Published in final edited form as:

Am J Physiol Regul Integr Comp Physiol. 2007 September ; 293(3): R1444–R1451. doi:10.1152/ajpregu.00294.2007.

Glucocorticoids increase salt appetite by promoting water and sodium excretion

Robert L. Thunhorst^{1,4}, Terry G. Beltz¹, and Alan Kim Johnson^{1,2,3,4}

¹ Department of Psychology, University of Iowa, Iowa City, Iowa

² Department of Integrative Physiology, University of Iowa, Iowa City, Iowa

³ Department of Pharmacology, University of Iowa, Iowa City, Iowa

⁴ Department of Cardiovascular Center, University of Iowa, Iowa City, Iowa

Abstract

Glucocorticoids [e.g., corticosterone and dexamethasone (Dex)], when administered systemically, greatly increase water drinking elicited by angiotensin and sodium ingestion in response to mineralocorticoids [e.g., aldosterone and deoxycorticosterone acetate (DOCA)], possibly by acting in the brain. In addition, glucocorticoids exert powerful renal actions that could influence water and sodium ingestion by promoting their excretion. To test this, we determined water and sodium intakes, excretions, and balances during injections of Dex and DOCA and their coadministration (DOCA+Dex) at doses commonly employed to stimulate ingestion of water and sodium. In animals having only water to drink, Dex treatment greatly increased water and sodium excretion without affecting water intake, thereby producing negative water and sodium balances. Similar results were observed when Dex was administered together with DOCA. In animals having water and saline solution (0.3 M NaCl) to drink, Dex treatment increased water and sodium excretion, had minimal effects on water and sodium intakes, and was associated with negative water and sodium balances. DOCA treatment progressively increased sodium ingestion, and both water and sodium intakes exceeded their urinary excretion, resulting in positive water and sodium balances. The combination of DOCA+Dex stimulated rapid, large increases in sodium ingestion and positive sodium balances. However, water excretion outpaced total fluid intake, resulting in large, negative water balances. Plasma volume increased during DOCA treatment and did not change during treatment with Dex or DOCA+Dex. We conclude that increased urinary excretion, especially of water, during glucocorticoid treatment may explain the increased ingestion of water and sodium that occurs during coadministration with mineralocorticoids.

Keywords

thirst; urine volume; dexamethasone; deoxycorticosterone acetate; food intake

Systemically administered mineralocorticoids, such as aldosterone and deoxycorticosterone acetate (DOCA), stimulate vigorous sodium ingestion by activating mineralocorticoid receptors in the brain. Glucocorticoids, such as corticosterone, greatly increase this salt appetite response when coadministered with mineralocorticoids (2,17,33,35). Systemically administered glucocorticoids also increase water drinking in response to peripheral and central administration of ANG II (9,27). The potentiation of salt appetite and thirst by

glucocorticoids has been postulated to arise from glucocorticoid actions within the brain, affecting either mineralocorticoid (17,35) or ANG II (3,6,8,22) receptors. For example, high levels of glucocorticoids are proposed to increase type I mineralocorticoid receptors and thus mineralocorticoid binding in the brain (17,35). Additionally, glucocorticoids may increase the number of ANG II receptors in the brain (22) and thereby augment the central actions of ANG II (which, in turn, may interact with mineralocorticoid receptors).

Glucocorticoids also have systemic effects that could facilitate ingestion of water and sodium. For example, they increase glomerular filtration rate in animals and humans at physiological and pharmacological levels (1). Glucocorticoids increase urine volume (1,11,18,28,34), urinary sodium excretion (1,11,18,28,34), and potassium excretion (1,11) and can cause negative water balance (11,28). Glucocorticoids interfere with the sodium-retaining properties of mineralocorticoids (14). By promoting water and sodium excretion during tests of thirst and salt appetite, glucocorticoids might indirectly affect these behavioral responses (9,27,33). The possibility that the potentiation of salt appetite and thirst by glucocorticoids is related to the renal effects of the steroids has received scant experimental attention. Therefore, in the present work, we tested these ideas by examining water and sodium intakes, excretions, and balances and changes in plasma volume during systemic administration of the glucocorticoid agonist dexamethasone (Dex), alone or together with DOCA, at doses routinely employed to increase thirst (9,27) and salt appetite (15,17,20,22).

METHODS

Animals

Male Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN). They were housed singly in hanging stainless steel cages in a temperature-controlled room (23°C) on a 12:12-h light-dark cycle. The rats received standard Teklad Laboratory diet and water from bottles. All procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

Drugs

DOCA and Dex in crystalline form were obtained from Sigma-Aldrich (St. Louis, MO). They were dissolved in propylene glycol (Fisher Scientific, Fair Lawn, NJ) and administered subcutaneously. Dex in injectable form (4 mg/ml) was obtained from American Regent (Shirley, NY) and was administered intraperitoneally.

Experiment 1: water intake and urinary water and electrolyte excretions and balances after Dex injections

On the morning of testing, rats were weighed and placed in standard metabolism cages with stainless steel funnels beneath. No food was available during testing. Separate groups of rats were injected intraperitoneally with Dex (injectable form, 2 mg/kg; $n = 6$ rats) or isotonic saline (vehicle, 2 ml/kg; $n = 6$ rats). The dose and manner of delivery of Dex were similar to those shown to increase drinking in response to ANG II (9,27). Burettes of water with sipper spouts were then attached to cage fronts. Urine was collected into Nalgene tubes. Feces were trapped and did not fall into the collected urine. Water intakes and urine volumes were recorded to the nearest 0.1 ml after 6 h. The burettes were then replaced with 100-ml graduated cylinders (1-ml resolution) of water, and preweighed glass beakers were placed under the funnels. The following morning (24 h after injections), water intakes and urine volumes were recorded to the nearest milliliter, and the rats were reweighed. The urine volume was calculated as 1 g = 1 ml. Samples of urine were collected at 6 and 24 h and

refrigerated for later analyses of sodium and potassium content by ion-specific electrode (NOVA 1Plus; Biomedical, Waltham, MA).

