Inhibition of IMCD 11β-Hydroxysteroid Dehydrogenase Type 2 by Low pH and Acute Acid Loading

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Abstract. Mineralocorticoid receptors in the inner medullary collecting duct (IMCD) are protected from glucocorticoid binding by an enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). To study the role of 11β-HSD2 in acid-base homeostasis, 11β-HSD2 activity was measured in rat IMCD-enriched cell suspensions. Homogenates of cell suspensions were incubated in buffers ranging in pH from 6.00 to 8.15 in the presence of 1 μCi of 3H-corticosterone (CS) and 400 μM NAD+. Enzyme activity was expressed as the amount of 3H-CS converted to 3H-11-dehydrocorticosterone (DHCS). IMCD 11β-HSD2 activity at pH 6.5 was 49% of activity at pH 7.5; 22.5 versus 11.0 fmol/μg of protein per h. Experiments also were performed on intact cell suspensions at pH 7.5 and 6.5. There was a 42% inhibition in the IMCD cell suspension conversion rate of 3H-CS to 3H-11-DHCS at pH 6.5; 13.1 versus 7.6 fmol/μg per h (P < 0.005). In cell suspensions at pH 7.5, 1-day acid loading caused a 26% inhibition in conversion rate, 13.2 versus 9.9 fmol/μg per h (P < 0.05), when compared with controls. These results suggest that during acute metabolic acidosis, IMCD 11β-HSD2 is inhibited and may allow access to the mineralocorticoid receptors by glucocorticoids. (J Am Soc Nephrol 8: 530–534, 1997)

Adrenal steroids are known to play a role in maintaining acid-base homeostasis. Adrenalectomized (ADX) acid-loaded animals have a blunted net acid-excretion response (1). When acutely acid-loaded ADX rats were supplemented with glucocorticoid (GC) analogues, net acid excretion was completely restored, but when they were supplemented with mineralocorticoid (MC) analogues, net acid excretion was only 60% that of intact animals (2).

Glucocorticoids circulate in the blood at concentrations 50 to 400 times higher than that of mineralocorticoids (3). They do not normally bind to mineralocorticoid receptors (MR) in MC-sensitive tissues, even though they have high affinity for the MR because they are enzymatically inactivated (4,5). The inactivation occurs via 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which has been found to colocalize in tissues that have mineralocorticoid receptors (6,7). 11β-HSD2 oxidizes corticosterone (CS), the chief GC in rats, to 11-dehydrocorticosterone (11-DHCS) (4). 11-DHCS does not effectively bind the MR (4). There are two isoforms of 11β-HSD in the rat kidney. Type 1, found in proximal tubules, is bidirectional and utilizes NADP+/NADPH as a cofactor, and type 2, found in distal tubules and collecting ducts, is a unidirectional oxidizer and utilizes NAD+ as a cofactor (8,9).

Recently, Brown et al. found that the activity of 11β-HSD2 in placental homogenates was inhibited by low pH (9). During antidiuresis, inner medullary interstitial pH as low as 6.4 has been reported (10). Those facts led us to hypothesize that during acid loading, the activity of 11β-HSD2 may be inhibited, allowing GC to bind MC receptors and thus stimulate IMCD proton secretion. In response to an acid load, proton pumping in the IMCD is stimulated by aldosterone, contributing to increased NH₄⁺ excretion (11). The purpose of this study was to determine if 11β-HSD2 in inner medullary collecting ducts is inhibited at low pH and by acute acid loading.

Materials and Methods

Animals

Male Sprague-Dawley rats ranging in weight from 200 to 250 g were used in all experiments. In the first series of experiments to test the pH sensitivity of IMCD 11β-HSD2, cells were harvested from rats that were not pretreated. To test the effects of acute acid loading on 11β-HSD2 activity, rats were first acclimated for 2 days in metabolic cages, during which time they had free access to water and were fed a food slurry containing 15 g of finely ground rat chow to which 20 mL of an aqueous solution containing 7.2 mEq of NaCl was added. On the third day, acid-loaded rats were fed a solution containing 7.2 mEq of NH₄Cl instead of NaCl, and control rats were fed as during the first 2 days. Rats ate the food ration immediately after it was offered and continued to have free access to water. Rats were euthanized 24 h after their final meal.

