Biochimica et Biophysica Acta, 641 (1981) 20—35

BBA 79108

MECHANISMS OF ACTIVATION OF RENAL ($Na^+ + K^+$)-ATPase IN THE RAT

EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF DEXAMETHASONE

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(Received June 9th, 1980)

Key words: ($Na^+ + K^+$)ATPase; Dexamethasone; P_i intermediate; p-Nitrophenylphosphatase; (Rat kidney)

Summary

The mechanisms of activation of renal ($Na^+ + K^+$)-ATPase by administration of the synthetic glucocorticoid hormone, dexamethasone, have been investigated in adrenalectomized rats. Chronic treatment with dexamethasone (1—5 mg/100 g body wt. daily for 5 days) stimulated ($Na^+ + K^+$)-ATPase specific activity in crude homogenates and microsomal fractions of renal cortex (by approx. 100—150%) and renal medulla (by approx. 100%). Acute treatment with dexamethasone (0.5—10 mg/100 g body wt.) also stimulated enzyme activity in crude homogenates and microsomal fractions of renal cortex and medulla (by approx. 40—50%). Stimulation was dose dependent and occurred within 2 h after hormone treatment. In vitro addition of dexamethasone (10^{-4}—10^{-8} M) to microsomal fractions did not modify the specific activity of ($Na^+ + K^+$)-ATPase. Stimulation of ($Na^+ + K^+$)-ATPase activity by acute and chronic administration of the hormone was demonstrated whether specific activities were expressed as a function of cellular protein or cellular DNA. Dexamethasone treatment increased the ratios protein:DNA and, to a lesser extent, the ratios RNA:DNA. However, these effects were mainly due to a reduction in the renal contents of DNA, which suggests that the observed enzyme activation is not due to an action of the hormone on renal hypertrophy. Dexamethasone also reduced cellular DNA contents in the liver. The characteristics of the activation process were essentially similar after treatment with single or multiple doses of the hormone. There were increases in the value for Na^+ (approx. 100%), K^+ (approx. 40%) and ATP (approx. 160%). The K_m values for Na^+ (approx. 17 mM) and K^+ (approx. 1.8 mM) were unchanged and there was

a small increase in the K_m value for ATP (0.7 mM as against 1.7 mM). There was no difference in the Hill coefficients for the three substrates. The levels of the high-energy P_i intermediate of the $(Na^+ + K^+)$-ATPase reaction were augmented by dexamethasone treatment and the increased levels were quantitatively correlated with the observed stimulation of $(Na^+ + K^+)$-ATPase specific activity. The apparent turnover numbers of the reaction remained unchanged. The specific activity of the ouabain-sensitive *p*-nitrophenylphosphatase increased proportionally to the increase in $(Na^+ + K^+)$-ATPase specific activity. Enzyme activation by acute dexamethasone treatment occurred in the absence of changes in glomerular filtration rate and tubular Na^+ excretion.

These results indicate that $(Na^+ + K^+)$-ATPase activation by acute and chronic dexamethasone treatment represents an increase in the number of enzyme units with little or no change in the kinetic properties (affinity, cooperativity) of the enzyme. In addition, the information presented suggests a direct regulatory effect of glucocorticoid hormones on the activity of renal $(Na^+ + K^+)$-ATPase and is inconsistent with the concept that changes in Na^+ loads mediate the effects of these hormones on enzyme activity. Instead, the results suggest a primary role for glucocorticoid hormones in the renal regulation of Na^+ homeostasis.

Introduction

A large body of evidence indicates that the Na^+- and K^+-activated adenosine triphosphatase (ATP phosphohydrolase, EC 3.6.1.3) is the enzymatic equivalent of the system responsible for active transmembrane sodium transport [1—3]. In the kidney, as in other epithelial tissues, the enzyme is localized on the basolateral membrane of the epithelial cells [1], and it appears to be involved in the process of transtubular sodium reabsorption [2]. Adrenalectomy lowers the specific activity of renal $(Na^+ + K^+)$-ATPase and the enzymatic activity can be restored to normal by administration of glucocorticoid hormones (corticosterone, hydrocortisone) [4]. In addition, chronic administration of synthetic glucocorticoid hormones (e.g., methylprednisolone) stimulates $(Na^+ + K^+)$-ATPase specific activity in the renal cortex and medulla of normal and adrenalectomized animals [5] but the mechanisms underlying this stimulation have not been defined. Since this stimulatory effect appears to coincide with increases in glomerular filtration rate and tubular sodium reabsorption [6], it has been suggested that the changes in enzyme specific activity that occur during prolonged treatment with these hormones are secondary to hemodynamic events (e.g., changes in cardiac output and glomerular filtration rate) that lead to increases in tubular sodium reabsorption and, accordingly, that the sodium load (rather than the hormone) may regulate enzyme activity [6].

The demonstration of specific receptors in rat kidney for two glucocorticoid hormones (type II receptors for dexamethasone, type III receptors for corticosterone) [7], raises the question of whether the glucocorticoid stimulation of $(Na^+ + K^+)$-ATPase specific activity may represent a direct hormonal effect on the steady-state levels or on the kinetic properties of the transport enzyme [4]. If this were the case, the increased rates of tubular sodium transport associated

with chronic administration of glucocorticoid hormones might be the consequence rather than the cause of enzyme activation.

The hypothesis that the stimulatory effect of chronic glucocorticoid administration on $(Na^+ + K^+)$-ATPase is mediated by changes in filtered loads of Na^+ [6] can be tested by examining the effects of acute administration of glucocorticoid hormone on enzyme activity. There is some evidence that the glomerular filtration rate does not increase within a few hours after administration of a single dose of glucocorticoids [10]; hence, a change in $(Na^+ + K^+)$-ATPase specific activity under these experimental conditions could not support a primary role for Na^+ loads in enzyme regulation. This inference can be further explored by analysis of the kinetic properties and the partial reactions of the $(Na^+ + K^+)$-ATPase after acute and chronic administration of glucocorticoids. The information thus obtained may also provide insight into the mechanisms that the kidney utilizes to regulate the steady-state levels of this transport enzyme and any potential role for glucocorticoid hormones on the renal regulation of Na^+ excretion.

