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Role of plasma arginine vasopressin in the impaired water diuresis of isolated glucocorticoid deficiency in the rat

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Role of plasma arginine vasopressin in the impaired water diuresis of isolated glucocorticoid deficiency in the rat. The role of antidiuretic hormone in the impaired water excretion of isolated glucocorticoid deficiency was investigated in the thyroxin-replaced, anterior hypophysectomized rat. Hypophysectomized rats demonstrated marked impairment in the excretion of an oral water load (32 ± 4% vs. 94 ± 4%), plasma hypoosmolality (281 ± 2 vs. 289 ± 2 mOsm/kg), and hyponatremia (129 ± 1 vs. 140 ± 1 mEq/liter) compared to controls (all $P < 0.001$). These defects were associated with increased levels of plasma arginine vasopressin (3.05 ± 0.45 vs. 1.38 ± 0.11 pg/ml; $P < 0.001$). Following physiologic corticosterone replacement in hypophysectomized rats, the percentage of the water load excreted, free water clearance, plasma sodium, plasma osmolality, and circulating levels of plasma arginine vasopressin were all restored to control values. This correction of water diuresis occurred in the absence of changes in GFR or solute excretion. The defect in water excretion and the elevation of plasma arginine vasopressin could not be corrected by chronic extracellular volume expansion in the absence of glucocorticoid replacement. It is concluded that (*a*) increased secretion of vasopressin plays an important role in the impaired water diuresis of isolated glucorticoid deficiency; (*b*) physiologic corticosterone replacement corrects both the impaired water excretion and the increased secretion of vasopressin associated with glucocorticoid deficiency; and (*c*) a volume-independent nonosmotic stimulus to vasopressin secretion may be activated by the chronic absence of glucocorticoid hormones.

Rôle de l'arginine vasopressine du plasma dans l'incapacité de diluer l'urine au cours du déficit isolé en glucocorticoïdes chez le rat. Le rôle de l'hormone antidiurétique dans l'incapacité à diluer l'urine au cours du déficit isolé en glucocorticoïdes a été étudié chez des rats ayant subi une hypophysectomie antérieure et compensés par de la thyroxine. Les rats hypohysectomisés ont un déficit important de l'excrétion d'une charge en eau (32 ± 4% contre 94 ± 4%), une hypoosmolalité plasmatique (281 ± 2 contre 289 ± 2 mOsm/kg), et une hyponatrémie (129 ± 1 contre 140 ± 1 mEq/liter), par comparaison aux contrôles ($P < 0,001$). Ces modifications sont associées à des concentrations plasmatiques élevées d'arginine vasopressine (3,05 ± 0,45 contre 1,38 ± 0,11 pg/ml; $P < 0,001$). L'administration de corticostérone aux rats hypophysectomisés ramène aux valeurs contrôles le pourcentage de la charge d'eau excrétée, la clearance de l'eau libre, la natrémie, l'osmolalité plasmatique et la concentration circulante d'arginine vasopressine. Cette restauration de la diurèse aqueuse est observée en l'absence de modifications du débit de filtration glomérulaire ou du débit urinaire de substances dissoutes. Le déficit d'excrétion de l'eau et l'augmentation de l'arginine vasopressine du plasma ne peuvent pas être corrigés par une expansion chronique du volume extra-cellulaire en l'absence de traitement substitutif par les glucocorticoïdes. Il est conclu que: (*a*) l'augmentation de la sécrétion de vasopressine joue un rôle important dans l'incapacité de diluer les urines observée au cours du déficit en glucocorticoïdes; (*b*) un traitement substitutif par des doses physiologiques de corticostérone corrige à la fois le déficit d'excrétion de l'eau et la sécrétion excessive de vasopressine associés au déficit en glucocorticoïdes; et (*c*) un stimulus, non osmotique et indépendant du volume extracellulaire, de la sécrétion de vasopressine peut être activé par l'absence chronique d'hormones glucocorticoïdes.

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Impaired excretion of water by the kidney is a long-recognized characteristic of primary and secondary adrenal insufficiency [1–6]. Although this phenomenon has been investigated extensively [5–17], the precise pathophysiology of this defect has not been defined. Major controversy concerning the primary causal factor has focused on the possible role of increased secretion of antidiuretic hormone (ADH) versus a direct effect on water permeability of the distal nephron.

The recent observation that ethanol administration restores water excretion to normal in patients with anterior hypopituitarism and glucocorticoid deficiency has provided indirect evidence of increased secretion of ADH [5, 6]. Measurements of plasma vasopressin by bioassay in patients and animals with adrenal insufficiency have yielded, however, directly conflicting results [7, 8, 10]. Alternatively, it has been postulated that glucocorticoid deficiency impairs the renal excretion of water by directly increasing the permeability to water of distal nephron segments [7, 11–13]. The demonstration of a defect in water excretion in adrenalectomized

rats with congenital absence of ADH (Brattleboro strain) has been interpreted as indirect evidence in favor of this hypothesis [15].

