Adrenocortical Steroids Increase Renal Thiazide Diuretic Receptor Density and Response

Zuofang Chen, Duke A. Vaughn, Patricia Blakely, and Darrell D. Fanestil

ABSTRACT
The density of the rat renal pharmacologic receptor for thiazide-type diuretics, as quantitated by the maximal specific binding of [3H]metolazone, decreased to one-third normal after adrenectomy. Selective glucocorticoid (dexamethasone or RU-28362) replacement increased thiazide receptor density to or above the normal level over the dose range of steroid that decreased thymus weight, which served as a bioassay for glucocorticoid activity. Mineralocorticoid (fludrocortisone or aldosterone), in doses that did not decrease thymus weight, also increased thiazide diuretic receptor density to or above normal. The addition of glucocorticoid (RU-28362) to maximal aldosterone increased thiazide receptor above that produced by aldosterone alone and to threefold normal. Similarly, the addition of aldosterone to high-dose RU-28362 also increased thiazide receptor density above that produced by the glucocorticoid alone and to threefold normal. Hence, the effects of glucocorticoids and mineralocorticoids appeared to be additive. The increase in renin thiazide receptor density produced by fludrocortisone, at a dose that elicited both mineralocorticoid and glucocorticoid effects, was unrelated to the basal (prethiazide) renal excretion of sodium, potassium, chloride, or calcium. However, fludrocortisone-pretreated animals responded to bendroflumethiazide with a greater natriuresis than did controls. In addition, the magnitudes of the thiazide-elicited natriuresis and chloruresis correlated significantly with thiazide receptor. It was concluded that both the density of the renal thiazide receptor and the quantity of sodium and chloride reabsorbed by the thiazide-sensitive Na-Cl cotransporter in the kidney are under adrenocortical regulation.

Key Words: Mineralocorticoid, glucocorticoid, carbenoxolone, adrenalectomy, aldosterone

The benzothiadiazine ("thiazide") class of diuretics has been used clinically for over 30 yr (1). Their diuretic action is exerted on the "cortical diluting segment" in the kidney, as deduced from renal clearance studies (2). Direct demonstration that thiazides inhibit sodium reabsorption by acting from the luminal but not the peritubular fluid of the distal convoluted tubule was achieved by Costanzo and Windhager (3). Thiazides enhance the reabsorption of calcium in the same tubular segment (4). A series of demanding micropuncture studies led to the conclusion that thiazides inhibit an electroneutral Na-Cl cotransport process in the early distal convoluted tubule (5-8). In marked contrast to these and other impressive studies (reviewed in References 8 and 9) about the thiazide-sensitive process, the relevance of the thiazide-sensitive Na-Cl reabsorptive process to the physiologic regulation of the thiazide receptor is probably the result of the shortness of the segment in the distal tubule in which thiazides act, difficult access to this segment by micropuncture, and delayed understanding of the axial heterogeneity in the types of cells along the nephron between the macula densa and the collecting ducts (11).

Specifically, with respect to the studies reported here, we are aware of no reports from other laboratories on the relationship between adrenocortical status and the renal thiazide-sensitive Na-Cl cotransporter. Hence, we examine this relationship here by determining the density of the renal receptor for thiazide diuretics, using saturation analysis of the specific binding of [3H]metolazone (12,13) in animals with varied adrenocortical status. In preliminary reports, we indicated that the density of the thiazide receptor was decreased by adrenalectomy and restored to or above control by the administration of corticosteroids (10,14-16). In this report, we examine in detail the effect of adrenalectomy and the effect of treatment with glucocorticoid and mineralocorticoid steroids on thiazide receptor density and we test for a relationship between steroid-dependent renal thiazide receptor density and the pharmacologic effect of a thiazide on the renal excretion of ions.

METHODS
Experimental Protocols

Sprague-Dawley male rats were purchased from two different vendors. This was necessitated by the detection of a rodent virus in the colony of the vendor used for the first sets
of studies in this report. The later studies in this report were on animals from a second vendor. One study, where indicated in the Results, used male rats of the Wistar-Kyoto (WKY) strain. All study protocols were approved by the Animal Subjects Committee of the University of California, San Diego. Animals were maintained in the AAALAC-approved animal care facility and were provided free access to tap water and to rat chow until the morning of the day of study.

