JCI The Journal of Clinical Investigation

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J Clin Invest. 1981;68(6):1514-1521. https://doi.org/10.1172/JCI110405.

Research Article

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Secondary Effect of Aldosterone on Na-K ATPase Activity in the Rabbit Cortical Collecting Tubule

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ABSTRACT The possibility that mineralocorticoids have a direct influence on renal Na-K ATPase activity has been the focus of intense research effort and some controversy for a number of years. Early studies were hindered by an inability to differentiate between possible glucocorticoid vs. mineralocorticoid effects on this enzyme within the multitude of cells that comprise the heterogeneous mammalian nephron. This study attempts to circumvent this problem by monitoring Na-K ATPase activity in the rabbit renal cortical collecting tubule (CCT), a proposed target epithelium for mineralocorticoids. Using an ultramicro assay, Na-K ATPase activity was measured in CCT from normal, adrenalectomized (adx), and adx rabbits subjected to one of several corticosteroid treatment protocols. The results indicate that Na-K ATPase activity in the CCT decreased by 86% subsequent to adrenalectomy. Injection of physiological doses of aldosterone (10 μg/kg) but not dexamethasone (100 μg/kg) restored CCT Na-K ATPase activity in adx rabbits to normal levels within 3 h after injection. An insignificant rise in activity was observed 1.5 h after aldosterone treatment. In addition, spirolactone SC 26304, a specific mineralocorticoid antagonist, blocked the action of aldosterone on Na-K ATPase. Therefore an acute increase in Na-K ATPase activity participates in the action of aldosterone on Na transport in this segment. To differentiate between primary vs. secondary activation of this enzyme, adx animals were treated with amiloride before the injection of aldosterone with the intent of blocking luminal membrane Na entry into CCT. In these animals, pretreatment with amiloride blocked the increase in CCT Na-K ATPase activity seen with aldosterone alone at 3 h. Thus the increase in activity with aldosterone appears to be a secondary adaptation that is dependent on an aldosterone-enhanced increase in the passive entry of Na across the luminal membrane. The subcellular mechanism by which Na modulates Na-K ATPase activity remains obscure.

INTRODUCTION

Numerous reports have appeared in recent years concerning the subcellular action of aldosterone in the mammalian kidney. Three main postulates have been formulated to explain the mechanism(s) by which aldosterone enhances transepithelial Na transport. The first of these, termed the permease theory, suggested that the increase in Na transport after treatment with aldosterone was a consequence of an aldosterone-induced increase in the Na permeability of the apical membrane (1–3). The second, or energy theory, held that aldosterone enhanced ion transport primarily by inducing the synthesis of key enzymes involved in the production of ATP, a necessary substrate for active Na transport (4–6). The third theory, known as the pump theory, maintained that aldosterone augmented the cellular capacity to transport Na by increasing the activity of Na-K ATPase (7–9).

Whereas each of these theories has significant experimental support, none of them has gained universal acceptance and each theory has attracted criticism from various sources. Much of the data have been generated using the toad urinary bladder as a model mineralocorticoid target tissue. Although the bladder preparation has many similarities to the mammalian cortical collecting tubule [a proposed renal mineralocorticoid

Portions of this work were presented at meetings of the Southern Section of the American Federation for Clinical Research, 15–17 January 1981, New Orleans, La. at the national meetings of the American Federation for Clinical Research, 25–27 April 1981, San Francisco, Calif., and at the VIII International Congress of Nephrology, 8–12 June 1981, Athens, Greece.

Received for publication 10 April 1981 and in revised form 31 July 1981.

[1] *Abbreviations used in this paper*: adx, adrenalectomized; aldo, aldosterone; amil, amiloride; BSA, bovine serum albumin; CCT, cortical collecting tubule; dex, dexamethasone; and MKH, moles P_i liberated per kilogram dry tissue weight per hour.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/81/12/1514/08 $1.00*
Volume 68 December 1981 1514–1521

target epithelium (10–12)], it nevertheless is not certain that all of the data derived from toad bladder studies can be extended to the mammalian kidney.

