Ultrastructure of Rat Initial Collecting Tubule

Effect of Adrenal Corticosteroid Treatment

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Abstract

This study examines the effects of adrenalectomy and physiological replacement of mineralocorticoids and glucocorticoids on the cellular ultrastructure of the rat initial collecting tubule (late distal tubule). Animals were adrenalectomized (ADX) and for 10 d received by osmotic minipump either: vehicle, aldosterone (0.5 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$), aldosterone (2.0 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$), dexamethasone (1.2 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$), or aldosterone (0.5 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$) with dexamethasone (1.2 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$). Radioimmunoassay revealed that the low dose of aldosterone restored plasma aldosterone to control levels. The higher dose of aldosterone increased plasma levels by threefold. Morphometric techniques were used to measure membrane length of individual principal and intercalated cells in each condition. The basolateral membrane length of principal cells decreased by 35% in ADX animals. Low dose aldosterone replacement (0.5 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$) in ADX animals maintained membrane length at control values; at a higher level of aldosterone (2.0 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$) membrane length increased by 111% compared with control. Dexamethasone treatment, at a level that restored glomerular filtration rate to normal, had no effect on cellular ultrastructure. Combined aldosterone and dexamethasone replacement had no greater effect on basolateral membrane length than aldosterone alone. The length of the luminal membrane of the principal cell type was not affected by ADX or hormone treatment. Intercalated cell membrane length was not affected by ADX or hormone replacement. Thus, chronic aldosterone levels have an important, selective effect on the basolateral membrane of the principal cell. The correlation between these morphological results and the steroid hormone effects on renal electrolyte excretion, reported in the companion paper (15), suggests that basolateral membrane length is an important factor controlling the rate of sodium and potassium transport by the initial collecting tubule.

Introduction

A growing body of evidence indicates that cells in the mammalian nephron can regulate ion transport by altering membrane area in response to specific stimuli. Chronic treatment of animals with large doses of desoxycorticosterone acetate (DOCA)[1] and dexamethasone, which stimulate electrolyte transport (1), increases the basolateral membrane area of the rabbit cortical collecting tubule (1). In rat and rabbit, long-term dietary loading with potassium (potassium adaptation) increases plasma aldosterone concentration, stimulates potassium and sodium transport, and increases the membrane area of initial collecting (2), cortical collecting (3), and medullary collecting tubule cells (4). These changes in membrane area are specific for the principal cell type and are selectively localized to the basolateral membrane with little (2) or no effect (3, 4) on the luminal membrane. All of the maneuvers described above also lead to a profound increase in basolateral Na-K ATPase activity (3, 5–9). These studies suggest that ion transport along the distal nephron can be enhanced by amplification of the basolateral membrane of principal cells.

Previous morphological studies have employed pharmacological doses of DOCA and dexamethasone (1). Therefore, it has not yet been established whether more physiologic levels of mineralocorticoid or glucocorticoid activity will lead to similar ultrastructural changes. Because of significant cross-over binding between the mineralocorticoid and glucocorticoid receptors (10–12) with the high corticosteroid doses employed, it was not possible to determine which receptor type was responsible for the morphological changes. The interpretation of the morphological effects of potassium adaptation is complicated by another consideration. Although potassium adaptation in the rat is associated with a sharp increase in aldosterone levels (2, 13), it is possible that elevated dietary intake of potassium may have additional important renal effects unrelated to aldosterone (14).

The purpose of this study was to examine the effects of adrenalectomy and selective chronic replacement of physiological amounts of mineralocorticoids and glucocorticoids on the cellular ultrastructure of the initial collecting tubule in rats maintained on a constant dietary potassium intake. Membrane length, surface density, and cell area were measured by morphometry. Results indicate that mineralocorticoids but not glucocorticoids influence the basolateral membrane area of principal cells in the initial collecting tubule. The functional data reported in a companion paper demonstrate that the variation in basolateral membrane area elicited by the selective hormone replacements is closely paralleled by changes in sodium and potassium transport (15).

Methods

Male Sprague-Dawley rats, initially weighing between 200 and 225 g, were used in all experiments. 10 d before the kidneys were prepared

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1. *Abbreviations used in this paper:* A, cell area; ADX, adrenalectomized; B_{BLM}, basolateral membrane length; B_{LM}, luminal membrane length; DOCA, desoxycorticosterone acetate; PEG, polyethelene glycol 400; S_VBLM, surface density of the basolateral membrane; S_VLM, surface density of the luminal membrane.

for electron microscopy, animals were either sham-operated or adrenalectomized (ADX) and given physiological replacement doses of aldosterone or dexamethasone by osmotic minipump (#2002; Alza Corp., Palo Alto, CA). The protocol of adrenalectomy, hormone replacement, and dietary maintenance was identical to that reported in a previous study (15). Administration of steroid hormone by osmotic pump (fluid delivery rate, 11 $\mu l \cdot d^{-1}$) has been shown to achieve constant plasma hormone levels for 14 d (16).

