

RAPID COMMUNICATIONS

MODULATION OF CELL MEMBRANE AREA IN RENAL COLLECTING TUBULES BY CORTICOSTEROID HORMONES

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ABSTRACT

Isolated renal cortical collecting tubules obtained from rabbits treated chronically with desoxycorticosterone acetate (DOCA) have been found to possess elevated transepithelial potential differences and a greatly increased capacity for ion transport. Structural examination of tubules from rabbits exposed to either DOCA or dexamethasone for 11–18 d reveals a marked increase in basolateral cell membrane area in these tubules. Morphometric analysis shows that this effect is specifically on the basolateral membrane area of only one of the two cell types found in this nephron segment. Increases of >140% and 90% are found for the basolateral membrane area of the principal cells for DOCA and dexamethasone, respectively, but no change could be detected in the basolateral membrane area of the intercalated cells found in this nephron segment. No significant changes were found in luminal membrane area, cell number, or cell volume for either cell type. These observations demonstrate that significant changes in membrane area can occur in differentiated epithelia and suggest that this may be an important mechanism for modulating epithelial transport capacity.

KEY WORDS cell membrane area · desoxycorticosterone acetate · dexamethasone · renal cortical collecting tubule · Na⁺-K⁺ transport

The development of techniques for the isolation and perfusion *in vitro* of individual renal tubules (1) has contributed significantly to a more detailed functional and structural characterization of many segments of the nephron. Because both luminal and peritubular media as well as other conditions can be closely controlled, it has been possible to study specific changes elicited by acute hormone exposure *in vitro* (7, 10, 12, 14). In addition, the transport capacity of isolated epithelia *in vitro* may be significantly influenced by the previous physiological status of the animal. In the case of renal cortical collecting tubules, recent studies have shown that the electrolyte composition of the diet or chronic exposure to corticosteroid hormone in the weeks before the sacrifice of the

animal can significantly increase the transepithelial potential difference and rates of Na⁺ and K⁺ transport measured *in vitro* (6, 11, 25, 27).

Because alterations in transport persist for hours *in vitro* in the absence of exogenous corticosteroid hormone, we have examined the possibility that the increase in transport capacity might be explained, at least in part, by morphological changes induced during the weeks of hormone exposure *in vivo*. Structurally, the mammalian cortical collecting tubule consists of two cell types: principal cells (PC), which have also been called "light" cells, and a second, less numerous class of cells which are called intercalated cells (IC) or "dark" cells. Thus it was also of interest to determine whether any changes were specific for one of these cell types. In this report, we demonstrate that long-term treatment of animals with corticosteroid hormones results in a dramatic and

specific amplification of the basolateral membrane area of the PC.

MATERIALS AND METHODS

New Zealand white female rabbits weighing 1.1–2.5 kg (~6–12 wk old) were maintained on standard Purina laboratory rabbit chow (Purina Chows, Ralston Purina Co., St. Louis, Mo.) (170 mEq Na/kg and 360 mEq K/kg) and tap water ad libitum. The rabbits were divided into three groups. The first group (control group) was untreated. The second group (desoxycorticosterone acetate [DOCA] group) received daily intramuscular injections of DOCA (Organon, Inc., West Orange, N. J.), 5 mg/d, for 11–18 d before sacrifice just as in a previous physiological study (25). A third group (dexamethasone group) received intramuscular injections of dexamethasone (Azium, Schering Corp., Kenilworth, N. J.) twice a day, 5 mg/d, for the same time period. Isolated segments of renal cortical collecting tubules (1.0- to 2.6-mm long) dissected from the inner to mid cortex were mounted on glass pipets at both ends and perfused at room temperature by the methods of Burg et al. (1) and as we have previously described (25). All tubules were perfused with (in mM): 115 NaCl, 35 Na isethionate, 2.25 K_2HPO_4 , 0.5 KH_2PO_4 , 1.0 $MgSO_4$, and 1.0 Ca lactate at pH 7.4. The bathing solution was identical except that 25 mM $NaHCO_3$ and 10 mM Na acetate was substituted for the Na isethionate and 5.5 mM glucose and 5% (vol/vol) calf serum was added. Bathing solution was gassed with 5% CO_2 -95% O_2 . Tubules were allowed to equilibrate in the bathing media for 2–4 h and were usually perfused for 1–3 h during this equilibration period. The transepithelial voltage (V_{tr}) was monitored via the perfusion pipet, and the steady-state values of V_{tr} recorded. After steady state was achieved, tubules were fixed in situ by simultaneously exchanging the bathing and perfusing solutions with quarter-strength Karnovsky's fixative (16) diluted with 0.2 M sucrose. Care was taken to avoid any pressure or flow variations, and microscope observation of the tubules during fixation verified that epithelial cell volume was not altered during fixation. After 30 min of fixation, the tubules were removed from the perfusion apparatus and left in fixative for an additional 30 min. Tubules were washed with 0.16 M Na cacodylate buffer and embedded initially in 2% agar to facilitate handling during the subsequent embedding for electron microscopy.