Adrenalectomy. Animals were adrenalectomized under pentobarbital anesthesia (50 mg/kg ip) via a single dorsal skin incision, which allowed access for bilateral adrenal removal via the transumbar approach. Control animals were sham operated at the same time. Animals received standard rat chow and 0.9% NaCl (adrenalectomized) or tap water (sham) until euthanasia under pentobarbital anesthesia, at which time the kidneys were rapidly removed and frozen in crushed dry ice for subsequent assay. Adrenocortical hormones, in the doses indicated in the individual experiments in the Results (osule infra), were administered by placement of steroid in denolized drinking water (in the case of fludrocortisone) or by Alzet osmotic minipumps. In the latter instances, the minipumps were placed sc in an interscapular area via a dorsal incision at the time the animals were anesthetized for adrenalectomy or sham adrenalectomy. Control animals received diluent via the minipumps. The weight of the thymus gland was determined in many studies for use as an internal bioassay for the glucocorticoid effect of steroids (17). The thymus was identified by its pale pink color, excised with iris scissors, and weighed to the nearest milligram.

Binding of [3H]lmetolazone. [3H]lmetolazone was custom synthesized by Amer sham (Arlington Heights, IL) (12). The binding assay for [3H]lmetolazone was conducted as previously described in detail (12). Briefly, whole kidneys were homogenized in 10 mL of ice-cold 50 mM Tris-P04 buffer (pH 7.4). Membranes were prepared by centrifugation for 5 min at 600 x g, and the resulting supernatant was centrifuged twice at 45,000 x g for 20 min. The final pellet was evenly suspended in 10 mL of buffer and diluted to achieve a final concentration of 0.8 to 1.0 mg of protein/mL in the binding assay. The binding of [3H]lmetolazone to each membrane preparation was quantitated at six concentrations of [3H]lmetolazone, ranging from 0.313 to 10 nM. The specific binding of [3H]lmetolazone, as defined by displacement with 10^-4 M hydroflumethiazide, was analyzed by the method of Scatchard to calculate the density and the dissociation constant (Kd) of the binding by use of the EBDA program of McPherson (18). Protein was determined by the Bradford Coomassie blue method (19) with bovine gamma globulin as the standard.

Urine Excretion of Electrolytes. The general protocol was as previously described (20). Urine was collected during 2-h periods before and after the ip administration of hydroflumethiazide at 0.3 mg/kg body wt, a dose that produces a maximal chlorothiazide, natriuretic, and diuretic response (20).

Sodium and potassium, ionized calcium, and ionized magnesium were measured by ion-sensitive electrodes (NOVA). Chloride, calcium, and creatinine were measured by colorimetry with commercially available kits (Sigma Chemical Co., St. Louis, MO). The rate of the urinary excretion of ions was calculated from their respective urinary concentrations and the urine flow rate. Aldosterone was radioimmunoassayed with commercially available materials (Diagnostic Products Corporation, Los Angeles, CA). Aldosterone, dexamethasone, and fludrocortisone were purchased from Sigma; RU-28362 was the gift of Roussel-UCLAF (Romainville, France).

Statistical Analysis

Data are expressed as mean values ± SE. Statistical significance was assessed by the use of the Statview® statistical program (Abacus Concepts, Inc., Berkeley, CA). A t test for paired samples was used when excretion after bendroflumethiazide was compared with excretion before the drug. A t test for unpaired samples was used when steroid-treated animals were compared with untreated animals. When three or more groups were compared, analysis of variance was followed by Fisher's post-hoc Protected Least Significant Difference (PLSD) test for multiple comparisons. Stepwise and multiple regression analyses were conducted with the Statview® program.