The animals used in this study were divided into six groups. Group 1, control, were sham-ADX and served as adrenal-intact controls. Since the steroids given to animals in groups 2–6 were dissolved in polyethelene glycol 400 (PEG, J. T. Baker, Phillipsburg, NJ), this group received PEG alone by minipump (16). Group 2, adrenalectomy, received vehicle alone (PEG). Group 3, adrenalectomy and dexamethasone, received dexamethasone (Sigma Chemical Co., St. Louis, MO) 1.2 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$. Group 4, adrenalectomy and aldosterone, received d-aldosterone (Sigma Chemical Co.) 0.5 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$. Group 5, adrenalectomy and aldosterone, received d-aldosterone, 2.0 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$. Group 6, adrenalectomy, dexamethasone and aldosterone, received the same dose of dexamethasone (1.2 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$) as animals in group 3 and the same dose of aldosterone (d-aldosterone, 0.5 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$) as animals in group 4.

The animals were pair-fed laboratory chow (#5010; Ralston Purina Co., St. Louis, MO), 18–20 $g \cdot d^{-1}$. Controls had distilled water to drink, while all ADX animals received 0.9% NaCl in 1.25% dextrose, ad libitum.

After 10 d of hormone replacement, animals were anesthetized with Inactin (100 $mg \cdot kg^{-1} \cdot$ body wt, i.p.) and the left kidney was fixed and processed for electron microscopy as described previously (2). Plasma aldosterone was kindly measured by Dr. Patrick Mulrow (Medical College of Ohio, Toledo, OH) by radioimmunoassay.

Initial collecting tubules[2] that were in direct contact with the capsule were examined. Previous microperfusion and electron microscopy studies have shown that the initial collecting tubule secretes potassium (2, 17) and that ultrastructural changes induced by potassium adaptation were specific for the principal cell type in this segment; intercalated cell structure was unchanged (2). In the present study, 2–4 initial collecting tubules that were cut in cross section from each block of tissue (three randomly selected blocks from each kidney) were selected, and thin-sectioned. In preliminary studies intercalated cells were photographed and examined; adrenalectomy and subsequent hormone replacement had no significant effect on luminal or basolateral membrane length, surface density, or cell area. Subsequently, only principal cells were photographed.

To eliminate possible observer bias, we coded all animals, tissue blocks, and micrographs. The code was broken only after all counts and calculations were made. For each animal, an average of 14 principal cells, which were cut in cross section and had an intact basement membrane with visible tight junctions, were photographed with an electron microscope (10B; Carl Zeiss Inc., Thornwood, NY) and enlarged during printing to a magnification of 10,000. The results of each animal represent a single observation. The following parameters were measured for each cell by methods described previously (1, 2): surface density (the ratio of membrane area to cell volume) of the basolateral (S_VBLM) and luminal membrane (S_VLM); boundary length of the luminal membrane (B_{LM}), boundary length of the basolateral membrane (B_{BLM}), and cell area (A).

Preliminary inspection of the data was done by a one-way analysis of variance. If there was a significant value of F by analysis of variance at $P < 0.05$, the Least Significant Difference test was used to identify statistical significance between means. If the variances between groups were significantly different, Dunnett's test was used to determine statistical significance between means (18). Levels of significance are expressed based on Least Significant Difference or Dunnetts test comparisons where appropriate. All data are expressed as the mean±SEM.

Results

Examples of typical principal cells in initial cortical collecting tubules from control and ADX animals are illustrated in Figs. 1 and 2, respectively. Qualitatively, the absence of adrenal hormone for 10 d is not associated with a remarkable alteration in ultrastructure. However, morphometric analysis of these groups reveals that adrenalectomy resulted in a statistically significant drop in the S_VBLM and B_{BLM} compared with control animals (Table I). Surface density decreased by more than 30%, from 3.05±0.12 $\mu m^2/\mu m^3$ in control to 2.11±0.13 $\mu m^2/\mu m^3$ ($P < 0.01$) in adrenalectomy. B_{BLM} decreased by 35% from 111.8±5.4 μm in control to 72.4±7.9 μm in adrenalectomy ($P < 0.01$). This effect is a very specific reduction, because S_VLM, B_{LM}, and A were not affected significantly by adrenalectomy (Table I).

The principal cells of ADX animals that received aldosterone replacement (illustrated in Figs. 3 and 4) had dramatically increased basolateral membrane compared with adrenalectomy. The lowest replacement dose of aldosterone given (0.5 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$) increased S_VBLM from 2.11±0.13 $\mu m^2/\mu m^3$ in adrenalectomy to 3.31±0.30 $\mu m^2/\mu m^3$ ($P < 0.01$), a value that is not significantly different from that of control animals, 3.05±0.12 $\mu m^2/\mu m^3$ (Table I). Basolateral membrane length also increased with this low dose of aldosterone from 72.4±7.9 μm to 121.4±7.4 μm ($P < 0.01$). This value is also similar to that observed for control animals, 111.8±5.4 μm. This dose of aldosterone also restored plasma aldosterone to values that were not significantly different from those levels observed in intact controls (7.2±1.3 ng/dl in control vs. 4.4±1.9 ng/dl in low dose aldosterone, P = NS) (15). S_VBLM and basolateral membrane area were further increased and reached values significantly greater than those observed in control animals by the administration of aldosterone at the higher dose of 2.0 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$. Basolateral membrane surface density increased from a control value of 3.05±0.12 $\mu m^2/\mu m^3$ to 3.87±0.13 $\mu m^2/\mu m^3$ ($P < 0.05$) with the high dose of aldosterone, while B_{BLM} also increased ($P < 0.01$) from 111.8±5.4 μm to 236.1±26.6 μm in control and high aldosterone, respectively. Plasma aldosterone was 15.5±1.4 ng/dl in the high dose aldosterone group, a value that was significantly greater than that observed in controls (7.2±1.3 ng/dl, $P < 0.01$) and low dose aldosterone replacement (4.4±1.9 ng/dl, $P < 0.01$).

