Enhancement of Electrogenic Na^+ Transport across Rat Inner Medullary Collecting Duct by Glucocorticoid and by Mineralocorticoid Hormones

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Abstract

We have investigated the effect of steroid hormones on Na^+ transport by rat renal inner medullary collecting duct (IMCD) cells. These cells, grown on permeable supports in primary culture, grow to confluence and develop a transmonolayer voltage oriented such that the apical surface is negative with respect to the basal surface. The results of these experiments demonstrate that this voltage is predominantly (or exclusively) the result of electrogenic Na^+ absorption. Na^+ transport can be stimulated two- to fourfold by exposure to either dexamethasone or aldosterone (100 nM). Experiments using specific antagonists of the glucocorticoid and mineralocorticoid receptors indicate that activation of either receptor stimulates electrogenic Na^+ transport; electroneutral Na^+ transport is undetectable. Two other features of the IMCD emerge from these studies. (*a*) These cells appear to have the capacity to metabolize the naturally occurring glucocorticoid hormone corticosterone. (*b*) The capacity for K^+ secretion is minimal and steroid hormones do not induce or stimulate conductive K^+ secretion as they do in the cortical collecting duct. (*J. Clin. Invest.* 1990. 86:497–506.) Key words: aldosterone • cell culture • corticosterone • dexamethasone • ion transport • steroid antagonists

Introduction

The renal inner medullary collecting duct (IMCD)[1] is the last structure within the kidney to modify the composition of the urine. The process by which Na^+ is absorbed by this segment has been the subject of considerable study, and evidence to date suggests that there may be several mechanisms. There is clear evidence from in vivo measurements that Na^+ can be absorbed (1–3), but isolated tubules perfused in vitro have demonstrated only low (if any) rates of transport (4, 5). These low transport rates have made it difficult to be certain that we have a complete understanding of the major mode(s) of Na^+ transport.

The collecting duct has long been recognized as a target for mineralocorticoid hormone. Although its action vis à vis Na^+ transport is heterogeneous along the collecting duct (6), mineralocorticoid hormone may play a role in stimulating Na^+ absorption by the IMCD (7–9). The question of whether steroids play a role in Na^+ transport by this nephron segment has been brought into sharper focus by the recent reports on the transport properties of isolated perfused IMCD segments. When these segments are removed from rats pretreated with mineralocorticoid hormone, the Na^+ transport rate is not significantly different from IMCD segments taken from untreated rats (4, 5, unpublished observations). These results stand in sharp contrast to the effects of mineralocorticoid pretreatment on the Na^+ transport rates of the cortical collecting duct (CCD). In the CCD, prior treatment greatly enhances the rate of Na^+ transport by the isolated perfused tubule (6, 10–13).

As part of an effort to understand the mechanisms of Na^+ transport by the IMCD, we have utilized primary cultures of this segment grown on permeable supports (14). In the present series of experiments we address three questions: (*a*) Do steroid hormones stimulate Na^+ transport? (*b*) Which Na^+ transport process(es) is enhanced? and (*c*) Can more than one steroid receptor be involved in the process?

Methods

Pathogen-free Wistar rats (100–150 g) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The IMCD cells were prepared for primary culture using techniques previously described (14). Briefly, rats were anesthetized with ether and killed by decapitation, and the kidneys were rapidly removed. They were rinsed in a phosphate-buffered saline solution (PBS) which contained 151 mM NaCl, 4.5 mM KH_2PO_4, 2.5 mM NaOH, pH = 7.2. To this PBS rinse we added 2 μg/ml amphotericin B, 500 U/ml penicillin, and 500 μg/ml streptomycin to reduce the risk of fungal and bacterial contamination. The kidneys were then opened with a sterile scalpel and the inner medullae were dissected and minced. The minced tissue was incubated in 0.1% collagenase (Worthington Biochemical Corp., Freehold, NJ) in Kreb's buffer which contained 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.8 mM $MgSO_4$, 1.8 mM KH_2PO_4, and 14 mM glucose. The mixture (two to three papillae per 5 ml) was incubated 2–3 h at 37°C in a 5% CO_2 atmosphere to maintain pH at 7.3. The tissue was agitated at 15-min intervals using a 10-ml pipette during the last 1–1½ h of incubation. The cells were then subjected to hypotonic lysis by adding 2 vol of distilled water, gentle centrifugation, resuspension, and centrifugation in PBS containing 10% albumin, and resuspension in culture medium. This process, using 2–10 papillae is one "isolation."

Cells from the isolation were seeded onto filter-bottom cups at a density of ~ 350,000 cells/cm^2. The cups were constructed by gluing a polycarbonate filter (13 mm, 0.8 μm pore, Nuclepore, Pleasanton, CA, or Poretics, Livermore, CA) to a plastic cylinder (PC-2, ADAPS, Dedham, MA). The filter-bottom cups were sterilized and the filters were coated with collagen as previously described (14). The cylinders were suspended in 24-well tissue culture dishes and both surfaces were bathed in the appropriate medium.

