

Aldosterone Decreases UT-A1 Urea Transporter Expression via the Mineralocorticoid Receptor

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Abstract. Adrenalectomy in rats is associated with urinary concentrating and diluting defects. This study tested the effect of adrenal steroids on the UT-A1 urea transporter because it is involved in the urine-concentrating mechanism. Rats were adrenalectomized and given normal saline for 14 d, after which they received (1) vehicle, (2) aldosterone, or (3) spironolactone plus aldosterone. Adrenalectomy alone significantly increased UT-A1 protein in the inner medullary tip after 7 d, whereas aldosterone repletion reversed the effect. Spironolactone blocked the aldosterone-induced decrease in UT-A1, indicating that aldosterone was working via the mineralocorticoid receptor. For verifying that glucocorticoids downregulate UT-A1 protein through a different receptor, three groups of adrenalectomized rats were prepared: (1) vehicle, (2) adrenalectomy plus dexamethasone, and (3) adrenalectomy plus dexamethasone and spironolactone. Dexamethasone significantly reversed UT-A1 protein abundance increase in the inner medullary tip of adrenalectomized rats. When spironolactone was given with dexamethasone, it did not affect the dexamethasone-induced decrease in UT-A1. There was no significant change in serum vasopressin level, aquaporin 2, or Na⁺-K⁺-2Cl⁻ co-transporter NKCC2/BSC1 protein abundances or UT-A1 mRNA abundance in any of the groups. In conclusion, either mineralocorticoids or glucocorticoids can downregulate UT-A1 protein. The decrease in UT-A1 does not require both steroid hormones, and each works through a different receptor.

Adrenalectomy reduces urine-concentrating ability (1–3) but also inhibits acute water diuresis (4). Both glucocorticoids and mineralocorticoids must be administered to reverse the inhibition of acute water diuresis (4). Valtin and colleagues (4) showed that giving aldosterone to adrenalectomized rats after an acute water load corrected their diluting ability, whereas prednisolone corrected urine flow. These findings suggest that both adrenal steroids are involved in the regulation of urinary concentration and dilution.

Urea plays an important role in the urine-concentrating mechanism (reviewed in (5)). We previously studied the effect of glucocorticoids (dexamethasone) on UT-A1 protein abundance in adrenalectomized rats. We showed that glucocorticoids decrease the protein abundance of the UT-A1 urea transporter in the inner medullary tip of adrenalectomized rats (6) and in conditions associated with excess glucocorticoid, such as uncontrolled diabetes (7, 8). Presumably, in catabolic states in which glucocorticoid levels are increased, UT-A1 is downregulated to permit more nitrogenous waste to be excreted into the urine and to prevent excessive reabsorption of urea across the terminal inner medullary collecting duct (IMCD).

The effect of mineralocorticoids (aldosterone) on UT-A1 protein abundance in adrenalectomized rats has not been studied. Ohara *et al.* (9) studied mineralocorticoid deficiency, induced by adrenalectomy with glucocorticoid replacement, and showed that it had no effect on UT-A1 protein abundance, regardless of whether the rats were given normal saline or plain water to drink. However, the presence of glucocorticoids may have altered any effect that would have resulted from mineralocorticoids alone. We showed that UT-A1 mRNA levels do not differ between adrenalectomized rats, sham-operated rats, or adrenalectomized rats given glucocorticoids (6). Therefore, the goal of this study was to determine whether mineralocorticoids alone have an effect on UT-A1 protein or mRNA abundance by administering aldosterone to adrenalectomized rats.

Materials and Methods

Animal Preparation

All animal protocols were approved by the Emory University Institutional Animal Care and Use Committee. Pathogen-free male Sprague-Dawley rats (100 to 150 g; Charles River Labs, Wilmington, MA) were kept in cages with autoclaved bedding and received free access to water and a standard diet (Diet 5001; Purina). Rats were adrenalectomized as described previously (6), then allowed to recover for 14 d. All adrenalectomized rats were given 0.9% normal saline to drink. After 14 d, osmotic minipumps (Alzet, Palo Alto, CA) were implanted with aldosterone at varying concentrations and for different durations (see the Results section). Aldosterone (2 mg) was dissolved in 100 μ l of DMSO and diluted with 1.9 ml of normal saline. A total of 200 μ l was loaded into each minipump. Control animals received minipumps that contained vehicle (DMSO) only. The DMSO level did

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not exceed 10%, and, when possible, 1% DMSO was used. Spironolactone (100 mg) was suspended in 1 ml of olive oil. Rats received a subcutaneous injection of 200 μ l of spironolactone per day for 8 d. Another group of rats underwent adrenalectomy and received subcutaneous dexamethasone for 7 d at a dose (20 μ g/d) designed to approximate a high physiologic glucocorticoid level (10). Some of these rats also received spironolactone as described above.

Rats were killed, at which time blood and urine were collected for plasma aldosterone, corticosterone, vasopressin, and urine osmolality (model 5500 vapor pressure osmometer; Wescor, Logan, UT). Plasma aldosterone and corticosterone levels were determined by RIA (Coat-A-Count Aldosterone; DPC, Los Angeles, CA). Plasma vasopressin levels were determined by EIA (Assay Designs, Inc., Ann Arbor, MI).

