed at a relatively high level in the kidney (6, 14).

Address for reprint requests and other correspondence: W. W. Souba, Dept. of Surgery, Milton S. Hershey Medical Ctr., Pennsylvania State Univ. College of Medicine, PO Box 850, H051, Hershey, PA 17033 (E-mail:wsouba@psu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajprenal.org 0363-6127/02 $5.00 Copyright © 2002 the American Physiological Society
The studies reported here strongly suggest that SN1 is the glutamine transporter responsible for increased renal uptake of glutamine during acidosis and that its expression is regulated as part of the coordinated physiological response to an acid load.

METHODS

Animals. Animals were treated in accordance with regulations of the Institutional Animal Use and Care Committee. Male Sprague-Dawley rats (250–300 g; Charles River, Wilmington, MA) were maintained in a 12:12-h light-dark cycle with unrestricted access to rat chow and water or water containing 1.5% NH₄Cl. Metabolic acidosis was maintained for varying lengths of time, up to 8 days. In some experiments, acidic rats were returned to drinking tap water for up to 3 days of recovery. The rats readily adjusted to the acid load received. After an initial 2- to 3-day period of no weight gain, acidic rats started to gain weight, although at a somewhat slower rate (~5 g/day) than that of control rats (~7 g/day). For most experiments reported here, rats were made acidic for 7 days. In some experiments, animals were killed at shorter time points, and, in others, additional animals were killed at 8 days acidosis. In general, results for 7- or 8-day acidic animals did not differ and have been pooled.

Rats were killed after being anesthetized with ketamine, and their kidneys were either freeze-clamped in tongs kept at the temperature of liquid nitrogen or immediately processed for isolation of basolateral or brush-border membrane vesicles (BLMV or BBMV, respectively). At the time of death, blood was drawn from the descending aorta and the renal vein for measurement of blood gases, pH, and plasma glutamine concentration.

Measurement of blood gases, pH, and glutamine concentration. Arterial blood pH and P CO₂ were measured with an IL 803 blood-gas machine (model 1420, Instrumentation Laboratories). Blood HCO₃⁻ concentration was automatically calculated from the measured pH and P CO₂. Plasma glutamine levels were measured in triplicate by a modified spectrophotometric assay using a colorimetric assay kit (Boehringer Mannheim, Mannheim, Germany) adapted to a 96-well plate format with a microplate spectrophotometer. Glutamine extraction was calculated as (arterial – renal vein) glutamine concentration/arterial glutamine concentration and expressed as a percentage.

Kidney dissection. Kidney dissection was carried out under approximately threefold magnification. The decapsulated kidney was first cut lengthwise into two symmetrical halves. For dissection of cortex, a thin slice (1.5–2 mm thick) was first cut from the curved outer surface. Additional cortex was dissected with fine scissors by trimming away the outer 1.5–2 mm, using the visible arcuate arteries as a guide to the junction of the cortex and medulla. The outer and inner stripes of the outer medulla and the inner medulla are clearly discernible. These regions were dissected from the remainder of the kidney and carefully trimmed of visible remaining tissue from other regions.

Northern blot analysis. Total RNA was isolated from whole kidney or dissected kidney regions by using the Totally RNA system (Ambion, Austin, TX). No pooling of RNA from different rats was necessary to obtain sufficient RNA for blots. Twenty micrograms of RNA were separated on a 1% formaldehyde gel, transferred to nylon membrane (Genescan, New England Nuclear), and hybridized with a SN1-specific oligonucleotide probe (5'-GTGCAGAAGGCTCGACGACTGT-CAGTTTGG-3') by using the method of Henderson et al. (16). The oligonucleotide was radioactively 3' end labeled using terminal transferase. For quantification of SN1 mRNA, autoradiographs were scanned with a laser densitometer (Dynamic Biosystems). GAPDH and β-actin were both found to be unsuitable for RNA loading normalization. Acidosis increased mRNA levels of both GAPDH and β-actin, and β-actin mRNA levels varied among kidney regions, increasing from cortex to inner medulla. Therefore, raw scanning data were used for relative quantitation of SN1 mRNA. Ethidium bromide-stained gels showed that the RNA was intact and that loading differences did not account for the wide variations observed in SN1 mRNA levels on the Northern blots. A cDNA closely related to SN1 has recently been described and designated system N2 (SN2) (22). The amino acid sequence of rat SN2 is 63% identical to that of rat SN1, and SN2 mRNA is expressed in the kidney. The SN1 probe is complementary to a region of the SN1 cDNA in which the SN1 and SN2 proteins differ completely. Basic Local Alignment Search Tool 2 sequence alignment found no significant similarity between the SN1 probe and the SN2 cDNA. Therefore, it is extremely unlikely that the SN1 probe hybridizes with SN2 mRNA under the stringent conditions used here for Northern blot analysis.

