Glucocorticoids have a role in renal cortical expression of the SNAT3 glutamine transporter during chronic metabolic acidosis

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KARINCH AM, LIN C-M, MENG QH, PAN M, SOUBA WW. Glucocorticoids have a role in renal cortical expression of the SNAT3 glutamine transporter during chronic metabolic acidosis. Am J Physiol Renal Physiol 292: F448–F455, 2007. First published September 5, 2006; doi:10.1152/ajprenal.00168.2006.—Glucocorticoids are involved in many aspects of regulation of acid-base homeostasis, including the stimulation of renal ammoniagenesis during chronic metabolic acidosis. Plasma glutamine is the principal substrate for ammoniagenesis under these conditions. Expression of the System N glutamine transporter SNAT3 is increased in the renal proximal tubules during acidosis. In vivo studies in rats using 1) sham and adrenalectomized rats, 2) the glucocorticoid receptor antagonist RU486, and 3) dexamethasone treatment demonstrated involvement of glucocorticoids in regulation of SNAT3 expression. Adrenalectomy attenuated the acidosis-induced increase in renal cortical SNAT3 mRNA ~40%, and treatment with dexamethasone (1 mg·kg−1·day−1 sc) partially reversed this effect. RU486 also blunted the acidosis-induced increase in SNAT3 expression ~50%. Chronic dexamethasone treatment (0.1 mg·kg−1·day−1 sc, 6 days) of normal rats slightly increased SNAT3 expression. In all cases, renal glutamine arteriovenous difference mirrored SNAT3 expression and activity in the proximal tubules, suggesting that SNAT3 regulates glutamine uptake during acidosis. These studies indicate that glucocorticoids regulate acid-base homeostasis during metabolic acidosis in part by regulating expression of the System N transporter SNAT3.

dexamethasone; System N expression; ammonium chloride acidosis; RU486

THE KIDNEY PLAYS A VITAL ROLE in maintaining acid-base homeostasis during chronic metabolic acidosis. Under acidic conditions, a number of adaptive changes occur throughout the kidney, acting in concert to reduce the acid load and restore acid-base balance. The renal proximal convoluted tubule is the site of remarkable coordinated changes that result in increased ammoniagenesis that is sustained for the duration of acidosis (16, 30). These changes include enhanced uptake and metabolism of glutamine, the principal substrate for renal ammoniagenesis during acidosis (39). Asymmetric secretion from the proximal tubule of ammonium ions into the urine and bicarbonate ions into the blood functions to return acid-base balance toward normal. Ammonium and bicarbonate ions are both products of glutamine metabolism, a single molecule of glutamine producing two ammonium and two bicarbonate ions. Under normal conditions, the kidney releases low levels of glutamine into the blood whereas during acidosis >40% of the glutamine presented to the kidney in the blood is extracted (36, 38), so that the kidney becomes the body’s principal consumer of glutamine.

Studies using adrenalectomized (ADX) rats suggest that glucocorticoids are involved in acid-base homeostasis during metabolic acidosis. This observation is consistent with elevated plasma corticosterone concentration during acidosis in rats (28, 42). ADX rats that are challenged with an acid load have a reduced ability to increase renal ammoniagenesis (33, 46). During metabolic acidosis, the kidneys of these animals cannot increase glutamine uptake from the blood or increase glutamine metabolism to the same degree as adrenal-intact rats (46). Glucocorticoid treatment of ADX rats before induction of acidosis partially restores their ability to extract glutamine from the blood (46). Glucocorticoids also increase ammonia excretion in the intact animal (19) and ammonia production by perfused kidneys (44). These studies suggest that glucocorticoids play a direct or indirect role in regulation of acidosis-induced glutamine metabolism.