With regard to the pump theory, it has been reported that aldosterone does not increase Na-K ATPase activity in unfractionated toad bladders within a time frame in which maximal effects on Na transport are seen (13, 14). In addition, the reported increases in renal Na-K ATPase activity in adrenalectomized animals treated with corticosteroids (7–9, 15–20) have been open to alternative interpretations for a variety of reasons. Evaluation of those studies was obscured due to the use of pharmacological doses of mineralocorticoids, the lack of appropriate glucocorticoid controls, and the cellular heterogeneity of the renal fraction examined that might have masked the hormone responses in a small subpopulation of cells. Thus, the purpose of this study was to overcome some of these shortcomings and was designed to address the following specific issues: (*a*) Can a single physiological dose of aldosterone increase Na-K ATPase activity in a putative mammalian target epithelium? (*b*) If so, is the response specific for mineralocorticoids as opposed to glucocorticoids? (*c*) Can changes in Na-K ATPase activity be attributed to a direct induction of the enzyme or are they secondary to mineralocorticoid-enhanced Na entry at the luminal membrane? To answer these questions, an ultramicro assay for Na-K ATPase activity was used to monitor enzyme levels in cortical collecting tubules (CCT).[1]

METHODS

Female New Zealand white rabbits weighing 1–2 kg were used in all experiments. With the exception of the normal group of rabbits, all animals were bilaterally adrenalectomized through a midline abdominal incision after anesthesia was induced with an injection of 100 mg/kg i.m. of Ketaset (ketamine HCl, Bristol Myers Company, Syracuse, N. Y.) plus 1 mg/kg of acepromazine (acepromazine maleate, Ayerst Labs, Inc., New York). Care was taken during each surgical procedure to insure that the adrenal capsule remained intact and that no ectopic adrenal tissue was present. Adrenalectomized rabbits each received a 0.1-mg i.m. injection of dexamethasone (decadron phosphate, Merck, Sharp and Dohme, Canada Ltd, Montreal, Quebec Canada) immediately following surgery and another 0.1 mg the next day. The animals received no other steroids beyond this point except as indicated below. In this preparation, plasma levels of corticosterone are undetectable when measured by radioimmunoassay at 1 wk postadrenalectomy (21). All rabbits were fed standard rabbit chow containing 170 and 360 meq/kg of Na and K, respectively. Normal rabbits were allowed to drink tap water while adrenalectomized animals were maintained on a 0.9% NaCl drinking solution. Adx animals were studied 8–14 d after surgery. Eight groups of rabbits were used: group 1: normal, 5 rabbits, adrenal glands intact; group 2: adrenalectomized (adx), 6 rabbits; and group 3: adx + aldosterone (aldo), 6 rabbits. Aldo (D-aldo, chromatographic grade, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) was dissolved in absolute ethanol at a concentration of 200 μg/ml. An appropriate aliquot (40–70 μl) of this solution was added to 1.0 ml of saline, injected into an ear vein, and the animal was killed 1.5 h later. The dose of aldo was 10 μg/kg body wt i.v. for all animals in this and subsequent groups in which this steroid was given.

Group 4: adx + aldo, 6 rabbits. The protocol for this group is identical to that for group 3 except that the animals in this group were killed 3 h after the injection of aldo.

Group 5: adx + spirolactone (spiro) + aldo, 4 rabbits. Spiro (SC 26304, G. D. Searle & Co., Des Plaines, Iowa) was dissolved at a concentration of 4 mg/ml in a solution of ethanol/propylene glycol/saline (2:5:5 by vol). Animals received 1.5 mg/kg i.p. spiro 30 min before and were killed 3 h after the injection of aldo.

Group 6: adx + dexamethasone (dex), 5 rabbits. Dex (Sigma Chemical Co., St. Louis, Mo.) was dissolved at a concentration of 2 mg/ml in absolute ethanol. A small aliquot (40–70 μl) of this solution was diluted with 1 ml of saline so that the dose was 100 μg/kg i.v. The animals were killed 3 h after injection.

Group 7: adx + amiloride (amil) + aldo, 6 rabbits. Adx animals in this group were placed on a sodium-free diet (ICN Nutritional Biochemicals, Cleveland, Ohio) and tap water for 18 to 24 h before experimentation in order to restrict Na intake. 3.5 h before death, a polyethylene (PE-50) catheter filled with heparinized saline was inserted into an ear vein and the animals received a loading dose (2 mg/kg) of amiloride (amiloride HCl, Merck, Sharp and Dohme, Canada Ltd.) dissolved in 5% glucose (wt/vol) at a concentration of 1 mg/ml. Subsequent to this, three maintenance doses (1 mg/kg) were given at 1-h intervals following the loading dose so that each animal received a total of 5 mg/kg amil over the 3.5-h period preceding death. Aldo was injected 30 min after the loading dose of amil and animals were killed 3 h after aldo injection.