RESULTS

Effects of Corticosteroid Therapy

Fludrocortisone, a steroid with potent mineralocorticoid activity (about 125-fold that of cortisol) and more modest glucocorticoid activity (about 10-fold that of cortisol) (21), was used in initial studies to assess the effects of time and dose of fludrocortisone. In the time course study, nonadrenalectomized animals from the first vendor were weighed, divided into six groups, and provided drinking water supplemented with 20 mg of fludrocortisone per liter, beginning 1, 2, 4, 7, or 14 days before euthanasia. The effects of the fludrocortisone, as shown in Table 1, included failure to gain weight normally and a decrease in thymus weight that was concurrent with an increase in renal thiazide receptor density. Thiazide receptor density was maximal by 7 days of fludrocortisone treatment, although body weight and thymus weight continued to deteriorate relative to control between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about twofold between 7 and 14 days. In the dose response study, increasing access to fludrocortisone was achieved by varying the concentration of fludrocortisone from 0 to 50 mg per liter of drinking water for 7 days. The results, summarized in Figure 1, show that renal thiazide receptor density increased about two-
TABLE 1. Time course of effects of fludrocortisone$^a$

<table>
<thead>
<tr>
<th>Days of Treatment</th>
<th>$N$</th>
<th>Body Wt$^b$</th>
<th>Thymus Wt</th>
<th>Thiazide Receptor Density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Delta</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>308 ± 33</td>
<td>380 ± 30</td>
<td>73 ± 7.5</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>300 ± 40</td>
<td>372 ± 32</td>
<td>73 ± 11$^c$</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>303 ± 5.2</td>
<td>364 ± 4.7</td>
<td>62 ± 2.4$^c$</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>298 ± 14</td>
<td>347 ± 18</td>
<td>50 ± 4.1$^c$</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>306 ± 37</td>
<td>341 ± 28</td>
<td>35 ± 10$^{cd}$</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>305 ± 37</td>
<td>300 ± 37</td>
<td>−5 ± 5.4$^d$</td>
</tr>
</tbody>
</table>

$^a$ Values are mean ± SE. Fludrocortisone was available at 20 mg/liter of drinking solution. Thiazide receptor density in picomoles per milligram of membrane protein. $N =$ number of Sprague-Dawley male rats from first vendor.

$^b$ All weights in the Table are in grams. "Pre" refers to body weight at the start of the 14 days before euthanasia. "Post" refers to body weight at the time of euthanasia.

$^c$ Significantly different from Day 14 at $P < 0.05$.

$^d$ Significantly different from Day 0 at $P < 0.05$.

---

adrenalectomized animals with aldosterone, at a dose that is reported to produce a plasma level of aldosterone that is intermediate between that observed in animals on a normal versus a low-salt diet (22), significantly increased receptor density to 62% of control ($P < 0.001$), although the level was still significantly less than control ($P < 0.001$). The replacement of adrenalectomized animals with dexamethasone, at a dose that produces a maximal decrease in thymus weight (17), also significantly increased receptor density to 72% of control ($P < 0.001$), although this level was also significantly less than control ($P = 0.01$).

Effects of Increasing Doses of Glucocorticoid

RU-28362, a synthetic steroid with potent and highly selective glucocorticoid activity (23), when administered to adrenalectomized male animals for 7 days by osmotic minipump at 20 μg/100 g per day, increased thiazide receptor density from the adrenalectomized level to a value not significantly different from the sham controls (Table 2). At a dose of 70 μg/100 g per day, RU-28362 increased thiazide receptor density to a value twofold greater than sham control (Table 2). Other expected effects of treatment with glucocorticoid for 7 days were observed: body weight was less than control, and thymus weight was markedly decreased (Table 2). Although this experiment did not define the upper limit of the dose response range with respect to the increase in thiazide receptor, the 70-μg RU-28362 dose is approaching a maximally effective dose, as assessed by the profound effects produced on body weight and thymus weight (Table 2).