Previous studies of primary adrenal insufficiency in man and adrenalectomized animals have been limited by problems inherent in the study of combined mineralocorticoid and glucocorticoid deficiency [7–10, 13–15]. In some of these earlier studies [9–11, 13, 15, 17], the use of pharmacologic replacement doses of glucocorticoids, which possess significant mineralocorticoid activity, and the failure to replace mineralocorticoids [8, 10, 14] have further confused identification of the separate effects of the two classes of adrenal steroids on renal water handling. In a more recent study, Boykin et al have shown that following withdrawal of glucocorticoids, adrenalectomized dogs replaced with mineralocorticoids have a marked impairment of water excretion that correlates closely with increased circulating levels of plasma arginine vasopressin [18]. The observed tachycardia and reduced stroke volume, which may have initiated a baroreceptor-mediated release of vasopressin in these animals, was attributed to glucocorticoid deficiency. In this model, the possibility must be considered that combined epinephrine-glucocorticoid deficiency contributed to the observed alteration in hemodynamics because removal of the adrenal medulla decreases circulating levels of plasma epinephrine [19, 20].

In the present study, we have used the thyroxin-replaced anterior hypophysectomized rat to provide another model of glucocorticoid deficiency. Because anterior hypophysectomy also results in the loss of other pituitary hormones, whose effects on water metabolism are unknown, we examined the ability of physiologic doses of corticosterone (which is the naturally occurring glucocorticoid in the rat) to reverse specifically the defect in water metabolism. Because pituitary insufficiency is the most common cause of glucocorticoid deficiency in man [21], the present model would be expected to parallel more closely the abnormalities in water metabolism most commonly encountered in clinical medicine.

Methods

Male Sprague-Dawley rats, each weighing 170 to 190 g, were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) 6 days following anterior hypophysectomy with a retroesophageal surgical approach. This method permits removal of the anterior hypophysis without damage to the posterior lobe [22]. Unoperated litter-mates served as controls. All rats were kept in individual metabolic cages and were allowed free access to a standard laboratory diet (Purina Rat Chow, Ralston Purina Co., St. Louis, Missouri) and tap water. Beginning 7 days following surgery, we replaced all hypophysectomized rats daily with thyroxin (2 μg/100 g body wt, i.p.; L-thyroxin, Sigma Chemical Co., St. Louis, Missouri). Body weight was recorded in all animals every 7 days.

Determining baseline renal water excretion following anterior hypophysectomy. On day 14, a baseline measurement of water excretion was determined in 32 thyroxin-replaced hypophysectomized rats. In this and subsequent studies, an oral water load (5% body weight of a 1% dextrose-in-water solution) was administered at 8 A.M. to conscious rats by gastric intubation. Urine was collected under oil, and urine flow and osmolality were measured at hourly intervals from 1 hour before until 3 hours after the water load. Water excretion was expressed as the percent of the water load excreted within 3 hours. Five normal rats were similarly studied at the same time to control for normal variation in water excretion. Hypophysectomized rats excreted 34 ± 3% of the water load in 3 hours compared to 101 ± 4% in controls ($P < 0.001$).

Effects of glucocorticoid deficiency and physiologic corticosterone replacement on renal water excretion, plasma AVP, and plasma corticosterone in hypophysectomized rats (groups 1 to 4). Following baseline studies, all 32 hypophysectomized and 12 control rats were placed in four groups: *group 1*, 12 normal control rats; *group 2*, 18 hypophysectomized rats receiving only thyroxin; *group 3*, 7 hypophysectomized thyroxin-replaced rats receiving 300 μg/100 g body wt corticosterone (Sigma Chemical Co., St. Louis, Missouri) given in two divided doses; *group 4*, 7 hypophysectomized thyroxin-replaced rats receiving 600 μg/100 g body wt corticosterone given in two divided doses.

Hypophysectomized rats were allocated to each of three groups such that mean baseline water excretion (± SEM) was similar in groups 2, 3, and 4. The replacement doses of corticosterone, the predominant naturally occurring glucocorticoid in this species [23], were derived from data indicating a basal corticosterone secretory rate in the rat of 1150 to 1650 μg/100 g body wt per 24 hours [24]. Corticosterone was administered i.p. every 12 hours at 7 A.M. and 7 P.M. to animals in groups 3 and 4. These dosages of corticosterone were calculated intentionally to be in the low physiologic range to minimize the possibility of excessive plasma con-

centrations of corticosterone at the time of water loading (60 to 240 min following the 7 A.M. dose).

