Concentrating Defect in the Adrenalectomized Rat

ABNORMAL VASOPRESSIN-SENSITIVE CYCLIC ADENOSINE MONOPHOSPHATE METABOLISM IN THE PAPILLARY COLLECTING DUCT

BRIAN A. JACKSON, JULIE L. BRAUN-WERNESS, EIJI KUSANO, and THOMAS P. DOUSA,
Nephrology Research Unit, Mayo Clinic and Foundation, Mayo Medical School, Rochester, Minnesota 55901; Department of Physiology and Biophysics, College of Medicine, University of Kentucky, Lexington, Kentucky 40536

ABSTRACT Among other defects in water metabolism, adrenal insufficiency is associated with an inability to concentrate urine maximally in both man and experimental animals. Recent studies in the rabbit cortical collecting tubule have suggested indirectly that this defect may result from impaired cyclic AMP (cAMP) formation in response to antidiuretic hormone stimulation. In the present study, we examined key elements of arginine vasopressin (AVP)-dependent cAMP metabolism in the papillary collecting duct (PCD), microdissected from 8-d adrenalectomized (ADX) and sham-operated control rats.

AVP-sensitive adenylate cyclase (ADC) activity in PCD did not differ between control and ADX rats. cAMP-phosphodiesterase activity (cAMP-PDIE), measured at 10^{-6} M cAMP substrate concentration, was significantly higher (Δ + 31.6%) in PCD of ADX rats compared with controls. Incubation of intact PCD from ADX rats with AVP resulted in an accumulation of cAMP (Δ − 48.5%) significantly lower than observed in control PCD. Chronic administration of dexamethasone reduced cAMP-PDIE activity in PCD of ADX rats to levels close to or below those observed in control rat PCD, and also resulted in a restoration of AVP-stimulated cAMP accumulation to levels approaching control values.

Results indicate that the impaired maximal urinary concentrating ability associated with adrenal insufficiency may be due, at least in part, to a reduced accumulation of cAMP in response to AVP in the PCD. This decreased cAMP accumulation results from increased cAMP-PDIE activity in the PCD of ADX rats and can be corrected by administration of glucocorticoid.

INTRODUCTION

Adrenal insufficiency is associated with a number of impairments in renal function, including decreased urinary concentrating ability. Although less frequently recognized than the impairment in diluting capacity, this concentrating defect has been observed in both man (1) and experimental animals (2–4). Recent microperfusion studies using the rabbit cortical collecting tubule have suggested that this concentrating defect may at least in part be due to a blunted hydroosmotic response to vasopressin (4). Since cyclic AMP (cAMP) analogues elicited normal hydroosmotic responses in tubules from adrenalectomized (ADX)[1] rabbits, it was suggested that impaired responsiveness resulted from a reduced accumulation of cAMP in response to the hormone.

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[1] *Abbreviations used in this paper:* ADX, adrenalectomized; AVP, [8-arginine]-vasopressin; cAMP-PDIE, cyclic AMP-phosphodiesterase; PCD, papillary collecting duct; U_{osM}, urine osmolality.

Adrenal steroids potentiate [8-arginine]-vasopressin (AVP)-dependent water flow and cAMP accumulation in the toad bladder (5–7). In contrast, reports concerning the effects of corticosteroid deficiency and adrenal steroid administration on AVP-dependent cAMP metabolism in the mammalian kidney remain equivocal. For example, AVP-dependent adenylate cyclase activity has been reported to be both decreased (8) and unchanged (9) in cell-free membrane preparations of renal medulla from ADX rats compared with controls. Inconsistent effects of adrenalectomy on AVP-dependent cAMP accumulation in renal medullary slices have also been reported; whereas basal cyclic AMP levels were found to be higher in ADX rats in two studies (10, 11), cAMP accumulation in response to AVP was found to be higher in renal medullary preparations (10), but lower in papillary preparations (11) of ADX rats compared with controls.

We have recently shown that the examination of key elements of AVP-dependent cAMP metabolism in specific microdissected segments of the nephron facilitates detection of specific alterations in this system (12–14), which had gone undetected in previous studies using heterogeneous renal tissue preparations.

Consequently, in the present study, we examined key elements of AVP-dependent cAMP metabolism in the papillary collecting duct (PCD), a critically important component of the renal concentrating mechanism, microdissected from 8-d ADX and sham-operated control rats, and, furthermore, we have examined the effects of glucocorticoid replacement on this system in ADX animals.