Materials and Methods

Female Sprague-Dawley rats weighing 150—200 g were adrenalectomized by a lumbar approach, under chloral hydrate anesthesia (36 mg/100 g body wt., intraperitoneally) and maintained on standard Purina Rat Chow and 0.9% saline solution as drinking fluid. They were allowed to recover from surgery for a period of 5 days prior to administration of hormones. Two different approaches were used to assess the adequacy of adrenalectomy: (a) adrenalectomized rats gained 40—50% of the weight gain of sham-operated controls during the recovery period (mean weight gain in controls: 38 ± 2 g/5 days; in adrenalectomized rats: 19 ± 1 g/5 days; $n = 12$); (b) adrenalectomized rats had consistently lower plasma sodium (131 ± 2 as compared to 143 ± 2 mequiv./l) and higher plasma potassium concentrations (6.7 ± 0.6 as against 4.8 ± 0.1 mequiv./l) than sham-operated controls ($n = 12$). Body weight was determined immediately prior to and serum electrolytes immediately after killing the animals. Rats which did not meet the above criteria for adrenalectomy were excluded from the study. For the acute experiments, adrenalectomized rats were injected, intraperitoneally, with a single dose (0.5—10 mg/100 g body wt.) of dexamethasone. Control animals received an identical volume of vehicle (0.15% $Na_2S_2O_3$, 1% benzyl alcohol N.F.). 2 h later, the rats were anesthetized with chloral hydrate and killed by exsanguination from the abdominal aorta. The kidneys were perfused with 20 ml of ice-cold saline, quickly removed and placed in ice-cold saline solution. For the chronic experiments, adrenalectomized rats were injected, intraperitoneally, with 1—5 mg/100 g body wt. of dexamethasone daily for 5 days; controls received daily injections of an identical volume of vehicle. At the end of this period, the animals were killed by exsanguination and the kidneys handled as described above.

Tissue preparation. Renal cortical and medullary slices were obtained with a Stadie-Riggs microtome. Tissue adjacent to the boundaries between the zones was discarded. The tissue segments were gently blotted on filter paper, weighed and homogenized in a 10 : 1 (v/w) solution containing: 250 mM sucrose, 30

mM DL-histidine, pH 7.4, 5 mM EDTA, 0.01 mM Cleland's reagent and 0.1% sodium deoxycholate (added fresh). Homogenization was carried out in a glass homogenizer with a Teflon pestle by 10 strokes at top speed. A sample of this suspension was used for the determination of DNA, RNA, protein and ATPase activity. The remainder of the homogenate was used to prepare a microsomal fraction by differential centrifugation, as previously described [11]. The final enzyme suspension from cortex contained 7—10 mg protein/ml and from medulla 3—5 mg protein/mg.

Assay of $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities. $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities were determined by a radiochemical method utilizing [^{32}P]ATP [11,12]. Total ATPase was determined in reaction mixtures containing: 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA, 100 mM Tris-HCl, pH 7.4, and appropriate quantities (about 5—10 μg) of enzyme suspension, in a total volume of 0.1 ml. Reactions were started by the addition of 3 mM Tris-ATP, containing 0.02—0.04 μCi of [γ-^{32}P]ATP (specific activity $\simeq$ 3000 Ci/mmol) and the mixtures were incubated for 5—15 min at 37°C. Incubations were terminated by cooling the mixtures down to 0—4°C and denaturing the protein with 0.5 ml of 190 mM $HClO_4$ containing 0.25 mM KH_2PO_4. The $^{32}P_i$ released was then precipitated with a mixture of 6.2 mM $(NH_4)_2MoO_4$ and 7.7 mM triethylamine hydrochloride, pH 5 [12]. The precipitate was separated by centrifugation, washed, resuspended in 5% NH_4OH and counted as previously described [11]. $(Na^+ + K^+)$-ATPase activity was computed from the difference in activity when potassium was present or absent in the assay media or in the presence of 1 mM ouabain [4,13]. These reaction conditions are optimal for assay of renal $(Na^+ + K^+)$-ATPase activity [11].

Assay of the specific activity of p-nitrophenylphosphatase. The activity of *p*-nitrophenylphosphatase was assayed in a solution of 10 mM Tris-HCl, pH 7.7, 5 mM $MgCl_2$, 10 mM KCl and 5 mM *p*-nitrophenyl phosphate in a final volume of 1 ml. Following incubation at 37°C for 15 min, the reaction was stopped by cooling the tubes in ice and adding 0.25 ml of 1 M NaOH. The amount of *p*-nitrophenol released was quantitated as previously described [11] and the specific activity was expressed as μmol *p*-nitrophenol/mg protein per h. The ouabain-sensitive phosphatase was determined in the presence of 1 mM ouabain in the incubation medium [11].

Determination of the levels of the high-energy phosphorylated intermediate (P_i intermediate). The levels of P_i intermediate were determined as previously described [11]. In brief, the assay medium contained: 50 mM NaCl, 100 mM imidazole, pH 7.4, 3 mM $MgCl_2$, 1 mM EDTA, 0.005 mM [γ-^{32}P]ATP and appropriate quantities of enzyme suspension in a final volume of 0.1 ml. Tandem tubes contained 5 mM KCl and served to measure non-specific phosphorylation [11,14]. Incubations were carried out at 0—4°C for 15 s and the ^{32}P radioactivity associated with trichloroacetic acid-insoluble materials was determined by scintillation counting. We have previously validated that these assay conditions are optimal for determination of the levels of P_i intermediate in kidney enzyme [11]. The levels of P_i intermediate are expressed as pmol/mg protein.