Effect of Mineralocorticoid Plus Glucocorticoid

An experiment in two sections tested the possibility that the combined effects of mineralocorticoid and
TABLE 2. Effects of Increasing doses of RU-28362a

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body Wt</th>
<th>Kidney Wt</th>
<th>Thymus Wt</th>
<th>Thiazide Receptor Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Control</td>
<td>5</td>
<td>256 ± 7.3</td>
<td>1.37 ± 0.05</td>
<td>0.52 ± 0.06b</td>
<td>0.437 ± 0.02</td>
</tr>
<tr>
<td>Adx</td>
<td>3</td>
<td>300 ± 17.3</td>
<td>1.41 ± 0.07</td>
<td>0.93 ± 0.05b</td>
<td>0.167 ± 0.03b</td>
</tr>
<tr>
<td>Adx + 20 μg of RU-28362</td>
<td>6</td>
<td>235 ± 7.1</td>
<td>1.22 ± 0.04</td>
<td>0.37 ± 0.03b</td>
<td>0.534 ± 0.04</td>
</tr>
<tr>
<td>Adx + 70 μg of RU-28362</td>
<td>6</td>
<td>210 ± 6.5p</td>
<td>1.27 ± 0.03</td>
<td>0.12 ± 0.01b</td>
<td>0.909 ± 0.05b</td>
</tr>
</tbody>
</table>

a Values are mean ± SE. Dose of RU-28362 is per 100 g body wt per day. Body weight and kidney weight (single kidney) are in grams. Thiazide receptor density is in picomoles per milligram of membrane protein. N = number of Sprague-Dawley male rats from first vendor. Adx, adrenalectomized.
b Values are significantly different from all other groups in the column at P ≤ 0.05 by analysis of variance plus Fisher’s LSD post-hoc test.

Effect of Fludrocortisone on Renal Response to Bendroflumethiazide

The treatment of normal animals with a thiazide diuretic increases thiazide receptor density within 60 min (24). Hence, we determined whether this thiazide-induced acute up-regulation could occur in animals in which the density of the thiazide receptor had already been increased by prior treatment with corticosteroid. Animals (males of the WKY strain in this experiment) were divided into two groups—one drinking deionized water and the other drinking a solution containing 20 mg of fludrocortisone per liter. Half of each group were then administered bendroflumethiazide (0.3 mg/kg body wt) 60 min before euthanasia. Thiazide receptor density was increased in both the control and the fludrocortisone groups (Table 4). These data indicate that fludrocortisone pretreatment does not eliminate the increase in receptor density elicited by acute treatment with thiazide.

Pretreatment with fludrocortisone for 7 days (in WKY animals who did not subsequently receive thiazide) resulted in a 54% decrease in thymus weight, suggesting significant glucocorticoid effect, and in both a significant increase in plasma sodium concentration and significant decreases in plasma concentrations of potassium and chloride (Table 4). Of the measured plasma variables, only potassium concentration significantly correlated with thiazide receptor...

Figure 3. Dose response to aldosterone or RU-28362 on renal thiazide receptor and thymus weight in animals with basal levels of alternate steroid. Number of animals in each group and other parameters relating to these animals are shown in Table 3. All animals were implanted for 7 days with minipumps containing 50% DMSO. Shaded bar: thiazide receptor density. Hatched bar: thymus weight. Sham: sham adrenalectomy plus no administered steroid. ADX: adrenalectomy plus no administered steroid. A0, A0.1, A1, and A10 = adrenalectomy plus 7 μg of RU-28362 (RU) and 0, 0.1, 1, or 10 μg of aldosterone, respectively, per 100 g body wt per day. R0, R1, R10, and R100, adrenalectomy plus 1 μg of aldosterone and 0, 1.0, or 100 μg of RU-28362, respectively, per 100 g body wt per day.
TABLE 3. Dose response to aldosterone and RU-28362a