Group 8: normal + amil, 5 rabbits. Animals in this group were treated in an identical manner to those in group 7 except that diluent was injected instead of aldo.

With the exception of the animals in groups 7 and 8, all adx rabbits had free access to food and saline drinking solution during the period following the injection of steroid.

In a separate set of experiments, adx rabbits (4 animals per group) were subjected to the protocols applied to the rabbits in groups 2, 4, and 7 and at the time of sacrifice a sample of urine was withdrawn from the bladder. Urinary Na and K concentrations were determined by flame photometry (flame photometer 343, Instrumentation Laboratory, Inc., Lexington, Mass.).

Dissection and lyophilization of tubule segments

Rabbits were killed by decapitation, the left kidney was rapidly excised, and a coronal slice (1–2 mm thick) was placed in a dish kept on ice containing a simulated ultrafiltrate solution of the following composition (in millimolar): NaCl 115, KCl 5, $NaHCO_3$ 25, NaH_2PO_4 2.3, Na acetate 10, $MgSO_4$ 1, glucose 8, alanine 5, $CaCl_2$ 1.8. The dissection solution had an osmolality of 290–300 mosmol/kg and was maintained at pH 7.4 by bubbling with 95% O_2/5% CO_2.

Freehand dissection of each renal slice was carried out within a period of 30–45 min without the use of collagenase or other added enzymes. Individual CCT 1.5–2.5 mm in length were identified by predetermined criteria (22) so that all tubules included only those segments distal to the superficial branch point and proximal to the corticomedullary junction. After dissection, individual CCT were removed from

the dissection dish and rapidly rinsed with distilled water to remove excess solutes. The tubules were then placed into a droplet (3–5 μl) of distilled water on a glass slide and immediately frozen over a block of dry ice. Lyophilization was carried out at −40°C for 18–24 h (23). This procedure is known to render the tissue permeable to various solutes upon rehydration (24). Lyophilized tubule segments were stored under vacuum (0.01 Torr) at −70°C until assay. There was no detectable loss of ATPase activity over a 3-wk period with these storage conditions.

Ultramicro ATPase assay for tubule segments

All procedures for cutting and weighing tubule fragments have been reported (23), and only a brief description will be given below. Vacuum flasks were warmed to room temperature and individual CCT were further cut into small segments, 75–200 microns in length. Each segment was weighed on a quartz fiber balance (23) with a sensitivity of 0.5 ng. Segments weighed between 10 and 30 ng and anywhere from 6 to 14 pieces were cut from each CCT. Each piece was transferred into a 270-nl droplet of either solution A or solution A′ in which the constituents were present at twice the final concentration. For total ATPase measurement, the final concentrations of constituents in solution A were (in millimolar): Tris-HCl (pH 7.4) 100, NaCl 53, KCl 5, $MgCl_2$ 3, EDTA 0.1, and 0.1% bovine serum albumin (BSA). For the determination of Mg-dependent ATPase activity, final concentrations in solution A′ were (in millimolar): Tris-HCl (pH 7.4) 100, NaCl 58, $MgCl_2$ 3, EDTA 0.1, ouabain 1 and 0.1% BSA. We found in preliminary kinetic studies using membrane fractions that optimal activity for Na-K ATPase from rabbit kidney could be attained with Na, K, and ATP concentrations of 50, 5, and 3 mM, respectively. The sample was contained within a well drilled into a Teflon block and was immediately covered with mineral oil to prevent evaporation. When all of the wells were filled, the Teflon block was cooled on ice for 5 min.

Step A: ATPase reaction. The ATPase reactions were initiated by rapidly adding 270 nl of 6 mM disodium ATP (vanadium-free, Sigma Chemical Co.) to the original 270-nl droplet of solution A or A′ containing the tubule segment so that the final ATP concentration was 3 mM. The Teflon blocks were then quickly transferred to a 37°C water bath for 10 min, and the reaction was terminated by heating the plates at 100°C for 4 min (Multi-blok heater no. 2093, Lab-Line Instruments, Inc., Melrose Park, Ill.). Under the conditions of this assay no more than 5% of the total amount of ATP was hydrolyzed by both enzymatic and nonenzymatic processes.