Stereological techniques (33) were employed to quantitatively evaluate micrographs taken with a Zeiss 10B electron microscope. For each tubule, at least three complete cross-sections from regions separated by $>50 \mu m$ were examined. Sections were perpendicular to the axis of cylindrical tubules. Quantitation was carried out both for the tubule overall and for each of the cell types found in this nephron segment. Intercalated cells (IC, Fig. 1) were identified by their denser cytoplasm and greater number of cytoplasmic vacuoles compared to adjacent principal cells (PC, Fig. 1).

The test grid of connected semicircles designed by Merz (24) with diam 1 cm was used on micrographs enlarged to a final magnification of $\times 6,000$ ($d = 10^4 \mu m/6 \times 10^3$). Because this grid is isotropic, the intersections between test grid lines and membrane (I_i) are a measure of membrane boundary length (B) in microns irrespective of the anisotropic orientation of membranes in the collecting tubule. For this test grid: $B = I_i d$. Cell area (A) in μm^2 including both cytoplasm and nuclei was estimated from the point count (P_T) with a square grid (point separation = 1 cm, d as above) so that $A = P_T d^2$. Tubular wall volume in μm^3 per mm tubular length was estimated directly from the cell area because nearly perfect cross-sections were used. Membrane surface density (S_V) in $\mu m^2/\mu m^3$ for these grids is obtained from membrane intersections and cell point count by the relationship: $S_V = 4 I_i/\pi d P_T$. It is also possible to estimate luminal and basolateral cell membrane surface (S) per length of tubule in $\mu m^2/mm$ from the membrane boundary length per cross section (B) as: S/mm of tubule length = $\frac{4}{\pi} B$.

Values obtained for tubules from corticosteroid-treated animals were compared to control values by one-way analysis of variance except where noted.

RESULTS

As shown in Table I, long-term treatment of animals with either DOCA or dexamethasone resulted in an increase in the measured V_{tr} of collecting tubules isolated from these animals. Structural examination of these tubules revealed that compared to control tubules (Fig. 1a) there is a striking increase in basolateral membrane associated with DOCA (Fig. 1b) or dexamethasone (Fig. 1c) exposure. Qualitatively, these micrographs also indicate that this change in basolateral membrane occurs in the PC but not in the IC of this nephron segment.

Morphometric evaluation of these tubules demonstrates that for the tubule as a whole the S_V for basolateral membrane is significantly increased by hormone exposure (Table I). Moreover, this effect is specifically on basolateral membrane because S_V for luminal membrane is not significantly changed by these hormones.

Quantitative comparison of the two cell types present in the collecting tubule shows that S_V of the luminal membrane does not differ significantly between cell types (Table I). However, S_V for basolateral membrane of PC is $>40\%$ greater than that of IC ($P < 0.05$ by paired t test). This difference is further accentuated by hormone exposure because basolateral membrane S_V of PC was increased significantly relative to controls by