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body Wt</th>
<th>Kidney Wt</th>
<th>Thymus Wt</th>
<th>Thiazide Receptor Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Control</td>
<td>4</td>
<td>270 ± 20</td>
<td>1.30 ± 0.077</td>
<td>0.53 ± 0.064</td>
<td>0.429 ± 0.006</td>
</tr>
<tr>
<td>Adx</td>
<td>4</td>
<td>278 ± 13</td>
<td>1.38 ± 0.033</td>
<td>0.88 ± 0.067b</td>
<td>0.216 ± 0.011b</td>
</tr>
<tr>
<td>Adx + RU-7</td>
<td>+ Aldo-0</td>
<td>4</td>
<td>263 ± 9.5</td>
<td>1.31 ± 0.64</td>
<td>0.58 ± 0.029c</td>
</tr>
<tr>
<td>+ Aldo-0.1</td>
<td>4</td>
<td>227 ± 9.3</td>
<td>1.24 ± 0.077</td>
<td>0.56 ± 0.036cd</td>
<td>0.269 ± 0.014bc</td>
</tr>
<tr>
<td>+ Aldo-1</td>
<td>4</td>
<td>253 ± 13</td>
<td>1.32 ± 0.048</td>
<td>0.61 ± 0.054cd</td>
<td>0.633 ± 0.032b-d</td>
</tr>
<tr>
<td>+ Aldo-10</td>
<td>4</td>
<td>255 ± 11</td>
<td>1.49 ± 0.063b</td>
<td>0.65 ± 0.037cd</td>
<td>0.637 ± 0.031b-d</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>263 ± 9.5</td>
<td>1.31 ± 0.64</td>
<td>0.58 ± 0.029c</td>
<td>0.202 ± 0.013bc</td>
</tr>
<tr>
<td>Fludrocortisone</td>
<td>4</td>
<td>227 ± 9.3</td>
<td>1.24 ± 0.077</td>
<td>0.56 ± 0.036cd</td>
<td>0.269 ± 0.014bc</td>
</tr>
<tr>
<td>BFTZ</td>
<td>4</td>
<td>253 ± 13</td>
<td>1.32 ± 0.048</td>
<td>0.61 ± 0.054cd</td>
<td>0.633 ± 0.032b-d</td>
</tr>
<tr>
<td>Adx + Aldo-1</td>
<td>+ RU-0</td>
<td>4</td>
<td>250 ± 19</td>
<td>1.35 ± 0.076</td>
<td>0.74 ± 0.049bc</td>
</tr>
<tr>
<td>+ RU-1</td>
<td>4</td>
<td>258 ± 14</td>
<td>1.35 ± 0.077</td>
<td>0.68 ± 0.049cd</td>
<td>0.612 ± 0.082b-d</td>
</tr>
<tr>
<td>+ RU-10</td>
<td>4</td>
<td>253 ± 18</td>
<td>1.34 ± 0.043</td>
<td>0.52 ± 0.13cd</td>
<td>0.669 ± 0.069b-e</td>
</tr>
<tr>
<td>+ RU-100</td>
<td>4</td>
<td>255 ± 11</td>
<td>1.49 ± 0.063b</td>
<td>0.65 ± 0.037cd</td>
<td>0.637 ± 0.031b-d</td>
</tr>
</tbody>
</table>

a Values are mean ± SE. Weights are in grams. Thiazide receptor density is in picomoles per milligram of membrane protein. N = number of Sprague-Dawley males. Adx, adrenalectomized. For Aldo-10 or RU-10: X refers to the micrograms of steroid (Aldo, aldosterone; RU, RU-28362) administered by osmotic minipump per 100 g body wt per day for 7 days.

By analysis of variance and Fisher’s LSD post-hoc test:
- Significantly different from Sham Control at P ≤ 0.05
- Significantly different from (Adx + Aldo-1 + RU-100) at P ≤ 0.05
- Significantly different from Adx at P ≤ 0.05
- Significantly different from Adx + Aldo-1 at P ≤ 0.05
- Significantly different from (Adx + Aldo-1 + RU-1) at P ≤ 0.05