Step B: Coupling reaction. After termination of the ATPase reaction, 3,890 nl of solution B was added to each well. The composition of solution B was: imidazole-HCl (pH 6.9) 50 mM, NADP+ 30 μM, 5′-AMP 10 μM, EDTA 1 mM, $MgCl_2$ 0.5 mM, glycogen 0.08%, glucose-6-phosphate dehydrogenase 3 μg/ml, phosphoglucomutase 9 μg/ml, glycogen phosphorylase 100 μg/ml and BSA 0.05%. The efficiency of solution B in the conversion of P_i to an equivalent amount of NADPH was tested before each assay by adding 8×10^{-9} moles of KH_2PO_4 to a 450-μl aliquot of solution B and the change in OD was measured at 340 nm in a cuvette maintained at 37°C. A conversion of 80% of the P_i (equivalent to a Δ OD $\cong$ 0.08) or more was considered acceptable, and for most assays the conversion was $\geq$90%. The coupling reaction was allowed to proceed to completion (45–60 min) at 37°C so that P_i was quantitatively converted to an equivalent amount of NADPH. Following this, 3,890 nl of 0.17 N NaOH was added to each well and the excess NADP+ from the coupling reaction was destroyed by heating at 80°C for 30 min. NADPH remains stable under these conditions (25).

Step C: Enzymatic cycling reaction. For the cycling reaction, a 280-nl aliquot of the resultant reaction mixture containing NADPH was transferred to a 1.5-ml conical Beckman centrifuge tube kept on ice. To each tube was quickly added 30 μl of solution C containing: Tris-HCl (pH 8.0) 100 mM, alpha-ketoglutarate 5 mM, glucose-6-phosphate 1 mM, NH_4 acetate 20 mM, 5′-ADP 0.3 mM, glutamate dehydrogenase 150 μg/ml, glucose-6-phosphate dehydrogenase 35 μg/ml, and BSA 0.02%. The time and temperature of incubation for the cycling reaction were chosen so that reaction products would accumulate at final concentrations >3,000–4,000 times the concentration of NADPH entering into this amplification step. The cycling reaction was initiated by centrifuging the tubes for a few seconds (Beckman microfuge, Beckman Instruments, Inc., Fullerton, Calif.) to insure that all reactants were mixed. The tubes were placed into a 32°C water bath for 45 min and the reactions were terminated by heating the tubes for 4 min in a 100°C water bath.

Step D: Indicator reaction. After cooling to room temperature, the indicator reaction was carried out by adding 1.0 ml of solution D to each tube. The composition of solution D was: Tris-HCl (pH 8.0) 20 mM, EDTA 0.1 mM, NH_4 acetate 60 mM, $MgCl_2$ 3 μM, NADP+ 0.1 mM, 6-phosphogluconate dehydrogenase 1 μg/ml, and BSA 0.05%. The tubes were incubated for 30 min at 37°C and the resultant fluorescence of NADPH was measured in a Zeiss PMQ III spectrophotometer-fluorometer (Carl Zeiss, Inc., New York) with an excitation wavelength of 365 nm and an emission filter of 460 nm. For each assay, a standard curve for P_i was established in duplicate by adding from 6.75 to 108×10^{-12} mol of KH_2PO_4 to step A. Nonenzymatic ATP hydrolysis was also monitored during each assay by adding (in duplicate) a 270-nl aliquot of 6 mM ATP to an equivalent volume of solution A or A′ in which tubule segments had been deleted. In all experiments, the amount of P_i produced from the nonenzymatic hydrolysis of ATP was always <0.5% of the total amount of ATP present. This amount was subtracted from the total amount of P_i produced by each piece of tissue in order to determine the amount of enzymatically liberated P_i.

Total or Mg ATPase activity was measured sequentially in alternate segments from each tubule such that half of the segments from each CCT were used to measure total ATPase, while the other half were used to determine Mg ATPase activity. An average activity for both total and Mg ATPase was calculated for each tubule and Na-K ATPase activity in each CCT was calculated by subtracting Mg ATPase activity from total ATPase activity. Na-K ATPase activity was taken to be zero in those few instances in which the mean Mg ATPase activity slightly exceeded the mean total ATPase activity. All activities are expressed as moles P_i liberated per kilogram dry tissue weight per hour (MKH) at 37°C±SEM.

All enzymes, cofactors, and substrates used in the assay were obtained either from Boehringer Mannheim Biochemicals, Indianapolis, Ind. or Sigma Chemical Co. All other solutes were reagent grade. Glass-distilled water was used in the preparation of all solutions.