Renal water excretion following an oral water load was measured again in all groups on day 21, following 7 days of corticosterone replacement in groups 3 and 4. Twenty-four hour urine collections for the determination of creatinine clearance and sodium excretion were obtained on day 22. On day 23, a third water load was administered. In this study, urine was collected for 90 min, at which time animals were rapidly decapitated by guillotine during water diuresis. Blood issuing from the vessels of the trunk was collected in chilled heparinized tubes and centrifuged at 0° C. Aliquots of plasma were analyzed for sodium and creatinine concentrations and osmolality. The remaining plasma was stored at −20° C and subsequently assayed for corticosterone, thyroxin, and arginine vasopressin. After the animals were sacrificed, both adrenal glands were removed and weighed on an analytical balance (Sartorius 2474, Brinkman Instruments, Inc., Westbury, New York).

Effect of combined administration of mineralocorticoids and glucocorticoids on renal water excretion, GFR, and plasma AVP in hypophysectomized rats (group 5). To exclude the possibility that partial mineralocorticoid deficiency was contributing to the defect in water excretion, we studied a group of 7 thyroxin-replaced hypophysectomized rats during oral water-loading on three different occasions: while receiving no steroid replacement (14 days following hypophysectomy); following 7 days of replacement with corticosterone alone (600 μg/100 g body wt); and following 7 days of combined corticosterone and deoxycorticosterone acetate (250 μg/day) (DOCA; Organon Inc., West Orange, New Jersey) administration (day 28). At the termination of this period, animals were sacrificed during water diuresis, and urine and plasma determinations were performed as described for groups 1 to 4.

Effects of chronic extracellular volume expansion on renal water excretion, plasma AVP, and GFR in glucocorticoid deficient rats (group 6). To assess the effect of extracellular fluid volume expansion alone on water excretion and plasma AVP in glucocorticoid deficiency, we subjected a group of 7 thyroxin-replaced hypophysectomized rats (group 5) to identical oral water loading on two separate occasions: before (day 14) and after (day 28) 2 weeks of extracellular volume expansion. Volume expansion was accomplished by replacing drinking water with normal saline in 2.5% dextrose in water and by the administration of DOCA, 250 μg/day. On day 30, rats were guillotined at 90 min following an oral water load. Plasma and urine samples were processed in a manner identical to groups 1 to 4.

Analytical methods. Plasma and urine osmolality was measured by freezing-point depression (Advanced Instruments, Inc., Needham Heights, Massachusetts); plasma and urine sodium concentration, by flame photometry (Instrumentation Laboratory, Lexington, Massachusetts); and plasma and urine creatinine, by an autoanalyzer (Technicon Corp., Tarrytown, New York) with the Stevens and Sheggs modification of the method of Folin and Wu [25]. Total serum thyroxin was measured by a competitive protein-binding assay in groups 1, 2, and 4 [26]. Plasma corticosterone was measured with a competitive binding radioassay by the method of Murphy [27]. Plasma arginine vasopressin was measured on coded samples by radioimmunoassay [28] with a highly specific antibody to arginine vasopressin that does not crossreact with oxytoxin. Arginine vasopressin in rat plasma is immunologically stable when stored for 4 to 6 weeks under the specific conditions of this study. This assay can measure plasma AVP at concentrations as low as 0.5 pg/ml in sample volumes of 1.0 ml or less.

All statistical computations were performed on an Olivetti desktop computer (model p602). All values represent the mean ± SEM. Data were compared by using the paired and unpaired Student's *t* test as indicated in the Results section.

Results

Baseline water excretion following anterior hypophysectomy. Fourteen days following hypophysectomy, mean baseline water excretion (percent of water load excreted in 3 hours) was 32 ± 3% in hypophysectomized rats receiving only thyroxin replacement. Hypophysectomized animals were then randomly assigned to groups 2 to 4. The mean baseline excretion of the water load was 32 ± 5%, 31 ± 7%, and 32 ± 6% in groups 2, 3, and 4, respectively.

Thyroid and adrenal function and body weight in control and hypophysectomized rats (groups 1 to 4). Serum thyroxin was 5.6 ± 0.3, 6.9 ± 0.7, and 7.7 ± 0.7 μg/dl in groups 1, 2, and 4, respectively. Adrenal weight was 13.3 ± 0.5 mg/100 g body wt in controls (group 1) and decreased to 3.8 ± 0.1 mg in group 2 ($P < 0.001$). A similar reduction in adrenal weight was observed in rats of groups 3 to 6. Plasma corticosterone concentration was 24.6 ± 2.9 μg/dl in control (group 1) compared to 0.8 ±0.2 μg/dl in group 2 rats ($P < 0.001$). Following 1 week of corticosterone replacement in groups 3 and 4, plasma

corticosterone increased to 7.7 ± 2.3 and 8.2 ± 0.9 μg/dl, respectively (both $P < 0.001$); both values are within the normal range of plasma corticosterone (5.35 ± 1.71 mg/dl) at 10 A.M. in the unstressed rat [29]. Control rats (group 1) gained 6 ± 1% body wt per week, whereas hypophysectomized rats (group 2) showed no gain in weight (1 ± 1% weight gain per week). A similar failure to gain weight was also observed in hypophysectomized rats in groups 3 to 6.