TABLE 4. Effects of fludrocortisone in WKY animalsa

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Fludrocortisone</th>
<th>BFTZ</th>
<th>Fludrocortisone + BFTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Wt</td>
<td>296 ± 5.2</td>
<td>284 ± 4.4</td>
<td>293 ± 5.6</td>
<td>283 ± 8.8</td>
</tr>
<tr>
<td>Kidney Wt</td>
<td>1.15 ± 0.027</td>
<td>1.13 ± 0.027</td>
<td>1.09 ± 0.028</td>
<td>1.11 ± 0.040</td>
</tr>
<tr>
<td>Thymus Wt</td>
<td>0.317 ± 0.014</td>
<td>0.147 ± 0.013bc</td>
<td>0.280 ± 0.011</td>
<td>0.146 ± 0.017bc</td>
</tr>
<tr>
<td>Thiazide Receptor Density</td>
<td>0.685 ± 0.044</td>
<td>1.05 ± 0.080b</td>
<td>1.19 ± 0.071b</td>
<td>1.51 ± 0.108b-d</td>
</tr>
<tr>
<td>Plasma Sodium (mM)</td>
<td>147 ± 0.360</td>
<td>150 ± 0.644b</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Plasma Potassium (mM)</td>
<td>4.0 ± 0.180</td>
<td>3.3 ± 0.051b</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Plasma Chloride (mM)</td>
<td>91.9 ± 0.753</td>
<td>89.6 ± 0.057b</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Plasma Ionized Calcium (mM)</td>
<td>1.29 ± 0.009</td>
<td>1.29 ± 0.013</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Drinking solution was deionized water (Control and BFTZ groups) or contained 20 mg/L fludrocortisone (Fludrocortisone and Fludrocortisone + BFTZ groups) for seven days before exposure to no treatment (Control and Fludrocortisone groups) or 0.3 mg/kg bendrofluamide. N = 8 WKY males in each group, total N = 24. Values are mean ± SE. Weights are in grams. ND, not determined; BFTZ, bendrofluamide.

By analysis of variance and Fisher’s LSD post-hoc test:
- Significantly different from Control at P ≤ 0.05
- Significantly different from BFTZ at P ≤ 0.05
- Significantly different from Fludrocortisone at P ≤ 0.05

Density. The inverse correlation (slope = -0.656) was significant at P = 0.0109, r = 0.656, and r^2 = 0.430.

Effect of Fludrocortisone on Renal Excretory Response to Bendroflumethiazide

The effects of fludrocortisone on the renal excretory responses evoked by thiazide were examined in Sprague-Dawley males at the end of 7 days of access to steroid (fludrocortisone at 20 mg/L of drinking solution). Blood was obtained at the time the animals were euthanized (1 day after the treatment with bendroflumethiazide). The steroid-treated animals had lower body and thymus weights and higher plasma sodium and lower plasma potassium concentrations than did the controls (Table 5). As in the prior experiment, only plasma potassium concentration (among the plasma constituents analyzed) significantly correlated with thiazide receptor density. The inverse correlation was also similar to that found in the prior experiment with P = 0.009, r = 0.647, and r^2 = 0.419.

There was no correlation between the urinary excretion rate of sodium, potassium, chloride, or calcium or the ratio of the concentrations of urinary sodium to urinary potassium in the period before the administration of bendroflumethiazide and the subsequently determined thiazide receptor density (Table 6). As expected, thiazide administration significantly increased the urinary excretion of sodium, potassium, and chloride in both control and steroid-treated animals (Table 7). However, the magnitude of increase...
was greater in the fludrocortisone-treated animals than in the controls only in the case of sodium (Table 7). Regression analysis of renal thiazide receptor density versus the urinary excretion data (Table 6) demonstrated that the thiazide receptor correlated significantly with the quantity of sodium ($P = 0.0019$) or chloride ($P = 0.0075$) excreted in the 2 h after the administration of bendroflumethiazide. The correlation between thiazide receptor and sodium excretion after thiazide is shown in Figure 4. The increase in the rate of the excretion of sodium or chloride (shown in Table 7 as BFTZ Delta) also correlated with thiazide receptor density, but the levels of statistical significance were less.

**DISCUSSION**

This discussion will focus on five issues related to the renal density of the thiazide receptor: regulation by adrenocortical status; regulation by glucocorticoids versus mineralocorticoids; whether corticosterone act directly or indirectly on nephron cells containing the thiazide receptor; effects of thiazide diuretic administration; and the relationship to effects produced by a thiazide diuretic.