The principal cells in animals treated with the high maintenance dose of aldosterone (Fig. 4) also showed a statistically significant increase in A (Table I). Hence, S_VLM actually decreased after high aldosterone compared with adrenalectomy ($P < 0.05$) and controls ($P < 0.01$). However, since luminal membrane boundary length was unchanged compared with control values, the decrease in S_VLM after high levels of aldosterone was clearly due to the 66% increase in cell area (Table I).

A typical principal cell from an animal that received dexamethasone replacement is illustrated in Fig. 5. This dose of dexamethasone restored glomerular filtration rate (15), and plasma insulin and glucose concentrations in ADX animals to control values (Stanton, B., G. Klein-Robbenhaar, G. Giebisch,

2. We have chosen to refer to the late distal tubule as the initial collecting tubule after Woodhall and Tisher (43) to distinguish this segment of the nephron from the cortical collecting tubule, which also contains principal and intercalated cells.

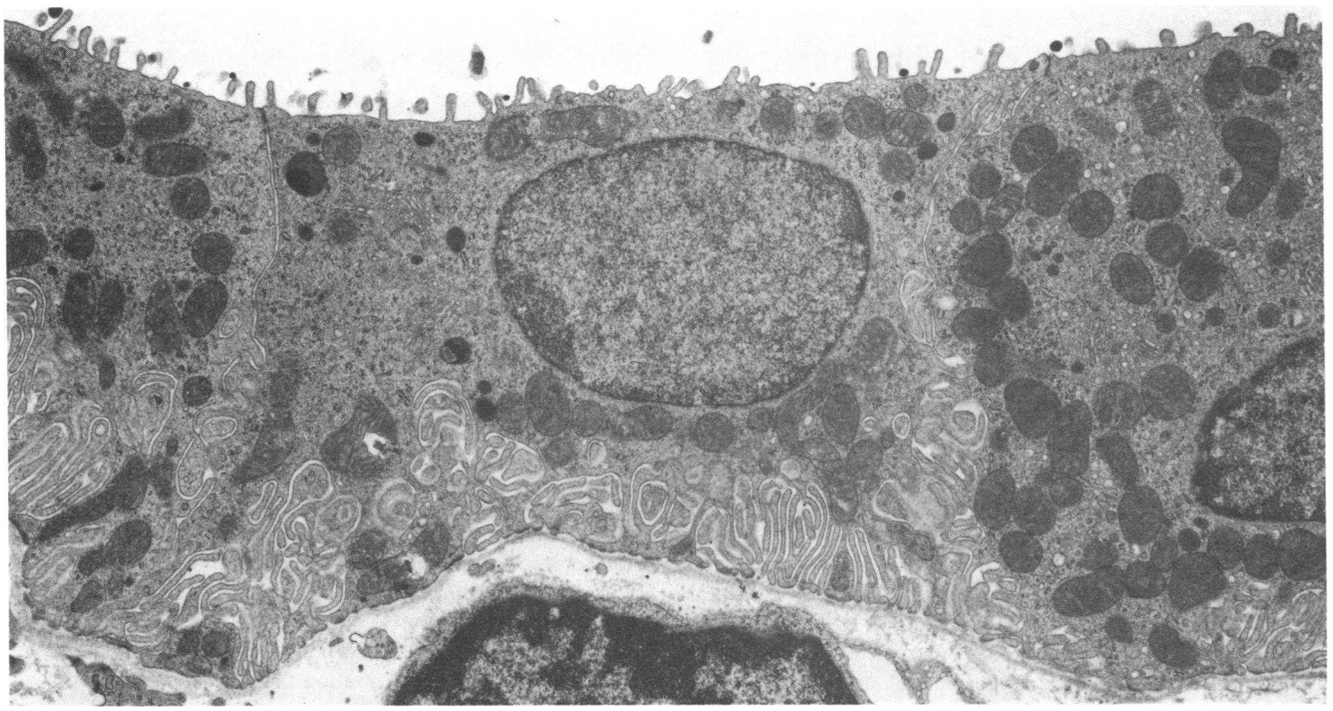

Figure 1. Principal cell, control. Tubular lumen, *top;* blood, *bottom.* Note the modest invaginations of the basolateral membrane (× 10,000).

J. Wade, and R. DeFronzo, unpublished observations). However, compared with adrenalectomy, this dose of dexamethasone had no significant effect on basolateral membrane length or any other morphological parameter that was examined (Table I). Thus, ADX animals with dexamethasone replacement still displayed a specific reduction in basolateral membrane area.