Effects of glucocorticoid deficiency and physiologic corticosterone replacement on renal water excretion and renal function (groups 1 to 4, Table 1, Figs. 1 and 2). At 21 days following hypophysectomy, glucocorticoid-deficient animals (group 2) excreted 32 ± 4% of the oral water load within 3 hours, in contrast to 94 ± 4% in control rats ($P < 0.001$). Minimum urine osmolality was 343 ± 55 mOsm/kg in group 2 compared with 98 ± 7 mOsm/kg in controls ($P < 0.001$) (Table 1). During water diuresis, plasma sodium concentration and osmolality were reduced in group 2 animals (129 ± 1 mEq/liter and 281 ± 2 mOsm/kg) compared to controls (140 ± 1 mEq/liter and 289 ± 3 mOsm/kg; both $P < 0.001$). Creatinine clearance (Table 1) decreased following hypophysectomy from 930 ± 53 μl/min/100 g body wt in controls to 651 ± 33 in group 2 ($P < 0.001$). Twenty-four hour sodium excretion was similar in control and group 2 rats. The impairment in water excretion in group 2 rats was associated with a marked reduction in free water clearance from 20 ± 2 μl/min/100 g body wt in controls to −3 ± 2 ($P < 0.001$). Similar changes in fractional free water clearance were observed. Osmolal clearance decreased from 22 ± 2 μl/min/100 g body wt in controls to 13 ± 1 μl/min in group 2 ($P < 0.001$). No reduction was observed, however, when

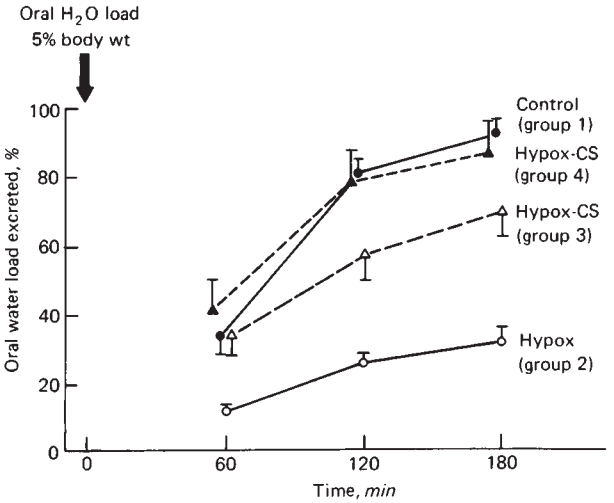

Fig. 1. *Time course of excretion of an oral water load in controls (group 1,* closed circles*), in hypophysectomized rats receiving no glucocorticoid replacement (group 2,* open circles*), and in hypophysectomized rats receiving corticosterone at 300 μg/100 g body wt (group 3,* open triangles*) and 600 μg/100 g body wt (group 4,* closed triangles*).* Group 4 animals were not statistically different from controls at both 120 and 180 min. See Fig. 2 for statistical comparisons between groups at 180 min. *P* values were obtained by unpaired Student's *t* test.

Table 1. Renal function and parameters of water excretion following an oral water load in control and hypophysectomized animals (groups 1 to 4)[a]

Group	*N*	P_{Osm} *mOsm/kg*	P_{Na} *mEq/liter*	Oral water load excreted in 180 min *%*	C_{H_2O} *μl/min/100 g body wt*	C_{H_2O}/GFR *μl/min/ml GFR*	C_{Osm} *μl/min/100 g body wt*	C_{Osm}/GFR *μl/min/ml GFR*	Minimum U_{Osm} *mOsm/kg*	C_{Cr} *μl/min/100 g body wt*	$U_{Na}V$ *μEq/min/100 g body wt*
1 (controls)	12	289 ± 2	140 ± 1	94 ± 4	20 ± 2	23 ± 3	22 ± 2	23 ± 2	98 ± 7	930 ± 53	0.66 ± 0.03
2 (hypox, no CS)	18	281 ± 2	129 ± 1	32 ± 4	−3 ± 2	−4 ± 3	13 ± 1	20 ± 2	343 ± 55	651 ± 33	0.63 ± 0.02
3 (hypox, 300 μg CS)	7	290 ± 2	140 ± 1	71 ± 8	14 ± 3	21 ± 4	18 ± 2	26 ± 3	203 ± 26	690 ± 58	0.74 ± 0.06
4 (hypox, 600 μg CS)	7	294 ± 3	140 ± 2	87 ± 9	16 ± 2	33 ± 3	15 ± 2	34 ± 7	162 ± 14	516 ± 60	0.54 ± 0.06
P values between groups											
1 vs. 2		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	NS
3 vs. 2		<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	NS	<0.05	NS	NS
4 vs. 2		<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS	<0.01	NS	NS
3 vs. 1		NS	NS	<0.025	NS	NS	NS	NS	<0.005	<0.01	NS
4 vs. 1		NS	NS	NS	NS	<0.05	<0.05	NS	<0.005	<0.001	NS

[a] Data are presented as the mean ± SEM; *P* values were obtained using the unpaired Student's *t* test. *N* denotes number of rats. Abbreviations are: hypox, hypophysectomized rats; CS, corticosterone given (per 100 g body wt); P_{Osm}, plasma osmolality; U_{Osm}, urine osmolality.