We reported that the acidosis-induced increase in renal glutamine uptake is accomplished by increased expression of the System N transporter in the proximal convoluted tubule of acidic rats (23). This observation was recently confirmed by others (37). The System N transporter, previously known as SN1, has been renamed SNAT3 in recognition of its identity as a member of the SCL38 gene family of sodium-coupled neutral amino acid transporters (25). SNAT3 is located in the basolateral membrane of proximal tubule epithelial cells (37) and transports one glutamine molecule and one sodium ion in exchange for one proton (7, 10). The transporter mediates glutamine flux in both directions, depending on substrate gradients (7) and is associated with an uncoupled proton conductance that can serve to minimize the proton flux produced by a transport-coupled proton antiport (7, 10).

We hypothesized that glucocorticoids enhance ammoniagenesis during metabolic acidosis in part by regulation of expression of the SNAT3 transporter. We studied regulation of renal cortical SNAT3 expression by glucocorticoids during chronic metabolic acidosis in vivo using normal and ADX rats and the glucocorticoid receptor antagonist RU486. Data from our studies suggest that glucocorticoids maintain acid-base homeostasis, in part, by altering renal glutamine uptake via regulation of expression of the SNAT3 transporter.

MATERIALS AND METHODS

Animals. Animals were treated in accordance with regulations of the Institutional Animal Use and Care Committee. Male Sprague-
Dawley rats (Charles River, Wilmington, MA), weighing 250–300 g, 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 6 days. ADX (bilateral) rats and sham-operated controls purchased from Charles River were used for some experiments. Both sham and ADX rats were maintained on 0.9% saline substituted for their drinking water before experimentation. We followed a modification of the protocol of Welbourne and colleagues (46) that adjusts the NH₄Cl concentration because ADX rats drink approximately twice the volume that intact rats drink. ADX rats were given 0.6% NH₄Cl in saline, and sham acidoic rats were given 1.2% NH₄Cl in saline so that they consumed approximately equal acid loads. ADX rats were exposed to acidosis for only 2 days because of significant mortality in acidoic ADX rats after 3 days. For some experiments, normal rats were treated acutely (5 mg/kg sc, once) or chronically (100 μg/rat sc, once a day for up to 6 days) with dexamethasone. The body weight of control rats increased steadily during the experimental period (259 ± 4 g at day 0, 295 ± 4 g at day 6), whereas the body weight of chronically treated rats decreased for the first 3 days and then stabilized (255 ± 3 at day 0, 240 ± 4 g at day 6).

Rats were killed after anesthesia with ketamine/acepromazine/xylazine (80:1:12 mg/kg body wt). The kidneys were removed, and the renal cortex was immediately dissected out and processed for RNA isolation or membrane preparation. 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 PCO₂ were measured using an IL BG3 blood-gas machine (model 1420, Instrumentation Laboratory, Lexington, MA). Blood HCO₃⁻ concentration was automatically calculated from the measured pH and PCO₂. Plasma glutamine levels were measured in triplicate by a modified spectrophotometric assay using a colorimetric assay kit (Boeringer Mannheim, Mannheim, Germany) adapted to a 96-well plate format with a microplate spectrophotometer. Glutamine arteriovenous (AV) difference was calculated as (arterial − renal vein) plasma glutamine concentration.

**Kidney dissection.**

Kidney dissection was carried out under approximately 1.5–2 mm thick) was first cut from the curved outer surface. Additional cortex was dissected using 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.

**Northern blot analysis.**

Total RNA was isolated from the renal cortex using the Totally RNA system (Ambion, Austin, TX) or from cultured cells using an RNeasy Mini kit (Qiagen, Valencia, CA). RNA was separated on a 1% formaldehyde gel, transferred to nylon membrane (Genescreen, New England Nuclear), and hybridized with a SNAT3-specific oligonucleotide probe. The SNAT3 oligonucleotide sequence was 5'-GTGCAGAAGGCTTCAGCAGTGTCAGGTTGG-3'. The oligonucleotide was radioactively 3'-end labeled using terminal transferase and α-[³²P]dATP. For quantification of SNAT3 mRNA, autoradiographs were scanned using a laser densitometer (Dynamic Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were both found to be unsuitable for RNA loading normalization because acidosis increased mRNA levels of both. The 18S ribosomal RNA subunit was therefore used for normalization.

