Effects of fludrocortisone on water and sodium intake of C57BL/6 mice

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1Department of Psychological and Brain Sciences, University of Iowa, Iowa City, Iowa; 2Department of Health and Human Physiology, University of Iowa, Iowa City, Iowa; 3Department of Pharmacology, University of Iowa, Iowa City, Iowa; and 4François M. Abboud Cardiovascular Center, University of Iowa, Iowa City, Iowa

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Johnson RF, Beltz TG, Johnson AK, Thunhorst RL. Effects of fludrocortisone on water and sodium intake of C57BL/6 mice. Am J Physiol Regul Integr Comp Physiol 309: R247–R254, 2015. First published May 27, 2015; doi:10.1152/ajpregu.00033.2015.—Little is known about steroidal control of thirst- and salt-appetite behaviors of mice. The current study investigates effects of fludrocortisone acetate (FCA), a steroid with potent glucocorticoid and mineralocorticoid effects, on thirst- and salt-appetite responses of C57BL/6 mice. Treatment with FCA produced dose-dependent (5, 10, and 25 mg/kg) increases in both magnitude and duration of water and sodium intake. Chronic elevation of water and saline intake was achieved with daily injections of FCA. Daily injection of FCA, when only 0.9% saline was available, produced a remarkably rapid increase in saline intake. A single injection of FCA stimulated brisk diuresis and natriuresis in fluid-restricted animals. This work is the first to demonstrate copious water drinking by mice in response to FCA. The results are discussed in terms of the possibility that the renal effects of FCA promote increases in water and sodium turnover and thereby, increases in water and sodium ingestion.

MICE HAVE BECOME INCREASINGLY important in the investigation of the homeostatic regulation of body fluids because of their range of genetic diversity and the relative ease of manipulating the genome of mice compared with most other mammalian species. There is an evolving body of research about body fluid regulation in mice [reviewed in Blair-West et al. (4), Coble et al. (6), Denton et al. (7), Johnson et al. (14), and Rowland and Fregly (22, 23)], including several reports on the steroidal regulation of salt intake. Several studies have reported that mineralocorticoid (MC), deoxycorticosterone acetate (DOCA), does not induce salt intake in mice (20, 22, 27), but other studies report that DOCA increases salt intake but not water intake (4), or it increases both water and sodium intake (2). In one case, the DOCA effect on salt intake in mice has been suggested to be due to an, as yet poorly understood, interaction of environmentally induced glucocorticoid (GC) elevation and exogenous MCs (21). Even more perplexing, complete adrenalectomy—a procedure that typically induces compensatory salt intake in rats—has little effect on salt intake or salt-taste sensitivity of mice (13, 22).

An insight into the regulation of salt intake in mice may be gained from reports that fludrocortisone acetate (FCA), a synthetic corticosteroid, produces reliable salt intake in mice (27). FCA, also called 9α-fluorohydrocortisone acetate, has both major GC and MC effects; FCA is ∼11 times more potent than cortisone in a liver glycogen assay and approximately five times more potent than DOCA in sodium retention (10). However, the effects of FCA have not been studied extensively in rodents. Early studies report that FCA administration increased water and NaCl intake in rats (30), decreased salt appetite in adrenalectomized rats (9), and increased blood pressure and plasma volume of rats (11). Today, FCA is used therapeutically, mainly for adrenocortical insufficiency and as a first-line treatment for idiopathic orthostatic hypotension (1, 19, 28). When prescribed in the former case, it is generally used in low doses and combined with administration of a specific GC receptor agonist.

The purpose of the present set of studies was to examine the effects of FCA on fluid ingestion and urinary excretion of mice. In agreement with earlier studies (27), the present data confirm that FCA induces salt intake in mice. In addition, we discovered that FCA is a very potent dipsogen in mice, i.e., inducing water drinking in the absence of saline to drink or sodium in the diet. The dose, time course, and chronic treatment parameters are detailed in the present data for the effects of FCA on both water and saline intake. Lastly, FCA produces a brisk diuresis that included increased sodium and potassium excretion in water-restricted mice.