METHODS

Surgically ADX and sham-operated (control) male Sprague-Dawley rats (200–250 g) were purchased from Johnson Laboratories, Inc., Bridgeview, IL. In steroid treatment experiments (see Results), dexamethasone or saline-filled osmotic minipumps (Alza Corp., Palo Alto, CA) were implanted intraperitoneally at the time of surgery. After at least 24 h recovery, the animals were shipped by air to Rochester, MN. All animals were maintained on standard rat diet (Purina Laboratory Rat Chow, Ralston Purina Co., St. Louis, MO) and ad lib. 0.9% sodium chloride drinking solution for a total of 8 d before being killed. Plasma corticosterone concentrations were measured on arterial blood samples drawn at random from both ADX and control rats when they were killed to confirm successful adrenalectomy. The mean arterial blood corticosterone concentration in control rats was 36±2.4 μg/100 ml (mean±SEM; n = 8), compared with a value of 0.9±0.4 μg/100 ml (n = 12) in ADX rats, 8 d after surgery.

In vivo studies. Maximal urinary concentrating ability was assessed in sham-operated, ADX, and dexamethasone-treated ADX rats. Dexamethasone-filled minipumps were designed to deliver ~100 μg/d i.p. Sham-operated and ADX rats were implanted with 0.9% saline-filled minipumps. All animals were adapted to metabolic cages for at least 2 d before the start of urine collection. Control urine collections were obtained on day 6 postoperative. To test maximal urinary concentrating ability, animals were first dehydrated for 24 h (day 7) by withholding all drinking fluid. Dehydration was continued on day 8, and in addition, 0.5 U vasopressin tannate was injected subcutaneously. Urine volume and U_{osM} (urine osmolality) was measured for each 24-h collection period.

Papillary osmolality was determined as described recently by Stoff et al. (15). Control (sham-operated) and ADX rats were anesthetized with pentobarbital (6 mg/100 g body wt). The kidney was removed and sectioned along the horizontal plane with a razor blade; the papilla, medulla, and cortex were dissected free. Wet tissue weight was rapidly determined in preweighed test tubes. The test tubes with the tissue were placed in a boiling water bath and 1 ml of 100°C triple-distilled water was added to each test tube. The whole procedure from removal of the kidney to placing tissue in a 100°C water bath took from 3 to 5 min. The tissue electrolytes and nonelectrolyte solutes were extracted for 1 h at 100°C after the method of Appelbloom et al. (16). After extraction at 100°C for 1 h, the tissue plus extract was cooled to room temperature, reweighed, then spun at 3,500 *g* for 30 min in a Beckman J2-21 centrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). The supernatant was aspirated and osmolality was determined using a Fiske model 230D osmometer (Fiske Associates, Inc., Burlington, MA). The supernatant was recombined with the tissue pellet and dried at 100°C overnight and then reweighed for determination of dry weight.

In vitro studies. PCD were microdissected from the inner medulla (papilla) of both ADX and control rats according to the procedure described in detail by Edwards et al. (17). Briefly, under light pentobarbital anesthesia (6 mg/100 g body wt), the left kidney was perfused with 20 ml of collagenase medium (for composition, see below) containing sodium heparin (20 U/ml). Thin corticomedullary tissue strips were then incubated in aerated collagenase medium for 60 min at 35°C. The tissue strips were thoroughly rinsed in microdissection medium (for composition, see below) and transferred to petri dishes containing the same medium. Microdissection and all subsequent procedures were performed at 0–4°C. Isolated PCD were transferred in a small volume of microdissection fluid to 5-mm-diam glass cover slips (Bellco Glass, Inc., Vineland, NJ) seated in the well of concave bacteriological slides. All tubular samples were photographed for subsequent length measurements.

Enzyme assays, i.e., adenylate cyclase and cAMP-phosphodiesterase (cAMP-PDIE), were performed on disrupted tubular segments. Tubular disruption (or permeabilization) was achieved as follows: The microdissection medium was aspirated off each sample and replaced with 0.5 μl (for adenylate cyclase assay) or 0.25 μl (for cAMP-PDIE assay) of hypoosmotic medium (for composition, see below). All samples were then frozen rapidly on dry ice and stored at −80°C overnight. Preliminary experiments (data not shown) confirmed that storage under these conditions does not affect adenylate cyclase activity. We have previously reported that cAMP-PDIE activity is not affected by overnight storage (18). Samples were allowed to thaw at 4°C before being assayed for enzyme activities.