IMCD Cell Preparation

Suspensions of IMCD cells were prepared using a previously described protocol. (12). Rats were decapitated, and their kidneys were removed. Each rat's two renal papillae were dissected, minced using a razor blade, and immediately placed in 5 mL of ice-cold bicarbonate buffer solution, pH 7.5, containing 0.2% collagenase (Boehringer Mannheim, Indianapolis, IN) and 0.2% hyaluronidase (Boehringer Mannheim). The bicarbonate buffer contained 118 mM...
NaCl, 5 mM KCl, 4 mM Na₂HPO₄, 24 mM NaHCO₃, 2 mM CaCl₂, 1.2 mM MgSO₄, 5.5 mM glucose, and 5 mM sodium acetate. To dissociate the cells, the tissue was incubated at 37°C and constantly gassed with 95% O₂/5% CO₂. Throughout the incubation, the cells were aspirated through a Pasteur pipette 10 to 12 times every 15 min until there were no visible cell clumps. DNAse (0.001%; Sigma Chemical Co., St. Louis, MO) was added after the first aspiration to cell suspension obtained as described above. Cell viability was assessed by trypan blue exclusion.

To separate IMCD cells from other medullary cells, the suspension was centrifuged three times at 16,000 g for 2 min, with the supernatant pipetted off and discarded after each centrifugation. The pellet was then resuspended in bicarbonate buffer solution plus 0.001% DNAse after each centrifugation, except the last. The supernatant of the last centrifugation was poured off, and the pellet was resuspended in either 1 mL of Krebs-Ringer buffer solution (10 mM glucose, 1.6 mM MgCl₂, 4.6 mM KCl, 83 mM NaHCO₃, 1.4 mM Na₂HPO₄, and 1.9 mM Na₂HPO₄, Sigma) for the homogenate studies or in 2 mL of bicarbonate buffer for the cell-suspension studies. IMCD cell enrichment was confirmed visually using interference and phase-contrast microscopy. A crude cell suspension, obtained by centrifuging the initial cell suspension at 175 g for 8 min was compared with a purified cell suspension obtained as described above. Cell viability was assessed by trypan blue exclusion.

Homogenates

To prepare homogenates, IMCD-enriched cell suspensions were ground in a Dounce tissue grinder. Protein concentration of both homogenates and cell suspensions were determined by Markwell assay (13).

Assay of 11β-HSD2 Activity

Homogenates. A 250-μL aliquot of homogenate was placed in Eppendorf tubes containing 750 μL of Krebs-Ringer buffer/steroid solution adjusted to pH ranging from 6.0 to 8.15. Included in each tube was 1 μCi of [1,2,6,7-N³H] CS (81 Ci/mmol; Amersham, Arlington Heights, IL), 400 μM NAD⁺ (Sigma), and 0.2% albumin (Sigma). Tubes were incubated at 37°C in a shaking water bath at 35 rpm for 4 h. Medium pH was determined at the beginning and at the end of the incubation.

Cell suspensions. Enriched IMCD cell suspensions were aliquoted into Eppendorf tubes. A 250-μL aliquot of cell suspension was placed into 750 μL of the bicarbonate buffer/steroid solution at pH 7.5 and 6.5. Included in each bicarbonate buffer was 1 μCi of [1,2,6,7-N³H] CS. Suspensions were incubated in a 37°C shaking water bath at 35 rpm for 4 h. The pH of the incubation solutions was measured at the beginning and at the end of the reaction. Controls for both homogenate and cell-suspension experiments were done by including all incubation components except the homogenate or cell-suspension aliquot.