The cells were grown in medium based on a 1:1 mixture of DME and Ham's F-12. The following additions were made: 50 μg/ml genta-

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1. *Abbreviations used in this paper:* CCD, cortical collecting duct; EC_{50}, concentration of an agonist required to produce 50% of its maximal effect; I_{sc}, short-circuit current; IC_{50}, concentration of an inhibitor required to produce 50% of its maximal effect; IMCD, inner medullary collecting duct; R_T, transmonolayer resistance; V_T, transmonolayer voltage.

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micin, 5 pM triiodothyronine, 50 nM hydrocortisone, 5 μg/ml transferrin, 5 μg/ml bovine insulin, 10 nM sodium selenite, and 1% wt/vol bovine albumin (Armour Pharmaceutical Co., Tarrytown, NY). After incubation in this medium for 3 d, the cells were usually confluent (as evidenced by a measurable transmonolayer resistance, R_T) and the medium was changed to one from which albumin and hydrocortisone had been omitted. After 48 h of incubation, the medium was changed to one containing the appropriate concentration of steroid hormone and/or inhibitor, or to a control medium that contained only vehicle (ethanol).

Measurement of the transmonolayer electrical parameters was done under sterile conditions in DME/F-12 medium (without additives). The filter-bottom cups were transferred to a water jacketed (37°C) lucite chamber (Jim's Instruments, Iowa City, IA) where transmonolayer voltage (V_T) was measured and the short-circuit current (I_{sc}) was measured after clamping the V_T to 0 mV (Department of Bioengineering, University of Iowa). The orientation of V_T is with respect to the basolateral solution. A positive I_{sc} is thus equivalent to a flow of positive charge from apical to basolateral solution. The R_T was calculated by imposing a voltage (0.5–5 mV) across the monolayer for 2 s and by dividing the imposed voltage by the resulting change in current. The fluid and filter resistance were subtracted so that the reported R_T represents only that of the cell layer. I_{sc} and R_T are expressed per unit area.

Na^+ uptake across the apical membrane was measured using a slight modification of the general procedures previously reported (14). The filter-bottom cups having confluent cell monolayers were placed in a well of a 24-well tissue culture plate containing uptake medium (37°C) to which furosemide (1 mM) and ouabain (1 mM) had been added. Uptake medium contained 115.2 mM NaCl, 10 mM NaHepes, 10 mM HHepes, 7.8 mM glucose, 5.4 mM tetramethyl ammonium chloride, 1.8 mM $CaCl_2$, 1 mM NaH_2PO_4, 1 mM Na pyruvate, 0.8 mM $MgSO_4$. Uptake medium (200 μl), to which 10 μCi/ml $^{22}Na^+$ (Amersham Corp., Arlington Heights, IL) and 10 μCi/ml dialyzed [^{3}H]inulin (New England Nuclear, Boston, MA) had been added, was placed on the apical side of the monolayer. After incubation for the appropriate amount of time (20–60 s), the monolayer was washed with an ice-cold stop solution of 150 mM tetramethyl ammonium chloride containing 1 mM amiloride, 1 mM ouabain, and 1 mM furosemide. The filter was then rapidly cut from the cup, rinsed again in ice-cold stop solution, and placed in a scintillation vial containing 0.5 ml of 0.1 N HNO_3. After overnight extraction of the radioactivity, 10 ml scintillation fluid was added and the samples were counted. Uptake was corrected for extracellular contamination using the inulin marker. This procedure yields a residual extracellular volume of < 40 nl/cm^2. $^{22}Na^+$ uptake was linear for up to 90 s.

K^+ and Na^+ concentrations in the apical and basolateral solutions were measured by flame photometry. Tissue culture reagents, hormones, and antagonists were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise noted. RU38486 was a generous gift from Roussel Uclaf (Romainville, France). Values are reported as mean±standard error of the mean. Statistical analysis was conducted using paired or unpaired *t* test, or analysis of variance with subsequent application of the Newman-Keuls or Bonferroni test, as indicated. A significant difference was concluded when $P < 0.05$.

Results

Steroid hormone effects on electrical properties. Table I displays the effects of a representative glucocorticoid hormone, dexamethasone, and the prototype mineralocorticoid hormone, aldosterone, on the electrical properties of IMCD monolayers. Also displayed are the effects of other representative steroid hormones: corticosterone, hydrocortisone, and progesterone. No steroid had a significant effect on monolayer resistance. Dexamethasone and aldosterone produced substantial increases in V_T and I_{sc}, whereas the other hormones produced smaller or insignificant effects. The relative effects of each hormone on the I_{sc} are displayed in Fig. 1, where the magnitude of the I_{sc} is normalized to the control value for the same isolation.

Table I. Effect of Steroids on Transmonolayer Electrical Parameters of Cultured IMCD Cells

	Filters; isolations	I_{sc}	Resistance	Voltage
	n	$\mu A/cm^2$	$\Omega \cdot cm^2$	*mV*
Control	77; 11	6.9±0.6	304±19	−3.2±0.3
Dexamethasone	76; 11	23.3±1.7*	325±19	−11.5±1.1*
Aldosterone	77; 11	17.0±1.4*	274±15	−7.9±0.8*
Corticosterone	34; 7	10.2±1.3‡	270±27	−4.1±0.6‡
Hydrocortisone	12; 3	17.4±3.3	210±24	−9.7±2.4
Progesterone	18; 4	9.7±1.2	179±26	−4.4±0.6

Cells grown for 3 d in serum-free medium which contained 1% albumin (see Methods). Albumin and all steroids were removed for 48 h before the addition of the indicated steroid (100 nM). Measurements were made after 24 h of exposure to steroid. * Values larger than control from same isolations, $P < 0.01$; ‡ values larger than control, $P < 0.05$ by ANOVA and Bonferroni test.