In situ hybridization. Kidneys were decapsulated and carefully frozen in an isopentane/dry ice bath. In situ hybridization was carried out as described by Campbell and Hess (5). Serial 20-μm sagittal sections were hybridized with 35S-labeled antisense and sense SN1 riboprobes synthesized by in vitro transcription from linearized Bluescript vector containing rat SN1 cDNA (a gift of Dr. Robert H. Edwards). Slides were exposed to X-ray film (Cronex 4, Sterling Diagnostic Imaging, Newark, DE) for 1 wk and then dipped in Kodak emulsion and developed 3–4 wk later. Slides were counterstained with hematoxylin/eosin. Sections from a total of 10 kidneys (5 from control and 5 from acidic rats) were hybridized in two separate experiments. Hybridized sections were viewed with an Olympus B-Max 50 microscope, and images were captured by using a cooled charge-coupled device camera (Photometrics, Tucson, AZ) interfaced with IPLab scientific imaging software (Scanalytics, Fairfax, VA).

Isolation of cortical BLMV. BLMV were isolated from renal cortex by Percoll density gradient centrifugation basically as described by Sacktor et al. (31) and modified by Edwards et al. (9). All steps were carried out on ice. Renal cortices were initially homogenized in homogenization buffer using 10 strokes with a loose plunger in a glass Dounce homogenizer, diluted with the same buffer and processed with a polytron (2 bursts of 15-s duration at setting 6; Brinkmann Instruments, Westbury, NY). The homogenization buffer contained 250 mM sucrose, 10 mM triethanolamine, pH 7.6, 0.1 mM phe- nylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, and 2 ml/l protease cocktail stock (P2714, Sigma, St. Louis, MO). The homogenate was centrifuged at 2,500 g for 15 min at 4°C. The resultant supernatant was centrifuged at 20,000 g for 20 min, and the upper fluffy layer of the pellet was resuspended in homogenization buffer with 10 strokes of a tight Dounce plunger. Percoll was added to 13% (vol/vol), and 36-mL gradients were centrifuged at 48,000 g for 30 min. The top clear 6 ml of the gradient were discarded, and the next 6 ml were harvested and diluted with intravesicular buffer (100 mM KCl, 100 mM mannitol, 12 mM Tris/HEPES, pH 7.5, protease inhibitors as above). The vesicles were washed three times at 48,000 g for 15 min with intravesicular buffer and resuspended in the same buffer to a concentration of ~1 mg/ml. Protein was measured by using the Bradford assay with BSA as a standard. BLMV were prepared in pairs of one control and one 7-day acidic rat (5 separate experiments) or one control and one 2-day recovered rat (3 separate experiments).
Renal cortices from three to six animals were pooled for each BLMV preparation. Freshly isolated or liquid nitrogen-frozen BLMV were used for glutamine transport assays. Transport activity was similar in fresh and frozen vesicles. Vesicle relative enrichment was estimated using ouabain-inhibitable ATPase activity in BLMV compared with homogenate (13). The enrichment factor was 23.0 ± 4.2 for control vesicles (1.4 ± 0.2 vs. 29.5 ± 2.1 μmol P;·mg⁻¹·30 min⁻¹, homogenate vs. BLMV) and 22.5 ± 4.5 for acidotic vesicles (1.5 ± 0.3 vs. 31.7 ± 3.6 μmol P;·mg⁻¹·30 min⁻¹).