**Isolation of cortical basolateral membrane vesicles.** Basolateral membrane vesicles (BLMV) were isolated from renal cortex by Percoll density gradient centrifugation as previously described (23). Vesicles were resuspended in intravesicular buffer [100 mM KCl, 100 mM mannitol, 12 mM Tris/HEPES, pH 7.5, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 2 μl/ml protease cocktail stock (Sigma P2714, Sigma, St. Louis, MO) to a concentration of ~1 mg/ml]. Protein was measured using the Bradford assay with BSA as a standard. BLMV were prepared at the same time from all groups to be compared in a specific experiment to minimize variation among the preparations. Renal cortices from two to five animals were pooled for each BLMV preparation. Vesicle relative enrichment was estimated using ouabain-inhibitable ATPase activity in BLMV compared with the homogenate (14). The relative enrichment did not vary significantly among preparations for the RU486 experiments (control 16 ± 2, acidosis 27 ± 9, acidosis+RU486 18 ± 3-fold increase over homogenate; P = 0.3, ANOVA) or the chronic dexamethasone experiments (control 17 ± 3, dexamethasone 14 ± 3-fold increase over homogenate; P = 0.5, t-test). Vesicles frozen in liquid nitrogen were used for glutamine transport assays.

**Glutamine transport.**

For transport studies in renal cortical BLMV, Na⁺−dependent glutamine uptake was measured at room temperature using a rapid mixing/filteration technique described previously (23). Basolateral glutamine transport was measured in cells grown to tight contact on Transwell plates. Following treatment, transport was measured in choline chloride or NaCl uptake buffer (in mM: 137 NaCl or choline Cl, 10 HEPES/Tris, pH 7.4, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.5 CaCl₂) containing [³H]glutamine (4 μCi/ml) and 50 μM unlabeled l-glutamine. Uptake was terminated after 5 min by washing with ice-cold NaCl uptake buffer without [³H]glutamine. Part of the cell lysate was counted in a TopCount scintillation spectrophotometer (Packard, Meriden, CT), and part was used for protein determination. The rate of glutamine transport was linear at 5 min and expressed as nanomoles per milligram protein per minute. Where indicated, 5 mM histidine was added to the uptake buffer to allow identification of SNAT3-mediated transport in BLMV and GR101 cells.

**Western blot analysis.**

Equal amounts of cortical BLMV (10 μg) were separated by SDS-PAGE on precast polyacrylamide gels (ISC BioExpress, Kaysville, UT) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Blots were incubated with a polyclonal antibody raised against a SNAT3-GST fusion protein containing the NH₂-terminal 71 amino acids of SNAT3 followed by a horseradish peroxidase-conjugated goat anti-rabbit antibody (Rockland Immunochemicals, Gilbertsville, PA). SNAT3 protein was detected by enhanced chemiluminescence (Upstate, Lake Placid, NY) following the manufacturer’s instructions.

**Cloning of the rat SNAT3 promoter.** A rat genomic library (Clontech, Mountain View, CA) was screened using a SNAT3-specific probe. One positive plaque was identified and isolated by limiting dilution. The purified plasmid was analyzed for an insert of ~12 kb, all of which represent a genomic sequence from the SNAT3 gene, based on comparison with the published rat genomic sequence (GenBank NW_047801.1). A 3.7-kb XhoI restriction fragment of the rat genomic clone that contains ~2.4 kb of the 5'-flanking sequence, exon 1 ( untranslated) and ~1.3 kb of intron 1 was subcloned into Bluescript and sequenced (Molecular Genetics Core Facility at the Pennsylvania State University College of Medicine). The Transcription Factor Search program TFSearch (Yutaka Akiyama: “TFSEARCH: Searching Transcription Factor Binding Sites”; http://www.rwcp.or.jp/papui/) was used to identify potential transcription factor binding sites in the 5'-flanking region. A series of SNAT3 promoter fragments (5'-boundaries −2,414 − 815, −132, −78 with a common 3’ boundary at +20 with respect to the published rat SNAT3 cDNA sequence, GenBank AF273025) was amplified by PCR using the cloned phage DNA as a template and cloned into the multiple cloning site of the pCAT3-Basic reporter vector (Promega, Madison, WI). Reporter constructs were verified by sequencing by the Molecular Genetics Core Facility.