In the last group of animals, aldosterone and dexamethasone were administered together. Although the A for this group was greater than observed with the low dose of aldosterone, there were no statistical significant differences between the effects of aldosterone alone or aldosterone with dexamethasone on membrane surface density or B_{BLM} (Table I). These results are not unexpected since dexamethasone itself had no significant effect on the principal cell type in this study. The reason for the increase in cell area in animals treated with aldosterone and dexamethasone (group 5) is unknown.

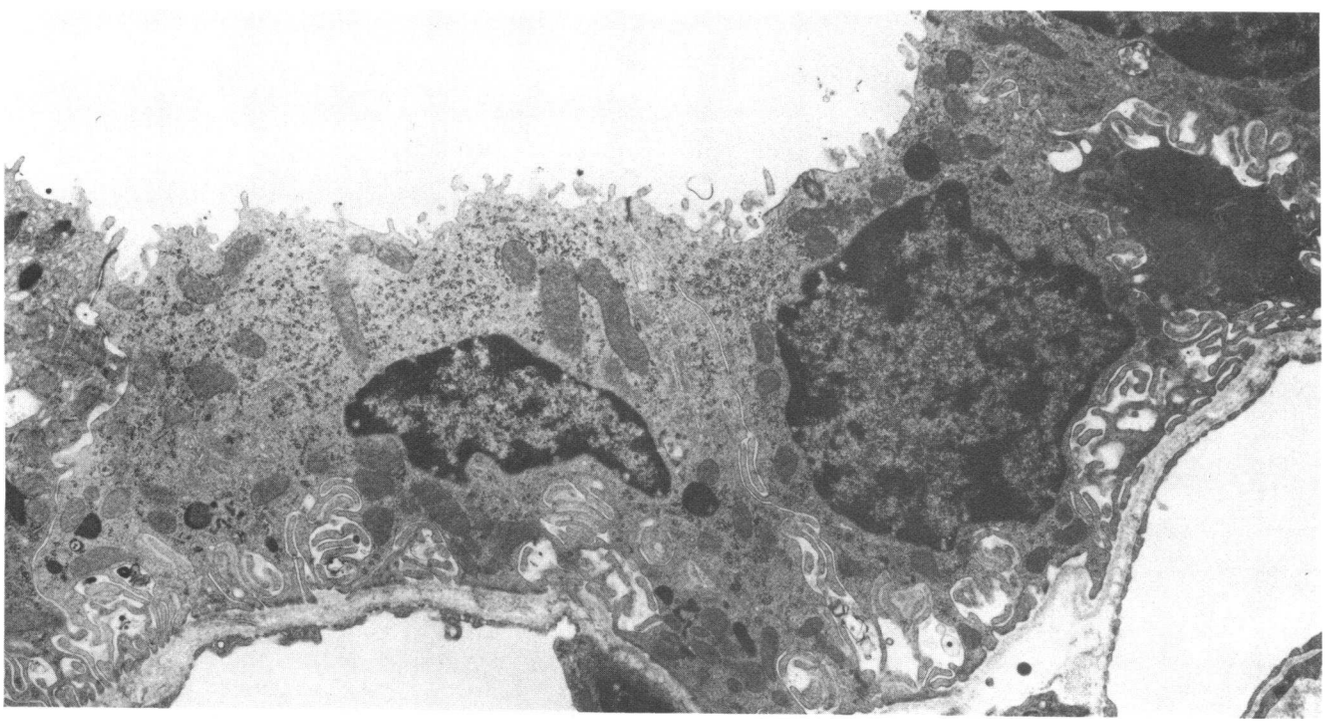

Figure 2. Principal cell, adrenalectomy. The basolateral membrane has fewer invaginations, especially in the basal portion, than principal cells from control. Note the numerous ribosomes in the cytoplasm (× 10,000).

Table I. Summary of Morphometric Analysis of Principal Cells

Group	S_VBLM	S_VLM	B_{BLM}	B_{LM}	Area
	$\mu m^2/\mu m^3$	$\mu m^2/\mu m^3$	μm	μm	μm^2
1 (Control, n = 8)	3.05±0.12‡	0.59±0.04	111.8±5.4‡	21.2±1.1	46.3±1.7
2 (ADX, n = 6)	2.11±0.13*	0.52±0.04	72.4±7.9*	17.2±1.0	43.4±4.0
3 (ADX + Dex, n = 7)	2.25±0.15*	0.50±0.02	84.0±6.2*	18.8±1.8	47.6±3.4
4 (ADX + Aldo, 0.5 μg/100 g/d, n = 6)	3.31±0.30‡	0.49±0.03	121.4±7.4‡	18.4±1.6	47.6±3.5
5 (ADX + Aldo, 2 μg/10 g/d, n = 5)	3.87±0.13*‡‖	0.42±0.04*	236.1±26.6*‡‖	25.0±2.2	76.9±7.8*§
6 (ADX + Aldo + Dex, n = 6)	2.82±0.15‡	0.37±0.03*	150.3±12.2*‡	19.8±1.4	69.0±5.3*§

Statistical comparisons between groups based on Least Significant Difference or Dunnetts test. Means are considered significantly different if $P < 0.05$. Aldo, aldosterone; Dex, dexamethasone; S_V, surface density (membrane area/cell volume). * vs. group 1; ‡ vs. groups 2 and 3; § groups 5 and 6 vs. groups 1–4; ‖ vs. groups 1–4 and 6.