Determination of the kinetic parameters of the $(Na^+ + K^+)$-ATPase reaction. The kinetic parameters (K_m, $K_{1/2}$, V) for the three substrates of the $(Na^+ + K^+)$-

ATPase reaction were determined in enzyme suspensions of cortex and medulla obtained from four to five pairs of rats. For determination of the kinetic parameters for ATP, the assay mixture contained 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA, 100 mM Tris-HCl, pH 7.4, and different concentrations of Tris-ATP (0.3—1 mM) with [γ-^{32}P]ATP (0.02—0.04 μCi). Incubations were carried out for 5 min at 37°C. As previously discussed, these reaction conditions eliminate the need for an ATP-regenerating system [11].

For determination of the kinetic parameters for Na^+, the concentration of this ion was varied in the range 5—30 mM and for K^+ in the range 0.5—2.5 mM. Incubations were carried out for 0—15 min at 37°C. Other reaction conditions were as previously described [11].

Chemical determinations. The levels of serum electrolytes, blood urea nitrogen, glucose, phosphate, and creatinine were measured as previously described [11]. For determination of nucleic acids, samples of tissue homogenates were extracted by using the method of Schneider [15]. DNA was assayed according to the method of Burton [16] and RNA according to that of Munroe and Fleck [17]. Protein was determined by using the method of Lowry et al. [18].

All of the results are given as means ±S.E. The significance of the differences between control and experimental animals was estimated by the non-paired Student's *t*-test; *P* values less than 0.05 were considered to be significant.

Materials. Tris-ATP from equine muscle (vanadium-free), *p*-nitrophenyl phosphate (dicyclohexyl ammonium salt) and deoxycholic acid (sodium salt) were purchased from Sigma Chemical Co., St. Louis, MO. [γ-^{32}P]ATP was purchased from New England Nuclear Corp. (Boston, MA). Dexamethasone (hexadrol phosphate) was purchased from Organon Incorporated, W. Orange, NJ. Dexamethasone vehicles were prepared by dissolving 0.15% $Na_2S_2O_3$ and 1% benzyl alcohol in 0.9% saline solution and adjusting the pH to 7.4 with NaOH. All other chemicals were purchased from different suppliers without apparent effects on the results obtained.

Results

Effects of acute and chronic administration of dexamethasone on blood chemistries

Chronic administration of dexamethasone restored the plasma levels of sodium and potassium to normal in adrenalectomized rats (Table I). This response may reflect expression of mineralocorticoid effects, since it has been shown that dexamethasone, at appropriate concentrations, can occupy aldosterone receptor sites (type I receptors) in rat kidney and hence trigger expression of mineralocorticoid effects [7]. Chronic dexamethasone administration produced a clear-cut increase in the plasma levels of glucose as expected from the well known effect of glucocorticoids on hepatic and renal gluconeogenesis [19]. Likewise, the plasma levels of phosphate and creatinine increased significantly whereas the levels of blood urea nitrogen did not change. The significance of these changes remains to be elucidated.

Administration of a single dose of dexamethasone increased the plasma levels of sodium but did not affect the plasma levels of potassium (Table I).

TABLE I

EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF DEXAMETHASONE ON BLOOD CHEMISTRIES IN ADRENALECTOMIZED RATS

Adrenalectomized rats received a single injection (5 mg/100 g body wt.) (acute) or multiple injections (5 mg/100 g body wt. daily for 5 days) (chronic) of dexamethasone. Control rats received single or multiple injections of vehicle. The animals were killed 2 h after the 'single' injection (acute) or 2 h after the 'last' injection (chronic) of dexamethasone and blood chemistries were determined. The values for control animals (single or multiple injections) have been pooled because the results of these two groups were essentially similar. The rats were non-fasted. The values are the means ±S.E. of 6—12 animals in each group. Values for Na^+ and K^+ are expressed in mequiv./l; values for the other components are given as mg/dl. n.s., not significant.

	Na^+	K^+	Glucose	Phosphate	Creatinine	Blood urea nitrogen
Control	130 ± 1	5.7 ± 0.23	255 ± 16	5.8 ± 0.26	0.38 ± 0.02	22.8 ± 1.8
Acute	135 ± 1	6.1 ± 0.38	235 ± 26	8.3 ± 0.40	0.39 ± 0.03	24.7 ± 1.1
Chronic	138 ± 2	4.7 ± 0.27	343 ± 27	7.1 ± 0.35	0.54 ± 0.02	19.1 ± 1.3
P control vs. acute	<0.05	n.s.	n.s.	<0.01	n.s.	n.s.
P control vs. chronic	<0.01	<0.05	<0.01	<0.02	<0.01	n.s.

The latter occurred in spite of the fact that administration of a single dose of dexamethasone (5 mg/100 g body wt.) to an adrenalectomized rat produced an increase in the fractional excretion of K^+ from 12.5 ± 0.26% to 19.0 ± 0.27% without changes in glomerular filtration rate or Na^+ excretion. Similar results have been reported by others [10]. The fact that the plasma levels of K^+ did not change may reflect a large increase in the body stores of this cation in adrenalectomized rats. Unlike the situation with chronic administration of dexamethasone, acute administration of the hormone did not change the plasma levels of glucose or creatinine, but the levels of serum phosphorus were significantly increased; blood urea nitrogen remained unchanged (Table I). The animals in these experiments were non-fasted. This may be important in interpreting the results obtained with plasma phosphate and glucose.

TABLE II

EFFECTS OF ACUTE AND CHRONIC DEXAMETHASONE ADMINISTRATION ON THE SPECIFIC ACTIVITIES OF RENAL ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase IN MICROSOMAL FRACTIONS FROM RENAL CORTEX AND RENAL MEDULLA

Experimental conditions were as described in the legend to Table I. The values are the means ±S.E. The numbers of animals are shown in parentheses. Specific activities are expressed as μmol P_i/mg protein per h.

	($Na^+ + K^+$)-ATPase		Mg^{2+}-ATPase	
	Cortex	Medulla	Cortex	Medulla
Control (6)	10.4 ± 1.0	15.6 ± 1.4	17.6 ± 0.3	2.2 ± 0.91
Acute (10)	15.1 ± 0.85	23.4 ± 5.5	15.6 ± 0.35	3.5 ± 0.77
P	<0.02	<0.02	n.s.	n.s.
Control (7)	10.5 ± 1.4	25.6 ± 1.4	7.6 ± 1.5	2.2 ± 0.01
Chronic (12)	27.6 ± 1.5	55.1 ± 8.3	16.6 ± 0.8	2.4 ± 0.98
P	<0.01	<0.01	<0.01	n.s.