Ionic basis for the short circuit current. The fact that aldosterone and dexamethasone increased I_{sc} indicates that ion transport across the monolayer was altered (increased). Based on the well-established action of mineralocorticoid hormones on distal nephron epithelia together with the direction of the I_{sc}, it seemed most likely that Na^+ absorption was being stimulated. To evaluate the extent to which this possibility was correct, we examined the effect of amiloride, an inhibitor of Na^+ channels, on the I_{sc}. Fig. 2 demonstrates the concentration–

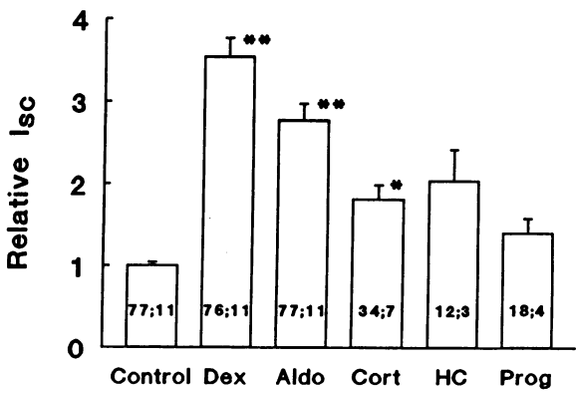

Figure 1. Effect of steroids on I_{sc} of rat IMCD cells in primary culture grown on permeable supports. IMCD cultures were grown for 3 d in albumin media (see Methods) and for 2 d in medium from which albumin and steroid hormones had been removed. On the fifth day, 100 nM dexamethasone (*Dex*), aldosterone (*Aldo*), corticosterone (*Cort*), hydrocortisone (*HC*), or progesterone (*Prog*) were added to the medium, and I_{sc} was measured after 24 h of incubation. Raw data are displayed in Table I and the relative I_{sc} is displayed here corrected for the appropriate control from the same isolation. Numbers in the bars represent number of filters and isolations respectively. **$P < 0.01$ compared with control; *$P < 0.05$ compared with control by ANOVA of log transformed data with Bonferroni test.

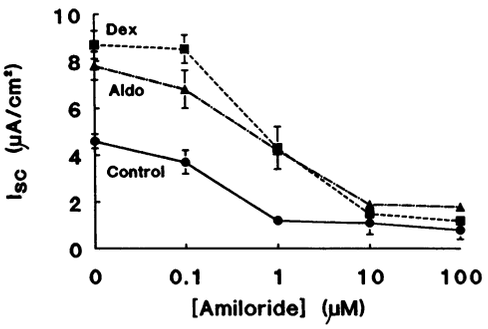

Figure 2. Inhibition of I_{sc} by amiloride. Primary cultures of IMCD cells were prepared as in Fig. 1 and exposed to 100 nM dexamethasone (*Dex*), aldosterone (*Aldo*), or vehicle (*Control*) for 24 h. Amiloride was applied to the luminal surface; addition to the basolateral solution produced no effect. $n = 6$ filters for each group.

response curves. The effect was apparent only when the drug was applied to the apical (luminal) solution; there was no effect of 100 μM amiloride added to the basolateral solution. Concentrations of 10 μM or higher inhibited most of the I_{sc} (except for 1–2 μA/cm²). Although we are not certain of the nature of this amiloride-insensitive I_{sc}, it is completely inhibited by ouabain (2 mM) applied to the basolateral solution (data not shown). No monolayers had a ouabain-insensitive I_{sc}.

The concentration of amiloride required to produce a 50% reduction (IC_{50}) in (amiloride-sensitive) I_{sc} is displayed in Table II. The IC_{50} was the same (~ 0.7 μM) for all groups, suggesting that the kinetic nature of the amiloride inhibition was similar. Table III displays the effects of amiloride on R_T in representative monolayers exposed to dexamethasone or aldosterone. There was a small but statistically significant increase in R_T. The data are consistent with the notion that dexamethasone, aldosterone, and corticosterone stimulate a similar or identical electrogenic Na^+ transport system.

We next conducted a series of experiments aimed at quantitating the extent to which the I_{sc} could be accounted for by Na^+ transport. Fig. 3 demonstrates values for the I_{sc} and the ^{22}Na uptake across the apical membrane measured in the same monolayer. For ease of comparison, both parameters have been expressed in the same flux units. There is good agreement between the two values, irrespective of whether the monolayers had been exposed to dexamethasone, aldosterone, or

Table II. Amiloride Inhibition of the I_{sc}

	IC_{50}	
	Isolation 1	Isolation 2
	μM	
Control	0.13–2.08	0.16–2.14
Dexamethasone	0.33–3.75	0.22–2.69
Aldosterone	0.15–1.53	0.34–3.67
Corticosterone	—	0.19–2.85

Values are 95% confidence limits.
$n = 6$ filters in each group. Combined means of $IC_{50} = 0.72$ μM (95% confidence limits 0.56–0.93 μM). There is no difference between groups by ANOVA.