METHODS

Animals and Housing

Adult male C57BL/6J mice (60 days old) were purchased from The Jackson Laboratories (Bar Harbor, ME). Mice were housed individually in plastic basket cages (26 × 20 cm × 13 cm deep) in a temperature (22°C)- and light-controlled room (light:dark, 12:12 h; light onset time, 0600). The plastic basket cages had stainless steel, barred tops with an indented food hopper and a slot for the regular water bottle. Except where noted, the mice had water and food (7013 NIH-31 Modified diet, Teklad; Harlan Laboratories, Indianapolis, IN; i.e., Na+-deficient chow) ad libitum. In some studies, the standard chow was replaced with a sodium-deficient chow (Sodium Deficient Diet Rat Modified; ICN Biomedicals, Aurora OH; i.e., Na+-deficient chow), and tap water was replaced with deionized water. All mice were adapted to the colony for at least 1 wk before experimentation. All experiments were conducted in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and were approved by the University of Iowa Animal Care and Use Committee.

Water- and Saline-Intake Measures

Measures of water and saline intake were read directly from graduated burettes (22 ml capacity with 0.1 ml resolution), with attached stainless steel sipper spouts that protruded ∼5 cm into the cage. To avoid leakage, care was taken so that the tips of the sipper tubes did not contact the litter at the cage bottom. Whenever saline intake was measured, at least 1-day exposure to the saline was allowed before measurement to avoid any novelty effect.
Behavioral Manipulations

General procedures and preliminary studies. The FCA (Sigma-Aldrich, St. Louis, MO) was suspended in sesame oil by sonication and vortexing at a concentration of 0.5–2.5 mg/100 μl and injected 1 μl/g body wt sc (doses of 5–25 mg/kg), using 50- to 100-μl Hamilton syringes fitted with a Luer Lock, 26-gauge, 0.5-in. needle. Care was taken to leave the needle in place and to massage the injection site for several seconds to allow dispersion of the viscous injectate and avoid reflux.

For the preliminary study, the baseline daily (i.e., 24 h) saline (3% NaCl wt/vol) and water intakes of a group of mice (n = 8) were established over 3 days, then a single injection of FCA (25 mg/kg) was given, and intakes were monitored for 6 days. The mice were switched to water and 1.8% saline and allowed 4 days to establish a baseline at the new saline concentration. Then another single injection (25 mg/kg) of FCA was given, and intakes were monitored for 9 days. Mice were then given only water (no saline solution) and placed on a Na+–deficient diet (see above) for 6 days, then 1.8% saline was returned, intakes were monitored for 7 days to establish a baseline, and two injections of FCA (25 mg/kg) were given, 48 h apart, and intakes monitored for 4 days beginning at the first injection.

Latency of onset and time course studies. To establish the time course of the increased ingestion after a single injection of FCA, two new groups of mice (FCA or vehicle, n = 6 each), adapted to water and 1.8% saline, were all initially given a single 25 mg/kg injection of FCA for adaptation to its effects, and 9 days were allowed for recovery. Then the groups received either single injections of FCA or vehicle, 3 h before light offset, and detailed measures of intakes were monitored for 48 h. After 8 days of recovery, the procedure was repeated on the same groups except that the single injections were given 1 h before light offset, and intakes were again monitored for 48 h. Data were analyzed as rate of intakes (i.e., milliliters/hour) for the individual periods using a two-factor mixed ANOVA with time as the repeated measure and treatment as the between factor, followed by mean comparisons between FCA and vehicle-treated group means.

There were separate analyses for the two injection times.

Time-dose response studies. Three separate groups of mice received single injections of FCA to establish the dose and long-term response characteristics of FCA treatments. After baseline daily intakes of water and 1.8% saline for 2 days were established, mice received FCA at different doses (5, 10, and 25 mg/kg, n = 24, 23, and 20, respectively). Daily water and saline intakes were monitored until they apparently recovered to baseline mean intakes. The daily intake data were first analyzed with a two-factor mixed ANOVA (repeated measures on time). If there was a significant interaction, then the data were partitioned by dose. A repeated-measures ANOVA was then conducted to establish significance of FCA effects over time for each dose. This was followed by comparisons of each postinjection, daily mean intake with the respective mean baseline intake.