Effects of acute and chronic administration of dexamethasone on the specific activities of ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase

It has long been recognized that chronic administration of synthetic glucocorticoids (methylprednisolone) [4,6,20] and naturally occurring glucocorticoids (hydrocortisone, corticosterone) [5] to normal and adrenalectomized animals stimulates the specific activity of ($Na^+ + K^+$)-ATPase in renal cortex and medulla [4,6]. Table II shows that chronic administration of dexamethasone (5 mg/100 g body wt. daily for 5 days), under our experimental conditions, significantly increased the specific activity of renal ($Na^+ + K^+$)-ATPase in the renal medulla and renal cortex. A 5-fold decrease in the daily dose of dexamethasone (1 mg/100 g body wt.) produced comparable stimulation of enzyme activity in cortex and medulla (results not shown). This dose range is similar to that used in other studies on activation of renal ($Na^+ + K^+$)-ATPase by synthetic glucocorticoids [4,6,20].

The effect of single doses of synthetic glucocorticoids on the specific activity of renal ($Na^+ + K^+$)-ATPase has not been studied. As shown in Table II, 2 h after the administration of a single dose (5 mg/100 g body wt.) of dexamethasone to adrenalectomized rats, there was a substantial increase in the specific activity of ($Na^+ + K^+$)-ATPase in both the renal cortex and the medulla. In vitro addition of dexamethasone to microsomal fractions from renal medulla had no effect on enzyme activity. ($Na^+ + K^+$)-ATPase specific activity in the presence of a high concentration of dexamethasone (10^{-4} M) was 50 ± 2 μmol P_i/mg protein per h as against 49 ± 3 μmol P_i/mg protein per h in vehicle-containing controls (n = 3). Similar results were obtained with a low concentration (10^{-8} M) of dexamethasone: vehicle-containing control. 58 ± 3 μmol P_i/mg protein per h and dexamethasone treated 55 ± 2 μmol P_i/mg protein per h. Likewise, pre-incubation of microsomal fractions in the presence of dexamethasone (10^{-4}—10^{-8} M) for 2 h prior to enzyme assay had no demonstrable effect on ($Na^+ + K^+$)-ATPase specific activity. The specific activity of Mg^{2+}-ATPase

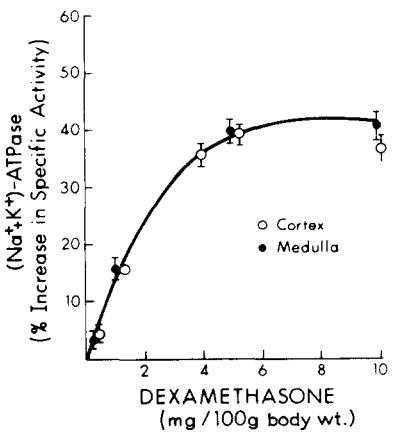

Fig. 1. Dose dependence of the stimulation of ($Na^+ + K^+$)-ATPase specific activity by acute administration of dexamethasone. Adrenalectomized rats were injected intraperitoneally with a single dose (0.1—10 mg/100 g body wt.) of dexamethasone. Enzyme activity was determined 2 h after injection of the hormone. Control rats received identical volumes of vehicle. The results are plotted as the percent change over control. n = 6—10 rats for each group.

changed slightly (Table II). The dose dependence of the stimulatory effect on $(Na^+ + K^+)$-ATPase by acute administration of dexamethasone was examined by investigating the effects of different doses of the steroid. The results of these experiments are depicted in Fig. 1. The dose-dependent curve shows that maximal activation of $(Na^+ + K^+)$-ATPase occurred with single intraperitoneal doses of 4—5 mg/100 g body wt. In a separate set of experiments, glomerular filtration rate and urinary Na^+ excretion were determined in three adrenalectomized, non-anesthetized rats at 30 min intervals 1 h before and 2 h after administration of a single dose of 5 mg/100 g body wt. of dexamethasone. Glomerular filtration rate was 2.1 ± 0.1 ml/min before and 2.3 ± 0.2 ml/min after dexamethasone treatment. Likewise, fractional excretion of Na^+ was unchanged by dexamethasone administration (2.3 ± 0.1% before and 2.5 ± 3% after hormone treatment). Hence, the stimulation of $(Na^+ + K^+)$-ATPase by a single dose of dexamethasone occurs in the absence of demonstrable changes in the filtered loads and tubular reabsorption of Na^+.

Effects of acute and chronic administration of dexamethasone on body weight, kidney weight and the ratios protein:DNA and RNA:DNA

Acute administration of dexamethasone produced no change in total body weight. In contrast, chronic administration of dexamethasone resulted in a 15% decrease in body weight (Table III). A similar weight loss has been reported in normal rats chronically treated with cortisone [21], an effect probably due to the catabolic effects of glucocorticoid hormones [22—24]. Both acute and chronic administration of dexamethasone increased kidney weight and the ratio of kidney to body weight (Table III); the effect being more apparent in chronically treated animals. The results raised the question of whether the stimulation of renal $(Na^+ + K^+)$-ATPase by glucocorticoid administration was mediated by an effect of the hormone on renal growth, since compensatory renal hyper-

TABLE III

CHANGES IN BODY WEIGHT AND KIDNEY WEIGHT AFTER ACUTE AND CHRONIC ADMINISTRATION OF DEXAMETHASONE TO ADRENALECTOMIZED RATS

Experimental conditions were as described in the legend to Table I. The values for control animals (single or multiple injections of vehicle) have been pooled because the results of these two groups were essentially similar. Initial body weights in g (obtained at the end of the recovery period form surgery) were: 204 ± 4 in control, 209 ± 8 in acute and 209 ± 9 in chronic animals. Final body weights in g (obtained at the time of killing) were: 214 ± 5 in control, 212 ± 7 in acute and 175 ± 7 in chronic animals. The kidney weight is the average weight of the two kidneys. The final body weight was used for computation of the ratio kidney wt.: body wt. The contents of protein and nucleic acids were determined in samples of crude homogenates prior to centrifugation. The values are the means ±S.E. of 6—12 animals for each group.