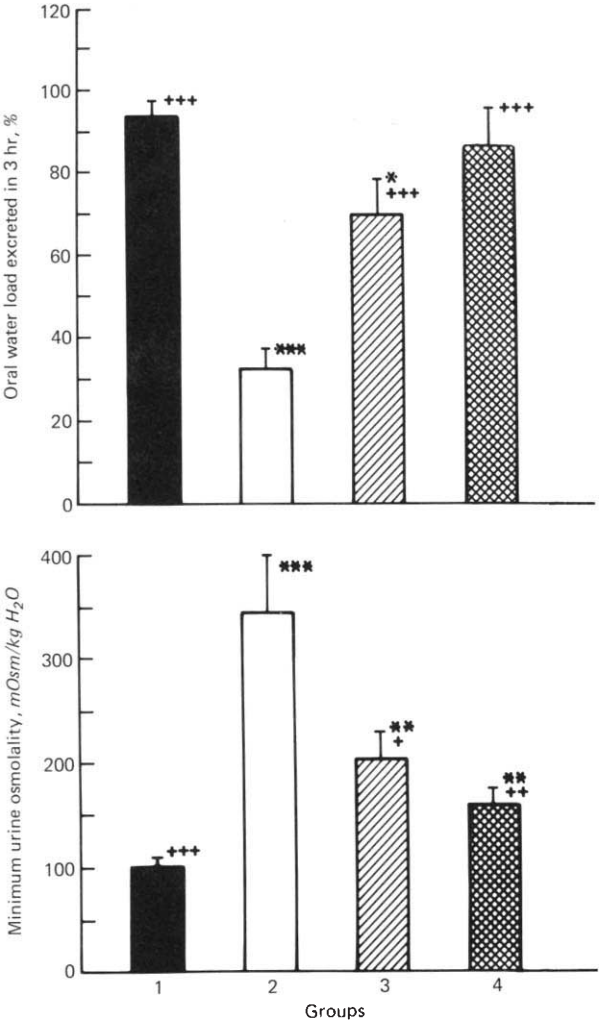

Fig. 2. *Excretion of oral water load and urinary dilution at 180 min in controls (group 1), in hypophysectomized rats receiving no glucocorticoid replacement (group 2), and in hypophysectomized rats receiving corticosterone at 300 μg/100 g body wt (group 3) and 600 μg/100 g body wt (group 4).* Asterisks indicate statistical comparison to group 1 rats (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). *Crosses* indicate statistical comparison to group 2 rats (+ = $P < 0.05$, ++ = $P < 0.01$, +++ = $P < 0.001$). P values were obtained by unpaired Student's t test.

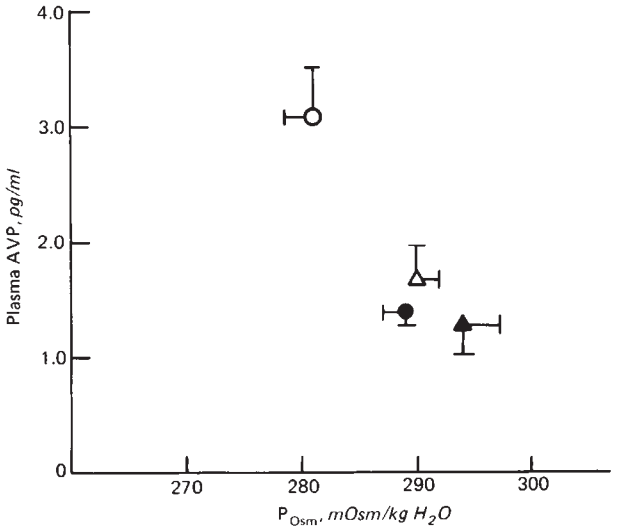

Fig. 3. *Relationship of plasma AVP to plasma osmolality during water diuresis (90 min following water load) in control rats (●), in hypophysectomized rats receiving no glucocorticoid replacement (○), and in hypophysectomized rats receiving corticosterone at 300 μg/100 g body wt (△) and 600 μg/100 g body wt (▲).* A significant elevation of plasma AVP was observed in glucocorticoid-deficient rats compared to controls ($P < 0.001$). Plasma AVP was not significantly different from controls in corticosterone-replaced hypophysectomized rats.

osmolal clearance was corrected for differences in GFR.