Quantification of ³H-Corticosterone and ³H-11-Dehydrocorticosterone

Quantification was performed as described previously (14). Assays of enzyme activity in both homogenates and cell suspensions were stopped by adding 100 μL of trichloroacetic acid. The tubes were then centrifuged at 1500 g for 20 min to pellet tissue debris. The supernatant was collected and stored at −70°C until steroid extraction. Labeled CS and 11-DHCS were extracted from thawed supernatant by passing it through a Sep Pak column (Waters Associates, Milford, MA), eluting the steroid with methanol, and evaporating the eluent to dryness under N₂ gas. The extracted steroids were then redissolved in 100 μL ethanol and spotted on a silica-gel thin-layer chromatography plate (ultraviolet [UV], 254 nm; Beckmann, Palo Alto, CA) in parallel with unlabeled CS (10 μg; Sigma) and 11-DHCS (10 μg; Sigma). The chromatogram ran for 1 h in an air-tight glass chamber, with 100 mL of 92% chloroform and 8% ethanol (95% ethanol, 5% H₂O) solvent. The steroids were visualized using UV light and were scraped directly into vials, eluted with ethanol, extracted into 10 mL of biodegradable scintillation fluid (Amersham), and counted in a Packard Tri-Carb liquid scintillation counter (Hewlett Packard, Wilmington, DE) for 1 min. The activity of 11β-HSD2 was expressed as the amount of ³H-CS converted to ³H-11-DHCS in fmol/μg per h.

Statistical Analysis

Results are presented as means ± SE. Differences between groups were judged significant at P < 0.05 using the t test.

Results

Interference and phase-contrast microscopy demonstrated a greater than twofold purification from crude to purified cell suspension (Figure 1, A and B). Virtually the only cell type left
after the three low-speed spins was that comprising sheets of IMCD cells. Almost all individual cells were removed when the supernatant was poured off after each low-speed centrifugation. Trypan blue experiments demonstrated a greater than 98% viability before the incubation period and a greater than 95% cell viability for cell-suspension experiments after the incubation period. All controls for both homogenates and cell suspensions had less than 1% total conversion of 3H-CS to 3H-11-DHCS for all of the 4-h incubation periods ($N = 24$). The range of total conversion of 3H-CS to 3H-11-DHCS for the experimental conditions was 1 to 12% ($N = 52$).

**Effect of pH on IMCD 11β-HSD2 Activity**

11β-HSD2 activity, as measured by the conversion of 3H-CS to 3H-11-DHCS, was sharply inhibited at low pH (Figure 2). The pH of buffers did not change by more than 0.03 pH units before and after the metabolism assay. 11β-HSD2 activity ranged from 26.5 ± 2.96 fmol/μg per h at pH 8.15, to 7.3 ± 0.53 fmol/μg per h at pH 6.00 ($N = 4$ at each pH tested).

**Effect of pH on IMCD Cell Suspension Conversion Rate**

IMCD cell suspension conversion rate was significantly inhibited by a drop in buffer pH from 7.5 to 6.5 as measured by the conversion of 3H-CS to 3H-11-DHCS ($P < 0.005, N = 8$). Buffer pH values did not change by more than 0.03 pH U over the course of the metabolism assay. IMCD cell suspension conversion rate was 13.1 ± 1.06 fmol/μg per h at pH 7.5 and 7.6 ± 0.31 fmol/μg per h at pH 6.5 (Figure 3).

**Effect of Acute Acid Loading on IMCD Cell Suspension Conversion Rate**

IMCD cell suspension conversion of 3H-CS to 3H-11-DHCS, measured at buffer pH 7.5, was significantly inhibited ($P < 0.05, N = 8$) by 1-day acid loading (Figure 4). Buffer pH values did not change by more than 0.03 pH U over the period of the metabolism assay. IMCD cell suspension conversion rate was 13.2 ± 1.01 fmol/μg per h for controls and 9.9 ± 1.07 fmol/μg per h for 1-day acid-loaded animals.

**Discussion**

**Effect of Medium pH on IMCD 11β-HSD2 Activity**

A reduction in medium pH from 7.5 to 6.5 caused a greater than 51% inhibition of 11β-HSD2 activity in homogenates (Figure 2) and a 42% inhibition of corticosterone oxidation in cell suspensions (Figure 3). We believe that these results, especially the inhibition of 11β-HSD2 in cell suspensions, are physiologically relevant because IMCD cells are exposed to acidic surrounding fluids in vivo. Luminal pH in the IMCD drops below 6.0 during acid loading, and inner medullary interstitial fluid pH has the potential to fall to 6.4 (10). In our *in vitro* system, an extracellular pH of 6.5 caused a significant
reduction in 11β-HSD2 activity within only a few hours. If cells respond over a similar time course in vivo, GC binding to MR could increase rapidly.