Table III. Effects of Amiloride and Ba^{2+} on Transmonolayer Resistance

		R_T		
	n	Control	Amiloride	Ba^{2+}
		Ω·cm²		
Dexamethasone	11	418±60	430±61*	422±63
Aldosterone	11	373±30	383±31*	380±32

Monolayers grown as described in Methods, withdrawn from all steroids for 48 h, and then exposed to 100 nM steroid for 24 h. Amiloride (100 μM) and Ba^{2+} (5 mM) applied to the apical solution. * $P < 0.01$ compared with control by paired analysis. There was no effect of Ba^{2+} on resistance.

neither agent. The most straightforward explanation for these data is that most or all of the I_{sc} is accounted for by electrogenic Na^+ transport. The small discrepancy (~ 20%) could be secondary to technical matters involving the precise conditions or to temporal effects on the measurements. There also could be a small I_{sc} that is not related to Na^+ absorption (Fig. 2).

As a further test of the relationship between the I_{sc} and Na^+ uptake via apical membrane Na^+ channels, we conducted additional experiments where the I_{sc} was first measured, and then 50 μM amiloride was applied to the apical surface and ^{22}Na influx was measured. From the data in Fig. 2, we know that 50 μM amiloride has a maximal inhibitory effect on I_{sc}. This concentration, although adequate to inhibit Na^+ channels, is relatively ineffective at inhibiting other Na^+ transporters (15). Fig. 4 shows a representative experiment demonstrating that amiloride blocked all detectable Na^+ uptake irrespective of whether the I_{sc} was unstimulated or stimulated with dexamethasone, aldosterone, or corticosterone. These experiments, when considered together, lead us to conclude that the great majority (if not all) of the I_{sc} is the result of electrogenic Na^+ transport. Furthermore, we can find no measurable Na^+ transport across the apical membrane by an amiloride insensitive (electrically silent) mechanism.

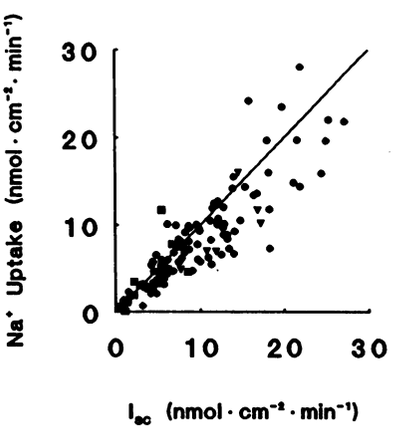

Figure 3. Comparison of I_{sc} and tracer Na^+ uptake across the apical membrane of cultured IMCD cells. Both Na^+ uptake and I_{sc} were measured in the same monolayer and are expressed in the same units of flux. Control (untreated) monolayers ($n = 18$, ■), dexamethasone-treated monolayers ($n = 101$, ●), and aldosterone-treated monolayers ($n = 12$, ▲). The slope of the regression through all points was $y = (0.81 \pm 0.01)x + (0.46 \pm 0.76)$. Line is the line of identity. The slopes of the regression lines for the three groups were not different.

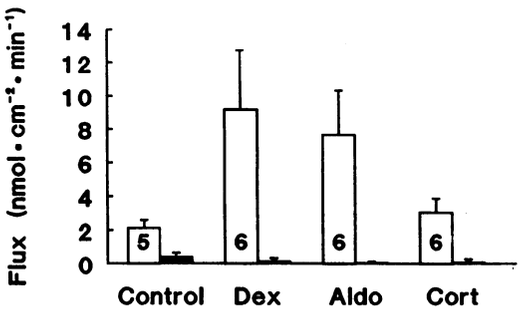

Figure 4. Effect of 50 μM amiloride on Na^+ uptake. Amiloride inhibited Na^+ uptake to values not different from zero in control as well as monolayers treated with 100 nM dexamethasone (*Dex*), aldosterone (*Aldo*), or corticosterone (*Cort*). I_{sc} before treatment with amiloride is shown in the open bars. Na^+ uptake in the presence of amiloride is shown in the solid bars. n = number of filters in this representative isolation.

One of the hallmarks of the CCD is its ability to secrete K^+, a process that is greatly enhanced by mineralocorticoid hormone (10, 12, 13, 16, 17). To evaluate the possibility that the IMCD possessed a similar capability, we performed two sets of experiments. First, we examined the effects of the K^+-channel inhibitor, Ba^{2+}, on R_T. In the CCD, the application of Ba^{2+} to the lumen produces a dramatic increase in R_T, especially in the presence of amiloride (10, 12, 18), owing to blockade of the apical membrane K^+ channels. The results of experiments examining the effect of Ba^{2+} on R_T in IMCD monolayers treated with amiloride are displayed in Table III. Ba^{2+} had no significant effect on R_T. This result makes it unlikely that there is a measurable apical membrane K^+-conductive pathway, even in monolayers stimulated by steroid hormones. However, this result does not eliminate the possibility that K^+ could be secreted by an electrically silent pathway, or a conductive pathway insensitive to Ba^{2+}.