As an extension of the dose-response studies, a modification of the dose-response protocol was conducted on another four groups of mice (n = 12 each) adapted to water and 1.8% saline. Each group received a single injection of FCA (0, 5, 10, and 25 mg/kg). The saline tube was then removed for 24 h, and water intake was measured for this period. Then saline was returned, and 2-h intakes of water and saline were measured. The 2- and 24-h intakes were analyzed by one-factor ANOVA. During the 2-h test, the saline intake was measured further at 30, 60, and 120 min, and these data were analyzed as rate of intake, i.e., milliliters/30 min, by two-factor ANOVA with time as the repeated factor.

Chronic treatment studies. Three studies were conducted to establish if FCA treatment chronically increased water or saline intake. The first study used two groups receiving bi-daily injections (i.e., injections every other day at about the same time) of FCA (6 mg/kg) or vehicle (n = 6 each), and daily intake of water and 1.8% saline was measured for 9 days. The second study used two new groups receiving daily injections of FCA (10 mg/kg) or vehicle (n = 6 each), with daily water and 1.8% saline intake measured for 8 days. Then the FCA dosage was halved for days 9–10 in an attempt to stabilize intake. A third study used two new groups receiving daily injections of FCA (10 mg/kg) or vehicle (n = 6 each), with only 0.9% saline available to drink for 5 days for blood pressure analyses not included in this study. Separate, two-factor, mixed-design ANOVAs (repeated on the daily measures) were conducted for the water and saline intakes, followed by independent t-tests comparing the FCA and vehicle groups at each daily intake mean.

Sodium deprivation study. This study was performed to determine whether the marked increase in water intake after FCA treatment was secondary to increased sodium intake. Two groups of mice received a single injection of FCA (25 mg/kg) or vehicle (n = 6 each) after 1 day of sodium deprivation (i.e., access only to water and sodium-deficient chow), and daily water intake was monitored for 2 days. Water intakes on the day before (preinjection) and the day after (postinjection) treatment were assessed by two-factor ANOVA with time as the repeated factor.

Urinary excretion study. This study was performed to determine if FCA affected urinary excretion. Mice were placed in metabolic chambers for the collection of urine. Two groups (n = 4 each) received a single injection of FCA (25 mg/kg) or vehicle (isotonic saline), and urine was collected periodically for 24 h. Food, water, and saline were not available during the entire 24 h. Samples of urine were collected and analyzed for sodium and potassium content by ion-specific electrode (1Plus; Nova Biomedical, Waltham, MA). At the end of the collection period, burettes filled with water were attached to the cages, and intakes were measured for 1 h. Data were analyzed as rate of excretion (i.e., milliliters or micromoles/2 h) for the individual periods using a two-factor mixed ANOVA, with time as the repeated measure and treatment as the between factor, followed by mean comparisons between FCA and vehicle-treated group means. Cumulated data were analyzed by one-factor ANOVA.

RESULTS

Preliminary Studies

The results indicated that a single injection of FCA (25 mg/kg) increased average daily intakes from baseline for both water (P < 0.05) and 3.0% saline (P < 0.05; Table 1). The effect on saline intake was larger if 1.8% saline was used. When mice were placed on a Na+-free regimen, the daily intake of both water and 1.8% saline increased dramatically after two bi-daily injections of FCA (25 mg/kg, both P < 0.05). Treatment was stopped after 4 days, because two of eight mice became sick (i.e., appeared lethargic) and died. We also note that after 6 days on the Na+-free regimen (no 1.8% saline), 3/9 injections of FCA (i.e., every other day) of FCA (10 mg/kg) or vehicle (n = 6 each), with daily water and 1.8% saline intake measured for 8 days. Then the FCA dosage was halved for days 9–10 in an attempt to stabilize intake. A third study used two new groups receiving daily injections of FCA (10 mg/kg) or vehicle (n = 6 each), with only 0.9% saline available to drink for 5 days for blood pressure analyses not included in this study. Separate, two-factor, mixed-design ANOVAs (repeated on the daily measures) were conducted for the water and saline intakes, followed by independent t-tests comparing the FCA and vehicle groups at each daily intake mean.