**Renal Thiazide Receptor Density Is Regulated by Adrenocortical Status**

Adrenalectomy reduces the receptor to about one-third the normal level (Table 2 and Figure 2). When animals are treated with steroid (fludrocortisone), the maximal increase in thiazide receptor occurred by 7 days (Table 1). Although body weight and thymus weight deteriorated further during a second week of fludrocortisone therapy, renal thiazide receptor density did not change further. These preliminary experiments formed the basis on which all subsequent experiments were conducted with 7 days of steroid replacement (or diluent for controls).

**Renal Thiazide Receptor Density Is Regulated by Both Glucocorticoids and Mineralocorticoids**

Glucocorticoid regulation of the thiazide receptor is evidenced by the increase in the density of the receptor after the treatment of adrenalectomized animals with dexamethasone, a selective glucocorticoid (17) (Figure 2), or with RU-28362, a highly selective glu-
Thiazide receptor density is in picomoles per milligram of muscle protein. Other data regarding these animals are shown in Table 7. Male Sprague-Dawley rats were provided deionized water with 0 (circles) or 20 mg of fludrocortisone (squares) per liter for 7 days. Urinary excretion is in micrograms per 100 g of body weight per 2 h after the ip administration of 0.3 mg of bendroflumethiazide per kilogram body weight. Thiazide receptor density is in picomoles per milligram of renal membrane protein. Sodium Excretion = 0.081 + (79.7 × Thiazide Receptor Density) with r = 0.731, R^2 = 0.508, and P = 0.0019.

corticosteroid (23) (Table 2 and Figure 3). Moreover, the increase in thiazide receptor density induced by these potent glucocorticoids occurred over the same dose ranges that produced a decrease in thymus weight (Tables 2 and 3 and Figure 3). Because thymus weight change has been proposed as a surrogate marker of glucocorticoid effect in vivo (17), the concordance of dependent increases in the delivery of sodium and chloride to the distal nephron is consistent with both effects being mediated by glucocorticoid action. The mineralocorticoid regulation of the receptor is evidenced by: (1) the increase in the density of the receptor after the treatment of adrenalectomized animals with doses of aldosterone that do not elicit a decrease in thymus weight (Table 3 and Figure 3); and (2) the increase in receptor density in adrenal intact animals by doses of fludrocortisone (up to 10 mg/L of drinking water) that lack a glucocorticoid effect on thymus weight (Figure 1).

Are the stimulatory effects on renal thiazide receptor density produced by glucocorticoids and mineralocorticoids mutually exclusive or additive? Several lines of evidence suggest that the effects are additive. First, the dose response relationship between fludrocortisone and thiazide receptor density (Figure 1) demonstrates that the increase in receptor density occurs across doses of the steroid that might be expected to elicit both mineralocorticoid and glucocorticoid effects (21). Second, 1 μg of aldosterone per 100 g body wt per day increases thiazide receptor in adrenalectomized animals to as great an extent as does 10-fold more aldosterone (Figure 3). However, the addition of the highly selective glucocorticoid RU-28362 to 1 μg of aldosterone produces a dramatic further increase in thiazide receptor density, which is coordinate with a decrease in thymus weight (Figure 3). This experiment provides strong support for the interpretation that the addition of glucocorticoid to maximal mineralocorticoid produces an additive (or nearly additive) further increase in thiazide receptor to approximately three times normal.

Are the Actions of Corticosteroids on Thiazide Receptor Density Direct or Indirect?