Sample Preparation

Renal medulla was dissected into outer medulla, inner medullary base, and inner medullary tip as described previously (6). These tissues were placed into an ice-cold isolation buffer (triethanolamine 10 mM, sucrose 250 mM [pH 7.6], leupeptin 1 μ g/ml, and PMSF 2 mg/ml), homogenized, and sheared with a 25-G needle, and SDS was added to a final concentration of 1%. Total protein in each sample was measured by a modified Lowry assay (DC Protein Assay Kit; Bio-Rad, Richmond, CA).

Western Blot Analysis

Proteins (10 μ g/lane) were size separated by SDS-PAGE using 10 or 15% polyacrylamide gels. Proteins were blotted to polyvinylidene difluoride membranes (Gelman Scientific, Ann Arbor, MI). Next, blots were incubated for 30 min at room temperature with blocking buffer: 5% nonfat dry milk suspended in Tris-buffered saline (TBS; 20 mM Tris HCl, 0.5 M NaCl [pH 7.5]), then with primary antibody overnight at 4°C (6). Primary antibodies included our polyclonal antibodies to UT-A1, aquaporin 2 (AQP2), and the Na⁺-K⁺-2Cl⁻ co-transporter NKCC2/BSC1 and a commercial antibody to 11- β hydroxysteroid dehydrogenase type II (11- β HSD2; Chemicon, Temecula, CA). Blots were washed three times in TBS with 0.5% Tween-20 (TBS/Tween), then incubated with either horseradish peroxidase-linked goat anti-rabbit IgG (Amersham, Arlington Heights, IL) or goat anti-rabbit IgG linked to Alexa 680 fluorescent dye (Molecular Probes, Eugene, OR) for 2 h at room temperature. Blots were washed twice with TBS/Tween, then the bound secondary antibody was visualized using either chemiluminescence (horseradish peroxidase-linked secondary antibody, ECL kit; Amersham) or infrared laser detection (Alexa-linked secondary antibody; LICOR Odyssey gel scanning system, Lincoln, NE). Laser densitometry was used to quantify the intensity of the resulting bands. Results are expressed as arbitrary units/ μ g protein loaded. In all cases, parallel gels were stained with Coomassie blue and showed uniformity of loading (data not shown).

Northern Blot Analysis

Tissue samples were homogenized in 500 μ l of Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA). RNA was isolated using 200 μ l of chloroform/1 ml Trizol. The samples were centrifuged, and the pellet was dissolved with diethyl pyrocarbonate water. The RNA concentration was measured with Genequant spectrophotometry (Pharmacia Biotech, Cambridge, England). One percent agarose gels were prepared by adding 1.5 g of agarose to 150 ml of northern running buffer (Ambion, Austin, TX). The gels were poured into a horizontal gel electrophoresis system (Life Technologies, Grand Island, NY) and immersed in running buffer (Ambion). RNA was

prepared by placing 5 μ g of sample in a tube with the corresponding amount of glyoxal load dye (Ambion). Samples were incubated for 30 min in a 50°C water bath and loaded onto the gel. The gel was run at 90 volts for approximately 3 h, then transferred to a blot transfer system (Life Technologies, Gaithersburg, MD) and allowed to soak overnight to allow transfer of the mRNA to a nylon transfer membrane. Ultrahyb solution (Ambion) was preheated to 65°C, and the blotted membranes were soaked in water in a hybridization tube. Tris chloride (20 mM) was added to the bottles that contained the membrane. The membrane was rotated in a hybridization oven for 10 min. Ten milliliters of ultrahyb and 200 μ g of fish DNA were added to the tubes. The tubes were prehybridized for 2 h in a preheated roller oven. A ³²P-labeled cDNA probe to UT-A1 (11) was created using a megaprime DNA labeling protocol from Amersham (Buckinghamshire, UK). The cDNA probe was denatured at 100°C for 5 min, then 25 μ l of probe was added to the membrane and it was hybridized overnight in a 65°C roller. The membranes were washed two times with SSC and 0.1% SDS and two times with 0.1 SSC and 0.1% SDS. The membranes were exposed at -80°C for 24 h.

Statistical Analyses

All data are presented as mean \pm SEM, and *n* = number of rats. To test for statistical significance, an ANOVA was used, followed by Tukey protected *t* test (12) to determine which groups were significantly different. The criterion for statistical significance was *P* < 0.05.

Results

Effect of Adrenalectomy on UT-A1

To ensure that any residual effect of adrenal steroid hormones had worn off, we determined the time course of response for UT-A1 after adrenalectomy. In the inner medullary tip, UT-A1 protein is typically detected as 97- and 117-kD bands; both bands represent glycosylated forms of UT-A1 (13). There was no significant change in UT-A1 protein abundance in the inner medullary tip at 3 or 5 d after adrenalectomy (Figure 1). However, UT-A1 protein abundance was significantly increased at 7 and 14 d after adrenalectomy. In contrast, both plasma mineralocorticoids (aldosterone) and glucocorticoids (corticosterone) are reduced to background level within 3 d of adrenalectomy (Figure 2). All subsequent experiments used rats at 14 d after adrenalectomy.