**Cell culture.** LLC-PK1-GR101 (GR101) cells are porcine epithelial-like LLC-PK₁ cells that express rat glucocorticoid receptors (41). GR101 were grown in low-glucose DMEM (Invitrogen, Carlsbad, CA) containing 800 ng/ml hygromycin B (Calbiochem, La Jolla, CA) to maintain glucocorticoid receptor expression. These cells were generously provided by Dr. S. R. Price of Emory University.
Transient transfection. GR101 cells were transiently transfected using Lipofectin (Invitrogen) following the manufacturer’s protocol. After cotransfection with pCAT.SNAT3 constructs (1 μg) and pSV40-β-Gal (1 μg, Promega), cells were treated with control or dexamethasone (0.01 or 0.1 μM) medium and harvested after 48 h. The dexamethasone-responsive plasmid pGREtkCAT was used as a positive control for glucocorticoid responsiveness. This plasmid contains two inverted repeats of a glucocorticoid response element sequence followed by the thymidine kinase promoter that drives expression of the chloramphenicol acetyl transferase (CAT) gene. pGREtkCAT was provided by Dr. S. S. Simons, Jr., of National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. CAT and β-galactosidase assays were carried out as described in the Promega protocols.

Statistics. Data were analyzed using a t-test, paired t-test, or one-way analysis of variance followed by Bonferroni or Dunnett posttests for multiple comparisons, as appropriate.

RESULTS

ADX rats are less able than sham-operated rats to maintain acid-base homeostasis during chronic metabolic acidosis. Five groups of rats were studied: 1) sham-operated, 2) ADX, 3) 2-day acidic sham-operated, 4) 2-day acidic ADX, and 5) 2-day acidic ADX pretreated for 2 days and throughout acidosis with the synthetic glucocorticoid dexamethasone (1 mg·kg⁻¹·day⁻¹·sc). Table 1 shows the arterial (systemic) pH and bicarbonate concentration and renal glutamine AV difference in each group of rats. The effect of adrenalectomy on the acid-base status and renal glutamine AV difference of normal and acidic rats was similar to that previously reported (11, 30, 46). After 2 days of ingesting ammonium chloride, both sham and ADX rats became acidic, but ADX rats were more severely acidic than sham rats. Chronic acidosis also increased glutamine AV difference in both sham and ADX rats, but the increase was significantly attenuated in ADX rats. Dexamethasone treatment improved the ability of acidic ADX rats to compensate for the acid load, as indicated by increased blood pH and serum bicarbonate concentration. In addition, glutamine AV difference in these rats reverted toward the level in acidic sham rats.

Plasma glutamine concentration is altered by adrenalectomy, metabolic acidosis, and dexamethasone. Figure 1 shows the arterial plasma glutamine concentration of all animal groups in the studies presented in Figs. 1-3. Acidosis decreases (Fig. 1, A and B) and dexamethasone increases (Fig. 1, A and C) plasma glutamine concentration. The decline in plasma glutamine during acidosis is principally the result of greatly enhanced renal glutamine utilization for ammoniagenesis. Plasma glutamine increases in animals treated with dexamethasone, reflecting increased glutamine synthetase (GS) activity in muscle and lung (1, 27) and increased proteolysis, principally in muscle (17). Treatment of acidic rats with the glucocorticoid receptor antagonist RU486 did not alter systemic glutamine concentration (Fig. 1B).