Isolation of cortical BBMV. BLMV were isolated from renal cortex by MgCl₂ precipitation basically as described by Edwards et al. (9). All steps were carried out on ice. Renal cortices were homogenized with a polytron (3 bursts of 20-s duration at setting 6) in 50 mM mannitol, 12 mM Tris/HEPES, pH 7.5, and protease inhibitors as above. MgCl₂ was added to a final concentration of 10 mM, and the homogenate was stirred for 20 min. The homogenate was centrifuged for 15 min at 35,000 g. The loosely packed BBMV layer was gently washed off the pellet and recentrifuged an additional three times as described. The BBMV were resuspended in intravesicular buffer to a protein concentration of 1 mg/ml and stored in liquid nitrogen. Renal cortices from three animals were pooled for each BBMV preparation. Vesicle relative enrichment was estimated using alkaline phosphatase activity in BBMV compared with homogenate, by using a commercially available kit (Sigma). The enrichment factor was 12.6 ± 0.9 for control vesicles (332 ± 8 vs. 4,181 ± 210 units alkaline phosphatase activity mg⁻¹·15 min⁻¹, homogenate vs. BBMV) and 13.3 ± 2.7 for acidotic vesicles (290 ± 28 vs. 3,791 ± 403 units alkaline phosphatase activity mg⁻¹·15 min⁻¹).

Glutamine transport in renal cortical BLMV and BBMV. For transport studies, Na⁺-dependent glutamine uptake was measured in BLMV and BBMV from control or acidotic rats. Glutamine transport was evaluated at room temperature by a rapid mixing/filtration technique described previously (24). Uptake was initiated by mixing 10 μl of vesicles (10 μg membrane protein) with 40 μl Na⁺ or K⁺ uptake buffer (75 mM NaCl or KCl, 100 mM mannitol, 12 mM Tris/HEPES, pH 7.9, 25 μM glutamine containing a tracer amount of [³H]glutamine). Termination of uptake was accomplished by addition of 1 ml ice-cold wash buffer (uptake buffer without glutamine) followed by rapid filtration through vacuum through a 0.45-μm membrane filter (GN-6 grid, Gelman Laboratory) and four additional washes with 1 ml wash buffer. The filter was incubated in 10 ml Scintiscan 30% for liquid scintillation counting (Beckman LS 1801, Beckman Instruments, Palo Alto, CA). Preliminary studies showed that glutamine transport was linear at 5 s under these assay conditions. Therefore the 5-s time point was chosen for transport experiments. Transport activity was expressed as micromoles glutamine per milligram protein per minute by multiplying the 5-s uptake by 12. l-[³H]glutamine was purchased from PerkinElmer Life Sciences, Boston, MA. Where indicated, 5 mM histidine, serine, or α-(methylamino)isobutyric acid (MeAIB) were added to the uptake buffer as inhibitors of glutamine transport to allow identification of the transport systems present in BLMV and BBMV.

Western blot analysis. Equal amounts of cortical BLMV (10 μg) and BBMV (20 μg) were separated by SDS-PAGE on precast polyacrylamide gels (ISC BioExpress, Kaysville, UT) and transferred to polyvinylidene difluoride membrane (Millipore). Blots were incubated with primary and secondary (horseradish peroxidase-conjugated donkey-anti-rabbit, Jackson ImmunoResearch Laboratories, West Grove, PA) antibodies, and the SN1 protein was detected by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, Piscataway, NJ) following the manufacturer’s instructions. The antibody used to detect the SN1 transporter was a polyclonal antibody generously provided by Dr. Robert H. Edwards.

Statistics. Data were analyzed with paired Student’s t-test or one-way analysis of variance followed by the Bonferroni or Dunnett posttests for multiple comparisons, as appropriate. In addition, a generalized linear model controlling for batch fixed effects was used to test for differences in Na⁺-dependent glutamine uptake in BLMV and BBMV in the inhibition profile experiments. When significant differences were detected, Duncan’s multiple range test was used for post hoc analyses of uptake in the presence of MeAIB, serine, or serine plus histidine.