Discussion

The results of the present experiments demonstrate that aldosterone, within the physiological range, has an important influence on basolateral membrane length of the principal cell type in the initial collecting tubule. Adrenalectomy resulted in more than a 35% decrease in basolateral membrane length. Chronic aldosterone replacement, in ADX animals with a dose that restored plasma aldosterone to levels observed in intact controls, also returned basolateral membrane surface density to control levels (3.31 $\mu m^2/\mu m^3$ in aldosterone vs. 3.05 $\mu m^2/\mu m^3$ in control). A larger aldosterone replacement dose, which significantly increased circulating plasma aldosterone levels above control, led to a further increase in S_VBLM compared with low dose aldosterone (3.31 $\mu m^2/\mu m^3$ in low dose aldosterone vs. 3.87 $\mu m^2/\mu m^3$ in high dose aldosterone). The increase in membrane surface density produced by the high dose aldosterone replacement was smaller than previously reported by us after potassium adaptation (2). In this later study surface density increased from 3.28 $\mu m^2/\mu m^3$ to 4.77 $\mu m^2/\mu m^3$ (2). The more pronounced effect produced by potassium adaptation is likely related to the observation that plasma aldosterone was significantly higher in potassium adapted animals (23.5±0.4 ng/dl, unstressed basal values) compared with animals treated with the high dose of aldosterone (15.5±1.4 ng/dl, $P < 0.01$). The effect of corticosteroid replacement on membrane area seems to be specific to mineralocorticoids since chronic glucocorticoid replacement with dexamethasone had no effect on B_{BLM} compared with adrenalectomy. Furthermore, when dexamethasone was administered to aldosterone replaced animals

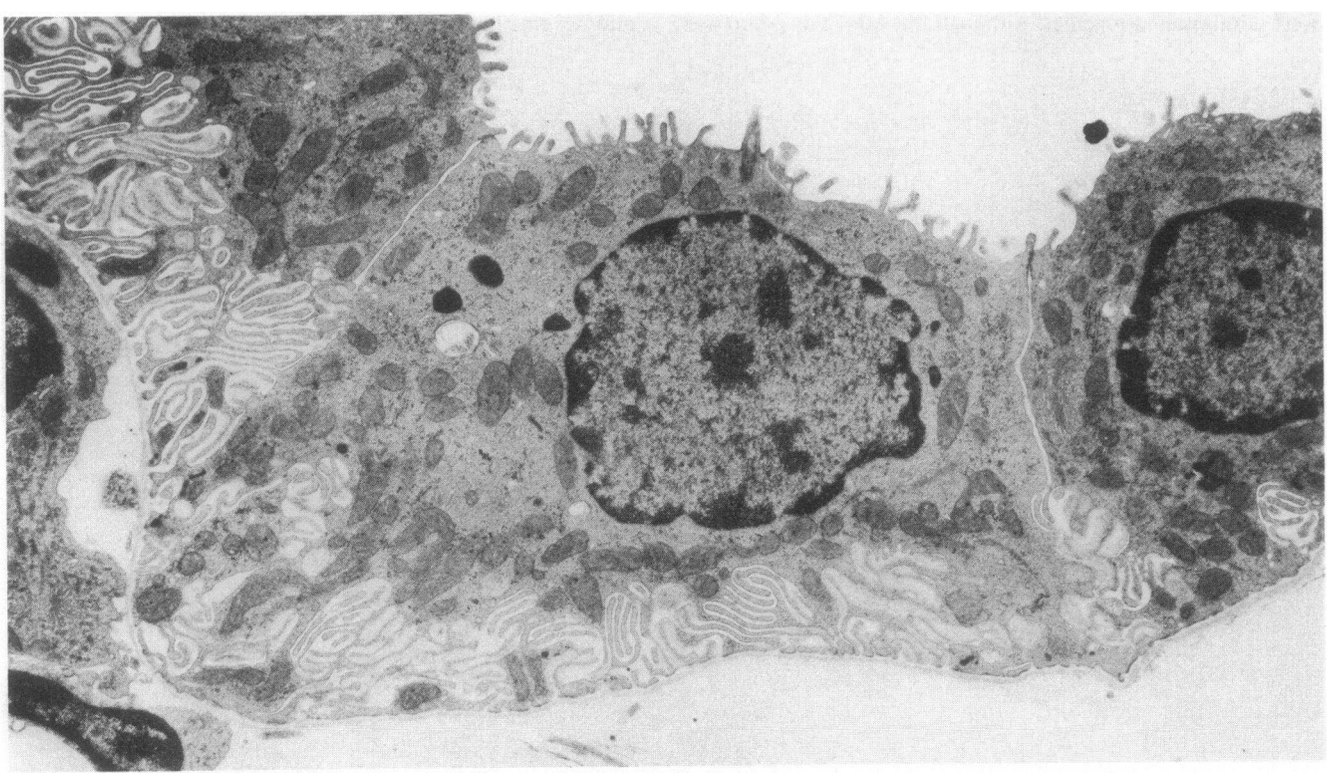

Figure 3. Principal cell, aldosterone replacement (0.5 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$). Compare the invaginations of the basolateral membrane with control (Fig. 1) and adrenalectomy (Fig. 2). (× 10,000).