Adrenalectomy attenuates the acidosis-induced increase in renal cortical SNAT3 mRNA and decreases renal glutamine AV difference. Northern blot analysis was used to measure the relative levels of renal cortical SNAT3 mRNA in the same five groups as above and in ADX rats treated with dexamethasone (Fig. 2A). Metabolic acidosis significantly increased SNAT3 mRNA in both sham and ADX rats compared with sham and ADX rats not exposed to acidosis (P < 0.001 for both). However, adrenalectomy blunted the acidosis-induced increase in SNAT3 mRNA (sham + acidosis 20.6 ± 4.1 vs. ADX + acidosis 11.9 ± 1.4 arbitrary units, P < 0.05) and dexamethasone partially reversed the effect of adrenalectomy (15.9 ± 3.4 arbitrary units). Glutamine AV difference in these groups followed a similar pattern of response (Fig. 1B).

Table 1. Selected blood parameters from sham-operated and adrenalectomized rats

<table>
<thead>
<tr>
<th>Blood pH</th>
<th>Bicarbonate, mM</th>
<th>Renal Glutamine AV Difference, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>7.38 ± 0.01</td>
<td>25.0 ± 0.5</td>
</tr>
<tr>
<td>ADX</td>
<td>7.29 ± 0.02</td>
<td>23.2 ± 0.6</td>
</tr>
<tr>
<td>Sham + acidosis</td>
<td>7.22 ± 0.02</td>
<td>16.9 ± 0.4</td>
</tr>
<tr>
<td>ADX + acidosis</td>
<td>7.17 ± 0.02</td>
<td>14.9 ± 0.5</td>
</tr>
<tr>
<td>ADX + acidosis + Dex</td>
<td>7.30 ± 0.02</td>
<td>20.4 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12–27 animals/group. ADX, adrenalectomized. Animals were treated as described in MATERIALS AND METHODS. Dexamethasone (Dex) concentration was 1 mg·kg⁻¹·day⁻¹. pH and bicarbonate values are from arterial blood. Glutamine renal arteriovenous (AV) difference is (arterial – renal vein) plasma glutamine concentration. *P ≤ 0.001 vs. sham. **P ≤ 0.05 vs. sham + acidosis. ***P ≤ 0.001 vs. sham + acidosis. ****P ≤ 0.001 vs. ADX + acidosis. *P ≤ 0.01 vs. ADX + acidosis (ANOVA).
Dexamethasone partially reversed the repressive effect of adrenalectomy (0.11 ± 0.02 mmol/l). There was a trend for dexamethasone to increase SNAT3 and glutamine AV difference in ADX rats, although the increase did not reach statistical significance. We reported that, in normal rats, increased renal glutamine uptake during chronic metabolic acidosis rats is associated with increased expression of the System N transporter SNAT3 in renal proximal tubules (23). Figure 2 suggests that this association also occurs in ADX rats. We have used renal AV difference (arterial − renal venous glucose plasma concentration) as a broad indicator of glutamine uptake, while recognizing that this approach has limitations (see DISCUSSION).