To evaluate the extent to which K^+ is secreted, we measured the K^+ concentration in apical and basolateral solutions 24 h after exposure to dexamethasone, aldosterone, and in control monolayers. We also measured V_T in the same monolayers. Table IV displays the electrical parameters and the apical and basolateral Na^+ and K^+ concentrations for control monolayers and those exposed to steroids for 24 h. Several points are apparent: (*a*) the apical solution Na^+ concentration is lower than the basolateral solution Na^+ concentration; (*b*) the apical solution K^+ concentration is higher than the basolateral solution K^+ concentration; and (*c*) the sum of Na^+ and K^+ concentrations is higher in the basolateral solution than the apical solution; each of these differences is larger in steroid treated monolayers. These apical-to-basolateral gradients can be ascribed qualitatively to electrogenic Na^+ transport which, by virtue of the resultant voltage, raise the apical K^+ concentration. Quantitatively, the apical-to-basolateral concentration differences are small with stimulated monolayers having K^+ gradients of only ~ 1.5 mM. This gradient is substantially smaller than the 5–20 mM gradient readily obtained in stimulated cortical collecting ducts (13, 16). Nevertheless, the measured K^+ gradient is 0.6–0.7 mM larger than can be explained by voltage alone (using the Nernst equation). Taken together, these results indicate that there is little or no apical membrane K^+ conductance, but there may be a small pathway through which K^+ secretion can occur.

Specificity of steroid hormone action. To evaluate the extent to which dexamethasone was acting through glucocorti-

Table IV. Effect of Steroids on Na^+ and K^+ Gradients across Cultured Rat IMCD Cells

	Control	Dexamethasone	Aldosterone
Filters; isolations (*n*)	28; 5	57; 7	51; 6
Resistance ($\Omega \cdot cm^2$)	220±28	280±23	283±27
Voltage (*mV*)	−0.66±0.13	−5.01±0.63	−4.94±0.71
Apical concentration (*mM*)			
Na^+	152.7±3.1	152.9±1.9	152.9±2.0
K^+	4.50±0.07	5.38±0.13	5.32±0.10
Na^+ and K^+	157.2±3.1	158.3±1.9	158.2±2.0
Basolateral concentration (*mM*)			
Na^+	155.7±3.3	159.7±1.9	159.7±2.0
K^+	4.14±0.08	3.83±0.08	3.86±0.08
Na^+ and K^+	159.9±3.4	163.5±1.9	163.6±2.1
Apical − basolateral (*mM*)			
Na^+	−3.0±0.7	−6.7±0.6	−6.8±0.6
K^+	0.35±0.04	1.55±0.18	1.46±0.16
Na^+ and K^+	−2.7±0.7	−5.2±0.5	−5.4±0.6
Apical/basolateral			
Na^+ (measured)	0.981±0.004	0.958±0.004	0.957±0.004
K^+ (measured)	1.090±0.012	1.467±0.066	1.426±0.055
Na^+ or K^+ (expected from voltage)	1.026±0.005	1.241±0.035	1.243±0.045
Expected apical [K^+] from voltage and basolateral [K^+] (*mM*)	4.25±0.09	4.67±0.10	4.71±0.14
Measured − expected [K^+] (*mM*)	0.24	0.71	0.61

Na^+ and K^+ concentrations measured in apical and basolateral solutions after 24 h of exposure to the indicated steroid (100 nM) or control. Expected apical [K^+] calculated using the Nernst equation.

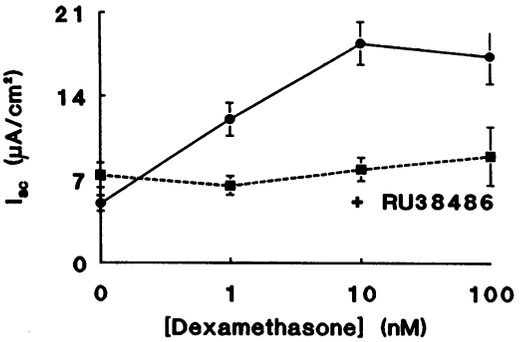

Figure 5. Dexamethasone concentration–response curve. Solid line represents the I_{sc} of monolayers treated with a concentration of dexamethasone indicated on the abscissa. Dashed line indicates the I_{sc} of monolayers that, in addition, were incubated with the glucocorticoid antagonist RU38486. The concentration of RU38486 was 1 μM except when dexamethansone concentration was 100 nM where the RU38486 concentration was 10 μM. n = 18 monolayers for each group.

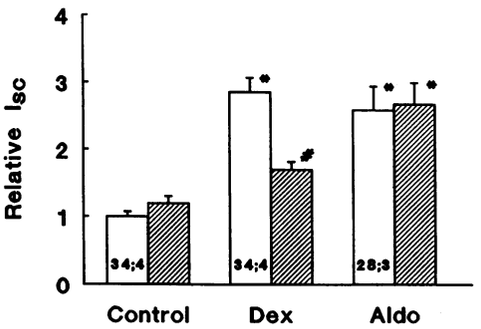

Figure 7. Effect of the glucocorticoid antagonist RU38486 on steroid-stimulated I_{sc}. Dexamethasone (*Dex*) or aldosterone (*Aldo*) applied at concentrations of 100 nM increased the relative I_{sc}. Values in bars indicate number of monolayers and isolations respectively for each group (Control, Dex, Aldo). Open bars are the control or steroid-treated groups; hatched bars are monolayers to which RU38486 was added. 10 μM RU38486 had no effect on the I_{sc} when applied alone. RU38486 had no effect on aldosterone-treated monolayers. *, $P < 0.01$ compared with control; #, $P < 0.05$ compared with DEX alone by ANOVA with Bonferroni test.