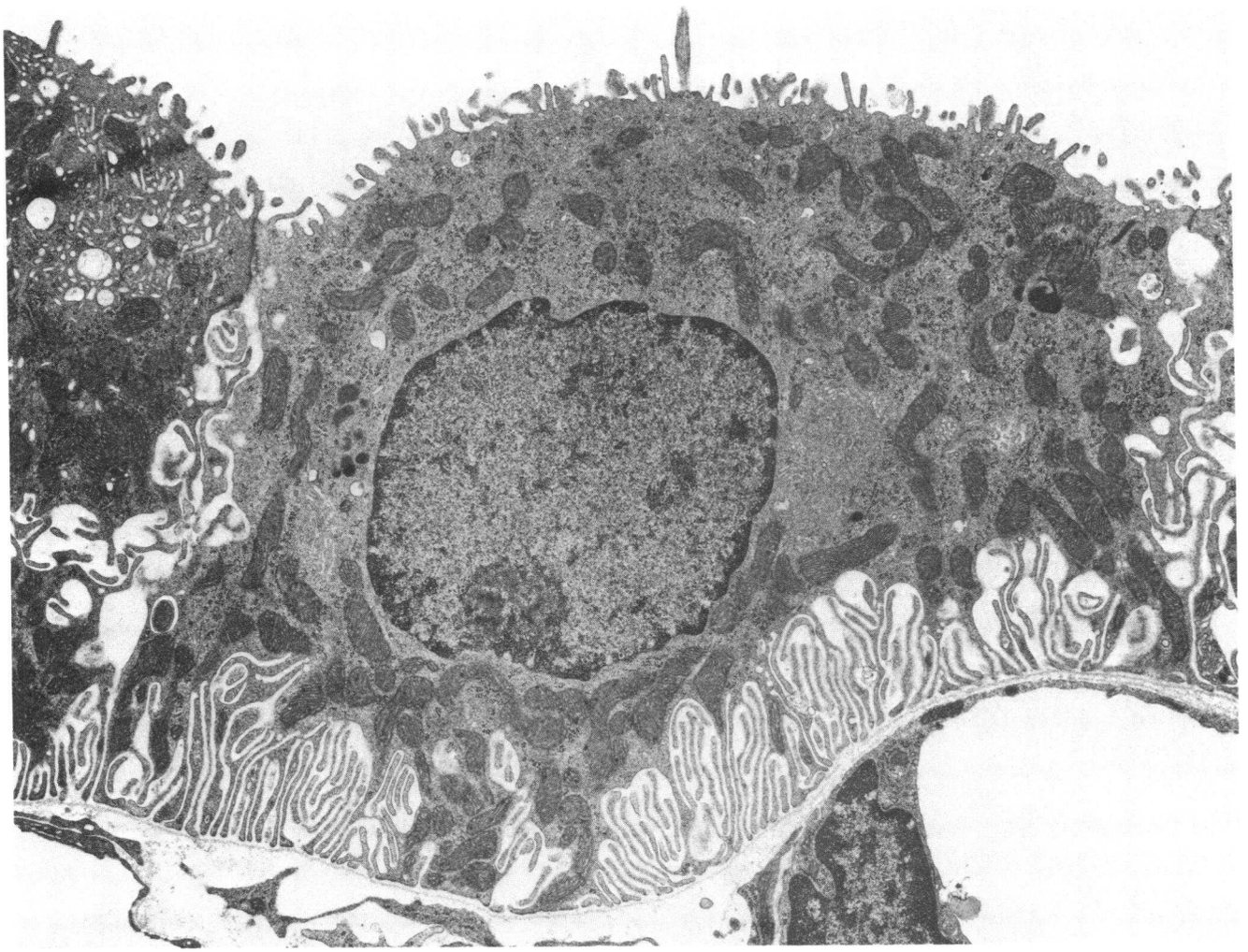

Figure 4. Principal cell, aldosterone replacement (2.0 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$). The higher dose of aldosterone enhanced basolateral membrane amplification (compare with Fig. 3). (× 10,000).

(group 6), no effect of dexamethasone on B_{BLM} was detected compared with the effects due to aldosterone alone.

Note that all animals received the same potassium diet. Thus, we can demonstrate that aldosterone affects basolateral membrane length in the absence of changes in dietary potassium intake. It is also clear that elevation in plasma potassium per se cannot alone be responsible for the increase in B_{BLM} since plasma potassium was normal when B_{BLM} was restored by aldosterone (15). Furthermore, in the ADX animals, plasma potassium was actually elevated (15), yet basolateral membrane length was reduced. These alterations in B_{BLM} occurred at a time when urinary potassium excretion was similar in all groups of animals (15). Thus, it is unlikely that changes in potassium transport per se led to the alterations in the basolateral membrane observed in this study.