**Inhibition of glucocorticoid activity depresses the acidosis-induced increase in cortical SNAT3 expression and renal glutamine AV difference.** The glucocorticoid antagonist RU486 was used to further explore a role for glucocorticoids in regulating renal cortical SNAT3 expression and renal glutamine uptake. Rats were exposed to metabolic acidosis for 6 days with or without administration of RU486 (12.5 mg/kg sc, twice/day). Control rats were injected with vehicle (ethanol). BLMV isolated from control, acidic, and RU486-treated acidotic rats were used to measure SNAT3-mediated glutamine transport and for Western blot analysis. Acidosis increased SNAT3 expression and glutamine transport in BLMV (Fig. 3, A–C). RU486 treatment significantly attenuated acidosis-induced renal cortical SNAT3 mRNA (acidosis 1.0 ± 0.08 vs. acidosis+RU486, 0.5 ± 0.1 arbitrary units, P < 0.05) (Fig. 3A), SNAT3 protein (acidosis 4.05 ± 0.75 vs. acidosis+RU486, 1.88 ± 0.19 arbitrary units, P < 0.05) (Fig. 3B), and SNAT3-dependent transport in BLMV (acidosis 409 ± 20 pmol·mg⁻¹·min⁻¹ vs. acidosis+RU486, 214 ± 74 pmol·mg⁻¹·min⁻¹, P < 0.05, repeated measures ANOVA) (Fig. 3C). Renal glutamine AV difference was blunted but not significantly decreased by RU486 (acidosis 0.24 ± 0.02 mmol/l vs. acidosis+RU486, 0.18 ± 0.03 mmol/l) (Fig. 3D). Treatment of normal rats with RU486 did not affect SNAT3 mRNA or glutamine AV difference (not shown).

**Chronic glucocorticoid treatment increases renal cortical SNAT3 expression.** To obtain more direct evidence of a role for glucocorticoids in SNAT3 expression in the renal cortex, we treated normal rats with dexamethasone, acutely (5 mg/kg sc for 1–6 h) or chronically (100 μg/day sc for 1–6 days). Acute treatment with dexamethasone did not increase renal cortical SNAT3 mRNA. In chronically treated animals, SNAT3 mRNA increased slowly to approximately twofold at day 3 (P ≤ 0.05) and remained at that level at day 6 (P ≤ 0.001) (Fig. 4A). SNAT3 protein and SNAT3-mediated glutamate transport exhibited a small but not statistically significantly increase (Fig. 4, B and C). Glutamine AV difference was modestly increased at day 6 (−0.02 ± 0.01 mmol/l control vs. 0.02 ± 0.01 mmol/l day 6, P = 0.03, t-test). Dexamethasone stimulation of renal GS activity in the dexamethasone-treated rats would tend to decrease apparent AV difference (see DISCUSSION), thus minimizing the apparent increase in glutamine uptake.

**Dexamethasone increases SNAT3 mRNA and SNAT3-mediated glutamine transport but does not activate the SNAT3 promoter in renal epithelial cells.** SNAT3 mRNA and SNAT3-mediated basolateral glutamine transport (control 214 ± 88 nmol·mg⁻¹·min⁻¹, dexamethasone, 558 ± 96 nmol·mg⁻¹·min⁻¹) are increased 50 (P = 0.01) and 250% (P = 0.01), respectively, by 0.01 μM dexamethasone treatment of glucocorticoid-responsive GR101 cells in culture (Fig. 5A). GR101 cells are porcine epithelial-like LLC-PK1 cells that express rat glucocorticoid receptors (41). To determine whether glucocorticoids increased SNAT3 expression via increased transcription of the SNAT3 gene, we cloned and sequenced 2.4 kb of the 5′-flanking region of the rat SNAT3 gene from a rat genomic library. Analysis of the 5′-flanking sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements. The promoter contains no TATA or CCAAT box sequences, but a GC-rich sequence using the TFSearch program identified a number of potential glucocorticoid response elements.