Adenylate cyclase activity was measured according to the method described in detail previously (12, 17–19), with minor modifications. Tubular samples (1–2-mm total tubular length) were incubated for 30 min at 30°C in a final volume of 5.5 μl consisting of the following (final concentrations): 0.25 mM (α-^{32}P) ATP (4 × 10^6 cpm/sample), 1 mM cAMP, 3.8 mM $MgCl_2$, 0.25 mM EDTA, 100 mM Tris HCl, 20 mM

phosphocreatine, 1 mg/ml creatine kinase (pH 7.4). Additionally, NaCl and urea were added to this incubation cocktail in a 1:2 molar ratio to attain a final osmolality of 800 mosM. The reaction was stopped by placing the slides on dry ice, resulting in almost instantaneous freezing of the incubation fluid. Each coverslip plus frozen sample was transferred to individual 12 × 75-mm glass test-tubes located in crushed dry ice followed by 150 μl of stop solution consisting of 3.3 mM ATP, 5 mM cAMP, 50 mM Tris HCl (pH 7.6), and ^{3}H-cAMP (8 × 10^3 cpm/sample) to monitor recovery. Samples were thawed and diluted with 1 ml of distilled water. cAMP was separated from other products with Dowex-50 and aluminum oxide columns according to the method of Salomon et al. (20).

cAMP-PDIE activity was measured according to the method described previously (12, 14, 18, 21), but again with minor modifications. Tubular samples (1–2 mm total tubular length) were incubated for 15 min at 37°C in a final volume of 2.5 μl consisting of the following (final concentrations): 10 mM $MgSO_4$, 0.1 mM EDTA, 50 mM Tris HCl, pH 8.0 (Mg-EDTA-Tris buffer), with a final substrate concentration of either 10^{-6} M or 10^{-4} M ^{3}H-cAMP (10^5 cpm).

The reaction was stopped by placing the slides on dry ice, followed by transfer of each sample plus coverslip to individual precooled 12 × 75-mm glass test-tubes. 100 μl of Mg-EDTA-Tris buffer was added to each tube while still frozen. All tubes were then immersed in a boiling water bath for 3 min, cooled on ice, and incubated for a further 15 min at 37°C in the presence of 50 μl of 5′-nucleotidase (snake venom from *Crotalus atrox*, Sigma Chemical Co., St. Louis, MO: 1 mg/ml). Nucleotides were separated from nucleosides on QAE-Sephadex columns as described by Wells et al. (22).

To determine the effect of in vitro addition of dexamethasone on cAMP-PDIE, freshly microdissected tubular samples were preincubated in 5 μl of microdissection medium containing 50 pM dexamethasone or vehicle as appropriate for 90 min at 37°C. After preincubation, the medium was removed, the samples were subjected to freezing in hypoosmotic medium, and cAMP-PDIE was measured as described above.

Basal and AVP-stimulated cAMP accumulation was measured on freshly microdissected nephron segments as described in detail previously (12, 14, 17, 23). For these incubations, 3–4-mm total tubule length was required to constitute one sample. After transfer to glass cover slips seated on concave bacteriological slides, followed by photography, excess microdissection medium was aspirated off and replaced with 2.5 μl of modified Krebs-Ringer Buffer containing NaCl and urea in a 1:2 molar ratio to achieve a final osmolality of ~800 mosM (for composition, see below) and also with or without AVP. All samples were incubated for 20 min at 30°C and the reaction stopped by placing slides on dry ice. Samples and cover slips were transferred while still frozen into precooled 12 × 75-mm glass test tubes and 100 μl of 50 mM sodium acetate added to each. All tubes were placed in a boiling water bath for 3 min. Preliminary experiments confirmed that this rapid method of cAMP extraction is as efficient as extraction with 10% TCA, a technique recently applied to microdissected tubules by Torikai et al. (24).

Tubule samples and standards were acetylated and cAMP content determined by radioimmunoassay (RIA) as described in detail previously (12, 14, 17, 23). Since the tubules and incubation medium were transferred directly from incubation chambers into RIA test tubes along with the cover slips on which they were incubated, and, since all subsequent steps (solubilization, acetylation, and RIA) were performed in these tubes, recovery of cAMP was assumed to be complete (12, 14, 17, 23).