Mineralocorticoid Effects on UT-A1

Aldosterone administration to adrenalectomized rats significantly reduced UT-A1 protein abundance in the inner medullary tip at 3 to 5 d but not at 2 d (Figure 3). In contrast, UT-A1 protein abundance in the inner medullary base was not affected by aldosterone (Figure 3).

Spironolactone was used to test whether the decrease in UT-A1 protein by aldosterone was through interaction with the mineralocorticoid receptor. Urine osmolality was significantly higher in the adrenalectomy plus aldosterone rats than in the adrenalectomy alone or adrenalectomy plus aldosterone plus spironolactone rats (Figure 4A). The plasma aldosterone levels in the two groups of rats that received aldosterone were significantly higher than in the adrenalectomy alone rats but were not significantly different from each other (Figure 4B). There

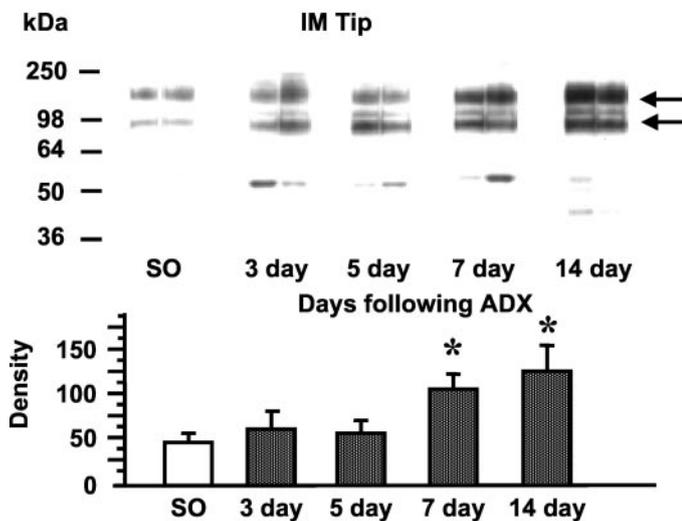


Figure 1. UT-A1 abundance in the inner medullary (IM) tip of adrenalectomized (ADX) rats. Rats were adrenalectomized and given normal saline for the number of days indicated on the *x* axis and compared with sham-operated (SO) control rats. (Top) Representative Western blots visualized with enhanced chemiluminescence (ECL) from a single gel showing progressive increase in UT-A1 (arrows point to the 97- and 117-kD UT-A1 bands). Each lane represents a different rat. (Bottom) Densitometry of UT-A1 protein abundance ($n = 9$ rats at each time point). Data presented as mean \pm SEM; * $P < 0.05$.

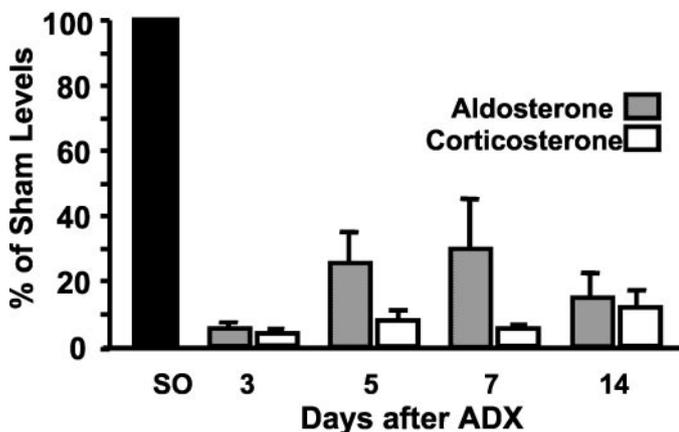


Figure 2. Adrenal steroid hormone levels after adrenalectomy. The graph shows plasma levels of aldosterone (■) and corticosterone (□) determined by RIA in rats at 3, 5, 7, and 14 d after adrenalectomy, expressed as a percentage of the level in plasma of SO rats (100%; ■). Data are presented as the mean \pm SEM from three rats at each time point.

was no significant difference in plasma vasopressin levels between any of the groups of rats (Figure 4C). There were no significant differences in AQP2 (Figure 5) or NKCC2/BSC1 (Figure 6) protein abundances between any of the groups.

Administering aldosterone to adrenalectomized rats significantly decreased UT-A1 protein abundance by 78%, compared with adrenalectomy alone (Figure 7). UT-A1 protein abundance was significantly increased in the adrenalectomized rats