	Kidney weight (g)	Kidney wt.: body wt. (g) ($\times 10^{-3}$)	Protein:DNA		RNA:DNA	
			Cortex	Medulla	Cortex	Medulla
Control	0.725 ± 0.027	3.21 ± 0.14	50 ± 11	41 ± 7	1.31 ± 0.13	1.65 ± 0.23
Acute	0.812 ± 0.024	3.78 ± 0.08	91 ± 3	71 ± 4	1.74 ± 0.20	1.56 ± 0.12
Chronic	0.844 ± 0.048	4.80 ± 0.20	70 ± 9	65 ± 11	1.66 ± 0.08	1.67 ± 0.11
P control vs. acute	n.s.	<0.05	<0.05	<0.01	n.s.	n.s.
P control vs. chronic	<0.05	<0.01	n.s.	n.s.	n.s.	n.s.

TABLE IV

EFFECT OF CHRONIC DEXAMETHASONE ADMINISTRATION ON RENAL AND HEPATIC CONTENTS OF DNA, RNA AND PROTEIN

Adrenalectomized rats received daily intraperitoneal injections of dexamethasone (5 mg/100 g body wt.) for 5 days. Controls were injected with identical volumes of vehicle. The animals were killed 2 h after the last injection of hormone or vehicle. Slices of kidney or liver were blotted, weighed (wet weight), dried (120°C for 3 days) and reweighed (dry weight). The remaining tissue was weighed and homogenized as described under Materials and Methods. The crude homogenates were assayed for DNA, RNA and protein. The ratios of wet weight to dry weight were used to compute nucleic acids and protein contents per mg of dry tissue weight. The values are the means ±S.E. The numbers of animals are shown in parentheses. Values are expressed as μg/mg dry weight.

	DNA	RNA	Protein
Kidney			
Control (n = 6)	12.8 ± 1.0	17.6 ± 1.1	533 ± 35
Dexamethasone (n = 6)	10.1 ± 1.0	17.8 ± 1.3	582 ± 80
P	<0.02	n.s.	n.s.
Liver			
Control (n = 6)	5.9 ± 0.3	27.4 ± 0.5	428 ± 9
Dexamethasone (n = 6)	3.4 ± 0.1	27.6 ± 1.4	411 ± 16
P	<0.01	n.s.	n.s.

trophy (e.g., after unilateral nephrectomy) is usually accompanied by stimulation of the activity of this transport enzyme [6].

The potential effects of dexamethasone on renal growth were further examined by determining the ratios of protein:DNA and RNA:DNA in samples of crude homogenates (Table III). Acute or chronic administration of dexamethasone increased the ratios protein:DNA in renal cortex and medulla, suggesting that the increase in kidney weight might reflect an increase in the cellular content of proteins throughout the kidney. The ratios RNA:DNA were slightly increased in the cortex and unchanged in the medulla by acute and chronic administration of dexamethasone (Table III). To ascertain whether the observed differences in the ratios were due to changes in the cellular contents of DNA and/or proteins, we examined the effects of steroid hormone treatment on nucleic acids and protein contents expressed as a function of dry tissue weight. As shown in Table IV, dexamethasone administration reduced the renal contents of DNA and produced a small increase in protein content without changes in RNA. Hence, the increased protein:DNA ratios do not entirely reflect an overall increase in the protein content of the kidney. The adverse effect of dexamethasone on the cellular contents of DNA is not unique to the kidney; an even greater effect occurs in the liver (Table IV). Taken together, these results suggest that renal hypertrophy does not appear to occur to a significant extent after dexamethasone treatment and hence cannot account for the observed stimulation of renal ($Na^+ + K^+$)-ATPase.

In view of the apparent effect of the hormone on the cellular contents of protein, it was of interest to determine whether the observed increase in ($Na^+ + K^+$)-ATPase specific activity in the microsomal fraction could also be demonstrated when expressed as a function of another parameter, such as DNA. To answer this question, we examined the effects of acute and chronic administra-

tion of dexamethasone on $(Na^+ + K^+)$-ATPase specific activity assayed in crude homogenates. Preliminary experiments showed that the concentration of deoxycholate used for homogenization (0.1%) yielded maximal activation of $(Na^+ + K^+)$-ATPase in crude homogenates. As shown in Table V, a significant increase in $(Na^+ + K^+)$-ATPase specific activity per mg DNA was observed after acute or chronic administration of dexamethasone in both cortex and medulla. These results suggest that the increase in $(Na^+ + K^+)$-ATPase activity after treatment with dexamethasone represents enhanced enzyme activity per cell. Likewise, the demonstration of an effect of the hormone on enzyme activity in crude homogenates, prior to differential centrifugation, excludes the possibility that the stimulation observed in microsomal fractions (Table II) is due to the preferential enrichment of microsomal membranes with a high activity of $(Na^+ + K^+)$-ATPase in fractions prepared from dexamethasone-treated rats.

Taken together, the results presented indicate that stimulation of renal $(Na^+ + K^+)$-ATPase can be shown by acute and chronic administration of dexamethasone. In both experimental situations, enzyme activation appears to be a specific event reflecting a true increase in the cellular activities of the enzyme. In addition, stimulation of the transport enzyme by acute dexamethasone treatment can be dissociated from changes in glomerular filtration rate and tubular Na^+ transport. To explore in more detail the activation mechanism, the next set of experiments was performed.