Table 1. Daily water and saline intake after injection of fludrocortisone acetate (FCA) in preliminary work

<table>
<thead>
<tr>
<th>Tmt</th>
<th>Water, ml/day</th>
<th>Saline, ml/day</th>
<th>Water, ml/day</th>
<th>Saline, ml/day</th>
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<tr>
<td></td>
<td></td>
<td>FCA 1</td>
<td></td>
<td>FCA 2</td>
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<tr>
<td></td>
<td></td>
<td>(a)</td>
<td></td>
<td>(b)</td>
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<td></td>
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<td>n = 4</td>
<td>n = 3</td>
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<tr>
<td></td>
<td></td>
<td>0.35 ± 0.2</td>
<td>0.8 ± 0.2*</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b)</td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ± 0.1</td>
<td>0.2*</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c)</td>
<td>(d)</td>
<td>(e)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 (9)</td>
<td>0 (6)</td>
<td>0.3 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.6* ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f)</td>
<td>(g)</td>
<td>(h)</td>
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<tr>
<td></td>
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<td>0.1 (9)</td>
<td>0 (6)</td>
<td>0.6 (10)</td>
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<tr>
<td></td>
<td></td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.6* ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. The treatments (Tmt) include the following: (a) a single injection of FCA with 3% NaCl as saline, (b) a single injection of FCA with 1.8% NaCl as saline, and (c) 2 injections of FCA (i.e., every other day) with 1.8% NaCl as saline. The number of days comprising baseline and post-FCA periods is in parentheses. *P < 0.05, significantly different from baseline values.
saline, Na⁺-deficient diet), the baseline daily intake of 1.8% saline was higher than baseline daily intakes observed when the mice had access to a regular diet (i.e., 0.33% Na⁺ content).

**Onset Studies**

Analysis of the raw intakes after the two injection times (i.e., 3 and 11 h, respectively, before light offset) revealed significant treatment × time interactions both for water ($F_{10, 100} = 5.64, P < 0.001$; $F_{11, 110} = 5.31, P < 0.001$) and saline ($F_{10, 100} = 4.19, P < 0.001$; $F_{11, 110} = 2.78, P < 0.01$) for both injection times (period intakes; Fig. 1). A comparison of means indicated that the effect of FCA for both water and saline intake occurred more robustly sooner after injection at 11 h, compared with 3 h, before light offset. The increase in water intake most clearly preceded the increase in saline intake for the injections made 11 h before light offset. Examination of the plots of the raw water and saline intakes after injections at the two times showed a circadian fluctuation, both in control and FCA groups.

**Dose-Response Studies**

For both daily water and daily saline intake, the main effects of dose (both $F_{2, 64} = 9.89, P < 0.001$) and days (both $F_{5, 310} = 22.78, P < 0.001$) were significant, as were the dose × days interactions (both $F_{10, 310} = 5.26, P < 0.001$). After single injections of FCA, both daily water and saline intake peaked within 1–2 days (Fig. 2). The greatest increase was seen with 25 mg/kg, which also had the most prolonged effect. Although both the 5 and 10 mg/kg doses significantly increased daily intakes of water and saline, there were no clear differences of dose or duration of effect for the two doses. However, at 2 days postinjection, the doses produced significantly different amounts of both water and saline intake.