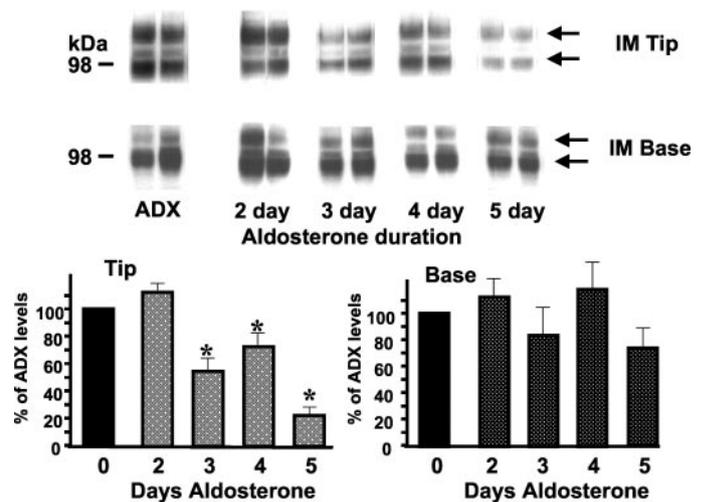


Figure 3. UT-A1 abundance in the IM tip and base of ADX rats after aldosterone administration. Rats were adrenalectomized and given normal saline for 14 d before aldosterone was administered. (Top) Representative Western blots visualized with ECL showing progressive decrease in UT-A1 protein up to 5 d after aldosterone administration in the IM tip. Both the 97- and 117-kD UT-A1 bands (arrows) are reduced by aldosterone. Each lane represents a different rat. (Middle) Representative Western blots visualized with ECL showing no change in UT-A1 protein up to 5 d after aldosterone administration in the IM base. (Bottom) Densitometry of UT-A1 protein abundance ($n = 9$ rats at each time point). Data presented as mean \pm SEM; * $P < 0.05$.

that received spironolactone and aldosterone, compared with aldosterone alone, indicating that spironolactone blocked the aldosterone-induced decrease in UT-A1 protein. There were no significant differences in UT-A1 mRNA abundance between any of the groups (Figure 8).

Glucocorticoid Effects on UT-A1

In the absence of 11- β HSD2, glucocorticoids can act through the mineralocorticoid receptor. Our previous study showed that UT-A1 protein abundance is reduced by glucocorticoids (6). Therefore, we measured 11- β HSD2 protein levels in rats that underwent sham operation, adrenalectomy alone, and adrenalectomy plus aldosterone. The 11- β HSD2 protein was present in all regions of the kidney but in varying abundance. However, adrenalectomy did not alter 11- β HSD2 protein abundance in any region of the kidney (Figure 9).

To verify that glucocorticoids were not decreasing UT-A1 protein abundance through the mineralocorticoid receptor, we determined the effect of administering dexamethasone to adrenalectomized rats, in both the presence and absence of spironolactone. Administering dexamethasone to adrenalectomized rats significantly decreased UT-A1 protein abundance, compared with adrenalectomy alone, consistent with our previous study (6). However, spironolactone did not block the dexamethasone-induced decrease in UT-A1 protein (Figure 10). There were no significant differences in UT-A1 mRNA abundance between any of the groups (Figure 8C).

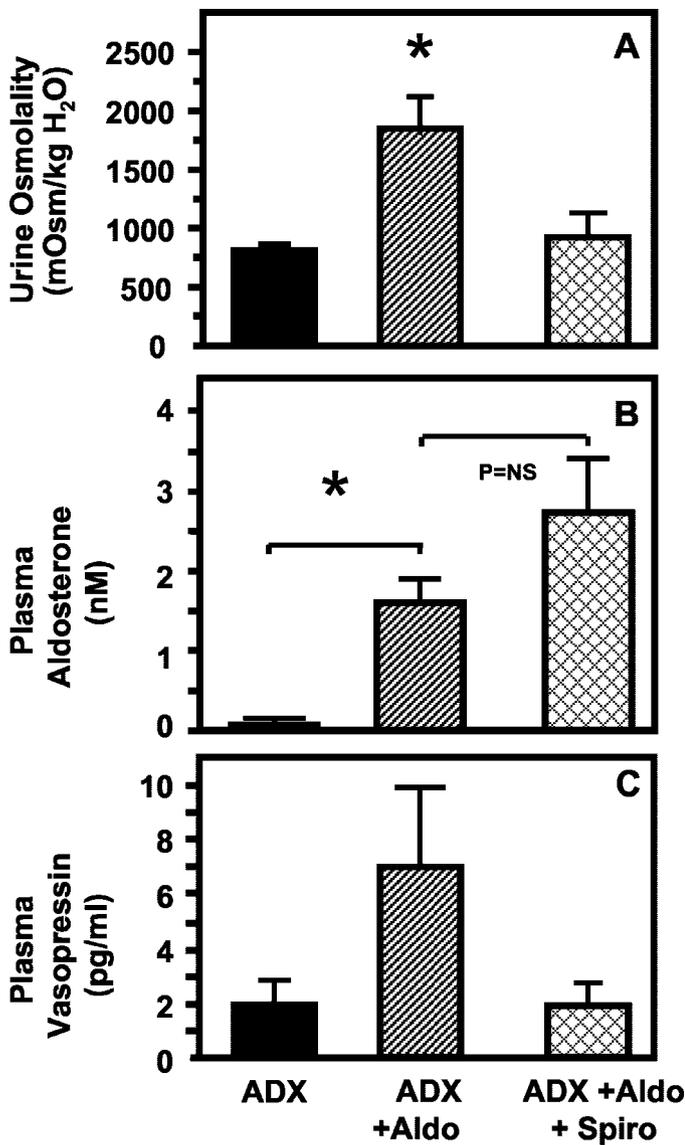


Figure 4. Urine osmolality (A), plasma aldosterone level (B), plasma vasopressin (C) of adrenalectomized rats given vehicle only (ADX), aldosterone (ADX + Aldo), or aldosterone and spironolactone (ADX + Aldo + Spiro). Urine and blood were collected when the rats were killed. *n* = 6 at each time point. Data are presented as mean ± SEM; **P* < 0.05.