Effects of acute and chronic administration of dexamethasone on the kinetic parameters (K_m, $K_{1/2}$, V) of the $(Na^+ + K^+)$-ATPase reaction

The increased $(Na^+ + K^+)$-ATPase activity observed after glucocorticoid administration may represent recruitment of additional enzyme units ('sodium-pump' units) or simply the activation of a fixed number of pre-existing sodium-pump sites [11]. Activation of pre-existing pump sites may be associated with changes in the relative affinities of the enzyme for one or all of the substrates (sodium, potassium, ATP), whereas an increase in the number of sodium-pump

TABLE V

EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF DEXAMETHASONE ON THE SPECIFIC ACTIVITIES OF $(Na^+ + K^+)$-ATPase AND Mg^{2+}-ATPase IN CRUDE HOMOGENATES FROM RENAL CORTEX AND RENAL MEDULLA

Enzyme activities and DNA contents were determined in samples of crude homogenates prior to differential centrifugation. Other experimental conditions were as described in the legend to Table I. The values are the means ±S.E. The numbers of animals are shown in parentheses. Specific activities are expressed as μmol P_i/mg DNA per h.

	$(Na^+ + K^+)$-ATPase		Mg^{2+}-ATPase	
	Cortex	Medulla	Cortex	Medulla
Control (6)	87 ± 8	189 ± 12	281 ± 5	257 ± 7
Acute (10)	139 ± 6	243 ± 8	273 ± 3	235 ± 3
P	<0.01	<0.05	n.s.	n.s.
Control (5)	76 ± 6	169 ± 15	300 ± 15	340 ± 35
Chronic (6)	124 ± 11	237 ± 18	311 ± 13	363 ± 11
P	<0.05	<0.05	n.s.	n.s.

TABLE VI

EFFECTS OF DEXAMETHASONE ADMINISTRATION ON THE KINETIC PARAMETERS OF RENAL MEDULLARY ($Na^+ + K^+$)-ATPase

Adrenalectomized rats received daily intraperitoneal injections of dexamethasone (5 mg/100 g body wt.) for 5 days. Controls were injected with identical volumes of vehicle. The animals were killed 2 h after the last dose of hormone or vehicle and microsomal fractions were prepared for enzyme assays. Kinetic parameters were calculated by linear regression from Eadie-Hofstee plots (K_m and $K_{1/2}$, slopes; V,y intercepts). K_m and $K_{1/2}$ values are expressed in mmol/l and V values in μmol P_i/mg protein per h. The values are the means ±S.E. of four to five pairs of rats for each substrate.

	Na^+		K^+		ATP	
	V	$K_{1/2}$	V	$K_{1/2}$	V	$K_{1/2}$
Control	31.6 ± 4.2	16.4 ± 1.0	47.4 ± 1.1	2.4 ± 0.11	37.2 ± 1.0	0.71 ± 0.0
Dexamethasone	64.0 ± 1.5	17.4 ± 0.8	67.0 ± 7.1	1.95 ± 0.39	97.7 ± 4.1	1.68 ± 0.0
P	<0.01	n.s.	<0.02	n.s.	<0.01	<0.01

sites would be expected to result in an increase of the V value for the three substrates with or without a corresponding change in their affinities [11,13]. The kinetic parameters for sodium, potassium and ATP of the ($Na^+ + K^+$)-ATPase reaction were examined in microsomal fractions prepared from kidneys of control rats and rats receiving multiple injections of dexamethasone (Fig. 2, Table VI). The changes in the kinetic parameters following dexamethasone treatment were similar in cortex and medulla. Fig. 2 depicts the changes in enzyme specific activity as a function of the concentrations of the three substrates analyzed by Eadie-Hofstee plots. Chronic dexamethasone treatment produced an increase in the V values for sodium (100%), potassium (43%) and ATP (160%). In addition, the affinities for sodium and potassium were unchanged and the apparent affinity for ATP was slightly decreased by chronic dexamethasone treatment (Fig. 2, Table VI). This kinetic behavior is consistent with an increase in the number of enzyme units (sodium-pump sites) and excludes a substantial role of changes in the affinity of pre-existing or new enzyme units as the basis for the observed increase in ($Na^+ + K^+$)-ATPase

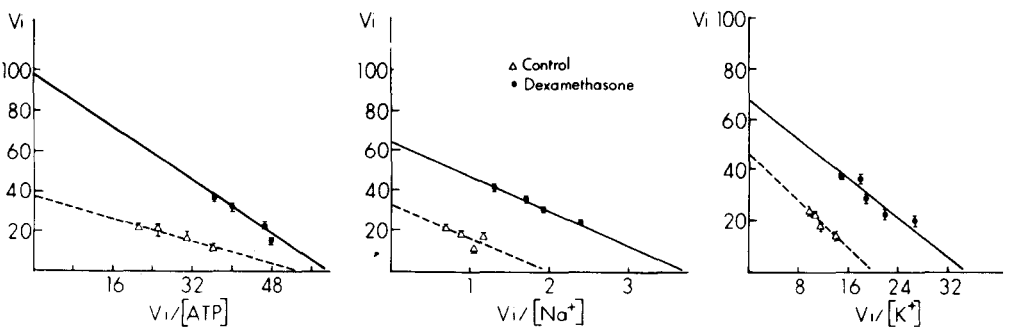

Fig. 2. Dependence of renal medullary ($Na^+ + K^+$)-ATPase on the concentration of ATP, Na^+ and K^+ in control rats (Δ) and in rats receiving multiple injections of dexamethasone (5 mg/100 g body wt. daily for 5 days) (•). The data were analyzed by Eadie-Hofstee plots. Vi, ($Na^+ + K^+$)-ATPase specific activity in μmol P_i/mg protein per h. The points and vertical lines represent the means ±S.E. n = 4—5 pairs of rats for each substrate.

specific activity after chronic administration of dexamethasone. Similar changes in the kinetic parameters of the $(Na^+ + K^+)$-ATPase reaction were observed, after acute administration of dexamethasone (data not shown). Neither acute nor chronic dexametasone treatment produced demonstrable changes in the Hill coefficients for any of the three substrates, thus indicating that in neither situation would changes in cooperative interactions with substrates [5] account for the observed increase in enzyme specific activity.