Corticosterone replacement in hypophysectomized rats resulted in complete correction of both the percentage of the water load excreted and free water clearance with restoration of plasma sodium concentration and osmolality to control values (Table 1, Figs. 1 and 2). Group 3 rats receiving low-dose corticosterone excreted 71 ± 8% of the water load in 3 hours ($P < 0.001$ vs. group 2). Whereas this value was statistically lower than that of control rats ($P < 0.025$), water excretion was restored completely to control values in group 4 animals receiving the higher replacement dose of corticosterone. In group 4 animals, the excretion of an oral water load was identical to controls at both 120 and 180 min following water loading (Fig. 1). This improvement in urine flow was associated with an increase in free water clearance to 14 ± 3 and 16 ± 2 μl/min/100 g body wt in groups 3 and 4, respectively (Table 1). Urinary diluting ability (minimum urine osmolality) improved significantly following corticosterone replacement in group 3 (203 ± 26 mOsm/kg) and in group 4 (162 ± 14 mOsm/kg); these values, however, remained slightly elevated compared to control rats. Creatinine clearance was reduced to 690 ± 58 and 516 ± 60 μl/min/100 g body wt in groups 3 and 4, respectively, values not different from glucocorticoid deficient rats (group 2). Urinary sodium excretion in groups 3 and 4 was similar to values observed in groups 1 and 2.

Plasma AVP in groups 1 to 4 (Fig. 3). During water diuresis, plasma AVP was significantly elevated to 3.05 ± 0.45 pg/ml in group 2 compared to 1.38 ± 0.11 pg/ml in controls ($P < 0.001$). This elevation of plasma AVP occurred despite a lower plasma osmolality in group 2 animals: 281 ± 2 vs. 289 ± 2 mOsm/kg ($P < 0.01$). Corticosterone replacement resulted in a significant decrease in plasma AVP during water diuresis in both groups 3 (1.73 ± 0.33

pg/ml; $P < 0.05$) and group 4 (1.34 ± 0.24 pg/ml; $P < 0.005$) compared to group 2 rats. These values were not statistically different from controls.

Effect of combined administration of mineralocorticoids and glucocorticoids on renal water excretion, GFR, and plasma AVP in hypophysectomized rats (group 5, Table 2). The persistent mild defect in the minimum urine osmolality observed in glucocorticoid-replaced hypophysectomized rats (groups 3 and 4) was not abolished by the combined administration of mineralocorticoid and glucocorticoid hormones (group 5). Following corticosterone replacement alone, water excretion increased in this group from 19 ± 5% to 103 ± 6% of the oral water load, and the minimum urine osmolality fell from 427 ± 88 to 157 ± 13 mOsm/kg. It is of note, however, that no further improvement in these parameters was observed when both DOCA and corticosterone were given together to this same group of rats. Creatinine clearance was 710 ± 31 μl/min/100 g body wt during combined mineralocorticoid and glucocorticoid administration, a value significantly lower than that of control rats (group 1; $P < 0.005$). Plasma AVP during water diuresis was 1.5 ± 0.2 pg/ml during combined replacement, a value not different from control rats (group 1) or hypophysectomized rats receiving only corticosterone (groups 3 and 4).

Table 2. Water excretion in hypophysectomized rats before and during corticosterone and DOCA replacement (group 5) and before and during chronic volume expansion (group 6)[a]

	Oral water load excreted in 180 min %	Minimum U_{Osm} *mOsm/kg*
Group 5 ($N = 7$)		
(*a*) GC deficient	19 ± 5	427 ± 88
(*b*) CS replaced	103 ± 6	157 ± 13
(*c*) CS + DOCA replaced	113 ± 5	142 ± 7
P values		
a vs. b	<0.001	<0.02
b vs. c	NS	NS
Group 6 ($N = 7$)		
(*a*) GC deficient	19 ± 4	480 ± 93
(*b*) DOCA + NaCl	36 ± 6	230 ± 42
P values		
a vs. b	<0.02	<0.05

[a] Data are presented as the mean ± SEM. *P* values were obtained with the paired Student's *t* test. *N* denotes number of rats. Subentries under groups are defined as: glucocorticoid (GC) deficient, hypophysectomized rats studied while receiving no corticosterone; CS replaced, hypophysectomized rats studied following 7 days of corticosterone (600 μg/100 g body wt); CS + DOCA, hypophysectomized rats studied following 7 days of combined corticosterone (600 μg/100 g body wt) and DOCA (250 μg/day); DOCA + NaCl, hypophysectomized rats studied following 14 days of DOCA (250 μg/day) and normal saline.