Discussion

The major finding in this study is that aldosterone decreases UT-A1 protein abundance in the inner medullary tip of adrenalectomized rats. Aldosterone exerts its effect through the mineralocorticoid receptor. Glucocorticoids also decrease UT-A1 protein abundance in the inner medullary tip of adrenalectomized rats ((6) and the present study) but not by interacting with the mineralocorticoid receptor. Presumably, glucocorticoids exert their effect through the glucocorticoid receptor. Neither aldosterone nor glucocorticoids alter UT-A1 protein abundance in the inner medullary base of adrenalectomized rats ((6) and the present study). The terminal IMCD, located in the inner medullary tip, contains a unique cell type,

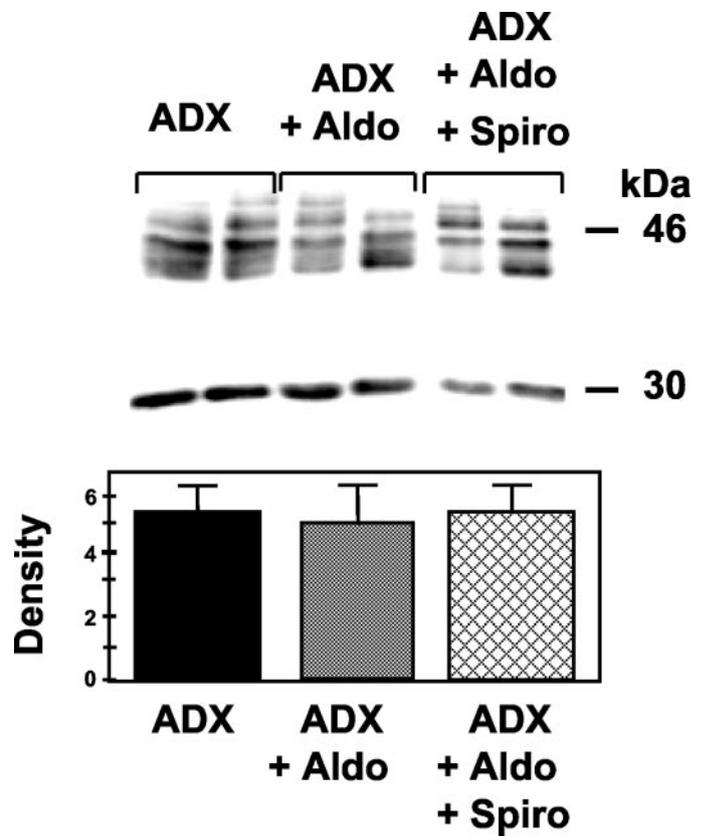


Figure 5. Aquaporin 2 (AQP2) protein abundance in the IM tip of ADX rats. Rats were adrenalectomized, then treated with vehicle (ADX), aldosterone (ADX + Aldo), or aldosterone and spironolactone (ADX + Aldo + Spiro). (Top) Representative Western blots visualized with LICOR from a single gel showing no change in AQP2 protein between any of the groups. Each lane represents a different rat. (Bottom) Densitometry of AQP2 protein abundance from nine rats at each time point. Data presented as mean ± SEM.

the IMCD cell, which is not present in other portions of the collecting duct (14, 15). This suggests that the IMCD may be unique in its response to adrenal steroids, at least in terms of their effect on UT-A1 protein abundance.

Although plasma aldosterone and corticosterone levels were depleted at 2 to 3 d after adrenalectomy, UT-A1 abundance did not change until 7 d after adrenalectomy. This finding suggests that the half-life of UT-A1 protein is days and/or that the biologic half-life of adrenal steroids is considerably longer than their half-life in plasma. Regardless of the mechanism, the present finding emphasizes the need to allow sufficient time after adrenalectomy for the pre-adrenalectomy level of UT-A1 to reach its new value before making experimental manipulations.

We found no change in UT-A1 mRNA abundance between adrenalectomy and either mineralocorticoid or glucocorticoid replacement. The lack of change with glucocorticoids is consistent with our previous study (6). These findings suggest that the change in UT-A1 protein abundance occurs by a posttranscriptional mechanism.