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**Fig. 1.** Effect of fludrocortisone acetate (FCA) on water and saline intake after a single injection of 25 mg/kg at time 0 h, i.e., 3 h (B, top) or 11 h (B, bottom) before light offset. The beginning of the dark phase (light:dark, 12:12 h) is indicated by the dark bars above the x-axes. For all panels, comparisons of data means are made between groups receiving FCA (closed symbols) or vehicle (open symbols), either 3 h before light offset (squares) or 11 h before light offset (circles). **A:** intakes of water; **B:** intakes of 1.8% saline. A: cumulative intakes. There is a significant divergence (see RESULTS) of cumulative intake over time between FCA and vehicle-treated groups for both water and saline intake at both times of injection. **B:** noncumulative rate of intakes for the measurement periods, individually for the 2 injection times. *P* < 0.05, FCA mean compared with corresponding vehicle mean.
intake by the 3rd day of treatment (days × treatment interaction, both $F_{12, 120} = 11.31$, $P < 0.001$). Furthermore, whereas the bi-daily injection protocol caused a sustained increase in water intake, with a marked increase on the day following each injection, the saline intake increased significantly only on days following the injections and was not detectably different from control intake on days between the injections (Fig. 3).

When mice received daily injections of FCA (10 mg/kg), a significant, sustained, progressive increase of water intake occurred by the 3rd day, and a significant increase of saline intake was detected by the 6th day (days × treatment interaction, both $F_{12, 120} = 5.57$, $P < 0.001$). Both patterns of increase continued until the 8th day postinjection when the daily dosage was halved and the increase in intakes moderated (Fig. 3).

When mice were given the same daily regimen of FCA (10 mg/kg) but with only 0.9% saline available for fluid intake, a very rapid increase in fluid intake occurred (days × treatment interaction, $F_{7, 70} = 59.85$, $P < 0.001$). It should be noted that the attenuation of intake, apparent on days 4 and 5 after the start of daily injections, is an artifact, since the mice had a daily consumption that reached the capacity of their drinking tubes (Fig. 3).

**Sodium Deprivation Study**

When two groups of mice were placed on a Na+-free regimen for 1 day and then received a single injection of FCA (25 mg/kg) or vehicle, the FCA-injected group showed a significantly greater increase in water intake compared with the control group, 1 day after the injection ($F_{1, 10} = 55.15$, $P < 0.001$; Fig. 4).

**Urinary Excretion Study**

When two groups of mice received a single injection of FCA (25 mg/kg) or vehicle, the FCA-injected group had significantly increased rates of urinary excretion of water ($P < 0.001$), sodium ($P < 0.001$), and potassium ($P < 0.01$) for the first 6 h (time × treatment interactions, all $F_{8, 12} = 11.00$; Fig. 5), accompanied by significantly increased urinary sodium ($P < 0.01$) and potassium ($P < 0.005$) concentrations, compared with the control group. The FCA-injected group also had significantly increased, cumulative amounts of water ($P < 0.05$) and potassium ($P < 0.001$) excretion for the entire 24-h period (both $F_{1, 6} = 7.60$; Table 1) compared with the control group. When water was offered at the end of the excretion portion of the test, the FCA-injected group drank significantly more in 1 h than did the control group (main effect, $F_{1, 6} = 25.00$, $P < 0.005$; Table 2).

**DISCUSSION**

In the only other study of fludrocortisone effects on fluid ingestion in mice, Underwood et al. (27) found that FCA dose dependently stimulated ingestion of an aversive saline solution. The present work extended those findings to include in-depth analysis of the phenomenon and also included measures of water drinking and urinary excretion. These results showed, for the first time, that FCA produces copious water intake in mice. When water and saline were offered together, the onset of increased water drinking preceded the onset of increased salt intake. The pattern of both FCA-induced water and FCA-induced saline intake exhibited circadian fluctuations. Both the
magnitude and duration of FCA-induced water and saline intake were dose dependent. The dipsogenic effect did not require the intake of salt. Finally, the present work showed that FCA readily stimulates urinary excretion of water and electrolytes in mice.