coid receptors and aldosterone through mineralocorticoid receptors, we examined concentration–response relationships and used specific antagonists of the respective receptors. Fig. 5 shows that dexamethasone produced a concentration-dependent increase in I_{sc} which appeared to be maximal at 10 nM. The concentration necessary to produce 50% of maximal stimulation (EC_{50}) was < 1 nM. This value is in good agreement with generally recognized values for the K_d for dexamethasone binding to the glucocorticoid receptor (19, 20). The glucocorticoid antagonist RU38486 (21) at a concentration of 1 μM produced no significant effect on the I_{sc}. In concentrations of at least 100-fold excess, RU38486 prevented the stimulation of I_{sc} produced by dexamethasone (Fig. 5).

The concentration response of the I_{sc} to aldosterone is shown in Fig. 6. Although aldosterone produced a clear stimulation of I_{sc} at a concentration of 1 nM and greater, the value did not plateau between 10 and 100 nM as it did with dexamethasone. Thus, we were not able to calculate an exact EC_{50} value. The reason for this apparent unsaturation is not clear.

The next series of experiments was designed to determine whether aldosterone might be producing a portion of its effect by binding to glucocorticoid receptors. Fig. 7 demonstrates

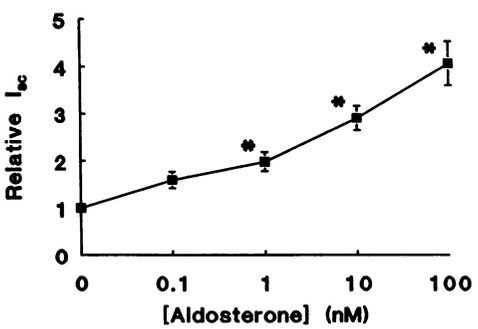

Figure 6. Concentration response to aldosterone. The I_{sc} is plotted relative to the control values for each isolation (n = 18 filters from three isolations for each point). *$P < 0.05$ compared with control by ANOVA of log transformed data with Bonferroni test.

that the glucocorticoid antagonist RU38486 (10 μM) had no effect on the ability of 100 nM aldosterone to stimulate I_{sc}. In this series of experiments, as in those displayed in Fig. 5, RU38486 alone had no effect on I_{sc}, but produced a significant inhibition of the effect of 100 nM dexamethasone.

We evaluated the possibility that dexamethasone could be producing a portion of its effect by interacting (partially) with the mineralocorticoid receptor by using the mineralocorticoid antagonist spironolactone. Fig. 8 *a* demonstrates that, as expected, 10 μM spironolactone inhibited the aldosterone stimulation of the I_{sc}. It also demonstrates that spironolactone produced a modest stimulation of the I_{sc} compared with control. This mild agonist capability has been previously described for

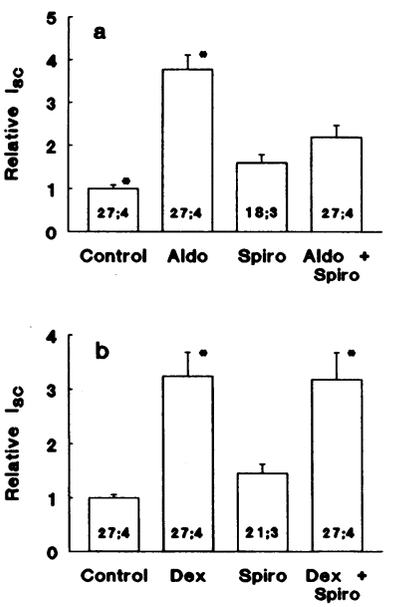

Figure 8. Effects of 10 μM spironolactone (*Spiro*) on steroid-stimulated I_{sc}. (*a*) Aldosterone (*Aldo;* 100 nM) or spironolactone, either alone or in combination, were incubated with IMCD monolayers. *, $P < 0.05$ vs. all other groups by ANOVA and subsequent Newman-Kuel's test. (*b*) similar experiments conducted with dexamethasone (*Dex,* 100 nM) and spironolactor alone and in combination. Values in bars indicate the number of filters and isolations, respectively. *, $P < 0.05$ vs. control or spironolactone by ANOVA and subsequent Newman-Kuel's test.

spironolactone (22) and may explain, in part, the lack of a complete blockade by spironolactone of the aldosterone-stimulated I_{sc}.

The effect of spironolactone on the dexamethasone-stimulated I_{sc} is displayed in Fig. 8 *b*. Spironolactone produced no detectable reduction in the I_{sc} stimulated by dexamethasone. These results provide evidence that supports the idea that dexamethasone stimulates I_{sc} predominantly or exclusively via interaction with the glucocorticoid receptor. As a corollary, these data do not support the idea that dexamethasone produces its effect via binding to mineralocorticoid receptors.