Renal function, water excretion, and plasma AVP before and after extracellular fluid volume expansion in glucocorticoid-deficient rats (group 6, Table 2). Following 14 days of extracellular volume expansion with a high sodium chloride intake and DOCA, 24-hour urinary sodium excretion was 4.7 ± 0.7 μEq/min/100 g body wt in glucocorticoid-deficient rats, a sevenfold increase compared to groups 1 to 4 (all $P < 0.001$) and indicative of mineralocorticoid escape. The natriuresis was accompanied by an increase in creatinine clearance to 910 ± 43 μl/min/100 g body wt, a value not different from controls.

Prior to volume expansion, hypophysectomized rats again demonstrated a profound impairment of water diuresis with excretion of 19 ± 4% of the water load and a minimum urine osmolality of 480 ± 93 mOsm/kg. Following extracellular volume expansion and restoration of GFR to normal, there was a small although significant increase in the percentage of the water load excreted to 36 ± 6% ($P < 0.02$) and a decrease in the minimum urine osmolality to 230 ± 42 mOsm/kg ($P < 0.05$).

Despite chronic volume expansion and restoration of GFR, during peak water diuresis plasma AVP was 3.2 ± 0.8 pg/ml in group 6 rats, a value similar to that observed in the nonvolume expanded glucocorticoid-deficient rats (group 2) and significantly higher than that in control animals ($P < 0.05$).

Discussion

The results demonstrate that the defect in renal water excretion resulting from selective glucocorticoid deficiency in the rat is associated with an elevation of immunoreactive plasma AVP. Physiologic glucocorticoid replacement resulted in a restoration of renal water excretion, correction of hyponatremia and hypoosmolality, and suppression of plasma AVP to control values. These findings suggest an important role for increased secretion of ADH in the pathogenesis of impaired water excretion in glucocorticoid deficiency.

The role of ADH in the defect in water excretion in glucocorticoid deficiency has been a source of controversy since Gaunt, Birnie, and Eversole [1] in 1949 first identified increased amounts of antidiuretic substance in serum of adrenalectomized rats. In 1960, Dingman and Despointes [9] reported that patients with Addison's disease were hypersensitive to the antidiuretic action of nicotine and sug-

gested that glucocorticoids modulate the release of vasopressin from the neurohypophysis. More recent studies have demonstrated that ethanol, a potent inhibitor of ADH release, can restore renal water excretion to normal in patients with anterior hypopituitarism and glucocorticoid deficiency [5, 6]. This observation provided indirect evidence of increased secretion of ADH. Attempts, however, to quantitate plasma vasopressin directly by bioassay yielded conflicting results. Kleeman, Czaczkes, and Cutler [7] found undetectable levels of ADH in the plasma of patients and animals with adrenal insufficiency during water diuresis. In contrast, Ahmed et al [10], using a different bioassay, found markedly elevated levels of plasma AVP in patients with primary and secondary adrenal insufficiency. These discrepant results may be due to the lack of sensitivity and reproducibility of the bioassay techniques used [30, 31]. In the present studies, a highly specific and sensitive radioimmunoassay for plasma AVP was used [28]. Previous studies have confirmed the reproducibility of this assay under a variety of conditions that inhibit or stimulate ADH release [28, 32–34]. The present data reveal a significant increase in plasma AVP during water diuresis in glucocorticoid-deficient rats, at a time when plasma hypoosmolality should have inhibited AVP secretion. Replacement of the naturally occurring glucocorticoid in physiologic doses resulted in suppression of plasma AVP to control values. These results are in agreement with a recent report by Boykin et al [18], who also observed increased levels of plasma AVP by radioimmunoassay in adrenalectomized dogs replaced with physiologic doses of mineralocorticoid.

Previous investigations of the mechanism of impaired water excretion in glucocorticoid-deficient animals have used bilateral adrenalectomy [3, 7, 8, 15, 16, 18]. Limitations of this model include the production of coexistent mineralocorticoid deficiency with the requirement for exogenous replacement of mineralocorticoids and the creation of a state of epinephrine deficiency. Deficiency of mineralocorticoids, by itself, with its attendant decrease in extracellular fluid volume, can impair water excretion both by reducing GFR and by increasing the secretion of ADH [16, 35]. The present model using the thyroxin-replaced anterior hypophysectomized rat, permitted examination of the specific effect of glucocorticoid deficiency on renal water excretion without concomitant epinephrine or mineralocorticoid deficiency. Previous studies in rats [24, 36], dogs [37], and man [38, 39] have shown that hypophysectomy results in glucocorticoid deficiency whereas aldosterone secretion remains unaffected. In the present experiments, glucocorticoid deficiency in hypophysectomized rats was documented by marked reduction in adrenal weight and plasma corticosterone concentration. Furthermore, corticosterone levels in glucocorticoid-replaced rats were within the normal range of unstressed rats of the same strain [29]. The elevation of plasma corticosterone above normal in group 1 (control) rats can be attributed to stress-induced ACTH release during the 4-hour water-loading procedure in animals with an intact pituitary adrenal axis. The lower plasma corticosterone concentrations observed in the corticosterone-replaced hypophysectomized animals are accounted for by the inability to secrete ACTH following anterior hypophysectomy.