Experiment 2: water intake and urinary water and electrolyte excretions and balances after Dex and DOCA injections

The procedures followed those of *experiment 1* above. Separate groups of rats were injected subcutaneously with Dex ($n = 4$), DOCA ($n = 4$), or their combination (DOCA+Dex; $n = 4$). The steroids were in crystalline form dissolved in propylene glycol, and the dose of each was $2 \text{ mg} \cdot 0.33 \text{ ml}^{-1} \cdot \text{rat}^{-1}$. The dose and manner of delivery of Dex were similar to those shown to increase sodium ingestion in response to DOCA (15,17,20,22).

Experiment 3: water and sodium intakes, excretions, and balances after repeated daily injections of Dex and DOCA

Starting at least 5 days before testing, rats were provided access to 0.3 M NaCl and distilled water. At least 3 days before testing, pelleted sodium-deficient diet (MP Biomedicals, Aurora, OH) was substituted for the standard diet. At the start of testing, the rats were placed in standard stainless steel metabolism cages as described above. Ground sodium-deficient diet was available from a stainless steel cup through an opening in the back of the cage. Graduated cylinders with distilled water and 0.3 M NaCl were attached to cage fronts. Stainless steel funnels were situated under the cages, and urine was collected into preweighed beakers.

Baseline measures for each group were obtained for 5–6 days followed by treatment measures for several days. Treatment consisted of daily subcutaneous injections of Dex ($n = 5$ rats), DOCA ($n = 6$ rats), or their combination (DOCA+Dex; $n = 5$ rats). The dose of each steroid was $2 \text{ mg} \cdot 0.25 \text{ ml}^{-1} \cdot \text{rat}^{-1}$. The treatment conditions were run serially using separate groups of animals. Rats receiving DOCA also received additional testing with the combination of DOCA+Dex for 4 days. Daily measurements were obtained between 0800 and 0900. Each morning, the water and saline intakes were read, the urine beaker and food cup were collected, and the rat was weighed. Distilled water was used to rinse the floor of the cage, and a funnel was used to collect residual salt. This wash volume was collected into a separate preweighed beaker. A clean funnel was then placed underneath the cage with a new beaker. The food cup was weighed, “topped off” with fresh ground diet, and reweighed before being placed back in the cage. Food spillage was collected and considered in the daily measures. The rat was returned to the cage, and water and saline cylinders were reattached.

Urine and wash volumes were determined by weight. Samples were refrigerated for later analyses. The urine and wash were analyzed for sodium and potassium content. Sodium and potassium excretions included the amounts of sodium and potassium lost in the urine plus the wash. Water balance was calculated as total fluid intake (i.e., water + saline) minus urine volume. Sodium balance was calculated as 0.3 M NaCl intake minus urinary excretion.

Experiment 4: daily water and sodium intakes and plasma volumes after Dex and DOCA injections

The fourth experiment examined daily water and sodium intakes and hematocrits during daily subcutaneous injections of Dex ($n = 5$ rats), DOCA ($n = 6$ rats), or their combination ($n = 4$ rats) at the doses used in *experiment 3*. Testing occurred in the home cage. Rats were given pelleted sodium-deficient diet (MP Biomedicals) and distilled water and 0.3 M NaCl from 100-ml graduated cylinders as above. After a few days, rats were implanted with jugular venous catheters under Equithesin anesthetic (0.33 ml/100 g body wt). The catheters were made of silicone tubing (0.047 in. OD; Helix Medical, Carpinteria, CA) and were

tunneled under the skin to emerge at the back of the neck. The catheters were filled with 200 U/ml heparin solution and plugged with 18-gauge obturators when not in use. After a day of recovery from surgery, rats underwent daily blood sampling. The rat was held in the tester's hand while ~0.25 ml of blood was withdrawn from the catheter by syringe. The blood was immediately transferred to microhematocrit tubes for centrifugation and measurement of hematocrit in triplicate. Baseline measures were obtained for 3–4 days followed by treatment measures for 3–4 days. Water and 0.3 M NaCl intakes and body weights were measured daily.

Statistical analysis

Data were analyzed by repeated-measures ANOVA. Planned comparisons were made with Fisher's least significant difference tests when the global F ratio was significant. Values are significant at $P < 0.05$.

RESULTS

Experiment 1: water intake and urinary water and electrolyte excretions and balances after Dex injections