Tubular protein content was determined by a micromodification of the colorimetric method of Lowry, as described in detail previously (12, 21). Since mean tubular protein content of PCD microdissected from control rat kidneys (0.139±0.006 μg/mm; mean±SEM; n = 15) did not differ from that of PCD microdissected from 8-d ADX rats (0.146±0.008 μg/mm; mean±SEM; n = 12), all enzyme activities and cAMP levels were subsequently expressed relative to tubular length.

Because of known variations between experiments, PCD microdissected from control and experimental animals were always processed and assayed simultaneously. The data were evaluated statistically by t test for either paired or group comparisons as appropriate and as specified in Results. P values < 0.05 were considered to be nonsignificant.

Solutions and materials. The collagenase medium consisted of medium 199 (Gibco Laboratories, Grand Island, NY) plus collagenase (0.1%), hyaluronidase (0.1%), and bovine serum albumin (0.1%). Microdissection medium was medium 199 without the above additives and also without phenol red indicator. Hypoosmotic medium contained (in final concentrations): 1 mM $MgCl_2$, 0.25 mM EDTA, 0.1% bovine serum albumin, and 1 mM Tris HCl (pH 7.4). Modified Krebs ringer buffer consisted of 175 mM NaCl, 350 mM urea, 5 mM KCl, 1.2 KCl, 1.2 mM $MgSO_4$, 0.8 mM $CaCl_2$, 10 mM sodium acetate, 10 mM glucose, 20 mM Tris, 2.0 mM NaH_2PO_4, pH 7.4.

Hyaluronidase (type 1), bovine serum albumin, cAMP, and 5′-nucleotidase (from *Crotalus atrox*) were products of Sigma Chemical Co. Collagenase (Type 1, 125–250 U/mg) was purchased from Worthington Biochemical Corp., Freehold, NJ. Sodium heparin (100 U. S. Pharmacopeia U/ml) was a product of Abbott Laboratories, North Chicago, IL. Synthetic AVP (385 U/mg), was purchased from Calbiochem-Behring Corp., La Jolla, CA. [α-^{32}P]ATP (25 Ci/mmol) was purchased from ICN Pharmaceuticals, Inc., Irvine, CA. ^{3}H-cAMP (30–50 Ci/mmol) and RIA kits for the measurement of cAMP were purchased from New England Nuclear, Boston, MA. All other compounds and reagents were of the highest quality available from standard suppliers.

RESULTS

In vivo studies. Maximal urinary concentrating ability was assessed by measuring U_{osM} in animals subjected to 48 h drinking-fluid deprivation combined with a single subcutaneous injection of 0.5 U vasopressin tannate given after the first 24 h of dehydration (for details, see Methods). Maximum U_{osM} of 11 ADX rats was significantly lower (Δ − 41.9%; $P < 0.001$; group t test) than that of sham-operated control animals (Fig. 1). Tissue osmolality, determined in the papilla of hydropenic ADX rats (935±80 mosM/kg H_2O; mean±SEM; n = 4), did not differ significantly from that of corresponding control animals (1,136±162 mosM/kg H_2O; mean±SEM; n = 4). In contrast, maximum U_{osM} in ADX rats receiving dexamethasone intraperitoneally via osmotic minipump (designed to deliver ~100 μg dexamethasone/d) was significantly

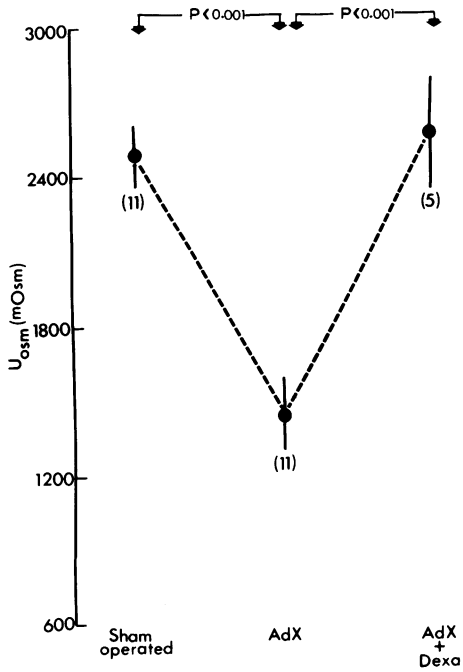

FIGURE 1 Effect of adrenalectomy (8 d: ADX) and dexamethasone (Dexa) treatment on maximal urinary concentrating ability. Maximum U_{osM} (in milliosmoles) was determined after 48 h of fluid deprivation combined with a single subcutaneous injection of vasopressin tannate (0.50 U/250 g rat) (for details, see Methods). Dexamethasone was delivered to ADX rats intraperitoneally via osmotic minipump implanted on day 1 (at the time of adrenalectomy). Each rat received ~100 μg dexamethasone/d. ADX and sham-operated control rats were implanted with 0.9% saline-filled pumps. Each point represents mean±SEM for each group. Numbers in parentheses, total number of observations. $P < 0.05$ or less represents a significant difference (group t test) between respective groups.