For statistical purposes, the number of individual CCT from each group of rabbits was used as the value for n. All statistical analyses were performed on a DEC-System 10 computer using both the Student's t test and Newman-Keuls multiple comparison procedures. Results were considered significantly different only when values of $P < 0.05$ were obtained by both statistical methods.

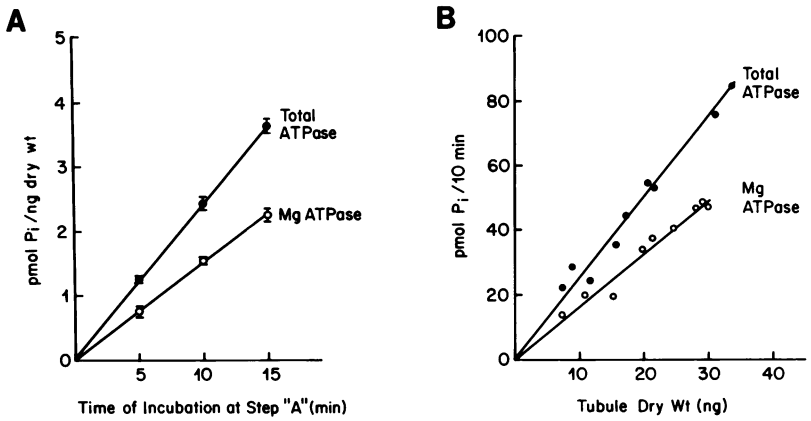

FIGURE 1 Dependence of P_i production on time of incubation and tubule dry weight. A. Segments of CCT taken from normal rabbits were incubated for 5, 10, or 15 min at step A. Each point represents the mean±SEM of 8–12 segments for either total (closed circles) or Mg ATPase (open circles). B. Individual tubule segments of various weights from normal CCT were incubated for 10 min and the amount of P_i was measured. Each point represents a single piece of tubule in which either total (closed circles) or Mg ATPase (open circles) was determined.

RESULTS

The dependence of P_i production on time of incubation and dry tissue weight of CCT from normal rabbits is shown in Fig. 1. As shown in Fig. 1a, P_i production for both total and Mg ATPase was linear up to a time of 15 min for the incubation at step A. Because all incubations at this step were for 10 min, the amount of liberated P_i was maintained within the appropriate range of the assay. The amount of P_i generated by any given segment was also linearly proportional to the dry weight of the segment (Fig. 1b).

The ATPase activities in CCT from the first seven groups of rabbits are shown in Table I and Fig. 2. Na-K ATPase activity decreased from 5.6 to 0.8 MKH U in the CCT after adrenalectomy ($P < 0.001$). These activities were comparable to those previously reported by Horster et al. (9) in CCT from normal and adx rabbits. After the administration of aldo to adx rabbits, there was an increase in the activity of Na-K ATPase that varied depending on the time interval over which the steroid was allowed to exert its effects. At 1.5 h, there was an insignificant increase in CCT Na-K ATPase activity (from 0.8 to 1.6 MKH U). Because we saw an equivocal effect of aldo on Na-K ATPase activity at 1.5 h, we elected to extend this time interval to 3 h. Over this time interval, Na-K ATPase activity in the CCT was restored to a level (4.9 MKH U), which was not significantly different from the level in CCT from normal rabbits but was significantly greater than the activity in adx rabbits ($P < 0.001$, group 2 vs. group 4). The antinatriuretic and kaliuretic effects of aldo were demonstrated by the urinary Na/K ratio of adx rabbits which declined from 5.70±1.66 to 0.80±0.09 ($P = 0.026$) 3 h after the injection of aldo.

To determine if the effect of aldo on Na-K ATPase at 3 h was specific for mineralocorticoid, enzyme activity was measured in two other groups of rabbits. In one of these groups (group 5), adx animals were pretreated with spiro SC 26304, a specific competitive antagonist of aldo at the level of the cytoplasmic mineralocorticoid receptor (26). This maneuver resulted in a complete abolition of the effect of aldo on Na-K ATPase seen at 3 h (0.4 vs. 4.9 MKH U, group 5 vs. group 4, $P < 0.001$),

TABLE I
ATPase Activities in the Rabbit CCT (MKH±SEM)