Effects of acute and chronic dexamethasone administration on the levels of the high-energy phosphorylated (P_i) intermediate

The inference that the increase in $(Na^+ + K^+)$-ATPase specific activity after dexamethasone treatment was primarily due to an increase in the number of enzyme units (sodium-pump sites) was further tested by quantitating the levels of the high-energy P_i intermediate. If the interpretation of the kinetic data is correct, it may be predicted that dexamethasone administration should be associated with a substantial increase in the levels of the P_i intermediate [11, 13,26]. As shown in Table VII, stimulation of cortical and medullary $(Na^+ + K^+)$-ATPase by chronic dexamethasone administration was accompanied by quantitatively similar increases in the levels of the P_i intermediate. This is reflected in the fact that the apparent turnover numbers of the enzymatic reaction were virtually unchanged by dexamethasone treatment, thus implying that

TABLE VII

EFFECTS OF CHRONIC AND ACUTE DEXAMETHASONE ADMINISTRATION ON THE SPECIFIC ACTIVITY OF RENAL $(Na^+ + K^+)$-ATPase, THE LEVELS OF P_i INTERMEDIATE AND THE APPARENT TURNOVER NUMBERS OF THE REACTION

Adrenalectomized rats were injected intraperitoneally with 5 mg/100 g body wt. of dexamethasone daily for 5 days (chronic), or with a single dose of 10 mg/100 g body wt. of dexamethasone (acute). Control rats received comparable injections of identical volumes of vehicle. The rats were killed 2 h after the last or single injection and microsomal fractions were prepared for enzyme assays. Apparent turnover numbers were calculated from the specific activities of $(Na^+ + K^+)$-ATPase and the corresponding values for the high-energy P_i intermediate. This computation assumes that the phosphorylation step is a stoichiometric reaction [11,25]. The numbers of animals are shown in parentheses. The values are the means ±S.E.

	$(Na^+ + K^+)$-ATPase (μmol P_i/mg protein per h)		P_i intermediate (pmol/mg protein)		Apparent turnover numbers ($\times 10^5$) (h^{-1})	
	Cortex	Medulla	Cortex	Medulla	Cortex	Medulla
Control (6)	11.2 ± 1.6	26.4 ± 2.5	20.0 ± 1	85.0 ± 7	5.60 ± 0.6	3.10 ± 0.3
Chronic dexamethasone (6)	28.7 ± 1.3	57.8 ± 3.1	61.0 ± 18	141.0 ± 18	4.70 ± 0.5	4.10 ± 0.5
P	<0.01	<0.01	<0.01	<0.02	n.s.	n.s.
Control (5)	10.4 ± 1.0	13.8 ± 0.5	11.4 ± 2.6	14.3 ± 1.8	1.07 ± 0.26	1.05 ± 0.12
Acute dexamethasone (6)	14.7 ± 1.3	21.0 ± 0.9	14.7 ± 1.2	23.9 ± 3.7	1.05 ± 0.12	0.95 ± 0.07
P	<0.01	<0.01	<0.02	<0.05	—	—

TABLE VIII

EFFECTS OF CHRONIC DEXAMETHASONE ADMINISTRATION ON THE SPECIFIC ACTIVITIES OF $(Na^+ + K^+)$-ATPase AND *p*-NITROPHENYLPHOSPHATASE

Adrenalectomized rats were injected intraperitoneally with 5 mg/100 g body wt. of dexamethasone daily for 5 days. Controls received identical volumes of vehicle. The animals were killed 2 h after the last injection of hormone or vehicle and microsomal fractions were prepared for enzyme assays. The numbers of animals are shown in parentheses. The values are the means ±S.E. %Δ, denotes percent change of dexamethasone-treated animals as compared to control.

	$(Na^+ + K^+)$-ATPase (μmol P_i/mg protein per h)		*p*-Nitrophenylphosphatase (μmol *p*-nitrophenol/mg protein per h)	
	Cortex	Medulla	Cortex	Medulla
Control (6)	12.5 ± 1.2	19.6 ± 1.3	1.33 ± 0.18	2.65 ± 0.21
Dexamethasone (9)	27.6 ± 1.5	38.4 ± 4.2	2.87 ± 0.27	4.87 ± 0.39
P	<0.01	<0.02	<0.01	<0.01
%Δ	120↑	96↑	122↑	84↑

the increase in $(Na^+ + K^+)$-ATPase specific activity can be explained by an increase in the number of enzyme units. In addition, analysis of the individual values for $(Na^+ + K^+)$-ATPase and P_i intermediate from the different animals under control and chronic dexamethasone administration showed a statistically significant correlation in both the cortex ($r = 0.92$, $P < 0.05$) and medulla ($r = 0.80$, $P < 0.05$) of the organ. Essentially, similar results were obtained after administration of a single dose of dexamethasone (Table VII). These data are consistent with the information obtained from kinetic analysis and strengthen the conclusion that the activation mechanism with chronic or acute dexamethasone administration is an increase in the number of enzyme units with little or no change in the kinetic properties (affinity, cooperativity) of the activated enzyme.

Effects of acute and chronic dexamethasone administration on the specific activity of p-nitrophenylphosphatase

Kinetic analysis of ouabain binding in rat kidney is hampered by the low affinity of the glycoside for enzyme preparations from renal cortex and renal medulla [1,4,25,30].