Detailed measures showed that the increase in water intake following FCA treatment preceded the increase in saline intake by several hours, which was most clearly seen when FCA treatment occurred 11 h before light offset (Fig. 1). Furthermore, FCA stimulated ingestion of both substances during the time of day (i.e., during the light phase) when mice are least likely to ingest fluids. We note that these mice received repeated dosing with FCA, and therefore, the earlier onset of FCA effects when administered 11 h before the light offset may also have reflected order effects. The fact that little effect of FCA was seen after injections 3 h before light offset, i.e., just before the first dark phase, was unexpected, since the dark phase is the active ingestive period for mice [reviewed in Johnson et al. (14)]. In this regard, the effects of FCA appeared to wax and wane in a circadian pattern of the sort that has been observed for several dipsogenic agents in rats (15, 16). There were peaks of ingestion for both water and saline intake in the dark phase that were exaggerated in the animals receiving FCA. However, there was also a sustained increase in the rate of ingestion during the light phase for FCA-treated mice, which is unusual for mouse ingestive behavior. Interestingly, FCA treatment did not affect the diurnal rhythm of water intake in the rat, although it did change the diurnal rhythm of water and sodium excretion in the rat (11).

It is clear that a single dose of 25 mg/kg produced not only a potent effect but also a very prolonged effect. The biological half-life of FCA in humans is 18–36 h (28), so one might expect slow offset. The slow rise to the peak effect (at 48 h), seen in the dose-response curves, may have been due to the administration of FCA as a subcutaneous suspension in oil (Fig. 2). Most notable of the prolonged effect were the relatively small standard errors in water intake near the peak effect, which increased markedly during the decline of the effect. The latter is indicative of a narrow variation of the onset of effect and a wide variation in the time for recovery, presumably due to individual differences in the clearing of the steroid from the system or other experiential effects. The administration of FCA just before a period of 24 h of saline deprivation effectively stimulated acute salt intake in amounts comparable with those seen after treatment with diuretics and overnight salt deprivation (14) (Fig. 2). However, there was little difference in the efficacy of different doses. For example, when the saline intake during the 2-h test was examined more closely, all doses produced maximal ingestion of saline in the first 30 min of access. In the 24-h period without saline to drink, FCA essentially doubled the water intakes compared with mice treated with vehicle, again, with little difference between the doses.

The first attempt (i.e., preliminary studies; Table 1) to induce a chronic increase in water and salt intake using bi-daily injections of FCA (25 mg/kg), while the mice were on a Na⁺-free diet, produced a rapid and dramatic increase in both

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Fig. 3. The response to chronic FCA injections is shown. Left: mice received bi-daily injections of FCA (6 mg/kg) or vehicle, starting on the day indicated by the arrow. Significant differences of mean intake of experimental vs. control group are indicated at *P < 0.05 (only water intake differs; **water and saline intake differ from control). Middle: mice received daily injections of FCA (10 mg/kg) or vehicle beginning on the day indicated by the arrow. Significant differences are indicated as indicated for left. Right: effect of daily injections of FCA (10 mg/kg) or vehicle beginning on the day indicated by the arrow in mice that had adapted to 0.9% saline as the only available fluid. Significant differences are indicated (*P < 0.05).

Fig. 4. Effect of a single injection of FCA (25 mg/kg) or vehicle on water intake after 1 day of sodium deprivation. The average daily intake for the day before (Pre) and day after (Post) injection is shown. Experimental mean differs significantly from the control mean for *P < 0.05.
water and saline intake. The induced saline intake of nearly 7 ml/day was higher than that seen for any other manipulation, even manipulations that induced similar levels of water intake (e.g., after chronic daily injection of 10 mg/kg). However, although producing striking effects on intake, this manipulation was also physiologically detrimental, since two of eight mice died by the 5th day.

The reduction of the bi-daily dosage to one-quarter (6 mg/kg) and the allowance of access to a regular diet were ineffective at producing chronically high intake (Fig. 3). However, there appeared to be a slow accumulation of effect over the course of the bi-daily injections. This cumulative effect was more clearly evident in the data from the regimen in which mice received daily injections (10 mg/kg). This regimen produced a relatively slow, progressive increase in both water and saline intake that could be maintained and finally stabilized by reducing the daily dosage by one-half. It remains to be shown empirically whether the above procedure might be used to select a particular level of chronic intake.

