The Requirement of Cortisol for the Inhibitory Effect of Norepinephrine on the Antidiuretic Action of Vasopressin* (37095)

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(Introduced by Alvin Sellers)

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Introduction. Evidence exists indicating that norepinephrine (NE) abolishes the antidiuretic effect of vasopressin (ADH) both in normal humans (1) and experimental animals (2). As a consequence of this action, it seemed appropriate to administer norepinephrine to adrenal-insufficient animals receiving a water load. A significant improvement in water excretion would provide indirect evidence that ADH is involved in the impaired water diuresis of the glucocorticoid-deficient state. However, in preliminary studies in our laboratory, it was found that NE did not improve the defect in water excretion of adrenalectomized dogs and did not inhibit the effect of exogenously administered vasopressin to these animals. These observations suggested that cortisol may be required for the process through which NE inhibits the action of ADH on the nephron. The present study was undertaken to test this hypothesis.

Methods. Six female mongrel dogs (12–15 kg) were subjected to episiotomy and were, then, trained to stand quietly in a canvas sling and to tolerate insertion of Foley catheter into the urinary bladder via the urethra. Permanent polyvinyl catheters (Tygon S50 HL i.d. 1/32 o.d. 3/32 in.) were inserted into the descending aorta via the carotid artery and into the right atrium via the right external jugular vein by methods previously reported (3); such catheters could remain functional for periods of several months. The free ends of the catheters were retained in a protective, specially designed, nylon jacket.

Bilateral adrenalectomy was performed through a midline incision under pentobarbital anesthesia. On the day of surgery and prior to adrenalectomy, the animals were given an intravenous injection of 50 mg hydrocortisone phosphate, CpF, (Merck, Sharp, and Dohme, West Point, Pa.); during surgery, the dogs received an intravenous infusion of one liter of normal saline containing 50 mg of CpF. After a period of stabilization, the animals were maintained with intramuscular injection of 25 mg of CpF and diet supplying 5 g NaCl per day. Studies were carried out in the awake state, 15 to 18 days after adrenalectomy at a time when the animals were apparently healthy, had good appetite, and maintained a stable weight.

Three days prior to each study, CpF treatment was discontinued and the animals received intramuscular injection of 10 mg desoxycorticosterone acetate and 3 g of NaCl supplementation over their dietary intake. This regimen produced a weight gain of 0.7–1.0 kg. This protocol was used to insure adequate urine flow. Water diuresis is impaired in glucocorticoid-deficient dogs and, therefore, such animals are poor preparations for the study of the effect of exogenous ADH. When the extracellular fluid volume in these animals is moderately expanded by the treatment with DOCA and NaCl, their ability to excrete a water load is improved and adequate urine flow could be achieved (4).

On the third day following the administration of DOCA and sodium chloride, clear-

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TABLE I. Effect of Arginine Vasopressin With and Without Norepinephrine on Urinary Volume and Osmolality in Glucocorticoid-Deficient Dogs and Adrenalectomized Dogs Receiving Cortisol.[a]

	BP mm Hg	C_{Cr} ml/min	C_{PAH} ml/min	UV ml/min	C_{H_2O} ml/min	Uosm mOsm/kgH_2O
I. *Glucocorticoid deficient dogs*						
Control	91 ± 1.5	45.3 ± 3.3	159 ± 21.5	2.66 ± 0.55	0.03 ± 0.27	248 ± 32
AVP	87 ± 1.0	45.8 ± 3.8	150 ± 23.9	0.98 ± 0.23	−0.93 ± 0.29	513 ± 42
AVP + NE	96 ± 1.0	54.7 ± 4.8	146 ± 17.8	0.74 ± 0.14	−0.75 ± 0.00	620 ± 61
II. *ADX dogs receiving CpF*						
Control	95 ± 1.4	48.3 ± 4.0	167 ± 22.9	4.48 ± 0.59	1.99 ± 0.76	151 ± 34
AVP	97 ± 2.3	47.3 ± 2.3	150 ± 11.1	0.69 ± 0.11	−0.94 ± 0.22	633 ± 69
AVP + NE	110 ± 3.7	55.0 ± 4.3	197 ± 25.0	4.63 ± 0.55	2.42 ± 0.63	135 ± 27

[a] Abbreviations: BP = blood pressure; C_{Cr} = exogenous creatinine clearance; C_{PAH} = paraaminohippurate clearance; Uv = urine volume, C_{H_2O} = free water clearance; U_{OSM} = urinary osmolality, AVP = arginine vasopressin; NE = norepinephrine; ADX = adrenalectomized; and CpF = cortisol.

Each volume is the mean ± SE from 6 dogs.

ance studies were carried out in the awake state. Urine was collected from an indwelling Foley catheter and the bladder was washed with air at the end of each clearance period to assure completeness of collection. Blood samples were obtained from the arterial catheter at the midpoint of the clearance periods. Glomerular filtration rate was determined by exogenous creatinine clearance and renal plasma flow by the clearance of paraaminohippurate (PAH) utilizing the standard priming dose and sustaining infusion technique, and 60 min were allowed for equilibration of the priming dose.