Evidence for metabolism of corticosterone. The experiments displayed in Fig. 1 show that 24-h exposure to corticosterone produces a smaller stimulation of I_{sc} than does dexamethasone or aldosterone. One possible explanation for this phenomenon is that, in contrast to the other two steroids, corticosterone is extensively metabolized by the IMCD cells to an inactive compound(s). Such metabolism has been recently demonstrated in kidney tissue (23) and in toad urinary bladder (24, 25). In these tissues, metabolism of corticosterone can be substantially slowed by adding an inhibitor of 11-OH steroid dehydrogenase. We tested the effect of such an inhibitor, 10 μM glycyrrhetinic acid, on the ability of corticosterone to stimulate I_{sc}. As displayed in Fig. 9, corticosterone alone stimulated I_{sc} modestly, consistent with the results displayed in Fig. 1. Glycyrrhetinic acid alone had no stimulatory effect, but when combined with corticosterone, the stimulatory effect was comparable to that produced by dexamethasone or aldosterone. These results are consistent with the idea that the metabolism of corticosterone reduces its ability to stimulate I_{sc}, and that inhibition of corticosterone metabolism produces an enhanced ability to stimulate I_{sc}.

Discussion

Mechanism of Na^+ transport. The present results demonstrate that Na^+ absorption by primary cultures of rat IMCD cells is predominantly, if not exclusively, electrogenic. Conversely, most, if not all, of the I_{sc} is caused by Na^+ transport; other currents are small or absent. In contrast to the CCD, active K^+ secretion is small and there is no detectable Ba^{2+}-sensitive K^+ conductance on the apical membrane.

These cells thus appear to transport Na^+ in the fashion described by Koefoed-Johnsen and Ussing (26) in the frog skin. In this process, Na^+ enters the cell via an apical membrane channel and is extruded by the basolateral membrane Na^+–K^+ ATPase. The frog skin (26, 27), toad urinary bladder (28), rabbit urinary bladder (29), and the turtle colon (30) exemplify this simple type of electrogenic Na^+ transport. The kidney distal nephron, and particularly the collecting duct, also possess an electrogenic Na^+ transport system but the processes are more complex. For example, the rat distal nephron has an electroneutral NaCl cotransport system in addition to an electrogenic system (31). The CCD contains an electrogenic H^+ secretory system (32), and a large apical membrane K^+ conductance (10, 12, 33–35), both of which contribute to the measured I_{sc}. The absence of these confounding electrogenic transport systems in this model of the IMCD render the interpretation of the changes in I_{sc} straightforward.

The lack of an apical membrane K^+ transport pathway in the IMCD suggests that, in contrast to the CCD, regulation of Na^+ transport serves primarily to regulate Na^+ homeostasis. In the CCD, altered Na^+ transport is usually coupled to alterations in K^+ secretion (6). The deduction that Na^+ absorption and not K^+ secretion is the major transport system is consistent with results obtained using in vivo measurements (1–3, 8), and in vitro perfused IMCD (5) experiments. The lack of apical membrane K^+ channels in the IMCD (as opposed to the CCD) is not likely explained by technical differences relating to cultured cells. In this regard, Naray-Fejes-Toth (36, 37) has demonstrated steroid hormone stimulation of K^+ secretion in cultured CCD cells. Thus, the available data support the idea that a major difference between the CCD and the IMCD relates to their capacity to secrete K^+.

The present results also address a previous uncertainty regarding the mechanism of Na^+ entry across the apical membrane. Previous results from this laboratory have suggested that there is more than one such mechanism (14). The differences between the previous and present results probably relate to (*a*) the large difference in the rate of Na^+ transport, (*b*) differences between the species of rat used to obtain the primary cultures, and (*c*) differences in the technique used in the Na^+ uptake measurement. The deduction that there is an amiloride-sensitive Na^+ channel on the apical membrane is in good agreement with a considerable body of data utilizing electrophysiological (5, 38), immunocytochemical (39), and tracer uptake (40) techniques. However, there have been several studies that suggest other mechanisms of Na^+ uptake. Such mechanisms include Na^+/H^+ exchange (41–43) and $Na^+/K^+/2Cl^-$ cotransport (44–46). Our results indicate that these transport processes are small or absent in the apical membrane of cultured IMCD cells. We presume that if they are present in the cultured IMCD cell they are on the basolateral membrane, a deduction supported by preliminary data from other laboratories (47, 48).

Steroid hormone effects. Na^+ transport by IMCD cells can be stimulated by either dexamethasone or aldosterone. There is precedent for such an effect of aldosterone in vivo (7) and recent evidence indicates that IMCD Na^+/K^+ ATPase activity is stimulated by dietary NaCl restriction and by injection of mineralocorticoid hormone (9). Despite this evidence supporting a role for mineralocorticoid hormone stimulation of Na^+ transport by IMCD, efforts to demonstrate such a stimulation in IMCDs dissected from desoxycorticosterone (DOC)-treated rats and perfused in vitro have been unsuccessful (4, 5, unpublished observations). The reason(s) for the discrepancy is (are) not clear. Extensive experimentation with both rat and rabbit CCDs perfused in vitro has demonstrated that pretreatment of the animal with desoxycorticosterone greatly stimulates Na^+ transport by that segment (6, 11, 13, 49). The failure to demonstrate stimulation by the IMCD using similar proto-