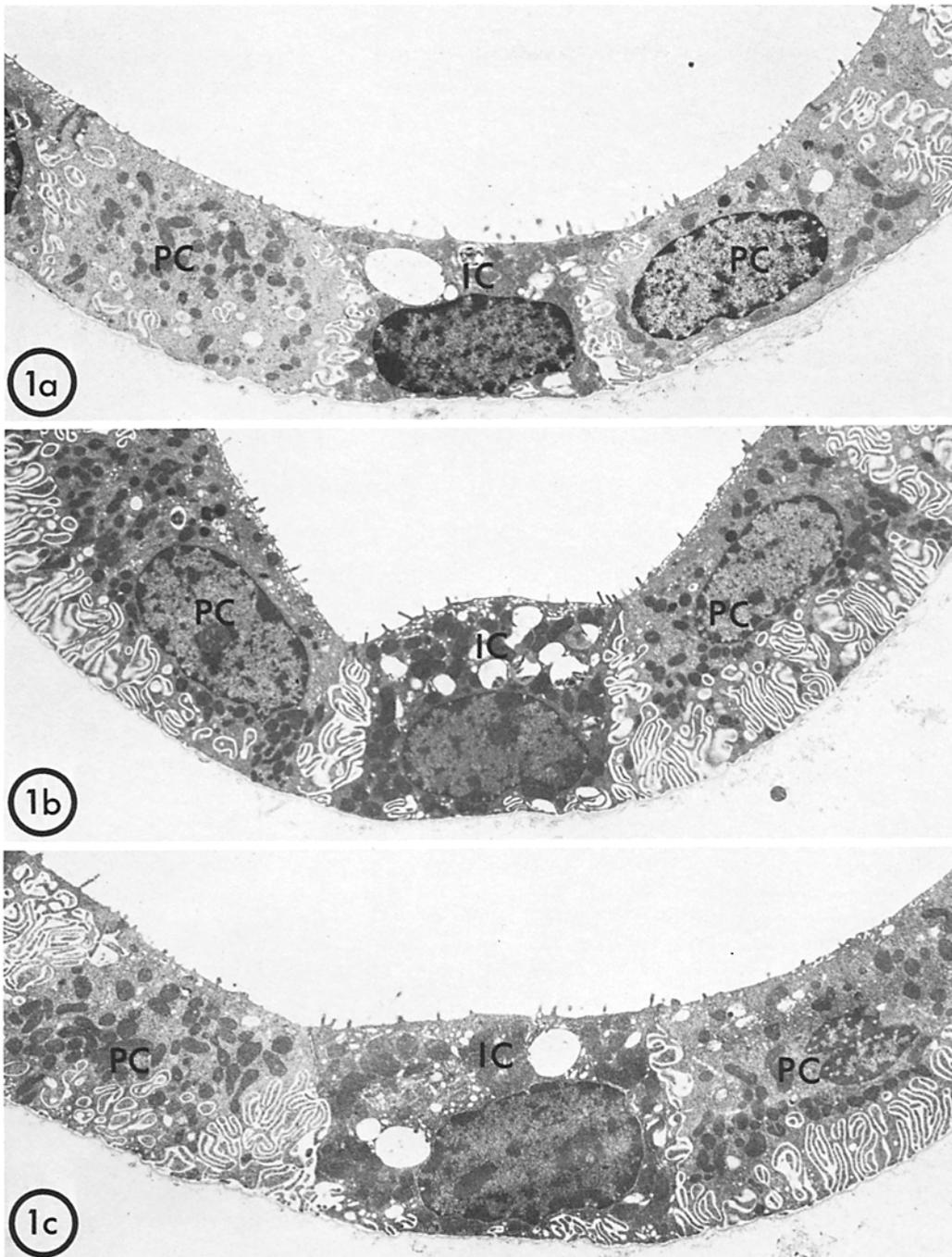


FIGURE 1 Isolated renal cortical collecting tubules showing principal cells (PC) and intercalated cells (IC). (a) Tubule from control rabbit. (b) Tubule from DOCA-treated rabbit. (c) Tubule from dexamethasone-treated rabbit. $\times 6,500$.

both DOCA and dexamethasone treatment while the basolateral membrane of IC was unchanged by hormone treatment (Table I). While the above

quantitation used only cross-sections of tubules, comparable specific changes in S_V were also measured in longitudinal sections of three tubules from

TABLE I
Effect of Corticosteroids on Transepithelial Potential (V_{te}) and Cell Surface Density (S_V)

	Control	DOCA	Dexamethasone
Number of tubules examined	6	7	6
V_{te} (mV)	-2.8 ± 4.8	$-41.8 \pm 3.1^*$	-22.3 ± 8.5
Overall S_V ($\mu\text{m}^2/\mu\text{m}^3$)			
Luminal membrane	0.38 ± 0.03	0.36 ± 0.02	0.42 ± 0.04
Basolateral membrane	3.01 ± 0.29	$5.17 \pm 0.45^*$	$5.07 \pm 0.53^*$
Principal cell S_V ($\mu\text{m}^2/\mu\text{m}^3$)			
Luminal membrane	0.40 ± 0.03	0.33 ± 0.03	0.40 ± 0.05
Basolateral membrane	3.20 ± 0.36	$5.88 \pm 0.57^*$	$5.41 \pm 0.56^*$
Intercalated cell S_V ($\mu\text{m}^2/\mu\text{m}^3$)			
Luminal membrane	0.34 ± 0.04	0.40 ± 0.08	0.53 ± 0.09
Basolateral membrane	2.23 ± 0.12	2.29 ± 0.21	2.15 ± 0.18

Values are means \pm SE.