The analysis used time (2 points) as the within-subject repeated measure and drug (vehicle or Dex) as the between-subject measure. Intakes and excretions were analyzed as rates (i.e., ml or $\mu\text{mol/h}$). Cumulative values are presented in Fig. 1. Rats that received Dex weighed significantly more at the start but significantly less by morning than rats that received vehicle (interaction, $F_{1,10} = 116.46$, $P < 0.001$) because they lost more weight (44 ± 1 vs. 26 ± 1 g, respectively; $t_{10} = 10.79$, $P < 0.01$). By 6 h, Dex caused fivefold increases in urine volume (interaction, $F_{1,10} = 57.29$, $P < 0.001$) and sodium excretion (interaction, $F_{1,10} = 38.78$, $P < 0.001$) and a tripling of potassium excretion (interaction, $F_{1,10} = 142.34$, $P < 0.001$), compared with that shown with vehicle. Dex also greatly reduced water balance by this time (interaction, $F_{1,10} = 82.17$, $P < 0.001$). In the overnight hours (7–24 h after injection), Dex significantly increased sodium excretion but reduced potassium excretion compared with vehicle. Cumulative urine volume, sodium excretion, and potassium excretion were greater after Dex treatment, whereas cumulative water balance was reduced after Dex treatment. A main effect of time indicated that the rats drank more in the overnight period than in the first 6 h of testing ($F_{1,10} = 24.27$, $P < 0.001$), but there were no effects involving drug. Thus the greater weight loss after Dex treatment is attributable to greater water loss as Dex increased urine volume without increasing water intake. Urinary sodium concentration did not differ between groups but was higher at 0–6 h (93 ± 17 and 81 ± 4 mmol/l with vehicle and Dex, respectively; means $\pm$ SE) than at 7–24 h (27 ± 5 and 47 ± 3 mmol/l with vehicle and Dex, respectively; time main effect, $F_{1,10} = 43.40$, $P < 0.001$). Main effects of drug ($F_{1,10} = 10.87$, $P < 0.01$) and time ($F_{1,10} = 28.13$, $P < 0.001$) showed that urinary potassium concentration was higher after vehicle than after Dex treatment and was higher in the first 6 h (130 ± 21 mmol/l with vehicle vs. 73 ± 4 mmol/l with Dex) than overnight (57 ± 9 mmol/l with vehicle vs. 31 ± 2 mmol/l with Dex).

Experiment 2: water intake and urinary water and electrolyte excretions and balances after Dex and DOCA injections

The data were analyzed as above, using time as the within-subject repeated measure and drug (Dex, DOCA, or both) as the between-subject measure. Cumulative values are presented in Fig. 2. Initial body weight differed across groups by a small amount (~6 g). By morning, all groups lost weight, and both groups receiving Dex (i.e., Dex and DOCA+Dex) lost significantly more weight (~41 g for both groups) than the group receiving DOCA by itself (~19 g; interaction, $F_{2,11} = 28.20$, $P < 0.001$). This greater weight loss for both groups receiving Dex is accounted for by significantly greater urine volumes (main effect, $F_{2,9} =$

6.87, $P < 0.05$) and significantly reduced water balances (interaction, $F_{2,9} = 8.96$, $P < 0.01$) than in the group receiving DOCA by itself. Dex caused significantly greater reductions in water balance by 6 h than in the other groups ($P < 0.01$). DOCA caused water retention as shown by positive water balance. Both groups that received Dex had significant (13-fold) increases in sodium excretion (main effect, $F_{2,9} = 23.14$, $P < 0.001$) compared with rats that received only DOCA. In addition, Dex caused more sodium excretion at 6 h and cumulatively when administered alone than when combined with DOCA. DOCA treatment nearly eliminated sodium excretion. Both Dex-treated groups had significantly greater potassium excretion (interaction, $F_{2,9} = 20.84$, $P < 0.001$) in the first 6 h and cumulatively than rats that received only DOCA. DOCA-treated animals excreted nine times more potassium than sodium, whereas both Dex-treated groups excreted the solutes in roughly similar amounts. The rats increased water intake overnight compared with that shown in the first 6 h (time main effect, $F_{1,9} = 24.01$, $P < 0.001$), but there were no drug effects.

Rats that received only Dex had significantly higher urinary sodium concentrations over 0–6 h than both other groups, and both groups that received Dex had significantly higher urinary sodium concentrations overnight than the DOCA group (interaction, $F_{2,9} = 8.76$, $P < 0.01$). Overall (i.e., 0–24 h), urinary sodium concentrations (mean $\pm$ SE) were 63 ± 3 mmol/l for Dex, 10 ± 3 mmol/l for DOCA, and 48 ± 5 mmol/l for DOCA+Dex. DOCA-treated rats had significantly higher urinary potassium concentrations overnight than the other groups (interaction, $F_{2,9} = 8.64$, $P < 0.01$). Overall, urinary potassium concentrations (mean $\pm$ SE) were 54 ± 5 mmol/l for Dex, 97 ± 28 mmol/l for DOCA, and 55 ± 7 mmol/l for DOCA+Dex.

Experiment 3: water and sodium intakes, excretions, and balances after repeated daily injections of Dex and DOCA

In this experiment, animals had continuous access to water, 0.3 M NaCl, and sodium-deficient diet for several days. The analysis used baseline/treatment condition and days as within-subject repeated measures and drug (DOCA, Dex, DOCA+Dex) as the between-subject measure. Rats that received Dex, either alone or in combination with DOCA, lost considerable weight during testing, and the treatments were stopped after 4 and 5 days, respectively. For statistical purposes, the analyses used data from the last 4 days of baseline and first 4 days of treatment.

Measurements over all days of testing are presented in Figs. 3–5. Baseline measurements for all variables were equivalent between the groups unless otherwise indicated. For example, rats that received Dex by itself were heavier at the start; however, the DOCA and DOCA+Dex groups, which comprise the most important contrast for the present purposes, had equivalent starting weights. The starting weights (mean $\pm$ SE) were 402 ± 13 g for Dex, 350 ± 11 g for DOCA, and 358 ± 14 g for DOCA+Dex. During the baseline period, all groups gained weight daily (~ 1.4 g/day for all groups). During the treatment phase, animals that received DOCA continued to gain weight at a rate of 3.1 g/day, whereas both Dex-treated groups lost weight at a rate of ~ 12 g/day (baseline/treatment $\times$ drug interaction, $F_{2,13} = 146.52$, $P < 0.001$; Fig. 3). DOCA-treated animals gained significantly more weight, cumulatively, over 4 days of treatment than they did over 4 days of baseline (3-way interaction, $F_{6,39} = 50.28$, $P < 0.001$; Fig. 5). Both Dex-treated groups lost significant weight on the first day of treatment, and the weight loss was progressive over 4 days of treatment.