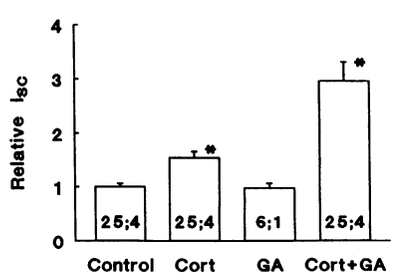

Figure 9. Effect of 10 μM glycyrrhetinic acid (*GA*) on I_{sc}. Monolayers were treated with either corticosterone (Cort) 100 nM or glycyrrhetinic acid alone or in combination. *$P < 0.05$ vs. all other groups by ANOVA and Newman-Kuel's test.

cols is, at present, unexplained. The present data indicate that the cellular machinery enabling the IMCD cell to respond to mineralocorticoid hormone is present. The simplest possibility is that some specific condition(s) present in vivo renders the IMCD resistant to mineralocorticoid hormone.

That dexamethasone stimulates electrogenic Na^+ transport in the kidney is not generally recognized. Dexamethasone, in contrast to mineralocorticoid hormone, does not alter the electrophysiological properties of the CCD (12) or the rate of Na^+ transport (13). Neither does it increase citrate synthase activity (50) nor Na^+-K^+ ATPase activity (51, 52). In contrast to this data suggesting no effect of dexamethasone on electrogenic Na^+ transport by the kidney, there is some evidence that there might be an effect. Wade et al. (53) determined that dexamethasone increased the basolateral membrane area of CCD principal cells. This effect is similar to that produced by mineralocorticoid hormone and is generally believed to accompany the increased Na^+-K^+ ATPase activity that develops with an enhancement in Na^+ transport. Recent preliminary experiments have shown a dexamethasone stimulation of Na^+ transport by CCD cells in primary culture (37). Taken together, the data suggest that dexamethasone could have an effect on electrogenic Na^+ transport by the CCD, but that the expression of its effect may be regulated by conditions that are currently unknown.

In contrast to the poorly understood effects of dexamethasone on renal Na^+ transport, there is considerably more data regarding the effects of dexamethasone on Na^+ transport by the colon. Three important concepts emerge from this literature: (*a*) Na^+ transport by the colon occurs by more than one transport system (54); (*b*) both mineralocorticoid and glucocorticoid hormones can alter Na^+ transport (55); and (*c*) different steroid hormone effects can be mediated via specific glucocorticoid and mineralocorticoid receptors (55, 56). The notion is evolving that electrogenic Na^+ transport in the colon is stimulated via mineralocorticoid receptors and that electroneutral Na^+ transport is stimulated via glucocorticoid receptors (57, 58).

The present data strongly support the idea that in the IMCD, in contrast to the colon, activation of the glucocorticoid receptor can stimulate electrogenic Na^+ transport. The dose response for the dexamethasone effect (Fig. 5) is close to that predicted from the kinetics of dexamethasone binding to the glucocorticoid receptor (19, 20); the dexamethasone effect is blocked by the glucocorticoid receptor antagonist RU38486 (21) (Figs. 5 and 7); and, importantly, the dexamethasone effect is not inhibited by the mineralocorticoid receptor antagonist, spironolactone (Fig. 8 *b*). Two features deserve emphasis: (*a*) "crossover" activation of mineralocorticoid receptors does not seem to be necessary for the glucocorticoid effect (59), and (*b*) there is no detectable development of apical membrane Na^+-H^+ exchange, as occurs in the frog distal nephron (60) and the colon (57, 58).

The present data also strongly support the notion that binding of agonist to mineralocorticoid hormone receptors is sufficient to stimulate electrogenic Na^+ transport. Crossover binding to glucocorticoid receptors does not seem to be required for the stimulation of Na^+ transport, as the glucocorticoid antagonist had no effect on the aldosterone-stimulated I_{sc} (Fig. 7).

Thus, our data indicate that binding of the appropriate agonists to either the glucocorticoid or the mineralocorticoid receptor can stimulate electrogenic Na^+ transport. Several points serve to place these observations in perspective. First, the demonstration that a single class of steroid receptor can stimulate Na^+ transport does not mean that mineralocorticoid or glucocorticoid hormones produce their effects only via a single class of receptors in vivo. To the contrary, there is evidence from the toad bladder that some of aldosterone's effects may be the result of binding to a second (non-mineralocorticoid) class of receptors (59). Secondly, the present experiments do not address the question of how each receptor might stimulate the various components of electrogenic Na^+ transport. We infer from the results that the rate of Na^+ entry across the apical membrane is enhanced. However, we do not as yet know the mechanism of this increased permeability. Neither do we know whether the other components of Na^+ transport, the Na^+-K^+ ATPase and metabolic capacity, are also stimulated (61). There may be important differences in the mechanism of action of these steroid hormones.

Finally, the results that suggest steroid hormone metabolism (Fig. 9) raise the possibility that the steroid hormone regulation of Na^+ transport in this tissue may be more complex than previously envisioned. If the IMCD cells contain the enzymes to metabolize the naturally occurring "glucocorticoid hormones," then corticosterone metabolism becomes a potentially important variable in the regulation of Na^+ transport. The regulation of corticosterone metabolism may have implications pertaining not only to the specific actions of corticosterone and aldosterone on these cells, but also to the possible effects of its metabolites.

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