RESULTS

Effect of chronic metabolic acidosis on selected physiological parameters. Chronic metabolic acidosis was induced in rats by adding 1.5% NH₄Cl to their drinking water. This is a well-characterized model of chronic metabolic acidosis in rats (15, 26, 36). Selected physiological parameters were measured in control, acidotic, and 7-day acidotic rats that were returned to drinking tap water for 1 or 3 days. Decreased blood pH (7.40 ± 0.01 control vs. 7.34 ± 0.02 acidosis, P < 0.05) and HCO₃⁻ concentration (24.3 ± 0.53 control vs. 20.9 ± 0.89 mM acidosis, P < 0.05) confirmed that the rats were acidotic. The degree of acidosis was mild, reflecting the action of renal and extrarenal compensatory mechanisms that minimize acid-base imbalance. After 7 days of acidosis, the kidney, which extracts relatively little glutamine from the plasma under normal conditions, becomes a major organ of glutamine consumption (2.8 ± 0.4 glutamine extraction control vs. 35.6 ± 6.5% extraction acidosis, P < 0.01). These acidosis-induced changes in blood parameters rapidly returned to, or overshot, control values within 24 h of the return of the animals to drinking tap water, and glutamine uptake changed to glutamine release (pH, 7.45 ± 0.01; HCO₃⁻ concentration, 30.30 ± 0.68 mM; glutamine extraction, -4.0 ± 3.7%).

Chronic metabolic acidosis increases renal expression of the SN1 mRNA. Northern blot analysis was used to determine the effect of acidosis on expression of SN1 amino acid transporter mRNA in whole kidney. Figure 1A is a representative Northern blot of whole kidney RNA isolated from control, acidotic, and 1- and 3-day recovered acidotic rats. The result of scans of blots from three separate experiments is shown in Fig. 1B. In acidotic rats, the level of SN1 mRNA is ~10-fold higher than in controls (P < 0.001). The response to an acid load is rapid; within 24 h of induction of acidosis, the SN1 mRNA level is already approximately fourfold that of controls (not shown). When the rats were returned to drinking tap water, SN1 mRNA approached control levels after 24 h.

**AJP-Renal Physiol • VOL 283 • NOVEMBER 2002 • www.ajprenal.org**
Chronic metabolic acidosis alters the localization of SN1 RNA in rat kidney. In situ hybridization was used to localize SN1 mRNA in the rat kidney and to determine whether the distribution was altered in response to acidosis. Figure 2D shows autoradiographs of sagittal sections of kidneys from two control and two acidotic rats, hybridized with an antisense SN1 riboprobe. There is strong hybridization in the outer stripe of both control and acidotic kidneys. No hybridization is detected in the inner stripe or inner medulla. A response to acidosis is evident in the renal cortex in which expression of SN1 mRNA is induced (arrows). Equivalent regions of cortex of control and acidic kidney (Fig. 2, A and B, respectively) hybridized with the SN1 riboprobe are shown. Silver grains indicating the presence of SN1 mRNA are distributed over tubular structures in Fig. 2B but are absent from Fig. 2A. Induction of SN1 mRNA expression in the cortex was not uniform but tended to occur in patches distributed throughout the cortex. Distribution of silver grains shows that SN1 mRNA is expressed in proximal tubules but is not expressed in distal tubules or glomeruli (Fig. 2C) or collecting ducts or blood vessels (not shown). No specific hybridization was seen on sections hybridized with a sense SN1 riboprobe.

To verify the regional differences in SN1 mRNA expression, Northern blot analysis was carried out by using total RNA isolated from the cortex, outer and inner stripes of the outer medulla, and the inner medulla of control and acidic rats (Fig. 3A). Dramatic induction of SN1 mRNA occurs in the cortex, in which expression is almost undetectable in control kidney but achieves a high level during acidosis (Fig. 3B). It is difficult to obtain an accurate measurement of the increase of induction in the cortex because of the vanishingly small amount present in control cortex. Under control conditions, the SN1 mRNA level is highest in the outer stripe in which, in response to acidosis, the
markedly increased glutamine extraction during metabolic acidosis reflects augmented uptake from the blood across the basolateral membrane (36). Second, in the liver, SN1 is located in the basolateral membrane of hepatocytes rather than in the canicular membrane (22). Because the largest induction in SN1 mRNA occurs in the cortex and because we expected the transporter to be in the basolateral membrane, we first isolated BLMV from renal cortices of normal, acidoic and 2-day-recovered rats and used these vesicles for glutamine uptake studies and for Western blot analysis.