Glucocorticoids are degraded by 11-β HSD2 to corticosterone. In conditions in which this enzyme is inhibited, intracel-

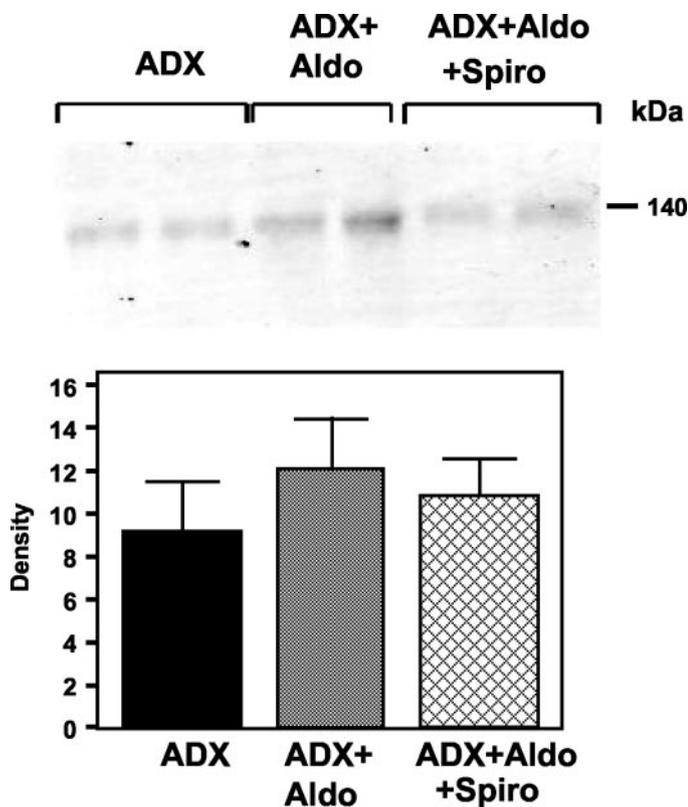


Figure 6. NKCC2/BSC1 protein abundance in the outer medulla of ADX rats. Rats were adrenalectomized, then treated with vehicle (ADX), aldosterone (ADX + Aldo), or aldosterone and spironolactone (ADX + Aldo + Spiro). (Top) Representative Western blots visualized with LICOR from a single gel showing no change in NKCC2/BSC1 protein abundance in any of the groups. Each lane represents a different rat. (Bottom) Densitometry of NKCC2/BSC1 protein abundance from nine rats at each time point. Data presented as mean \pm SEM.

lular concentrations of glucocorticoids are high enough to act at the mineralocorticoid receptor. Fenton *et al.* (16) recently showed that Dahl salt-sensitive rats have a marked increase in UT-A1 protein abundance and 11- β HSD2 activity compared with control rats. Because glucocorticoids decrease UT-A1 transcription (17) and the increase in 11- β HSD2 activity will decrease the intracellular glucocorticoid level, the increase in UT-A1 protein in the Dahl salt-sensitive rat may result from a decrease in glucocorticoid-mediated repression of UT-A1 transcription (16). Wang *et al.* (18) showed that aldosterone-induced extracellular volume expansion results in a decrease in UT-A1 protein abundance. In the present study, if adrenalectomy had resulted in a reduction of 11- β HSD2 protein, then glucocorticoid administration could have reduced UT-A1 protein through the mineralocorticoid receptor. However, we found no change in 11- β HSD2 protein after adrenalectomy. More important, we found no change in UT-A1 protein abundance when rats were given both glucocorticoids and spironolactone, compared with glucocorticoids alone. Thus, glucocorticoids are acting through a distinct mechanism that is unrelated to the mineralocorticoid receptor, most likely through the glucocorticoid receptor.

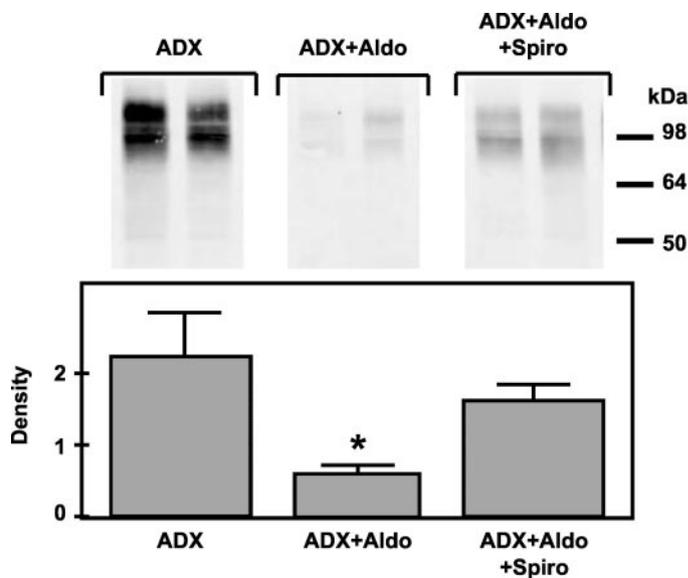


Figure 7. UT-A1 protein abundance in the IM tip of ADX rats. Rats were adrenalectomized, then treated with vehicle (ADX), aldosterone (ADX + Aldo) or aldosterone and spironolactone (ADX + Aldo + Spiro). (Top) Representative Western blots visualized with LICOR from a single gel showing that the decrease in UT-A1 after aldosterone repletion is blocked by spironolactone for both the 97- and the 117-kD UT-A1 bands. Each lane represents a different rat. (Bottom) Densitometry of UT-A1 protein abundance from nine rats at each time point. Data presented as mean \pm SEM; * $P < 0.05$.