Water diuresis was induced by the rapid intravenous infusion of 2.5% dextrose in water (30 ml/kg body weight); the infusion was then maintained at a rate exceeding urine volume by 1 ml/min until a stable urine flow was achieved when the infusion rate was adjusted to equal urine flow. Each study consisted of two parts:

(*a*) *Evaluation of the effect of vasopressin with and without norepinephrine in the glucocorticoid-deficient animals.* After 3 to 5 control clearance periods, a solution of ADH (bovine arginine vasopressin[3]) in 2.5% dextrose in water was infused at a rate of 0.5 ml/min to deliver 15–25 mU/hr. After 15 min of the start of the vasopressin administration, several clearance collections of 15 min duration were obtained. Norepinephrine (Winthrop Laboratories, New York, N.Y.) was then added to the vasopressin infusion in adequate quantity to deliver 30–40 μg/kg/hr, and urine was collected for several additional clearance periods; the vasopressin-norepinephrine infusion was then discontinued.

(*b*) *Evaluation of the effect of CpF on the responses to vasopressin with and without NE.* In 4 dogs, CpF (50 mg) was given intravenously immediately after completion of the first part of the study as noted above. Sixty to ninety minutes later, three control clearance periods of 15 min duration were obtained. The animals then received vasopressin and NE as described above. In 2 dogs, 50 mg of CpF was given intravenously at the end of the first part of the study, and the following morning each animal received an additional 25 mg of CpF intravenously prior to the second part of the study. The protocols for the induction of water diuresis and the drug doses and sequence of their administration were the same as described above.

Urine and blood specimens were analyzed for osmolality by freezing point depression with Precision Osmometer, and for creatinine and PAH by the Technicon Autoanalyzer. Arterial blood pressure was continuously monitored by mercury manometer connected to an indwelling aortic catheter.

[3] The bovine arginine vasopressin (AVP) was standardized by Parke, Davis Company against the USP standard and contained 52 pressor U/mg powder.

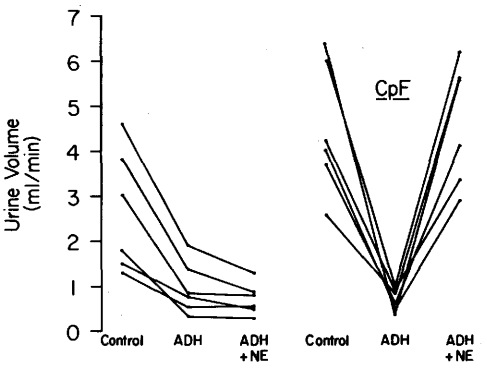

FIG. 1. The effect of the administration of arginine vasopressin (ADH) with and without norepinephrine (NE) on urine volume in the glucocorticoid-deficient dogs shown in the left panel, and in the same animals treated with cortisol (CpF) shown in the right panel. Each line represents one animal.

Results. The changes in urine volume and osmolality in all animals are shown in Figs. 1 and 2 and the summary of the data from all studies is presented in Table I. Glomerular filtration rate did not vary significantly in the various parts of the study. The clearance of PAH increased with the infusion of NE in the dogs receiving CpF, and the mean value was not significantly different from that observed during the administration of NE to the glucocorticoid-deficient dogs. In the control periods of the studies in the DOCA-expanded, glucocorticoid-deficient dogs, urine volume ranged between 1.5–4.5 (2.66 ± .55 (SE)) ml/min; C_{H_2O}, between —0.71–1.1 ml/min and only three animals excreted free water; and minimal urinary osmolality ranged between 151 to 357 (248 ± 32) mOsm/kg H_2O. The intravenous infusion of AVP caused antidiuresis in every animal (Figs. 1 and 2). Urine volume decreased to a mean value of 0.98 ± 0.23 ml/min ($p < 0.01$) and urinary osmolality increased to 513 ± 43 mOsm/kg H_2O ($p < 0.01$). The addition of NE did not abolish the antidiuretic effect of AVP and both urine volume and urinary osmolality during AVP + NE were not significantly different from values observed during AVP infusion alone (Figs. 1 and 2).

The administration of CpF to the DOCA-expanded adrenalectomized dogs resulted in a marked improvement of water excretion. The control urine volumes ranged between 2.6–6.4 ml/min with the mean value of 4.48 ± 0.59 ml/min which is significantly different ($p < 0.01$) from the control values observed in the same animals in the absence of glucocorticoids. Also, free water was excreted by all animals after CpF administration, and the mean value for C_{H_2O} (1.99 ± 0.76 ml/min) was significantly different from that noted in the glucocorticoid-deficient state. The infusion of AVP into the CpF-treated dogs produced antidiuresis with a fall in urine volume from 4.48 ± 0.59 to 0.69 ± .11 ml/min ($p < 0.01$) and a rise in urinary osmolality from 151 ± 34 to 663 ± 69 mOsm/kg H_2O ($p < 0.01$). In contrast to the failure of NE to abolish the antidiuretic effect of AVP in the glucocorticoid-deficient dogs, this drug completely blocked the effect of AVP when the animals were pretreated with CpF; urine volume increased and urinary osmolality decreased and the values returned toward the levels observed prior to the administration of AVP.