The three-way interactions for water, saline, and total fluid (i.e., water + saline) intakes were significant (all $F_{6,39} \geq 3.21$; $P < 0.05$). Rats that received only Dex drank significantly less water on day 3 of treatment, and those that received DOCA+Dex drank significantly more water on day 4 of treatment (data not shown). All drug treatments significantly increased

daily saline intakes compared with their respective baseline intakes, and the treatment saline intakes were significantly different from each other (sodium intake in mmol is found in Fig. 4). Dex significantly increased saline intake only on the first treatment day. The greatest increases were observed for rats given the combination of DOCA+Dex, which showed a fivefold increase in saline consumption on the first day of treatment, from a baseline average of 5.2 ± 1.9 ml/day to 27.2 ± 5.4 ml. DOCA progressively increased saline intakes to match those caused by DOCA+Dex after 5 days. Because the drug effects on water intake were small, the group differences in total fluid intake were due mainly to the group differences in 0.3 M NaCl intake. All groups increased total fluid intake on the first treatment day compared with their respective baseline days (Fig. 3). The first day increases in total fluid intake were solely as a result of increased saline intake and thus were significantly greater for DOCA+Dex rats than for the other groups. By *day 4* of treatment, total daily fluid intake was unchanged from baseline measures for rats that received only Dex, had nearly doubled for rats that received only DOCA, and had more than doubled for rats that received the combination of drugs.

Urine volume increased significantly above baseline measures on at least some days during all drug treatments (3-way interaction, $F_{6,39} = 3.94$, $P < 0.01$; Fig. 3). The increase in urine volume was significant on the first day of treatment for both groups that received Dex and by the second day of treatment for rats that received only DOCA. Urine volume was increased significantly more during DOCA+Dex treatment than for the other treatments. During baseline, average daily water balance was slightly, but significantly, higher in the DOCA+Dex group (baseline/treatment $\times$ drug interaction, $F_{2,13} = 118.49$, $P < 0.001$). During DOCA treatment, average daily and cumulative water balances were increased significantly compared with average baseline values as total daily fluid ingestion increased more than daily urine volume. For both groups that received Dex, average daily and cumulative water balances were significantly reduced compared with average baseline values and compared with those of rats that received only DOCA as daily urine volumes increased more than total daily fluid intake. Dex treatment reduced average daily and cumulative water balances significantly more than the combination of DOCA+Dex treatment.

Sodium intake, excretion, and balance are presented in Fig. 4. Sodium intake in the form of saline solution was described above. All drugs significantly increased sodium excretion from baseline values on some treatment days, and there were significant differences among the treatment excretions (3-way interaction, $F_{6,39} = 7.21$, $P < 0.001$). Dex increased sodium excretion compared with all baseline days on treatment *days 1* and *2* and compared with DOCA on treatment *day 1*. The combination of DOCA+Dex also increased sodium excretion on all treatment days compared with baseline levels and increased sodium excretion compared with that shown with DOCA. During DOCA treatment, sodium excretion was higher than that shown with predrug baseline levels. During baseline, there was an overall difference in levels of sodium balance between animals in the Dex group and those in the DOCA group (3-way interaction, $F_{6,39} = 2.63$, $P < 0.05$). During Dex treatment, sodium excretion was greater than sodium ingestion, and the resulting sodium balances were significantly reduced from baseline values on the first day of treatment. On subsequent days of Dex treatment, daily sodium balance returned to basal levels, but cumulative sodium balance remained below basal levels (Fig. 5). During DOCA treatment, sodium excretion was always less than sodium ingestion, and the resulting daily and cumulative sodium balances were always positive. During DOCA+Dex treatment, sodium excretion was generally less than sodium ingestion, and the sodium balances were significantly greater on *days 2* and *3* of treatment. Animals in the DOCA+Dex group developed the most positive cumulative sodium balance, which was significantly greater than the other groups on treatment *days 2–4*.

All groups increased potassium excretion on the first day of treatment. DOCA-treated animals maintained daily potassium excretion above baseline levels compared with the other groups, whereas both groups of Dex-treated rats decreased daily potassium excretion to levels lower than baseline (baseline/treatment $\times$ drug interaction, $F_{2,13} = 20.49$, $P < 0.001$; data not shown).

Both groups of rats that received Dex treatment had significantly reduced food intake levels compared with levels shown at baseline and compared with rats that received only DOCA (3-way interaction, $F_{6,39} = 11.23$, $P < 0.001$). The reductions in food intake began on the second day of treatment (Fig. 4).

Rats treated with DOCA were permitted to establish stable saline intakes (*days 5–7*) and then received daily injections of Dex along with DOCA. All of the major effects of injecting DOCA+Dex from the start were observed after addition of Dex injections to animals already receiving DOCA and thus are in effect a replication. Notably, urine output immediately spiked and was greater than the increase in total fluid ingestion, so water balance was reduced. Body weight also declined on the first day of adding Dex to DOCA treatment and declined throughout combined treatment.