**Fig. 3. Effect of chronic metabolic acidosis on regional expression of SN1 mRNA.** A: Northern blot of total RNA (20 μg) isolated from dissected regions of kidney from a control (C) and 7-day acidic (A) rat. The blot was hybridized with an SN1-specific probe. Also shown is the ethidium bromide-stained gel before transfer. B: regional increase of induction of SN1 mRNA in kidney regions in response to metabolic acidosis, on the basis of the densitometric scan of the blot in A.

level of SN1 mRNA increases about ninefold. A low level of SN1 mRNA is apparent in the inner stripe (Fig. 3A). However, in situ hybridization shows that SN1 mRNA is not expressed in the inner stripe (Fig. 2D), so the SN1 mRNA detected on the Northern blot probably represents outer stripe contamination during dissection. SN1 mRNA is not expressed in the inner medulla.

A cDNA closely related to SN1 has recently been described and designated SN2 (22). The amino acid sequence of rat SN2 is 63% identical to rat SN1, and SN2 mRNA is expressed in kidney. All Northern blots presented in this paper were hybridized with an SN1-specific oligonucleotide probe complementary to a region of the SN1 cDNA in which the SN1 and SN2 proteins differ completely (see METHODS). The expression and induction shown here on Northern blots represent only SN1 mRNA.

**Chronic metabolic acidosis increases glutamine transport in renal cortical BLMV.** Two lines of evidence suggested to us that the SN1 transporter is present in the basolateral membrane of cells. First, studies of glutamine uptake and utilization conclude that the markedly increased glutamine extraction during metabol-
control, acidotic, and 2-day-recovered rats (Fig. 5A) and BBMV from control and acidotic rats (Fig. 5B). The molecular mass of SN1, predicted from the cDNA, is 56 kDa. Under control conditions, no SN1 protein is detected in BLMV or BBMV. However, these membranes in acidotic rats contain high levels of transporter protein. After 2 days of recovery from acidosis, SN1 protein is barely detectable in BLMV membranes. As noted above, SN2, a cDNA closely related to SN1, has recently been described (22). The antibody used for the Western blot analyses shown in Fig. 5 was raised against the NH₂ terminus of SN1 (6) in which the amino acid sequences of SN1 and SN2 differ completely (22).

Renal cortical BLMV and BBMV contain system ASC transport activity. To determine what transport system(s) is responsible for “normal” glutamine transport, we also measured transport in the presence of 5 mM MeAIB (transported by system A) and 5 mM serine (transported by system ASC) (Fig. 6). The inhibition profiles for BLMV (Fig. 6A) and BBMV (Fig. 6B) are quite similar. The failure of MeAIB to inhibit glutamine uptake indicates that system A is not a major basolateral membrane carrier of glutamine in the renal cortex. Figure 6B suggests that it may play a role in the brush border during acidosis but not under control conditions. This is consistent with in situ hybridization data (30) showing that ATA2 mRNA is not expressed in the renal cortex of normal rats. Serine reduced transport in the BLMV and BBMV of both control and acidotic animals. Inhibition by cysteine, also transported by system ASC, was similar to serine (not shown). These results suggest that system ASC is, at least in part, responsible for cortical glutamine transport under normal conditions. The combination of serine and histidine, as expected, reduces normal transport and eliminates the difference between control and acidic membrane vesicles (Fig. 6).

**DISCUSSION**

Our laboratory has been studying the regulation of the altered glutamine metabolism that is characteristic of pathophysiological states for years (20, 25). The central role of glutamine metabolism in the coordinated response to acid loading has been the subject of much research. During chronic metabolic acidosis, renal glutamine extraction increases from <3% to up to 35% (Ref. 36 and this study), greatly exceeding the extraction of any other amino acid (34, 36). However,
The outer stripe consists predominantly of straight proximal tubules, the thick ascending limb of distal tubules, and collecting ducts. The bulk of filtered glutamine is reabsorbed by epithelial cells lining the proximal convoluted tubule (35) so that filtrate entering the straight segment of the proximal tubule is depleted of glutamine. The pattern of SN1 expression in control animals may reflect the means of supplying this region of the kidney with glutamine for normal cell function. The deep cortex and outer stripe are also the site of glutamine synthase expression (32), so in this region, SN1 may function to export newly synthesized glutamine. The inner regions of the kidney in which SN1 is not expressed may be served by system A, which is expressed in the renal medulla (30).