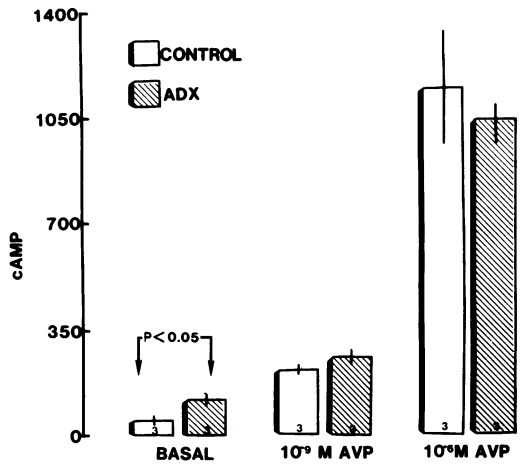

FIGURE 2 Effect of adrenalectomy (8 d: ADX) on basal and AVP-stimulated adenylate cyclase activity in PCD. Responses to both a maximal (10^{-6} M) and submaximal (10^{-9} M) stimulatory concentration of AVP are presented. Each column represents the mean±SEM of values expressed as femtomoles cAMP per 30 min per millimeter of tubule length; numbers at the base of the columns represent the number of animals; 8–10 samples were assayed from each animal for each condition. $P < 0.05$ represents a significant difference (group t test) between control and ADX groups.

higher than that of untreated ADX rats (Δ + 78.5%; $P < 0.001$; group t test) and in fact did not differ from the maximum U_{osM} observed in control sham-operated animals without dexamethasone treatment (Fig. 1).

In vitro studies. Basal adenylate cyclase activity was slightly, but significantly higher ($P < 0.05$; group t test) in PCD of ADX rats compared with controls (Fig. 2). However, AVP-stimulated adenylate cyclase activity in PCD did not differ between control and ADX rats when assayed in the presence of either a maximal (10^{-6} M) or submaximal stimulatory concentration (10^{-9} M) of AVP (Fig. 2). Responses to other submaximal concentrations of AVP (10^{-8} and 10^{-7} M AVP) also did not differ between groups (data not shown).

cAMP-PDIE activity, measured at a cAMP substrate concentration of 10^{-6} M was significantly higher (Δ + 31.6%; $P < 0.02$; group t test) in PCD of ADX rats compared with controls (Fig. 3). No difference in the activity of cAMP-PDIE measured at a cAMP substrate concentration of 10^{-4} M could be detected between groups (Fig. 3).

Basal cAMP levels in intact PCD did not differ between ADX and sham-operated control animals. How-

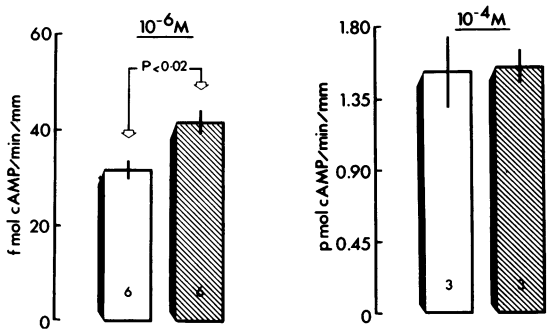

FIGURE 3 Effect of adrenalectomy (8 d: ADX) on cAMP-PDIE activity in PCD. Enzyme activity was measured at two different substrate concentrations: 10^{-6} cAMP (low K_m–high affinity; *left* panel) and 10^{-4} cAMP (high K_m–low affinity; *right* panel). Each column represents mean±SEM of values expressed as femtomoles cAMP hydrolyzed per minute per millimeter of tubule length: Numbers at the base of columns represent the number of animals; 8–10 samples were assayed from each animal for each condition. White bars, sham-operated controls; striped bars, 8-d ADX. $P < 0.05$ or less represents a significant difference (group t test) between control and ADX groups.

ever, cAMP accumulation in response to 10^{-6} M AVP was markedly reduced (Δ − 48.5%; $P < 0.005$; group t test) in PCD of ADX rats compared with controls (Fig. 4).