The ouabain-sensitive *p*-nitrophenylphosphatase activity is believed to correspond to the potassium catalytic site for dephosphorylation of the $(Na^+ + K^+)$-ATPase reaction [27,28]. In this regard, this reaction is thought to probe a site on the enzyme similar to the site studied by examining the rates of ouabain binding [28]. The limitations of this approach in analyzing the mechanism of activation of $(Na^+ + K^+)$-ATPase have been discussed [11]. If the observed activation of renal $(Na^+ + K^+)$-ATPase by acute and chronic treatment with dexamethasone does indeed represent an increase in the number of enzyme units, it then follows that the activity of the ouabain-sensitive *p*-nitrophenylphosphatase should be proportionally increased following hormone treatment. As shown in Table VIII, after chronic dexamethasone administration the increase in $(Na^+ + K^+)$-ATPase specific activity was accompanied by quantita-

tive increases in the activity of *p*-nitrophenylphosphatase in the cortex and medulla of the organ. As in the case of the P_i intermediate, there was a good correlation between the activities of $(Na^+ + K^+)$-ATPase and those of *p*-nitrophenylphosphatase in control and chronically treated rats (cortex; $r = 0.86$, $P < 0.05$; medulla; $r = 0.75$, $P < 0.05$). Essentially, similar results were obtained in rats receiving a single dose of dexamethasone. These results are consistent with an activation mechanism based primarily on an increase in the number of enzyme units [11].

Discussion

The results of these experiments confirm previous observations that chronic administration of synthetic glucocorticoids to adrenalectomized rats increases the specific activity of $(Na^+ + K^+)$-ATPase in the renal cortex and medulla [4,5, 9,20]. The specific activity of Mg^{2+}-ATPase increased significantly in the cortex but not in the medulla; this is different from other studies in which the activity of this enzyme was unchanged [4,5] or did not increase significantly [20] after prolonged treatment with glucocorticoids. It should be pointed out, however, that in those studies the synthetic hormone used (methylprednisolone), the route of administration (intramuscular), the sex of the animals (male) and the assay method (chemical) employed were different from those of our studies. We elected to use dexamethasone (a fluorine derivative of methylprednisolone) because of the evidence for specific binding proteins for this steroid in rat kidney [7], which suggests that this particular steroid may have direct renal effects.

The results of the kinetic parameters (K_m, $K_{1/2}$, V, apparent turnover numbers, Hill coefficients for the three substrates) and the partial reactions (P_i intermediate, *p*-nitrophenylphosphatase of the $(Na^+ + K^+)$-ATPase enzymatic reaction are consistent with the interpretation that stimulation of renal $(Na^+ + K^+)$-ATPase specific activity by chronic dexamethasone treatment in adrenalectomized rats represents an increase in the number of enzyme units with little or no change in the kinetic properties of the activated enzyme. This interpretation is consistent with the reported decreases in V for Na^+, K^+ and ATP of renal $(Na^+ + K^+)$-ATPase after adrenalectomy [5] and also with the observation that chronic administration of methylprednisolone to guinea-pig appears to increase the binding of radioactive ouabain to guinea-pig kidney microsomes [4]. Stimulation of $(Na^+ + K^+)$-ATPase by chronic potassium loading [11,29] and thyroid hormone [12,26,30] appears to be mediated by similar mechanisms.

The results of our experiments also demonstrate that acute administration of dexamethasone stimulates the specific activity of renal $(Na^+ + K^+)$-ATPase in renal cortex and renal medulla in a dose-dependent manner. This effect, like the one observed with chronic hormone treatment, appears to represent a true increase in the cellular activities of the transport enzyme. Furthermore, since enzyme activation occurred in the absence of demonstrable changes in glomerular filtration rate and tubular Na^+ loads, these results suggest that dexamethasone may exert a direct effect on renal $(Na^+ + K^+)$-ATPase independent of changes in the filtered load of sodium. A similar dissociation between changes

in glomerular filtration rate and enzyme activation has been observed after multiple doses of glucocorticoid hormones [9] and single doses of thyroid hormones [31].

The kinetic parameters and the partial reactions (P_i intermediate *p*-nitrophenylphosphatase) of the ($Na^+ + K^+$)-ATPase enzymatic reaction are consistent with the interpretation that acute dexamethasone treatment also results in an increase in the number of enzyme units. Hence, the mechanisms underlying enzyme activation after acute and chronic administration of dexamethasone appear to be similar. The rapid activation (2 h) of ($Na^+ + K^+$)-ATPase specific activity does not necessarily preclude an increase in the number of Na^+-pump sites as the underlying mechanism. Although the half-life of ($Na^+ + K^+$)-ATPase is prolonged (96 h) [26], it may be possible to increase rapidly the available number of enzyme units by unmasking pre-existing pump sites. Alternatively, the hormone may, under acute conditions, modify the rates of enzyme degradation. The present experiments do not provide information as to whether acute and chronic dexamethasone treatment increases the number of enzyme units by differential effects on the cellular synthesis and/or catabolism of renal ($Na^+ + K^+$)-ATPase. To answer this question will require investigation of the rates of synthesis and degradation of the transport enzyme.

The results of our experiments provide biochemical evidence for a direct regulatory effect of renal ($Na^+ + K^+$)-ATPase by glucocorticoid hormones. These observations and the presence of high-affinity glucocorticoid receptor sites in the kidney are inconsistent with the interpretation that the stimulatory effect of these hormones on renal ($Na^+ + K^+$)-ATPase is an indirect one and mediated by changes in the rates of tubular Na^+ transport. Instead, they suggest that glucocorticoid hormones directly increase the number of Na^+-pump sites in the kidney. This interference is supported by the observation that cortisol treatment of the American eel increases the specific activity of ($Na^+ + K^+$)-ATPase in gill tissue without a corresponding change in sodium transport [8]. Likewise, enzyme activation after chronic glucocorticoid administration can be demonstrated in the kidney of rats with chronic reduction in glomerular filtration and tubular reabsorptive rates [9]. In view of the critical role of ($Na^+ + K^+$)-ATPase in the presence of Na^+ reabsorption, our results raise the possibility that glucocorticoid hormones (like mineralocorticoid hormones) may play an essential role in the renal regulation of Na^+ homeostasis.

Acknowledgments

Financial support for this work was provided by U.S. Public Health Service NIAMDD program project Grant AM-09976 and Grant AM-07126. We thank Mrs. Sue King, Mrs. Claire Pederson and Ms. Margaret Reich for their help with the blood chemistries; Dr. Mabel L. Purkerson and Doris B. Rolf for performing clearance studies; and Mrs. Mary Brown, Ms. Linda Malawy and Ms. Sandra Maul for their secretarial assistance.

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