It is interesting to note that both the homogenate and intact cell experiments show similar decreases in conversion rate of CS to 11-DHCS when medium pH was changed from 7.5 to 6.5. It is unlikely that ICF pH fell to 6.5 in the cell-suspension experiments, so it is reasonable to postulate that the homogenate and intact cell studies are inhibiting 11β-HSD2 through different mechanisms.

Effect of Acute Acid Loading on IMCD Cell Suspension Conversion Rate

Cell suspensions prepared from 1-day acid-loaded rats and assayed at pH 7.5 showed a 26% inhibition in conversion of $^3$H-CS to $^3$H-11-DHCS when compared with controls (Figure 4), demonstrating that 24-h acid loading had a significant inhibitory effect on conversion, exclusive of any effects of assay medium pH. In an in vivo situation, it is possible that the activity of IMCD 11β-HSD2 is also directly inhibited by low extracellular fluid pH. The mechanism by which IMCD cell suspension conversion rate was inhibited could be either by direct inhibition of 11β-HSD2 or by downregulation of the enzyme in IMCD cells because of systemic acidosis.

Role of the IMCD in Acute Metabolic Acidosis

Micropuncture and microcatheterization studies have shown that the role of the IMCD in net acid and NH$_4^+$ excretion differs during acute and chronic metabolic acidosis. During chronic metabolic acidosis, the IMCD accounts for only 12% of total NH$_4^+$ excreted and 16% of net acid excreted (15). However, during acute metabolic acidosis, the IMCD is particularly important, accounting for 36% of the total NH$_4^+$ excreted and 54% of net acid excreted (16). During the early stages of acute metabolic acidosis, the initial increase in NH$_4^+$ excretion results in part from increased proton pumping in the IMCD, leading to increased ammonia partitioning. Aldosterone increases proton secretion in the IMCD (2,11,17). The increase in ammonia partitioning is followed by an increase in proximal tubule ammoniagenesis. Messenger RNA coding for proximal tubule ammoniagenic enzymes have been found to increase 10 h after acid loading (18). The results presented here suggest that during the acute response to an acid load, glucocorticoids may have access to IMCD MR. In vivo studies in rats using 11β-HSD2 inhibitors have shown “mineralocorticoid-like” effects in ADX rats that were supplemented with corticosterone (19). These “mineralocorticoid-like” effects were inhibited by RU28318, an MR inhibitor (19).

The role of adrenal steroids in the renal response to acute metabolic acidosis has been investigated in two earlier studies. Wilcox et al. used ADX rats supplemented with aldosterone and dexamethasone, which were injected 1 h before the rats were acid-loaded by gavage with an NH$_4$Cl solution (2). They measured net acid excretion, NH$_4^+$ excretion, and urine pH for 4 h after gavage and found that supplementing acutely acid-loaded rats with dexamethasone returned net acid and NH$_4^+$ ion excretion to levels seen in sham-operated acid-loaded controls, even though dexamethasone-supplemented rats were not able to acidify their urine to the same degree as controls. ADX acid-loaded rats supplemented with aldosterone were not able to excrete net acid and NH$_4^+$ at control levels but were able to acidify their urine.

Because dexamethasone has virtually no binding affinity for the MC receptor (20), the finding of Wilcox et al. (2) that dexamethasone alone restored normal NH$_4^+$ excretion in ADX rats does not support our hypothesis that GC binding to MC receptors in the IMCD helps stimulate NH$_4^+$ excretion. However, in a recent in vivo study using rats (21), we found that blocking MR with RU28318 caused a 36% inhibition of NH$_4^+$ excretion during the first 4 h after an acid load when compared with acid-loaded controls. Six hours after acid loading, NH$_4^+$ excretion by RU28318-treated rats was 34% less than that of acid-loaded controls.

In conclusion, we found that IMCD 11β-HSD2 activity measured in vitro is inhibited by low medium pH and is also depressed in IMCD cells isolated from acid-loaded rats. Because corticosterone circulates at concentrations 50 to 400 times that of aldosterone, if corticosterone is not being deactivated efficiently by 11β-HSD2, corticosterone may bind the aldosterone receptor during acute acidosis and stimulate urinary NH$_4^+$ excretion.

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