Since administration of dexamethasone restored concentrating ability in ADX rats in vivo, subsequent experiments examined the effects of dexamethasone treatment (delivered for 8 d via osmotic minipump intraperitoneally, as before) on key elements of AVP-dependent cAMP metabolism in PCD of ADX rats.

As in the previous groups of animals, cAMP-PDIE activity (measured at 10^{-6} M cAMP substrate) was significantly higher (Δ + 25.5%; $P < 0.05$; group t test) in PCD of ADX rats compared with controls (Fig. 5). Chronic dexamethasone treatment reduced cAMP-PDIE activity in ADX rats to values similar to, or somewhat lower than, those measured in PCD of control, sham-operated rats (Fig. 5). In contrast, incubation of PCD microdissected from ADX rats with 50 pM dexamethasone added in vitro did not decrease cAMP-PDIE activity (Table I).

Moreover, as observed in the first series of experiments, AVP-stimulated cAMP accumulation was significantly lower (Δ − 55.6%; $P < 0.05$; group t test) in PCD of ADX rats compared with sham-operated controls (Fig. 6). Dexamethasone treatment increased AVP-dependent cAMP accumulation in PCD of ADX rats to levels that were significantly greater (Δ + 68.0%; $P < 0.001$; group t test) than those in PCD of non-dexamethasone-treated ADX rats and that approached AVP-stimulated cAMP levels observed in PCD of sham-operated, control rats.

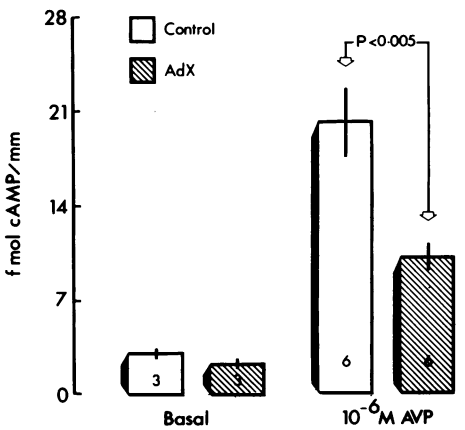

FIGURE 4 Effect of adrenalectomy (8 d: ADX) on basal and AVP (10^{-6} M)-stimulated cAMP-accumulation in PCD. Each column represents the mean±SEM of values expressed as femtomoles cAMP per millimeter of tubule length. Numbers at the base of columns represent the number of animals; 8–10 samples were assayed from each animal for each condition. $P < 0.05$ or less represents a significant difference (group t test) between control and ADX groups.

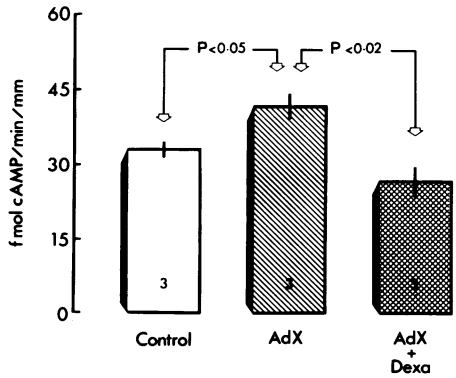

FIGURE 5 Effect of dexamethasone (100 μg/d: DEXA) on cAMP-PDIE (10^{-6} M cAMP) activity in PCD of ADX (8 d: ADX) rats. Dexamethasone-filled osmotic minipumps were implanted intraperitoneally at the time of adrenalectomy. Sham-operated controls and ADX rats were implanted with 0.9% saline-filled pumps. Each column represents mean±SEM of values expressed as femtomoles cAMP hydrolyzed per minute per millimeter of tubule length. Numbers at the base of columns represent the number of animals; 8–10 samples were assayed from each animal for each condition. $P < 0.05$ or less represents a significant difference (group t test) between respective groups.

While dexamethasone treatment reduced cAMP-PDIE activity, and enhanced AVP-dependent cAMP accumulation in PCD of ADX rats (Figs. 5 and 6), dexamethasone treatment had no effect on AVP-stimulated adenylate cyclase activity in this group of animals (Table II).