Group	n*	Total ATPase	Mg ATPase	Na-K ATPase
1 normal	8	15.9±0.7	10.3±1.2	5.6±0.7
2 adx	11	9.4±0.8‡	8.8±0.6§	0.8±0.3‡
3 adx + aldo¶	14	9.1±0.5‡	8.3±0.8§	1.6±0.3‡
4 adx + aldo**	11	13.2±0.9‖	8.3±0.6§	4.9±0.7§
5 adx + spiro + aldo**	10	8.1±1.0‡	8.2±0.8§	0.4±0.2‡
6 adx + dex**	11	10.2±0.5‡	10.7±0.7§	0.7±0.3‡
7 adx + amil + aldo**	18	9.9±1.3‡	10.1±1.1§	1.2±0.4‡
8 normal + amil	13	16.2±0.5§	10.7±0.4§	5.5±0.6§

* n = number of individual CCT.
‡ $P < 0.001$ vs. groups 1 and 4.
§ P = N.S. vs. group 1.
‖ $P < 0.05$ vs. group 1.
¶ Killed 1.5 h after steroid injection.
** Killed 3 h after steroid injection.

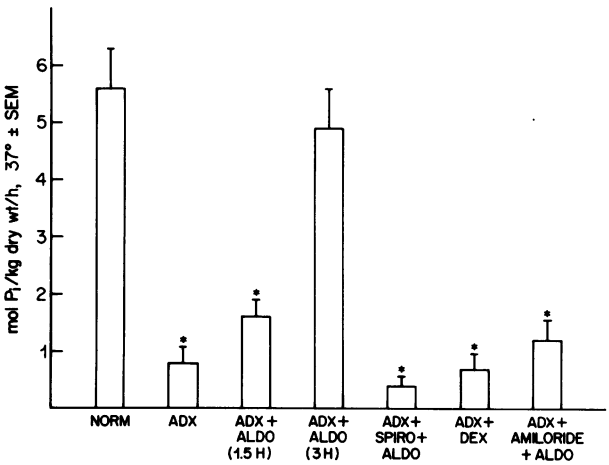

FIGURE 2 Na-K ATPase activities in CCT from the first seven groups of rabbits. Values are the same as those displayed in Table I (MKH±SEM)*$P < 0.001$.

which would imply that the increase in enzyme activity with aldo was mediated through a specific mineralocorticoid receptor-dependent pathway. In the other group of rabbits (group 6), dex, a potent glucocorticoid, was given at a dose that was tenfold higher than the dose of steroid used in the aldo-treated groups of animals (groups 3, 4, 5, and 7). As shown in Table I and Fig. 2, this dose of dex was ineffective in restoring Na-K ATPase activity over a time interval in which aldo restored the activity to normal levels (0.7 vs. 4.9 MKH U, group 6 vs. group 4, $P < 0.001$). This indicates that the effect on the enzyme at 3 h was not mediated through a glucocorticoid induction pathway but did in fact occur through a specific mineralocorticoid-stimulated system.

The results for the first six groups of rabbits do not indicate whether aldo acted directly or indirectly on Na-K ATPase. In an attempt to address this issue, amiloride, a diuretic that blocks the luminal entry of sodium in various epithelia (27–29), was administered to adx rabbits before the injection of aldo. The Na intake of these rabbits was also restricted for 18–24 h before experimentation in an effort to reduce the amount of Na delivered to distal nephron segments. As shown in Table I and Fig. 2, treatment with amil abolished the 3-h effect of aldo on Na-K ATPase activity (1.2 vs. 4.9 MKH U, group 7 vs. group 4, $P < 0.001$). The enzyme activity following treatment with amil + aldo was not significantly different from the activities in the adx-, spiro-, and dex-treated rabbits (groups 2, 5, and 6). This effect of amil was not due to a direct inhibitory influence of the diuretic on Na-K ATPase because the activity of this enzyme in CCT from normal rabbits was the same with or without amil treatment (5.5 vs. 5.6 MKH U, group 8 vs. group 1, P = NS). In addition, the efficacy of amil in altering urinary electrolyte excretion was demonstrated by an increase in the urinary Na/K ratio, which rose from 5.70±1.66 in adx rabbits to 13.40±1.57 in amil + aldo-treated adx rabbits ($P = 0.015$). Thus if it is assumed that amil acts principally by blocking the entry of sodium from the lumen into cells of the CCT, the effect of aldo on Na-K ATPase would appear to be dependent on an aldo-enhanced entry of Na across the luminal membrane. It should be noted that changes in Na-K ATPase activity were independent of changes in other ATPase activities because Mg ATPase activity was statistically the same in all groups of rabbits.