In other words, do corticosteroids act directly via specific glucocorticoid and mineralocorticoid receptors in the target cells in the distal nephron? Or do the steroids produce their primary effects on other cells or organs, the combined effects of which result in indirect increases in thiazide receptor density in the kidney? Relevant to these questions is the statistically significant correlation in fludrocortisone-treated animals between the renal density of the thiazide receptor and the plasma concentration of potassium. Although the density of the receptor correlated inversely with the plasma concentration of potassium, it is unlikely that the hypokalemia occurred secondarily to an increase in the reabsorption of sodium and chloride in the distal convoluted tubule, because hypokalemia is normally the consequence of the blockade of this reabsorption by thiazide diuretics. The importance, if any, of the steroid-induced alterations in plasma electrolytes to changes in renal thiazide receptor cannot be addressed with these data. An alternate explanation for an indirect mechanism by which the steroids might increase thiazide receptor is that the increase in thiazide receptor density occurs as a result of steroid-dependent increases in the delivery of sodium and chloride to the distal convoluted tubule. We have previously provided evidence that increasing the delivery of sodium and chloride to the distal nephron (by blocking reabsorption in the thick ascending limb with furosemide) resulted in an increase in renal thiazide receptor density (24). Because we did not collect evidence that allows a direct examination of whether the steroids increased NaCl delivery to the distal convoluted tubule in these experiments, we cannot comment conclusively on this attractive possibility.

Do the Effects of Thiazide Diuretics on Thiazide Receptor Density Occur in Animals in Which the Receptor Has Already Been Increased by Corticosteroids?

Corticosteroids increase thiazide receptor density, as shown in this study. Similarly, acute (1-h) treatment with a thiazide increases thiazide receptor density (24). Hence, we determined whether the effect of one of these stimuli affected the second. Bendroflumethiazide treatment increased receptor density in both control and fludrocortisone-treated animals (Table 4). The percent increase in receptor density caused by
fludrocortisone in the absence of thiazide treatment (53%) was nearly twice that in the thiazide-treated group (27%). However, when the data are expressed in absolute terms (picomoles per milligram of protein), the increases in thiazide receptor density caused by fludrocortisone treatment were similar with and without acute thiazide administration (0.324 pmol/mg with thiazide: 0.364 pmol/mg without thiazide). Thus, pretreatment with fludrocortisone did not eliminate the increase in receptor density elicited by acute treatment with bendroflumethiazide. Additionally, these results indicate that, after the administration of bendroflumethiazide, the diuretic will act on kidneys that have a greater thiazide receptor density when the animal has been pretreated with fludrocortisone.

The Corticosteroid-Dependent Increase in Renal Thiazide Receptor Density Results in an Increase in the Excrelory Response Elicited by a Thiazide Diuretic

In a final experiment on fludrocortisone pretreated animals, there was no effect of fludrocortisone on the basal (that is, before diuretic) rate of excretion of sodium, potassium, chloride, or calcium (Table 7). Bendroflumethiazide, as expected, increased the rates of the excretion of sodium, potassium, and chloride in both control and steroid-treated animals. However, only the diuretic-induced increase in the excretion of sodium was significantly greater in fludrocortisone than in control. The possibility that all or some of these findings are the result of the increased delivery of NaCl to a distal tubule that reabsorbs more NaCl in a load-dependent fashion has not been eliminated. However, the absolute rates of the excretion of sodium and chloride, as well as the magnitude of the increase in the rates of their excretion, did significantly correlate with renal thiazide receptor density (Table 6 and Figure 4). This constellation of findings (Tables 6 and 7 and Figure 4) can be interpreted to indicate that: (1) the quantity of sodium and chloride reabsorbed by the thiazide-inhibitable Na-Cl cotransporter in fludrocortisone pretreated animals was greater than that in control animals before the administration of thiazide diuretic; and (2) the blockade of the Na-Cl cotransporter with bendroflumethiazide resulted in natriuresis and chloruresis that were greater in fludrocortisone pretreated animals in proportion to the density of the renal receptor for thiazide-type diuretics.

ACKNOWLEDGMENTS

These studies were supported by NIH Program Project Grant PHS HL35015, Program Director Dr. Morton Printz, and NIH Program Physican Scientist Award DK01408, Program Director Dr. Daniel Steinberg. References 14 through 16 contain some of these data in preliminary format. Dr. Kevin Beaumont contributed important encouragement and advice. The able assistance of Pat Spindler is gratefully acknowledged.

REFERENCES