The defect in water excretion in glucocorticoid-deficient rats was characterized by an inability to excrete an oral water load, a reduction in absolute and fractional free water clearance, and an increase in the minimum urine osmolality. Following 7 days of corticosterone replacement (group 4), both the ability to excrete a water load and the free water clearance were restored to normal; urinary diluting ability improved substantially, although the minimum urine osmolality remained slightly higher than that of controls. The ability of physiologic replacement doses of corticosterone to restore the excretion of a water load indicated that the primary cause of the impairment in water metabolism was ACTH-mediated glucocorticoid deficiency itself and not the loss of some other anterior pituitary hormone. The inability to restore the minimum urine osmolality completely to normal may be due to the deficiency of some other anterior pituitary hormone or to the persistent reduction in GFR, which has been shown to impair renal diluting capacity even in the absence of ADH [40]. The persistent reduction in GFR could indicate that the corticosterone replacement dosages selected were not physiologic with respect to GFR. Alternatively, because aldosterone secretion was not measured directly in our study, it is conceivable that partial deficiency of this hormone might have impaired urinary dilution by reducing sodium reabsorption at some site in the distal nephron. This possibility is unlikely because the combined administration of DOCA and corticosterone to hypophysectomized rats failed to improve urine diluting ability to a greater degree than that achieved with corticosterone replacement alone (group 5).

Reduction of GFR is a characteristic of both primary and secondary adrenal insufficiency and has been considered to contribute to the defect in water

excretion observed in both states [1, 2]. Decreased GFR was not a primary determinant of the defect in water excretion observed in the present study because glucocorticoid replacement restored water excretion without increasing GFR above that observed in glucocorticoid-deficient animals. Furthermore, increasing GFR to normal with extracellular fluid volume expansion (group 6) during glucocorticoid deficiency resulted in only a small improvement in water excretion. These results are in agreement with previous reports demonstrating that volume expansion alone does not restore water excretion in the absence of glucocorticoids [41] and that improvement in water excretion following steroid administration correlates poorly with changes in GFR and solute excretion [3, 6, 11, 17].

Another hypothesis that has been proposed to explain the defect in water excretion is that glucocorticoid deficiency directly increases the permeability to water of distal nephron epithelial cells in the absence of ADH, resulting in enhanced back diffusion of water [7, 11-13]. The evidence cited in favor of this mechanism has been indirect. First, Kleeman et al [7], using a bioassay, failed to detect elevated levels of plasma ADH. Second, Green, Harrington, and Valtin [15] demonstrated a defect in water excretion in adrenalectomized rats with congenital absence of ADH (Brattleboro strain), suggesting that ADH is not essential in the pathogenesis of the defect. Third, binding studies have demonstrated the presence of specific glucocorticoid receptors in the kidney [42, 43], specifically along the cortical portion of collecting tubules [44]. In contrast, in vitro studies in the toad urinary bladder have demonstrated that glucocorticoids increase, not decrease, vasopressin-stimulated water transport [45]. Recently, Rayson, Ray, and Morgan [46] have provided direct evidence against an increased tubular sensitivity to vasopressin in glucocorticoid deficiency. These workers have shown no differences in basal or vasopressin-stimulated diffusional water permeability in collecting ducts of adrenalectomized and control rats using an isolated perfused renal papilla preparation. Thus, the available data are conflicting and do not allow any unequivocal statement concerning a direct effect of glucocorticoid deficiency on water permeability in the distal nephron. The present experimental design was not constructed to examine this question. The results do indicate that elevated levels of plasma AVP contribute, at least in part, to the impairment in water excretion observed in glucocorticoid deficiency.

The mechanism by which glucocorticoid deficiency results in increased secretion of ADH is unknown. Because plasma osmolality was significantly decreased in glucocorticoid-deficient rats in the present study, an osmotic stimulus to vasopressin release can be excluded. It is unlikely that volume depletion can account for the elevated levels of vasopressin because chronic extracellular fluid volume expansion failed to suppress plasma AVP. This latter finding is consistent with previous studies demonstrating that mineralocorticoid replacement alone in adrenal insufficiency was capable of restoring both blood [17] and extracellular fluid volume [18] to normal, despite a persistent defect in water excretion. Therefore, it is likely that glucocorticoid deficiency modulates ADH secretion through a nonosmotic mechanism that is independent of plasma or extracellular volume. Such an effect could result from hemodynamic or humoral activation of peripheral afferent pathways originating in the arterial and cardiac baroreceptors [33, 34, 47] or from a direct effect of glucocorticoid hormones on central afferent neurons that interact with neurosecretory cells.

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