DISCUSSION

Decreased concentrating ability is a distinct, but less frequently studied abnormality of renal water metabolism associated with adrenal insufficiency (1–4). This

TABLE I
cAMP-PDIE Activity in PCD: Effect of Dexamethasone Added In Vitro

Experiment	Control	Dexamethasone
1	29.1±4.1* (6)	56.7±6.4 (6)
2	52.2±6.9 (5)	57.4±3.7 (6)
3	57.9±5.1 (6)	56.9±2.9 (6)
Mean±SEM	46.4±8.9	57.0±0.2

PCD microdissected from ADX rats were preincubated without (Control) or with 50 pM dexamethasone for 90 min and cAMP-PDIE was then determined as described in Methods. cAMP-PDIE activity is expressed in femtomoles cAMP hydrolyzed per minute per millimeter tubule length.

* Mean±SEM; numbers in parentheses denote number of samples.

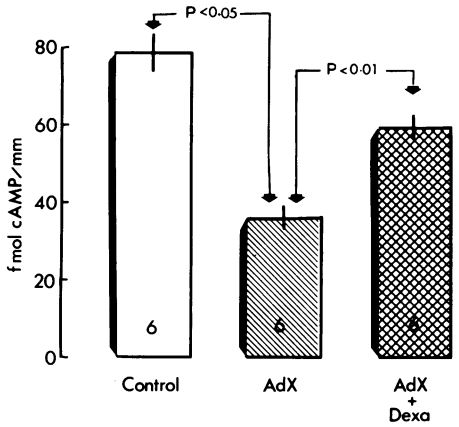

FIGURE 6 Effect of dexamethasone (100 μg/d: DEXA) on AVP (10^{-6} M)-stimulated cAMP accumulation in PCD of ADX (8 d: ADX) rats. Dexamethasone-filled osmotic minipumps were implanted intraperitoneally at the time of adrenalectomy. Sham-operated controls and ADX rats were implanted with 0.9% saline-filled pumps. Each column represents mean±SEM of values expressed as femtomoles cAMP per millimeter of tubule length. Numbers at the base of columns represent the number of animals; 8–10 samples were assayed from each animal for each condition. $P < 0.05$ or less represents a significant difference (group t test) between respective groups.

defect was originally attributed, at least in part, to an observed decrease in medullary tonicity which would diminish the driving force for water absorption in collecting tubules (2, 3). In the present study, while U_{osM} was markedly diminished, by ~1,000 mosM (Fig. 1), tissue osmolality determined in the papilla of hydropenic ADX rats tended to be only slightly lower than that of corresponding sham-operated control rats. Thus, although potentially a minor contributing factor, relatively small decreases in interstitial osmolality could not fully explain the observed decreases in maximum U_{osM}. Direct evidence for a reduced functional responsiveness to AVP in the collecting tubule system was recently provided by the microperfusion studies of Schwartz and Kokko (4), in which hydroosmotic responses to AVP in cortical collecting tubules dissected from ADX rabbits were lower than those from corresponding control tubules. This study (4) also pointed to the possibility that the decreased hydroosmotic responses of adrenal-steroid depleted epithelia to AVP may be due to increased cAMP breakdown, as first suggested by Handler et al. (7) and Stoff et al. (25), based on studies of toad urinary bladder.

Previous studies of the effects of adrenalectomy on AVP-dependent cAMP metabolism in the mammalian kidney (in particular, the medulla and/or papilla) have yielded inconsistent results (9–11). These inconsistencies may have been due to the heterogeneous nature of the renal medullary tissue preparations used in these studies, especially in view of the fact that this zone of the kidney contains at least two nephron segments with an AVP-responsive cAMP system, i.e., the thick ascending limb of Henle's loop as well as the collecting tubule (12, 13, 21, 26).

Our present study clearly documents an AVP-dependent renal concentrating defect in the ADX rat (Fig. 1) and identifies the PCD as a site of abnormal AVP-dependent cAMP metabolism. Specifically, AVP-dependent cAMP accumulation is markedly decreased in PCD of ADX rats compared with controls (Fig. 4). Since adenylate cyclase activity is not diminished in response to either maximal or submaximal stimulatory concentrations of AVP (Fig. 2), decreased cAMP accumulation is most likely due to the enhanced activity of cAMP-PDIE detected in this nephron segment (Fig. 3). We have shown in our previous studies on PCD (15) that the extent of AVP-induced cAMP accumulation increases markedly with increased osmolality of the medium. Since papillary osmolality tends to be