* Significant difference ($P < .01$) compared to control.

TABLE II
Tubular Diameter, Cell Number, and Cell Volume

	Control	DOCA	Dexamethasone
Number of tubules examined	6	7	6
Inner diameter (μm)	19 ± 1.0	17 ± 0.9	20 ± 1.1
Outer diameter (μm)	27 ± 1.3	29 ± 0.9	29 ± 1.7
Overall tubule			
Number of cells per tubular cross section	10.1 ± 0.6	9.9 ± 0.5	9.1 ± 0.5
Tubular wall volume $\left(\frac{\mu\text{m}^3}{\text{mm tubular length}}\right) \times 10^5$	2.64 ± 0.25	3.29 ± 0.22	2.85 ± 0.42
Principal cells (PC)			
Number of PC per cross section	7.9 ± 0.5	8.0 ± 0.5	7.4 ± 0.4
PC volume $\left(\frac{\mu\text{m}^3}{\text{mm tubular length}}\right) \times 10^5$	2.02 ± 0.25	2.67 ± 0.23	2.35 ± 0.34
Intercalated cells (IC)			
Number of IC per cross section	2.2 ± 0.2	1.9 ± 0.3	1.7 ± 0.3
IC volume $\left(\frac{\mu\text{m}^3}{\text{mm tubular length}}\right) \times 10^5$	0.62 ± 0.06	0.61 ± 0.11	0.53 ± 0.11

Values are means \pm SE. None of the values are significantly different from control by analysis of variance.

DOCA-treated rabbits when compared to similarly sectioned control tubules.

The possible effect of hormone administration on the incidence of each cell type and on cell volume was also evaluated. As shown in Table II, these parameters are not significantly altered by the hormones administered. However, because the volume of PC tends to be larger with DOCA administration, this factor may reduce the magnitude of changes in membrane surface area as assessed by changes in S_V .

From the boundary length of membrane seen in cross-sections of the tubules, it is possible to estimate membrane surface area per length of tubule. As shown in Table III, statistically significant changes in membrane area occur only in the

basolateral membrane, increasing overall tubule basolateral area by 120% for DOCA treatment and by 74% for dexamethasone treatment. However, when broken down by cell type, the entire effect is on PC, with the result that basolateral membrane area of this cell type is increased by 146% in the case of DOCA treatment and by 94% for tubules from dexamethasone-treated rabbits.

An alternative view of these observations can be provided by considering the degree to which membrane folds amplify the surface over that of a cylinder with the inner tubular diameter given in Table II. The microvilli of the collecting tubule amplify the luminal surface by only 1.9-fold in contrast to a 13-fold amplification by the basolat-

TABLE III
Cell Surface Area per mm Tubular Length

	Control	DOCA	Dexamethasone
Number of tubules examined	6	7	6
Overall tubule $\left(\frac{\mu\text{m}^2}{\text{mm tubular length}}\right) \times 10^5$			
Luminal membrane area	0.98 ± 0.07	1.22 ± 0.10	1.11 ± 0.05
Basolateral membrane area	7.71 ± 0.65	17.00 ± 1.75*	13.39 ± 0.83*
Principal cells $\left(\frac{\mu\text{m}^2}{\text{mm tubular length}}\right) \times 10^5$			
Luminal membrane area	0.77 ± 0.07	0.93 ± 0.11	0.85 ± 0.02
Basolateral membrane area	6.35 ± 0.66	15.61 ± 1.75*	12.29 ± 0.74*
Intercalated cells $\left(\frac{\mu\text{m}^2}{\text{mm tubular length}}\right) \times 10^5$			
Luminal membrane area	0.20 ± 0.02	0.30 ± 0.10	0.26 ± 0.06
Basolateral membrane area	1.35 ± 0.11	1.40 ± 0.24	1.10 ± 0.23

Values are means ± SE.

* Significant difference ($P < .01$) compared to control.

eral membrane. While exposure to corticosteroids did not influence amplification at the luminal surface significantly, the amplification by the basolateral membrane is increased to 32-fold and 21-fold by DOCA and dexamethasone treatment, respectively.