Conditions that increase basolateral membrane area of the principal cell type such as potassium adaptation (2–4) or DOCA treatment (1), have been associated with sharp and parallel increases in the activity of Na-K ATPase in the cortical collecting tubule (3, 5–8). Since movement of potassium and sodium across the basolateral membrane is mediated by the ATPase-driven sodium-potassium pump, a primary regulatory site of transepithelial transport in the initial and cortical collecting tubule (19–21), it is possible that variation in basolateral membrane area provides a mechanism whereby aldosterone levels may regulate potassium and sodium transport. Clearly, this does not exclude an additional luminal site of action of mineralocorticoids. Electrophysiological studies have provided strong evidence that mineralocorticoids increase the sodium and potassium permeability of the luminal membrane of cells in the cortical collecting tubule (22).

Although we have not measured the activity of Na-K ATPase in the present experiments, data by Mujais et al. (9) indicate that in chronically adrenalectomized rats Na-K ATPase activity in the cortical collecting tubule decreases by 52%. Chronic aldosterone administration (0.8 $\mu g \cdot 100\ g^{-1} \cdot d^{-1}$) restores enzyme activity to control values. In contrast, when corticosterone replacement was provided to ADX animals at a dose that restored hormone levels to control values, there was no effect on ATPase activity. Although the dose of aldosterone used by Mujais et al. (9) was somewhat higher than that used in this study, and corticosterone rather than dexamethasone was used, these measurements of Na-K ATPase closely parallel our morphological results. Acute doses of aldosterone have also been shown to increase ATPase activity in the cortical collecting tubule (23, 24), whereas dexamethasone

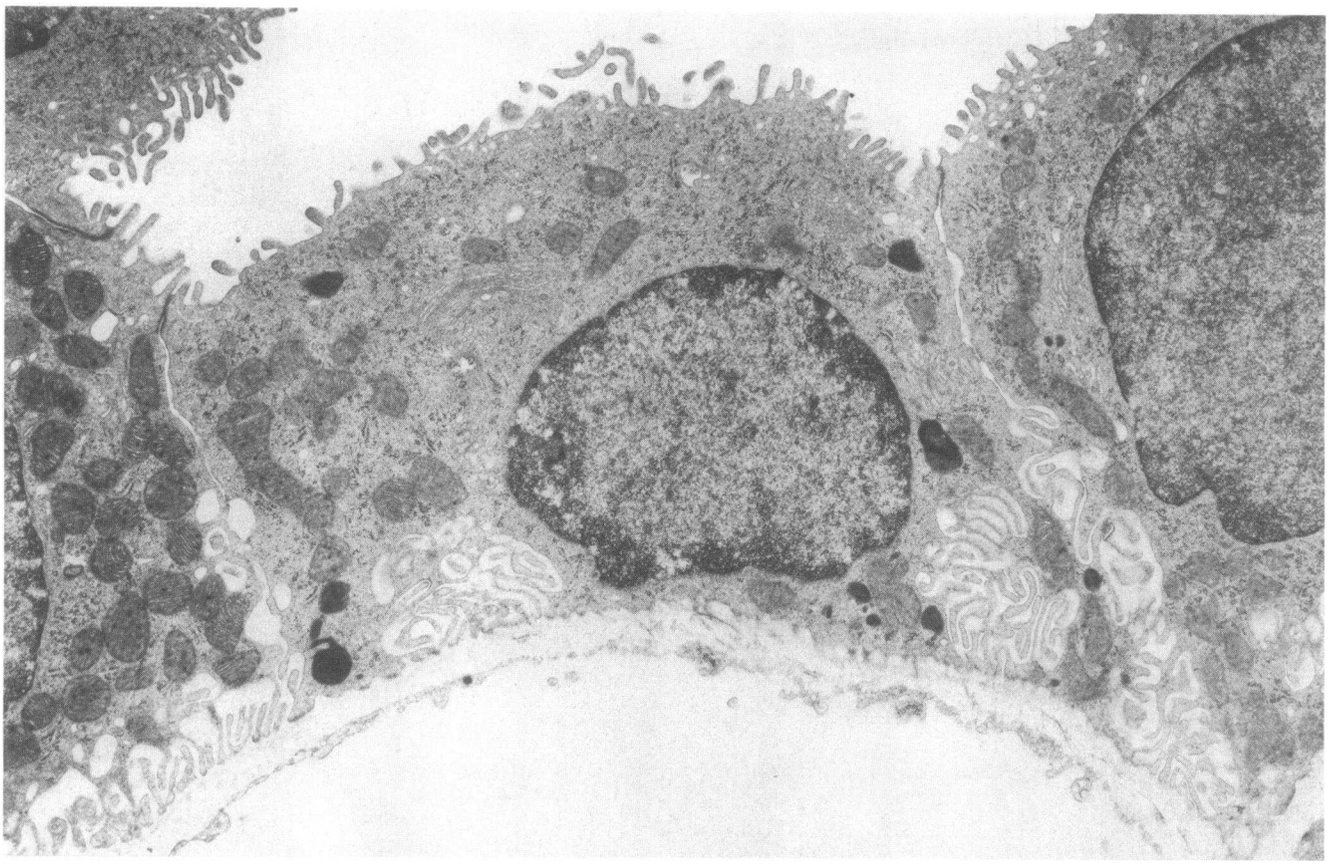

Figure 5. Principal cell, dexamethasone replacement. The basolateral membrane is reduced compared with control (Fig. 1) and similar to cells in ADX unreplaced animals (Fig. 2). (× 10,000).

has no effect (23). Together, these results suggest that chronic alterations of aldosterone levels, within the physiological range, have a potent effect on principal cell basolateral membrane area and Na-K ATPase activity. Glucocorticoids, in low doses, have no significant effect on membrane area or on ATPase activity in the collecting tubule.