Another observation was the failure of NE to elevate blood pressure in the absence of glucocorticoids. In the glucocorticoid-deficient dogs the mean systolic blood pressure was 91 ± 1.5 mm Hg before the infusion of NE and 96 ± 1.0 mm Hg during the administration of the drug. In contrast, blood pressure increased significantly during the infusion of NE in CpF-treated dogs, from 95 ± 1.4 to 110 ± 3.7 mm Hg ($p < 0.01$).

Discussion. It has been shown that the antidiuretic effect of vasopressin is abolished

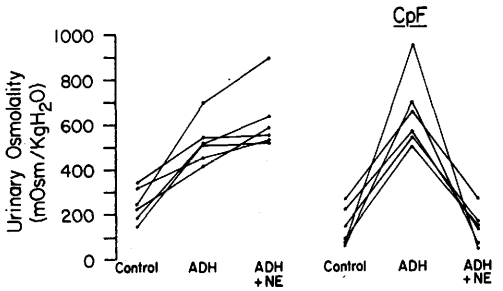

FIG. 2. The effect of the administration of arginine vasopressin (ADH) with and without norepinephrine (NE) on urinary osmolality in the glucocorticoid deficient dogs shown in the left panel, and in the same animals treated with cortisol (CpF) shown in the right panel. Each line represents one animal.

by norepinephrine both in normal humans (1) and dogs (2). The mechanisms underlying this phenomenon have not been elucidated. However, current data indicate that the inhibitory effect of NE on the antidiuretic action of AVP is not related to hemodynamic changes produced by NE (2). It has been suggested that NE inhibits the cellular action of vasopressin (5) possibly by inhibiting the activation of adenylcyclase sites by the antidiuretic hormone.

The results of the present study demonstrate that the ability of NE to block the effect of AVP on the dog kidney is dependent on an adequate circulating or tissue level of adrenal glucocorticoids. The exact mechanism(s) through which cortisol exerts its effect on the interaction between NE and AVP on the kidney is not clear. At least two explanations should be considered. First, it is possible that systemic or renal hemodynamic effects produced by NE in the cortisol-treated animals can influence water excretion. However, it is unlikely that the rise in blood pressure and the statistically insignificant increase in renal plasma flow produced by NE in these animals could completely antagonize the effect of AVP. Klein *et al.* have shown that the inhibitory effect of NE on AVP action occur in the normal dogs without observable changes in blood pressure or renal hemodynamics (2). Second, it is plausible that cortisol plays a permissive role for the interaction between NE and AVP on the renal tubule. Although the present study does not provide direct support for such a postulate, evidence exists indicating that glucocorticoids are necessary for a variety of physiological actions of catecholamines. Fritz and Levine (6) demonstrated that blood vessels of the mesoappendix of adrenalectomized rats are not responsive to norepinephrine. Also, the hypotension of adrenalcortical insufficiency is not corrected by NE. Recently, Maas and Mednieks reported that the uptake of NE by slices of cerebral cortex is enhanced by the incubation of these slices with hydrocortisone (7). In a detailed account Ramey and Goldstein (8) reviewed the literature pertinent to the presence of an interaction between catecholamines and glucocorticoids and concluded that "these amines function as a physiological unit with cortisol." The results of our study provide further support for such contention.

Summary. Cortisol (CpF) is required for various physiological actions of norepinephrine (NE). In humans and animals, NE abolishes the antidiuretic action of arginine vasopressin (AVP). This study evaluates whether CpF is necessary for the interaction between NE and AVP on the kidneys. Six trained adrenalectomized (ADX) dogs were maintained with desoxycorticosterone acetate DOCA and 8 g of NaCl/day but without CpF for 3 days prior to study which was carried out in the awake animal. In this state, the dogs were volume expanded with weight gain of 0.7 to 1 kg and moderate water diuresis could be induced. With intravenous infusion of AVP (15–25 mU/hr) urine volume (UV) fell from 2.66 ± 0.55 (SE) to 0.98 ± 0.23 ml/min and urinary osmolality (Uosm) rose from 248 ± 32 to 513 ± 42 mOsm/kg H_2O. Addition of NE (360–600 μg/hr) did not abolish the effect of AVP. Intravenous injection of CpF (50 mg) increased water diuresis in the control periods, and the infusion of AVP to CpF and DOCA-treated ADX dogs also produced a fall in UV from 4.48 ± .50 to .69 ± .11 ml/min and a rise in Uosm from 151 ± 34 to 663 ± 69 mOsm/kg H_2O. Addition of NE to these animals abolished the action of AVP with both UV and Uosm returning to control values, 4.68 ± 0.55 ml/min and 135 ± 27 mOsm/kg H_2O, respectively. The results show that the inhibitory effect of NE on AVP action on kidney requires CpF.

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