Experiment 4: daily water and sodium intakes and plasma volume changes after Dex and DOCA injections

The analysis used baseline/treatment condition and days as within-subject repeated measures and drug (DOCA, Dex, DOCA+Dex) as the between-subject measure. Catheter patency was difficult to maintain over extended testing. Therefore, treatment periods were truncated at 3 days; for statistical purposes, the last 3 days of baseline were used for comparisons with the 3 days of treatment. The results for fluid intake and body weight were similar to those found in *experiment 3*. Baseline body weights, water intakes, and 0.3 M NaCl intakes were equivalent among groups. During treatment, body weights of both Dex-treated groups were significantly reduced compared with baseline values and to body weights of DOCA-treated animals (3-way interaction, $F_{4,24} = 56.48$, $P < 0.001$; Fig. 6). Rats that received only Dex lost more weight by *days 2* and *3* of treatment than rats that received DOCA+Dex. A significant three-way interaction indicated that all groups increased ingestion of 0.3 M NaCl on some days of treatment compared with baseline days ($F_{4,24} = 9.30$, $P < 0.001$). Dex administered by itself significantly increased 0.3 M NaCl intakes on the first day of treatment. DOCA administered by itself increased 0.3 M NaCl intakes compared with that shown at baseline on all days and compared with intakes of Dex-treated animals on *days 2* and *3* of treatment. Intakes of 0.3 M NaCl during DOCA+Dex were significantly increased compared with that shown at baseline and compared with intakes of DOCA-treated animals on all treatment days. Water intakes were increased on the first day of treatment when collapsed across groups (interaction, $F_{2,24} = 4.26$, $P < 0.05$). Otherwise, there were no significant effects on water ingestion. A significant interaction revealed differences in hematocrit across groups during baseline ($F_{2,12} = 4.67$, $P < 0.05$). Average baseline hematocrits for DOCA, Dex, and DOCA+Dex groups were 45.4 ± 1.1 , 44.3 ± 1.9 , and $42.9 \pm 1.7\%$, respectively. Subsequently, hematocrit was significantly reduced during DOCA treatment but was not affected by Dex administered either alone or together with DOCA. On the basis of hematocrit level (31,32), DOCA increased relative plasma volume by 11.3% on the first day of treatment and by an average of 15.3% across 3 days of treatment. Relative plasma volume increased only 2.7 and 3.1% during treatment with Dex and DOCA+Dex, respectively, compared with average baseline values.

DISCUSSION

Systemic injections of the glucocorticoid agonist Dex greatly increased urinary excretion of water and sodium in animals that had access only to water to drink and with no food available as a source of sodium. Under these conditions, Dex treatment resulted in negative water and sodium balances and weight loss, even when coadministered with DOCA. In animals with 0.3 M NaCl solution to drink, systemic injections of Dex transiently stimulated salt appetite and produced negative water and sodium balances because of greatly increased water and sodium excretion. However, when Dex was coadministered with DOCA, the resulting salt appetite was greater than that observed after DOCA alone. This “potentiation” of the salt appetite was not because of net sodium loss, as sodium balances remained positive. However, urinary excretion of water outpaced the increased fluid ingestion, resulting in negative water balance and weight loss, indicating loss of water from the body. Lastly, measurements of hematocrit levels indicated expansion of plasma volume during DOCA treatment but not when Dex was administered with DOCA or alone. This suggests the possibility that glucocorticoid treatment permits greater ingestion of water, and especially sodium, by limiting volume expansion that occurs with DOCA alone.

Dex treatment had significant effects on urinary excretion in both experiments (*experiments 1 and 2*) in which animals were without food and had only water to drink. The large increases in water, sodium, and potassium excretions appear to be the direct effect of Dex on renal function. Urine volume increased within hours of administration, whereas water intake remained unchanged. There were no sources of sodium and potassium, so increased excretion of all three substances was not secondary to their increased ingestion. These results are in line with the known ability of glucocorticoids to increase glomerular filtration rate (1). Notably, greatly increased urinary excretion of all three substances was also observed when Dex was coadministered with DOCA, and the effects were nearly as great as those observed after Dex alone. Thus, in animals without food to eat and only water to drink, the effect of Dex treatment, with or without DOCA treatment, is loss of water, sodium, and potassium, resulting in substantial negative balances for all three substances. The lack of food availability, resulting in reduced need to drink water, probably accounts for some of the weight loss during testing, but Dex and DOCA+Dex treatments caused significantly greater weight loss than vehicle or DOCA alone. Therefore, most of the acute weight loss after treatments involving Dex is attributable to reduced body water after increased water excretion.

In *experiments 3 and 4*, involving repeated daily injections of drugs and continuous access to water, 0.3 M NaCl solution, and sodium-deficient diet, there were clear treatment effects on handling of water and sodium. During Dex treatment, there was only a transient increase in sodium consumption. Sodium and water ingestion were less than their excretion, so both balances were negative. These animals lost weight. Daily injections of DOCA caused a typical progressive increase in salt appetite. Sodium and water ingestion were greater than their excretion, so both balances were positive and the animals gained weight. During DOCA+Dex treatment, there was an immediate fivefold increase in sodium intake that exceeded sodium loss. However, overall water ingestion from both water and saline was less than water excretion. Therefore, although sodium balance remained positive, water balance was always negative. These animals also lost weight. The data do not indicate whether the greatly increased ingestion of sodium during DOCA+Dex treatment followed, or preceded, increased excretion of water and sodium. However, the results from the first two experiments suggest that increased renal excretion of water and sodium likely began within hours after the first injection.

Suppression of food intake is a well-known consequence of Dex treatment and is partly responsible for the observed weight loss (9,10,16,25). However, weight loss after Dex treatment precedes the suppression of food intake (*experiment 3*; Refs. 9,10) and is much greater than the weight loss of pair-fed controls (16), suggesting that other mechanisms, including catabolism of muscle tissue (16), contribute to the weight loss. In this regard, the pronounced diuresis and negative water balance within hours of Dex treatment likely explain the large initial reductions in body weight (also see Ref. 11), with later reductions in weight due to a combination of reduced food intake, catabolism, and continuing negative water balance.