Our failure to observe any effect of glucocorticoids on membrane length deserves further comment. There is considerable evidence that glucocorticoids increase renal Na-K ATPase activity (25–27) and membrane area (1). However, in all of these studies (1, 25–27), pharmacological doses of glucocorticoids were employed. It is likely, therefore, that the alterations in ultrastructure and ATPase activity in these previous studies resulted, in large part, from nonspecific binding to mineralocorticoid receptors in the cortical collecting tubule. This conclusion is substantiated by the fact that spironolactone, a competitive inhibitor of mineralocorticoids, interferes with the dexamethasone-induced stimulation of ATPase (24). Furthermore, low physiological doses of glucocorticoid have been shown not to have an effect on ATPase activity in the cortical collecting tubule (9, 23, 26). It is also likely that the effects of dexamethasone on ATPase in renal homogenates (25–27) represent an effect on the proximal tubule and loop of Henle, nephron segments that have glucocorticoid receptors (24, 26, 28). For example, Rayson and Lowther (26) and El Mernissi and Doucet (24) have shown that dexamethasone stimulates ATPase activity in the thick ascending limb of Henle's loop, an effect that is insensitive to spironolactone (24). Together, these studies indicate that ATPase activity in the collecting tubule is regulated by aldosterone. This effect is mediated by mineralocorticoid receptors. Glucocorticoids, on the other hand, in the physiological range act on ATPase in the proximal tubule and the thick ascending limb, an effect mediated by glucocorticoid receptors.

In a parallel study (15) we report renal clearance experiments on a similar group of animals. It is of interest to examine the relationship between the cellular ultrastructure of the initial collecting tubule in the present study and the rate at which the kidneys can excrete potassium since the rate of urinary potassium excretion is largely determined by transport events in the initial and cortical collecting tubule (reviewed in 19, 21). We found that there is a close relationship between the amount of basolateral membrane of principal cells and the rate of potassium excretion during an acute intravenous infusion of potassium. This conclusion is based on the following key observations. Adrenalectomy sharply reduced both membrane area and the ability of the kidney to excrete potassium. Aldosterone replacement restored membrane area to control levels and improved potassium excretion but not to levels observed in intact control animals. As discussed previously (15), the failure of chronic aldosterone replacement to restore potassium excretion to levels observed in intact animals during intravenous infusion of potassium was due to the inability of ADX animals to increase plasma aldosterone concentration in response to the acute potassium infusion. When we mimicked this acute rise in plasma aldosterone in ADX rats that were chronically given aldosterone, potassium excretion rose to levels observed in controls. These findings are compatible with

a dual role of aldosterone in the regulation of potassium transport. First, aldosterone may regulate transport by influencing the amount of basolateral membrane. This effect may require long-term exposure to aldosterone due to the low turnover rate of the Na-K ATPase molecule (29). It may correspond to the "late" mineralocorticoid response reported for amphibian epithelia (30). Second, acute elevations in plasma aldosterone may stimulate transport in the distal tubule and cortical collecting duct through several additional mechanisms, including an increase in apical membrane sodium and potassium permeability (19, 22) and an increase in the Na-K pump activity (23, 24, 26). Future studies will have to determine whether acute stimulation involves insertion of additional pump units into the basolateral membrane.

Mineralocorticoids, in addition to their effects on potassium transport, also influence sodium transport in the kidney (1, 2, 17, 20, 31, 32). The results of our companion study confirm these findings and demonstrate that sodium reabsorption is inhibited by adrenalectomy and stimulated by chronic and acute aldosterone replacement (15). The precise relationship between sodium reabsorption and potassium secretion in inducing principal cell basolateral membrane amplification needs further explanation. Several lines of evidence indicate that the mineralocorticoid-induced increase in ATPase activity depends on increased luminal sodium entry since it can be blocked by amiloride (23, 34). Whether such increased sodium entry after mineralocorticoid administration is also responsible for membrane amplification, is presently not known.

Because the dexamethasone-stimulated kaliuresis has been shown to be closely related to an increase in urine flow (15, 33, 35), and because dexamethaseone was without effect upon principal cell B_{BLM} we conclude that glucocorticoids most likely do not have a direct effect at the level of the initial collecting tubule. This view is supported by a recent microperfusion study from our laboratory. Thus, when tubular flow rate was kept constant by pump-perfusion (33), potassium secretion was not affected by dexamethasone administration (33).

In the last several years it has also become evident that cells in other epithelia can regulate ion transport by altering membrane area in response to specific transport stimuli. Relevant examples are the gastric mucosa (36), turtle bladder (37, 38), chloride cells in the teleost gill (39), avian salt gland (40), and colon (41). Furthermore, intercalated cell luminal membrane area in the renal medullary collecting duct is enhanced by respiratory acidosis, which is associated with an increase in acid secretion (42). Our present observations provide strong evidence that the mammalian initial collecting tubule is also able to adapt structurally in response to specific hormonal stimuli.

Acknowledgments

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