DISCUSSION

Transport physiologists recognize both that measured rates of membrane transport are a function of membrane area and that membrane area is often greatly amplified at the ultrastructural level by features such as the microvilli and basolateral membrane interdigitations of epithelia. Nevertheless, for practical reasons measurements of transport are often factored by gross estimates of membrane area such as the area of tissue mounted in a chamber or the length of tubule studied. This assumes that the membrane surface area of cells in a given epithelium is proportional to apparent tissue area. While it has been realized that the relationship between true and apparent area varies between different epithelia, the results of this study demonstrate that it is possible to dramatically increase the membrane area within a particular epithelium. Moreover, the changes in membrane area reported in this paper were found specifically in the basolateral membrane of PC. No significant changes were found in the luminal membrane area of PC or in the area of either the luminal or basolateral membrane of IC.

The present results are the first demonstration of a specific response of the basolateral membrane of PC to steroid hormones and raise the possibility

that effects of these hormones on transport may involve specifically this cell type. Thus, previous observations demonstrating that chronic exposure to corticosteroid hormone increases V_{te} (11, 25, 27), transport rates (25, 27), and the activity of Na-K-ATPase in the kidney (2, 23) may, at least in part, be explained by the observation that the basolateral membrane surface area of PC, whether expressed per cell volume or per mm tubule length, is significantly increased by such treatment. Indeed, it is remarkable that the increase in overall basolateral membrane area of 120% with DOCA is comparable to the 131% increase in sodium transport previously reported for tubules from similarly treated rabbits (25). It is likely that the increase in basolateral membrane surface area with DOCA treatment results in an increase in the number of sodium pumps. Whether this view is supported by similar changes in transport and enzyme activity with dexamethasone treatment has not been established. However, a previous study using methylprednisolone-treated rats found an increase in Na-K-ATPase activity per mg protein in kidney homogenates but no change in ATPase activity per mg protein in a plasma membrane preparation (23). These authors concluded that glucocorticoids increase the amount of cell membrane per tubular mass rather than the quantity of enzyme per membrane area. It is also interesting to note that both the measured basolateral membrane area of PC and the V_{te} for tubules from dexamethasone-treated animals tend to be intermediate in value relative to the control and DOCA groups. Furthermore, in pre-

liminary measurements we have observed a net rate of potassium secretion in tubules from dexamethasone-treated animals of -2.62 ± 0.18 pEq $\text{sec}^{-1} \text{cm}^{-1}$ ($N = 3$, unpublished observations) which is greater than rates observed for control and lower than the rate reported previously for rabbits treated with DOCA for 11–18 d (25). It should be noted, however, that doses of dexamethasone (0.8 mg/d) lower than those used in the present study have not been found to elicit a significant change in V_w , sodium reabsorption, or potassium secretion (27).

While this study correlates the effects of corticosteroid hormones on transport function and membrane area, it has become apparent in recent years that corticosteroid hormones have a wide range of effects on epithelia. A number of changes have been reported in association with effects on net transport such as changes in transepithelial ion fluxes and conductances (13, 25), changes in luminal membrane sodium permeability (3, 4, 29), the induction of specific proteins (19, 28), changes in enzyme activity (18, 20, 22, 31), changes in membrane phospholipids (9, 21), and alterations in the sodium pump (2, 8, 26). Although many of these effects are much more rapid responses to corticosteroid treatment than the chronic response described in this paper, they may be interrelated. For example, increases in membrane area could be secondary to alterations induced on a short-term basis such as an increase in luminal membrane permeability which could affect intracellular ion activity and in turn promote cell membrane development.

The fact that the changes in membrane area reported here are specific for PC does not mean that IC are incapable of adaptive response. Recent observations in the rat indicate that the luminal membrane of IC in medullary collecting ducts can be radically modified by dietary potassium depletion (30).

While more work is required to determine whether the capacity to modulate membrane area is a widespread property of transporting epithelia, recent studies of the rat colonic mucosa and collecting ducts of the renal medulla suggest that the basolateral membrane area of these epithelia can be increased by corticosteroid exposure or variation in the electrolyte composition of an animal's diet (17, 32). These observations, taken together with the present report and findings in other vertebrate epithelia (5, 15, 34, 35), suggest that regulation of membrane surface area may be

an important mechanism for modulating epithelial transport capacity.

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