At the end of the chronic studies involving daily injections, the mice were ingesting approximately their body weight in daily fluid intake. This is in the range of intake seen with the bi-daily injection of 25 mg/kg, a dosage that was detrimental. However, the ingestion was also very high with the daily 10 mg/kg dose, and these mice all survived without apparent adverse effects. Thus it may not have been the volume of intake per se that was pathogenic in the former case. Furthermore, when given access only to 0.9% saline to drink, FCA-treated mice showed a very rapid increase in daily intake without apparent negative, short-term effects. Both of these observations suggest that the mice, even in the face of greatly increased salt and water ingestion, were able to maintain adequate renal function and electrolyte balance.

In both of the chronic FCA administration regimens, the increase in ad libitum salt intake lagged several days behind the increase in water intake (Fig. 3). This may be partly a statistical artifact: the increase in salt intake was a smaller volumetric effect and harder to detect statistically than a change in water intake. There appeared to be a trend of increased saline intake that occurred earlier in the chronic treatment regimens than was detected statistically. It should be noted that a significant increase in both water and saline intake occurred at 24 h in the dose-response studies, at dosages equivalent to the chronic regimen studies, but the dose-response studies were based on four times the number of mice and were therefore statistically more powerful. Nevertheless, the increase in water intake was more than would be expected to be required to dilute the increase in 1.8% saline intake to physiological levels in any of the present studies. When mice were deprived of both dietary sodium and access to 1.8% saline, treatment with FCA still more than doubled the water intake, indicating that water intake was not secondary to a need to dilute ingested sodium (Fig. 4). Given the size of the increase in water intake, it is also difficult to argue that the water intake was secondary to a need to dilute body fluids from excess renal retention of sodium. These results differ from those observed from rats treated with FCA. In rats, FCA causes robust ingestion of hypertonic saline (30), but its effects on water intake are more equivocal (11, 30). Wolf et al. (30) found that in the absence of ambient sodium for ingestion, FCA had no effect on water intake of rats and suggested that the tendency of FCA-treated rats to increase daily water intake was entirely secondary to increased sodium ingestion. Haack et al. (11) observed small decreases in daily water intake of rats during FCA treatment. In contrast, our mice had greatly increased water intake in all circumstances, including in the absence of ambient sodium for ingestion. Therefore, water intake in response to FCA in the mouse cannot simply be due to increased sodium ingestion.

Injections of FCA rapidly produced urinary excretion of water, sodium, and potassium in mice not permitted to drink and therefore, without the confounding influence of ingested water (Fig. 5). The acute urinary response to FCA was striking: fully one-half of the volumes of water, sodium, and potassium was collected in the first 6 h after administration. These volumes constituted a fourfold increase in water excretion and

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Table 2. Cumulative 24-h urinary water, sodium, and potassium excretion after a single injection of FCA and water drinking after the test

<table>
<thead>
<tr>
<th>Tmt</th>
<th>n</th>
<th>24-h UVol, ml</th>
<th>24-h UNaV, µmol</th>
<th>24-h UKV, µmol</th>
<th>Water intake, ml</th>
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<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>1.50 ± 0.1</td>
<td>103 ± 13</td>
<td>156 ± 8</td>
<td>0.10 ± 0.1</td>
</tr>
<tr>
<td>FCA</td>
<td>4</td>
<td>1.94 ± 0.1*</td>
<td>125 ± 12</td>
<td>213 ± 10*</td>
<td>0.35 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Water intake is for 1 h immediately after the urine collection period. UVol, urine volume; UNaV, urinary sodium excretion; UKV, urinary potassium excretion. *P < 0.05, significantly different from controls (main effect of treatment).
eightfold increases in sodium and potassium excretion compared with measures from vehicle-treated mice. The time course of these urinary effects indicated that FCA-treated mice were in negative water balance relative to vehicle-treated mice throughout the 24-h test. The increased potassium loss of FCA-treated mice suggests that the negative water balance was largely due to loss of intracellular water. Notably, Haack et al. (11) provide evidence in the rat that FCA causes negative water balance and a compartmental shift of water from the intracellular to extracellular space. Therefore, it seems reasonable to conclude that the increased water intake by FCA-treated mice at the end of the test likely reflected increased cellular dehydration-induced thirst.