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tested (~2.4 kb) exhibited a 15-fold increase in basal activity, indicating that this 5'-flanking region of the SNAT3 promoter contains a functional promoter. The active promoter constructs did not, however, respond to dexamethasone treatment (Fig. 5B). The pCAT.SNAT3 vector containing only the proximal 78 bases of the flanking region was transcriptionally inactive, presumably because three Sp1 binding sites between 101 and 78 are absent from this construct. Glucocorticoid responsiveness of the GR101 cells was verified by transfection with the dexamethasone-responsive pGREtkCAT plasmid that contains two inverted glucocorticoid response elements followed by the thymidine kinase promoter. CAT activity of pGREtkCAT increased approximately fivefold in response to 100 nM dexamethasone. The SNAT3 promoter constructs used in these studies (except the inactive 78-bp construct) are activated by incubation in acidic medium. CAT activity of the constructs...
In our studies using sham-operated and ADX rats, renal cortical SNAT3 mRNA was increased by metabolic acidosis in sham animals. This response was attenuated in ADX animals, and treatment of ADX animals with dexamethasone partially reversed the effect of adrenalectomy (Fig. 1A), suggesting that glucocorticoids have a role in acidosis-induced activation of SNAT3 expression. The observation that the glucocorticoid receptor antagonist RU486 also blunts the acidosis-induced increase in SNAT3 expression (Fig. 2) provides additional evidence of glucocorticoid involvement. Dexamethasone also increased SNAT3 expression in the absence of acidosis, although the increase was much smaller than that induced by acidosis. In all the in vivo studies reported here, renal glutamine AV difference mirrored SNAT3 expression, reinforcing the suggestion that glutamine uptake and expression of SNAT3 in the renal cortex are linked. These results are consistent with previous observations that glucocorticoids play a role in acid-base homeostasis in part via regulation of renal glutamine utilization (33, 46).

We have used renal AV difference as a broad indicator of glutamine uptake. AV difference is a net measurement that is a function of arterial glutamine concentration, renal GS activity, and renal blood flow. 1) Figure 1 shows the arterial glutamine concentration in all experimental groups. 2) GS is present in the outer medulla of the kidney (35). GS activity, mRNA, and protein in the kidney are unchanged (8, 35, 47) by chronic acidosis so that the impact of renal glutamine synthesis on AV difference in acidotic rats is negligible. Glucocorticoids increase GS activity and gene expression in muscle and lung (1). In the kidney, glucocorticoids have no effect (1) or modestly increase (26, 43) GS activity. The effect of increased GS activity would be to increase the renal venous concentration of glutamine, thus decreasing the apparent glutamine uptake in those groups treated with dexamethasone. 3) Renal blood flow or glomerular filtration rate either is not altered (15, 24, 32) or is increased (45, 46) by metabolic acidosis. Welbourne and colleagues (45, 46) reported that renal plasma flow in ADX and acidic ADX rats decreased compared with normal animals and increased in acidic rats and in ADX rats supplemented with glucocorticoid. They studied experimental groups similar to ours and observed a pattern of renal glutamine AV differences in these groups the same as that shown in Fig. 2, except for their ADX + acidosis + glucocorticoid group, where the AV difference recovered to or above the sham acidosis level, whereas we observe only partial recovery. This difference may result from the use of different glucocorticoids and doses in our studies. In those studies, the pattern of glutamine extraction (renal plasma flow × AV difference) and AV difference for each group was the same, suggesting that, in these experimental conditions, AV difference broadly reflects glutamine extraction.

ADX animals are deficient in both glucocorticoids and mineralocorticoids. Both glucocorticoids and mineralocorticoids act in the kidney to increase elimination of protons in the urine of acidic animals, but their sites and mechanisms of action differ. Glucocorticoids increase acid excretion in the form of ammonia excretion in response to an acid load with little effect on urine acidification, whereas mineralocorticoids increase acid secretion and acidify the urine with a limited effect on ammoniagenesis (20, 48). Glucocorticoid receptors are enriched in the proximal tubule, whereas mineralocorticoid
receptors are enriched in more distal regions (12, 29, 34). The differential distribution of these receptors is consistent with the restriction of ammoniagenesis induction to the proximal tubules. In addition, other acidosis-regulated transporters demonstrate a role for glucocorticoids, but not mineralocorticoids, in the response to acidosis (2, 5, 13). For these reasons, we have focused on glucocorticoid regulation of SNAT3 expression in the studies reported here. However, a role for mineralocorticoids is not excluded.