The present study cannot distinguish between a direct effect of aldosterone on UT-A1 *versus* a response to some other change that occurs after adrenalectomy and/or aldosterone administration. One mechanism to consider is a change in serum vasopressin levels, because administering vasopressin to Brattleboro rats for 5 d decreases UT-A1 protein abundance (19). However, we found no significant difference in vasopressin levels. Measuring vasopressin levels in rats can be problematic because any factor that causes the rat to become anxious can result in a large and rapid release of vasopressin from the posterior pituitary. Therefore, we also measured the protein abundance of AQP2 and NKCC2/BSC1, two proteins whose abundances are increased by vasopressin (20, 21), but we found no significant difference in either AQP2 or NKCC2/BSC1 protein abundance. Therefore, it seems that changes in vasopressin levels did not contribute to the aldosterone-induced decrease in UT-A1 protein abundance.

In the present study, all adrenalectomized rats drank normal saline to maintain effective circulating volume in the absence of any adrenal steroids. Ohara *et al.* (9) compared glucocorticoid-replaced adrenalectomized rats that were given normal saline *versus* water to vary their volume status but found no change in UT-A1 protein between rats that were given saline *versus* water. Ohara *et al.* also showed that the mineralocorticoid-deficient rats that were given water had a significant increase in NKCC2/BSC1, but when the same rats were given normal saline in an effort to preserve intravascular volume, there was no change in NKCC2/BSC1 abundance (9). In the

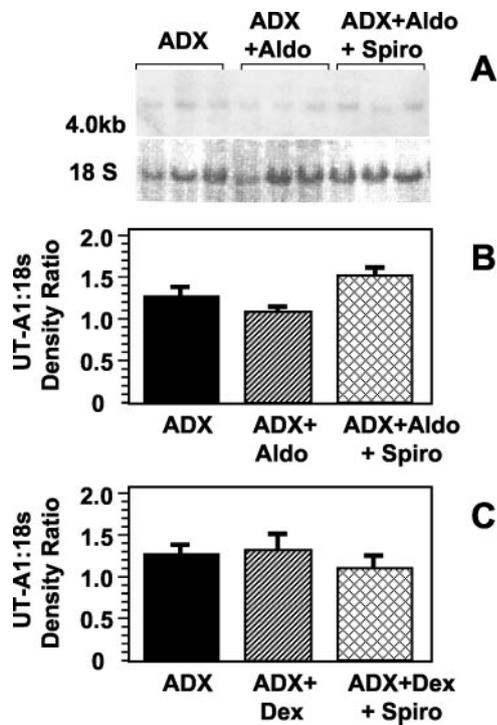


Figure 8. UT-A1 mRNA abundance in the IM of ADX rats. Rats were adrenalectomized, then treated with vehicle (ADX), aldosterone (ADX + Aldo), or aldosterone and spironolactone (ADX + Aldo + Spiro). (A) Representative Northern blots showing no change in mRNA level in any of the groups of the 4.0-kb mRNA band (top) and the corresponding 18S band (loading control; bottom). Each lane represents a different rat. (B) UT-A1:18s density ratio for 18 rats/group. Data presented as mean ± SEM. (C) UT-A1:18s density ratio (*n* = 18 rats/group) for rats that received vehicle, dexamethasone, or dexamethasone + spironolactone. Data presented as mean ± SEM.

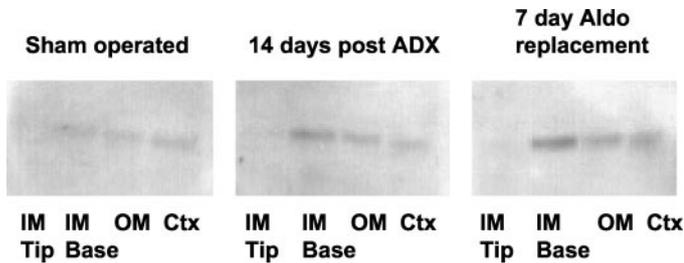


Figure 9. 11β-Hydroxysteroid dehydrogenase protein in the IM, outer medulla (OM), and cortex (Ctx). Blots shown are from sham operation (left), 14 d after adrenalectomy (ADX; middle), and 7 d after aldosterone (Aldo) repletion (right). Adrenalectomy did not alter the protein levels in any part of the rat kidney in sham-operated, adrenalectomized, or aldosterone-repleted groups. Data are from Western blots visualized with ECL. Each lane represents a combined sample from five to six rats.

present study, all adrenalectomized rats received normal saline, so the lack of change in NKCC2/BSC1 that we observed is consistent with the results of Ohara *et al.* (9) and suggests that the rats in the present study were not hypovolemic. Thus, it seems unlikely that hypovolemia contributed to the aldoste-