Hematocrit was significantly reduced over 3 days of DOCA treatment (*experiment 4*). The hematocrit data indicate a substantial (~15%) increase in plasma volume during DOCA treatment, presumably due to retention of water and sodium. Neither group of animals that received Dex had significant changes in hematocrit or, presumably, plasma volume during treatment. The apparent loss of body water evidenced by decreased water balance, without reduction in plasma volume, probably means water shifted from the cells to the extracellular space during Dex treatment. In an experiment that used procedures and doses similar to those employed here, Shelat et al. (22) measured hematocrit in rats after 5 days of DOCA, Dex, and DOCA+Dex administration. Their results indicated plasma volume expansion after DOCA, plasma volume contraction after Dex, and no change in plasma volume after DOCA +Dex. Thus, in both their study and ours, repeated administration of DOCA resulted in plasma volume expansion that was not seen when Dex was coadministered with DOCA. It seems likely that Dex treatment prevents increases in plasma volume when coadministered with DOCA by promoting increased urine volume that may, in part, counter the usual water and sodium retention during DOCA (14).

In all experiments, Dex administered by itself increased water and sodium excretion, resulting in negative water and sodium balances and substantial weight loss. The loss of water and sodium and reduction in body weight observed during testing are consistent with volume contraction. Indeed, volume contraction is a consequence of long-term glucocorticoid administration (13,21,29). Combining Dex with DOCA at the current doses also resulted in water and sodium loss and reduced body weight in animals lacking an additional source of sodium for ingestion. This contrasts markedly with the comparative water and sodium retention after DOCA by itself (*experiment 2*). When sodium was available for drinking, rats receiving the combination of DOCA+Dex drank enough sodium to maintain positive sodium balance. Dex treatment did not stimulate more than a transient salt appetite when saline solution was available for drinking. These results suggest that the stimulus for salt appetite was provided by the mineralocorticoid DOCA and that the glucocorticoid Dex merely augmented the response but did not itself stimulate salt appetite.

In these chronic experiments, Dex greatly increased urinary water excretion with little change in water intake, resulting in negative water balance. Sometimes, the negative water balances were large and persistent. It seems surprising that Dex-treated animals did not increase water intake to compensate for the increased urinary excretion of water. Increased excretion of water without change in water intake during glucocorticoid administration has been noted by others (9,11,27). In experiments lasting <24 h, it has been suggested that the glucocorticoid-induced diuresis does not sufficiently reduce extracellular volume to trigger reflex drinking (27). For chronic experiments lasting days, we can speculate that the presumed shift of water from the cells to the extracellular space during glucocorticoid treatment somehow blunts generation of a significant thirst stimulus.

In short-term experiments, systemic administration of Dex increases water drinking in response to ANG II administered either systemically or centrally (9,27). This increased

drinking response is a specific glucocorticoid-mediated effect, is dose related, and is maximal when ANG II is administered 3–6 h after Dex. Sumners et al. (27) suggested that increased urinary excretion after Dex treatment might promote drinking in response to ANG II. They noted that Dex-treated animals excreted somewhat more water than they drank in response to ANG II during a 2-h test. They did not measure urine in the hours after Dex injection and before ANG II. The present data (*experiments 1* and *2*) indicate that Dex also likely caused significant urinary excretion in the hours before the ANG II test, thus bolstering their idea that increased water loss from Dex administration promotes additional water drinking to ANG II.

Hypovolemic treatments that stimulate thirst and salt appetite are associated with increased levels of circulating corticosterone in addition to aldosterone and angiotensin (19,26). Pharmacological doses of glucocorticoids increase sodium ingestion by mineralocorticoid-treated animals (8,17,22,33,35). It has been postulated that the powerful influence of glucocorticoids on salt appetite elicited by mineralocorticoids reflects a synergy between these classes of steroid hormones at the cellular level (3,8). Thus the “synergy” hypothesis of salt appetite (4,7) now includes prominent roles for glucocorticoids and their receptors (6,8). In the brain, glucocorticoids increase ANG II receptor binding while amplifying ANG II-related intracellular signaling processes (3,6,22–24,27) and also increase binding to type I mineralocorticoid receptors (8,17,35). Dex in combination with DOCA increases ANG II binding at angiotensin type 1 (AT₁) receptors in brain areas involved in mediating responses to circulating ANG II, including the subfornical organ, area postrema, and paraventricular nucleus (22). However, at the cellular level, no synergy between glucocorticoids and mineralocorticoids affecting AT₁ binding has been found (24).

The present results support another explanation for the ability of glucocorticoids to potentiate thirst and salt appetite. By promoting water and sodium excretion and reducing water balance, Dex appears to limit or prevent volume expansion during DOCA treatment. Volume-related signals have been shown to inhibit water drinking and sodium ingestion (12,30). Wolf (33) first suggested that glucocorticoids might increase sodium intake by facilitating the excretion of ingested sodium. Accordingly, the accumulation of body sodium, as occurs with DOCA, might progressively inhibit sodium ingestion; thus facilitation of sodium excretion by Dex would diminish this inhibitory effect. DOCA-treated animals in the present studies clearly retained water and sodium and became volume expanded. This was reflected by their increased water and sodium balances, weight gain, and reduced hematocrit. With the addition of Dex to DOCA treatment, there was a net loss of water and no significant change in plasma volume levels and, therefore, little volume-related “braking” mechanism to slow ingestive behavior. Note that it is not necessary for Dex to produce negative sodium balances to permit greater sodium ingestion. Indeed, previous work by Ma et al. (17) noted that glucocorticoid enhancement of sodium ingestion is not accompanied by net sodium loss. Rather, sodium balance remains positive during combined administration of DOCA+Dex. The present results (*experiment 3*) confirm this observation.