The studies presented here provide some insight into the mechanism(s) by which glucocorticoids regulate SNAT3 expression. The effects of H⁺ and glucocorticoids cannot be separated in normal rats in vivo because the plasma concentration of glucocorticoids increases in response to acidosis (28, 42). Experiments using ADX rats provide insight into the relative roles of glucocorticoids and H⁺ in regulating cortical SNAT3 expression. A comparison of Figs. 2A, 3A, and 4A shows that expression of SNAT3 mRNA is more strongly induced by H⁺ than by dexamethasone in both ADX and adrenal-intact rats. Figure 2A demonstrates that the role of glucocorticoids in regulating SNAT3 expression is not permissive because SNAT3 mRNA is induced by acidosis in ADX rats that lack endogenous glucocorticoids. The data in Fig. 2A also suggest that, for SNAT3 mRNA, the inductive effects of H⁺ and glucocorticoids may be additive. There is no evidence of synergistic interaction between H⁺ and glucocorticoids in the induction of SNAT3 expression.

The ability of RU486 to partially block the stimulation of SNAT3 expression by acidosis suggests the involvement of the glucocorticoid receptor in the regulation of SNAT3 gene transcription. Dexamethasone treatment increased SNAT3 mRNA in control, ADX, and acidic ADX rats (Figs. 2A and 4A), although to a lesser degree than chronic acidosis. The 5'-flanking region of the SNAT3 gene contains several potential glucocorticoid response elements that could be involved in glucocorticoid activation of SNAT3 transcription. However, transcriptionally active reporter expression vectors containing up to 2.4 kb of the SNAT3 5'-flanking sequence did not respond to dexamethasone treatment when transiently transfected into glucocorticoid-responsive GR101 cells (Fig. 5B). The lack of response suggests that glucocorticoids do not regulate SNAT3 directly by binding of the activated glucocorticoid receptor to a response element within the promoter, but indirectly perhaps by interaction with or induction of an unknown factor or by message stabilization. It is also possible that an essential glucocorticoid-regulatory element lies outside the cloned 2.4 kb of promoter used for transfection.

In addition to SNAT3, expression of a number of enzymes and transporters important in the response to acid-base imbalance is regulated in the kidney by both H⁺ and glucocorticoids (3, 4, 21, 31). In some cases, for example, the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and the Na⁺/H⁺ antiporter NHE3, the mechanism of glucocorticoid action has been elucidated in considerable detail and has proven to be very complex. Both acidosis and glucocorticoids regulate phosphoenolpyruvate carboxykinase transcription through complex interactions of nuclear factors with two independent domains in the promoter (9). Regulation of NHE3 involves both transcription and transcription-independent mechanisms (trafficking, phosphorylation) that differ in acute and chronic acidosis (6, 22, 40). Regulatory mechanisms for other acidosis- and glucocorticoid-regulated transporters have yet to be elucidated.

The studies reported here demonstrate that glucocorticoids, in addition to H⁺, directly or indirectly regulate expression of the SNAT3 transporter during chronic metabolic acidosis. In addition, the close association between SNAT3 expression and renal glutamine uptake as reflected by AV difference under a number of experimental conditions makes a strong argument for a central role for SNAT3 in the adaptive ammoniagenic response to acidosis. When rats are chronically exposed to metabolic acidosis, a series of compensatory adaptations occur in the kidney so that blood pH and bicarbonate concentration are restored to normal values within 7 days despite continued acid ingestion (30). Ammoniagenesis, however, remains elevated despite the normalization of acid-base status, suggesting that factors other than H⁺ are responsible for maintenance of activation of adaptive processes, including SNAT3 upregulation and renal glutamine uptake. Blood glucocorticoids, in contrast, remain elevated during acidosis (28, 42). Because of their widespread actions, glucocorticoids may be important in orchestrating the systemic and renal response to metabolic acidosis and in maintaining activation of key homeostatic activities.

REFERENCES

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