The renal effects of FCA may aid in explaining some aspects of the behavioral results observed in the present work. For example, FCA may have stimulated increases in water drinking before increases in sodium ingestion because water was preferentially excreted under the influence of FCA, as shown by the initial large volumes of dilute urine after FCA (Fig. 5). The increased water drinking by sodium-restricted mice in two studies may similarly reflect increased urinary water loss following FCA (Figs. 2 and 4). The increased water drinking of FCA-treated mice when ambient sodium was not available indicates that there is something more to the response than simply drinking to dilute excess sodium from consumption. Given that the urinary loss of water caused by FCA is relatively greater than loss of solute, the increased water drinking is likely to be, in part, secondary to increased urinary water loss. We cannot exclude the possibility that the increased water drinking is completely due to the increased water loss during FCA. Although somewhat speculative, it is reasonable to posit that renal actions of FCA were responsible for the increased fluid turnover of mice, daily ingesting nearly their body weight in water and saline during chronic FCA treatment. By increasing urinary excretion of water and sodium, the renal effects of FCA may have minimized inhibition of drinking behavior from volume-related signals, thereby permitting further increased ingestion of both.

Part of the difficulty in determining the nature of the steroidal effects is the ambiguous nature of many of the adrenocortical steroids in terms of reactivity with MC and GC receptors. Underwood et al. (27) and others (20, 22) noted that several strains of the mouse are refractory to the salt appetite-inducing effects of DOCA that are readily observed in the rat (18, 23, 25, 26, 29). Accordingly, the ability of FCA to stimulate salt appetite—notably, in mice that lacked salt appetite in response to DOCA (27)—was ascribed to the relatively greater GC properties of FCA compared with DOCA. Indeed, it has been suggested that mice may require a summation or synergy of the actions of MCs with GCs to express salt appetite (27). In the rat, the coadministration of a GC compound (e.g., dexamethasone) greatly potentiates the salt-appetite response to DOCA (26, 29) and also to aldosterone (18). The “potentiation” of MC-induced salt appetite by GCs has been attributed to presumed central GC effects. For example, dexamethasone increases brain levels of MC and responsiveness to angiotensin II (8, 24, 25). Alternatively, GCs can effectively counter the sodium-retaining properties of MCs by greatly increasing the glomerular filtration rate (GFR) and urine volume (3, 12, 17, 26) and thereby, causing substantial renal loss of sodium and water (17, 26) and volume contraction (26). In this regard, FCA loses its antinatriuretic effects while stimulating an increased GFR and sodium excretion at high doses (5, 11). Notably, in the present work, a single injection of 25 mg/kg FCA rapidly stimulated diuresis with significant loss of sodium in mice. Thus FCA could promote salt appetite via renal effects, similarly to dexamethasone (26), although analogous studies in mice have yet to be performed.

**Perspectives and Significance**

The increasing use of mice to investigate the homeostatic regulation of body fluids invites comparison of their behavior with that of rats. In this context, it has seemed relatively more difficult to elicit salt appetite from mice by procedures (e.g., MC treatment, adrenalectomy) that readily stimulate salt appetite in rats. The present work confirmed that FCA potently stimulates salt appetite in C57BL/6 mice, a response that has been attributed to the pronounced GC effects of FCA (27). In addition, FCA produced copious water drinking that preceded, and did not depend on, increased salt intake. The marked increase in urinary excretion of water, sodium, and potassium after FCA administration is highly suggestive of a role for these renal effects in the exaggerated intake of both water and sodium by FCA-treated mice. Together, the results support the suggestion (27) that mice are relatively more dependent on the actions of GCs for the robust expression of salt appetite than are rats. These findings suggest the intriguing possibility that the failure of mice to increase salt ingestion following adrenalectomy (13, 22) may be due to impaired renal function from loss of endogenous GCs.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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