Perspectives

Glucocorticoids have been shown to possess powerful influences on the ingestion of water and sodium. The mechanisms for these effects have not been fully elucidated but have been suggested to involve actions on glucocorticoid receptors in the brain to affect central binding of ANG II and mineralocorticoids. The present work experimentally addresses an alternative mechanism by which glucocorticoids may potentiate fluid ingestion. The results support the idea that the powerful renal effects of glucocorticoids permit further ingestion of water and sodium by removing volume-related “braking” of water and sodium intake through increased excretion of both substances. Release from volume-related inhibition may also

explain why certain brain lesions permit greater salt appetite responses to DOCA (5), i.e., by disrupting central pathways essential to the volume-related inhibition of behavior.

Acknowledgments

GRANTS

This work was supported by National Institute on Aging Grant AG-025465 and National Institute of Mental Health Grant MH-59239 to R. L. Thunhorst and National Heart, Lung, and Blood Institute Grant HL-14388 and National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-066086 to A. K. Johnson.

References

1. Baylis C, Handa RK, Sorkin M. Glucocorticoids and control of glomerular-filtration rate. *Semin Nephrol* 1990;10:320–329. [PubMed: 2200095]
2. Braun-Menendez E. Aumento del apetito específico para la sal provocado por la desoxicorticosterona: sustancias que potencian o inhibiten esta acción. *Rev Soc Arg Biol* 1952;28:23–32.
3. Daniels D, Fluharty SJ. Salt appetite: a neurohormonal viewpoint. *Physiol Behav* 2004;81:319–337. [PubMed: 15159174]
4. Epstein AN. Mineralocorticoids and cerebral angiotensin may act together to produce sodium appetite. *Peptides* 1982;3:493–494. [PubMed: 7122274]
5. Fitts DA. Effects of lesions of the ventral ventral median preoptic nucleus or subfornical organ on drinking and salt appetite after deoxycorticosterone acetate or yohimbine. *Behav Neurosci* 1991;105:721–726. [PubMed: 1667732]
6. Fluharty, SJ. The neuroendocrinology of body fluid homeostasis. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain, and Behavior*. Vol. 1. Amsterdam: Academic; 2002. p. 525–570.
7. Fluharty SJ, Epstein AN. Sodium appetite elicited by intracerebroventricular infusion of angiotensin II in the rat. II. Synergistic interaction with systemic mineralocorticoids. *Behav Neurosci* 1983;97:746–758. [PubMed: 6639747]
8. Fluharty SJ, Sakai RR. Behavioral and cellular analysis of adrenal steroid and angiotensin interactions mediating salt appetite. *Prog Psychobiol Physiol Psychol* 1995;16:177–212.
9. Ganesan R, Sumners C. Glucocorticoids potentiate the dipsogenic action of angiotensin II. *Brain Res* 1989;499:121–130. [PubMed: 2804659]
10. Grigson PS, Johnson DF, Collier GH, Flaherty CF. The effect of dexamethasone-21-acetate on meal size, meal frequency, and macronutrient self-selection in rats. *Physiol Behav* 1989;46:211–216. [PubMed: 2602461]
11. Haack D, Mohring J, Mohring B, Petri M, Hackenthal E. Comparative study on development of corticosterone and DOCA hypertension in rats. *Am J Physiol Renal Physiol* 1977;233:F403–F411.
12. Kaufman S. Role of right atrial receptors in the control of drinking in the rat. *J Physiol* 1984;349:389–396. [PubMed: 6737298]
13. Kenyon CJ, Brown WB, Fraser R, Tonolo G, McPherson F, Davies DL. Effects of dexamethasone on body fluid and electrolyte composition of rats. *Acta Endocrinol* 1990;122:599–604. [PubMed: 2353557]
14. Kenyon CJ, Saccoccio NA, Morris DJ. Glucocorticoid inhibition of mineralocorticoid action in the rat. *Clin Sci (Colch)* 1984;67:329–335. [PubMed: 6380888]
15. Lucas LR, Pompei P, McEwen BS. Salt appetite in salt-replete rats: involvement of mesolimbic structures in deoxycorticosterone-induced salt craving behavior. *Neuroendocrinology* 2000;71:386–395. [PubMed: 10878500]
16. Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, Gonzalez-Cadavid N, Arias J, Salehian B. Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. *Am J Physiol Endocrinol Metab* 2003;285:E363–E371. [PubMed: 12721153]