DISCUSSION

The results of these experiments indicate that Na-K ATPase activity in the CCT is increased within 3 h after the administration of aldo to adx rabbits. This restoration of activity was a specific mineralocorticoid phenomenon as evidenced by the ability of spiro to block the response and by the inability of dex to restore activity. Furthermore, the acute effect of aldo on CCT Na-K ATPase activity was apparently secondary to an increased entry of Na across the luminal membrane because this effect was blocked by amil (Table I).

The dose of aldo used in this study was intended to provide near saturation of mineralocorticoid receptor sites with minimal binding to glucocorticoid receptors. Previous studies in the rat (30) and the rabbit (31) showed that the affinity of aldo for the mineralocorticoid receptor was similar in both species. Based on these findings and in view of the fact that 10 μg/kg of aldo produced maximal alterations in urinary electrolyte excretion patterns (32) and amino acid incorporation into specific renal proteins in the adx rat (33), this dose was given to the adx rabbits in our study.

The results of this study show that Na-K ATPase in the CCT responds acutely to a physiological dose of aldo administered in vivo. This finding supports the results of Horster et al. (9), although we found a longer time-course of activation of the enzyme (3 h as opposed to 60–90 min). In contrast to our results and those of Horster et al. (9) are some preliminary studies by Doucet and Katz (34). In these studies, high doses of aldo had no effect on Na-K ATPase in the adx mouse CCT when added either in vivo or in vitro following a 1- or 3-h treatment period. The reason for the discrepancy between those results and the results of this study is not clear and may be due either to species differences or to the use of different assay techniques.

Our experiments in which dex was given in an amount tenfold greater than the dose of aldo indicated that the acute enzyme response to aldo was not the result of a glucocorticoid effect. Although dex did not produce an acute increase in Na-K ATPase in our study, it is possible that chronic administration of glucocorticoids

may increase Na-K ATPase activity in various nephron segments, including the CCT, through a glucocorticoid receptor pathway.

The ability of spiro to block the response to aldo seen in this study suggests that the increase in Na-K ATPase was mediated through a mineralocorticoid receptor-dependent pathway because it is at the level of this receptor that spiro exerts its antagonistic properties (26). Thus these results give further credence to the postulate that the CCT is a target segment for mineralocorticoids in the mammalian kidney (10–12).

Our experiments in which the 3-h effect of aldo was blocked by amil suggest that the activation of Na-K ATPase by aldo was secondary to an increased entry of Na across the luminal membrane into the cell. The diuretic action of amil is thought to be due to the ability of this substance to block specifically Na channels not only in the CCT (28, 29) but also in other electrolyte transporting epithelia (27). When these Na channels in the CCT of adx animals were blocked by pretreatment with amil in the present study, the 3-h effect of aldo on Na-K ATPase was abrogated. Furthermore, the ability of amil to block the aldo response was not due to a direct inhibitory action on Na-K ATPase because there was no decrement in enzyme activity in CCT taken from normal rabbits treated with this diuretic (Table I). Therefore the aldo-induced increase in CCT Na-K ATPase in this study appears to be a response that was secondary to an increased entry of Na across the luminal membrane. Because earlier studies from this laboratory reported that aldo increased CCT citrate synthase levels at 1.5 h (12), the fact that CCT Na-K ATPase activation required 3 h instead of 1.5 h to manifest itself further implies that this was a secondary event. Studies by Handler et al. (35) using a toad kidney cell culture have also demonstrated an amil-sensitive rise in Na-K ATPase activity following 18-h exposure to steroids. This phenomenon of activation of Na-K ATPase subsequent to an increased entry of Na into the cell has also been observed in fibroblasts (36, 37), neuroblastoma (38), and HeLa cells (39).

Certain subcellular steps in the mechanism of aldo action have been well characterized (40, 41) and these events are depicted in Fig. 3. While it is generally agreed that a receptor-mediated stimulation of mRNA and protein synthesis occurs, some controversy arises concerning the identity of the proteins induced by aldo. As mentioned in the introduction, one theory proposes that aldo increases the Na permeability of the luminal membrane by increasing the number or capacity of Na channels. The results of our study support this model and give credence to the hypothesis that aldo-enhanced luminal Na entry into the CCT leads to a secondary increase in Na-K ATPase activity as first proposed by Jorgensen (18).