TABLE II

Effect of Dexamethasone (100 μg/d) on Basal and AVP (10^{-6} M)-stimulated Adenylate Cyclase Activity in PCD of 8-d-ADX Rats

	ADX Rats		ADX plus dexamethasone rats	
Experiment	Basal	10^{-6} M AVP	Basal	10^{-6} M AVP
1	101.2±7.7 (8)*	1,131.9±107.9 (8)	73.4±14.7 (6)	1,043.6±108.4 (8)
2	53.3±6.4 (7)	907.3±72.8 (8)	34.2±0.8 (4)	853.7±91.7 (6)
3	114.1±43.7 (4)	1,366.5±146.1 (6)	—	915.9±48.3 (6)
Mean±SEM	89.2±18.8	1,135.2±132.6	54.0±19.4	937.4±56.1

Enzyme activity is expressed in femtomoles cAMP per 30 min per millimeter tubule length (for details, see Methods).

* Mean±SEM; number of samples indicated in parentheses.

slightly lower in ADX animals compared with controls (2, 3), it is possible that the decrease in AVP-dependent cAMP accumulation seen in ADX tubules in vitro may be even more pronounced in vivo.

In addition, our studies have demonstrated that both the functional defect, i.e., the decreased maximal urinary concentrating response to AVP in vivo (Fig. 1), as well as decreased AVP-dependent cAMP accumulation in vitro (Fig. 6), can be corrected by treatment with the glucocorticoid dexamethasone. A comparison of the effect of dexamethasone on cAMP-PDIE (Fig. 5) and on cAMP levels (Fig. 6) suggests that the degree of inhibition of cAMP-PDIE is more pronounced than the extent to which AVP-dependent cAMP accumulation is restored. This feature might be due at least in part to the fact that cAMP-PDIE may be exposed to conditions in vivo that are not identical to those in vitro. Nevertheless, the directionality of the observed changes should be stressed; the decrease in cAMP-PDIE activity associated with an increase in cAMP accumulation is consistent with the concept that glucocorticoids influence cAMP metabolism by modulating the activity of cAMP-PDIE. Although the restoration of AVP-dependent cAMP accumulation (Fig. 6) and restoration of in vivo responsiveness to AVP (Fig. 1) after dexamethasone treatment is likely due to the observed suppression of cAMP-PDIE activity in PCD of ADX rats (Fig. 5), the possibility that other factors may be involved cannot be excluded. One such possibility that should be examined in future studies is the potential role of prostaglandins (27). Studies in the toad bladder (27) have demonstrated that adrenal steroids may modulate prostaglandin synthesis and specifically that prostaglandin synthesis is markedly increased in steroid-depleted bladders. Extrapolating from amphibian membranes to the mammalian nephron, elevated prostaglandin synthesis in the collecting tubule of the ADX rat might also potentially contribute to the observed attenuation of AVP-dependent cAMP accumulation. The effect of mineralocorticoid hormones on the concentrating defect in ADX animals also remains to be explored, but the present results suggest that glucocorticoid replacement alone is fully capable of correcting the functional defect as well as the biochemical abnormality in PCD of ADX animals.

The exact mechanism by which dexamethasone causes a decrease in cAMP-PDIE is not apparent from the present studies; in vitro addition of this compound was without effect (Table I). Such a comparison may indicate that only more prolonged exposure to the steroid and/or other as yet unidentified conditions are required. Alternatively, dexamethasone might have acted through some unknown indirect mechanism.

It is of interest to note that the cellular basis of this concentrating defect appears analogous to that observed previously in studies on a strain of mice with hereditary nephrogenic diabetes insipidus (12). As in ADX rats, impaired AVP-dependent cAMP accumulation in the medullary collecting tubule of NDI mice appeared to be due, at least in part, to increased cAMP-PDIE activity (12). Whether glucocorticoid would have similar effects in NDI mice to those observed in ADX rats, namely reduced cAMP-PDIE activity and restored AVP-dependent cAMP accumulation is of interest and awaits examination.

In conclusion, the present studies suggest that the decreased concentrating ability associated with adrenal insufficiency may be due to reduced AVP-dependent cAMP accumulation in the PCD, and that this reduced cAMP accumulation is due to enhanced cAMP-PDIE activity. Selective chronic glucocorticoid (dexamethasone) treatment reverses both the biochemical and functional impairments, in that it reduces elevated cAMP-PDIE activity, enhances depressed AVP-dependent cAMP accumulation on PCD, and restores maximal urinary concentrating ability in vivo.

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