17. Ma LY, McEwen BS, Sakai RR, Schulkin J. Glucocorticoids facilitate mineralocorticoid-induced sodium intake in the rat. *Horm Behav* 1993;27:240–250. [PubMed: 8349282]
18. Okuno T, Suzuki H, Saruta T. Dexamethasone hypertension in rats. *Clin Exp Hypertens* 1981;3:1075–1086. [PubMed: 7026195]
19. Rowland NE, Morian KR. Roles of aldosterone and angiotensin in maturation of sodium appetite in furosemide-treated rats. *Am J Physiol Regul Integr Comp Physiol* 1999;276:R1453–R1460.
20. Sakai RR, Ma LY, Zhang DM, McEwen BS, Fluharty SJ. Intracerebral administration of mineralocorticoid receptor antisense oligonucleotides attenuate adrenal steroid-induced salt appetite in rats. *Neuroendocrinology* 1996;64:425–429. [PubMed: 8990075]
21. Severino C, Brizzi P, Solinas A, Secchi G, Maioli M, Tonolo G. Low-dose dexamethasone in the rat: a model to study insulin resistance. *Am J Physiol Endocrinol Metab* 2002;283:E367–E373. [PubMed: 12110544]
22. Shelat SG, King JL, Flanagan-Cato LM, Fluharty SJ. Mineralocorticoids and glucocorticoids cooperatively increase salt intake and angiotensin II receptor binding in rat brain. *Neuroendocrinology* 1999;69:339–351. [PubMed: 10343175]
23. Shelat SG, Fluharty SJ, Flanagan-Cato LM. Adrenal steroid regulation of central angiotensin II receptor subtypes and oxytocin receptors in rat. *Brain Res* 1998;807:135–146. [PubMed: 9757019]
24. Shelat SG, Flanagan-Cato LM, Fluharty SJ. Glucocorticoid and mineralocorticoid regulation of angiotensin II type 1 receptor binding and inositol triphosphate formation in WB cells. *J Endocrinol* 1999;162:381–391. [PubMed: 10467229]
25. Simpson CW, Dicara LV, Wolf G. Glucocorticoid anorexia in rats. *Pharmacol Biochem Behav* 1974;2:19–25. [PubMed: 4828480]
26. Stricker EM, Vagnucci AH, McDonald RH Jr, Leenen FH. Renin and aldosterone secretions during hypovolemia in rats: Relation to NaCl intake. *Am J Physiol Regul Integr Comp Physiol* 1979;237:R45–R51.
27. Sumners C, Gault TR, Fregly MJ. Potentiation of angiotensin II-induced drinking by glucocorticoids is a specific glucocorticoid Type II receptor (GR)-mediated event. *Brain Res* 1991;552:283–290. [PubMed: 1913190]
28. Suzuki H, Handa M, Kondo K, Saruta T. Role of renin-angiotensin system in glucocorticoid hypertension in rats. *Am J Physiol Endocrinol Metab* 1982;243:E48–E51.
29. Tonolo G, Fraser R, Connell JM, Kenyon CJ. Chronic low-dose infusions of dexamethasone in rats: effects on blood pressure, body weight and plasma atrial natriuretic peptide. *J Hypertens* 1988;6:25–31. [PubMed: 2965178]
30. Toth E, Stelfox J, Kaufman S. Cardiac control of salt appetite. *Am J Physiol Regul Integr Comp Physiol* 1987;252:R925–R929.
31. Van Beaumont W. Evaluation of hemoconcentration from hematocrit measurements. *J Appl Physiol* 1972;32:712–713. [PubMed: 5038863]
32. Van Beaumont W. Red cell volume with changes in plasma osmolarity during maximal exercise. *J Appl Physiol* 1973;35:47–50. [PubMed: 4716160]
33. Wolf G. Effect of deoxycorticosterone on sodium appetite of intact and adrenalectomized rats. *Am J Physiol* 1965;208:1281–1285. [PubMed: 14301393]
34. Yoshida S, Yamada K, Tamura Y, Yoshida S. Role of the renal nerve in glucocorticoid hypertension. *Endocrinol Jpn* 1988;35:877–884. [PubMed: 3074918]
35. Zhang DM, Epstein AN, Schulkin J. Medial region of the amygdala: involvement in adrenal-steroid-induced salt appetite. *Brain Res* 1993;600:20–26. [PubMed: 8422586]

Fig. 1. Body weight, cumulative sodium and potassium excretions, urine volume, water balance, and water intake for 24 h after injections of vehicle (Veh) or dexamethasone (Dex) in rats. Values are means $\pm$ SE. *Significantly different from Veh, $P < 0.05$.

Fig. 2. Body weight, cumulative sodium and potassium excretions, urine volume, water balance, and water intake for 24 h after injections of deoxycorticosterone acetate (DOCA), Dex, or their combination [DOCA+Dex (“both”)] in rats. Values are means $\pm$ SE. *Significantly different from DOCA, $P < 0.01$. #Significantly different from DOCA+Dex (both), $P < 0.05$.

Fig. 3. Daily total fluid intake, urine volume, water balance (Wat bal), and change in body weight (ΔBW) during treatment with DOCA, Dex, or DOCA+Dex (both). The average water balance over 4 days of baseline was used as the estimate of relative zero balance for each animal. Total fluid intake = water + saline. Values are means $\pm$ SE. *Significantly different from baseline, $P < 0.05$. #Significantly different from baseline and DOCA treatment, $P < 0.05$.

Fig. 4.

Daily sodium intake, excretion, and balance and daily food intake during treatment with DOCA, Dex, DOCA+Dex (both). The average sodium balance over 4 days of baseline was used as the estimate of relative zero balance for each animal. Values are means $\pm$ SE.

*Significantly different from baseline average, $P < 0.05$. #Significantly different from baseline and from DOCA treatment, $P < 0.05$.

Fig. 5.

Cumulative sodium and water balance and cumulative body weight change during treatment with DOCA, Dex, DOCA+Dex (both). Values are means $\pm$ SE. *Significantly different from baseline, $P < 0.05$. #Significantly different from baseline and from DOCA treatment, $P < 0.05$.

Fig. 6.

Daily 0.3 M NaCl and water intake, change in hematocrit, and cumulative change in body weight during treatment with DOCA, Dex, or DOCA+Dex (both). Values are means $\pm$ SE.

*Significantly different from baseline, $P < 0.01$. #Significantly different from baseline and from DOCA, $P < 0.01$.