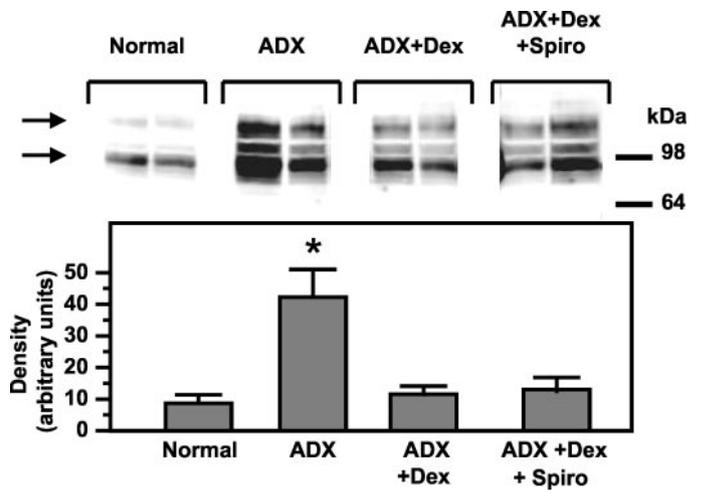


Figure 10. UT-A1 protein abundance in the IM tip of ADX rats that were given glucocorticoids. Rats underwent sham operation (Normal) or were adrenalectomized for 14 d and then treated with vehicle (ADX), dexamethasone for 7 d (ADX + Dex), or dexamethasone and spironolactone for 8 d (ADX + Dex + Spiro). (Top) Representative Western blot visualized with LICOR from a single gel of UT-A1 showing that spironolactone does not block the dexamethasone-induced decrease in UT-A1 protein in ADX rats. Each lane represents a different rat. (Bottom) densitometry of UT-A1 protein abundance from six rats at each time point. Data presented as mean ± SEM; **P* < 0.05.

rone-induced decrease in UT-A1 protein abundance in the present study.

Possible Physiologic Role of Aldosterone’s Effect on UT-A1

UT-A1 protein is upregulated in several conditions in which rats cannot maximally concentrate their urine (reviewed in (22)). In the present study, adrenalectomy decreased urine osmolality, and aldosterone administration increased it. UT-A1 protein abundance in the inner medullary tip increased when the concentrating defect was present and decreased when the defect was corrected with aldosterone. Administering either dexamethasone, a glucocorticoid, or aldosterone, a mineralocorticoid, corrected the adrenalectomy-induced concentrating defect and decreased UT-A1 protein abundance. These results indicate that both glucocorticoids and mineralocorticoids participate in the regulation of UT-A1 and that replacing either adrenal steroid is sufficient to reduce UT-A1 protein abundance to the level found in sham-operated rats.

Mineralocorticoid levels are typically elevated in situations in which an animal or a person would be required to preserve intravascular volume. In the present experiment, however, mineralocorticoids decreased the abundance of UT-A1, which would not facilitate the preservation of vascular volume. Thus, the decrease in UT-A1 may seem to be opposite to what one would have predicted. However, in the present study, we created a urine-concentrating defect without hypovolemia by adrenalectomy and giving the rats normal saline to drink, and this increased UT-A1, consistent with previous studies in

which a urine-concentrating defect was created (reviewed in (22)); mineralocorticoid repletion reversed the urine-concentrating defect and the increase in UT-A1.

Both glucocorticoids and mineralocorticoids play an important role in maintaining vascular stability in patients. Indeed, patients with adrenal insufficiency are frequently hypotensive, and aldosterone is an important contributor to the maintenance of effective circulating volume. Upregulation of UT-A1 in adrenal insufficiency would tend to blunt the tendency for hemodynamic instability by creating a hypertonic inner medullary interstitium that promotes water reabsorption. We recently showed that UT-A1 protein is upregulated in rats with uncontrolled diabetes at 10 to 20 d after streptozotocin injection (8). We suggested that this upregulation is part of a compensatory response to limit the loss of solute and water despite the ongoing osmotic diuresis (8). During the opposite physiologic condition, aldosterone-induced volume expansion, Wang *et al.* (18) showed that UT-A1 protein abundance is decreased. Thus, UT-A1 may be upregulated during adrenal insufficiency as part of a compensatory response to limit the loss of solute and water.

Our experiments may provide some insight into the findings that Valtin and colleagues made >3 decades ago (4). They concluded that aldosterone corrected the diluting ability of adrenalectomized rats (4). Downregulation of UT-A1 would tend to decrease the urea concentration in the inner medulla, thereby decreasing the ability to reabsorb water. In turn, this would contribute to the production of dilute urine.

Conclusion

Adrenalectomy reduces urine-concentrating ability (1–3) and also inhibits acute water diuresis (4). In part, adrenalectomized rats adapt by increasing UT-A1 protein abundance in the inner medullary tip. This adaptation is fairly specific, because neither AQP2 nor NKCC2/BSC1 protein abundances are altered. In addition, the regulation occurs via a posttranscriptional mechanism because UT-A1 mRNA levels did not change. When adrenalectomized rats are given back either aldosterone or dexamethasone, UT-A1 protein returns to the level found in sham-operated control rats. The effect of aldosterone on UT-A1 protein is blocked by a mineralocorticoid antagonist. However, the effect of dexamethasone on UT-A1 protein is not blocked by a mineralocorticoid antagonist. We conclude that either aldosterone or dexamethasone alone is sufficient to reverse the changes in urine osmolality and UT-A1 protein abundance caused by adrenalectomy, but mineralocorticoids and glucocorticoids act through distinct receptors.

Acknowledgments

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