The mediating events between aldo-enhanced Na

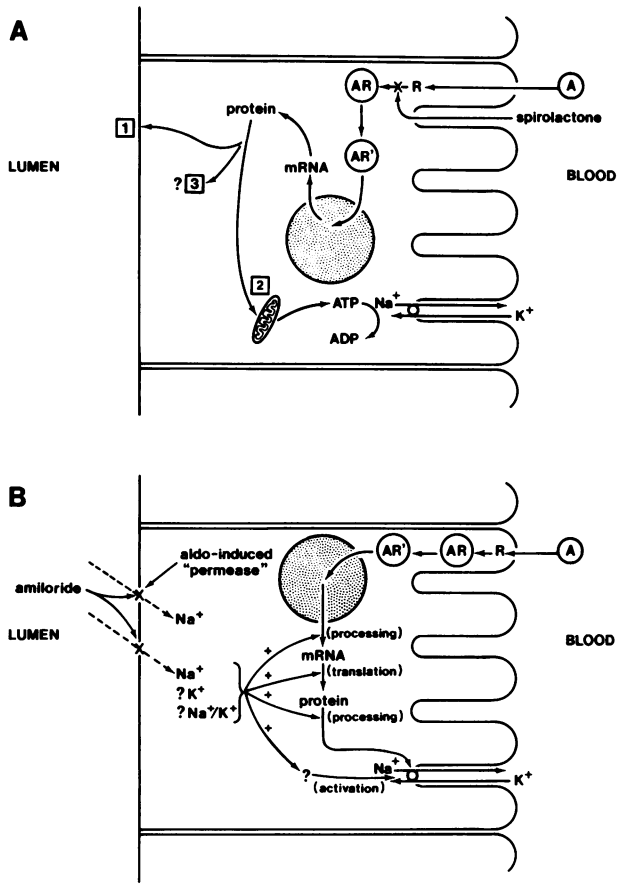

FIGURE 3 A. Classical model of primary steroid induction of specific proteins. (A) represents unbound aldo and R is the cytoplasmic mineralocorticoid receptor to which aldo binds (blocked by spiro). The steroid receptor complex activates specific regions of nuclear chromatin leading to the synthesis of mRNA. Transcription of specific mRNA is followed by translation into specific aldo-induced proteins. Specific proteins could include: 1) A "permease" to increase luminal membrane permeability to Na; 2) Enzymes involved in energy production; 3) Other proteins such as enzymes involved in lipid metabolism. B. Model for secondary enhancement of Na-K ATPase activity by steroid. Increased luminal membrane permeability to Na induced by aldo leads to increased entry of Na into the cell (blocked by amil). Changes in intracellular ion activity (Na, K, Na/K, or others) lead secondarily to an activation of Na-K ATPase perhaps by acting directly on the enzyme or indirectly by modulating the steps involved in the synthesis of the subunits of Na-K ATPase.

entry and activation of Na-K ATPase are less clear. As indicated in Fig. 3b, the increased influx of Na down its concentration gradient from the lumen into the cell would transiently alter the intracellular activity of Na. This in turn might lead to changes in the intracellular activity of K or other ions. It is possible that any one of these ionic alterations might lead to activation of "latent" Na pumps, or stimulate *de novo* synthesis of

one or more of the subunits of the Na pump (39). As depicted in Fig. 3b, the regulation of new pump synthesis could occur at the level of either transcription or posttranslational processing of protein. In any event, the results reported here are supportive of a mechanism in which acute changes in Na-K ATPase activity are brought about by an aldo-specific enhancement of Na entry across the luminal membrane. Such modifications could be expected to increase the overall capacity of the cell to transport Na and to participate in the physiological action of aldo in this segment.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent technical assistance of Mr. James Harrison, the helpful advice of Dr. H. R. Jacobson and Dr. W. E. Lombard, and the gift of SC 26304 from Dr. F. Radziolowski of G. D. Searle & Co., Des Plaines, Iowa. We are indebted to Kay Williams and Yvonne Wilburn for preparing the manuscript.

These studies were performed in partial fulfillment of the requirements for a Ph.D. degree in Physiology; University of Texas Health Science Center, Graduate School of Biomedical Sciences; Dallas, Texas (K. J. Petty). He was supported in part by funds from the Morgan-Good Foundation; Dallas, Texas. The studies were also supported by National Institutes of Health research grants R01-AM 21576 and R01-AM 14677. Diana Marver is an Established Investigator of the American Heart Association.

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