Na⁺-dependent glutamine transport was increased approximately threefold in BLMV from acidotic animals compared with controls and 50% in BBMV. Elimination of the acidosis-induced increase in transport activity in both BLMV and BBMV by histidine suggests that SN1 is the induced transporter. This conclusion is supported by the detection of SN1 protein in BLMV and BBMV that exhibit induced Na⁺-dependent uptake activity and the failure to detect SN1 in BLMV and BBMV lacking the induced activity (control and recovered animals). Two earlier studies that examined the effect of chronic acidosis on renal basolateral and brush-border glutamine uptake had differing results (12, 40). Windus et al. (40) observed a twofold increase in Na⁺-dependent uptake of glutamine into BLMV isolated from renal cortex of chronically acidotic dogs compared with BLMV from control dogs but found no difference in transport into BBMV. On the other hand, Foreman et al. (12) found no difference in glutamine transport in renal BLMV of acidic and control rats but observed an increase in transport into acidotic BBMV. In the latter study, BLMV were isolated from whole kidney.

Expression of SN1 mRNA is rapidly induced in rat kidney after exposure to an acid load, increasing fourfold within 24 h and 10-fold after 7 days. Induction of SN1 expression and transport activity was rapidly reversed on withdrawal of NH₄Cl and return to tap water. A similar pattern of response to acidosis has been established for a variety of physiological parameters and enzyme activities (7, 26). The rapid alterations in SN1 expression parallel those of other changes that make up the coordinated homeostatic response to disturbed acid-base balance. Activity of other transporters that facilitate adaptation to an acid load is elevated in proximal tubules during chronic acidosis. These include the apical Na⁺/H⁺ exchanger NHE3 (42) and the basolateral Na⁺−HCO₃⁻ cotransporter (29), promoting both removal of H⁺ from cell to tubular lumen and translocation of reabsorbed and newly synthesized HCO₃⁻ ions to the venous blood. Other alterations include rapid induction of the expression and activity of enzymes involved in glutamine utilization. These include mitochondrial glutaminase (phosphate-dependent glutaminase (PDG)) (8), GDH (32, 41), and phosphoenolpyruvate carboxykinase (4), although it has been appreciated that increased glutamine transport into renal tubule cells is a necessary feature of the homeostatic response to acidosis (38), few studies have specifically examined the effect of acidosis on renal membrane transport of glutamine (12, 15, 21, 40).

Three Na⁺-dependent amino acid transport systems transport glutamine across cell membranes. These are system A, system ASC (ATB0), and system N and were originally described on the basis of functional transport criteria. In recent years, cDNAs corresponding to these activities have been cloned and their tissue distribution determined (3, 6, 11, 14, 30, 39). System A (ATA2) (30), system ASC (ASCT2) (39), and system N (SN1) (6) mRNA are all expressed in rat kidney. In the studies reported here, we examine the expression of the SN1 amino acid transporter in the rat kidney and demonstrate its role in the renal homeostatic response to chronic metabolic acidosis.

In this study, the level of SN1 mRNA in whole kidney increased ~10-fold in response to acidosis and in situ hybridization, and Northern blot analysis of dissected kidney regions identified the renal cortex as the specific site of increased SN1 expression. Hybridization occurred specifically in the epithelial cells of the proximal convoluted tubules that make up ~80% of cortical cells (27). In control animals, SN1 mRNA is confined to the outer stripe and medullary rays that are projections of the outer stripe into the renal cortex.

![Fig. 6. Inhibition profiles of glutamine uptake in cortical BLMV and BBMV from control and acidic rats. Na⁺-dependent glutamine uptake was measured in BLMV (A) and BBMV (B) in the presence of no inhibitor (N), MeAIB (M), serine (S), or serine plus histidine (S+H) (all at 5 mM). Three BLMV and BBMV preparations are shown in each group (except for 2 S+H in BLMV). No significant differences were detected within BLMV groups: P = 0.091, control; P = 0.100, acidosis. For BBMV, *P < 0.05 vs. no inhibitor for each group (Duncan’s multiple range test).](http://ajprenal.physiology.org/)

---

**AJP-Renal Physiol • VOL 283 • NOVEMBER 2002 • www.ajprenal.org**
enzymes involved in ammoniagenesis and oxidation of α-ketoglutarate with formation of HCO₃⁻ ions. As we report for SN1, induction of these enzymes occurs exclusively in the proximal convoluted tubule. The specific mechanisms leading to increased activity of these enzymes vary. For example, PDG and GDH mRNA are stabilized in response to increased intracellular H⁺ concentration via binding of δ-crystallin/reduced NADP:quinone reductase to a pH-response element (an 8-base AU sequence) present in the 3'-untranslated region (UTR) (7). In contrast, transcription of the phosphoenolpyruvate carboxykinase gene is increased (18). The rapid increase in SN1 mRNA and protein level suggests that a pretranslational mechanism is involved in acidosis-induced augmented glutamine transport activity. Analysis of the nucleotide sequence of the 3'-UTR of SN1 shows that sequences corresponding to the pH response element in the 3'-UTR of PDG and GDH (7) are not present. However, further studies are required to determine whether mRNA stabilization and/or transcription activation causes the observed elevated SN1 mRNA concentration. Other regulatory mechanisms may also be involved.

We have demonstrated that chronic metabolic acidosis induces the expression of the system N transporter SN1 in the epithelia of cortical convoluted proximal tubules and that the transporter is present in both basolateral and brush-border membranes of these cells. Associated with the presence of the transporter protein is increased Na⁺-dependent glutamine transport. Expression of SN1 in cultured cells (6) and X. laevis oocytes (11, 14) has allowed functional characterization of SN1-mediated glutamine uptake. The SN1 transporter is an antipporter that exchanges one proton for one glutamine molecule and one Na⁺ ion (2). The direction of exchange is reversible so that SN1 can mediate glutamine influx or efflux depending on the gradients for H⁺ or Na⁺ ions. These characteristics have implications for the activity of SN1 during acidosis. If proximal tubule intracellular pH is lower than extracellular pH, as reported by Alpern and Chambers (1), glutamine uptake from both the blood and the glomerular filtrate will be facilitated. Under normal conditions, >95% of filtered glutamine is reabsorbed across the proximal tubule cell brush border (35) so that total glutamine uptake via this route has little room for increase during acidosis, despite the potential for increased uptake provided by additional SN1 transporters. However, during acidosis, glutamine reabsorbed by SN1 transporters in the brush border is accompanied by proton secretion into the urine. Early studies of renal glutamine uptake and utilization conclude that increased uptake from the blood across the basolateral membrane causes the markedly elevated glutamine extraction observed during metabolic acidosis (36). The antipporter activity of SN1 also dictates the secretion of a proton to the blood for each glutamine molecule taken up across the basolateral membrane. This action would seem to decrease the efficiency of removal of protons from the body, although the net effect is to remove H⁺ because two ammonium ions are produced from each glutamine molecule metabolized and two HCO₃⁻ ions are added to the blood. In the aggregate, the presence of SN1 transporters in both basolateral and brush-border membranes of proximal tubule epithelia during acidosis contributes to maintenance of acid/base homeostasis.

Parry and Brosnan (26) studied glutamine metabolism in the kidney during induction of and recovery from chronic NH₄Cl acidosis in rats. Acid-base status returned toward normal after 2 days of exposure to NH₄Cl when ammonia excretion had increased to a maximal level. The elevated rate of ammonia excretion was maintained, along with normal acid-base status, until the rats were returned to drinking tap water. Within 1 day of recovery from acidosis, plasma HCO₃⁻ concentration increased, plasma pH increased, and ammonia excretion and renal glutamine extraction returned to control levels (26). This study illustrates the adaptation to chronic acidosis made possible by increased renal ammoniagenesis. Increased expression of the SN1 glutamine transporter plays an essential adaptive role by supplying additional glutamine, the critical substrate for increased ammoniagenesis.

The authors are grateful to Dr. Steven W. Levison for assistance in capturing the in situ hybridization images and to Dr. Christopher S. Hollenbeak for assistance with the statistical analysis. This research was supported by National Heart, Lung, and Blood Institute Grant HL-44986 (W. W. Souba).

REFERENCES

11. Fei YJ, Sugawara M, Nakasaji T, Wang H, Prasad PD, Leibach FH, and Ganapathy V. Primary structure, genomic organization, and functional and electrogenic characteristics of SN1 TRANSPORTER REGULATION IN KIDNEY DURING ACIDOSIS

AJP-Renal Physiol • VOL 283 • NOVEMBER 2002 • www.ajprenal.org


